



**Sustainable Development of
Algal Biofuels
in the United States**

THE NATIONAL ACADEMIES PRESS

Sustainable Development of Algal Biofuels in the United States

Committee on the Sustainable Development of Algal Biofuels

Board on Agriculture and Natural Resources
Division on Earth and Life Studies

Board on Energy and Environmental Systems
Division on Engineering and Physical Sciences

NATIONAL RESEARCH COUNCIL
OF THE NATIONAL ACADEMIES

THE NATIONAL ACADEMIES PRESS
Washington, D.C.
www.nap.edu

THE NATIONAL ACADEMIES PRESS

500 Fifth Street, NW

Washington, DC 20001

NOTICE: The project that is the subject of this report was approved by the Governing Board of the National Research Council, whose members are drawn from the councils of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine. The members of the committee responsible for the report were chosen for their special competences and with regard for appropriate balance.

This study was supported by Contract/Grant No. DE-DT0001899 between the National Academy of Sciences and the Department of Energy. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the views of the organizations or agencies that provided support for the project.

International Standard Book Number 13: 978-0-309-26032-9

International Standard Book Number 10: 0-309-26032-9

Additional copies of this report are available for sale from the National Academies Press, 500 Fifth Street, NW, Keck 360, Washington, DC 20001; (800) 624-6242 or (202) 334-3313; <http://www.nap.edu/>.

Cover: Design by Anne Rogers. Photo courtesy of Sammy Boussiba, J. Blaustein Institute for Desert Research, Sde-Boker.

Copyright 2012 by the National Academy of Sciences. All rights reserved.

Printed in the United States of America

THE NATIONAL ACADEMIES

Advisers to the Nation on Science, Engineering, and Medicine

The **National Academy of Sciences** is a private, nonprofit, self-perpetuating society of distinguished scholars engaged in scientific and engineering research, dedicated to the furtherance of science and technology and to their use for the general welfare. Upon the authority of the charter granted to it by the Congress in 1863, the Academy has a mandate that requires it to advise the federal government on scientific and technical matters. Dr. Ralph J. Cicerone is president of the National Academy of Sciences.

The **National Academy of Engineering** was established in 1964, under the charter of the National Academy of Sciences, as a parallel organization of outstanding engineers. It is autonomous in its administration and in the selection of its members, sharing with the National Academy of Sciences the responsibility for advising the federal government. The National Academy of Engineering also sponsors engineering programs aimed at meeting national needs, encourages education and research, and recognizes the superior achievements of engineers. Dr. Charles M. Vest is president of the National Academy of Engineering.

The **Institute of Medicine** was established in 1970 by the National Academy of Sciences to secure the services of eminent members of appropriate professions in the examination of policy matters pertaining to the health of the public. The Institute acts under the responsibility given to the National Academy of Sciences by its congressional charter to be an adviser to the federal government and, upon its own initiative, to identify issues of medical care, research, and education. Dr. Harvey V. Fineberg is president of the Institute of Medicine.

The **National Research Council** was organized by the National Academy of Sciences in 1916 to associate the broad community of science and technology with the Academy's purposes of furthering knowledge and advising the federal government. Functioning in accordance with general policies determined by the Academy, the Council has become the principal operating agency of both the National Academy of Sciences and the National Academy of Engineering in providing services to the government, the public, and the scientific and engineering communities. The Council is administered jointly by both Academies and the Institute of Medicine. Dr. Ralph J. Cicerone and Dr. Charles M. Vest are chair and vice chair, respectively, of the National Research Council.

www.national-academies.org

COMMITTEE ON THE SUSTAINABLE DEVELOPMENT OF ALGAL BIOFUELS

JENNIE C. HUNTER-CEVERA, *Chair*, Hunter and Associates, Ellicott City, Maryland
SAMMY BOUSSIBA, J. Blaustein Institute for Desert Research, Sde-Boker
JOEL L. CUELLO, The University of Arizona, Tucson
CLIFFORD S. DUKE, Ecological Society of America, Washington, DC
REBECCA A. EFROYMSON, Oak Ridge National Laboratory, Tennessee
SUSAN S. GOLDEN, University of California, San Diego
JENNIFER HOLMGREN, Lanzatech, Roselle, Illinois
DONALD L. JOHNSON, Grain-Processing Corporation (retired), Muscatine, Iowa
MARK E. JONES, The Dow Chemical Company, Midland, Michigan
VAL H. SMITH, The University of Kansas, Lawrence
CAI STEGER, Natural Resources Defense Council, New York
GREGORY N. STEPHANOPOULOS, Massachusetts Institute of Technology,
Massachusetts
LARRY P. WALKER, Cornell University, Ithaca, New York
ERIC WILLIAMS, Rochester Institute of Technology, New York
PAUL V. ZIMBA, Texas A&M University, Corpus Christi

Staff

EVONNE P.Y. TANG, Study Codirector
K. JOHN HOLMES, Study Codirector
RUTH S. ARIETI, Research Associate
KATHLEEN REIMER, Senior Program Assistant
ROBIN A. SCHOEN, Director
JAMES ZUCCHETTO, Director

BOARD ON AGRICULTURE AND NATURAL RESOURCES

NORMAN R. SCOTT, *Chair*, Cornell University, Ithaca, New York (*Emeritus*)
PEGGY F. BARLETT, Emory University, Atlanta, Georgia
HAROLD L. BERGMAN, University of Wyoming, Laramie
RICHARD A. DIXON, Samuel Roberts Noble Foundation, Ardmore, Oklahoma
DANIEL M. DOOLEY, University of California, Oakland
JOAN H. EISEMANN, North Carolina State University, Raleigh
GARY F. HARTNELL, Monsanto Company, St. Louis, Missouri
GENE HUGOSON, Global Initiatives for Food Systems Leadership, St. Paul, Minnesota
MOLLY M. JAHN, University of Wisconsin-Madison
ROBBIN S. JOHNSON, Cargill Foundation, Wayzata, Minnesota
A.G. KAWAMURA, Solutions from the Land, Washington, DC
JULIA L. KORNEGAY, North Carolina State University, Raleigh
KIRK C. KLASING, University of California, Davis
VICTOR L. LECHTENBERG, Purdue University, West Lafayette, Indiana
PHILIP E. NELSON, Purdue University, West Lafayette, Indiana
KEITH PITTS, Marrone Bio Innovations, Davis, California
CHARLES W. RICE, Kansas State University, Manhattan
HAL SALWASSER, Oregon State University, Corvallis
ROGER A. SEDJO, Resources for the Future, Washington, DC
KATHLEEN SEGERSON, University of Connecticut, Storrs
MERCEDES VAZQUEZ-AÑON, Novus International, Inc., St. Charles, Missouri

Staff

ROBIN A. SCHOEN, Director
CAMILLA YANDOC ABLES, Program Officer
RUTH S. ARIETI, Research Associate
KAREN L. IMHOF, Administrative Coordinator
KARA N. LANEY, Program Officer
JANET M. MULLIGAN, Senior Program Associate for Research
KATHLEEN REIMER, Senior Program Assistant
EVONNE P.Y. TANG, Senior Program Officer
PEGGY TSAI, Program Officer

BOARD ON ENERGY AND ENVIRONMENTAL SYSTEMS

ANDREW BROWN, JR., *Chair*, Delphi Corporation, Troy, Michigan
WILLIAM F. BANHOLZER, The Dow Chemical Company, Midland, Michigan
MARILYN BROWN, Georgia Institute of Technology, Atlanta
WILLIAM CAVANAUGH III, Progress Energy (retired), Raleigh, North Carolina
PAUL DeCOTIS, Long Island Power Authority, Albany, New York
CHRISTINE EHLIG-ECONOMIDES, Texas A&M University, College Station
SHERRI GOODMAN, CNA, Alexandria, Virginia
NARAIN HINGORANI, Independent Consultant, Los Altos Hills, California
ROBERT HUGGETT, Independent Consultant, Seaford, Virginia
DEBBIE NIEMEIER, University of California, Davis
DANIEL NOCERA, Massachusetts Institute of Technology, Cambridge
MICHAEL OPPENHEIMER, Princeton University, New Jersey
DAN REICHER, Stanford University, California
BERNARD ROBERTSON, Daimler-Chrysler (retired), Bloomfield Hills, Michigan
GARY ROGERS, FEV, Inc, Auburn Hills, Michigan
ALISON SILVERSTEIN, Consultant, Pflugerville, Texas
MARK THIEMENS, University of California, San Diego
RICHARD WHITE, Oppenheimer & Company, New York City

Staff

JAMES ZUCCHETTO, Director
DANA CAINES, Financial Associate
ALAN CRANE, Senior Program Officer
K. JOHN HOLMES, Senior Program Officer
LANITA JONES, Administrative Coordinator
ALICE WILLIAMS, Senior Program Assistant
JONATHAN YANGER, Senior Project Assistant

Preface

The desire to develop energy sources that can provide greater environmental and security benefits has spurred research and investments in the development of alternatives to petroleum, the dominant source of liquid transportation fuels. Because of its high biomass (and oil productivity in some cases), algae and cyanobacteria (commonly referred to as blue-green algae) frequently have been considered a promising renewable feedstock for fuel production. We all were taught that petroleum and other fossil fuels formed on this planet from plant remains that were compressed for millions of years at high temperatures. It seems fitting that scientists would choose to study some of the most primitive life forms to develop large-scale biofuel replacements for such fossil fuels. Algae have been grown under a variety of conditions for the production of lipids and high-value products for several decades. Two factors that influenced the consideration of algal biofuel production in the past were the cost of a barrel of oil and the ability to cultivate algae and process them into transportation fuel at a reasonable cost. The U.S. Department of Energy (DOE) had a robust program to develop biofuels from algae from 1978 to 1996, when it was concluded that algal biofuel would not be cost competitive with petroleum soon. Fast forward to 2012, and with advances in genetics and engineering, we are back to the future in considering whether algae can be an economic and sustainable alternative source of liquid transportation fuels. Could it be that use of algae to produce biofuels is the answer to becoming less dependent on foreign oil?

At the request of DOE, the National Research Council (NRC) appointed a committee of 15 experts with diverse backgrounds and experience to examine the sustainability of algal biofuels. The committee reviewed many scientific papers and government and industry reports, and listened first hand to company representatives, academic experts, and government agency program managers who deal with production of algal biofuels. The committee also met three times and held regularly scheduled conference calls to deliberate and reach agreement as to how to best address the charge from DOE to identify potential sustainability concerns, mitigate environmental concerns, and identify indicators of sustainability and metrics that could be used to monitor progress as the technology advances on several fronts.

In its consideration of the task, the committee examined the algal biofuel supply chain from the characteristics of the species to the methods for cultivation and processing into fuels. It separated the potential pathways for deployment into four basic scenarios and used those scenarios to help assess the resource needs and environmental concerns resulting from the location and design of large-scale production. The outcome of the current knowledge available through literature and discussion by the committee is this report on sustainable development of algal biofuels. This report does not address economic analyses or comparative life-cycle analyses. However, it provides a framework for assessing sustainability as the DOE continues to invest in algal biofuel research and development.

I thank the committee members and NRC staff for the very stimulating and thought-provoking dialogue and for their many contributions to the writing of this report.

Jennie C. Hunter-Cevera
*Chair, Committee on Sustainable
Development of Algal Biofuels*

Acknowledgments

This report has been reviewed in draft form by persons chosen for their diverse perspectives and technical expertise in accordance with procedures approved by the National Research Council's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making its published report as sound as possible and to ensure that the report meets institutional standards of objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. We wish to thank the following for their review of this report:

Brenda Little, Naval Research Laboratory
James R. Katzer, Exxon Mobil Research and Engineering Company (retired)
Qiang Hu, Arizona State University
Paul DeCotis, Long Island Power Authority
Andres Clarens, University of Virginia
Paul Roessler, Algenol, LLC
Amha Belay, Earthrise Nutritional, LLC
LaReesa Wolfenbarger, University of Nebraska, Omaha
Jason Hill, University of Minnesota
Tryg Lundquist, California Polytechnic State Institute
Christopher R. Somerville, University of California, Berkeley, and Energy Biosciences Institute
Robert Haselkorn, University of Chicago
Barry Solomon, Michigan Technology University

Although the reviewers listed above have provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations, nor did they see the final draft of the report before its release. The review of this report was overseen

by coordinator, George M. Hornberger, appointed by the Division of Earth and Life Studies, and monitor, Mark R. Cullen, appointed by the NRC's Report Review Committee. The coordinator and monitor were responsible for making certain that an independent examination of this report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests entirely with the author committee and the institution.

Contents

SUMMARY	1
1 INTRODUCTION	11
Interest in Algal Biofuels, 11	
Sustainable Development of Biofuels, 13	
Tools and Methodologies for Assessing Sustainable Development of Algal Biofuels, 17	
Study Scope and Approach, 22	
Structure of Report, 23	
References, 23	
2 OVERVIEW OF ALGAL BIOFUEL SUPPLY CHAIN	27
Algal Feedstocks, 27	
Cultivation, 41	
Processing Algal Biomass into Fuels, 58	
Conclusions, 64	
References, 66	
3 PATHWAYS FOR ALGAL BIOFUEL PRODUCTION	77
Features of Biofuel Pathways, 78	
Reference Pathway–Raceway Pond Producing Drop-in Hydrocarbon, 80	
Alternative Pathway #1–Raceway Pond Producing Drop-in Hydrocarbon and Coproducts, 83	
Alternative Pathway #2–Raceway Pond Producing FAME, 86	
Alternative Pathway #3–Photobioreactors with Direct Synthesis of Ethanol, 88	
Alternative Pathway #4–Whole-Cell Processing, 92	
Other Potential Pathways, 95	

	Summary, 96	
	References, 96	
4	NATURAL RESOURCE USE	99
	Water, 100	
	Nutrients, 110	
	Land, 117	
	Energy, 126	
	Conclusions, 131	
	References, 133	
5	ENVIRONMENTAL EFFECTS	139
	Water Quality, 140	
	Land-Use Change, 147	
	Greenhouse-Gas Emissions, 151	
	Local Climate, 154	
	Air Quality, 154	
	Species Invasiveness and Aquatic Biodiversity, 159	
	Terrestrial Biodiversity, 164	
	Environmental Effects of Genetically Engineered Organisms, 168	
	Waste Products, 172	
	Pathogens and Toxins, 174	
	Mosquito-Borne Diseases, 177	
	Conclusions, 178	
	References, 180	
6	A FRAMEWORK TO ASSESS SUSTAINABLE DEVELOPMENT OF ALGAL BIOFUELS	191
	Summary of Resource Use and Environmental Effects of Different Algal Biofuel Production Pathways, 192	
	Tools for Assessing Overall Sustainability, 194	
	Framework for Integrated Assessment, 199	
	Opportunities for Algal Biofuels to Improve Sustainability, 203	
	References, 204	
APPENDIXES		
A	Biographical Sketches of Committee Members	209
B	Statement of Task	215
C	Presentations to the Committee	217
D	Glossary	221
E	Select Acronyms and Abbreviations	223
F	Conversion Factors	225
G	Economics of Coproduct Production from Large-Scale Algal Biofuel Systems	227

Summary

Domestic production of renewable fuels, including algal biofuels, has the potential to meet the dual goals of improving energy security and decreasing greenhouse-gas (GHG) emissions from the transportation sector in the United States. Biofuels produced from microalgae and cyanobacteria¹ offer potential advantages over terrestrial plant-based biofuels, such as high biomass productivity and the ability to grow in cultivation ponds or photobioreactors on non-arable lands using saline water or wastewater sources. However, along with potential environmental and social benefits, production of algal biofuels could result in significant resource inputs and in negative environmental and other detrimental effects, as is true of all forms of energy production.

At the request of the Department of Energy, Office of Energy Efficiency and Renewable Energy's (DOE-EERE) Office of Biomass Program, the National Research Council (NRC) convened a committee of 15 experts to examine the sustainable development of algal biofuels. (See Appendix A for biographical sketches of committee members.) The purpose of this study was to identify and anticipate potential sustainability concerns associated with a selected number of pathways for large-scale deployment of algal biofuels, discuss potential strategies for mitigating those concerns, and suggest indicators and metrics that could be used and data to be collected for assessing sustainability across the biofuel supply chain to monitor progress as the industry develops. (See Appendix B for the complete statement of task.) In addition, the committee was asked to identify indicators that are most critical to address or have the greatest potential for improvement through DOE intervention and to suggest preferred cost-benefit analyses that could best aid in the decision-making process.

"Sustainable development is development that meets the needs of the present without compromising the ability of future generations to meet their own needs."² The sustainability

¹Referred to as algal biofuels hereafter. Cyanobacteria, historically known as blue-green algae, are prokaryotes whereas algae are eukaryotes.

²Definition from United Nations. 1987. Report of the World Commission on Environment and Development: Our common future. Available online at <http://www.un-documents.net/ocf-02.htm>. Accessed August 21, 2012.

goals for developing algal biofuels are to contribute to energy security by providing domestically sourced fuels, to maintain and enhance the natural resource base and environmental quality, to produce fuel that is economically viable, and to enhance the quality of life for society as a whole. Although economics is an important aspect of sustainability, this report does not assess the economics or costs of algal biofuels, as specified in the statement of task. Heterotrophic approaches³ for algae cultivation are not considered in this report because DOE-EERE considers the production of biofuel using heterotrophic algae a biochemical pathway to convert another feedstock (a sugar source such as cellulosic biomass) rather than a pathway that uses algae as a feedstock for fuels.

The intent of this report is to help anticipate the major sustainability concerns associated with resource use and the potential environmental and societal consequences if commercial-scale algal biofuel production is widely deployed and to explore the opportunities for mitigating the concerns. However, the ultimate productivity of algal biofuels, some of their resource use and environmental concerns, and some strategies for mitigating the concerns might affect the economic viability of algal biofuels. This report makes reference to economics if there are synergies or trade-offs among economics, productivity, resource use, and environmental effects. This report also discusses tools for assessing the multi-attribute nature of sustainability of algal biofuels.

POTENTIAL SUSTAINABILITY CONCERNS

Assessing the sustainability of an algal biofuel requires an understanding of the individual components that make up an algal biofuel production system. An algal biofuel production system involves cultivating selected strain(s) of algae; collecting the biomass and dewatering it, if necessary; and processing the algal lipid, biomass, or secreted products into fuels and possibly other co-products. The production of fuels and energy from algae is not an established industry and a variety of production systems have been proposed. Figure S-1 is a simplified diagram that attempts to limit and group the potential steps in the algal biofuel production pathway. Each row of the diagram details a processing step or process option. Different combinations of cultivation and processing options have resulted in more than 60 different proposed pathways for producing algal biofuels.

Based on a review of literature published until the authoring of this report, the committee concluded that the scale-up of algal biofuel production sufficient to meet at least 5 percent of U.S. demand for transportation fuels⁴ would place unsustainable demands on energy, water, and nutrients with current technologies and knowledge. However, the potential to shift this dynamic through improvements in biological and engineering variables exists.

For some system designs analyzed, the energy outputs of algal biofuels (and co-products if they are produced) are less than the energy inputs for producing the fuel. Estimated values for energy return on investment range from 0.13 to 3.33. The estimated

³Some algae can grow heterotrophically in the absence of light by taking up organic molecules (such as glucose) as a source of carbon.

⁴U.S. consumption of fuels for transportation was about 784 billion liters in 2010. Five percent of the annual U.S. consumption of transportation fuels, which would be about 39 billion liters, is mentioned to provide a quantitative illustration of the water and nutrients required to produce algal biofuels to meet a small portion of the U.S. fuel demand.

Potential Variation in Each Step

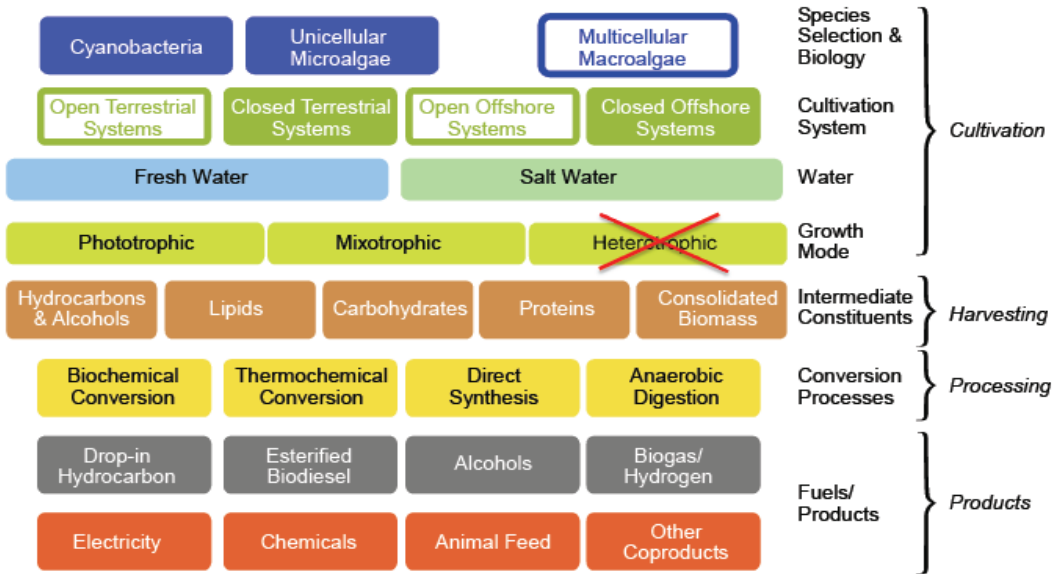


FIGURE S-1 Pathways for cultivating and processing algae to fuels and their products. Heterotrophic routes are outside the scope of this analysis.

consumptive use of fresh water for producing 1 liter of gasoline equivalent of algal biofuel is 3.15 to 3,650 liters, depending on whether the algae or cyanobacteria need to be harvested to be processed to fuels or if they secrete fuel products; whether fresh water, inland saline water, marine water, or wastewater is used as a culture medium; the climatic condition of the region if open ponds are used; and whether the harvest water from algae cultivation is recycled. In other words, at least 123 billion liters of water would be needed to produce 39 billion liters of algal biofuels or an equivalent of 5 percent of U.S. demand for transportation fuels. The estimated requirement for nitrogen and phosphorus needed to produce that amount of algal biofuels ranges from 6 million to 15 million metric tons of nitrogen and from 1 million to 2 million metric tons of phosphorus if the nutrients are not recycled or included and used in coproducts. Those estimated requirements represent 44 to 107 percent of the total nitrogen use and 20 to 51 percent of total phosphorus use in the United States.

Sustainable development of algal biofuels would require research, development, and demonstration of the following:

- Algal strain selection and improvement to enhance desired characteristics and biofuel productivity.
- An EROI that is comparable to other transportation fuels, or at least improving and approaching the EROIs of other transportation fuels.
- The use of wastewater for cultivating algae for fuels or the recycling of harvest water, particularly if freshwater algae are used.
- Recycling of nutrients in algal biofuel pathways that require harvesting unless coproducts that meet an equivalent nutrient need are produced.

Another resource that could pose a limit on the potential amount of algal biofuel that could be produced is land area and the number of suitable and available sites for algae cultivation. A number of site-specific factors—suitable topography, climate, proximity to sustainable water supplies (whether fresh water, inland saline water, marine water, or wastewater), and proximity to sustainable and economic nutrient supplies—would have to be matched carefully with algal cultivation systems to ensure the successful and sustainable production of algal biomass for fuels. Although the use of inland saline water, marine water, or wastewater has been suggested as a mitigation strategy for reducing freshwater use, information on the depth and accessible volume of saline aquifers is lacking, and the actual land area close to wastewater sources suitable for algae cultivation has not been assessed. If the sites are near urban or suburban centers or coastal recreation areas, the price of those lands could hinder their use for algae cultivation.

A national assessment of land requirements for algae cultivation that takes into account climatic conditions; fresh water, inland and coastal saline water, and wastewater resources; sources of CO₂; and land prices is needed to inform the potential amount of algal biofuels that could be produced economically in the United States.

The potential environmental effects listed in Box S-1 can be divided into three types:

- Effects that can be minimized or prevented by proper management of algal cultivation systems or mitigated by engineering designs—for example, accidental release and seepage of culture water, waste products from algal biofuel production, and mosquito-borne diseases.
- Potential effects that have not been assessed or reported extensively in the literature—for example, the effects of large-scale, open-pond algae cultivation on terrestrial wildlife, natural ecosystems, and local climate; potential adverse effects of genetically engineered algae; and presence of unknown or unidentified toxins. Large investments into researching these topics might not be necessary at this early stage of development, but some preliminary assessment now and periodic monitoring as the industry develops would be prudent.
- Effects that need to be assessed for each pathway for algal biofuel production or considered carefully before deployment of algal biofuels—for example, potential land conversion and its effects on GHG emissions; net GHG emissions; air emissions; and safety and nutritional quality of feedstuff coproducts if a pathway relies heavily on coproduct production to achieve high EROI, economic viability, or low resource use.

INNOVATION POTENTIAL

Algal biofuels have the potential to contribute to improving the sustainability of the transportation sector, but the potential is not yet realized. Additional innovations that require research and development are needed to realize the full potential of algal biofuels.

The use of algae offers the potential for sustainability benefits over petroleum-based fuels. The potential benefits stem from the ability to produce algal biofuels domestically, the inherently high photosynthetic productivities of algae relative to terrestrial plants, the use of alternative water sources to reduce the freshwater requirement, the ability to use non-arable lands, and the potential to remediate wastewater and use it as a nutrient and water

Box S-1 Potential Sustainability Concerns for Large-Scale Development of Algal Biofuels

This report identifies the following resource use and environmental effects as potential sustainability concerns for large-scale development of algal biofuels. The concerns of high importance are the ones that have to be addressed, if they are not being addressed already, for a sustainable development of algal biofuels. The concerns of medium importance generally reflect the ones that require some assessment or monitoring to ensure that they do not present serious sustainability concerns. The concerns of low importance are ones that are likely avoidable with proper management and good engineering designs.

Concerns of High Importance

- The quantity of water (whether fresh water or saline water) required for algae cultivation and the quantity of freshwater addition and water purge to maintain the appropriate water chemistry. Maintenance of water level and quality in open-pond systems or evaporative loss of cooling water if it is used to maintain temperature in photobioreactors could be a concern because of the potential for high net evaporative losses, particularly in arid regions where solar resources are most suitable for cultivation.
- Supply of the key nutrients for algal growth—nitrogen, phosphorus, and CO₂. Nutrient sources can include virgin sources and waste streams such as flue gas. Preparation and transport of these waste streams for reuse, nutrient recycling, production of coproducts, and fossil inputs required to produce necessary nutrients all affect the energy return and GHG emissions.
- Appropriate land area with suitable climate and slope, near water and nutrient sources (for example, a stationary source of CO₂ such as a coal-fired power plant or a wastewater source such as municipality, industry, or agriculture).
- Energy return on investment. Algal biofuel production would have to produce sufficiently more energy than is required in cultivation and fuel conversion to be sustainable.
- GHG emissions over the life cycle of algal biofuels. Algal biofuel production would have to produce a GHG benefit relative to other fuel options such as fossil fuels. Yet, estimates of life-cycle GHG emissions of algal biofuels span a wide range, and depend on many factors including the source of CO₂ and the disposition of coproducts.

Concerns of Medium Importance

- Presence of waterborne toxicants in cultivation systems that use flue gas as a source of CO₂ or wastewater as a source of culture water and nutrients, particularly if fertilizers or feedstuff are to be produced as coproducts.
- Effects from land-use changes if pasture and rangeland are to be converted to algae cultivation. Displacing pasture and rangeland could incur direct and indirect land-use changes (ILUC) that would affect the net GHG emissions of algal biofuels.
- Air-quality emissions over the life cycle of algal biofuels. Emissions from the processing facilities and tailpipe emissions will be regulated. The committee is not aware of any published studies that include measured emissions of air pollutants from open-pond cultivation.
- Potential effects on local climate. The introduction of large-scale algal cultivation systems in arid or semi-arid environments could alter the local climate of the area by increasing humidity and altering temperature extremes.
- Releases of cultivated algae to natural environments and potential alteration of species composition in receiving waters.
- Effects on terrestrial biodiversity from changing landscape pattern as a result of infrastructure development for algal biofuels.
- Potential adverse effects and unintended consequences of introduction of genetically engineered algae for biofuel production.
- Waste products from processing algae to fuels.
- Potential presence of pathogens if wastewater is used for algae cultivation.
- Potential presence of unknown, unidentified, or unexpected algal toxins.

continued

Box S-1 Continued**Concerns of Low Importance**

- Accidental releases of culture water and infiltration of nutrients and chemicals into soil or surrounding water.
- Seepage of culture water into the local groundwater system if clay-lined ponds are used or if plastic liners are breached through normal weathering or from extreme weather events.
- Potential presence of mosquitoes and mosquito-borne diseases around poorly managed open ponds.

source. If algal biofuels are to contribute a significant amount of fuels for transportation, the following are needed:

- Improvements in the algal strains used.
- Testing additional strains for desired characteristics.
- Advancements in the materials and methods used for algae cultivation and for processing algal biomass into fuels.
- Reductions in the energy requirements for cultivation, algae collection, and processing to fuels.

Algal strain development is needed to enhance traits that contribute to increasing fuel production per unit resource use, reducing the environmental effects per unit fuel produced, and enhancing economic viability. Improvements in biomass or product (lipid, alcohol, or hydrocarbons) yield, culture density, nutrient uptake, ease of harvest, and photosynthetic efficiency are some of the improvements that would improve sustainability of algal biofuels.

The strains used for large-scale algal biofuel production are being improved through selection and genetic approaches. Breakthroughs and innovations in areas such as increasing the capability of algae to use nutrients efficiently or engineering designs to reduce processing requirements have the potential to greatly improve the energy balance and enhance the overall sustainability of algal biofuels.

Engineering solutions to enhance algae cultivation, to facilitate biomass or product collection, and to improve processing of algae-derived fuels can increase the EROI and reduce the GHG emissions of algal biofuel production.

Lipid collection and conversion have dominated algal biofuel development for several decades. Processing improvements to reduce energy requirements and increase productivity continue to be proposed. Whole-cell processing of algae into fuels also has been investigated. Innovations focused on reducing energy use, nutrient requirements, water use, and land use are necessary for the sustainable development of algal biofuels. These innovations may require algal strain improvements, engineering solutions to improve hardware required for fuel production, and the interplay of the two.

A FRAMEWORK TO ASSESS SUSTAINABLE DEVELOPMENT

Given the multiple resource requirements and potential environmental effects, specific sustainability concerns cannot be viewed in isolation from others. Any one life-cycle assessment (LCA) for a single resource use or environmental effect is insufficient to determine the overall sustainability of an algal biofuel production system. Challenges arise regarding how to assess the overall environmental sustainability of algal biofuels holistically and how to balance the environmental objectives against the economic and social objectives of sustainable development. An overall and comparative assessment of sustainability is complicated by the fact that some sustainability objectives can be estimated on the basis of mass balance or engineering principles and compared across systems—for example, nutrient budgets, energy balances, and GHG emissions—while others are region specific. Other sustainability objectives are specific to region and maybe species, and the environmental effects in one region might not be directly comparable to another—for example, land-use change (LUC) and biodiversity.

The committee proposes a stepwise framework (Figure S-2) to aid DOE in its decision-making process that would help ensure sustainable development of algal biofuels. The framework uses a variety of tools for assessing overall sustainability including LCAs that integrate a particular aspect of sustainability through the supply chain, cumulative impact

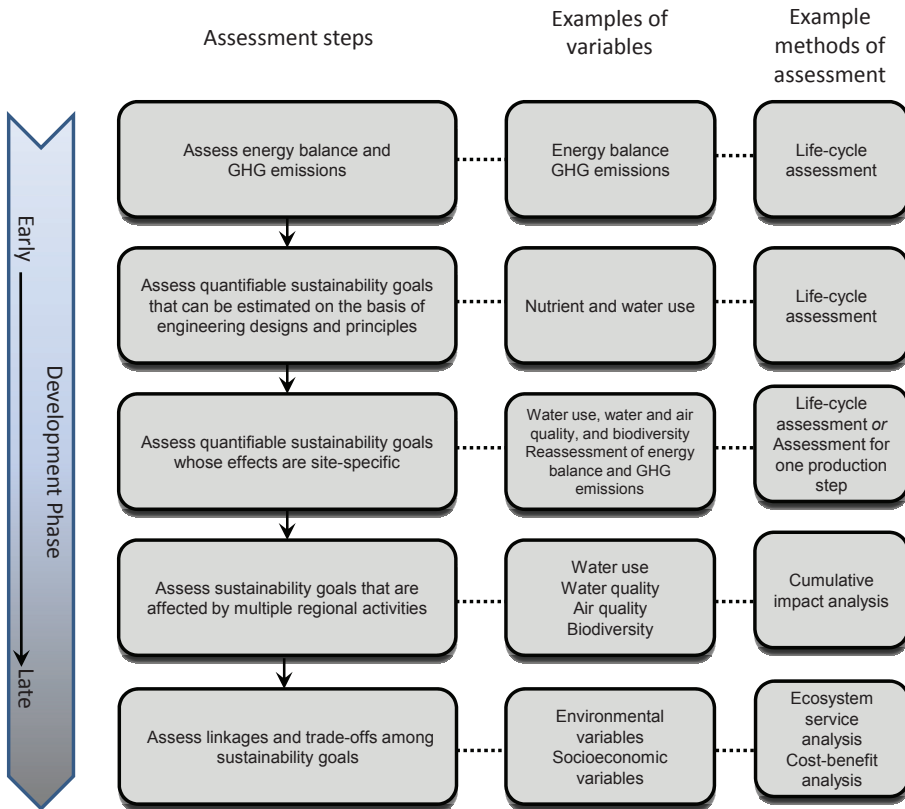


FIGURE S-2 A potential framework for assessing sustainability of algal biofuels during different stages of development.

analyses that examine the cumulative effects of a resource use or an environmental effect of algal biofuel production in addition to the existing activities in the production area, and cost-benefit analyses that integrate the monetized environmental costs of algal biofuel production with the monetized environmental benefits.

Determining the sustainability of algal biofuel production requires comparisons with fuels being used today to assess whether substituting algal biofuels for an existing option contributes to improving sustainability. The framework starts with assessing two of the primary goals for developing alternative liquid fuels—improving energy security and reducing GHG emissions. To be a sustainable source, any fuel produced needs to return more energy in use than was required for its production; therefore, EROI is a logical first step for assessment. Some authors suggest an EROI of less than 3 for any fuel to be considered unsustainable. Therefore, the EROIs of algal biofuels at least have to show progress toward a value that is within the range of EROIs of other transportation fuels. Ideally, the alternative fuel that is replacing petroleum-based fuels will improve energy security and contribute to reducing GHG emissions.

If algal biofuels show promise for achieving these two goals, then a few variables that reflect commonly agreed-upon sustainability objectives and that can be estimated from mass balance and engineering principles are assessed. For example, nitrogen and phosphorus inputs and freshwater use are sustainability objectives that can be assessed using LCAs. Avoiding competition for these resources between food and fuel production is a commonly agreed-upon objective. The estimated EROI, GHG emissions, nutrient, and freshwater requirements would have to be reassessed once the likely locations of deployment are determined. Then the productivities of algal feedstocks and fuel products and any potential land-use changes can be estimated with increased certainty, and the precision of the estimated resource requirements and GHG emissions can be improved. When the industry is further along in its development, direct measurements can be made in operating algal biofuel production systems to verify estimates. In addition, progressively comprehensive and regional assessments that include other variables can be made.

Though some resource use or emissions can be estimated quantitatively, some biological effects (for example, biodiversity) or the impact of some environmental effects (for example, air-quality emissions and water use) are location specific. For example, water use (coastal or inland saline water or fresh water) can be estimated over the life cycle of biofuel, but the effect of the water use has to be put into the context of regional availability. The effect of algal biofuel production on biodiversity cannot be assessed unless the specific location of deployment and the species present there are known. Some of these effects might be easily quantifiable. Other effects might require research and data collection before the effects can be understood and quantified.

The resource requirements and environmental effects also have to be assessed in the context of existing activities at the sites where algal biofuel production systems are to be developed. As the algal biofuel industry develops, the ability of different pathways for algal biofuel production to meet and balance productivity of fuel with the other environmental, economic, and social sustainability goals has to be assessed in a holistic manner. Such assessment by itself does not inform whether algal biofuels would contribute to improving sustainability of the transportation sector.

The environmental, economic, and social effects of algal biofuel production and use have to be compared with those of petroleum-based fuels and other fuel alternatives to determine whether algal biofuels contribute to improving sustainability. Such comparison

will be possible only if thorough assessments of each step in the various pathways for algal biofuel production are conducted.

Given the four aspects of sustainability and the multiple goals within each aspect, a participatory approach is necessary to develop a collective vision of the importance of various sustainability objectives relative to each other. An approach that involves different stakeholders (for example, algal biofuel producers, fuel consumers, environmental groups, and residents near areas to be developed for algae cultivation or biofuel refinery) from the beginning of a sustainability assessment would help ensure that trade-offs among sustainability goals would be acceptable to the various parties.

CONCLUSIONS

This report identified EROI; GHG emissions; water use; supply of nitrogen, phosphorus, and carbon dioxide; and appropriate land resources as potential sustainability concerns of high importance. The committee does not consider any one of these sustainability concerns a definitive barrier to sustainable development of algal biofuels because mitigation strategies for each of those concerns have been proposed and are being developed. However, all of the key sustainability concerns have to be addressed to some extent and in an integrative manner. Therefore, research, development, and demonstration are needed to test and refine the production systems and the mitigation strategies for sustainability concerns and to evaluate the systems and strategies based on the sustainability goals if the promise of sustainable development of algal biofuels has any chance of being realized.

1

Introduction

1.1 INTEREST IN ALGAL BIOFUELS

Petroleum-based fuels have been the primary type of transportation fuel in the United States for decades. Until the 1960s, domestic production of these fuels met the vast majority of the nation's demand. U.S. oil production peaked in the 1970s, but demand continued to grow. The desire to reduce reliance on foreign oil imports and to improve energy security sparked interests in research and development (R&D) of alternative fuels. In 1978, the U.S. Department of Energy's (DOE) Office of Fuels Development initiated the Aquatic Species Program whose goal is to produce renewable transportation fuels from algae (Sheehan et al., 1998). That program furthered the understanding of algae's potential as a feedstock for fuel through its development and characterization of a large collection of oil-producing algae, its research to improve understanding of the biological triggers for enhancing oil production in algae, and its work on demonstrating open-pond systems for large-scale algae cultivation (Sheehan et al., 1998). Biofuels derived from algae and cyanobacteria¹ were considered a promising alternative fuel for improving energy security for the following reasons:

- Microalgae, macroalgae,² and cyanobacteria convert solar energy to chemical energy for their growth and development through the process of photosynthesis. Some species also can be grown in heterotrophic conditions, where an exogenous source of organic carbon is provided.

¹Cyanobacteria, also called cyanoprokaryotes, were historically known as blue-green algae. For simplicity, biofuels derived from macroalgae, microalgae, and cyanobacteria grown under photosynthetic conditions all are referred to as algal biofuels.

²Macroalgae are multicellular algae that lack true roots and leaves. Macroalgae are found in fresh water and marine water, soil, and growing on other organisms.

- Unicellular algae and cyanobacteria have the advantage of being able to complete a reproductive cycle in a matter of hours or a few days. Therefore, they can be harvested on a daily or weekly basis.
- The oil productivity of many species of algae exceeds that of oil crops (Patil et al., 2008).

During its two decades of operation, the Aquatic Species Program built a collection of more than 3,000 species of oil-producing microalgae (Sheehan et al., 1998). Program research shed light on algal physiology and biochemistry and the relationship between oil content in cells and algal productivity. Efforts also were made to demonstrate the feasibility of large-scale cultivation of algae in open ponds (Sheehan et al., 1998). The Aquatic Species Program was terminated in 1996 when DOE was under budget pressure. At that time, the price of oil was less than \$20 per barrel (EIA, 1999). In contrast, a technoeconomic analysis conducted in 1982 estimated algal biofuels would cost about \$60 per barrel of oil equivalent under an optimistic scenario and about \$120 per barrel of oil equivalent under a conservative scenario (Benemann et al., 1982).

Volatile oil prices observed from 2000 to the present renewed interests in alternative fuels. In addition, mounting evidence of global climate change raised concern over the carbon footprint of using fossil fuels. Greenhouse gases (GHG)—such as carbon dioxide (CO₂), nitrous oxide, and methane—are heat-trapping gases that produce a warming effect on the Earth's atmosphere. CO₂ emissions from burning of fossil fuels account for a large portion of GHG emissions (NRC, 2011a). In the United States, the use of petroleum-based fuel in the transportation sector accounted for 30 percent of the nation's CO₂ emissions in 2009. Although using algal biofuels for transportation would produce tailpipe emissions comparable to those from using petroleum-based fuels, algae and cyanobacteria take up CO₂ during growth and thereby offset some of the CO₂ emissions (Brune et al., 2009). In addition, the net impact on CO₂ emissions also depends on the quantity of fossil fuels used throughout the algal biofuel production pathway. Life-cycle assessment (LCA), discussed later in this chapter, attempts to account for and aggregate the energy requirements and CO₂ impacts over the whole production pathway.

Domestic production of renewable fuels including algal biofuels has the potential to meet the dual goals of improving energy security and decreasing GHG emissions from the transportation sector. However, a dramatic decrease in foreign oil importation and reduction in GHG emissions in the United States will require the production and use of multiple alternative transportation fuels. Biofuels produced from algal feedstock could be one of the alternatives. The number of startup companies working on the development of algal biofuels has been increasing, and some oil companies are investing in algal biofuels (Mascarelli, 2009; Mouawad, 2009). The U.S. military is interested in substituting part of its fuel use with renewable energy sources including algal biofuel (Physorg.com, 2010), and the Defense Advanced Research Projects Agency funded projects for developing technologies to produce affordable algal biofuels (Lundquist et al., 2010). Given the interest in algal biofuels, the DOE Energy Efficiency and Renewable Energy's (DOE-EERE) Office of Biomass Program (OBP) held a workshop in 2008 "to discuss and identify the critical barriers currently preventing the economical production of algal biofuels at a commercial scale" (DOE, 2010). DOE and private companies are actively investing in R&D for algal biofuels to resolve technical barriers, improve feasibility of large-scale production, and reduce costs. In addition to developing production technologies, any developing industry also needs to consider sustainability. Addressing sustainability concerns and challenges as the industry develops can help ensure its success well into the future. Ignoring sustainability at the

BOX 1-1 Statement of Task

The committee is tasked to examine the promise of sustainable development of algal biofuels, identify potential concerns and unforeseen sustainability challenges and unintended consequences for a range of approaches to algal biofuel production, explore ways to address those challenges, and suggest appropriate indicators and metrics that can inform future assessments of environmental performance and social acceptance associated with sustainability. Although economics is an important aspect of sustainability, the study will not assess costs of algal biofuels. Algal biofuel production approaches and technical systems are still emerging, and facilities have not reached commercial scale. Public data on the economics of algal biofuel production is sparse. Therefore, it is premature for the committee to conduct generalized economic analyses of algal biofuels.

The committee will:

- Identify the potential sustainability concerns for commercial production (including larger centralized and smaller distributed facilities) of algal biofuels associated with a selected number of different pathways of biomass production and conversion. Potential concerns to be addressed could include the availability and use of land, water, and nutrient resources; human health and safety associated with feedstock cultivation and processing; potential toxicity associated with algal metabolites and their adverse impacts on downstream coproducts; and other impacts that are of social and environmental concern.
- Identify information or data gaps related to the impacts of algal biofuel production.
- Suggest indicators and metrics to be used to assess sustainability concerns across the algal biofuel supply chain and data to be collected now to establish baseline and to assess sustainability. Identify indicators that are most critical to address or have the greatest potential for improvement through DOE intervention. This input will inform DOE-EERE OBP's broader analysis of biofuels and bioenergy sustainability.
- Using selected approaches as illustrations, discuss whether any, or combinations of, the identified challenges could present major sustainability concerns. Are there preferred cost and benefit analyses that could best aid in the decision-making process, and could those decisions be performance based and technology neutral?

outset might exacerbate the sustainability issues for future generations and make it difficult for an industry to successfully scale-up (Azapagic and Perdan, 2000).

At the request of DOE-EERE's OBP, the National Research Council (NRC) appointed an independent committee to examine the sustainable development of algal biofuels. (See Appendix A for committee membership.) The purpose of this study is to identify and anticipate sustainability concerns associated with large-scale deployment of algal biofuels, discuss potential mitigation strategies, and suggest indicators and metrics that could be used and data that could be collected to evaluate sustainability across the biofuel supply chain to monitor progress as the industry develops (Box 1-1).

1.2 SUSTAINABLE DEVELOPMENT OF BIOFUELS

1.2.1 Defining Sustainable Development

“Sustainable development is development that meets the needs of the present without compromising the ability of future generations to meet their own needs” (United Nations, 1987). Most definitions of sustainability include and integrate an economic, an

environmental, and a social dimension (Hammond, 2000; IISD, 2011; United Nations, 2011). A recent NRC report identified four key societal sustainability goals for agriculture. Those goals are:

- “Satisfy human food, feed, and fiber needs, and contribute to biofuel needs.
- Enhance environmental quality and resource base.
- Sustain the economic viability of agriculture.
- Enhance the quality of life for farmers, farm workers, and society as a whole.” (NRC, 2010b; p.23)

In the context of algal biofuels, the goals of sustainable development can be framed as follows:

- Contribute to energy security, particularly the domestic supply of transportation fuel.
- Maintain and enhance the natural resource base and environmental quality.
- Produce fuel that is economically viable.
- Enhance the quality of life for society as a whole.

The four aspects of sustainability are interconnected in many ways, some of which are synergistic or mutually reinforcing, others of which might involve tradeoffs among goals. An example of synergy could be technological improvements in algae and cyanobacteria production and in processing the biomass to fuels. Those improvements would enhance fuel yield, contribute to energy security, increase resource use efficiency, and reduce cost of production, and therefore contribute to transportation fuel needs and improve environmental, economic, and social sustainability. An example of a tradeoff could be pollutant management, which would contribute to maintaining environmental quality and minimizing human-health impacts but could add to the cost of production.

1.2.2 Components of Sustainable Biofuel Development

As in the case of plant-based biofuels (NRC, 2011b), algal biofuels could provide opportunities to improve energy security, reduce GHG emissions, and maintain and enhance the resource base and environmental quality, but their production also could raise sustainability concerns. Whether those opportunities will be realized depends on how the industry develops. It is prudent to consider potential sustainability concerns that might arise and to avoid or mitigate them as the industry develops. Sustainability of plant-based biofuels has been discussed, and criteria for assessments have been developed by various entities over the past decade (ESA, 2008; Markevicius et al., 2010; NRC, 2010a,c). Examples of sustainability criteria are shown in Table 1-1. Many of the sustainability criteria apply to algal biofuels.

1.2.2.1 Energy Security

Whether and how much algal biofuels would contribute to energy security depends in part on the resources (for example, land and water) available for algal biofuel production, the productivity of algae cultivation, the yield of the processing of algae to fuel, and the ability to integrate the various components of algal biofuel production into one functional system and to scale it up. Resource limitations bound how much algal biofuel could be

TABLE 1-1 Examples of Sustainability Criteria Used to Evaluate Plant-Based Biofuels.

Sustainability Criteria	Explanation
Economic	
<ul style="list-style-type: none"> • Cost of production • Economic development • Fiscal effects • Employment 	<p>Cost competitiveness with respect to other fuel alternatives.</p> <p>Effects on the standard of living and economic health.</p> <p>Effects on fiscal balances.</p> <p>Employment creation.</p>
Resource Use and Environmental	
<ul style="list-style-type: none"> • Energy balance • Resource use including land and water • Pollutant emissions including GHG and criteria pollutants • Biodiversity 	<p>Energy output in fuel per unit of energy input to make the fuel over its life cycle.</p> <p>Land and water requirements to produce one unit of fuel.</p> <p>Emissions (for example, CO₂ and sulfur oxides) over the life cycle of one unit of fuel.</p> <p>Effects on ecological species and communities (for example, habitat destruction or enhancement).</p>
Social	
<ul style="list-style-type: none"> • Competition for resources being used for other human activities • Cultural acceptability • Visual impacts • Health effects 	<p>Effects of resource use (for example, water and nutrients) for biofuel production on other activities (for example, farming food crops and animals).</p> <p>Acceptability of the effects of biofuel production.</p> <p>Perception of landscape aesthetics.</p> <p>Effects of emissions (for example, air-quality emissions) on human health.</p>

produced, but technological progress could enhance the productivity of algal feedstock and fuel yield.

1.2.2.2 Economics

Cost of production is an important aspect of sustainable development that applies to all nonpetroleum-based alternative fuels including algal biofuels. Alternative fuels are not likely to penetrate the fuel markets if they are much more expensive for consumers than other fuel alternatives (NRC, 2008, 2011b). Although government policies and subsidies can facilitate and accelerate the market penetration of biofuels, the biofuels eventually would have to become economically viable without subsidy. Brazilian ethanol was heavily subsidized when Brazil's National Alcohol Program was initiated, but the government subsidies gradually were phased out in the 1990s. Sugar-cane ethanol has been economically viable in Brazil since 2003 (Solomon, 2010).

As discussed in Box 1-1, the committee was not asked to analyze costs of algal biofuels. Published estimates for costs of algal oil and algal biofuels span a wide range of about \$1-\$25 per gallon (Williams and Laurens, 2010; Gallagher, 2011; and references cited therein). The wide range reflects a number of factors including when the estimate was made. The cost estimates reported in the literature were not in constant dollars and therefore are not directly comparable. Some cost estimates were for algal oil before upgrading to fuels. A wide range of technologies could be used in an algal biofuel production system resulting in varying costs. This range in estimated costs reflects the immaturity of algal biofuel production and the uncertainties associated with a developing industry (Williams and Laurens, 2010). It is still premature to analyze and draw any conclusions about the economic sustainability of algal biofuels, particularly when costs likely will decrease with ongoing

technological developments. Although this report does not address costs of algal biofuels, it makes occasional reference to economics if there are known critical synergies or tradeoffs between economics, productivity, resource use, and environmental effects.

1.2.2.3 Resource Use and Environmental Effects

Land, water, and nutrients are required for cultivating plants and algae. Cropland acreage in the United States has been decreasing in the past few decades (Nickerson et al., 2011), and the water levels of some aquifers used for irrigated agriculture have been declining (NRC, 2001). Nutrient runoff from row-crop agriculture into surface water and its environmental effects has raised concerns (NRC, 2009). Some of these concerns for resource use and availability and for the environment might be alleviated by developing algal biofuels because the production of algae and cyanobacteria biomass does not require high-quality land resources, as in the case of the production of sugar cane or corn for ethanol, and soybean or other oilseeds for biodiesel (Schenk et al., 2008). Algae and cyanobacteria can be grown in saline waters or nutrient-rich wastewater that is not suitable for agriculture or human consumption (Woertz et al., 2009; Bhatnagar et al., 2010; Chinnasamy et al., 2010; Craggs et al., 2011). In addition, enriching algae and cyanobacteria cultures with CO₂ and other nutrients helps maximize photosynthetic algal biomass production on a large scale. One suggestion is to co-locate algal biomass production sites with stationary industrial CO₂ emission sources like fossil fuel-fired power plants to integrate the plant CO₂ emissions with the algal cultivation system. Another suggestion is to locate algal biomass production facilities near wastewater sources, such as municipal wastewater treatment plants. Algae cultivation systems can use the nutrients present in wastewater that has undergone primary or secondary treatment thereby serving as a nutrient removal component of wastewater treatment. An important issue then to assess is the number of potential sites for algae cultivation that are near both a source of CO₂, such as fossil-fired power plants, and a source of nonpotable water, such as wastewater or saline water. Resource use and maintaining the quality of the natural resource base necessary for developing algal biofuels will play a role in the sustainable development of algal biofuel. This report focuses on the sustainable development of algal biofuels with respect to resource use and effects on the environment.

1.2.2.4 Social Well Being

Although biomass production of algae and cyanobacteria is not likely to compete for high-quality arable land with crops, there could be social concerns about land use that need to be considered in the development of algal biofuels. For example, situating algae and cyanobacteria biomass production in the U.S. desert Southwest could be perceived as a good use of low-value land by some, but as an intrusion into pristine land by others. Similarly, the use of genetically engineered organisms in production systems could affect social acceptability. This report discusses how the resource use and environmental effects of large-scale algal biofuel production could affect the social acceptability of algal biofuels.

1.2.3 Sustainability of Transportation Fuel

The preceding section mentioned some potential sustainability concerns for large-scale development of algal biofuels (which will be discussed in detail in later chapters along with opportunities to mitigate them), but the sustainability of algal biofuels cannot be viewed in isolation and needs to be put into the broader context of the transportation-fuel sector

for two reasons. First, there is not one alternative fuel that can replace all the petroleum-based fuels used in U.S. transportation. Few options are available to reduce petroleum use (NAS-NAE-NRC, 2009), and algal biofuels could become a future option for reducing petroleum use and GHG emissions from the transportation sector. Second, every fuel source has its positive and negative effects on the resource base or other aspects of the environment. Therefore, the overall sustainabilities of different fuels have to be compared to assess whether replacing one fuel with another would contribute to improving sustainability. Therefore, the committee cautions that the report is not to be read as a mere list of sustainability concerns, but as a discussion of resource use and environmental effects that need to be compared with those of other fuels to see which fuel option is more sustainable or better balances the various sustainability objectives.

1.3 TOOLS AND METHODOLOGIES FOR ASSESSING SUSTAINABLE DEVELOPMENT OF ALGAL BIOFUELS

This section presents a brief overview of the tools and methodologies used for assessing the sustainability of algal biofuels in this report. The objective here is not to provide results from the application of these methodologies to algal biofuels, but to provide a brief description of the approaches used in this report and how they help meet the overall objectives of providing indicators and approaches to measuring the sustainability of algal biofuels. It focuses on several basic concepts: the systems analysis framework, indicators of sustainability, LCA, and futures or scenario analysis. Indicators are repeated measurements, observations, or model results that “are used to represent or serve as proxies for impacts of outcomes of concerns” (NRC 2010b, p.32). LCA and futures analysis are methodologies for estimating resource use and environmental effects. Systems analysis is an integrating conceptual approach for evaluating impacts of algal biofuels.

1.3.1 Systems Analysis Framework

As Holmes and Wolman (2001) have pointed out, the systems analysis approach emphasizes the development of comprehensive strategies and impact assessments by integrating all “critical physical, biological, socioeconomic, and engineering processes and constraints into a unified framework” (Figure 1-1). Typically quantitative models are used to define the most effective outcome or tradeoffs among multiple outcomes for a given set of system inputs. Historically, the application of this methodology involved “elucidating the objective(s) in the solution, developing a comprehensive description [of the system], formulating alternative solutions, and [quantitatively] analyzing the alternatives with respect to the magnitude and distribution of their consequences” (Holmes and Wolman, 2001, p. 177). The systems analysis framework is particularly applicable to algal biofuels. Of all of the current renewable energy alternatives, biofuels derived from algae and plant-based resources represent one of the most complex systems integration challenges. Part of the complexity is due to the diverse set of feedstocks, and logistical and conversion technologies that designers of bioenergy systems can select from as major components of a biofuel industrial ecology. In addition, many of these technologies are at different evolutionary stages of development ranging from an intriguing possibility to large-scale pilot demonstrations. Further adding to this complexity is the diverse way that these technologies can be integrated to design and implement advanced biofuel systems. This diversity in the mixing of technologies and the possible integration schemes is a driver for innovation as currently seen in the diverse commercial approaches to algal biofuel development. At the

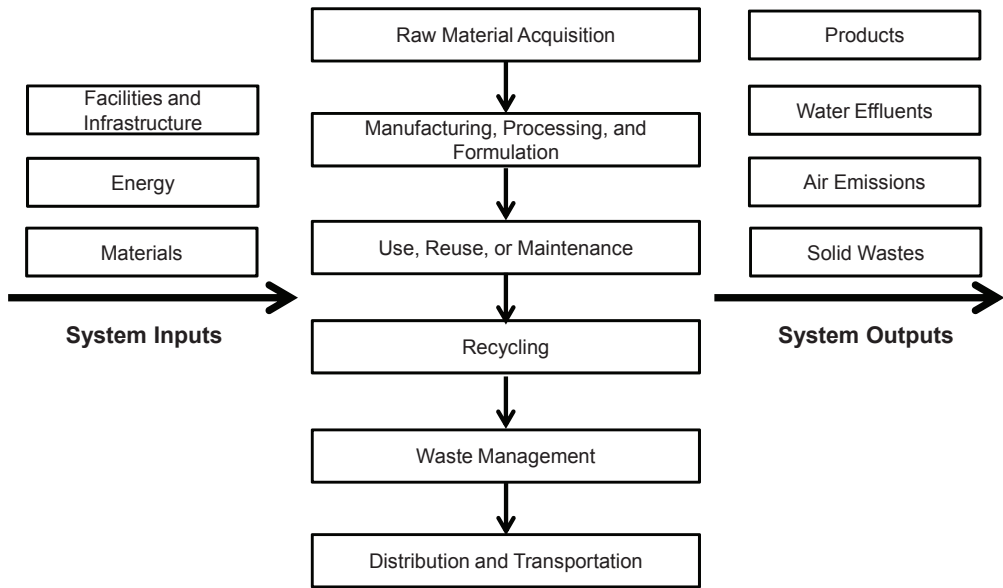


FIGURE 1-1 Schematic representation of a production system, including system inputs and outputs.

same time, this diversity creates challenges for documenting critical material, energy, and monetary flows needed to assess performance.

Understanding the performance of alternative designs for producing liquid fuels from algae requires the adoption of a systems framework for assessing alternative designs. The systems framework illustrates the interdependent nature of the individual supply chain components and the system inputs and outputs. The understanding developed from such a representation is fundamental for applying a wide array of sustainability tools such as LCA, engineering process modeling, and cost-benefit analysis.

1.3.2 Indicators

Biofuel sustainability indicators are metrics of defined aspects of sustainability that represent system status or progress toward sustainability goals. Some researchers and institutions distinguish between definitions of indicators and metrics, while others see substantial overlap in the concepts. The definition of an indicator used in this report is “a measure that is somehow indicative of some unmeasurable environmental goal such as environmental integrity, ecosystem health, or sustainable resources” (Suter, 2001). Indication of sustainable development of algal biofuels is indirect, through the union of metrics of resource use, other environmental impacts, social acceptance (all considered in this report), and economics and energy security (not considered in this report). Specific metrics of water quality or quantity or GHG emissions, for example, are viewed as indicators of sustainability or sustainable development.

Because sustainability includes environmental, economic, and social dimensions (in addition to energy and energy security, which may be classified separately), indicators also typically are divided among these categories. Categories of resource requirement indicators that have been discussed for biofuels include total and consumptive water use,

nutrient use, total land use, and net energy return, and categories of environmental indicators include net GHG emissions, water quality, and biodiversity. This report emphasizes the sustainability of the broad environment and thus presents categories of indicators of aspects of the environment that are pertinent to algal biofuels. This report also emphasizes the sustainability of resource use that determines the viability of the biofuel system. Indicators at this interface between environmental and economic sustainability also are presented and discussed in this report. Specific sustainability indicators pertaining to other aspects of the economy (for example, international trade, profitability, employment) are beyond the scope of this study, though clearly these will influence and be influenced by indicators of environmental sustainability. Social indicators of biofuel sustainability often are not derived or considered, but such potential indicators for algal biofuels could be developed. However, the focus of this study is on environmental sustainability and indicators related to environmental impacts and natural resource requirements.

Because sustainable development implies progress toward sustainability goals, it is important to understand baselines for indicators of sustainability. Moreover, the attribution of particular environmental and social effects to algal biofuel production requires an understanding of baseline and reference conditions. An appropriate definition of a baseline is conditions that would have prevailed in the absence of algal biofuel production. In principle, the baseline incorporates dynamic land-use and associated environmental changes in the region, but in practice it is often simpler and more certain to consider the conditions that prevailed prior to biofuel production.

The use of particular units can influence the way that sustainability indicators are interpreted (Turnhout et al., 2007; Corbière-Nicollier et al., 2011; Efroymson et al., 2012). Units may include volume or mass of resources required; concentrations, emissions, or loadings of chemicals to environmental media; and abundance of organisms or habitat area. The units may have denominators of land area, energy produced, or volume of fuel. Choosing a denominator such as land area or volume of fuel can facilitate comparisons between alternative land uses or fuels but also can add to the uncertainty associated with an indicator. For example, land area may include the area for infrastructure or the area for infrastructure plus a buffer. Including time as a factor in an indicator allows the duration of an environmental effect to be considered. Including coproduct quantities in the divisor of an indicator can imply that decision makers have determined that part of an effect should be formally attributed to the coproduct.³

Sustainability and sustainable development encompass diverse goals and targets that relate to dynamic human values. Movement toward sustainability cannot be assessed unless the specific goals are defined and targets and metrics for aspects of sustainability are selected. Many international organizations are developing sustainability indicators for biofuels. These include the Roundtable on Sustainable Biofuels (RSB), the G-8-endorsed Global Bioenergy Partnership (GBEP), and others (van Dam et al., 2008). Additional organizations have been created to promote sustainable biofuel industries, such as the Council on Sustainable Biomass Production in the United States (CSBP, 2010). The International Sustainability and Carbon Certification system for biomass and bioenergy has been implemented globally by 750 stakeholders from 45 countries (ISCC, 2012). Some organizations recommend a large number of sustainability indicators. For example, RSB (2011) recommends more than 200

³Coproducts are commercial products such as nutrient supplements, animal feedstuff, or chemical feedstocks that can be coproduced from the pathways that produce algal biofuels and marketed. For example, after lipids have been extracted from algal biomass, the lipid-extracted biomass might be processed to become animal feedstuff or animal feed supplements.

indicators and measures of biofuel sustainability. A challenge is to winnow generic lists of biofuel sustainability indicators to a suite that is appropriate for a particular assessment problem and is technically and economically practical (McBride et al., 2011; Efroymson et al., 2012). Many of the efforts to develop generic biofuel sustainability indicators have focused on plant-based biofuels—corn ethanol, cellulosic biofuels, and agricultural biodiesel. Therefore, some recommended indicators may not be pertinent to algal biofuels, and some important potential indicators may not appear on previously published lists.

Turnhout et al. (2007) suggested that the successful application of indicators is specific to each situation. What typically leads to a sustainability assessment is a decision or other purpose, combined with sustainability goals. Sustainability goals may include concepts such as efficient use of resources, maintenance of water quality, maintenance of biodiversity, and minimization of waste (Sydorovych and Wossink, 2008). Indicators would have to be selected to reflect goals. Moreover, the context of a biofuel sustainability assessment is important for selecting, measuring, and interpreting sustainability indicators (Efroymson et al., 2012). The context for the application of sustainability indicators includes the purpose of the assessment, the region, the scale of analysis, the relevant policies context, the decision context (including stakeholders), and available data on baselines and reference scenarios (Efroymson et al., 2012).

A sustainability assessment for algal biofuel production may entail comparing algal biofuels with business-as-usual scenarios for energy use (that is, using mostly petroleum-based gasoline in transportation as is done today), alternative energy sources (for example, other biofuels or other algal biofuel pathways), previous land uses or land uses that would have occurred in the absence of biofuel production, or alternative sites for the facility. These comparisons may lead assessors to prioritize various sustainability indicators differently and may lead to different measurement or modeling methods and units.

1.3.3 Life-Cycle Assessment

LCA is a set of methods, databases, and tools that aims to characterize the environmental impacts over a life cycle of a product or service. LCA is defined as “a systematic set of procedures for compiling and examining the inputs and outputs of materials and energy and the associated environmental impacts directly attributable to the functioning of a product or service system throughout its life cycle” (ISO, 2006). The life cycle in the context of algal biofuel production refers to a chain of activities that includes extraction of raw resources, producing materials, manufacturing, transportation, use, and disposal (Baumann and Tillman, 2004; EPA, 2006). Figure 1-1 shows a schematic of the chain of activities involved in a production system, and LCA attempts to account for and aggregate a resource requirement or an environmental impact over the whole pathway. However, it is generally infeasible to analyze every process in a life cycle. Data and knowledge limitations imply that LCA entails selection of a “system boundary” that delineates processes included in the analysis versus those excluded.

One approach to LCA involves numerical modeling of material flows in supply chains. The idea is to map a target product to a set of activities or processes (or sectors) and use input-output tables to estimate cumulative material flows per unit product. An input-output table delineates requirements of inputs to generate a set of outputs (for example, iron ore, coal, and electricity as inputs for crude steel as an output). This input-output approach has the advantage of being able to rely on economy sector level data to quantify the relationship between energy, resources, and the final products (Miller and Blair, 2009). However, given the nascent nature of algal biofuel production, the LCAs discussed in this study will focus

primarily on the process analysis approach to LCA that uses specific information on the energy, nutrients, and emissions associated with each component of the process, which is combined to get a complete LCA of resource requirements. The process approach to LCA is a bottom-up approach that builds a full supply-chain estimate through the examination of the individual components. In contrast, the economic input-output approach to LCA (EIO-LCA) (Bullard and Herendeen, 1975; Hendrickson et al., 2006) is a top-down approach that uses a holistic model of an economy divided into sectors, with the input-output table describing economic transactions between sectors (Leontief, 1970). To briefly address uncertainty in LCA models, the bottom-up process method suffers from variations in defining the system boundary when data on part of the supply chain are unavailable, while the EIO-LCA has error associated with the aggregation of processes into economic sectors (Williams et al., 2009). Hybrid LCA is a set of methods that aims to combine process and EIO-LCA methods to reduce uncertainty (for example, Bullard et al., 1978).

A second component of LCA, impact assessment, interprets life-cycle material flows in terms of environmental impacts. A major thrust of impact assessment is mapping flows to multiple types of impacts (for example, climate change, resource availability, and human toxicity) and developing ways to inform decision-making tools to navigate these multiple impacts (Baumann and Tillman, 2004; EPA, 2006). Many of the LCAs done for other biofuels are reviewed in the NRC report *Renewable Fuel Standard: Potential Economic and Environmental Effects of U.S. Biofuel Policy* (NRC, 2011b).

LCA can provide important insights into the sustainability of algal biofuels. Algal biofuels potentially have lower GHG emissions compared to petroleum-based fuels and they might not generate significant new negative impacts. Estimates of life-cycle GHG emissions for other biofuels span a wide range depending on the feedstock type, management practices used to grow feedstock, and whether any land-use changes were incurred. Algal biofuels thus need to be vetted with LCA and other approaches. Also, mass-scale agricultural systems induce significant material inputs of water and nutrients and emit various pollutants. LCA can help characterize material flows associated with such requirements for an algal biofuel industry.

There are challenges to using LCA to assess sustainability of algal biofuels. These challenges, including the issues associated with defining the system boundary for LCA analysis, are discussed in other publications (NAS-NAE-NRC, 2010; NRC, 2011b). LCA primarily is formulated as a retrospective description of existing supply chains. Algal biofuel production is in early development and there are limited historical data. In addition, technological progress and scale-up will affect future material flows but are challenging to forecast.

In addition to LCAs for assessing environmental variables, social LCAs are being developed to compare social impacts of products, processes, or companies, and to identify potential areas of improvements (Jorgensen et al., 2008). Social LCA is in early development stage, and consensus has yet to be reached on the impact categories to be included and how they would be measured (Dreyer et al., 2006; Jorgensen et al., 2008).

1.3.4 Scenario Analysis

A scenario is a characterization of a possible future. Scenarios take many different forms and can be constructed in many different ways (Chermack et al., 2001). Generally, scenarios “provide conceptual and quantitative frameworks to describe and assess” an activity or technology (NAS-NAE-NRC, 2010, p.292). Scenarios typically “use qualitative analysis and quantitative assumptions to integrate the environmental, technologic, economic, and deployment-related elements” into a framework to compare alternative possible outcomes

(NAS-NAE-NRC, 2010, p.292). Scenarios do not simply extrapolate historical data but try to develop internally consistent sets of conditions that are needed to occur to attain a given set of outcomes. For example, scenarios for algal biofuels might look at potential system design, resource requirements, and infrastructure needs required to reach a given percentage of the liquid fluids market. Scenarios can help define the environmental and resource sustainability issues that might accompany a greatly expanded algal biofuel production system.

Scenarios are a part of a more general future analysis. Elements of future analysis include trend projections, systems modeling, and scenarios; the analysis can combine these elements in different ways. Trend projection involves extrapolation of retrospective information to the future. A central element of the trend projection process is simply deciding on a functional form for the trend, such as linear, exponential, or some other relationship (Craig et al., 2002). Further, trend analysis is best for known systems where there is a large quantity of historical data, which is not true for algal biofuels. Systems modeling consists of identifying relationships between variables of interest (Ibid). For example, an econometric model finds the optimal statistical fit between variables that are assumed to be related by a predefined functional form. A systems dynamics model develops causal relationships between quantities of interest and evolves the future from some initial condition using these relationships. Future issues relevant to the sustainability of algal biofuels include: how individual technology elements will develop (for example, algae cultivation), how technology elements will combine to yield a fuel production system, and how the production system will link to natural systems (for example, salt versus fresh water).

1.4 STUDY SCOPE AND APPROACH

Algal biofuels can be produced from a variety of feedstocks (autotrophic microalgae and cyanobacteria, heterotrophic microalgae, and macroalgae) using different processing technologies (for example, transesterification of algal oil, thermochemical conversion of algal biomass such as gasification and pyrolysis, or direct synthesis of alcohol). Examining the promise of different combinations of feedstocks and processing technologies to sustainably develop algal biofuels within the timeframe of this study was not feasible. Therefore, the committee limited the scope of the report in three ways following the guidance of the study sponsor and the committee's expert judgment.

First, this study focuses on biofuel production systems that use autotrophic microalgae as a feedstock in the United States. Heterotrophic approaches for algae cultivation are excluded because DOE-EERE considers production of biofuel using heterotrophic algae as a biochemical pathway to convert another feedstock (a sugar source such as cellulosic biomass) rather than a pathway that directly produces fuels from algae (Pate, 2011). The exclusion of the heterotrophic pathways is not a judgment on the validity of these approaches. Second, the study sponsor indicated macroalgae as a feedstock was of lower priority for this study than microalgae and cyanobacteria and suggested that the committee could consider macroalgae if time and budget allowed. The committee could not fully address the sustainability of using macroalgae as a feedstock not only because of time and budget constraints, but also because of the sparse literature on this topic. The focus on microalgae also is consistent with the research and investment patterns in algal biofuels. Third, the study relies on published literature so that the well-studied topics are emphasized more often than less well-studied topics relative to others in the report.

The committee developed its report based on members' expertise and information gathered from the public record. In its examination of publicly available information, the

committee relied on peer-reviewed papers; reports produced by government agencies and other interested parties; and documents filed as part of regulatory activities, including patent applications and environmental-impact assessments. In addition, the committee gathered information through presentations at open committee meetings from government agencies, companies, and others involved in the algal biofuel supply chain, researchers from academia, and other groups. The information gathered at these public meetings was augmented by public webinars and solicitation of information from algal biofuel companies. The information gathered during these activities helped form the basis for the description of the algal biofuel supply chain, resource requirements, and impacts discussed in subsequent chapters. In analyzing this information, the committee relied on the methods described earlier.

1.5 STRUCTURE OF REPORT

The report addresses the statement of task in the following ways. Chapter 2 provides an overview of algal biofuel supply chain and examples of different cultivation, harvesting, dewatering, processing, and coproduction methods that could be used in producing algal biofuels. Chapter 3 introduces selected algal biofuel production systems as examples to illustrate challenges and sustainability concerns of algal biofuel production and possible tradeoffs among sustainability goals. Chapters 4 and 5 discuss potential concerns related to resource use (for example, availability of land, water, and nutrient resources) and environmental effects and how some of those concerns might affect social acceptability of algal biofuels, respectively. For each category of resource use and environmental effect, indicators and metrics to be employed and data to be collected to assess sustainability are suggested. Chapter 6 summarizes the sustainability challenges for each of the selected algal biofuel production systems introduced in Chapter 3 and uses them to illustrate benefits and tradeoffs of each system.

REFERENCES

- Azapagic, A., and S. Perdan. 2000. Indicators of sustainable development for industry: A general framework. *Process Safety and Environmental Protection* 78(4):243-261.
- Baumann, H., and A.M. Tillman. 2004. *The Hitch Hikers Guide to LCA*. Lund, Sweden: Studentlitteratur AB.
- Benemann, J.R., R.P. Goebel, J.C. Weissman, and D.C. Augenstein. 1982. *Microalgae as a Source of Liquid Fuels*. Fairfield, CA: EnBio, Inc.
- Bhatnagar, A., M. Bhatnagar, S. Chinnasamy, and K.C. Das. 2010. *Chlorella minutissima*—A promising fuel alga for cultivation in municipal wastewaters. *Applied Biochemistry and Biotechnology* 161(1-8):523-536.
- Brune, D.E., T.J. Lundquist, and J.R. Benemann. 2009. Microalgal biomass for greenhouse gas reductions: Potential for replacement of fossil fuels and animal feeds. *Journal of Environmental Engineering-ASCE* 135(11):1136-1144.
- Bullard, C.W., and R.A. Herendeen. 1975. Energy cost of goods and services. *Energy Policy* 3(4):268-278.
- Bullard, C., P. Pennter, and D. Pilati. 1978. Net energy analysis: Handbook for combining process and input-output analysis. *Resources and Energy* 1:267-313.
- Chermack, T.J., S.A. Lynham, and W.E.A. Ruona. 2001. A review of scenario planning literature. Available online at <http://www.cse.buffalo.edu/~peter/refs/DataAssimilation/Multihypothesis/ReviewofSP.PDF>. Accessed June 18, 2012.
- Chinnasamy, S., A. Bhatnagar, R.W. Hunt, and K.C. Das. 2010. Microalgae cultivation in a wastewater dominated by carpet mill effluents for biofuel applications. *Bioresource Technology* 101(9):3097-3105.
- Corbière-Nicollier, T., I. Blanc, and S. Erkman. 2011. Towards a global criteria based framework for the sustainability assessment of bioethanol supply chains. Application to the Swiss dilemma: Is local produced bioethanol more sustainable than bioethanol imported from Brazil? *Ecological Indicators* 11:1447-1458.
- Craggs, R.J., S. Heubeck, T.J. Lundquist, and J.R. Benemann. 2011. Algal biofuels from wastewater treatment high rate algal ponds. *Water Science and Technology* 63(4):660-665.

- Craig, P.P., A. Gadgil, and J.G. Koomey. 2002. What can history teach us? A retrospective examination of long-term energy forecasts for the United States. *Annual Review of Energy and the Environment* 27:83-118.
- CSBP (Council on Sustainable Biomass Production). 2010. Draft Provisional Standard for Sustainable Production of Agricultural Biomass. Dillon, CO: Council on Sustainable Biomass Production.
- DOE (U.S. Department of Energy). 2010. National Algal Biofuels Technology Roadmap. Washington, DC: U.S. Department of Energy, Energy Efficiency and Renewable Energy.
- Efroymson, R.A., V.H. Dale, K.L. Kline, A.C. McBride, J.M. Bielicki, R.L. Smith, E.S. Parish, P.E. Schweizer, and D.M. Shaw. 2012. Environmental indicators of biofuel sustainability: What about context? *Environmental Management* DOI: 10.1007/s00267-012-9907-5.
- EIA (U.S. Energy Information Administration). 1999. Annual Energy Review 1998. Washington, DC: U.S. Department of Energy Energy Information Administration.
- EPA (U.S. Environmental Protection Agency). 2006. Life Cycle Assessment: Principles and Practice. Washington, DC: U.S. Environmental Protection Agency.
- ESA (Ecological Society of America). 2008. Biofuel sustainability. Available online at <http://www.esa.org/pao/policyStatements/Statements/biofuel.php>. Accessed February 27, 2012.
- Gallagher, B.J. 2011. The economics of producing biodiesel from algae. *Renewable Energy* 36(1):158-162.
- Hammond, G.P. 2000. Energy, environment and sustainable development: A UK perspective. *Process Safety and Environmental Protection* 78(4):304-323.
- Hendrickson, C.T., L.B. Lave, and H.S. Matthews. 2006. Environmental Life Cycle Assessment of Goods and Services: An Input-Output Approach. Washington, DC: Resources for the Future Press.
- Holmes, K.J., and M.G. Wolman. 2001. Early development of systems analysis in natural resources management from man and nature to the Miami Conservancy District. *Environmental Management* 27(2):177-193.
- IISD (International Institute for Sustainable Development). 2011. What is sustainable development? Available online at <http://www.iisd.org/sd/>. Accessed July 7, 2011.
- ISCC (International Sustainability and Carbon Certification). 2012. ISCC PLUS Standard on Sustainability Requirements for the Production of Biomass. Cologne, Germany: International Sustainability and Carbon Certification.
- ISO (International Organization for Standardization). 2006. Environmental Management—Life Cycle Assessment—Principles and Framework. Geneva: International Organization for Standardization.
- Leontief, W. 1970. Environmental repercussions and economic structure—Input-output approach. *Review of Economics and Statistics* 52(3):262-271.
- Lundquist, T.J., I.C. Woertz, N.W.T. Quinn, and J.R. Benemann. 2010. A Realistic Technology and Engineering Assessment of Algae Biofuel Production. Berkeley, CA: Energy Bioscience Institute.
- Markevicius, A., V. Katinas, E. Perednis, and M. Tamasauskienė. 2010. Trends and sustainability criteria of the production and use of liquid biofuels. *Renewable and Sustainable Energy Reviews* 14(9):3226-3231.
- Mascarelli, A.L. 2009. Algae: Fuel of the future? *Environmental Science and Technology* 43(19):7160-7161.
- McBride, A.C., V.H. Dale, L.M. Baskaran, M.E. Downing, L.M. Eaton, R.A. Efroymson, C.T. Garten Jr, K.L. Kline, H.I. Jager, P.J. Mulholland, E.S. Parish, P.E. Schweizer, and J.M. Storey. 2011. Indicators to support environmental sustainability of bioenergy systems. *Ecological Indicators* 11(5):1277-1289.
- Miller, R.E., and P.D. Blair. 2009. *Input-Output Analysis: Foundations and Extensions*. New York: Cambridge University Press.
- Mouawad, J. 2009. Exxon to invest millions to make fuel from algae. Available online at <http://www.nytimes.com/2009/07/14/business/energy-environment/14fuel.html?pagewanted=all>. Accessed February 16, 2012.
- NAS-NAE-NRC (National Academy of Sciences, National Academy of Engineering, and National Research Council). 2009. *America's Energy Future: Technology and Transformation*. Washington, DC: The National Academies Press.
- . 2010. *Electricity from Renewable Resources*. Washington, DC: The National Academies Press.
- Nickerson, C., R. Ebel, A. Borchers, and F. Carriazo. 2011. *Major Uses of Land in the United States, 2007*. U.S. Department of Agriculture.
- NRC (National Research Council). 2001. *Envisioning the Agenda for Water Resources Research in the Twenty-First Century*. Washington, DC: National Academy Press.
- . 2008. *Transitions to Alternative Transportation Technologies—A Focus on Hydrogen*. Washington, DC: The National Academies Press.
- . 2009. *Nutrient Control Actions for Improving Water Quality in the Mississippi River Basin and Northern Gulf of Mexico*. Washington, DC: The National Academies Press.
- . 2010a. *Expanding Biofuel Production: Sustainability and the Transition to Advanced Biofuels: Summary of a Workshop*. Washington, DC: The National Academies Press.

- . 2010b. *Toward Sustainable Agricultural Systems in the 21st Century*. Washington, DC: The National Academies Press.
- . 2010c. *Transitioning to Sustainability Through Research and Development on Ecosystem Services and Biofuels: Workshop Summary*. Washington, DC: The National Academies Press.
- . 2011a. *America's Climate Choices*. Washington, DC: The National Academies Press.
- . 2011b. *Renewable Fuel Standard. Potential Economic and Environmental Effects of U.S. Biofuel Policy*. Washington, DC: The National Academies Press.
- Pate, R. 2011. U.S. Department of Energy, Office of Biomass Program (OBP) Sponsor Perspectives for NRC Study on Algae Biofuels Sustainability. Presentation to the the NRC Committee on Sustainable Development of Algal Biofuels on March 17.
- Patil, V., K.Q. Tran, and H.R. Giselrod. 2008. Towards sustainable production of biofuels from microalgae. *International Journal of Molecular Sciences* 9(7):1188-1195.
- Physorg.com. 2010. U.S. military to make jet fuel from algae. Available online at <http://www.physorg.com/news185521814.html>. Accessed February 6, 2012.
- RSB (Roundtable on Sustainable Biofuels). 2011. Principles and criteria. Available online at <http://rsb.epfl.ch/page-24929.html>. Accessed July 18, 2011.
- Schenk, P.M., S.R. Thomas-Hall, E. Stephens, U.C. Marx, J.H. Mussgnug, C. Posten, O. Kruse, and B. Hankamer. 2008. Second generation biofuels: High-efficiency microalgae for biodiesel production. *Bioenergy Research* 1(1):20-43.
- Sheehan, J., T. Dunahay, J. Benemann, and P. Roessler. 1998. *A Look Back at the U.S. Department of Energy's Aquatic Species Program: Biodiesel from Algae*. Golden, CO.: National Renewable Energy Laboratory.
- Solomon, B.D. 2010. Biofuels and sustainability. *Annals of the New York Academy of Sciences* 1185:119-134.
- Suter, G.W.I. 2001. Applicability of indicator monitoring to ecological risk assessment. *Ecological Applications* 11:101-112.
- Sydorovych, O., and A. Wossink. 2008. The meaning of agricultural sustainability: Evidence from a conjoint choice survey. *Agricultural Systems* 98(1):10-20.
- Turnhout, E., M. Hisschemoller, and H. Eijsackers. 2007. Ecological indicators: Between the two fires of science and policy. *Ecological Indicators* 7(2):215-228.
- United Nations. 1987. *Report of the World Commission on Environment and Development: Our common future*. Available online at <http://www.un-documents.net/ocf-02.htm>. Accessed August 21, 2012.
- United Nations. 2011. UN Department of Economics and Social Affairs. Division for Sustainable Development. Available online at http://www.un.org/esa/dsd/index.shtml?utm_source=OldRedirect&utm_medium=redirect&utm_content=dsd&utm_campaign=OldRedirect. Accessed July 7, 2011.
- van Dam, J., M. Junginger, A. Faaij, I. Jürgens, G. Best, and U. Fritsche. 2008. Overview of recent developments in sustainable biomass certification. *Biomass and Bioenergy* 32(8):749-780.
- Williams, E., C. Weber, and T. Hawkins. 2009. Hybrid approach to managing uncertainty in life cycle inventories. *Journal of Industrial Ecology* 15(6):928-944.
- Williams, P.J.L.B., and L.M.L. Laurens. 2010. Microalgae as biodiesel and biomass feedstocks: Review and analysis of the biochemistry, energetics and economics. *Energy and Environmental Science* 3(5):554-590.
- Woertz, I., A. Feffer, T. Lundquist, and Y. Nelson. 2009. Algae grown on dairy and municipal wastewater for simultaneous nutrient removal and lipid production for biofuel feedstock. *Journal of Environmental Engineering-ASCE* 135(11):1115-1122.

2

Overview of Algal Biofuel Supply Chain

Assessing the sustainability of algal biofuels requires an understanding of the individual components that make up potential supply chains. This chapter focuses on the basic processes of algal biofuel production from the biology and traits of the organisms, to methods for cultivation, and to processing into liquid fuels. It discusses algal strains and the attributes of those strains critical for biofuel production, the photoautotrophic methods for algae cultivation through open-pond and closed photobioreactor systems, the processes for collection and dewatering if necessary, and the processing of algal lipid, biomass, or secreted products into fuels. It provides the basic descriptions of the supply chain components used in later chapters and summarizes some critical process improvements that could enhance the overall sustainability of algal biofuels.

2.1 ALGAL FEEDSTOCKS

The organisms considered as potential feedstock for algal biofuel production belong to a vast and diverse assemblage of aquatic organisms that carry out oxygen-evolving photosynthesis and lack the stems, roots, leaves, and embryos of plants (Leliaert et al., 2011). The category includes eukaryotic species that are related to the plant lineage and can be further categorized as macroalgae that are large structured species (for example, kelps) or microalgae that are microscopic species (for example, *Nannochloropsis* spp.). In the context of biofuel, the term “microalgae” also includes cyanobacteria, a diverse prokaryotic lineage whose ancestor gave rise to the plant chloroplast (Keeling, 2010). More than 40,000 species of microalgae have been described, and they collectively cover a comprehensive spectrum of habitats and tolerances of ranges of pH, salinity, and temperature (Van den Hoek et al., 1995; Falkowski and Raven, 1997; Paerl, 2000). McKenzie (2011) estimated that prokaryotic and eukaryotic microalgae are responsible for more than 40 percent of net primary productivity on Earth. Algae can be a more appealing biofuel feedstock than land plants because of their faster biomass doubling cycle, their more accessible forms of stored carbon than

the lignocelluloses used for cellulosic biofuels, and their ability to thrive on water sources and on land sites that are unsuitable for terrestrial farming.

Microalgae contain diverse pigments and metabolites that are desirable as nutritional supplements and colorants. Examples of such products include astaxanthin, an antioxidant derived from the alga *Haematococcus*, and a high-protein powder derived from cyanobacterial species of *Spirulina* (*Arthrospira*) (Gershwin, 2008; Guedes et al., 2011). Commercial-scale algal ponds that grow these and other microalgae have operated for more than a decade (Del Campo et al., 2007). However, the scale of deployment for algae cultivation for fuel is expected to be much larger than the scale of algae cultivation for nutraceuticals or other specialty products currently available in the market.

Generating biofuels from algae requires exploiting and expanding the demonstrated commercial-scale growth of algal biomass, and harvesting the relatively accessible carbon stored therein. Carbon is stored within algal cells in various forms, and these molecules can be accessed by different technologies. Both eukaryotic and prokaryotic algal cells are rich sources of polar lipids that are associated with membranes; in some cases, the photosynthetic thylakoid membranes are extensive. Carbon is such a crucial element for algae that it is typical for them to store surplus carbon when cellular division is restricted by some factor other than carbon availability—this situation is termed unbalanced growth. In many eukaryotic microalgae, photosynthetic carbon fixation continues under unbalanced conditions. Under extended periods of environmental stress, the excess fixed carbon is stored in the form of neutral lipids called triacylglycerols (TAGs). TAGs are hydrocarbon chains terminated in a carboxylic acid group. The three carboxyl groups are bound to glycerol through an ester linkage. Biofuels containing hydrocarbon chains longer than six carbons are particularly valued because of their high heats of combustion, volatility, and compatibility with existing engines. As discussed later in this chapter, extracted TAGs can be converted to biodiesel using a number of technologies, including transesterification and hydrotreating. Even algal species that do not store large amounts of TAGs can be converted to biofuels through various chemical conversion technologies. For example, species that store polysaccharides can be fermented to yield ethanol, and other biomass processing technologies, such as gasification, pyrolysis, and hydrothermal liquefaction, have shown great utility for the conversion of whole biomass into biofuels.

The incipient algal biofuel industry is emerging and evolving from its early foundations in algae cultivation for fish feedstuff and for human nutraceuticals. Early technology development of processing algae to fuels emphasized the conversion of neutral lipids (TAGs) to biodiesel. Choices of algal feedstocks have been expanding to address the goals of fuel production rather than nutritional content and to exploit new technologies for processing biomass that extend beyond those that focus on TAGs. Ideal attributes for algal feedstock for fuels include rapid and dense growth; efficient use of nutrients, light, and carbon dioxide (CO₂) under a range of temperatures; resistance to pests and predators; accumulation of desirable macromolecules that can be processed into fuels; ease of harvest; and the absence of undesirable by-products.

Commercial and research interest in the United States has focused on microalgae, and these species are emphasized in this report. Microalgae have been reported to reach short-term maximum productivities of 50–60 g dry weight per square meter (m²) per day in CO₂-enriched open ponds in Hawaii and California (Sheehan et al., 1998). These and other data on productivity from laboratory-scale experiments have promoted the reputation of microalgae as prime candidates for providing cheap biomass feedstocks for food, feedstuff, or energy. Some authors have extrapolated values of maximal biomass productivity and combined them with maximal oil content to predict oil yields of 100 tonnes per hectare

(ha) per year. Such reports have spurred investment in intensive research on algal biofuel production. However, such high productivity projections have yet to be obtained in large-scale, long-term experiments. Serious barriers remain for reproducing optimal growth and productivity conditions at a commercial scale. They include maintaining the stability of the culture and delivering the required nutrients and other resources in an efficient manner at such scales. Current yields from large-scale operations range from 40–60 tonnes dry weight of algal biomass production per ha per year, and conservative projections anticipate up to 100 tonnes dry weight of biomass, or 30 tonnes of biodiesel per ha per year in subtropical or tropical, sunny climates (Scott et al., 2010). Estimated yields from a variety of cultivation systems are discussed later in the chapter.

2.1.1 Strain Diversity

The choice of strains for biomass production depends on the desired product and technology to be used for fuel production, the source, and the type of cultivation facility (open versus closed). Initial efforts using outdoor ponds focused on production of biodiesel by the transesterification of TAGs to produce fatty-acid methyl esters (FAME).¹ Therefore, strains that accumulate TAGs were selected. Five groups of microalgae were classified as high priority for biofuel production by the U.S. Aquatic Species Program (Sheehan et al., 1998): diatoms (Bacillariophyceae), green algae (Chlorophyceae), golden-brown algae (Chrysophyceae), prymnesiophytes or haptophytes (including Prymnesiophyceae), and eustigmatophytes (Eustigmatophyceae). Many strains and genera of eukaryotic microalgae are potential high-oil producers for large-scale culture (Sheehan et al., 1998; Rodolfi et al., 2009). These include species of *Tetraselmis*, *Dunaliella*, *Chlorococcum*, *Scenedesmus*, and *Chlorella*, and particularly *Neochloris oleoabundans* and *Botryococcus braunii* from Chlorophyta; the genera of *Amphora*, *Amphiprora*, *Cylindrotheca*, and *Navicula*, and the species of *Nitzschia dissipata*, *Phaeodactylum tricornutum*, and *Chaetoceros muelleri* from Bacillariophyta; the species of *Nannochloropsis ocalata* and *N. salina* from Eustigmatophyceae; and the genera of *Isochrysis* and *Pavlova* from Haptophyta.

Improvements of technologies that convert total biomass to yield drop-in fuels—such as those being pursued by companies such as Inventure (Inventure, 2012), Xtrudx (Xtrudx Technologies, 2012), and Solvent Rescue Limited (Solvent Rescue Limited, 2012) and academic institutions such as Old Dominion University (Hatcher, 2011)—are changing the scope of organisms that are being considered for biofuel production. All categories of algae are rich in polar lipids that can be recovered by such processes, and they have cellulose or other polysaccharide cell walls composed of sugars. Cyanobacteria store excess carbon as glycogen rather than TAGs, and cyanobacteria and macroalgae accumulate quantities of other complex polysaccharides. These and other macromolecules are all potential carbon sources for producing drop-in fuels if appropriate processing technologies are available. In addition, algal carbohydrate potentially can be a feedstock for fermentative fuel production processes that are based on heterotrophic organisms, such as those used by LS9, Inc. (LS9 Inc., 2011) and Solazyme (Solazyme, 2012). Cyanobacteria are used directly for ethanol production by Algenol (Chance et al., 2011a; Algenol Biofuels, 2012). As of 2012, a number of marine macroalgal species are being considered for biofuel production in India. An example

¹As Chapter 3 discusses, algal triacylglycerols are reacted with methanol to form fatty-acid methyl esters (FAME). Due to its higher viscosity compared to conventional liquid transportation fuels, FAME cannot be used as a drop-in fuel, but can be blended with conventional diesel.

is the red algal species *Kappaphycus alvarezii*, a species cultivated for its high carrageenan² content (Russell, 1983; Rodgers and Cox, 1999; Woo et al., 2000). Species of *Spirulina* have properties suitable for aquaculture, and they are grown at relatively large scales for sale as a nutritional supplement (Earthrise Nutritional, 2009a). Still, the spectrum of cyanobacteria that could be suitable for fuel production is largely unexplored. Prokaryotic algal species provide additional diversity in light harvesting, tolerance of growth habitat and pH, and facility of genetic modification.³ Moreover, some cyanobacterial species are diazotrophs; that is, they are able to fix atmospheric nitrogen (N). Although no current commercial operations rely on a nitrogen-fixing strain, several filamentous strains that have good light-harvesting properties and for which genetic methods are well developed are diazotrophic (Heidorn et al., 2011; Ruffing, 2011). The use of these strains as a biofuel feedstock or as a nitrogen provider for non-fixing strains (to reduce nutrient input) has received little attention.

Clear differences exist in carbon storage forms (important as fuel feedstock), dominant pigments (important for solar energy capture), and accessory pigments such as carotenoids (which can be valuable commercial products) among different algal divisions (Table 2-1). Furthermore, their pigmentation and composition are affected by growth conditions and environmental stress.

Emphasizing individual strains that are intended for monoculture discounts potential advantages that could be associated with mixed cultures. A recent study showed increased lipid production in algal cultures as a function of species diversity in mixed cultures under nutrient-limiting growth conditions (Stockenreiter et al., 2012). However, this effect has been demonstrated only at the laboratory scale or in low-density natural algal populations, and requires confirmation for extended periods of time and at relevant volumes. Moreover, lipid production of mixed algal culture could be different under the nutrient-replete conditions of ponds designed for maximal growth. Mixed cultures might facilitate cross-protection, diversity of products through product conversion, flocculation and harvesting improvements, and efficient use of light in the water column (Stomp et al., 2007). However, mixed cultures increase the heterogeneity of the potential product, which could affect the quality of yield and the ability to optimize the diverse characteristics of the mixture for a single product. The potential to enhance the supply chain of algal biofuel through growth of mixed cultures merits additional research to determine the effects on desirable product yield and biomass accumulation (see section Cultivation in this chapter). Because data are not available for large-scale, mixed-species systems, this report introduces the concept of mixed culture systems but focuses primarily on monoculture systems.

Among the biggest challenges for strain selection is the difficulty of translating desirable strain properties from the laboratory to the field. A desirable strain would have robust growth in open ponds under natural weather and cultivation conditions, and would retain attributes that are selected and measured in the controlled conditions of the laboratory. However, the ability to grow well and compete when exposed to environmental conditions is difficult to predict. Few strains are already proven to be robust in outdoor mass cultivation, and years of investment in time and process went into their commercial development.

²A gelatinous substance extracted from red algae and widely used as a stabilizing or thickening agent in industrial, pharmaceutical, and food products.

³Within the text of this report, the committee will distinguish whether it is discussing “genetic modification” or “genetic engineering” specifically. The committee considers genetic modification to be a general term and includes in its definition any organism whose genetic material has been altered through an array of approaches, including traditional cross breeding, mutagenesis, and genetic engineering. Genetic engineering is a modern technique that enables the introduction of a foreign gene or genes into the genome of an organism through recombinant DNA methods in an attempt to introduce a new trait into that organism.

TABLE 2-1 Characteristics of Photoautotrophic Algae^a

Division	Dominant Photosynthetic Pigment(s)	Accessory Pigments (Carotenoids)	Principal Energy Storage Compound	% Protein ^b	% Lipid ^b
Cyanoprokaryota (blue-green algae)	Phycobilins, Chlorophyll <i>a</i>	Zeaxanthin, <i>beta</i> -carotene, myxoxanthin, echinenone, canthaxanthin	Glycogen, other polysaccharides, polyhydroxyalkanoates, Lipid	10-70	1-20
Bacillariophyceae	Chlorophyll <i>a</i> , Chlorophyll <i>c</i>	Fucoxanthin, <i>beta</i> -carotene, diadinoxanthin, diatoxanthin		5-35	5-55
Haptophyceae	Chlorophyll <i>a</i> , Chlorophyll <i>c</i>	Beta-carotene	Chrysolaminaran	5-30	5-55
Chlorophyceae	Chlorophyll <i>a</i> , Chlorophyll <i>b</i>	Lutein, <i>beta</i> -carotene, violaxanthin, neoxanthin	Starch	5-30	5->50
Haptophyceae	Chlorophyll <i>a</i> , Chlorophyll <i>c</i>	Fucoxanthin	Starch	5-35	5-50
Raphidophyceae	Chlorophyll <i>a</i>	Diatoxanthin	Lipid	5-35	5-55
Rhodophyceae	Phycobilins, Chlorophyll <i>a</i>		Starch	5-15	5-15
Phaeophyceae	Chlorophyll <i>a</i>	Fucoxanthin	Starch	5-15	5-15
Chrysophyta	Chlorophyll <i>a</i> and <i>c</i>	Beta-carotene, fucoxanthin	Lipids (oil) Leucosin	20-30	30-40
Eustigmatophyta	Chlorophyll <i>a</i>	vialaxanthin, <i>beta</i> -carotene	Lipids (oil)	10-30	40-65

^a Table shows wide ranges in the percentage of lipids and proteins, reflecting that these and other parameters are dramatically affected by growth conditions.

^b Percentages are given as a percent of dry weight.

Successful mass cultivation of new strains likewise will require intensive work to commercialize, whether those strains are native, genetically modified, or bred for improved attributes.

2.1.2 Desirable Strain Properties

Regardless of the technology or strain, the goal is to maximize the quantity of a final product per unit time, area, or water volume. Further, the desire is to maximize the product output per unit input of energy, nutrients, and other resources. Biomass and lipid accumulation per unit time are two measures of productivity (see Rodolfi et al., 2009 for example). Many other criteria are important for selecting algal strains for commercial biofuel production, including variables that alter cost in the supply chain that are important for economic viability (for example, AQUAFUEL, 2009). Ideally, the criteria for strain selection are measurable. Among important selection criteria are:

- **Photosynthetic efficiency.** The most objective measure to compare productivity of algae with land crops is photosynthetic efficiency. Photosynthetic efficiency is defined as the percent of available light (energy) that is converted into biomass energy. However, this definition might not be the most relevant for a given supply chain, depending on how the biomass will be processed and what the final products and coproducts will be (Box 2-1).

BOX 2-1 Relevance of Photosynthetic Efficiency to Biofuel Production

The amount of biofuel produced per unit of land area is a key parameter in the evaluation of any biofuel production process. Photosynthetic efficiency, a measure of how efficiently light energy is converted to chemical energy, is one of the key determinants of overall biomass yield. The measure relevant to biofuel production is the amount of energy contained in biomass expressed as a ratio of the solar energy supplied (Blankenship et al., 2011). The calculation is performed for a typical area integrated over a year or a growing season. When done this way, values of up to 3 percent have been reported for microalgae (Wijffels and Barbosa, 2010). Some authors choose to calculate photosynthetic efficiency based on only the percentage of photosynthetically active radiation (PAR) present (Ort et al., 2011), or even only the PAR absorbed (Janssen et al., 2001). These calculations lead to considerably higher values and lead to some confusion around the potential for biofuel production from algae.

Further complicating this particular discussion is determination of the heat of combustion, or the heating value,^a to be used. For measures of total photosynthetic efficiency, the heat of combustion is generally taken to be the higher heating value of the dried biomass (Jenkins et al., 1998).

The critical feature for this discussion is not the exact efficiency, but rather that the value is far below what should be theoretically possible (Robertson et al., 2011). Indeed, many have lamented that photosynthesis uses one of the “slowest metabolic enzymes in the contemporary biosphere” (Parikh et al., 2006; p. 113). Considerable improvement in photosynthesis might be realized by any number of techniques of modern biology. Improvements in photosynthesis would lead directly to more prolific production of biofuels, which would consequently reduce the land, water, nutrient, and energy inputs required. Improvements to photosynthesis would directly improve the sustainability of algal biofuels.

^a “The higher heating value (also known as gross calorific value or gross energy) of a fuel is defined as the amount of heat released by a specified quantity (initially at 25°C) once it is combusted and the products have returned to a temperature of 25°C, which takes into account the latent heat of vaporization of water in the combustion products. The lower heating value (also known as net calorific value) of a fuel is defined as the amount of heat released by combusting a specified quantity (initially at 25°C) and returning the temperature of the combustion products to 150°C, which assumes the latent heat of vaporization of water in the reaction products is not recovered” (DOE-EERE, 2012a).

- **Quantity of final products.** This category includes the total amount of biomass, its composition, and the products to be refined, extracted, or excreted from the biomass:
 - Total caloric value of the biomass (for combustion or a total biomass processing technology).
 - Percent lipids and lipid composition (for biodiesel).
 - Percent starch and carbohydrate composition (for subsequent fermentation and to identify higher value by-products such as agar).
 - Percent protein and protein composition (soluble and insoluble protein for food and feedstuff).
 - Total secretion of desirable products.⁴
 - Presence of high-value coproducts.

⁴Some companies, such as Joule and Algenol, have taken a dramatically different approach, relying not on accumulation of biomass, but on the secretion of desirable products from stable algal cultures (Robertson, D.E., S.A. Jacobson, F. Morgan, D. Berry, G.M. Church, and N.B. Afeyan. 2011. A new dawn for industrial photosynthesis. *Photosynthesis Research* 107(3):269-277). In this paradigm that uses photobioreactors, the criteria for strain selection are different from those used for open ponds. Planktonic unicellular species that would be difficult to protect from grazers and to harvest from ponds, are desirable within bioreactors. Well-developed genetic model organisms that are amenable to genetic engineering (such as *Synechocystis* sp. strain PCC 6803, *Synechococcus* sp. strain PCC 7002, and *Synechococcus elongatus* PCC 7942; and the unicellular green alga *Chlamydomonas reinhardtii*) can be used in the controlled environment of photobioreactors.

- **Nutrient and other resource requirements.** These include the quantity of nutrients, such as CO₂, nitrogen, and phosphorus; the type and quality of the water supply; and siting requirements. Strains could be selected because of their nutrient-use efficiency. Strains also might be selected because of their ability to flourish in brackish or wastewater, which would reduce the demand on freshwater supplies, and in the climatic conditions of a particular site.
- **Robustness.** This term describes the overall stability of the crop, which depends on resistance to extremes of climate and environmental variables (for example, competitors, pathogens and predators, salinity and dissolved solutes, temperature, and pH). Tolerances to these variables vary widely within the diverse spectrum of microalgae. The ability to thrive in water with various salts, metals, and other solutes could become increasingly important as competition for freshwater use among different sectors increases. Resistance to high pH allows growth in alkaline conditions that favor a monoculture crop over sensitive predators and pathogens. Filamentous species or species with large cell size tend to be more resistant to grazers than unicellular species with small cell size (Tillmann, 2004). Tolerance to a broad range of temperatures could be important if the algae are cultivated in regions with high daily or seasonal fluctuation in temperature. To maintain year-round production, it might be desirable to rotate strains that have different temperature tolerance profiles. The wide spectrum of sites that are under consideration for production ponds will require organisms with different light, water quality, and climatic tolerances. Robustness might be assessed by scoring the strain success under a wide range of potentially relevant conditions such as in Evens and Niedz (2011).
- **Harvestability.** Harvesting cost and energy consumption can vary dramatically among different algal strains (Uduman et al., 2010). Contributing factors include the sedimentation rate and the capability for induced bioflocculation⁵ or auto-flocculation. Filamentous strains that can be seined, species with positive buoyancy, or species that settle out of the water column quickly once agitation ceases might not require centrifugation, and they can be harvested easily. Growing mat-forming algae or algal films could facilitate harvesting (Tang et al., 1997), but to the committee's knowledge, such approaches have not been scaled up. Strategies that rely on harvesting secreted products rather than biomass simplify the harvesting step, but such strategies require photobioreactors for algae cultivation to prevent contamination by microorganisms that would consume the product.
- **Processability and extractability.** This parameter includes factors that influence the ease of extracting algal oil or processing algal biomass to fuels, for example, cell volume, thickness and toughness of the cell wall, the presence of tough fibers (for example, cellulose and silica) or cell walls, and the moisture content (Brennan and Owende, 2010). A measure for processability and extractability could be the energy input per gram of dry weight necessary for fractionation and full recovery of all biomass components.
- **Added value of coproducts.** The algal biomass could be used to produce coproducts that have an intrinsic added value, such as carotenoids, phycobilins, docosahexaenoic acid, or eicosapentaenoic acid (Pal et al., 2011). Coproducts can offset some of the costs of the biofuel product. A specification of the compounds and their expected added value per gram of dry biomass needs to be indicated. However, the market value of coproducts could decrease under an excessive-supply and low-demand condition.

⁵Bioflocculation is the clumping together of microorganisms through biological interactions.

- **Local origin of strains.** Using locally selected strains could ease management and improve sustainability (RSB, 2011). Some governments have sought to restrict the importation of nonnative species, for example, the 81st Texas Legislature House Bill 3391 (2009). However, the cosmopolitan nature and wind-borne movements of algae make it unlikely that legislation can reasonably define species as native or nonnative. Regardless of legislation, local strains might have unique adaptations to the local climate, water, and possible parasites that imported or laboratory-grown strains might not have.
- **Non-toxic.** The selection of non-toxic algal strains will increase social acceptability and reduce the potential impacts related to occupational exposures and accidental releases.

2.1.3 Strain Development and Engineering

Modern agriculture has advanced primarily on the development of improved germplasm, and algae cultivation will likely advance using similar approaches. As with traditional agriculture, advances in breeding, mutagenesis, and genetic engineering are likely to play roles in algal germplasm enhancement. Domestication of algae potentially could change their phenotype dramatically because the desired characteristics for production are different from those that have evolved in the selective pressures of the wild and because hypereutrophic aquaculture conditions will support genotypes that would not be fit in natural environments. Breeding and engineering will enable the stacking of desirable traits within a single species or mixture of species. The definition of desirable traits, product type desired, choice of production organism, and specification of growth and harvesting methods will influence the needs for further development on a case-by-case basis.

The understanding of genetics, physiology, and metabolism at present is uneven across the spectrum of genera and species of algae that might have desirable features for algal biofuel production. Major hurdles include the need to develop genetic technologies for new species that have not been domesticated previously and that have desirable characteristics for large-scale cultivation. The application of genomic approaches could accelerate the analysis of new strains by addressing changes in gene expression for a given organism under various conditions and identifying conserved and nonconserved genes among organisms. Those approaches facilitate the identification of candidate genes that might be relevant for particular pathways of interest (Flaherty et al., 2011; Karpowicz et al., 2011; Lopez et al., 2011; Weckwerth, 2011). Cryogenic storage methods, such as those used at the Culture Collection of Algae at the University of Texas (UTEX, 2012), also may prove important to maintaining germplasm stocks and to replenishing pond inocula with a desired genotype after genetic drift of the crop population. Cultured algae, particularly cultures held for more than 10 years in selective media, have been shown to have reduced growth and production of unexpected secondary metabolites (Martins et al., 2004). A factor that might be overlooked in efforts to genetically engineer metabolic pathways in algae is that both eukaryotic and prokaryotic strains possess circadian clocks that time the peaks of daily rhythmic changes in physiological and metabolic functions (Suzuki, 2001; Ditty et al., 2003; Matsuo and Ishiura, 2010; O'Neill et al., 2011). The mechanisms and the physiological and metabolic consequences of circadian rhythms are insufficiently understood in these organisms.

At present, few eukaryotic algal species are readily amenable to breeding or genetic engineering. Published transformation methods are well developed for *Chlamydomonas reinhardtii* and *Phaeodactylum tricorutum*. Solazyme appears to rely on genetically engineered

Chlorella species for heterotrophic fermentation of algal oils. About 30 strains of eukaryotic microalgae have been transformed using biolistic bombardment, vigorous mixing with glass beads, electroporation, or deoxyribonucleic acid (DNA) transfer from *Agrobacterium tumefaciens*. Strains that have been transformed include representatives of green, red, and brown algae; diatoms; euglenoids; and dinoflagellates (Radakovits et al., 2010). However, in many cases the reported transformation is only transient (Radakovits et al., 2010), and these reports have not led to routine adoption and application for most of those strains. Nevertheless, the transformations demonstrate that developing genetic systems for diverse species is possible with focused effort.

Targeted gene inactivation by homologous recombination has been a long-standing challenge for manipulation of *Chlamydomonas* and other algal nuclear genes. However, Kilian et al. (2011) made progress in this area when they reported successful knockouts of *Nannochloropsis* sp. nuclear genes encoding nitrate reductase and nitrite reductase. Various genes have been suppressed successfully in *Chlamydomonas* by interfering ribonucleic acid (RNAs) (Cerutti et al., 2011). High-throughput methods to introduce interfering RNAs could provide an effective way for gene inactivation in diverse strains that do not exhibit homologous recombination of transgenic DNA. Another challenge for nuclear modification is that gene expression is often silenced when heterologous genes are inserted randomly into the *Chlamydomonas reinhardtii* nuclear genome (Fuhrmann et al., 1999). Manipulation of the chloroplast genome is facile in *C. reinhardtii*, but not in other algae (Radakovits et al., 2010). A report of stable chloroplast transformation in *Porphyridium* suggests that chloroplast transformation via homologous recombination might be a universally applicable approach (Lapidot et al., 2002). Waaland et al. (2004) reviewed macroalgal species as candidates for genomic research and concluded that the red alga *Porphyra yezoensis* exhibits numerous attributes conducive to further analyses. Extensive biochemical and physiological research has been conducted on the macroalgae because of their use in the food industry. Because there is extensive variation in the extent and type of genetic malleability among different algal species, technologies would have to be developed on a case-by-case basis for individual new algal types whose physiological and metabolic properties suggest their potential as production strains. Moreover, it will be highly desirable to develop methods that can be used to more rapidly develop a genetic system *de novo* in new strains or species as they are discovered.

Genetic manipulation is more straightforward among cyanobacteria than eukaryotic algae because prokaryotes are amenable to techniques of bacterial genetics (Figure 2-1); some species are naturally transformable and take up exogenous DNA without specific intervention (Heidorn, 2011; Ruffing, 2011). Figure 2-2 shows some of the biochemical pathways in cyanobacteria that can be engineered to produce different desired products. Methods for gene inactivation via homologous recombination and the stable expression of transgenes, from plasmids or integrated into the chromosome, are well established in at least a dozen diverse species (Ducat et al., 2011; Ruffing, 2011). However, the developed model organisms have been maintained in the laboratory for several decades and are not likely to be suitable for growth under outdoor cultivation conditions. The *Spirulina* species that grow robustly outdoors have proven recalcitrant to manipulation. Despite some reports of transgenic *Spirulina* (Toyomizu et al., 2001; Kawata et al., 2004), many laboratories have failed to achieve stable transformation of the organism. This failure is likely, at least in part, due to a host of restriction endonucleases that specifically cleave foreign DNA (Zhao et al., 2006). Steps that protect plasmids by methylation while they are in an *Escherichia coli* host and before they are introduced to the cyanobacterium by conjugation have facilitated genetic technologies for the nitrogen-fixing filamentous strains *Anabaena* (*Nostoc*) sp. PCC

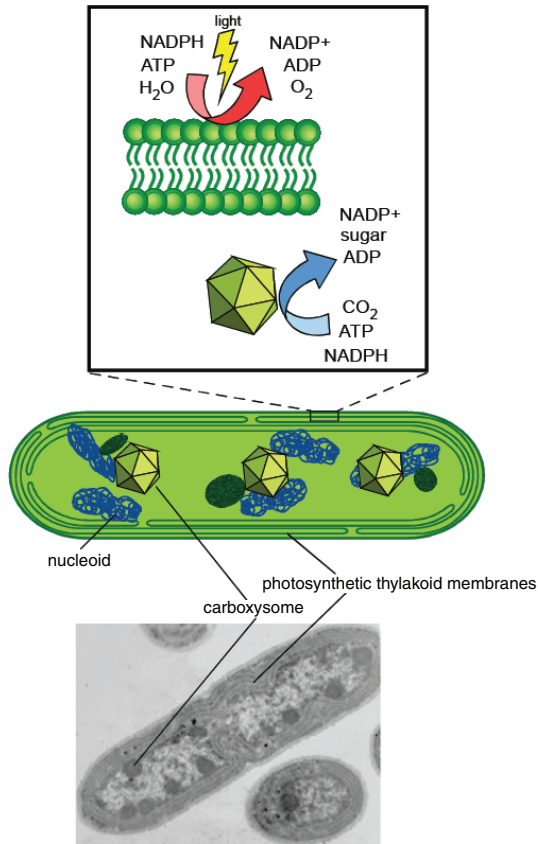


FIGURE 2-1 Overview of cyanobacterial organization.

NOTE: The cartoon diagram in the middle shows the longitudinal section of a representative cyanobacterium (modeled after *Synechococcus elongatus*). The major features are indicated on the cartoon diagram above and the electron micrograph below.

SOURCE: Adapted from Ducat et al. (2011). Micrograph image courtesy of and reprinted with permission from Lou Sherman, Purdue University.

7120, *Anabaena* sp. ATCC 29413, and *Nostoc punctiforme* ATCC 29133 (Elhai et al., 1997). Similar approaches are likely to work for other strains that initially resist transformation. A filamentous cyanobacterium isolated from an outdoor pond that has robust growth properties similar to *Spirulina* species has been found to be easily manipulated by conjugal introduction of transgenes and transposons (Taton et al., 2012). This finding suggests that diverse cyanobacterial model strains that are more relevant for biofuel development than current laboratory strains could be readily developed.

Genetic engineering holds the promise of transplanting completely novel pathways from heterologous sources and making products of tailored composition (Figures 2-1 and 2-2; Ruffing, 2011). Some demonstrations from genetically engineered cyanobacteria include the production of 1-butanol, isobutyraldehyde, N-alkanes, free fatty acids, and sugars from transformable species of *Synechococcus* (PCC 7002 and 7942), *Thermosynechococcus* (BP-1), and *Synechocystis* (PCC 6803) (Atsumi et al., 2009; Niederholtmeyer et al., 2010; Lan and Liao, 2011). Transgenic strains could play an important role in biofuel production, and some companies are making major investments in these technologies (for example, the Exxon Mobil alliance with Synthetic Genomics, Inc.; Marler, 2011; Roessler, 2011) even though

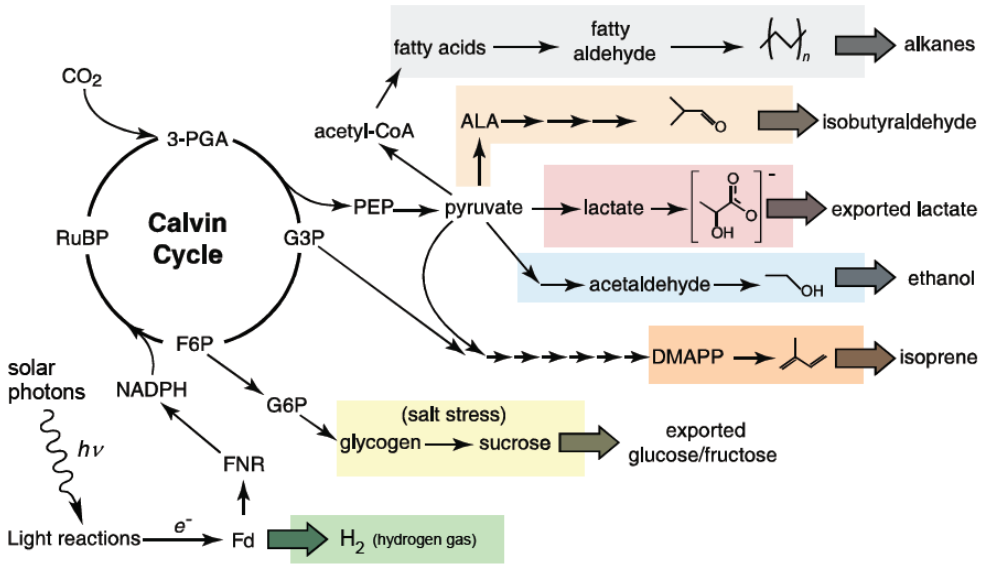


FIGURE 2-2 Schematic representation of engineered biochemical pathways in cyanobacteria. NOTE: Core metabolism of photosynthetic processes is shown in black text. Branch points used to produce various desired compounds are highlighted in colored boxes. Abbreviations: 3-PGA, 3-phosphoglycerate; FNR, ferredoxin NADP+ reductase. SOURCE: Adapted from Ducat et al. (2011).

strains have not been used in outdoor systems. The use of engineered strains in outdoor cultivation will be regulated according to the type of genetic modifications applied. The U.S. Environmental Protection Agency (EPA) under the Toxic Substances Control Act (TSCA) recognizes microorganisms that carry sequences from another genus as new organisms that require regulatory permitting (EPA, 2011). Under TSCA, organisms that are modified by technologies based solely on rearranging and reinserting endogenous genetic material into strains of interest are not categorized as genetically modified. Thus, self-cloned species can be used in open ponds without special oversight. Growing genetically modified algae in photobioreactors will follow the same regulatory standards that are common in the fermentation and biotechnology industries.

Irrespective of the algal strain cultivated and its end use, some areas of improvement in strain and cultivation are generally desirable. These include:

- Modulation of carbon allocation.
- Increases in culture density.
- Net increase in photosynthetic efficiency.
- Algal crop protection.
- Other enhancements.

2.1.3.1 Modulation of Carbon Allocation

The basic strategies to adapt microalgae to increased oil production for processing to diesel were summarized by Radakovits et al. (2010). A major target of genetic engineering

is production of algal strains that accumulate and maintain high amounts of oil under high growth rates in continuous cultivation systems. Most eukaryotic algae accumulate increased amounts of oil only in response to nutrient stress or in late exponential growth phase and do so at the expense of a reduced growth rate. Methods to enhance lipid accumulation in algae include enhancing certain enzymatic activities through genetic and transcription engineering approaches (Courchesne et al., 2009; Turchetto-Zolet et al., 2011). Research has focused on identifying the “nutrient stress trigger” that induces TAG accumulation in an effort to make TAG production constitutive. Strains that maintain elevated basal oil content might be produced by mutagenesis or genetic engineering. However, the pathways that regulate stress responses—and key enzymes—that initiate oil production are insufficiently understood at present. Understanding the metabolic regulatory networks that control carbon allocation to carbohydrates and lipid and identifying means to modulate these networks are necessary to achieve constitutively elevated oil yields under continuous growth. Assessments of whether metabolic modifications can be made without genetic tradeoffs that result in suboptimal performance in other aspects of the cells’ metabolism are important.

An array of techniques for improving lipid yields is described in the literature. A few examples are discussed in this section. Genetic manipulation of carbon allocation can enhance lipid production (Li et al., 2011). Starch production is blocked from the *sta6* mutant of *C. reinhardtii*, and its lipid body content increases 30-fold compared to 10-fold in the wild type (Wang et al., 2009). Modifications to improve oil yields have been achieved in oil-seed plants by altering the activities of dozens of genes, each of which results in an increase of a few percent in oil content (Thelen and Ohlrogge, 2002; Lardizabal et al., 2008; Clemente and Cahoon, 2009). Similarly, a broad approach of modifying several genes, which operate in both starch and lipid metabolism, could result in a substantial increase in oil content in algae.

Strategies that target steps in diverse metabolic pathways like starch metabolism, acetyl-coenzyme A (acetyl-CoA) and fatty-acid biosynthesis, and reactions of TAG assembly have shown significant effects on TAG accumulation in some organisms. For example, acetyl-CoA carboxylase overexpression led to “a 40 percent increase in the total fatty acid content of the non-oleaginous yeast *Hansenula polymorpha*” (Ruenwai et al., 2009). Mutants of *Arabidopsis* that are deficient in plastid pyruvate kinase had 60 percent less seed oil than the wild type, revealing a major role of this enzyme in pyruvate supply for acetyl-CoA biosynthesis (Baud et al., 2007). Reactions in the latter steps of TAG biosynthetic assembly might provide increased sink strength that could stimulate fatty-acid production (Thelen and Ohlrogge, 2002). Indeed, stimulation in seed oil content of *Arabidopsis* and rapeseed had been observed when a yeast long chain sn 2 acyltransferase was overexpressed (Zou et al., 1997). The overexpression of a diacylglycerol acyltransferase (DGAT), a committed and final step in TAG biosynthesis, increased seed oil content and seed weight in *Arabidopsis* (Jako et al., 2001) and tobacco leaves (Andrianov et al., 2009). A specific phenylalanine residue in DGAT was found to be a key determinant of oil content and composition in maize (Zheng et al., 2008), and the corresponding *Phaeodactylum* gene has been identified. A novel acyl-CoA:diacylglycerol acyltransferase 1-like gene (PtDGAT1) has been cloned and characterized from the diatom *P. tricornutum* (Guihéneuf et al., 2011) and will be tested in transgenic algae. Structural components of oil globules such as oleosin and caleosin might accelerate oil body formation in oil seeds of higher plants, but an oleosin gene has not been identified in algae. However, a major oil body protein of *Haematococcus* has been described (Peled et al., 2011).

Manipulating regulatory enzymes (such as transcription factors and signal transduction proteins) has been shown to enhance TAG accumulation in higher plants (Cernac and Benning, 2004) and might be effective in eukaryotic algae. Similar engineering can affect

glycogen accumulation in cyanobacteria (Osanai et al., 2005; Ehira and Ohmori, 2011). Several microRNAs that are differentially expressed in *C. reinhardtii*, under conditions in which lipid content is changed, were used to develop strains that produce 25 percent more oil than the wild type strain (Maor Sasson, TransAlgae Ltd., Israel, personal unpublished data). The redistribution of carbon from carbohydrates to lipids, higher alcohols, and hydrocarbons requires a better understanding of carbon regulation networks in these species.

2.1.3.2 Increases in Culture Density

The product yield (expressed, for example, as grams per liter per day, or as grams per square meter per day) of an outdoor algal culture is a function of its specific growth rate and its biomass concentration. Thus, maintaining an outdoor culture at its optimal biomass concentration is important to maximizing product yield. However, high cell density reduces light penetration and limits the growth rate of cells below the surface. Although counterintuitive, reducing the light-harvesting ability of individual cells could improve the light availability to the culture and increase overall photosynthetic activity. By reducing wasteful absorption and dissipation of light energy by cells at the surface, excess light is allowed to pass through to cells below. Thus, researchers have proposed selecting and developing strains with low pigmentation level (small light-harvesting antenna⁶ size) to increase the standing biomass of the culture (Benemann, 1989; Huesemann et al., 2009; Ort et al., 2011). This approach was evaluated in greenhouse conditions and shown to have a positive effect on productivity (Polle et al., 2003). The challenge is to isolate such mutants from the desired strain and to ensure they are stable under long-term outdoor cultivation. More complex culture strategies might facilitate achieving this goal, for example, layering strains that have different antenna sizes and spectra.

Whether factors other than light limit maximum culture densities is unknown. Little work has been done regarding cell-to-cell communication within a given species of microalgae, but evidence of quorum sensing⁷ (Teplitski et al., 2004; Sharif et al., 2008) and widespread interspecies allelopathic interactions have been reported (Gross, 2003). Endogenous mechanisms that limit population density might exist, in which case genetic modification may improve this aspect for aquaculture purposes.

2.1.3.3 Net Increase in Photosynthetic Efficiency

A long-time goal, as old as the techniques of genetic engineering itself, is to improve photosynthetic efficiency by such alterations as reducing losses from photorespiration, increasing the substrate selectivity of ribulose-1,5-bisphosphate carboxylase oxygenase (Rubisco), and enhancing photosystem stability and efficiency. However, 30 years of efforts in this area have not yielded any progress in higher plants or algae. Recent advances in synthetic biology, by fundamentally redesigning prokaryotic photosynthetic organisms to maximize the production of fuel molecules directly driven by photosynthesis (Chance et al., 2011b; Algenol Biofuels, 2012; Joule Unlimited, 2012), might provide some progress in this field. However, only laboratory-scale or small pilot-scale results have been presented. Atsumi et al. (2009) found that overexpression of Rubisco in transgenic *Synechococcus elongatus* PCC 7942 led to increased production of isobutyraldehyde without negatively

⁶ Light-harvesting antennae are protein-pigment complexes that capture sunlight and direct the radiant energy to the reaction centers.

⁷ Quorum sensing is the process of cell-to-cell communication in microorganisms that involves the production, release, and subsequent detection of chemical-signal molecules.

affecting photosynthetic oxygen evolution, suggesting that net improvements in carbon fixation are reasonable. Chen and Blankenship (2011) made the challenging proposal that photosynthetic capacity might be expanded by engineering cells to use different chlorophylls to capture a broader range of the light spectrum than non-engineered cells.

CO₂ abatement is a driver for developing algal biofuels. However, with current practices and species, CO₂ often is limited in production ponds and photobioreactors, and addition of a CO₂ source is a significant production expense. The effects of CO₂ concentrations on algal growth are discussed in the cultivation section later in this chapter, and CO₂ requirements and sourcing issues are discussed in Chapter 4. Improved carbon concentrating strategies would address this aspect of photosynthetic efficiency. The enzyme carbonic anhydrase is produced by several divisions of algae (Giordano et al., 2005). The enzyme converts bicarbonate to CO₂ that is released intracellularly for fixation by Rubisco. Most algae possess C3 metabolism. That is, the enzyme Rubisco is solely responsible for CO₂ fixation. The ability of some plants and microalgae (specifically diatoms and dinoflagellates) to use CO₂ directly during C4-intermediate metabolism offers promise for reducing bicarbonate limitation (Zimba et al., 1990; Raven, 2010). A November 2011 press release from Iowa State University reports that Spalding et al. increased algal biomass by 50 to 80 percent in *C. reinhardtii* by artificially increasing the expression of genes that encode components of the carbon-concentrating mechanism, which normally is induced only under low CO₂ conditions. The cells presumably continue to actively scavenge CO₂ even when it is at relatively abundant levels (Iowa State University, 2011).

2.1.3.4 Algal Crop Protection

Events in which the crop dies (pond “crash” or culture collapse) take a toll on resources and could threaten the economic sustainability and the future potential of the algal biofuel industry (see section Cultivation in this chapter). One cause of such culture collapse is the activity of predators on high-density biomass cultures (see section Contamination and Stability of Culture in this chapter). Simple genetic modifications that affect cell size can improve resistance to grazers and could improve harvesting properties at the same time (Jurgens et al., 1999). Focused screens to find mutations that confer resistance to specific pathogens and grazers are likely to improve crop protection. Because their carbon- and nutrient-allocating traits are the results of domestication, crop algae might carry a heavier metabolic burden than invading weed species. For these reasons, trait modification to instill resistance to herbicides, production of antifungals, and anti-grazer properties could be important. Indeed, at least one company has developed a genetically engineered algal strain for use in open ponds that is resistant to herbicides (IP Monitor, 2009; Aravanis, 2011). Some algae are known to increase lipid content when they are exposed to low levels of herbicide (Ma et al., 2002, 2006). However, if residual biomass is to be used for food or feedstuff, possible negative consequences of these traits would have to be considered. Algal species production of allelopathic chemicals could be exploited to enhance or inhibit growth of other organisms in crop cultures (Gross, 2003). The activities, pathways, and genes related to the secondary metabolites of strains of interest need to be characterized to harness the potential of intrinsic growth modulators.

2.1.3.5 Other Enhancements

The list of potential enhancements is open-ended and will expand as the specific algal species are chosen for cultivation and their attributes become apparent and as the

technologies to modify them increase. Some clearly desirable modifications would provide increases in tolerance to temperature, salinity, pH ranges, and metal concentrations. Tolerances to a range of conditions contribute to crop robustness. Prior demonstrations of such modifications include the conversion of freshwater cyanobacteria to use saline water sources (Waditee et al., 2002; Laloknam et al., 2006). Other aspects of the supply chain can be targeted through genetic modifications, including genetic engineering. For example, groups at Los Alamos National Laboratory have transplanted genes from magnetotactic bacteria. These genes direct the production of magnetic nanoparticles in green algae, which allows simple harvesting by magnetic collection of cells and reduces energy input for centrifugation and dewatering steps (Los Alamos National Laboratory, 2011).

2.2 CULTIVATION

Evaluating the sustainability of algal cultivation systems for biofuel production requires examining the various material and energy inputs needed for the cultivation systems to maintain scalable productivity, maximize system robustness, and minimize costs (Figure 2-3). Scalable productivity refers to a cultivation system’s ability to maintain productivities with respect to algal biomass and algal product (mass/area-time or mass/volume-time) from the laboratory scale to the commercial scale. System robustness refers to a cultivation system’s ability to reliably and dependably deliver consistent productivity and avoid system crashes or failures as a result of either biological or physicochemical causes. Costs pertain to capital and operating costs for a cultivation system.

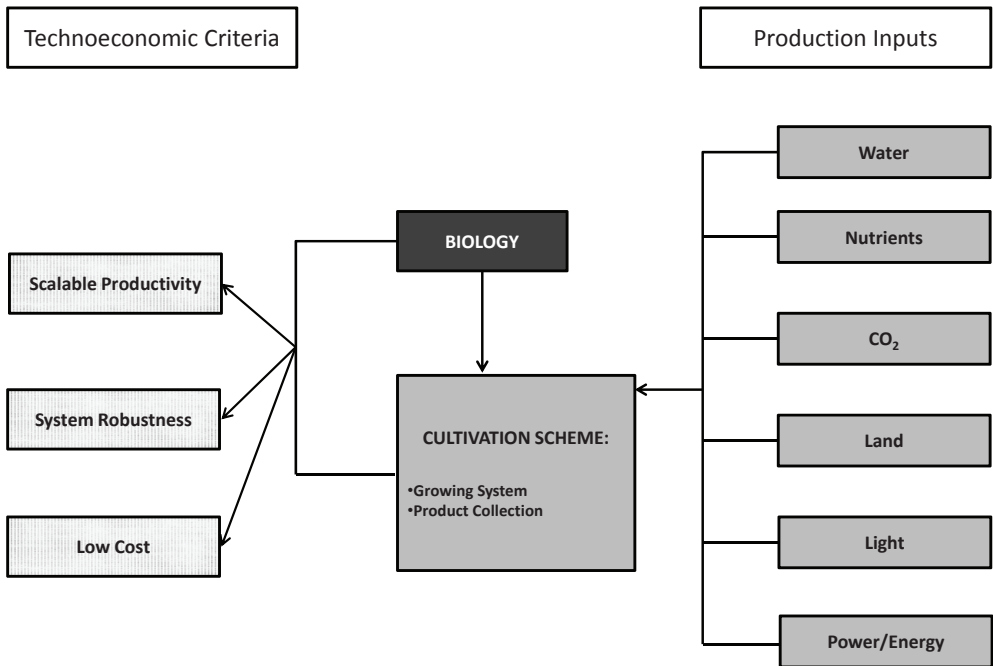


FIGURE 2-3 Material and energy inputs required by a cultivation scheme. Together with the biological scheme, these inputs determine the cultivation system’s productivity, robustness, and cost.

2.2.1 Overview of Algae-Growing Systems

The commercial large-scale cultivation of microalgae began in earnest in the 1960s with the cultivation of *Chlorella* in Japan (Tsukuda et al., 1977) and the use of phytoplankton as a feedstuff for animals reared in aquaculture (Duerr et al., 1998). In the 1970s, *Spirulina* was harvested from Lake Texcoco in Mexico (Durand-Chastel, 1980) and produced in Thailand (Kawaguchi, 1980). By 1980, 46 large-scale facilities operated in Asia producing more than 1,000 kg of microalgae each month (Kawaguchi, 1980). The global production of microalgal biomass was estimated to be more than 5,000 dry tonnes in the year 2005 with a value of more than U.S. \$1.25 billion, which excludes the value of processed products (Spolaore et al., 2006). About 3,000 dry tonnes of *Spirulina* are produced in China, India, Myanmar, the United States, and Japan; 2,000 dry tonnes of *Chlorella* are produced in Taiwan, Germany, and Japan; and 1,200 dry tonnes of *Dunaliella salina* are produced in Australia, Israel, the United States, and China (Spolaore et al., 2006). In 2008, the global production of microalgal biomass was estimated to be about 9,000 dry tonnes per year (Benemann, 2008).

In addition to algal biology and the intended algal products, numerous factors are considered in selecting the particular algal cultivation system to be used. These include the availability and cost of land, water, energy, nutrients, and labor, and the climate of the location (Borowitzka, 1992). The characteristics of each cultivation system, including its mixing or hydrodynamic characteristics, light utilization efficiency, ability to control temperature, ability to maintain a unialgal culture, and ease of scaling from laboratory to pilot and commercial scales also are considered (Borowitzka, 1999). The two general types of algal cultivation systems discussed in this report are open-pond systems and closed photobioreactor systems.

2.2.2 Open-Pond Systems

The majority of the large-scale microalgal production systems in commercial operation today are open-pond systems, mainly due to economic factors and ease of scale up. Most commercial-scale microalgal cultivation operations are for producing nutraceuticals, and none of them are for producing fuel. The number of microalgal species that can be grown effectively in open-pond systems is limited by the species' ability to thrive in particularly selective environments while the ponds remain relatively free of protozoan and other algal species contamination (Borowitzka, 1999; Milledge, 2011). For example, *Chlorella* is grown in a nutrient-rich medium, *Spirulina* at high pH and bicarbonate concentration, and *Dunaliella salina* at high salinity (Borowitzka, 1999; Milledge, 2011).

The two most common types of open-pond systems are circular ponds and raceway ponds. Circular ponds are round ponds, with depths of 30-70 centimeters (Moheimani and Borowitzka, 2006). They are typically agitated through a centrally pivoted rotating arm. Ponds up to 45 meters in diameter have been operated in Japan and Taiwan (Becker, 1994). *Oscillatoria* grown in a circular pond achieved a productivity of about 15 grams dry weight per m² per day (Sheehan et al., 1998). Mixing efficiency is poor in ponds with diameters greater than 50 meters (Shen et al., 2009). Raceway ponds (Figure 2-4 a-e) are constructed either as single units (Figure 2-4 b-e) or a group of continuous units that are joined together (Figure 2-4a). The raceway channels enable culturing algae in ponds with depths of 15-40 centimeters. The channels are constructed from concrete or compacted earth that might be lined with plastics. A paddle wheel, a propeller, or an air-lift pump operates at all times to agitate and circulate the mixture to prevent algae sedimentation (Becker, 1994; Chen et al., 2009). A key factor in open-pond design and operation is mixing, which evenly distributes

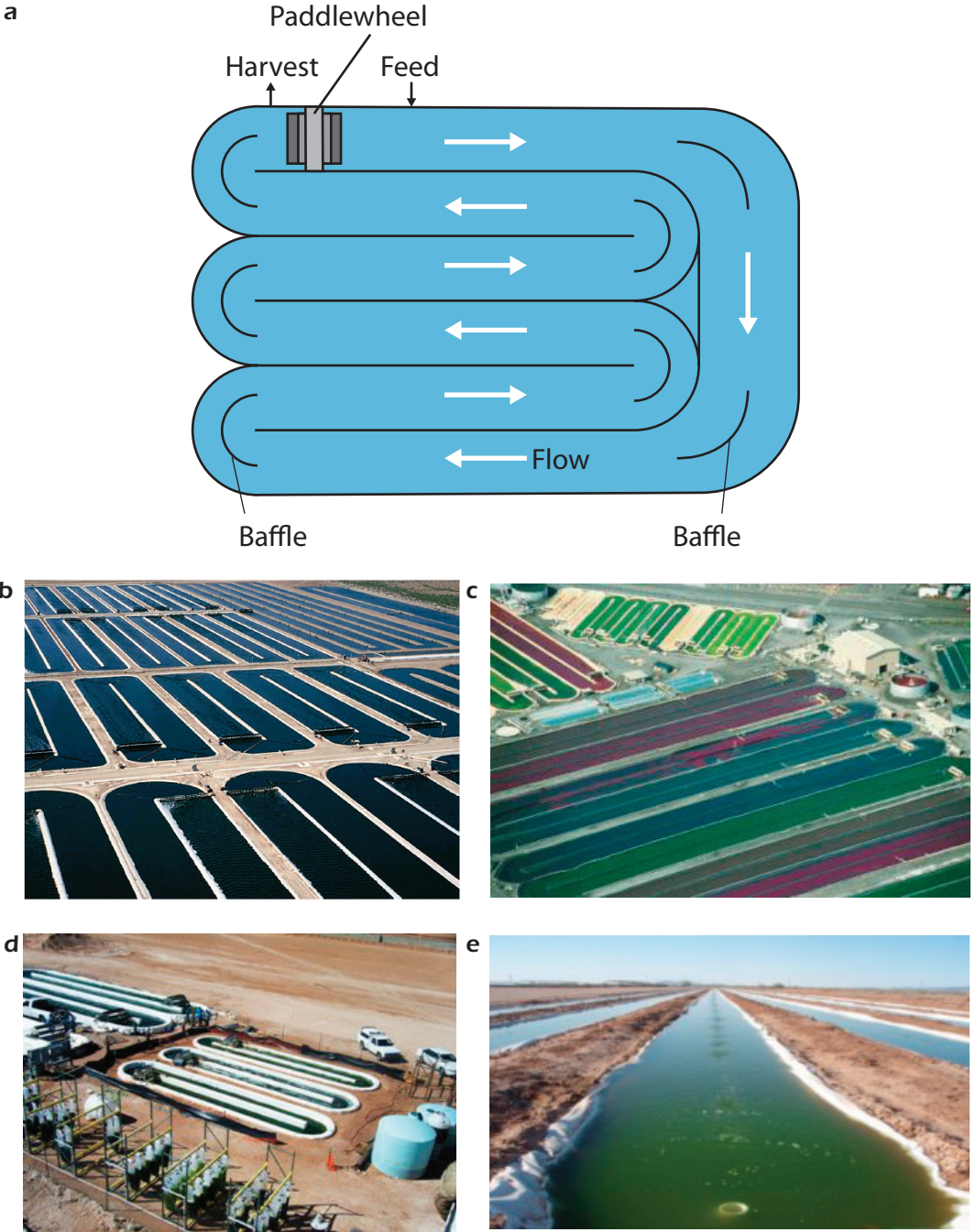


FIGURE 2-4 Open-pond designs for algae cultivation: schematic of raceway design (a), Earthrise raceways (b), Cyanotech raceways (c), Sapphire Energy raceways (d), and Phyco raceways (e).
 SOURCES: (a) Adapted from Spirulina Source (Spirulinasource, 1999).
 (b) Earthrise (Algae Energy, 2012a). Reprinted with permission from Algae Energy.
 (c) Cyanotech (2012). Reprinted with permission from Cyanotech.
 (d) Sapphire (Mveda, 2011). Reprinted with permission from Sapphire and Mveda.
 (e) Phyco Biosciences (Edwards, 2010). Reprinted with permission from Algae Industry Magazine.

nutrients and exposes algal cells to sunlight and CO₂. A velocity of 10-20 centimeters per second (cm/s) prevents algal cells from depositing and settling (Shen et al., 2009). Higher velocities are preferred, but a velocity greater than 30 cm/s could consume too much energy to be economically viable (Sheehan et al., 1998). Earthrise Nutritional, LLC, in California, and Cyanotech Corporation, in Hawaii, have some of the largest algal open ponds lined with plastic liners. Earthrise maintains 30 production ponds each about 5,000 m² and a series of research ponds (1,000 m², 200 m², and 50 m²) (Earthrise Nutritional, LLC, 2009b). Cyanotech has more than 60 ponds, each of which is about 2,900 m² (Lorenz, 2002; Enay, 2011). The depth of these ponds varies from 30 to 40 centimeters. For raceway ponds, a cell concentration of up to 1 gram dry weight per liter can be achieved, and productivities of 10 to 25 grams dry weight per m² per day have been reported (Shen et al., 2009). Table 2-2 shows algal productivities for open systems, which vary widely depending on numerous factors, including the type of open system and the algal species grown. Although a productivity of 50 to 60 g dry weight per m² per day is possible with open systems, achieving even 10 to 20 g dry weight per m² per day in large-scale systems is difficult on an annual basis because of operational conditions and seasonal variations in temperature and sunlight intensity (Shen et al., 2009).

In a raceway pond of 100 m², a paddle wheel driven by an electric motor has a power demand of 600 watts (W) (Becker, 1994). The overall energy requirement for paddle wheels in a pond with a roughness coefficient of 0.025 has been estimated at 20 kilowatt hour (kWh) per ha per day for a mixing velocity of 15 centimeters per second and 160 kilowatt

TABLE 2-2 Microalgae Productivities in Open Ponds

Pond Type	Volume (L)	Microalgal Species	Areal Productivity (g DW/m ² /d)	Volumetric Productivity (g DW/L/d)	Reference
Circular	1,960	<i>Chlorella</i> spp.	1.61–16.47	0.02–0.16	Kanazawa et al. (1958)
Circular		<i>Scenedesmus</i> spp.	2.43–13.52	0.03–0.13	Kanazawa et al. (1958)
Sloped (cascade)	1,970	<i>Oscillatoria</i>	15		Sheehan et al. (1998)
Slope	1,990	<i>Chlorella</i> spp.	25	10	Lee (2001)
		<i>Scenedesmus obliquus</i>	24.8		Becker (1994)
Raceway		<i>Spirulina</i>	9–13		Olguín et al. (2003)
		(<i>Arthrospira</i>) spp.			
Raceway	282	<i>Spirulina platensis</i>	14.47	0.183	Pushparaj et al. (1997)
Raceway	300	<i>Anabaena</i> spp.	9.4–23.5	0.031–0.078	Moreno et al. (2003)
Raceway	135,000	<i>Spirulina</i>	2–17	0.006–0.07	Jiménez et al. (2003)
		(<i>Arthrospira</i>) spp.			
Raceway		<i>Dunaliella salina</i>	1.6–3.5		García-González et al. (2003)
Raceway	750	<i>Spirulina platensis</i>	15–27	0.06–0.18	Richmond et al. (1990)
Raceway	4,150	<i>Phaeodactylum tricornutum</i>	2.4–11.3	0.0028–0.13	Laws et al. (1988)
Hybrid system (open ponds and closed photobioreactors)		unknown	30 (anticipated)		Phycal (2011)
Raceway (proprietary lined “Super Trough System”)		<i>Cyanobacteria</i> spp.	15.36 (anticipated)		Phyco BioSciences, Inc. (Cloud, 2011a,b)

SOURCE: Adapted from Chen et al. (2009).

hours per ha per day for a mixing velocity of 30 centimeters per second (Benemann, 1986). Other estimates of power requirements for large ponds (for example, Cyanotech's 2,900 m² ponds mentioned earlier) range from about 1,200-3,700 W/ha for mixing velocities of 20 to 30 centimeters per second (Pedroni et al., 2001; Frank et al., 2011). A raceway pond of 85 m² that uses an air-lift pump for circulation has a power consumption of 195 W based on a compressor efficiency of 70 percent and an air demand of 120 liters per second. Ponds in Chile and Brazil have used motor-driven drag boards as an alternative to paddle wheels; the energy requirement was reported to be only 20 percent of the energy needed for a comparable agitation with paddle wheels (Becker, 1994). Laws et al. (1983) introduced the concept of foils that create circular vortices to effectively mix the pond suspension from top to bottom. This is the type of agitation device that Algenol uses in its plastic and covered photobioreactor design (Chance et al., 2011b; see also Chapter 3).

2.2.3 Photobioreactors

Mass cultivation of microalgal species that lack pronounced environmentally selective advantages might require the use of photobioreactors (Milledge, 2011). Photobioreactors are transparent containers or vessels designed to have reduced light path to enhance the amount of available light to the algal cells, and the cultures within are continuously mixed to enhance nutrient distribution and gas exchange. Photobioreactors for microalgae production have an optimal thickness of about 2-4 centimeters (Borowitzka, 1999). The tubular and the flat-plate are the two most common types of microalgal photobioreactors.

All photobioreactors have large surface to volume ratio (SVR). Because of their widespread availability, tubes long have been used as a basic photobioreactor material. The geometric configurations of tubular photobioreactors span a wide range from straight horizontal, straight vertical, helical, to triangular configurations (Figures 2-5, 2-6). One of the world's largest photobioreactor facilities is in a greenhouse in Klotze, Germany. This facility consists of straight horizontal tubes stacked in vertical fence-like arrays (Figure 2-7). The facility has a total volume of 700 cubic meters (m³), occupies a total land area of 10,000 m², and produces 35-41 grams dry weight per m² per day or 120-140 dry tonnes per year. Algae wall adhesion, biofouling, large pressure drop, and gradients in pH, dissolved oxygen, or CO₂ can occur along the tube length. These factors are potential disadvantages of tubular photobioreactors (Chen et al., 2009), which might be resolved by innovative engineering designs.

Flat-plate (or flat-panel) photobioreactors are transparent rectangular containers (usually vertical or inclined) with a light path of 1-30 centimeters (Figure 2-8). Flat-plate photobioreactors mix substrate by vigorous air sparging from the bottom.

Productivities of algal biomass in photobioreactors vary with the type of geometric configuration used and the algal species grown (Table 2-3). Many novel production systems have been designed and currently are being developed and tested. The new production systems aim to lower construction and maintenance costs close to those of open-pond systems and maintain the high, stable productivity and reduced contamination risk of closed photobioreactors. These systems include the Solix, ACCORDION, Algenol, and the National Aeronautics and Space Administration's (NASA) Offshore Membrane Enclosure for Growing Algae (OMEGA), and Photon8's traveling wave system.

The Solix photobioreactor is an elongated (low height-to-length ratio) flat-panel photobioreactor made of plastics. It is designed to bridge the gap from flask to open raceway pond by serving as a controlled-environment test bed or as an algae inoculum scale-up device (Figure 2-9; Solix Biofuels, 2011). The Solix photobioreactor allows for open-pond deployment by using the water as a thermal regulator for open-air field applications. Air sparging for aeration and mixing occurs along the full length of each panel.

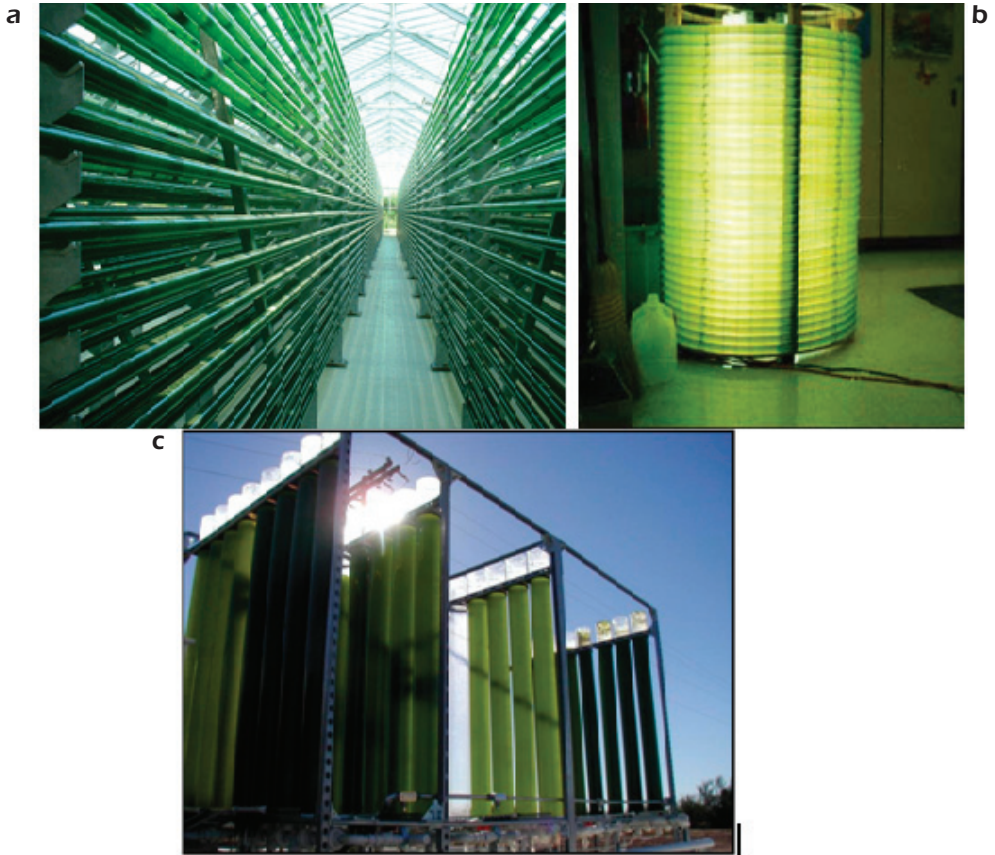


FIGURE 2-5 Tubular photobioreactors.

SOURCES: Clockwise from top left: (a) California Polytechnic State University;

(b) Kennedy et al. (1995); and

(c) NanoVoltaix (2012). Reprinted with permission from Qiang Hu and Arizona State University/ NanoVoltaix.

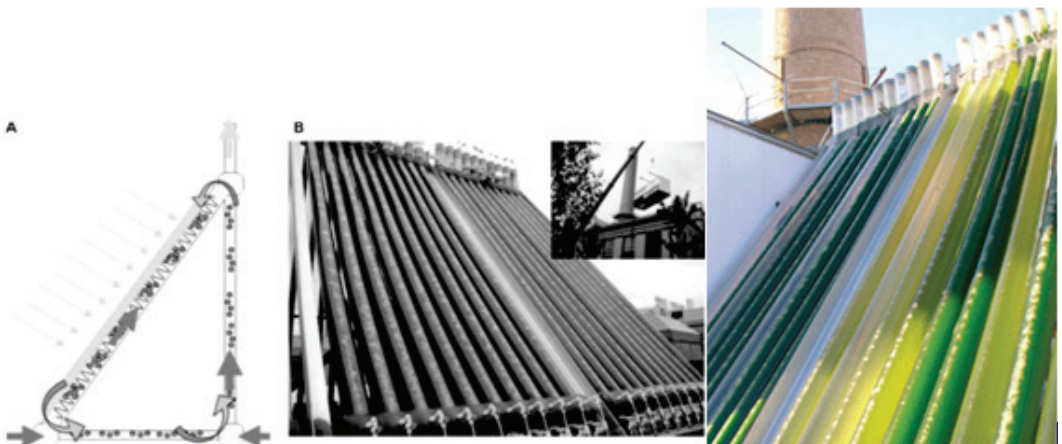


FIGURE 2-6 Triangular external air-lift tubular photobioreactors.

SOURCES: Vunjak-Novakovic et al. (2005). Reprinted with permission from American Chemical Society.



FIGURE 2-7 Tubular photobioreactors of straight horizontal tubes stacked in vertical fence-like arrays housed in a greenhouse in Klotze, Germany.
 SOURCE: Algomed. Reprinted with permission from AGU.

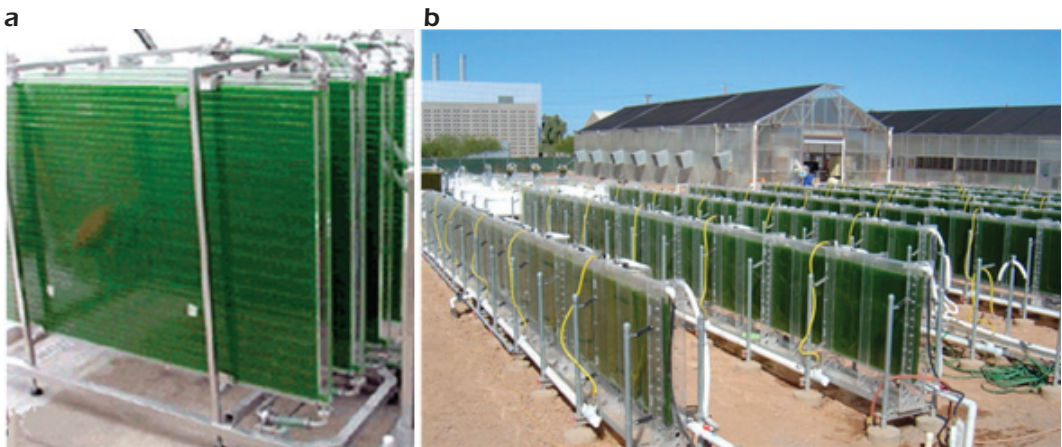


FIGURE 2-8 Flat-plate photobioreactors.
 SOURCES: (a) Algae Energy (2012b);
 (b) NanoVoltaix (2012). Reprinted with permission from Qiang Hu and Arizona State University/NanoVoltaix.

TABLE 2-3 Microalgae Productivities in Photobioreactors

Photobioreactor	Volume (L)	Microalgal Species	Productivity (g DW/L/d)	Reference
Airlift tubular	200	<i>Porphyridium cruentum</i>	1.50	Camacho Rubio et al. (1999)
Airlift tubular	200	<i>Phaeodactylon tricornutum</i>	1.20	Ación Fernández et al. (2001)
Airlift tubular	200	<i>Phaeodactylon tricornutum</i>	1.90	Molina et al. (2001)
Inclined tubular	6.0	<i>Chlorella sorokiniana</i>	1.47	Ugwu et al. (2002)
Undular row tubular	11	<i>Arthrospira platensis</i>	2.70	Carlozzi (2003)
Helical tubular	75	<i>Phaeodactylon tricornutum</i>	1.40	Hall et al. (2003)
Parallel tubular	25,000	<i>Haematococcus pluvialis</i>	0.05	Olaizola (2000)
Bubble column	55	<i>Haematococcus pluvialis</i>	0.06	López et al. (2006)
Flat plate	440	<i>Nannochloropsis</i> spp.	0.27	Cheng-Wu et al. (2001)
Flat plate	100	<i>Nannochloropsis</i> spp.	0.30	Rodolfi et al. (2009)
ACCORDION	60	<i>Monodus subterraneus</i>	0.40	Cuello and Ley (2011)

SOURCE: Adapted from Ugwu et al. (2007). Reprinted with permission from Elsevier.



FIGURE 2-9 Solix photobioreactor.
SOURCE: Reprinted with permission from Solix Biosystems.

NASA's OMEGA is a flat-panel photobioreactor made of plastic. Inserts of forward-osmosis membranes allow the exit flow of oxygen and water while the photobioreactor is laid down horizontally on a water surface (Figure 2-10; Trent, 2011). OMEGA is designed for deployment on the surface of bodies of saline water (for example, sea and ocean) where it exploits wave movements for mixing culture and regulating temperature. This photobioreactor currently is undergoing redesign to overcome technical challenges; the final design likely will be more complex than its original design and could include some significant deviations (Trent, 2011).

The ACCORDION photobioreactor is a vertical series of flat plastic panels through which the algal suspension is grown in batch or grown continuously recirculated in batch, semicontinuous, or continuous modes (Figure 2-11; Cuello and Ley, 2011). The adjustable alternating vertical and angled flat plates, or alternating angled flat plates, are designed to improve the light incidence on surfaces and enhance the mixing and flow patterns inside the plates. For example, a treatment for a 60-liter ACCORDION photobioreactor with 45° plate angle and liquid flow rate of 14 liters per minute resulted in algal productivity of 0.30 grams of dry weight per day that was statistically indistinguishable from that of a 1-liter shake-flask control. The ACCORDION photobioreactor is a modular design that can be scaled up by adding modules. This is equivalent to open raceway ponds achieving scale up by adding raceway units. The ACCORDION photobioreactor currently is undergoing further design and structural optimization (Cuello and Ley, 2011).

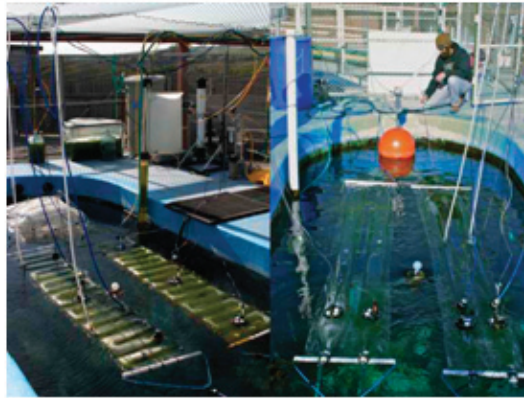


FIGURE 2-10 NASA's OMEGA photobioreactor.
SOURCE: Trent (2011).

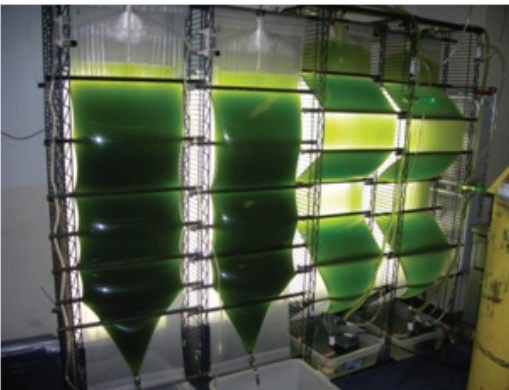


FIGURE 2-11 ACCORDION photobioreactor.
SOURCE: Cuello et al. (2011).

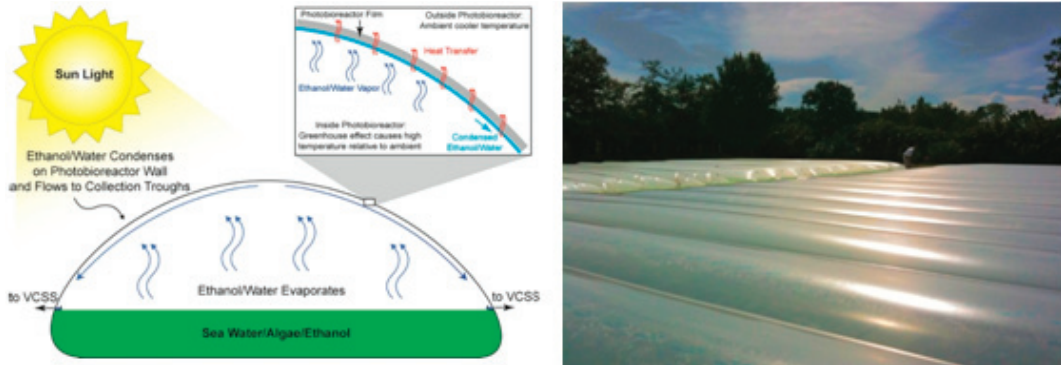


FIGURE 2-12 Algenol photobioreactor.

SOURCES: Chance et al. (2011b); Algenol Biofuels (2012). Reprinted with permission from Ron Chance, Algenol Biofuels.

The Algenol photobioreactor is a plastic, horizontal, half-cylinder vessel that uses a hydrofoil that moves back and forth along the longitudinal axis of the photobioreactor to mix substrate (Figure 2-12). The photobioreactor, which is designed for direct ethanol production, is used to culture enhanced cyanobacteria that excrete ethanol into an aqueous medium. The ethanol-water mixture evaporates to form liquid condensate on the photobioreactor's concave ceiling and flows down both the sides of its internal walls where plastic sleeves catch the ethanol-water condensate and convey it to a collection port at one end of the photobioreactor (Chance et al., 2011b).

2.2.4 Comparison of Open Systems and Closed Systems

Table 2-4 compares open-pond systems and closed photobioreactor systems for photoautotrophic microalgae production. Although outside the scope of this report, the low costs of construction and maintenance constitute one of the biggest advantages of open-pond

TABLE 2-4 Comparison of Open and Closed Algal Cultivation Systems

Parameter	Open System	Closed System	Reference
Cost	Lower	Higher	Shen et al. (2009)
Pumping energy	Lower	Higher	Becker (1994); Shen et al. (2009)
Ease of scale up	Greater	Lower	Shen et al. (2009)
Evaporative water loss	up to 10 L/m ² /day	Negligible where wind cooling is sufficient; 1-2 L/m ² /day when water-spray cooling is used; or similar to open systems if photobioreactors are submerged in ponds for cooling	Becker (1994)
Land area required	Higher	Lower	
Contamination risks	Higher	Lower	Borowitzka (1999); Milledge (2011); Shen et al. (2009)
Productivity	Lower	Higher	See Tables 2-1 and 2-2
Productivity stability	More variable	Less variable	Shen et al. (2009)
Sparged CO ₂ loss	Higher	Lower	Becker (1994); Shen et al. (2009)

systems compared to photobioreactors. The conventional view was that the use of open-pond cultivation is more likely to achieve the goal of technoeconomic feasibility for producing microalgae for biofuels than the use of photobioreactors. Because of their lower capital costs and simpler designs, open-pond systems are easier to scale up to increase production than photobioreactors. Most photobioreactor configurations are scaled up by multiplying units and by increasing the unit volume. Increasing unit volume of photobioreactors requires adjustments of physical variables to achieve appropriate flow dynamics within the new unit volume. Disadvantages of open-pond systems include losses of water to evaporation, risk of contamination by competing microorganisms, loss of algal biomass due to weather, and loss of introduced CO₂. (See Chapter 4 for details on evaporative water loss.)

Advantages of photobioreactors include significantly higher microalgal biomass productivity and greater production stability over time than open-pond systems. For example, the volumetric productivity of *Nannochloropsis* spp. in photobioreactors could exceed that in open raceways by as much as 16 times (Table 2-4). The risk of biological contamination is much greater in open-pond systems than in closed photobioreactor systems. With the exception of *Spirulina* and *Dunaliella salinas*, which are cultivated in open systems under highly selective growing conditions, the lack of competitive advantages of many of the microalgal species being tested for biofuel production in open ponds and their susceptibility to culture crashes are concerns. Thus, the low volumetric productivity and susceptibility to contamination could constitute a substantial risk to the economic sustainability of open-pond cultivation systems compared to closed photobioreactor systems.

The utilization efficiencies of some vital input resources in terms of production per unit input—particularly for water and land—are in general lower in open cultivation systems than in closed photobioreactors (Table 2-5; Davis et al., 2011). As noted above, evaporative water loss is of particular concern in open-pond systems. These losses could be as much as 10 liters per m² per day. Thus, a one hectare open pond could lose 100,000 L of water per day or 36,500,000 liters of water per year. When the cooling of photobioreactors is achieved through water-spray cooling or through submergence in open ponds, the evaporative water loss associated with photobioreactors also can be substantial and as much as in open systems. Table 2-5 further compares the land area requirement, energy consumption, net energy ratio, and other criteria for cultivating *Nannochloropsis* spp. in an open raceway, flat-plate photobioreactor, and tubular photobioreactor to produce 100,000 kg dry weight (DW) of algal biomass per year (Jorquera et al., 2010). The land area required for the open raceway exceeded that of the tubular photobioreactor by 241 percent and that of the flat-plate photobioreactor by 256 percent. The total energy consumption for the open raceway, flat-plate photobioreactor, and tubular photobioreactor were 3.72 watt per cubic meter (W/m³), 53 W/m³, and 2,500 W/m³, respectively. The resulting net energy ratios for oil production, defined as the total energy produced divided by the total energy requirement, were 3.05, 1.65, and 0.07 for the open raceway pond, flat-plate photobioreactor, and tubular photobioreactor, respectively. While the tubular photobioreactor had a net energy ratio of less than 1, and thus consumed more energy than it produced, the net energy ratios for flat-plate photobioreactors and open raceway ponds were both greater than 1. Therefore, the favorable energy balance might persist through mass cultivation of *Nannochloropsis* using either of these methods. However, the 2010 study by Jorquera et al. did not consider the harvest costs and the cost of oil extraction that add significantly to energy consumption. A thorough discussion of life-cycle assessment (LCA) of energy balance for algal biofuels is in Chapter 4. The Jorquera study was part of a meta-analysis that reanalyzed published data to provide an estimate of the energy requirement for fuel production (Liu et al., 2012). The true energy return may not fully be known until full-scale commercial production has been

TABLE 2-5 Comparison of Raceway Ponds, Flat-plate, and Tubular Photobioreactors in Cultivating *Nannochloropsis* spp. to Produce 100,000 kg DW Per Year

Variable	Raceway Ponds	Flat-Plate Photobioreactors	Tubular Photobioreactors
Annual biomass production (kg/year)	100,000	100,000	100,000
Volumetric productivity (g/L per day)	0.035	0.27	0.56
Illuminated areal ^a productivity (kg/m ² per day)	0.011	0.014	0.0081
Occupied areal ^b productivity (kg/m ² per day)	0.011	0.027	0.025
Occupied areal ^b productivity (t/ha per year)	39	99	93
Illuminated area ^a volume (per m ²)	300	50	14
Illuminated area ^a /volume ratio (per m)	3.3	19	69
Occupied area ^b /volume ratio (per m)	2.3	10	22
Biomass concentration (g/L)	0.35	2.7	1.02
Dilution rate (per day)	0.1	0.1	0.1
Area required for biomass production of 100,000 kg/yr (m ²)	26,000	11,000	11,000
Reactor volume required for biomass production of 100,000 kg/yr (m ³)	7,800	1,000	490
Flow rate required to maintain a 0.1 /day dilution rate (m ³ /day)	780	100	49
Hydraulic retention time (volume/flow rate)	10	10	10
Relative oil content (%)	30	30	30
Net oil yield (m ³ /year)	33	33	33
Oil yield per area (m ³ /ha per year)	13	32	31
Energy consumption (W/m ³)	3.7	53	2,500
Energy consumption required for accumulation of 100,000 kg/year biomass (W)	29,000	54,000	1,200,000
Total energy consumption (kWh/month)	8,700	16,000	370,000
Total energy consumption (GJ/year)	378	700	16,000
Energy produced as oil (GJ/year)	1,200	1,200	1,200
Total energy content in 100,000 kg biomass (GJ/year)	3,200	3,200	3,200
NER for oil production	3.1	1.7	0.07
NER for biomass production	8.3	4.5	0.20

^aIlluminated area refers to the surface area of a raceway pond or photobioreactor subject to illumination.

^bOccupied area refers to the land area occupied by the raceway pond or photobioreactor.

Note: Net Energy Ratio (NER) = total energy produced/total energy requirement.

SOURCE: Adapted from Jorquera et al. (2010). Reprinted with permission from Elsevier.

realized. Even then, uncertainty in the estimates of energy return might remain, as in the case of corn-grain ethanol (Hall et al., 2011). Where biofuel feedstocks consist of genetically modified organisms or other organisms of potential societal concern (for example, organisms that have been invasive in one or more environments), photobioreactors may be more acceptable to some communities or individuals.

In summary, open-ponds and closed photobioreactors each offer distinct advantages and disadvantages. Open-pond systems allow for larger scale units at lower capital investments, lower operating costs, and lower energy demands than closed photobioreactors. However, the open nature of such ponds makes them vulnerable to the natural elements, including loss of water through evaporation and invasion of undesirable species. Closed systems offer some protection of the cultivated algae from the natural elements. Because they have pipes and tubes, closed photobioreactors are more expensive to construct and require more energy to operate than open-pond systems. But closed photobioreactors can improve the sun exposure and take advantage of specialized species, thereby improving

productivity. It is premature to draw conclusions as to which system is preferable at this nascent state of the development of algal biofuels. Other aspects of sustainability (for example, economics) would have to be considered in selecting the cultivation systems for algal biofuel production.

2.2.5 Design Considerations for Algal Cultivation Systems

2.2.5.1 Supplemental Carbon Dioxide

Because supplemental gaseous CO_2 significantly enhances algal biomass growth rates, supplementation is recognized as a universal practice in mass cultivation (Ono and Cuello, 2004a,b, 2006; Williams and Laurens, 2010). Yue and Chen (2005) reported, for instance, that the maximum growth rate for *Chlorella* ZY-1 strain was achieved when an air flow enriched with 10 percent (v/v) CO_2 was used (Figures 2-13 and 2-14). The linear growth rate under that condition was about 1.17 g dry weight per liter per day (Figure 2-14), and the cell concentration reached 5.77 g dry weight per liter after 6 days of cultivation. Growth rates and cell concentrations were higher in cultures grown with 5, 20, 30, and 60 percent CO_2 with all CO_2 treatments delivered at a flow rate of 2.5 L/min than without CO_2 supplementation (Figure 2-13).

Supplemental carbon sources for algal cultures include bicarbonate dissolved in water or CO_2 gas. If carbon is supplied as CO_2 gas in open ponds, then optimizing the size of gas bubbles is critical to ensure that the CO_2 remains in the water and is taken up by algae. Open-pond cultivation has used sintered porous stones or PVC pipes with a line of fine holes on the upper part. In shallow ponds, however, these methods result in significant losses of CO_2 to the atmosphere because the aqueous algal suspension retains the gas bubbles only for a short time so that CO_2 cannot be absorbed completely unless counter-current carbonation sumps are used. Achieving a utilization rate of more than 10 percent of the supplied CO_2 under working conditions has been difficult (Becker, 1994). In addition, mounting pipes or tubes at the bottom of ponds is technically challenging, and porous

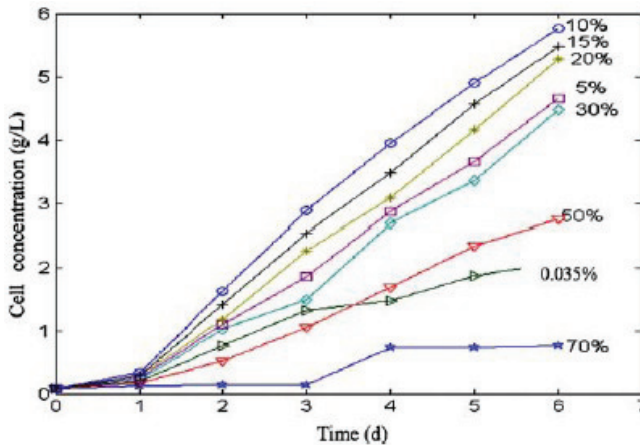


FIGURE 2-13 Variations in growth of *Chlorella* ZY-1 strain with CO_2 concentrations (v/v).

SOURCE: Yue and Chen (2005). Reprinted with permission from Elsevier.

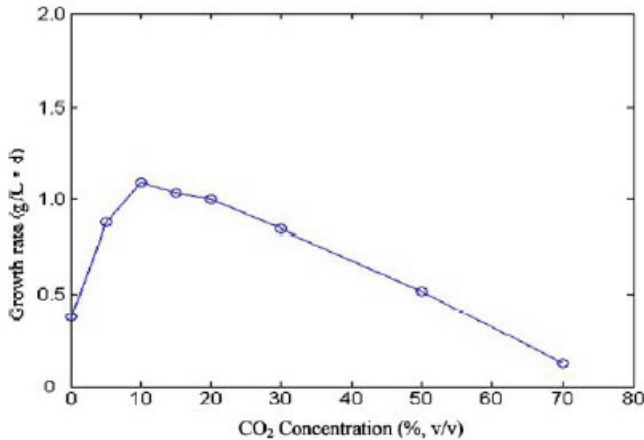


FIGURE 2-14 Variations in growth rate of *Chlorella* ZY-1 strain with CO₂ concentrations (v/v). SOURCE: Yue and Chen (2005). Reprinted with permission from Elsevier.

materials used for gas distribution tend to get clogged by algae and other debris and require regular cleaning (Becker, 1994). Weissman et al. (1988) demonstrated that the rate of CO₂ outgassing from an open raceway varied and could be reduced, based on pH, alkalinity, and ionic strength. An alternative is the use of a floating CO₂ injector, such as one developed in Peru (Vasquez and Heussler, 1985). The device consists of a floating compartment with a hollow enclosure (preferably made from sealed PVC pipes and covered with transparent sheeting). Flotation is achieved because of the gas cushion under the cover. Through a ball-valve system, the compartment is filled with pure CO₂. When the device emerges through its buoyancy, the valve is shut, and vice versa. The resulting CO₂ loss is as low as 4 percent of total CO₂ supply in some cases (Becker, 1994).

The amount of CO₂ offgassing from photobioreactors depends on the specific type of photobioreactor and how it is operated. Flat-plate photobioreactors, for instance, generally have much shorter gas paths than tubular photobioreactors (Sierra et al., 2008). Unlike in open raceways, CO₂ offgassing from photobioreactors could be minimized by recycling the effluent gas stream. If the recycled gas stream happens to have a high oxygen concentration, the challenge of stripping the oxygen to prevent inhibition of culture growth needs to be addressed.

2.2.5.2 Contamination and Stability of Culture

If the product yield (for example, algal oil or ethanol) depends on the growth of a particular cultivated algal species, then growing and maintaining that monoculture is critical. Citing Moheimani and Borowitzka (2006), Stephens et al. (2010) noted that some open-pond systems could be run over 6 months without significant levels of contamination. Nonetheless, maintaining dominance and high biomass productivity of the preferred cultivated species in open and non-aseptic algal monocultures remains one of the most formidable challenges in open-pond mass cultivation. Like monocultures of terrestrial crops, large algal monocultures tend to be invaded by undesirable pests and pathogens, and crop protection is a major challenge to algal pond sustainability (Hannon et al., 2010). The principal types

of contaminants in algal cultures include other algal species, bacteria, zooplankton, fungi, insects, and viruses. The types and populations of contaminants in algal cultures depend on the local environmental conditions, the algal species cultivated, and the specific cultivation system in use (Baptist et al., 1993; Becker, 1994; Mahan et al., 2005).

Contamination of open-pond algal cultures by other algal species is unavoidable because the growth conditions inevitably are suitable for the cultivated algal species and other local species. Even in the case of the cyanobacterium *Spirulina*, which is cultivated at highly selective growing conditions of high alkalinity and pH, contamination by another cyanobacterium *Oscillatoria* or by algae has been reported at suboptimal growth conditions such as at bicarbonate concentrations below 15 grams per liter (Becker, 1994). In extreme cases, an invading algal species could become dominant and overgrow the intended species. In cultures of *Scenedesmus* spp., the common contaminants include *Chlorella* spp., *Selenastrum* spp., and some species of diatoms (Becker, 1994). In Japan, it has been reported that maintaining *Chlorella* cultures required frequent start-up of the culture with uncontaminated inoculum (Becker, 1994). While it is impossible to keep other algal species away from an open-pond system, strategies can be adopted to keep the contamination at acceptable levels, including using high concentrations of the inoculum, periodic cleaning of the cultivation system, and implementing a specific nutrient or a combination of environmental conditions that favor the desired species (Becker, 1994).

Algal cultures contaminated by *Monas* spp. or other species of protozoans often are totally decimated within 12 to 18 hours after the corruption is first detected (Baptist et al., 1993). The fungi chytrids have been detected in several algal cultures and often occur as epidemics, which sometimes result in the complete loss of cultures. The fungus *Chytridium* spp. is the most dangerous fungus for cultures of Chlorophyceae, and often appears together with the zooflagellate *Aphelidium* spp. Infections of *Scenedesmus* cultures by these organisms have been detected practically worldwide. Such infection is characterized by heavy flocculation of the algal suspension, a brown color of the culture medium, and decreased oxygen evolution (Becker, 1994). By the time these symptoms appear in the algal culture, it is already too late to control the parasite. The only biological control for *Aphelidium* infection in an early stage is a many-fold dilution of the culture with fresh medium, enabling the algal population to remain in an exponential growth phase so that its multiplication rate exceeds that of the parasite. Infection of *Scenedesmus* spp. cultures with *Chytridium* spp. have been treated successfully in Israel by applying the fungicide Benomyl (methyl 1-butylcarbomoyl benzimidazolecarbamate) at a dosage of 1 milligram per liter (mg/L) (Becker, 1994). Fungal contamination of *C. reinhardtii* can be controlled using the fungicides carbendazim (1 mg/L), thiophanate-methyl (20 mg/L), and benomyl (20 mg/L) (Mahan et al., 2005). A combination of carbendazim and the antibiotics ampicillin and cefotaxime also has been shown to remove or reduce contamination of *C. reinhardtii* by three different bacteria and two different fungi tested (Kan and Pan, 2010).

There have been occasional reports of contamination of algal cultures by the zooplankton *Lycrymanis* spp., *Colpidium* spp., and *Vorticella* spp., though these organisms had negligible effects on algal growth (Becker, 1994). Contamination by a group of rotifers called *Branchionus*, however, may impede the growth of algal cells and in extreme cases could spoil the entire algal culture. The most effective control has been to lower the pH of the culture to about 3.0 by adding acid and keeping the culture at that pH for 1 to 2 hours before the pH is readjusted back to 7.5 with potassium hydroxide (KOH). The treatment effectively eliminates the rotifers without deleterious effects on the algal cultures (Becker, 1994).

2.2.5.3 Open-Pond Operations

Open-pond facilities for the large-scale production of algal biofuels likely will need to be managed as complex, bioengineered systems by applying known principles of population, community, and ecosystem ecology (cf. Graham and Smith, 2004; Smith et al., 2010a). Numerous configurations for open-pond systems can be envisioned, two of which are discussed below.

2.2.5.3.1 *Single species (target strain inoculum) hybrid system*

One hybrid facility design for algae cultivation involves the front-end use of a photobioreactor that is later linked to open ponds. The upstream photobioreactor would be a breeder or feeder system that provides an influx of high-density, target algae for production and harvest in downstream open-raceway systems (DOE, 2010). However, several key ecological factors potentially may complicate the stable long-term use of single-strain hybrid or single-strain all open-pond systems.

First, nominally single-strain hybrid systems would be subjected constantly to potential invasions by microflora and microfauna present in the local landscape. For example, resting stages or live individuals from taxonomically diverse groups of cyanobacteria and algae can be deposited onto the surface of the open ponds, either via the direct deposition of atmospheric particulates, or in association with rain and snowfall (for example, Brown et al., 1964). In addition, open ponds will be invaded by a diverse community of aquatic consumers, including rotifers, ciliates, insect larvae, and crustacean zooplankton. These animal invaders can be transmitted primarily via insects, migratory waterfowl, and other regionally mobile animals (for example, Frisch et al., 2007) and by wind and rainfall (Jenkins and Underwood, 1998). The taxonomic identities and total number of species that ultimately become resident in these ponds depend on the size and composition of regional species pools (Chase, 2003; Ptacnik et al., 2010), allelopathic interactions with the desired species, competition for available resources, and the productivity and surface area of the pond system (Hoffmann and Dodson, 2005; Smith et al., 2005). Some species of herbivorous invaders are highly undesirable because their unrestricted growth can strongly suppress algal growth (see the predator-prey dynamics discussion later in this chapter).

Second, the primary goal of the upstream photobioreactor is to provide a constant supply of the target algal strain. However, once invasions by cyanobacteria and algae occur in the downstream open pond, the target algal strain may not continue to dominate the algal community. This problem could exist whether the target strain is genetically modified or naturally occurring. Such invasions could reduce overall biomass yield and require costly interruptions of biomass production for system closure, cleaning, and strain re-establishment.

In the most desired case, the target strain would be highly competitive and would continuously remain the dominant primary producer in the pond while the hybrid cultivation system is in operation. However, strong temporal dynamics in species composition are observed in most polycultures. Individual species tend to exhibit major changes in relative abundance as conditions change over ecological time. Occasionally some species increase from nearly undetectable abundance to strong numerical dominance during a period of only weeks (for example, Reynolds, 1997). Such strong species dynamics are undesirable because the oscillation of target strain abundance could create strong instability in algal biofuel production rates via changes in algal biomass, lipid content and molecular composition, lipid extractability, and lipid harvestability. Zmora and Richmond (2003) summarized the production of *Nannochloropsis* for rotifers and as a direct aquaculture feed. They

reported that the highest lipid content in *Nannochloropsis* was observed in the summer, but the highest eicosapentaenoic acid level was observed in winter. Daily harvesting of 25 to 30 percent of the culture volume during the summer yielded the most biomass. Contaminants were controlled by pH shifts and use of low levels of chlorine.

2.2.5.3.2 Mixed species (natural plankton community inoculum) systems

Another facility design for algae cultivation uses open-pond systems that are inoculated with planktonic assemblages obtained from natural water bodies in the nearby landscape. Such inocula contain a representative subset of the indigenous species pool of microflora and microfauna. This species pool will include aquatic viruses, bacteria, and fungi; cyanobacteria and eukaryotic microalgae and macroalgae; and aquatic consumers such as rotifers, ciliates, insect larvae, and crustacean zooplankton. Just as in the hybrid ponds discussed earlier, mixed-species algal production systems potentially would be subjected to daily invasions by species of microflora and microfauna that might not have been in the original starting inoculum.

A mixed species assemblage provides potential advantages of improved biomass yield and increased culture stability. The agricultural literature has long demonstrated that the joint cultivation of multiple plant species (polyculture) typically provides a greater total biomass yield than a single crop. This phenomenon is known as “overyielding” (Bessler et al., 2009). Overyielding of biomass has been observed in algal assemblages (Weis et al., 2008), leading Smith et al. (2010b) to hypothesize that mixed-species cultures could produce higher yields of algal biomass and lipids than single-species algal cultures. Stockenreiter et al. (2012) tested this hypothesis in both natural and laboratory microalgal communities and found higher lipid production in diverse algal communities relative to algal monocultures grown under the same resource supply conditions. This study supports the suggestion that naturally occurring, multispecies, microalgal communities grown in open ponds potentially could store more solar energy than single species communities cultivated in closed photobioreactors (Smith et al., 2010b). Incorporating the ecological advantages of diversity-related, resource-use dynamics into algal biomass production might provide a cost-effective way to improve yield and the robustness of algae cultivation for biofuel production (Stockenreiter et al., 2012).

Another key ecological interaction that applies to commercial-scale algae production in open ponds is the predator-prey dynamic. Similar to their natural analogues, artificially constructed open-pond systems will develop diverse biological communities. In particular, because they will contain primary producers (algae) and primary consumers (herbivorous zooplankton), these pond communities will tend to exhibit predator-prey population oscillations similar to insect outbreaks that damage crop yields in terrestrial agriculture. In particular, unrestrained growth of large herbivorous zooplankton, such as *Daphnia pulex* or *Daphnia magna*, is analogous to placing an excess of grazing animals in a field. They can over-graze algal cells and result in order-of-magnitude reductions in algal biomass yields. Selective grazing of desirable species coupled with nutrient release from grazers can alter the composition of the resident algae to increased levels of undesirable forms. From a crop protection point of view, this is a highly undesirable outcome. However, aquatic food webs can be altered to lessen losses of algal biomass via top-down control (Carpenter and Kitchell, 1988). For example, the carnivorous mosquitofish (*Gambusia affinis*) can be added to pond production systems to remove large zooplankton and help maximize the pond-grown algal biomass for biofuel production (Smith et al., 2010b). The mixed community could contain variable nutritional value as taxonomic composition changes. Changes in composition likely would alter the lipid content and the potential quality of algal biomass for making fuels.

2.3 PROCESSING ALGAL BIOMASS INTO FUELS

Fuel production from algal biomass is most commonly assumed to involve cultivation of microalgal species that have high lipid productivity and the processing of the lipid to biodiesel. In this case, production of biofuel requires the algae to be concentrated and subsequently treated to cause the release of the intracellular lipids. The concentration, or harvest, step involves the separation and typically drying of the algal cells to prepare them for lipid collection. Lipid collection usually is accomplished by rupturing the algal cells. Subsequent extraction of the biomass might be required for economical oil recovery. Thus, biodiesel production from algae requires two distinct separation steps—harvest and product collection—regardless of whether growth occurs in open or closed photobioreactors.

The important feature in harvest and extraction is that the algae and the lipids are insoluble in water. The technical problem in the production of biodiesel is simply producing a pure, dry triacylglycerol stream for subsequent processing to biofuels. Because the algal biomass and the algal oils are immiscible in water, harvest can be completely spontaneous, and there is no key thermodynamic separation energy to be overcome. The constraints on the system are purely engineering-related, and better engineering can reduce the energy expenditure required for separating the algal biomass from the culture water and drying it for subsequent oil collection. Relatively low algal biomass concentrations and the small size of microalgae make separation challenging and energy intensive. A meta-analysis of published studies shows that more than 40 percent of the total energy required for biodiesel production can be attributed to harvest and product collection (Clarens et al., 2010). (See Chapter 4 for details on energy use.)

Purity of the algal lipid is an important parameter for processing into liquid transportation fuel. Inorganic materials that stay with the oil are a concern, and the method of harvest and collection can influence the impurity levels. Inorganic salts and phospholipids are two known impurities that could affect processing. Inorganic salts are in the culture medium and occur naturally in algae, but they also can be introduced as flocculants.

2.3.1 Harvesting and Dewatering Methods

Microalgal cultures are about neutrally buoyant suspensions of microscopic particles. As noted earlier in this chapter (see Tables 2-1, 2-2, and 2-4), algal cell biomass is most commonly reported to be up to about 0.4 grams per liter in open ponds and 3 g/L in photobioreactors, though concentrations up to 40 grams per liter have been reported (Brennan and Owende, 2010). These concentrations require that almost a liter of water be removed from the algae to produce a few grams of dry biomass. The pumping and processing of water are energy intensive, and reducing the energy required to collect the algae directly affects the sustainability of microalgae cultivation.

Microalgae are grown as insoluble particles in an aqueous medium. Furthermore, the lipids present in the algae are similarly immiscible in water. In principle, the separation of algal oils from the aqueous growth media can be spontaneous and require little energy. In practice, the separation of algae from the growth media and the separation of lipids from algal biomass in a timely manner is energy intensive. Reducing the energy required can be accomplished through improvements in the algal strains, through engineering improvements, and through favorable interplay of the two. As an example, improvements to algae that increase the density of cells in the culture, in principle, reduce the amount of water that has to be eliminated during recovery. Reducing the water processed would, all other things being equal, reduce the energy expended during algae collection.

Methods for harvest vary greatly depending on whether macroalgae or microalgae are grown. Though the focus of this report is on microalgae, macroalgae are harvested mechanically with relatively low-energy input today (Roesijadi et al., 2010). Harvesting macroalgae is a more than 400-year-old industry (McHugh, 2003) with innovations still being proposed to improve efficiency (Garthwaite, 2012; OneWater, 2012). For microalgae, the methods used for harvest rely on size exclusion or separation based on density (Table 2-6). These include filtration, centrifugation, sedimentation, flotation, flocculation (coagulation), and electrophoresis techniques (Uduman et al., 2010). Flocculation and gravity sedimentation are similar. Natural density separation can be sped up by adding agents that cause the microalgae to aggregate. Triggers for aggregation include changes in pH or other chemical triggers. Algae either may settle or will float to the surface of the liquid. The separation is a relatively low-energy decanting that produces a majority water phase and an algal phase. Inorganic or organic (synthetic) flocculants both are used, with the nature and disposition of these materials being the sustainability concerns.

Use of inorganic flocculants, such as ferric chloride and alum, pose environmental and processing concerns. Flocculants travel through the process with the algal biomass and would have to be accounted for in the process. High concentrations of metals present in residual algal biomass would limit its use as coproducts because of safety concerns. Organic flocculants may be susceptible to anaerobic digestion, removing them from the recycle stream. The presence of flocculants may affect the suitable uses for the algal biomass as coproducts.

Centrifugation and filtration can be used alone or in concert with a preliminary density-driven separation. Centrifugation rapidly concentrates organisms but requires high capital and operating costs. Filtration may be inefficient because the microscopic algal cells tend to clog the filter. Centrifugation and filtration are receiving considerable focus, and innovations are being reported (for example, Heaven et al., 2011; Milledge, 2011; Bhave et al., 2012). Other methods for harvest, such as acoustic manipulation of algal cells or electrophoresis techniques including electrolytic coagulation and electrolytic flocculation, have been reviewed, but their harvest rate and reliability vary (Sukenik and Shelef, 1984; Chen et al., 2009; Uduman et al., 2010; Vandamme et al., 2011; Leckey and Hinders, 2012). As noted earlier in this chapter, there also have been efforts to develop genetically engineered algae incorporating magnetic nanoparticles to reduce energy costs for harvesting and dewatering.

TABLE 2-6 Characteristics of Microalgae Harvesting Techniques.

Harvest Methods	Suspended Solids Concentration (%)	Operating Costs per Gallon of Water	Cell Harvesting Efficiency	Algal Species
Centrifuging	High (< 22%)	Very high (\$20 to \$50)	> 90%	Almost all except the very fragile
Filtration/screening	Medium to high (5% to 18%)	Medium to high (\$10 to \$20)	20% to 90%	Algae with large (> 5 μ m) cells
Flocculation	Low to medium (3% to 6%)	Low to medium (\$3 to \$10)	50% to 90%	Algae with low density
Bioflocculation	Low to medium (2% to 5%)	Low (\$0.20 to \$0.50)	About 90%	
Sedimentation/settling	Low (0.5% to 3%)	Low to medium (\$0.50 to \$1.50)	10% to 90%	Algae with high density

SOURCE: Adapted from Shen et al., 2009. Reprinted with permission from the American Society of Agricultural and Biological Engineers.

2.3.2 Extraction

Once the algal biomass has been harvested, oil needs to be extracted if lipids are the desired primary product. For processing methods that use whole cells, harvest might be all that is required for the next stage of fuel production. Biodiesel production is a technology that in most variants requires collection of the algal lipids for post processing. Extraction of oil from algal biomass has proven to be difficult. Unlike oil recovery from oilseed plants, there is no well-defined, commercial lipid extraction process for algae on the market. As in the case of oil recovery from seeds, extraction with organic solvents has been tested with algal systems. There are two differences between oil extraction from seeds and algae. First, simple milling of oilseeds creates an extractable meal. Second, the oilseed meal is about 90 percent solids. In contrast, algal biomass is high in water content, and cell membranes are not easily ruptured to yield readily extractable oil.

High levels of oil expression have been reported for algae grown heterotrophically. Levels reported up to 80 percent are said to be so high that only cell lysing is required for collection, avoiding extraction (Dillon, 2011). There are no reports indicating that photoautotrophic cultivation has attained such high oil fractions.

Oil extraction can be done with dried algae or with the wet paste from harvest. Drying is energy intensive, but yields a material that can be mechanically treated to open up access for oil extraction (Viswanathan et al., 2011). Once dried, oils are extracted. Characteristics of a desirable solvent include high solvent power, low toxicity, low specific heat, low heat of vaporization, low cost, high availability, and preferably nonflammable. Examples of solvents include hexane (most frequently cited as the solvent used for algal-oil extraction), chloroform, methanol, ethanol, butanol, ethyl acetate, and petroleum ether. The solvent is boiled away from the algal oil, recovered, and reused. Solvent recovery is high but is not 100 percent. A 0.5 to 5 percent loss can be assumed. The large volume of solvents and solvent vapors in the process can represent a fire and explosion risk.

Solvents used for wet extraction usually are immiscible with water to save on energy use in solvent recovery. The biomass generally is processed as whole cells because mechanical membrane rupture is difficult in the wet pastes. The solution of algal lipid in solvent is recovered for purification. The solvent is boiled away from the algal oil, as in oilseed processing.

2.3.3 Combined Harvest and Collection

Several methods that seek to use electric fields, heating, or other means to free oil from algae without having to harvest the algae are being developed. Origin Oil, Open Algae, and Diversified Technologies are a few of the companies that seek to substantially reduce the energy requirement of harvest and oil collection. The premise of those methods is to supply energy to an algal culture to rupture (lyse) the cells. Lipids in the cells then spontaneously separate from the biomass, rising to the surface while the biomass sinks. The anticipated result is a solid sediment, an aqueous layer, and a free oil layer so that simple, cost-effective, and energy-efficient gravity separation recovers the oil. These systems offer the promise of substantial reduction in energy use and the elimination of solvent use. A new extraction-harvest technique, microwave-assisted drying, uses microwaves to excite liquid within algal cells, causing the cell wall to rupture and release lipids. Solvents then are used to extract the lipids. Microwave-assisted drying can save time because algal biomass does not have to be dehydrated before the lipid extraction. Whether microwaving would diminish the quality of the lipids or the practicality of scale-up to quantities needed for liquid transportation fuel is unknown (Mercer and Armenta, 2011). Little information currently is available about the effectiveness and energy balance of these processes.

2.3.4 In-Situ Esterification

Treatment of wet algal biomass with alcohols can result in direct collection of fatty-acid methyl esters. To date, this technology has shown little benefit when compared with the more common oil recovery technologies (Ehimen et al., 2010).

2.3.5 Processing

The majority of the presentations to this committee and the published literature focus on producing fuels from lipid-producing microalgae (Benneman, 2011; Clarens, 2011). These, however, do not represent all available options for processing algae to fuels. Many of the processing options are studied but not described in the literature, making a thorough analysis difficult. Three key parameters influence the algal biofuel supply chain (Figure 2-15):

- Whether microalgae or macroalgae is cultivated for fuel production (micro versus macro).
- Whether the algae are cultivated for short duration and harvested for processing or cultivated in stable conditions while desired products emitted by algae are continuously harvested (short duration versus stable).
- Whether biochemical or thermochemical processes are used to produce biofuels from algae (biological versus chemical).

Although the sustainability challenges identified in this report frequently cannot be addressed without quality data, categorizing the algal biofuel production pathways by these three key parameters allows some general comments to be made about each combination of parameters (Figure 2-15).

The categories used to distinguish the processing depend on the nature of the algae, whether a stable culture is used, and whether the post processing is chemical or biological. Algae can be divided into microalgae or macroalgae, independent of whether the algae are fresh or salt water species. The dynamics of the culture are important and largely can be divided into whether the desire is for a time-stable culture or whether the algae are killed in the collection of the product. This designation, therefore, is largely determined by whether

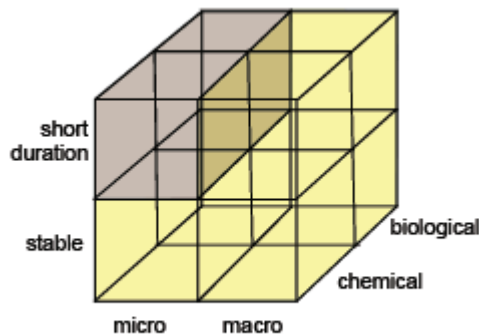


FIGURE 2-15 Matrix showing combinations of key parameters that define algal biofuel processing pathways. NOTE: The grey boxes indicate combinations of pathways that are not pursued, to best of the committee's knowledge.

the desired products are retained within the algal cell walls or emitted extracellularly. Currently all processes described that use stable cultures and emit desirable products use closed photobioreactors. Presentations to the committee raised doubts that extracellular products could be collected in open-pond systems because of potential microbial consumption of the product (Benemann, 2011). Chemical processes, including conventional extraction of oil from microalgae and processing the oil to biodiesel, are typically used. Biological processes, such as fermentation of microalgal biomass, have been demonstrated. Other combinations have been described as a means for producing algal fuels.

2.3.5.1 Microalgae Harvested with Product Collected for Chemical Processing

The harvest of lipid-producing microalgae cultivated for short duration and the chemical processing of algal oil into fuel represent the most commonly discussed method for production of algal biofuels. The expression level of the oil as a fraction of the total biomass determines what processing will be required. High oil-expression levels sufficient to avoid extraction were not found in published data. Extraction with volatile alkane, ester, or alcohol solvents will recover lipids and phospholipid fractions from the algal biomass. The lipid recovery begins by boiling away the solvent, leaving the lipids for subsequent processing (Sheehan et al., 1998). Oil collected then is subjected to degumming. Most degumming methods involve a water wash step, creating an aqueous waste stream, which is reported to be 10-30 kilograms per 1,000 kilograms of degummed oil for typical seed oil processes (Crown Iron Works, 2008). Other component additions may be required, but are similarly small. This step, while necessary, is not likely to have a big impact on the energy or raw material requirements of the process.

The degummed oil then can be processed in several ways. Two main products are commonly mentioned: traditional transesterified biodiesel and hydrotreated or so-called green diesel. In traditional biodiesel production, methanol and a base catalyst react with the triacylglycerol algal oil to produce a fatty-acid methyl ester. Homogeneous base catalysts, commonly in the form of sodium or potassium hydroxide, are being replaced by heterogeneous catalysts, reducing waste (Ondrey, 2004). Glycerol is produced as a coproduct in both methods.

Recent trends have been toward the production of hydrotreated diesels rather than esters. In hydroprocessing, hydrogen reacts with the raw algal oil to produce alkanes, propane, and water. Hydrotreated diesels are more similar to petroleum-based diesel and are said to offer better performance than esters (Kalnes et al., 2007; Pew Center on Global Climate Change, 2011). Hydrogenated diesels are assumed to be compatible with existing petroleum infrastructure (DOE-EERE, 2012b), and whether they are sufficiently similar enough to petroleum-based fuels to be considered drop-in fuels would have to be tested.

2.3.5.2 Extracellular Secretion of Products by Microalgae for Chemical Processing

Algenol and Joule Technologies are two companies exploiting the ability of algae to secrete products extracellularly (Algenol Biofuels, 2012; Joule Unlimited, 2012). The products collect in the growth media for subsequent recovery. Algenol addressed the committee and has described its production method in journal articles and patents (Chance et al., 2011b). Algenol uses cyanobacteria that directly produce ethanol. Joule Technologies has patented cyanobacteria that directly produce alkane (Reppas, 2012). Eukaryotic organisms also have been described (Ramachandra et al., 2009). Algae and cyanobacteria emit a range of materials that could be used for fuel production. The best-described process in the published

literature is Algenol's method of producing ethanol. Ethanol requires only purification and does not require subsequent processing. Recovery of ethanol from an aqueous solution is energy intensive, even with the solar still arrangement used by Algenol to provide primary concentration (Chance et al., 2011b).

The production methods described all use closed photobioreactors. This is likely a result of the desire to maintain stable producing cultures for long periods of time. Introduction of competing algal species or microbial contamination would be detrimental. Closed systems are a means to ensure culture purity and consistent product quality.

In principle, the energy required for separation in this mode of operation can be very low. Engineering organisms that express immiscible products would result in spontaneous separations that do not require energy input. Stable cultures can be maintained requiring minimal water inputs. Water clearly is required to replenish any water lost to photosynthesis and during processing. While the promise is large, the available published studies are insufficient for an accurate appraisal of the overall energy and LCAs to be performed.

2.3.5.3 Microalgae Harvested with Product Collected for Biological Processing

Although fermentation of microalgal biomass has been studied (Harun, 2010), it is not being developed at commercial scale at present. Microalgae provide carbon sources in the form of proteins and carbohydrates that can be exploited using fermentation. Some advantages include rapid growth rates, short harvesting cycles, and the absence of lignin. Like other biomass fermentations, a wide variety of products could be produced. There is a lack of detailed studies on this processing pathway.

2.3.5.4 Extracellular Emission of Products by Microalgae for Biological Processing

Proterro (2012) describes a method that uses microalgae in a photobioreactor to generate sugars that can be recovered for subsequent use as a feedstock for other fermentations. The sugar produced can be used for any fermentation process to produce fuels. Ethanol is currently the largest volume fuel produced by fermentation. Butanol (Butamax Advanced Biofuels, 2012; Gevo, 2012), farnesene (Amyris, 2012), alkanes (LS9 Inc., 2011; Solazyme, 2012), and other products also can be produced by sugar fermentation. Direct production of sugars requires that the algal culture be protected from opportunistic microorganisms and requires an environmentally sealed photobioreactor.

2.3.5.5 Macroalgae Harvested with Product Collected for Biological Processing

Bio Architecture Labs recently announced the development of a technology for the fermentation of macroalgal biomass (Wargacki et al., 2012). Use of macroalgae as feedstock for biochemical conversion is made possible by the development of organisms capable of metabolizing alginate polysaccharides. Organisms engineered for alginate transport and metabolism were further engineered for ethanol synthesis. This enables direct ethanol synthesis from macroalgae. Brown macroalgae are said to be attractive feedstocks because they do not require fertilizer input, arable land, and freshwater resources, and therefore do not compete with existing food crops for those resources. Sugars can be released in simple mechanical operations, like crushing and milling, because macroalgae do not contain lignin (Wargacki et al., 2012). Furthermore, cultivation methods are established because macroalgae are harvested for food ingredients, animal feedstuff, and fertilizers. *Saccharina japonica*

was demonstrated as a fermentation substrate. *Gracilaria salicornia* also has been shown to be a suitable substrate for microbial fermentation to ethanol (Wang et al., 2011).

2.3.5.6 Microalgae or Macroalgae Harvested for Whole-Biomass Processing

Interest is increasing in whole-biomass conversion for processing of terrestrial biomass (Marker et al., 2010; Wright et al., 2010). Pyrolysis of whole biomass yields an upgradeable biocrude. A recent review (Anex et al., 2010) shows that these routes have cost advantages relative to other biomass conversion technologies. The material produced potentially can be used in an existing refinery, saving capital relative to other options.

Several forms of pyrolysis have been explored. Hydropyrolysis is reported to be appropriate for use in processing of algal biomass (Marker et al., 2012). The low aromatic content in algal biomass (because of the absence of lignin) is said to make algal biomass a particularly good feedstock for hydropyrolysis.

The development of two pilot-scale alternatives has been reported, both producing fossil fuel-compatible materials (Hatcher, 2011). These synthetic crudes are stated to be compatible with existing refineries. In one of the processes, fertilizer is a coproduct.

Insufficient documentation is available for a detailed mass and energy balance of the processes. It can be presumed that the detailed studies on terrestrial biomass will yield similar results when algae are the feedstock. That is, whole-cell processing provides a potentially viable means of producing drop-in replacement fuels, taking advantage of existing refinery infrastructure to reduce risk and costs.

2.3.6 Fuel Products and Coproducts

The processes described above make many potential fuel components. Table 2-7 summarizes some of the dominant inputs and outputs for the technologies described.

2.3.7 Status of Algal Biofuel Production

Algal biofuel production is rapidly evolving, and as such, any status report is outdated at the moment of its completion. There are currently no operating algal biofuel production facilities that are comparable in scale to the average capacity of about 13.5 million gallons (51 million liters) per year for U.S. biodiesel refineries, much less rivaling the largest at about 100 million gallons (378 million liters) per year (NBB, 2012). Many projects are still in the research and development phase and exact production numbers are difficult to obtain.

2.4 CONCLUSIONS

An integrated coordination of biological (for example, algal strain selection and development of algae cultivation) and engineering processes (for example, reactor design, harvesting and dewatering methods, and processing) is needed to realize the potential of algal biofuels. However, the domestication of algae poses a special challenge as investigation into key biological and ecological aspects of algal biofuel production has lagged far behind the progress in feedstock processing design, system engineering, and life-cycle analyses over the past few decades. Nonetheless, increased core understanding of algae and their potential for improvements is fundamental to accelerating the entire algal biofuel enterprise. Relative to the vast and diverse spectrum of potentially available organisms, only a narrow range of currently cultivated microalgal strains are considered for commercial production

TABLE 2-7 Dominant Inputs and Outputs for Algal Processing Technologies.

Duration of Cultivation System	Type of Algae	Type of Processing	Product	Major Inputs for Processing	Coproducts
Short	Microalgae	Chemical	Hydrogenated diesel	Hydrogen, extraction solvent	Algal biomass, propane
Short	Microalgae	Chemical	Biodiesel	Methanol, base catalyst	Algal biomass, glycerol
Stable	Microalgae	Chemical	Ethanol		
Stable	Microalgae	Chemical	Alkanes		
Short	Macroalgae	Biochemical	Ethanol, other products		
Short	Microalgae	Biochemical	Ethanol, other products		
Short	Microalgae	Chemical	Pyrolysis oil		Char, off-gases
Short	Macroalgae	Chemical	Pyrolysis oil		Char, off-gases

of biofuels. Extensive new genomic analyses and physiological studies will be useful for screening and expanding the range of candidate species of microalgae and macroalgae that can be used for commercial-scale production of biofuels.

The presence of dramatically different photosynthetic efficiencies and chemistries across algal species underscores a critical need for basic and applied research to expand the spectrum of germplasm available for the enterprise. Research on expanding the light spectrum useful for photosynthesis, improving the distribution of incident light to various aquatic photosynthetic scale-up processes, and enhancing the efficiency of Rubisco or other basic physiological processes to better utilize carbon could lead to dramatic improvements in productivity. Additional new breakthroughs in areas such as the capability of algae to convert nutrients into biomass more efficiently or the reduction of processing costs associated with harvesting and dewatering (for example, via genetic enhancements that favor autoflocculation) also have the potential to further improve the energy balance and to enhance the overall sustainability of an expanding algal biofuel industry.

Equally important is crop protection research that focuses on reducing biomass losses to pathogens and grazers. Because contamination by other algal species is largely unavoidable, especially in open-pond algal cultures, improving the existing understanding of how algal biomass production systems can be managed as complex bioengineered systems would be helpful. This can be achieved in part via the application of principles of population, community, and ecosystem ecology. Identifying which ecophysiological parameters and genes best provide protection against grazers and pathogens at commercial-scale production levels also would be helpful.

Improvements in algae cultivation methods and the physical processes used to harvest, dewater, and convert algal biomass into fuels are as important to the sustainable development of algal biofuels as improvements in algal strains. New ways to reduce the energy requirements for converting cultivated algae in an aqueous solution into a dewatered state that can then be processed into fuel could be explored. Research and development in understanding how dewatered algae can be processed into a fuel and whether algae can produce a useful hydrocarbon directly without the need for harvest and dewatering and with minimal processing could be an important contributor to reducing production costs. Fundamentally, the questions involve integrating biology, ecology, and engineering into a systematic understanding and improvement of the entire algal biofuel enterprise (Box 2-2).

BOX 2-2 Research and Development for Enhancing Algal Biofuel Production

Research and development directed at domestication of algae for biofuel production is vitally important. This effort will require improving the functional understanding of the biology, physiology, and ecology of microalgae. This upstream research and development will help inform and guide downstream engineering methods and designs for cultivation and processing systems that will enhance the entire algal biofuel production chain. Thus, concerted, complementary efforts in algal domestication and biofuel production will include:

- Development of strategies to improve carbon fixation rates and yields of algal crops at commercial production-level scale.
- Development of algal strains or multi-species assemblages that achieve high productivity and high volumetric concentrations over a wide range of environmental conditions (including variations in temperature and light levels) and are as easily harvested and processed as possible.
- Evaluation and development of improved crop protection methods.
- Design and development of robust, low-cost, long-lasting production systems for algal strains or multi-species assemblages that demand minimal regulations and control of environmental parameters.
- Development of strains that excrete oil or other fuel precursors, especially immiscible products.
- Development of improved harvest technologies that reduce energy required during collecting and processing.
- Design and development of integrated biological and engineering production strategies that obviate algae harvesting, drying, and oil-extraction processes.
- Design and development of integrated biological and engineering production strategies that continually reuse the algae, water, and nutrients.
- Design and development of systems that can process whole biomass into fuels.

SUMMARY FINDING FROM THIS CHAPTER

Algal strain development is needed to enhance traits that contribute to increasing fuel production per unit resource use, reducing the environmental effects per unit fuel produced, and enhancing economic viability. Improvements in biomass or product (lipid, alcohol, or hydrocarbons) yield, culture density, nutrient uptake, ease of harvest, and photosynthetic efficiency are some of the improvements that would improve sustainability of algal biofuels.

REFERENCES

- 81st Texas Legislature House Bill 3391, Bill introduced by the Texas House of Representatives relating to the continuation and functions of the Parks and Wildlife Department; changing the elements of an offense. § Chapter 952. (Regular Session, June 19, 2009).
- Acien Fernández, F.G., J.M. Fernández Sevilla, J.A. Sánchez Pérez, E. Molina Grima, and Y. Chisti. 2001. Airlift-driven external-loop tubular photobioreactors for outdoor production of microalgae: Assessment of design and performance. *Chemical Engineering Science* 56(8):2721-2732.
- Algae Energy. 2012a. Open cultivation system for growing algae. Available online at http://algae-energy.co.uk/biofuel_production/cultivation/. Accessed May 15, 2012.
- . 2012b. Photobioreactors. Available online at http://algae-energy.co.uk/biofuel_production/pbrs/. Accessed May 15, 2012.

- Algenol Biofuels. 2012. Homepage. Available online at <http://algenol.com/>. Accessed June 16, 2012.
- Algomed. 2012. Microalgae cultivated in a 500 km long system of glass tubes—A German innovation. Available online at http://www.algomed.de/index.php?lang=eng&op=algenfarm_anlage. Accessed May 15, 2012.
- Amyris. 2012. Homepage. Available online at <http://www.amyris.com/>. Accessed June 17, 2012.
- Andrianov, V., N. Borisjuk, N. Pogrebnyak, A. Brinker, J. Dixon, S. Spitsin, J. Flynn, P. Matyszczuk, K. Andryszak, M. Laurelli, M. Golovkin, and H. Koprowski. 2009. Tobacco as a production platform for biofuel: Overexpression of *Arabidopsis* DGAT and LEC2 genes increases accumulation and shifts the composition of lipids in green biomass. *Plant Biotechnology Journal* 8:1-11.
- Anex, R.P., A. Aden, F.K. Kazi, J. Fortman, R.M. Swanson, M.M. Wright, J.A. Satrio, R.C. Brown, D.E. Daugaard, A. Platon, G. Kothandaraman, D.D. Hsu, and A. Dutta. 2010. Techno-economic comparison of biomass-to-transportation fuels via pyrolysis, gasification, and biochemical pathways. *Fuel* 89(Supplement 1):S29-S35.
- AQUAFUEL. 2009. Deliverable 1.4 Report on biology and biotechnology of algae with indication of criteria for strain selection. Available online at http://www.aquafuels.eu/attachments/079_D%201.4%20Biology%20Biotechnology.pdf. Accessed September 14, 2012.
- Aravanis, A. 2011. Near-Term Commercialization of Algal Biofuel. Presentation to the NRC Committee on the Sustainable Development of Algal Biofuels on June 13.
- Atsumi, S., W. Higashide, and J.C. Liao. 2009. Direct photosynthetic recycling of carbon dioxide to isobutyraldehyde. *Nature Biotechnology* 27(12):1177-1180.
- Baptist, G., D. Meritt, and D. Webster. 1993. Growing Microalgae to Feed Bivalve Larvae. North Dartmouth: University of Massachusetts, Dartmouth.
- Baud, S., S. Wuilleme, B. Dubreucq, A. de Almeida, C. Vuagnat, L. Lepiniec, M. Miquel, and C. Rochat. 2007. Function of plastidial pyruvate kinases in seeds of *Arabidopsis thaliana*. *Plant Journal* 52(3):405-419.
- Becker, E.W. 1994. *Microalgae: Biotechnology and Microbiology*. Cambridge, UK: Cambridge University Press.
- Benemann, J.R. 1986. *Microalgae Biotechnology: Products, Processes, and Opportunities*. Washington, DC: OMEC International Inc.
- . 1989. The future of microalgal biotechnology. In *Algal and Cyanobacterial Biotechnology*, edited by Cresswell R.C., N. Shah, and T.A. Rees. Harlow, England: Longman Scientific and Technical.
- . 2008. Opportunities and challenges in algae biofuel production. Available online at http://www.fao.org/uploads/media/algae_positionpaper.pdf. Accessed October 21, 2011.
- . 2011. Promise and Challenges in Sustainable Development of Algal Biofuels: Questions and Answers. Presentation to the NRC Committee on Sustainable Development of Algal Biofuels on July 5.
- Bessler, H., V.M. Temperton, C. Roscher, N. Buchmann, B. Schmid, E.D. Schulze, W.W. Weisser, and C. Engels. 2009. Aboveground overyielding in grassland mixtures is associated with reduced biomass partitioning to belowground organs. *Ecology* 90(6):1520-1530.
- Bhave, R., T. Kuritz, L. Powell, and D. Adcock. 2012. Membrane-based energy efficient dewatering of microalgae in biofuels production and recovery of value added coproducts. *Environmental Science and Technology* 46(10):5599-5606.
- Blankenship, R.E., D.M. Tiede, J. Barber, G.W. Brudvig, G. Fleming, M. Ghirardi, M.R. Gunner, W. Junge, D.M. Kramer, A. Melis, T.A. Moore, C.C. Moser, D.G. Nocera, A.J. Nozik, D.R. Ort, W.W. Parson, R.C. Prince, and R.T. Sayre. 2011. Comparing photosynthetic and photovoltaic efficiencies and recognizing the potential for improvement. *Science* 332(6031):805-809.
- Borowitzka, M.A. 1992. Algal biotechnology products and processes—matching science and economics. *Journal of Applied Phycology* 4(3):267-279.
- . 1999. Commercial production of microalgae: ponds, tanks, tubes and fermenters. *Journal of Biotechnology* 70(1-3):313-321.
- Brennan, L., and P. Owende. 2010. Biofuels from microalgae—A review of technologies for production, processing, and extractions of biofuels and coproducts. *Renewable and Sustainable Energy Reviews* 14(2):557-577.
- Brown, R.M., D.A. Larson, and H.C. Bold. 1964. Airborne algae—Their abundance and heterogeneity. *Science* 143(360):583-585.
- Butamax Advanced Biofuels, LLC. 2012. Homepage. Available online at <http://www.butamax.com/>. Accessed June 17, 2012.
- California Polytechnic State University. 2012. Tubular Photobioreactor. Available online at <http://brae.calpoly.edu/CEAE/biofuels.html>. Accessed May 15, 2012.
- Camacho Rubio, F., F.G. Ación Fernández, J.A. Sánchez Pérez, F. García Camacho, and E. Molina Grima. 1999. Prediction of dissolved oxygen and carbon dioxide concentration profiles in tubular photobioreactors for microalgal culture. *Biotechnology and Bioengineering* 62(1):71-86.

- Carlozzi, P. 2003. Dilution of solar radiation through "culture" lamination in photobioreactor rows facing south-north: A way to improve the efficiency of light utilization by cyanobacteria (*Arthrospira platensis*). *Biotechnology and Bioengineering* 81(3):305-315.
- Carpenter, S.R., and J.F. Kitchell. 1988. Consumer control of lake productivity. *Bioscience* 38(11):764-769.
- Cernac, A., and C. Benning. 2004. WRINKLED1 encodes an AP2/EREB domain protein involved in the control of storage compound biosynthesis in *Arabidopsis*. *Plant Journal* 40(4):575-585.
- Cerutti, H., X.R. Ma, J. Msanne, and T. Repas. 2011. RNA-mediated silencing in algae: Biological roles and tools for analysis of gene function. *Eukaryotic Cell* 10(9):1164-1172.
- Chance, R.R., B. McCool, and J. Coleman. 2011a. Questionnaire reply from Algenol Biofuels Inc. Received by the NRC Committee on Sustainable Development of Algal Biofuels on July 7.
- Chance, R.R., B. McCool, and J.D. Coleman. 2011b. A Cyanobacteria-Based Photosynthetic Process for the Production of Ethanol. Presentation to the NRC Committee on Sustainable Development of Algal Biofuels on June 13.
- Chase, J.M. 2003. Community assembly: when should history matter? *Oecologia* 136(4):489-498.
- Chen, M., and R.E. Blankenship. 2011. Expanding the solar spectrum used by photosynthesis. *Trends in Plant Science* 16(8):427-431.
- Chen, P., M. Min, Y.-F. Chen, L. Wang, Y. Li, Q. Chen, C. Wang, Y. Wan, X. Wang, Y. Cheng, S. Deng, K. Hennessy, X. Lin, Y. Liu, Y. Wang, B. Martinez, and R. Ruan. 2009. Review of the biological and engineering aspects of algae to fuels approach. *International Journal of Agricultural and Biological Engineering* 2(4):1-30.
- Cheng-Wu, Z., O. Zmora, R. Kopel, and A. Richmond. 2001. An industrial-size flat plate glass reactor for mass production of *Nannochloropsis* sp. (Eustigmatophyceae). *Aquaculture* 195(1-2):35-49.
- Clarens, A.F. 2011. Life cycle assessment of algae-to-energy technologies. Presentation to the NRC Committee on Sustainable Development of Algal Biofuels on June 13.
- Clarens, A.F., E.P. Resurreccion, M.A. White, and L.M. Colosi. 2010. Environmental life cycle comparison of algae to other bioenergy feedstocks. *Environmental Science and Technology* 44(5):1813-1819.
- Clemente, T.E., and E.B. Cahoon. 2009. Soybean oil: Genetic approaches for modification of functionality and total content. *Plant Physiology* 151(3):1030-1040.
- Cloud, B. 2011a. Questionnaire reply from Phyco Biosciences Inc. Received by the NRC Committee on Sustainable Development of Algal Biofuels on September 27.
- . 2011b. Questionnaire reply from Phyco BioSciences Inc. Received by the NRC Committee on Sustainable Development of Algal Biofuels on July 25.
- Courchesne, N.M.D., A. Parisien, B. Wang, and C.Q. Lan. 2009. Enhancement of lipid production using biochemical, genetic and transcription factor engineering approaches. *Journal of Biotechnology* 141(1-2):31-41.
- Crown Iron Works. 2008. MultiPure degumming/neutralizing system. Available online at <http://www.crown-iron.com/userimages/DegNeuN1.pdf>. Accessed June 16, 2012.
- Cuello, J., and J. Ley. 2011. ACCORDION Photobioreactor for Algae Production of Biofuels and Biochemicals. Presentation to the Conference EPA's GHG Emissions Standards for Power Plants and Oil Refineries on February 2.
- Cyanotech. 2012. Hawaiian Spirulina Pacifica® Available online at <http://www.cyanotech.com/spirulina.html>. Accessed April 5, 2012.
- Davis, R., A. Aden, and P.T. Pienkos. 2011. Techno-economic analysis of autotrophic microalgae for fuel production. *Applied Energy* 88(10):3524-3531.
- Del Campo, J.A., M. Garcia-Gonzalez, and M.G. Guerrero. 2007. Outdoor cultivation of microalgae for carotenoid production: Current state and perspectives. *Applied Microbiology and Biotechnology* 74(6):1163-1174.
- Dillon, H. 2011. Presentation in the session "Broadening the Bionergy Horizon." In DOE Biomass Summit: Replace the Whole Barrel, Supply the Whole Market, July 26, 2012, National Harbor, MD.
- Ditty, J.L., S.B. Williams, and S.S. Golden. 2003. A cyanobacterial circadian timing mechanism. *Annual Review of Genetics* 37:513-543.
- DOE-EERE (U.S. Department of Energy, Energy Efficiency and Renewable Energy). 2012a. Lower and higher heating values of fuels. Available online at http://hydrogen.pnl.gov/cocoon/morf/hydrogen/site_specific/fuel_heating_calculator?canprint=false. Accessed June 18, 2012.
- . 2012b. Drop-in biofuels. Available online at http://www.afdc.energy.gov/fuels/emerging_dropin_biofuels.html. Accessed August 21, 2012.
- DOE (U.S. Department of Energy). 2010. National Algal Biofuels Technology Roadmap. Washington, D.C.: U.S. Department of Energy, Energy Efficiency and Renewable Energy.
- Ducat, D.C., J.C. Way, and P.A. Silver. 2011. Engineering cyanobacteria to generate high-value products. *Trends in Biotechnology* 29(2):95-103.

- Duerr, E.O., A. Molnar, and V. Sato. 1998. Cultured microalgae as aquaculture feeds. *Journal of Marine Biotechnology* 6(2):65-70.
- Durand-Chastel, H. 1980. Production and use of *Spirulina* in Mexico. Pp. 51-64 in *Algae Biomass*. Amsterdam, The Netherlands: Elsevier/North-Holland Biomedical Press.
- Earthrise Nutritional, LLC. 2009a. Earthrise. Available online at <http://www.earthrise.com/>. Accessed February 9, 2012.
- . 2009b. About Earthrise: Our farm. Available online at <http://earthrise.com/farm.html>. Accessed August 26, 2012.
- Edwards, M. 2010. Phyco Biosciences super trough growing algae. Available online at <http://www.algaeindustry.com/abundance-food/>. Accessed May 15, 2012.
- Ehimen, E.A., Z.F. Sun, and C.G. Carrington. 2010. Variables affecting the in situ transesterification of microalgae lipids. *Fuel* 89(3):677-684.
- Ehira, S., and M. Ohmori. 2011. NrrA, a nitrogen-regulated response regulator protein, controls glycogen catabolism in the nitrogen-fixing cyanobacterium *Anabaena* sp strain PCC 7120. *Journal of Biological Chemistry* 286(44):38109-38114.
- Elhai, J., A. Veprikitskiy, A.M. MuroPastor, E. Flores, and C.P. Wolk. 1997. Reduction of conjugal transfer efficiency by three restriction activities of *Anabaena* sp. strain PCC 7120. *Journal of Bacteriology* 179(6):1998-2005.
- Enay, S. 2011. Hawaii's Natural Energy Laboratory fuels innovation. Available online at <http://www.hawaiibusiness.com/Hawaii-Business/November-2011/Hawaiis-Natural-Energy-Laboratory-fuels-innovation/>. Accessed August 26, 2012.
- EPA (U.S. Environmental Protection Agency). 2011. Microbial products of biotechnology: Final regulations under the Toxic Substances Control Act summary (fact sheet). Available online at http://www.epa.gov/biotech_rule/pubs/fs-001.htm. Accessed March 2, 2012.
- Evens, T.J., and R.P. Niedz. 2011. Mapping the fundamental niches of two freshwater microalgae, *Chlorella vulgaris* (Trebouxiophyceae) and *Peridinium cinctum* (Dinophyceae), in 5-dimensional ion space. *International Journal of Ecology* DOI:10.1155/2011/738035.
- Falkowski, P.G., and J.A. Raven. 1997. *Aquatic Photosynthesis*. Malden, MA: Blackwell Science.
- Flaherty, B.L., F. Van Nieuwerburgh, S.R. Head, and J.W. Golden. 2011. Directional RNA deep sequencing sheds new light on the transcriptional response of *Anabaena* sp strain PCC 7120 to combined-nitrogen deprivation. *BMC Genomics* 12:332.
- Frank, E.D., J. Han, I. Palou-Rivera, A. Elgowainy, and M.Q. Wang. 2011. Life-Cycle Analysis of Algal Lipid Fuels with the GREET Model. Oak Ridge, TN: U.S. Department of Energy.
- Frisch, D., A.J. Green, and J. Figuerola. 2007. High dispersal capacity of a broad spectrum of aquatic invertebrates via waterbirds. *Aquatic Sciences* 69(4):568-574.
- Fuhrmann, M., W. Oertel, and P. Hegemann. 1999. A synthetic gene coding for the green fluorescent protein (GFP) is a versatile reporter in *Chlamydomonas reinhardtii*. *Plant Journal* 19(3):353-361.
- García-González, M., J. Moreno, J.P. Cañavate, V. Anguis, A. Prieto, C. Manzano, F.J. Florencio, and M.G. Guerrero. 2003. Conditions for open-air outdoor culture of *Dunaliella salina* in southern Spain. *Journal of Applied Phycology* 15(2-3):177-184.
- Garthwaite, J. 2012. Unlocking Seaweed's Next-Gen Crude: Sugar. Available online at <http://green.blogs.nytimes.com/2012/01/23/unlocking-seaweeds-next-gen-crude-sugar/>. Accessed June 13, 2012.
- Gershwin, M.E., and A.B. Belay. 2008. *Spirulina* in human nutrition and health. Boca Raton: CRC Press.
- Gevo. 2012. Homepage. Available online at <http://www.gevo.com/>. Accessed June 17, 2012.
- Giordano, M., J. Beardall, and J.A. Raven. 2005. CO₂ concentrating mechanisms in algae: Mechanisms, environmental modulation, and evolution. *Annual Review of Plant Biology* 56:99-131.
- Graham, D.W., and V.H. Smith. 2004. Designed ecosystem services: Application of ecological principles in wastewater treatment engineering. *Frontiers in Ecology and the Environment* 2(4):199-206.
- Gross, E.M. 2003. Allelopathy of aquatic autotrophs. *Critical Reviews in Plant Sciences* 22(3-4):313-339.
- Guedes, A.C., H.M. Amaro, and F.X. Malcata. 2011. Microalgae as sources of high added-value compounds—A brief review of recent work. *Biotechnology Progress* 27(3):597-613.
- Guihéneuf, F., S. Leu, A. Zarka, I. Khozin-Goldberg, I. Khalilov, and S. Boussiba. 2011. Cloning and molecular characterization of a novel acyl-CoA:diacylglycerol acyltransferase 1-like gene (PtDGAT1) from the diatom *Phaeodactylum tricorutum*. *FEBS Journal* 278(19):3651-3666.
- Hall, C.A., B.E. Dale, and D. Pimentel. 2011. Seeking to understand the reasons for different energy return on investment (EROI) estimates for biofuels. *Sustainability* 3(12):2413-2432.
- Hall, D.O., F.G. Ación Fernández, E.C. Guerrero, K.K. Rao, and E.M. Grima. 2003. Outdoor helical tubular photobioreactors for microalgal production: Modeling of fluid-dynamics and mass transfer and assessment of biomass productivity. *Biotechnology and Bioengineering* 82(1):62-73.

- Hannon, M., J. Gimpel, M. Tran, B. Rasala, and S. Mayfield. 2010. Biofuels from algae: Challenges and potential. *Biofuels* 1(5):763-784.
- Harun, R., M.K. Danquah, and G.M. Forde. 2010. Microalgal biomass as a fermentation feedstock for bioethanol production. *Journal of Chemical Technology and Biotechnology* 85(2):199-203.
- Hatcher, P.G. 2011. The ODU algae to biodiesel project. Presentation to the NRC Committee on the Sustainable Development of Algal Biofuels on August 24.
- Heaven, S., J. Milledge, and Y. Zhang. 2011. Comments on "Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable." *Biotechnology Advances* 29(1):164-167.
- Heidorn, T., C. Daniel, H. Hsin-Ho, L. Pia, O. Paulo, S. Karin, and L. Peter. 2011. Synthetic biology in cyanobacteria: Engineering and analyzing novel functions. Pp. 539-579 in *Methods in Enzymology*, C. Voigt, ed. Academic Press.
- Hoffmann, M.D., and S.I. Dodson. 2005. Land use, primary productivity, and lake area as descriptors of zooplankton diversity. *Ecology* 86(1):255-261.
- Huesemann, M.H., T.S. Hausmann, R. Bartha, M. Aksoy, J.C. Weissman, and J.R. Benemann. 2009. Biomass productivities in wild type and pigment mutant of *Cyclotella* sp (Diatom). *Applied Biochemistry and Biotechnology* 157(3):507-526.
- Inventure, Inc. 2012. Homepage. Available online at <http://inventurechem.com/home.html>. Accessed June 16, 2012.
- Iowa State University. 2011. Iowa State University scientists genetically increase algae biomass by more than 50 percent. Available online at <http://www.news.iastate.edu/news/2011/nov/spaldingdario>. Accessed June 17, 2012.
- IP Monitor. 2009. Patent application no. 209333021—genetically engineered herbicide resistant algae. Available online at <http://www.ipmonitor.com.au/patents/case/2009333021>. Accessed June 15, 2012.
- Jako, C., A. Kumar, Y.D. Wei, J.T. Zou, D.L. Barton, E.M. Giblin, P.S. Covelto, and D.C. Taylor. 2001. Seed-specific over-expression of an *Arabidopsis* cDNA encoding a diacylglycerol acyltransferase enhances seed oil content and seed weight. *Plant Physiology* 126(2):861-874.
- Janssen, M., P. Slenders, J. Tramper, L.R. Mur, and R.H. Wijffels. 2001. Photosynthetic efficiency of *Dunaliella tertiolecta* under short light/dark cycles. *Enzyme and Microbial Technology* 29(4-5):298-305.
- Jenkins, B.M., L.L. Baxter, T.R. Miles Jr., and T.R. Miles. 1998. Combustion properties of biomass. *Fuel Processing Technology* 54(1-3):17-46.
- Jenkins, D.G., and M.O. Underwood. 1998. Zooplankton may not disperse readily in wind, rain, or waterfowl. *Hydrobiologia* 387:15-21.
- Jiménez, C., B.R. Cossío, and F.X. Niell. 2003. Relationship between physicochemical variables and productivity in open ponds for the production of *Spirulina*: A predictive model of algal yield. *Aquaculture* 221(1-4):331-345.
- Jorquera, O., A. Kiperstok, E.A. Sales, M. Embiruçu, and M.L. Ghirardi. 2010. Comparative energy life-cycle analyses of microalgal biomass production in open ponds and photobioreactors. *Bioresource Technology* 101(4):1406-1413.
- Joule Unlimited. 2012. Frequently asked questions. Available online at <http://www.jouleunlimited.com/faq>. Accessed March 9, 2012.
- Jurgens, K., J. Pernthaler, S. Schalla, and R. Amann. 1999. Morphological and compositional changes in a planktonic bacterial community in response to enhanced protozoan grazing. *Applied and Environmental Microbiology* 65(3):1241-1250.
- Kalnes, T., T. Marker, and D.R. Shonnard. 2007. Green diesel: A second generation biofuel. *International Journal of Chemical Reactor Engineering* 5(1): ISSN (Online) 1542-6580.
- Kan, Y., and J. Pan. 2010. A one-shot solution to bacterial and fungal contamination in the green alga *Chlamydomonas reinhardtii* culture by using an antibiotic cocktail. *Journal of Phycology* 46(6):1356-1358.
- Kanazawa, Z., C. Fujita, T. Yuhara, and T. Sasa. 1958. Mass culture of unicellular algae using the open pond circulation method. *Journal of General and Applied Microbiology* 4:135-139.
- Karpowicz, S.J., S.E. Prochnik, A.R. Grossman, and S.S. Merchant. 2011. The GreenCut2 resource, a phylogenomically derived inventory of proteins specific to the plant lineage. *Journal of Biological Chemistry* 286(24):21427-21439.
- Kawaguchi, K. 1980. Microalgae production systems in Asia. Pp. 25-33 in *Algae Biomass Production and Use*, G. Shelef and C.J. Soeder, eds. Amsterdam, The Netherlands: Elsevier/North Holland Biomedical Press.
- Kawata, Y., S. Yano, H. Kojima, and M. Toyomizu. 2004. Transformation of *Spirulina platensis* strain C1 (*Arthrospira* sp PCC9438) with Tn5 transposase-transposon DNA-cation liposome complex. *Marine Biotechnology* 6(4):355-363.
- Keeling, P.J. 2010. The endosymbiotic origin, diversification and fate of plastids. *Philosophical Transactions of the Royal Society B: Biological Sciences* 365(1541):729-748.

- Kennedy, C.A., K. Thurston, D. Gahl, T. Gordon, and S. Julian. 1995. The Biocoil Project—1994-95. Available online at <http://advbio.cascadeschools.org/94-95/biocoil.html>. Accessed May 15, 2012.
- Kilian, O., C.S.E. Benemann, K.K. Niyogi, and B. Vick. 2011. High-efficiency homologous recombination in the oil-producing alga *Nannochloropsis* sp. Proceedings of the National Academy of Sciences of the United States of America 108(52):21265-21269.
- Laloknam, S., K. Tanaka, T. Buaboocha, R. Waditee, A. Incharoensakdi, T. Hibino, Y. Tanaka, and T. Takabe. 2006. Halotolerant cyanobacterium *Aphanothece halophytica* contains a betaine transporter active at alkaline pH and high salinity. Applied and Environmental Microbiology 72(9):6018-6026.
- Lan, E.L., and J.C. Liao. 2011. Metabolic engineering of cyanobacteria for 1-butanol production from carbon dioxide. Metabolic Engineering 13(4):353-363.
- Lapidot, M., D. Raveh, A. Sivan, S. Arad, and M. Shapira. 2002. Stable chloroplast transformation of the unicellular red alga *Porphyridium* species. Plant Physiology 129(1):7-12.
- Lardizabal, K., R. Effertz, C. Levering, J. Mai, M.C. Pedroso, T. Jury, E. Aasen, K. Gruys, and K. Bennett. 2008. Expression of *Umbelopsis ramanniana* DGAT2A in seed increases oil in soybean. Plant Physiology 148(1):89-96.
- Laws, E.A., D.G. Redalje, D.M. Karl, and M.S. Chalup. 1983. A theoretical and experimental examination of the predictions of two recent models of phytoplankton growth. Journal of Theoretical Biology 105(3):469-491.
- Laws, E.A., S. Taguchi, J. Hirata, and L. Pang. 1988. Optimization of microalgal production in a shallow outdoor flume. Biotechnology and Bioengineering 32(2):140-147.
- Leckey, C.A.C., and M.K. Hinders. 2012. Viscous effects in the acoustic manipulation of algae for biofuel production. Journal of Applied Phycology 24(1):145-156.
- Lee, Y.K. 2001. Microalgal mass culture systems and methods: Their limitation and potential. Journal of Applied Phycology 13(4):307-315.
- Leliaert, F., H. Verbruggen, and F.W. Zechman. 2011. Into the deep: New discoveries at the base of the green plant phylogeny. BioEssays 33(9):683-692.
- Li, Y., D. Han, M. Sommerfeld, and Q. Hu. 2011. Photosynthetic carbon partitioning and lipid production in the oleaginous microalga *Pseudochlorococcum* sp. (Chlorophyceae) under nitrogen-limited conditions. Biore-source Technology 102(1):123-129.
- Liu, X., A.F. Clarens, and L.M. Colosi. 2012. Algae biodiesel has potential despite inconclusive results to date. Bioresource Technology 104:803-806.
- Lopez, D., D. Casero, S.J. Cokus, S.S. Merchant, and M. Pellegrini. 2011. Algal functional annotation tool: A web-based analysis suite to functionally interpret large gene lists using integrated annotation and expression data. BMC Bioinformatics 12:282.
- López, M.C.G.M., E.D.R. Sánchez, J.L. Casas López, F.G.A. Fernández, J.M.F. Sevilla, J. Rivas, M.G. Guerrero, and E.M. Grima. 2006. Comparative analysis of the outdoor culture of *Haematococcus pluvialis* in tubular and bubble column photobioreactors. Journal of Biotechnology 123(3):329-342.
- Lorenz, R. 2002. GRAS notification: "Spirulina" microalgae. Available online at http://www.accessdata.fda.gov/scripts/fcn/gras_notices/grn_101.pdf. Accessed August 26, 2012
- Los Alamos National Laboratory. 2011. LANL develops first genetically engineered "magnetic" algae. Los Alamos News Center: News, Releases, Video, Publications. Available online at http://www.lanl.gov/news/stories/magnetic_algae.html. Accessed February 8, 2012.
- LS9 Inc. (Life Sciences Sustaining 9 Billion). 2011. Homepage. Available online at <http://www.ls9.com/>. Accessed February 9, 2012.
- Ma, J.Y., S.F. Wang, P.W. Wang, L.J. Ma, X.L. Chen, and R.F. Xu. 2006. Toxicity assessment of 40 herbicides to the green alga *Raphidocelis subcapitata*. Ecotoxicology and Environmental Safety 63(3):456-462.
- Ma, J.Y., L.G. Xu, S.F. Wang, R.Q. Zheng, S.H. Jin, S.Q. Huang, and Y.J. Huang. 2002. Toxicity of 40 herbicides to the green alga *Chlorella vulgaris*. Ecotoxicology and Environmental Safety 51(2):128-132.
- Mahan, K.M., O.W. Odom, and D.L. Herrin. 2005. Controlling fungal contamination in *Chlamydomonas reinhardtii* cultures. Biotechniques 39(4):457-458.
- Marker, T., M. Linck, and L. Felix. 2010. Integrated hydrolysis and hydroconversion (IH2) process for direct production of gasoline and diesel fuel from biomass. Paper read at the BIOMASS 2010, March 30-31, Arlington, VA.
- Marker, T.L., L.G. Felix, M.B. Linck, and M.J. Roberts. 2012. Integrated hydrolysis and hydroconversion (IH2) for the direct production of gasoline and diesel fuels or blending components from biomass, Part 1: Proof of principle testing. Environmental Progress and Sustainable Energy 31(2):191-199.
- Marler, D. 2011. An Assessment of the Environmental Performance of Algal Biofuels. Presentation to the NRC Committee on Sustainable Development of Algal Biofuels on September 8.
- Martins, C.A., D. Kulis, S. Franca, and D.M. Anderson. 2004. The loss of PSP toxin production in a formerly toxic *Alexandrium lusitanicum* clone. Toxicon 43(2):195-205.

- Matsuo, T., and M. Ishiura. 2010. New insights into the circadian clock in *Chlamydomonas*. *International Review of Cell and Molecular Biology* 280(C):281-341.
- McHugh, D.J. 2003. *A guide to the seaweed industry*. Rome, Italy: FAO.
- McKenzie, F.T. 2011. *Our changing planet: An introduction to earth system science and global environmental change*, 4th edition. Upper Saddle, NJ: Prentice-Hall.
- Mercer, P., and R.E. Armenta. 2011. Developments in oil extraction from microalgae. *European Journal of Lipid Science and Technology* 113(5):539-547.
- Milledge, J.J. 2011. Commercial application of microalgae other than as biofuels: A brief review. *Reviews in Environmental Science and Biotechnology* 10(1):31-41.
- Moheimani, N.R., and M.A. Borowitzka. 2006. Limits to productivity of the alga *Pleurochrysis carterae* (haptophyta) grown in outdoor raceway ponds. *Biotechnology and Bioengineering* 96(1):27-36.
- Molina, E., J. Fernández, F.G. Acien, and Y. Chisti. 2001. Tubular photobioreactor design for algal cultures. *Journal of Biotechnology* 92(2):113-131.
- Moreno, J., M.A. Vargas, H. Rodríguez, J. Rivas, and M.G. Guerrero. 2003. Outdoor cultivation of a nitrogen-fixing marine cyanobacterium, *Anabaena* sp. ATCC 33047. *Biomolecular Engineering* 20(4-6):191-197.
- Mveda. 2011. Sapphire Energy photo. Available online at <http://www.mveda.com/blog/2011/03/sapphire-develops-research-center/>. Accessed May 15, 2012.
- NanoVoltaix. 2012. Photo-Bioreactors. Available online at <http://www.nanovoltaix.com/markets/algae.php>. Accessed May 15, 2012.
- NBB (National Biodiesel Board). 2012. Plants listings. Available online at <http://www.biodiesel.org/production/plants/plants-listing>. Accessed June 15, 2012.
- Niederholtmeyer, H., B.T. Wolfstader, D.F. Savage, P.A. Silver, and J.C. Way. 2010. Engineering cyanobacteria to synthesize and export hydrophilic products. *Applied and Environmental Microbiology* 76(11):3462-3466.
- O'Neill, J.S., G. Van Ooijen, L.E. Dixon, C. Troein, F. Corellou, F.Y. Bouget, A.B. Reddy, and A.J. Millar. 2011. Circadian rhythms persist without transcription in a eukaryote. *Nature* 469(7331):554-558.
- Olaizola, M. 2000. Commercial production of astaxanthin from *Haematococcus pluvialis* using 25,000-liter outdoor photobioreactors. *Journal of Applied Phycology* 12(3-5):499-506.
- Olguín, E.J., S. Galicia, G. Mercado, and T. Pérez. 2003. Annual productivity of *Spirulina (Arthrospira)* and nutrient removal in a pig wastewater recycling process under tropical conditions. *Journal of Applied Phycology* 15(2-3):249-257.
- Ondrey, G. 2004. Biodiesel production using a heterogeneous catalyst. *Chemical Engineering Journal*, Chemantator (October).
- OneWater, Inc. 2012. OneWater. Available online at <http://onewaterworks.com/>. Accessed June 14, 2012.
- Ono, E., and J.L. Cuello. 2004a. Design parameters of solar concentrating systems for CO₂-mitigating algal photobioreactors. *Energy* 29(9-10):1651-1657.
- . 2004b. Feasibility assessment of open-pond microalgal CO₂ mitigation technology with implementation of the Kyoto Protocol: A case study for Japan. *Environment Control in Biology* 42(3):161-168.
- . 2006. Feasibility assessment of microalgal carbon dioxide sequestration technology with photobioreactor and solar collector. *Biosystems Engineering* 95(4):597-606.
- Ort, D.R., X. Zhu, and A. Melis. 2011. Optimizing antenna size to maximize photosynthetic efficiency. *Plant Physiology* 155(1):79-85.
- Osanai, T., Y. Kanesaki, T. Nakano, H. Takahashi, M. Asayama, M. Shirai, M. Kanehisa, I. Suzuki, N. Murata, and K. Tanaka. 2005. Positive regulation of sugar catabolic pathways in the cyanobacterium *Synechocystis* sp PCC 6803 by the group 2 sigma factor sigE. *Journal of Biological Chemistry* 280(35):30653-30659.
- Pal, D., I. Khozin-Goldberg, Z. Cohen, and S. Boussiba. 2011. The effect of light, salinity, and nitrogen availability on lipid production by *Nannochloropsis* sp. *Applied Microbiology and Biotechnology* 90(4):1429-1441.
- Parikh, M.R., D.N. Greene, K.K. Woods, and I. Matsumura. 2006. Directed evolution of Rubisco hypermorphs through genetic selection in engineered *E. coli*. *Protein Engineering Design and Selection* 19(3):113-119.
- Paerl, H.W. 2000. Marine plankton. Pp. 121-148 in *The Ecology of Cyanobacteria: Their Diversity in Time and Space*, Whitton, B.A. and M. Potts, eds. Boston, M.A.: Kluwer Academic.
- Pedroni, P.M., J. Davison, H. Beckert, P. Bergman, and J. Benemann. 2001. A proposal to establish an international network on biofixation of CO₂ and greenhouse gas abatement with microalgae. Available online at http://www.netl.doe.gov/publications/proceedings/01/carbon_seq/p17.pdf. Accessed August 26, 2012.
- Peled, E., S. Leu, A. Zarka, M. Weiss, U. Pick, I. Khozin-Goldberg, and S. Boussiba. 2011. Isolation of a novel oil globule protein from the green alga *Haematococcus pluvialis* (chlorophyceae). *Lipids* 46(9):851-861.
- Pew Center on Global Climate Change. 2011. Advanced biohydrocarbon fuels. Available online at <http://www.c2es.org/docUploads/AdvancedBiohydrocarbonFuels.pdf>. Accessed August 21, 2012.
- Phycal. 2011. Questionnaire reply from Phycal. Received by the NRC Committee on the Sustainable Development of Algal Biofuels on July 6.

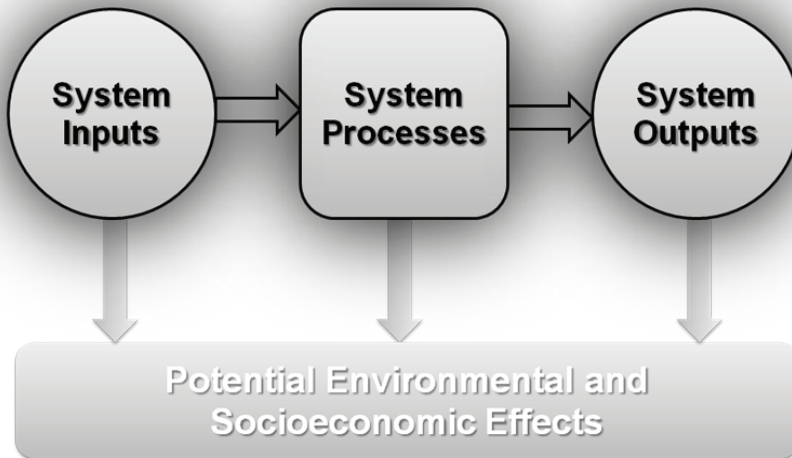
- Polle, J.E.W., S.D. Kanakagiri, and A. Melis. 2003. *tlal*, a DNA insertional transformant of the green alga *Chlamydomonas reinhardtii* with a truncated light-harvesting chlorophyll antenna size. *Planta* 217(1):49-59.
- Proterro. 2012. Homepage. Available online at <http://proterro.com/>. Accessed June 17, 2012.
- Ptacnik, R., T. Andersen, P. Brettum, L. Lepisto, and E. Willen. 2010. Regional species pools control community saturation in lake phytoplankton. *Proceedings of the Royal Society B-Biological Sciences* 277(1701):3755-3764.
- Pushparaj, B., E. Pelosi, M.R. Tredici, E. Pinzani, and R. Materassi. 1997. An integrated culture system for outdoor production of microalgae and cyanobacteria. *Journal of Applied Phycology* 9(2):113-119.
- Radakovits, R., R.E. Jinkerson, A. Darzins, and M.C. Posewitz. 2010. Genetic engineering of algae for enhanced biofuel production. *Eukaryotic Cell* 9(4):486-501.
- Ramachandra, T.V., D.M. Mahapatra, B. Karthick, and R. Gordon. 2009. Milking diatoms for sustainable energy: Biochemical engineering versus gasoline-secreting diatom solar panels. *Industrial and Engineering Chemistry Research* 48(19):8769-8788.
- Raven, J.A. 2010. Inorganic carbon acquisition by eukaryotic algae: Four current questions. *Photosynthesis Research* 106(1-2):123-134.
- Reppas, N.B. 2012. Methods and compositions for the recombinant biosynthesis of N-Alkanes. Patent US8183027, filed September 23, 2011, and issued May 22, 2012.
- Reynolds, C.S. 1997. *Vegetation processes in the pelagic: A model for ecosystem theory*. Vol. 9, Excellence in Ecology. Oldendorf, Germany: Ecology Institute.
- Richmond, A., E. Lichtenberg, B. Stahl, and A. Vonshak. 1990. Quantitative assessment of the major limitations on productivity of *Spirulina platensis* in open raceways. *Journal of Applied Phycology* 2(3):195-206.
- Robertson, D.E., S.A. Jacobson, F. Morgan, D. Berry, G.M. Church, and N.B. Afeyan. 2011. A new dawn for industrial photosynthesis. *Photosynthesis Research* 107(3):269-277.
- Rodgers, S.K., and E.F. Cox. 1999. Rate of spread of introduced rhodophytes *Kappaphycus alvarezii*, *Kappaphycus striatum*, and *Gracilaria salicornia* and their current distribution in Kane'ohe Bay, O'ahu, Hawai'i. *Pacific Science* 53(3):232-241.
- Rodolfi, L., G.C. Zittelli, N. Bassi, G. Padovani, N. Biondi, G. Bonini, and M.R. Tredici. 2009. Microalgae for oil: Strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. *Biotechnology and Bioengineering* 102(1):100-112.
- Roesijadi, G., S.B. Jones, L.J. Snowden-Swan, and Y. Zhu. 2010. *Macroalgae as a Biomass Feedstock: A Preliminary Analysis*. Richland, WA: Pacific Northwest National Laboratory.
- Roessler, P. 2011. *Algal Strain Selection and Development for Biofuel Production*. Presentation to the NRC Committee on Sustainable Development of Algal Biofuels on September 8.
- RSB (Roundtable on Sustainable Biofuels). 2011. Principles and criteria. Available online at <http://rsb.epfl.ch/page-24929.html>. Accessed July 18, 2011.
- Ruenwai, R., S. Cheevadhanarak, and K. Laoteng. 2009. Overexpression of acetyl-CoA carboxylase gene of *Mucor rouxii* enhanced fatty acid content in *Hansenula polymorpha*. *Molecular Biotechnology* 42(3):327-332.
- Ruffing, A.M. 2011. Engineered cyanobacteria: Teaching an old bug new tricks. *Bioengineered Bugs* 2(3):136-149.
- Russell, D.J. 1983. Ecology of the imported red seaweed *Euclima striatum* Schmitz on Coconut Island, Oahu, Hawaii. *Pacific Science* 37(2):87-107.
- Scott, S.A., M.P. Davey, J.S. Dennis, I. Horst, C.J. Howe, D.J. Lea-Smith, and A.G. Smith. 2010. Biodiesel from algae: Challenges and prospects. *Current Opinion in Biotechnology* 21(3):277-286.
- Sharif, D.I., J. Gallon, C.J. Smith, and E. Dudley. 2008. Quorum sensing in cyanobacteria: N-octanoyl-homoserine lactone release and response, by the epilithic colonial cyanobacterium *Gloethece PCC6909*. *ISME Journal* 2(12):1171-1182.
- Sheehan, J., T. Dunahay, J. Benemann, and P. Roessler. 1998. *A Look Back at the U.S. Department of Energy's Aquatic Species Program: Biodiesel from Algae*. Golden, CO: National Renewable Energy Laboratory.
- Shen, Y., W. Yuan, Z.J. Pei, Q. Wu, and E. Mao. 2009. Microalgae mass production methods. *Transactions of the ASABE* 52(4):1275-1287.
- Sierra, E., F.G. Acien, J.M. Fernandez, J.L. Garcia, C. Gonzalez, and E. Molina. 2008. Characterization of a flat plate photobioreactor for the production of microalgae. *Chemical Engineering Journal* 138(1-3):136-147.
- Smith, A.G., S.A. Scott, M.P. Davey, J.S. Dennis, I. Horst, C.J. Howe, and D.J. Lea-Smith. 2010a. Biodiesel from algae: Challenges and prospects. *Current Opinion in Biotechnology* 21(3):277-286.
- Smith, V.H., B.L. Foster, J.P. Grover, R.D. Holt, M.A. Leibold, and F. deNoyelles, Jr. 2005. Phytoplankton species richness scales consistently from laboratory microcosms to the world's oceans. *Proceedings of the National Academy of Sciences of the United States of America* (102):4393-4396.
- Smith, V.H., B.S.M. Sturm, F.J. deNoyelles, and S.A. Billings. 2010b. The ecology of algal biodiesel production. *Trends in Ecology and Evolution* 25:301-309.
- Solazyme. 2012. Homepage. Available online at www.solazyme.com. Accessed February 9, 2012.

- Solix Biofuels. 2011. The Lumian™ AGS4000: A high productivity algae growth system. Available online at <http://www.solixbiofuels.com/content/products/lumian-ags4000>. Accessed February 9, 2012.
- Solvent Rescue Limited. 2012. Homepage. Available online at <http://solventrescue.co.nz/>. Accessed June 16, 2012.
- Spirulinasource. 1999. Yaeyama farm in Southern Japan grows chlorella in circular ponds. New Ambadi farm in India grows spirulina in raceway style ponds. Available online at <http://www.spirulinasource.com/earth-foodch6c.html>. Accessed May 15, 2012.
- Spolaore, P., C. Joannis-Cassan, E. Duran, and A. Isambert. 2006. Commercial applications of microalgae. *Journal of Bioscience and Bioengineering* 101(2):87-96.
- Stephens, E., I.L. Ross, J.H. Mussgnug, L.D. Wagner, M.A. Borowitzka, C. Posten, O. Kruse, and B. Hankamer. 2010. Future prospects of microalgal biofuel production systems. *Trends in Plant Science* 15(10):554-564.
- Stockenreiter, M., A.K. Grabe, H. Haupt, and H. Stibor. 2012. The effect of species diversity on lipid production by micro-algal communities. *Journal of Applied Phycology* 24(1):45-54.
- Stomp, M., J. Huisman, L.J. Stal, and H.C.P. Matthijs. 2007. Colorful niches of phototrophic microorganisms shaped by vibrations of the water molecule. *ISME Journal* 1(4):271-282.
- Sukenik, A., and G. Shelef. 1984. Algal autoflocculation—Verification and proposed mechanism. *Biotechnology and Bioengineering* 26(2):142-147.
- Suzuki, L., and C.H. Johnson. 2001. Algae know the time of day: Circadian and photoperiodic programs. *Journal of Phycology* 37(6):933-942.
- Tang, E.P.Y., W.F. Vincent, D. Proulx, P. Lessard, and J. de la Noue. 1997. Polar cyanobacteria versus green algae for tertiary waste-water treatment in cool climates. *Journal of Applied Phycology* 9(4):371-381.
- Taton, A., E. Lis, D.M. Adin, G. Dong, S. Cookson, S.A. Kay, S.S. Golden, and J.W. Golden. 2012. Gene transfer in *Leptolyngbya* sp. Strain BL0902, a cyanobacterium suitable for production of biomass and bioproducts. *Plos One* 7(1):e30901.
- Teplitski, M., H. Chen, S. Rajamani, M. Gao, M. Merighi, R.T. Sayre, J.B. Robinson, B.G. Rolfe, and W.D. Bauer. 2004. *Chlamydomonas reinhardtii* secretes compounds that mimic bacterial signals and interfere with quorum sensing regulation in bacteria. *Plant Physiology* 134(1):137-146.
- Thelen, J.J., and J.B. Ohlrogge. 2002. Metabolic engineering of fatty acid biosynthesis in plants. *Metabolic Engineering* 4:12-21.
- Tillmann, U. 2004. Interactions between planktonic microalgae and protozoan grazers. *Journal of Eukaryotic Microbiology* 51(2):156-168.
- Toyomizu, M., K. Suzuki, Y. Kawata, H. Kojima, and Y. Akiba. 2001. Effective transformation of the cyanobacterium *Spirulina platensis* using electroporation. *Journal of Applied Phycology* 13(3):209-214.
- Trent, J. 2011. Offshore Membrane Enclosure for Growing Algae. Presentation to the NRC Committee on Sustainable Development of Algal Biofuels on September 8.
- Tsukuda, O., T. Kawahara, and S. Miyachi. 1977. Mass culture of *Chlorella* in Asian countries. Pp. 363-365 in *Biological Solar Energy Conversion*, A. Mitsui, S. Miyachi, A. San Pietro and S. Tamura, eds. New York: Academic Press.
- Turchetto-Zolet, A.C., F.S. Maraschin, G.L. de Morais, A. Cagliari, C.M.B. Andrade, M. Margis-Pinheiro, and R. Margis. 2011. Evolutionary view of acyl-CoA diacylglycerol acyltransferase (DGAT), a key enzyme in neutral lipid biosynthesis. *BMC Evolutionary Biology* 11:263.
- Uduman, N., Y. Qi, M.K. Danquah, G.M. Forde, and A. Hoadley. 2010. Dewatering of microalgal cultures: A major bottleneck to algae-based fuels. *Journal of Renewable and Sustainable Energy* 2(1): ISSN (Online) 1941-7012.
- Ugwu, C.U., H. Aoyagi, and H. Uchiyama. 2007. Photobioreactors for mass cultivation of algae. *Bioresource Technology* 99:4021-4028.
- Ugwu, C.U., J.C. Ogonna, and H. Tanaka. 2002. Improvement of mass transfer characteristics and productivities of inclined tubular photobioreactors by installation of internal static mixers. *Applied Microbiology and Biotechnology* 58(5):600-607.
- UTEX (The Culture Collection of Algae at the University of Texas at Austin). 2012. Cryopreservation of algae. Available online at <http://web.biosci.utexas.edu/utex/protocols.aspx>. Accessed June 16, 2012.
- Van den Hoek, C., D.G. Mann, and H.M. Jahns. 1995. *Algae: An Introduction to Phycology*. Cambridge, UK: Cambridge University Press.
- Vandamme, D., S.C.V. Pontes, K. Goiris, I. Foubert, L.J.J. Pinoy, and K. Muylaert. 2011. Evaluation of electrocoagulation-flocculation for harvesting marine and freshwater microalgae. *Biotechnology and Bioengineering* 108(10):2320-2329.
- Vasquez, V., and P. Heussler. 1985. Carbon dioxide balance in open air mass culture of algae. *Archiv für Hydrobiologie, Ergebnisse der Limnologie, Beiheft* 20:95-113.

- Viswanathan, T., S. Mani, K.C. Das, S. Chinnasamy, and A. Bhatnagar. 2011. Drying characteristics of a microalgae consortium developed for biofuels production. *Transactions of the ASABE* 54(6):2245-2252.
- Vunjak-Novakovic, G., Y. Kim, X. Wu, I. Berzin, and J.C. Merchuk. 2005. Air-lift bioreactors for algal growth on flue gas: Mathematical modeling and pilot-plant studies. *Industrial and Engineering Chemistry Research* 44(16):6154-6163.
- Waaland, J.R., J.W. Stiller, and D.P. Cheney. 2004. Macroalgal candidates for genomics. *Journal of Phycology* 40(1):26-33.
- Waditee, R., T. Hibino, T. Nakamura, A. Incharoensakdi, and T. Takabe. 2002. Overexpression of a Na⁺/H⁺ antiporter confers salt tolerance on a freshwater cyanobacterium, making it capable of growth in sea water. *Proceedings of the National Academy of Sciences of the United States of America* 99(6):4109-4114.
- Wang, X., X. Liu, and G. Wang. 2011. Two-stage hydrolysis of invasive algal feedstock for ethanol fermentation. *Journal of Integrative Plant Biology* 53(3):246-252.
- Wang, Z.T., N. Ullrich, S. Joo, S. Waffenschmidt, and U. Goodenough. 2009. Algal lipid bodies: stress induction, purification, and biochemical characterization in wild-type and starchless *Chlamydomonas reinhardtii*. *Eukaryotic Cell* 8(12):1856-1868.
- Wargacki, A.J., E. Leonard, M.N. Win, D.D. Regitsky, C.N.S. Santos, P.B. Kim, S.R. Cooper, R.M. Raisner, A. Herman, A.B. Sivitz, A. Lakshmanaswamy, Y. Kashiyama, D. Baker, and Y. Yoshikuni. 2012. An engineered microbial platform for direct biofuel production from brown macroalgae. *Science* 335:308-313.
- Weckwerth, W. 2011. Green systems biology—From single genomes, proteomes and metabolomes to ecosystems research and biotechnology. *Journal of Proteomics* 75(1):284-305.
- Weis, J.J., D.S. Madrigal, and B.J. Cardinale. 2008. Effects of algal diversity on the production of biomass in homogeneous and heterogeneous nutrient environments: A microcosm experiment. *Plos One* 3(7):e2825.
- Weissman, J.C., D.T. Tillett, and R.P. Goebel. 1989. Design and Operation of an Outdoor Microalgae Test Facility. Golden, CO: Solar Energy Research Institute.
- Wijffels, R.H., and M.J. Barbosa. 2010. An outlook on microalgal biofuels. *Science* 329(5993):796-799.
- Williams, P.J.L.B., and L.M.L. Laurens. 2010. Microalgae as biodiesel and biomass feedstocks: Review and analysis of the biochemistry, energetics and economics. *Energy and Environmental Science* 3(5):554-590.
- Woo, M., C. Smith, and W. Smith. 2000. Ecological interactions and impacts of invasive *Kappaphycus striatum* in Kane'ohe Bay, a tropical reef. Pp. 186-192 in *Marine Bioinvasions*, J. Pedersen, ed. Cambridge, MA: Massachusetts Institute of Technology, Sea Grant College Program.
- Wright, M.M., D.E. Daugaard, J.A. Satrio, and R.C. Brown. 2010. Techno-economic analysis of biomass fast pyrolysis to transportation fuels. *Fuel* 89:S11-S19.
- Xtrudx Technologies, Inc. 2012. Homeage. Available online at <http://www.xtrudx.com/about.html>. Accessed June 16, 2012.
- Yue, L., and W. Chen. 2005. Isolation and determination of cultural characteristics of a new highly CO₂ tolerant fresh water microalgae. *Energy Conversion and Management* 46(11-12):1868-1876.
- Zhao, F.Q., X.W. Zhang, C.W. Liang, J.Y. Wu, Q.Y. Bao, and S. Qin. 2006. Genome-wide analysis of restriction-modification system in unicellular and filamentous cyanobacteria. *Physiological Genomics* 24(3):181-190.
- Zheng, P., W.B. Allen, K. Roesler, M.E. Williams, S. Zhang, J. Li, K. Glassman, J. Ranch, D. Nubel, W. Solawetz, D. Bhatramakki, V. Llaca, S. Deschamps, G.Y. Zhong, M.C. Tarczynski, and B. Shen. 2008. A phenylalanine in DGAT is a key determinant of oil content and composition in maize. *Nature Genetics* 40(3):367-372.
- Zimba, P.V., M.J. Sullivan, and H.E. Glover. 1990. Carbon fixation in cultured marine benthic diatoms. *Journal of Phycology* 26(2):306-311.
- Zmora, O., Richmond, A. 2003. Microalgae production for aquaculture. Pp. 365-379 in *Handbook of Microalgal Culture, Biotechnology and Applied Phycology*. A. Richmond, ed. Boston, MA: Blackwell Publishing.
- Zou, J.T., V. Katavic, E.M. Giblin, D.L. Barton, S.L. MacKenzie, W.A. Keller, X. Hu, and D.C. Taylor. 1997. Modification of seed oil content and acyl composition in the brassicaceae by expression of a yeast sn-2 acyltransferase gene. *Plant Cell* 9(6):909-923.

3

Pathways for Algal Biofuel Production



The set of processes that lead from algae cultivation to collection and harvest, and finally to fuel conversion is termed the pathway for algal biofuel production. This chapter describes several contrasting pathways that lead from algae cultivation to fuel production. The pathways described here are used in subsequent chapters to provide a framework for understanding the sustainability impacts of different approaches for producing algal biofuels. The intent is to group pathways that share common processes to help contrast resource requirements and impacts of different approaches and to help clarify the key biological and engineering advances that are needed to improve sustainability.

3.1 FEATURES OF BIOFUEL PATHWAYS

Pathways for producing liquid biofuels share many common features regardless of the biomass feedstock being used. All have a cultivation step, a collection or harvest step, and a processing or finishing step. Some land-based crops are used or being considered for use as biofuel feedstock because of their ability to produce oils, their ability to produce carbohydrates that are readily converted to fuels by microbial action, or their ability to fix carbon with low input. Oil-producing crops, such as soybean, jatropha, and camelina, are harvested and the oil is separated for subsequent processing. Sugar in sugarcane and starch in corn grain can be converted efficiently to ethanol by yeast and bacteria. Dedicated energy crops, such as poplar, switchgrass, and *Miscanthus*, are selected because of their growth with low inputs of nutrients or their ability to store carbon in soil (Tilman et al., 2006; Pyter et al., 2009; NRC, 2011). The lignocellulosic biomass can be converted chemically, thermochemically, or biologically to liquid fuels (NAS-NAE-NRC, 2009). There are algal biofuel systems analogous to each of these feedstock types. Analysis of the options in the cultivation, collection, and processing of algae is complicated by the vast number and complexity of options. As discussed in more detail in Chapter 4, these options affect the resource requirements needed to produce fuels. Analyzing all possible pathways in this report is not practical. However, the pathways can be grouped by the main features they share that are affecting resource use and energy balance. A few representative pathways are analyzed to illustrate the current state of the technologies and where advances are needed to reduce the resource requirements.

Trends observed in the science and technologies for other biofuel production are likely to occur in algal biofuel production as the latter develops as an industry. These trends include improvements in biomass production and total biomass processing discussed in Chapter 2, and the increasing comparative analysis of the full life-cycle impacts and requirements for various sources of alternative liquid fuels through the use of life-cycle assessments (LCAs) discussed in Chapters 1, 4, and 5. An additional trend is the move toward drop-in fuels that are compatible with existing infrastructure for petroleum-based fuels. Ethanol and fatty-acid methyl esters (FAME; or commonly called biodiesel) have compatibility and performance issues in vehicles that hamper their adoption (NAS-NAE-NRC, 2009; NRC, 2011). Current trends are moving toward production of pure hydrocarbon fuels or blendstocks that are compatible with existing fuel infrastructure and vehicle technologies (NREL, 2006).

The production of fuels and energy from algae is not an established industry and a variety of production systems have been proposed. Figure 3-1 is a simplified diagram that attempts to limit and group the potential steps in the algal biofuel production pathway. Each row of the diagram details a processing step or process option. Different combinations of cultivation and processing options have resulted in more than 60 different proposed pathways for producing algal biofuels.

As noted in Chapter 1, this study focuses on algal production systems that rely directly on photosynthesis (see Figures 3-1 and 3-2). Heterotrophic cultivation is, by design, outside the scope of this report. The exclusion of heterotrophic production from this report is not a judgment on the validity of these approaches but a reflection of the requirement to study photosynthetic algae as a feedstock for fuel production. This report examines pathways for producing liquid transportation fuels from algae. Gaseous power generation and hydrogen production are not discussed. Proteins are considered only as coproducts.

From the perspective of this study, the large number of possible designs of an algal biofuel pathway means that a small number of the most likely designs need be chosen

Potential Variation in Each Step

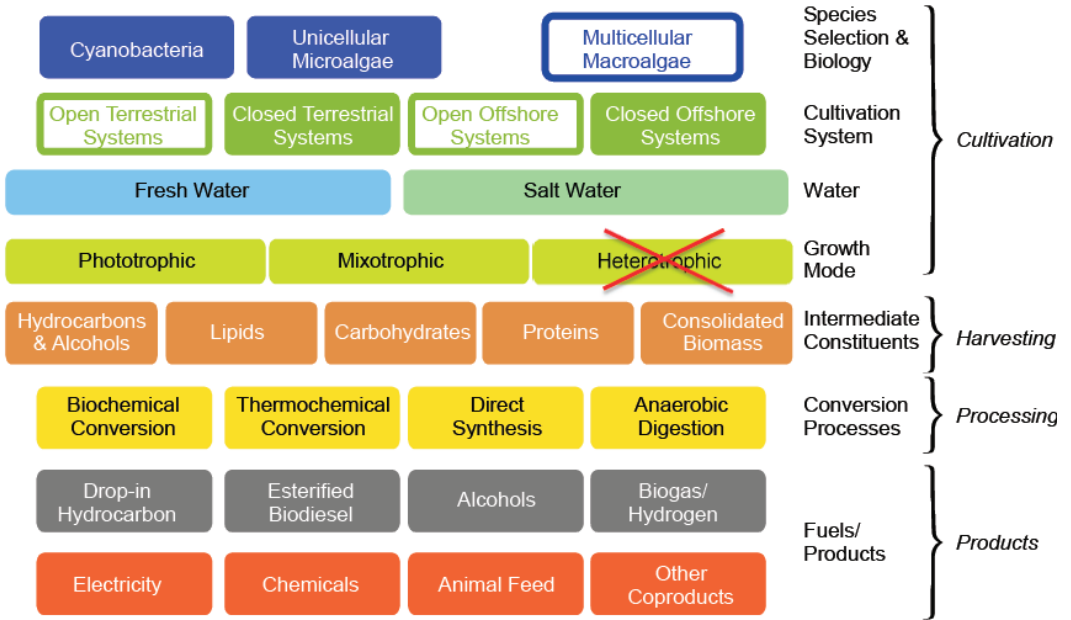


FIGURE 3-1 Pathways for cultivating and processing algae to fuels and their products. NOTE: Heterotrophic routes are outside the scope of this analysis.

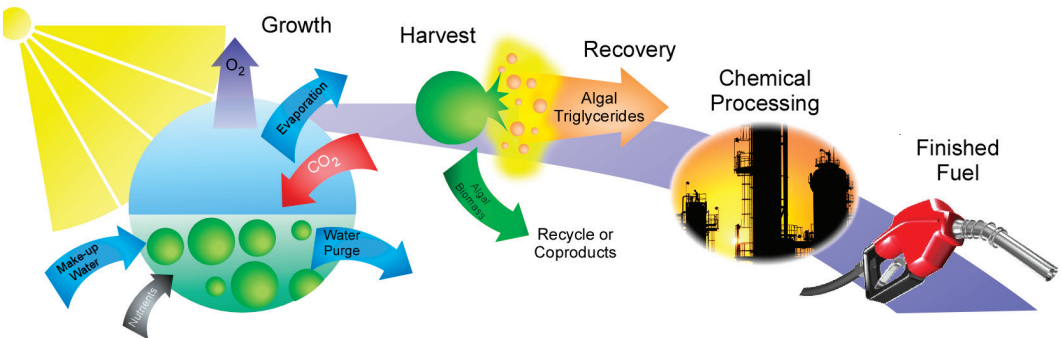


FIGURE 3-2 An example of processes involved in converting algae to fuels.

and used as a framework for the analysis of sustainability. The pathways help illustrate the resource requirements and potential impacts associated with greatly scaling up various approaches to produce algal biofuels. These pathways also allow different approaches to be compared and contrasted directionally, enabling conclusions to be drawn, pitfalls identified, and potential solutions drafted. The reference pathway is drawn from the recent National Renewable Energy Laboratory techno-economic analysis of algal biofuel production (Davis et al., 2011).

3.2 REFERENCE PATHWAY–RACEWAY POND PRODUCING DROP-IN HYDROCARBON

For the purpose of this discussion, the reference pathway assumes that microalgae are cultivated in saline water in an open raceway pond. Algae are harvested and lysed to release lipids, which are collected for further processing into green diesel¹ (also called renewable diesel), a drop-in hydrocarbon fuel (Figure 3-3).

Under the reference pathway, lipid-producing species are selected, and recovered lipids are converted by known chemical processes to yield hydrocarbon fuels. The chemical structures of these hydrocarbon fuels are oxygen-free and appropriate for use in aviation and as on-road fuels. Lipid recovery in the most often described processes requires that the cells be destroyed (lysed) and cell membranes ruptured to release intracellular oils. The algal triacylglycerol can be processed in several ways. Similarly, the remaining cellular mass can have different uses. Two of the uses being considered are recycling back to algae cultivation or selling as a coproduct. For the reference case, the biomass is treated anaerobically to produce biogas for power generation, with the effluent being returned to the culture to provide needed nutrients. According to Davis et al. (2011), this treatment recovers almost all of the biomass phosphate and much of the nitrogen (N) used during cultivation.

The reference pathway is further amplified in Figure 3-4, which shows the details of the processing steps. For this and subsequent figures, several conventions have been adopted. Yellow diamonds show the inputs to the system and orange the outputs. Green process steps represent those associated with algae cultivation, light blue with lipid collection, and

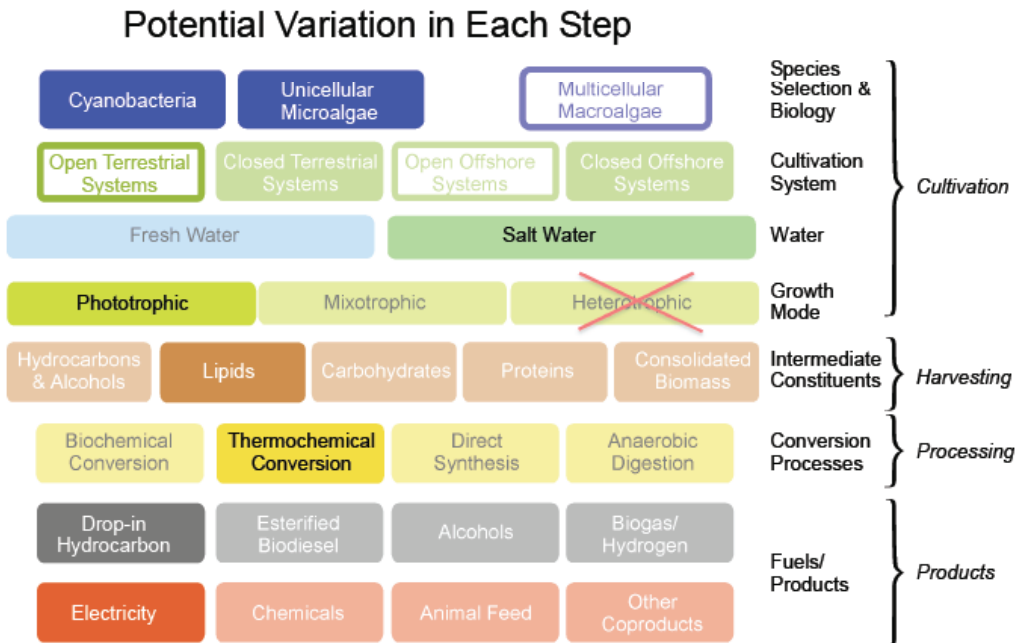


FIGURE 3-3 Reference pathway: Open raceway pond producing green diesel, a drop-in hydrocarbon fuel.

¹Green diesel is a product of hydrotreating of triglycerides.

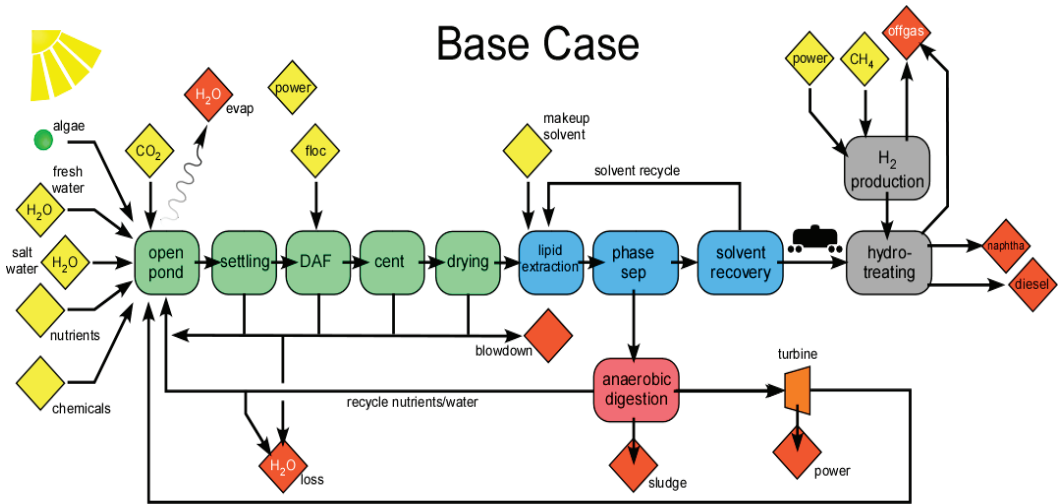


FIGURE 3-4 Inputs and outputs of the reference pathway.

NOTES: Reference pathway uses open raceway pond to produce algae for processing to green diesel. Tankcar symbol reflects only the option for separating the lipid production remote to the fuel processing. DAF is dissolved air flotation, cent is centrifugation.

grey with chemical processing. Algae are harvested and ruptured during the oil extraction, resulting in an oil and a lipid-extracted biomass. The reference pathway assumes that the algal biomass is first recovered by flocculation by adding a chemical agent such as chitosan (Davis et al., 2011). Material is recovered by dissolved air flotation (DAF). In DAF, air is introduced to lift the algae to the surface where it can easily be recovered. DAF increases the algal biomass to liquid ratio, but centrifugation is still required to reduce water content for subsequent oil recovery. The lipid-extracted biomass is digested anaerobically to produce high-quality energy, and its nutrient content is returned to the algal culture. The hydrotreating of algal lipids to produce pure hydrocarbon fuels is analogous to second generation biodiesel production, which is based on seed crops to produce green diesel (NREL, 2006). This pathway represents what the committee believes to be the one of the most probable pathways for producing drop-in fuels from algae based on the current state-of-the-art technologies for cultivation and processing.

Hydrotreating is used to convert raw triacylglycerol into a drop-in hydrocarbon fuel (Olusola et al., 2009; Serrano-Ruiz et al., 2012). Hydrotreating is a traditional refinery operation that serves to remove heteroatoms from incoming fuels, hydrogenate olefinic species to alkanes, and, potentially, to do some chain cleavage. Its main function with regard to lipids is to remove oxygen from the feedstock, thereby making alkanes out of the lipid chains, propane out of the glycerol backbone, and water out of all oxygen molecules (Davis et al., 2011). Processing algal lipids by hydrotreating offers several advantages. First, a drop-in replacement fuel is produced. Drop-in hydrocarbon fuels are increasingly desired, and their production is accomplished by collecting algal triacylglycerol as an intermediate product. This intermediate product can be shipped efficiently to refineries for inclusion in the conventional fuel pool. Triacylglycerol also can be processed near the cultivation facility. There are scale advantages in the hydrotreating that might favor transportation to a larger facility fed by many algae farms. Among them is the requirement for hydrogen. Supplying

2011). The high content of neutral oils also may eliminate the need for solvent extraction. These types of innovations are important for energy balance.

Phosphorus (P) within the organism in the form of phospholipids presents additional complexity in processing algae into fuels. These molecules are detrimental to downstream processing and in end-use can inhibit or poison catalysts used in fuel conversion and can damage vehicle catalytic converters (Fan et al., 2010). ASTM D6751-11b, which is the standard specification for biodiesel fuel blend stock for middle distillate fuels, specifies maximum P content allowable in biodiesel blend stock. P compounds are removed using the degumming technology developed for use with seed oils (Lurgi GmbH; AlfaLaval, 2010). Most commonly, phospholipids are converted to immiscible solids that are then removed by centrifugation. Methods of extracting phospholipids include water, acid, or enzymatic degumming. The most commonly used acid is phosphoric acid. Any of these methods produces a crude gum stream for disposal or alternative use. Acid and enzymatic degumming, while truly catalytic, require acid and enzyme additions.

3.3 ALTERNATIVE PATHWAY #1—RACEWAY POND PRODUCING DROP-IN HYDROCARBON AND COPRODUCTS

The next pathway, illustrated in Figures 3-6 and 3-7, assumes that the algal biomass has sufficient value so that it provides a significant revenue stream. This pathway assumes that the biomass has value in the unprocessed and dewatered state, and that subsequent processing to recover minor valuable components is not done.

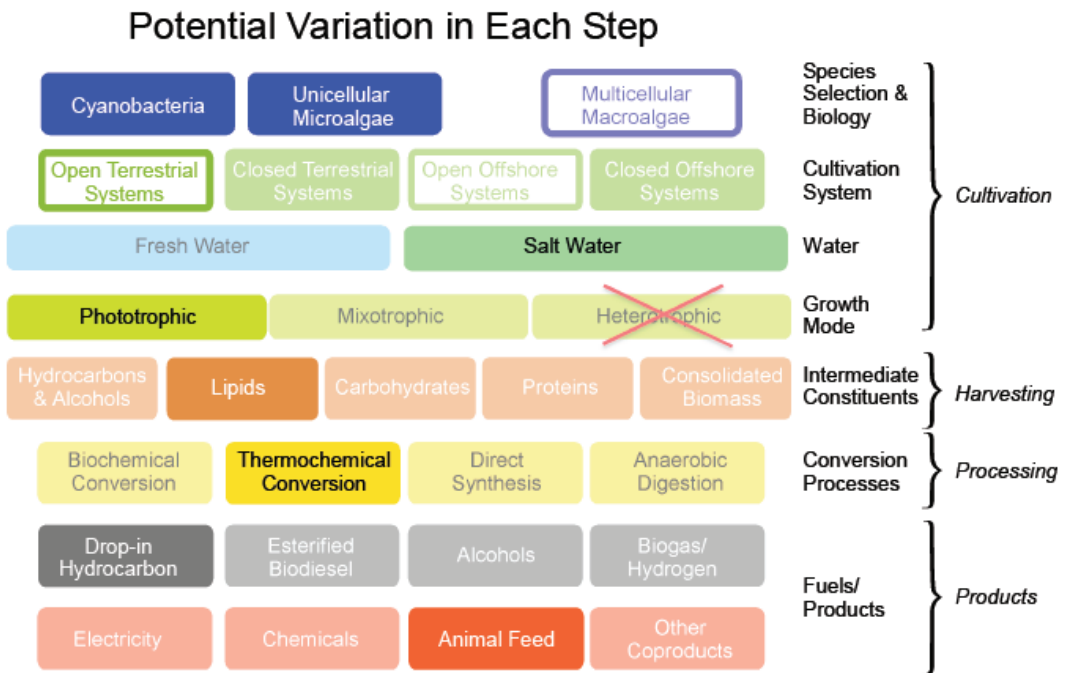


FIGURE 3-6 Alternative pathway #1: Open raceway pond producing green diesel, a pure hydrocarbon fuel with coproduct for sale.

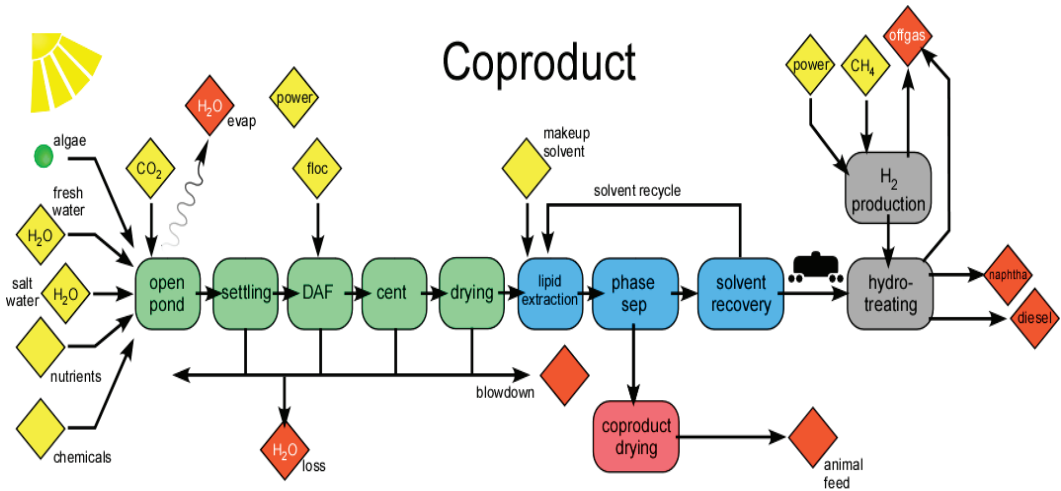


FIGURE 3-7 Inputs and outputs of the alternative pathway #1: Open raceway pond producing green diesel with fuel and animal feedstuff as coproducts.

This pathway results in the increased nutrient requirements for algae cultivation compared to the reference pathway because of the loss of biomass nutrients from direct coproduct sales. The scale of biofuel production has a large impact on the volume, and therefore the value, of coproduct streams. The committee believed that coproducing high-value products, such as chemical feedstocks, with biofuels would be viable only on a small scale. If large quantities of high-value algal products are coproduced with biofuels, the coproduct value likely decreases with market saturation. A coproduct that is likely to have a large enough market to absorb the large quantities produced is animal feedstuff. The coproduct value depends on the composition of the animal feedstuff and the characteristics of the market in which it would be sold.

Coproducing algal biofuels and high-value products has been suggested as a strategy to address the challenge of making algal biofuels economically viable. The strategy has proven to be contentious at several levels. Coproducts are strongly linked to the economics and LCAs of algal biofuel production. The economics of algal biofuel production are outside the scope of this analysis, but are a key reason for the importance of coproducts. Coproducts are proposed as a means to improve the economics of algal biofuel production. Economic benefit comes at a cost, however, and a simple analysis is presented to explain the impacts and potential concerns. First, some general comments can be made based on published works and presentations to the committee. Perhaps the strongest statements heard involved the strategy of LiveFuels, Inc. in testimony before the committee (Morgenthaler-Jones, 2011). The presenter believed that the economics of producing algal biofuels at a cost that is competitive with fossil fuels is impossible. The company's focus moved to fish production with a coproduct oil outlet. The majority of the revenue is now envisioned to come from fish, and all oils produced (fish oil for human or animal nutritional supplements and biofuels for transportation) are sold. Others have taken a more measured approach and have claimed that coproducts could contribute to the profitability of algal biofuels while their market develops, and the cost of algal biofuel production would decrease with efficiency improvements and economies of scale.

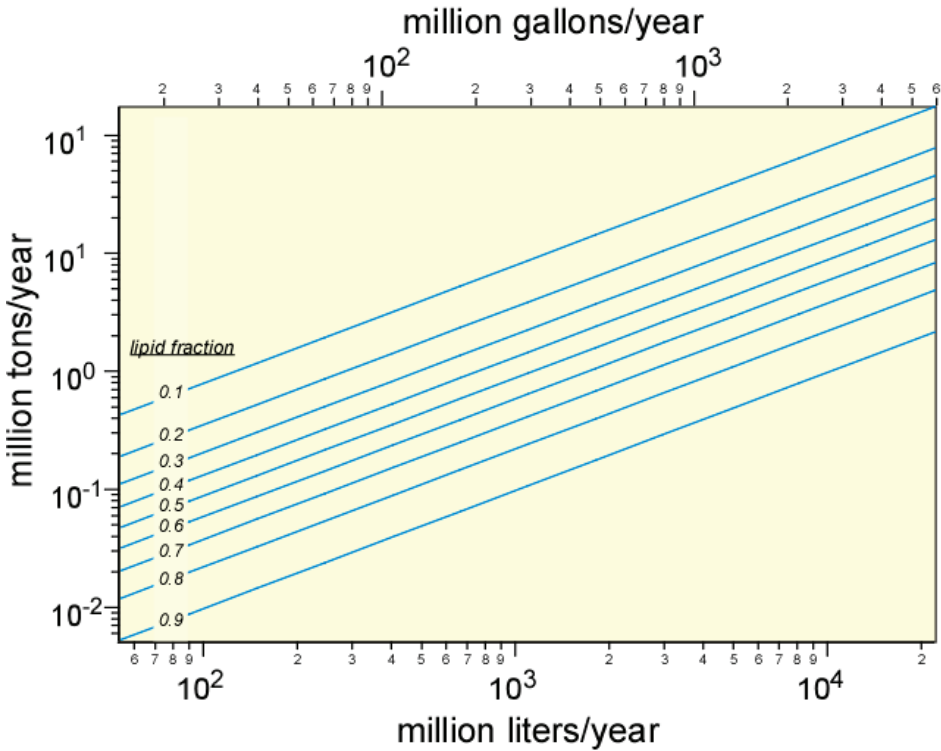


FIGURE 3-8 Annual residual biomass production, in million tonnes per year, shown as a function of annual fuel production and lipid fraction.
 NOTES: A single algal biofuel refinery is likely to have a capacity of 90 million liters per year. Current algae are cultivated in the 20 to 30-percent lipid mass fraction range. Five percent of annual U.S. consumption of transportation fuels is about 39 billion liters.

The amount of residual biomass increases quickly as the scale of biofuels increases. Residual biomass is a function of the amount of fuel produced and the fraction of the total dry biomass. Plotting residual biomass as a function of both lipid fraction and annual fuel production indicates the magnitude of the issue (Figure 3-8). An algal biofuel refinery sized equally to a typical 95 million liter (25 million gallon) transesterification biodiesel refinery yields more than 180 thousand tonnes of residual biomass at a 30 percent lipid fraction. If an industry capable of supplying 3.8 billion liters (1 billion gallons) was built, which is still only one-tenth of what the fuel ethanol is in the United States today, the residual biomass would reach 7.7 million tonnes. As discussed with respect to the reference pathway, this residual biomass can be used in anaerobic digestion to produce power, but some sludge would remain and require disposal. (Waste management is discussed in Chapter 5.) The alternative pathway #1 considers using this residual to produce coproducts such as animal feedstuff.

Although coproduction of fuel and other products can improve the economics of algal biofuels, it has limited potential and cannot be the single remedy to improving the economic viability of widespread and large-scale deployment of algal biofuels. (See Appendix G for details.) Markets tend to correlate scale and price of sale, which is the cost of production

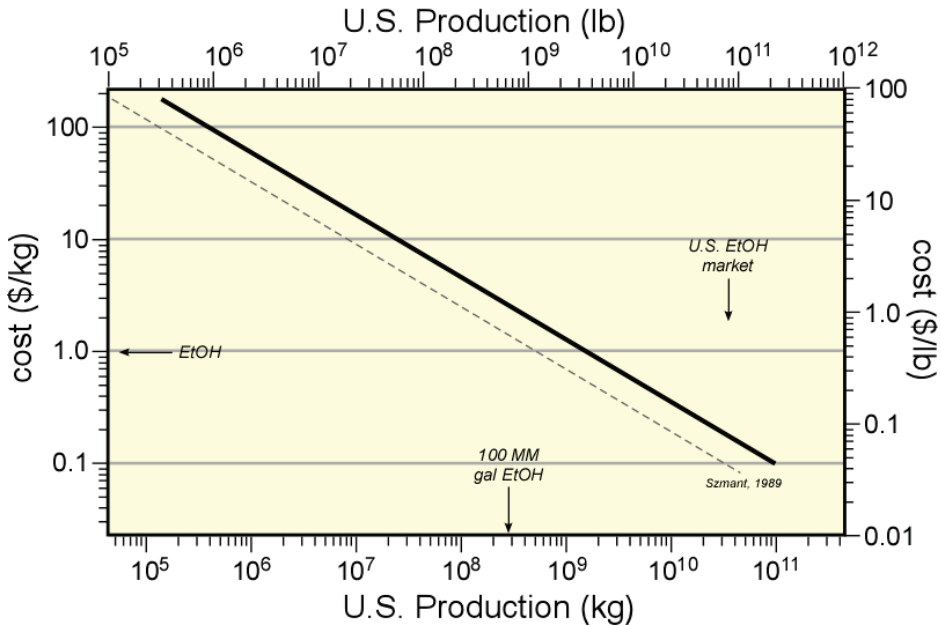


FIGURE 3-9 Chart showing the general power law dependence of a materials cost with production scale.

NOTES: As scale increases, price generally decreases. This is true both for fuel components and coproducts. The dotted line shows Szmant's original curve and the solid line is inflation corrected to 2010.

SOURCE: Adapted from Szmant (1989).

plus return on capital. This is frequently overlooked as coproducts are touted as a significant source of additional revenues for an economically suspect fuel production process. The correlation is somewhat poor across different products, but for a single product, scale and price are related by a power law (Figure 3-9). This means that doubling scale reduces price more than double. For materials intended to be sold into the massive fuels market, coproduct volumes swell rapidly with the scaling of fuel production unless a wide variety of coproducts for different markets are produced. From a resource sustainability perspective, the reference pathway described earlier closely represents the economic analyses and LCAs that have been completed. The use of anaerobic digestion to return nutrients to the algae cultivation and electrical power to the algal biofuel production system is a key component of alternative pathway #1. Removing the residual biomass as a coproduct, therefore, affects the energy balance of fuel production and the required nutrient load.

3.4 ALTERNATIVE PATHWAY #2—RACEWAY POND PRODUCING FAME

Most of the reports on algal biofuels assume that FAME is produced. FAME is not a hydrocarbon fuel, but an ester made by transesterification of the triacylglycerol. This pathway most closely approximates conventional biodiesel in the way that crude bio-oil is converted to a transportation fuel (Figure 3-10). Algal triacylglycerol are reacted with methanol to form FAME or so-called biodiesel (Figure 3-11; Van Gerpen, 2005). FAME

Potential Variation in Each Step

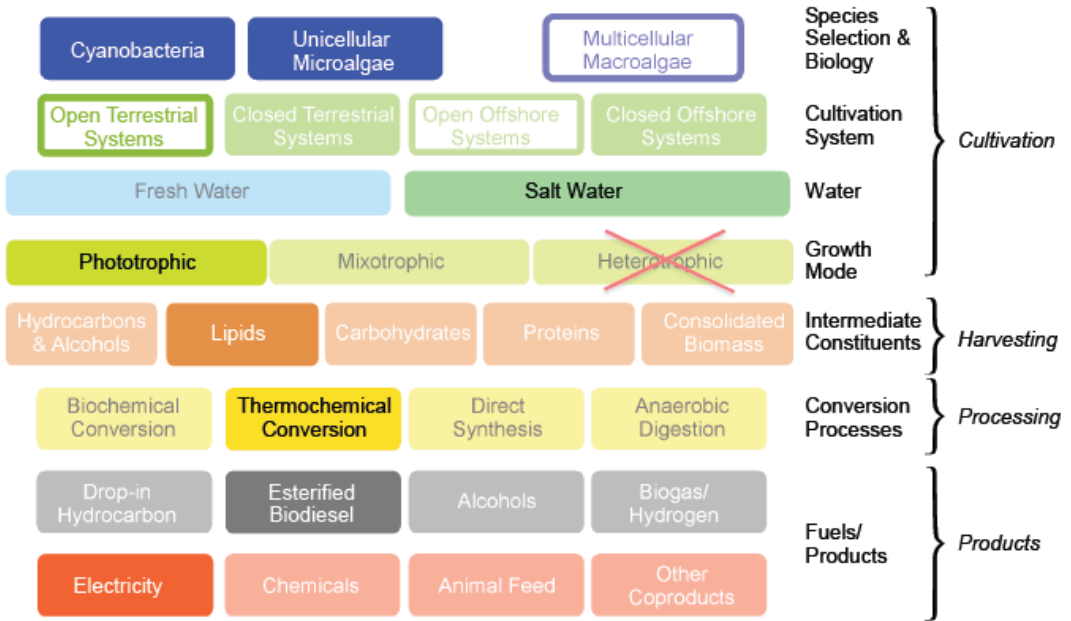


FIGURE 3-10 Alternative pathway #2: Open raceway pond producing esterified biodiesel, also known as fatty-acid methyl esters (FAME).

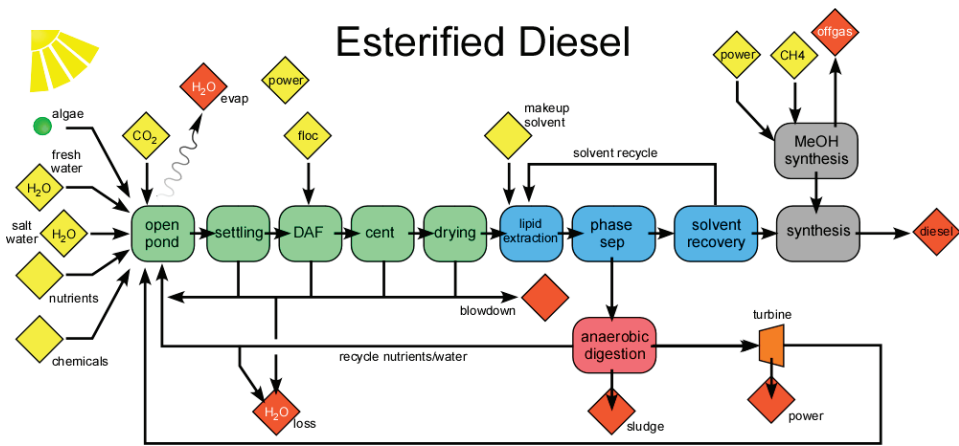


FIGURE 3-11 Inputs and outputs of the alternative pathway #2: Open raceway pond producing a fatty-acid methyl ester (FAME).

has poor cold-flow properties and cannot be used as pure components in cold environments. The increased viscosity relative to hydrocarbon diesel makes FAME difficult to pump. Even in mixtures, cloud point issues can occur when wax crystals begin to form. The wax crystals can lead to gel formation, which is incompatible with engine operation.

Biodiesel-containing mixtures have higher cloud points and pour points (the temperature at which the fuel has gelled so it no longer flows) than pure hydrocarbon diesels. Therefore, biodiesel usually is blended with petroleum-based diesel for final use. Because of its higher oxygen content, FAME has 10 percent lower energy content than hydrocarbon diesel; hence, it has reduced vehicle mileage per gallon in use. FAME biodegrades with long-term storage because of its chemical activity, and exposure to air and water accelerates the degradation (NRC, 2011). However, FAME can be made with relative efficiency at small scales so that algal processing and finished fuel production can occur at the same site. It also has low sulfur content and aromatics, and therefore results in low particulate emissions when the fuel is combusted. Coproduct glycerol also is produced in this pathway, but glycerol has a low market value.

3.5 ALTERNATIVE PATHWAY #3–PHOTOBIOREACTORS WITH DIRECT SYNTHESIS OF ETHANOL

Previously described processes for algal biofuel production have focused on open-pond systems for algae cultivation, and most analyses indicate that photobioreactor systems are cost prohibitive for the production of fuels (Williams and Laurens, 2010). At present, photobioreactor systems are used to produce algal biomass for high-value products, such as nutraceuticals and cosmetic ingredients (BioProcess Algae, 2011; Boussiba, 2011; Photon8, 2011; Thomas, 2011). The next pathway described assumes that a marine species of algae or cyanobacteria directly produce a valuable fuel product (Figure 3-12). Direct synthesis of fuel components virtually requires that the algae or cyanobacteria be cultivated in

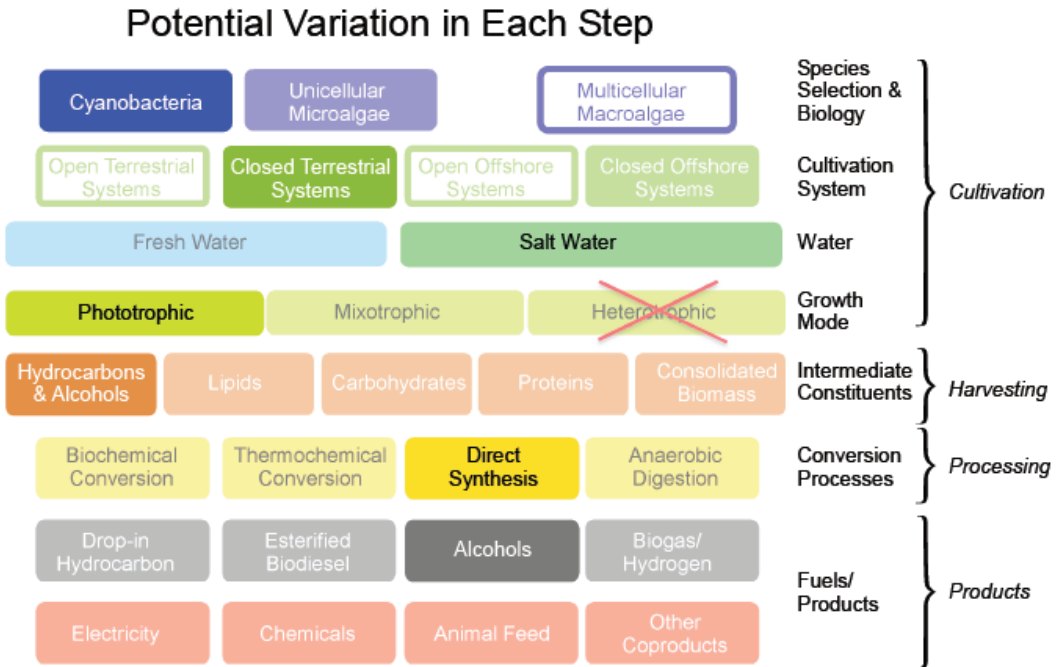


FIGURE 3-12 The Algenol direct synthesis pathway uses a closed reactor with an organism that directly produces alcohols.

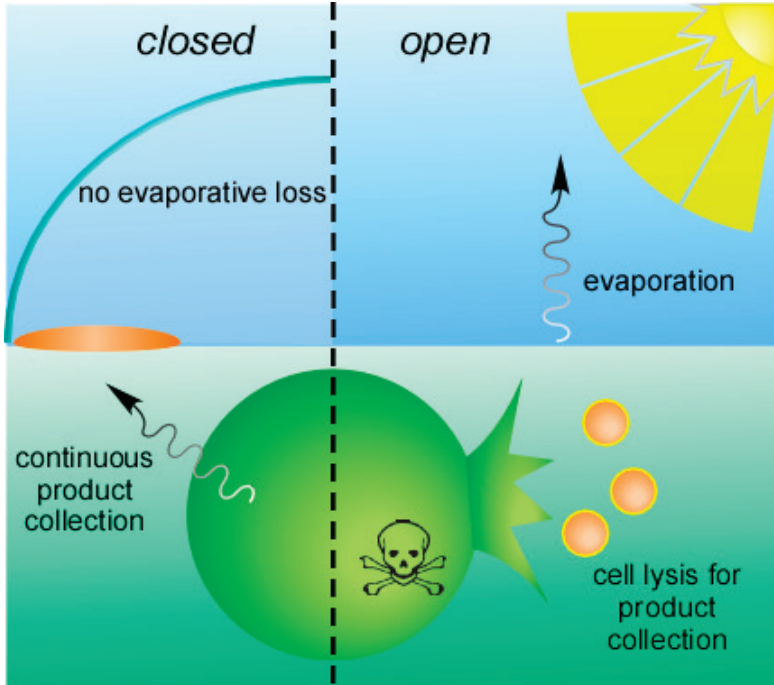


FIGURE 3-13 An illustration of the key differences between direct synthesis and the more common practice of cell lysis for lipid collection and why closed systems are required for continuous collection of volatile products.

a closed photobioreactor to prevent product degradation. This option differs dramatically from the production of lipids that require rupture of the cell membrane to harvest. Harvest is continual, with the organism releasing product into the media continuously. The algal or cyanobacteria culture can be stable for months. This pathway cannot be carried out in an open pond because the rate of fuel synthesis is believed to be about the same as the rate of microbial degradation. In addition, volatile products would be lost to evaporation (Figures 3-13 and 3-14).

The alternative pathway #3 is effectively the Algenol process, as it was described to the committee (Luo et al., 2010; Chance et al., 2011). Other companies known to be pursuing direct production are Joule Unlimited and Synthetic Genomics, Inc. Publications from Joule indicate that pure hydrocarbons are the company's preferred target product (Robertson et al., 2011; Joule Unlimited, 2012).

The Algenol process uses a marine species of cyanobacteria to directly produce ethanol (see also Figure 2-12 in Chapter 2). Algenol reactors are polyethylene bags. The cyanobacteria release ethanol into the supporting media, which then partitions between the liquid and photobioreactor headspace. Ethanol produced is trapped in the closed photobioreactor. Solar energy penetrating the bag forms a kind of "solar still." Primary collection of dilute ethanol is mostly solar driven, but subsequent purification steps require input of fossil-derived energy to produce an alcohol fuel product that meets fuel specifications.

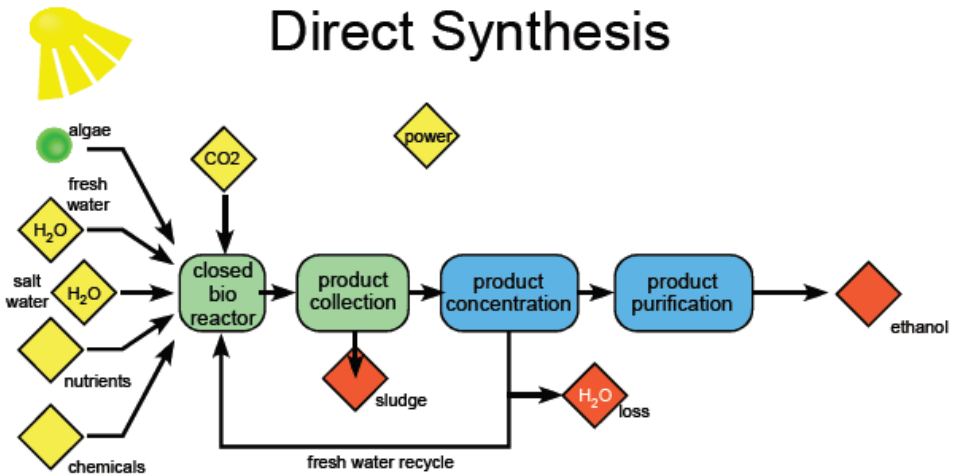


FIGURE 3-14 Inputs and outputs of the alternative pathway #3: Photobioreactors with direct synthesis of ethanol.

Algenol measured the concentration in the ethanol condensate in the photobioreactor and found that to be 0.5 to 2.0 percent (Chance et al., 2011). The energy required to recover the alcohol as useable fuel largely determines the energy return and, hence, the GHG emissions for the process. Figure 3-15 shows the impact of the ethanol concentration on the energy consumption of the entire process. A combination of separation methods (for example, vapor compression stream stripping, molecular sieve, vapor compression distillation, distillation, and membrane) is needed for ethanol separation. The energy balance results are generally favorable; that is, more energy is retained in the fuel than is required to make it (Luo et al., 2010). The energy return on investment of this pathway reported to the committee by a representative of Algenol is within the range of 1.2 widely reported for corn-grain ethanol and 8 reported for ethanol from sugarcane (Chance et al., 2011). However, these results and those for other algal biofuels systems were not obtained from fully scaled-up demonstration facilities.

Comparison of the Algenol results to other studies on algal biofuels is favorable in terms of energy and other resource requirements. Eliminating the need for dewatering reduces energy requirements and is a clear advantage of processes that directly produce fuel. Also inherent to a closed photobioreactor system are the advantages of lower water and nutrient consumption and reduced risk of contamination. The capital cost is a frequently cited concern for biofuel-producing closed photobioreactor systems (Benemann, 2008; Williams and Laurens, 2010), but it is not addressed in this report.

Water use can be significantly reduced in a closed bioreactor, but cannot be fully eliminated. Water is lost to photosynthesis and in processing. Irrespective of whether marine or freshwater algae are used, fresh water addition or water purge is required to maintain water level and key concentrations, such as salinity. Photosynthesis consumes water:



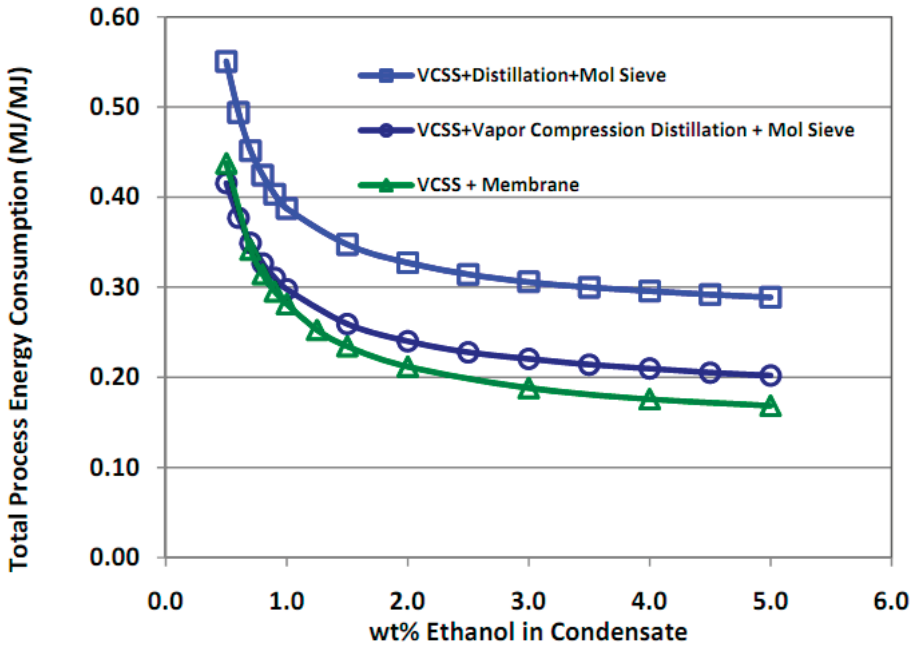


FIGURE 3-15 Process energy requirements as a function of the alcohol concentration in the condensate.

NOTE: The energy requirement is affected by the choice of subsequent purification technology. Vapor compression steam stripping (VCSS), molecular sieve (Mol Sieve), and vapor compression distillation are methods for ethanol separation.

SOURCE: Chance et al. (2011). Reprinted with permission from Ron Chance, Algenol Biofuels.

For every carbon fixed, at least one water molecule is reacted (Eq. 3-1). Even in the case of no evaporative loss and full recycle of process water, water is consumed because of photosynthesis. This amount can be estimated from the stoichiometry of photosynthesis and fuel production. The limiting cases for a closed system are shown in Figure 3-16.

The water consumed during photosynthesis depends on the apportioning of the fixed carbons in biomass or fuel component. In the case of the Algenol design referenced here, all the fixed carbons are effectively in the fuel component, which is ethanol that has a gram atomic weight of 46 grams/mole. The carbon average molecular weight is 46/2 or 23 grams/mole. The carbon average molecular formula for biomass is CH₂O, with a carbon average molecular weight of 30 grams/mole. Lipids have a carbon average molecular weight of approximately 16 grams/mole. The equation for the amount of water lost to photosynthesis per liter of fuel produced is:

$$\frac{L_{H_2O}}{L_{fuel}} = \frac{GFW_{H_2O} \times \rho_{fuel}}{GFW_{fuel}^* \times GFW_{biomass}^*} \left(\frac{100 \times GFW_{fuel}^*}{\%_{fuel}} + GFW_{biomass}^* \times GFW_{fuel}^* \right) \quad (\text{Eq. 3-2})$$

where the GFW* are the carbon average molecular weight of the fuel component or residual biomass (Eq. 3-2). Figure 3-17 shows the water to fuel volume ratio as a function of the

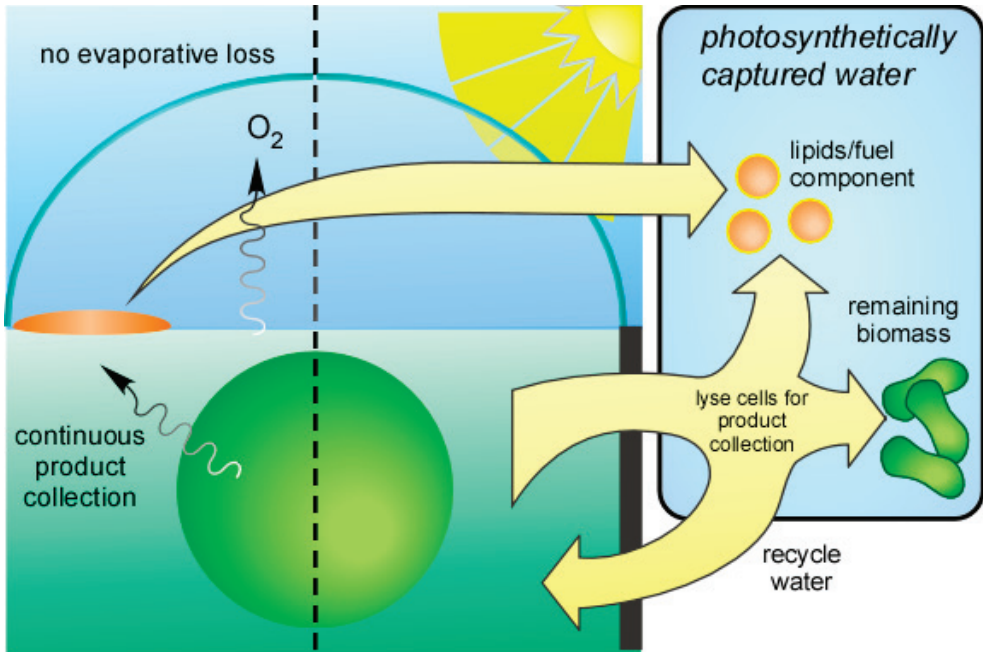


FIGURE 3-16 Water consumption by photosynthesis in the production of algal biomass for fuels.

NOTES: In the case of a stable culture that continuously secretes products for collection, there are no residual biomass carbons. In contrast, water was consumed fixing the carbons in both the residual biomass and the fuel component in the case where algal cells are destroyed to collect lipids or to be processed to fuel.

carbon average molecular weight of the fuel component and the fuel component mass ratio in the dry biomass. Using a closed photobioreactor to make ethanol from cyanobacteria consumes less than 1 liter of water per liter of ethanol for photosynthesis. As discussed in the previous chapter, water consumption through photosynthesis for lipid-forming algae is at least three times higher.

3.6 ALTERNATIVE PATHWAY #4—WHOLE-CELL PROCESSING

Thermochemical pathways for processing biomass to fuels have garnered interest as the focus shifts away from production of alcohols and esters and toward production of “drop-in” hydrocarbon fuels (Figure 3-18; Huber and Dale, 2009). Figure 3-19 shows the detail input and output of a thermochemical pathway—pyrolysis. Anaerobic heating of virtually any biomass causes thermal degradation that begins to fractionate the biomass into gaseous, liquid, and solid components (Mohan et al., 2006). Subsequently, liquid fractions can be upgraded by hydrotreating to yield a hydrocarbon fuel. The final fuel products are compatible with existing petroleum refinery infrastructure. An advantage of thermochemical technologies is that they are largely feedstock agnostic and can accept any type of biomass, including biomass of aquatic microalgal and macroalgal species. Pyrolysis is the only process discussed that easily accepts macroalgal species.

Lipid-producing microalgae are not required for fuel production in this pathway. Algal strains or mixed cultures are selected for their high biomass productivity and ability to fix

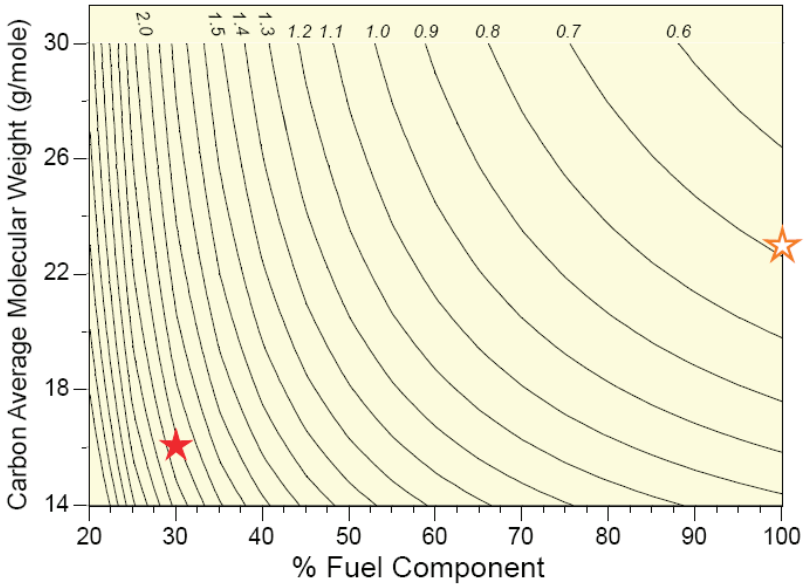


FIGURE 3-17 Curves showing the volume ratio of water consumed in photosynthesis per liter of fuel component produced assuming a fuel component density of 0.88 kg/L. NOTES: The orange star approximates the Algenol direct ethanol synthesis assuming that ethanol is produced in a stable culture that produces no residual biomass. The red star is shown for reference and approximates the case for a lipid forming algae at 30-percent lipid.

Potential Variation in Each Step

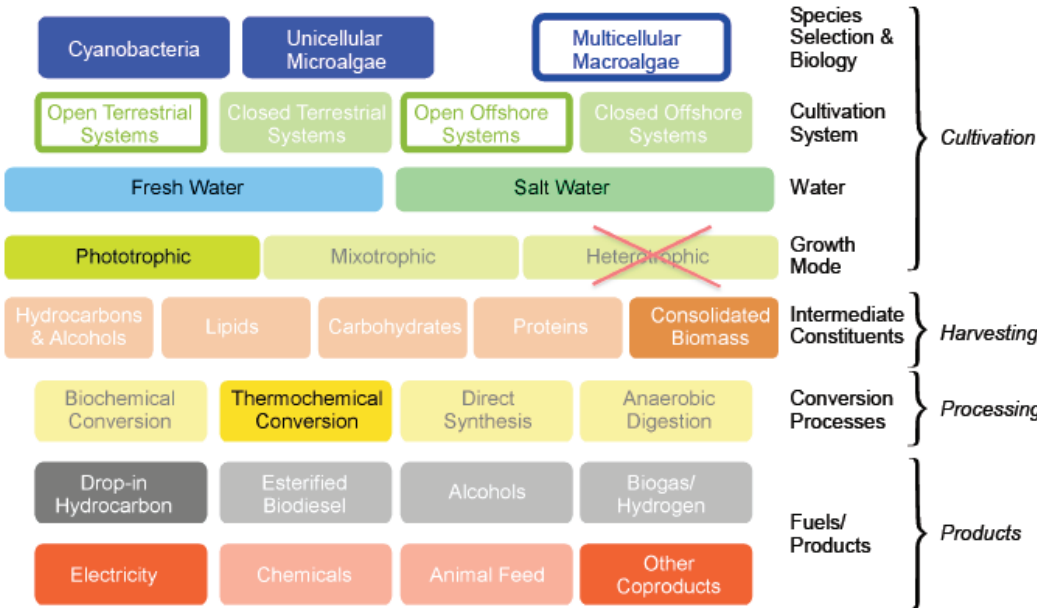


FIGURE 3-18 Overview of the whole-cell processing to make drop-in replacement fuels. The key feedstock is the entire cellular biomass.

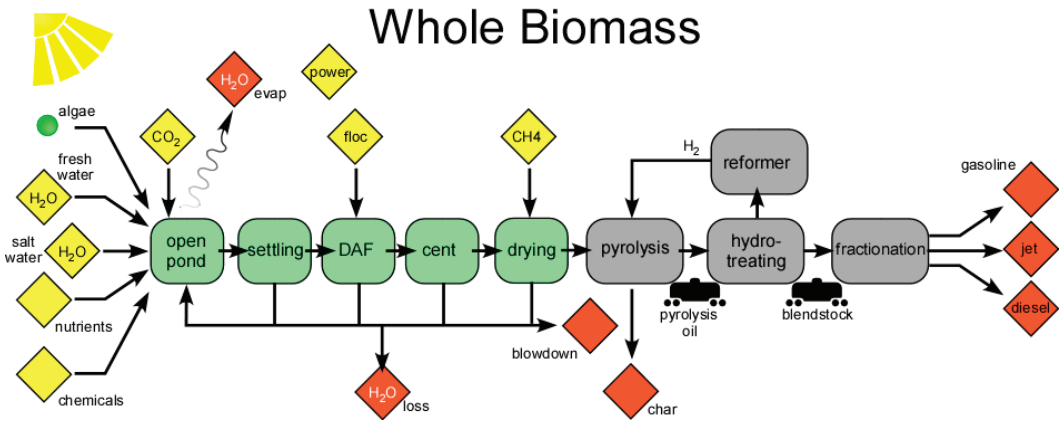


FIGURE 3-19 Inputs and outputs of the alternative pathway #4: Pyrolysis of cellular biomass and hydrotreating to yield hydrocarbon blendstock.

NOTES: Several options exist for the pyrolysis of algal biomass. Shown here is a two-stage conversion where tank cars indicate that it is possible to transport both pyrolysis oil and blendstock.

carbon. Algae would have to be harvested, dewatered, and likely dried for use as feedstock. Aquatic species present a challenge to a pyrolysis process because of the water they carry into the process. Water serves as a largely unreactive diluent that saps away heat during the pyrolysis step. As a diluent, water reduces efficiency of attempts to recover value from the gas streams.

Several options exist for the pyrolysis process (Huber et al., 2006). Pyrolysis oil intermediates pose some technical challenges. Pyrolysis oil is acidic and reactive, and storage without stabilization is a challenge (Mohan et al., 2006; Hatcher, 2011). Integrated hydro-pyrolysis and hydroconversion (IH₂) is a combined hydro-pyrolysis and hydrotreating process developed by the Gas Technology Institute and collaborators and funded by the Department of Energy. IH₂ has many promising attributes and has been claimed to successfully process algae at high yield (Marker et al., 2010; Sims, 2011). The particular combination of steps is claimed to avoid pyrolysis oil issues. The IH₂ technology uses low-pressure hydrogen together with a proprietary catalyst to remove virtually all of the oxygen present in the starting biomass (Figure 3-20). Production of exportable steam is possible, and is likely suitable for offsetting some of the energy requirements for drying. The hydrogen required for the process is produced through steam reforming of the off-gas stream of methane and other hydrocarbons from the process. Pilot-scale testing was promising and several demonstration units are now under construction. GHG reduction potential is reported to be lower than competing processes (Marker et al., 2010; Sims, 2011).

Literature on performance of this process is limited. Yields of product fuels have ranged from 26 to 46 percent on a dry, ash-free basis depending on feedstock. This represents more than 70 percent efficient energy conversion for the highest yield. This process was selected for comparison because of reports suggesting that supplemental energy and hydrogen are not required. As a result, there are no extra feed or effluent streams that would affect the analysis of the overall environmental footprint of fuel production. Projects are moving forward using this technology for the conversion of algal biomass.

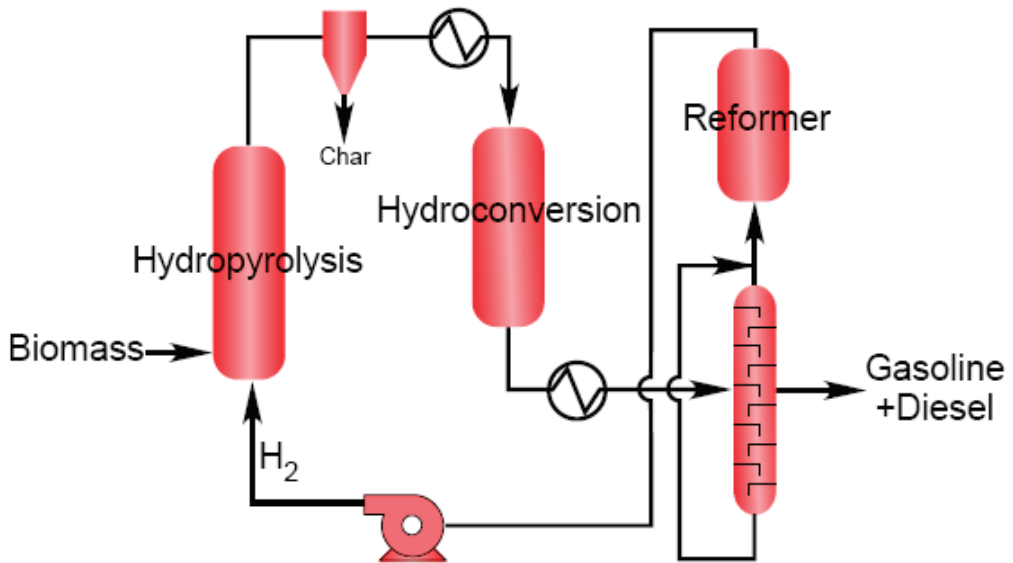


FIGURE 3-20 IH₂ process schematic.
SOURCE: Adapted from Marker et al. (2010).

3.7 OTHER POTENTIAL PATHWAYS

Many other processes for extracting fuel from microalgae are being discussed and investigated. Lack of published or available data on key sustainability metrics means that little can be said about the sustainability attributes of other potential pathways relative to pathways discussed earlier. In addition to the pyrolysis route described above, microalgal systems that use thermochemical transformation techniques to process whole algal cells are beginning to be tested. These systems appear to have energy requirements similar to other production techniques in which the cells are killed to harvest product. Clearly, thermochemical pathways can manufacture a range of fuel products. Whether the subsequent energy use in processing offers advantages from an energy or emissions perspective is unclear. These whole-biomass systems offer the advantage that cultivation is not limited to oleaginous species. Species can be grown at maximum carbon fixation rates to feed processes that retain high fractions of the fixed carbon in their final fuels. Examples of whole organism conversion technologies include (Gouveia, 2011; Hatcher, 2011):

- Fermentation of algal biomass to yield alcohols or hydrocarbons;
- Gasification and syngas conversion to alkanes, alcohols, or aromatics (through methanol and subsequent conversion);
- Gasification and syngas conversion to alcohols by conventional catalysis;
- Gasification and syngas conversion to alcohols by syngas fermentation;
- Anaerobic digestion to methane (making no liquid fuel); and
- Hydrous pyrolysis (Hatcher, 2011).

These techniques are not widely used and could be put into wider practice (Gouveia, 2011). High water content is not a desirable characteristic of feedstock for fuels. The

fundamentals of water removal from the product are critical in any discussions about large-scale fuel production. Laboratory-scale or pilot-scale techniques that use solar drying are relatively slow, require large land areas, and are not likely to scale up commercially.

3.8 SUMMARY

This chapter describes and contrasts pathways that lead from algae cultivation to fuel production. Many technical options exist for each individual component in the processing pathway (for example, algae can be cultivated in an array of open ponds or closed photobioreactor systems with different designs). This chapter illustrates how particular individual components are linked together to constitute the pathway for algal biofuel production and how categorizing these processes into several distinctive pathways can help with the analysis of the sustainability impacts of algal biofuels. This chapter further discusses the potential fuel products and coproducts from various production pathways. In concert with Chapters 4 and 5, the reference and alternate pathways demonstrate the sustainability issues for the photosynthetic methods of producing fuels from microalgae and highlight potential improvements that might alleviate critical sustainability concerns.

Though this chapter focuses on describing algal biofuel production pathways that are further considered in following chapters, it is the only part of the report that considers the value-added propositions associated with coproducts. The committee believed that coproducing high-value products, such as nutraceutical products, with algal biofuels would be viable only on a small scale. If large quantities of high-value algal products are coproduced with biofuels, the value of coproducts likely decreases with market saturation. Animal feedstuff is the only coproduct that is likely to have a large enough market to absorb the large quantities produced if algal biofuels are produced at commercial scale. The coproduct value depends on the composition of the animal feedstuff and the characteristics of the market in which it would be sold. In general, coproduct volumes swell with the scaling up of algal biofuel production, potentially saturating markets for these products unless a wide variety of coproducts for different markets are produced.

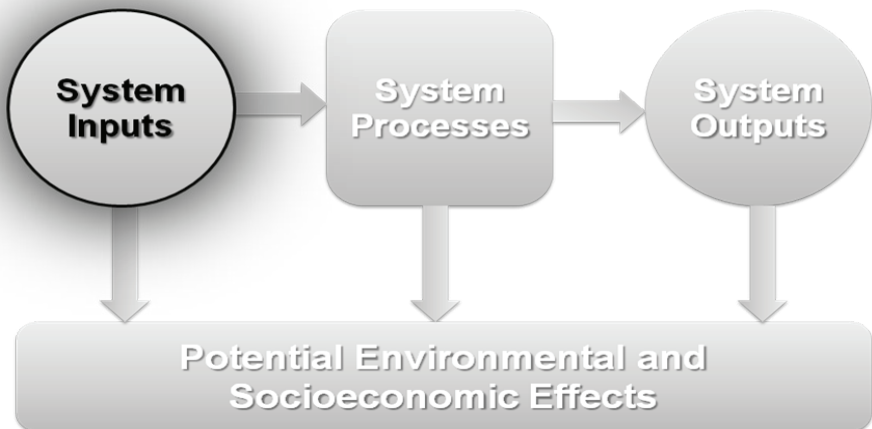
REFERENCES

- AlfaLaval. 2010. Multiple choice for fats and oils refining. Available online at http://local.alfalaval.com/en-us/key-industries/food/fatsandoils/Documents/PFT00355EN_Degumming_neutralization.pdf. Accessed December 21, 2011.
- Beal, C.M., R.E. Hebner, M.E. Webber, R.S. Ruoff, and A.F. Seibert. 2011. The energy return on investment for algal biocrude: Results for a research production facility. *Bioenergy Research*: DOI: 10.1007/s12155-011-9128-4.
- Benemann, J.R. 2008. Opportunities and challenges in algae biofuel production. Available online at http://www.fao.org/uploads/media/algae_positionpaper.pdf. Accessed October 21, 2011.
- BioProcess Algae. 2011. Questionnaire reply from BioProcess Algae. Received by the NRC Committee on the Sustainable Development of Algal Biofuels on September 27.
- Boussiba, S. 2011. Questionnaire reply from AlgaTechnologies, Kibbutz Qetura. Received by the NRC Committee on the Sustainable Development of Algal Biofuels on July 6.
- Chance, R.R., B. McCool, and J.D. Coleman. 2011. A Cyanobacteria-Based Photosynthetic Process for the Production of Ethanol. Presentation to the NRC Committee on Sustainable Development of Algal Biofuels on June 13.
- Davis, R., A. Aden, and P.T. Pienkos. 2011. Techno-economic analysis of autotrophic microalgae for fuel production. *Applied Energy* 88(10):3524-3531.
- Fan, X., R. Burton, and G. Austic. 2010. Conversion of degummed soybean oil to biodiesel: Optimization of degumming methods and evaluation of fuel properties. *International Journal of Green Energy* 7(6):593-599.
- Gouveia, L. 2011. *Microalgae as a Feedstock for Biofuels*: Springer. Available online at <http://www.springer.com/life+sciences/microbiology/book/978-3-642-17996-9>. Accessed December 21, 2011.

- Hatcher, P.G. 2011. The ODU algae to biodiesel project. Presentation to the NRC Committee on the Sustainable Development of Algal Biofuels on August 24.
- Huber, G.W., and B.E. Dale. 2009. Grassoline at the Pump. *Scientific American* (July):52-59.
- Huber, G.W., S. Iborra, and A. Corma. 2006. Synthesis of transportation fuels from biomass: Chemistry, catalysts, and engineering. *Chemical Reviews* 106(9):4044-4098.
- Joule Unlimited. 2012. Frequently asked questions. Available online at <http://www.jouleunlimited.com/faq>. Accessed March 9, 2012.
- Kalnes, T.N., T. Marker, D.R. Shonnard, and K.P. Koers. 2008. Green diesel production by hydrotreating renewable feedstocks. *Biofuels Technology* (October):7-11.
- Luo, D.X., Z.S. Hu, D.G. Choi, V.M. Thomas, M.J. Realff, and R.R. Chance. 2010. Life cycle energy and greenhouse gas emissions for an ethanol production process based on blue-green algae. *Environmental Science and Technology* 44(22):8670-8677.
- Lurgi GmbH. 2012. Edible oil technology—Oilseed extraction and edible oil refining. Available online at http://lurgi.com/website/fileadmin/user_upload/1_PDF/1_Broschures_Flyer/englisch/0314e_Edible_Oil_Technology.pdf. Accessed July 20, 2012.
- Marker, T., M. Linck, and L. Felix. 2010. Integrated Hydrothermal Liquefaction and Hydroconversion (IH2) Process for Direct Production of Gasoline and Diesel Fuel from Biomass. Paper read at the BIOMASS 2010, March 30-31, Arlington, VA.
- Marler, D. 2011. An Assessment of the Environmental Performance of Algal Biofuels. Presentation to the NRC Committee on Sustainable Development of Algal Biofuels on September 8.
- Mohan, D., C.U. Pittman Jr, and P.H. Steele. 2006. Pyrolysis of wood/biomass for bio-oil: A critical review. *Energy and Fuels* 20(3):848-889.
- Morgenthaler-Jones, L. 2011. Algae + fish: Food, fuel and more. Presentation to the NRC Committee on Sustainable Development of Algal Biofuels on September 8.
- NAS-NAE-NRC (National Academy of Sciences, National Academy of Engineering, and National Research Council). 2009. *Liquid Transportation Fuels from Coal and Biomass: Technological Status, Costs, and Environmental Impacts*. Washington, DC: The National Academies Press.
- NRC (National Research Council). 2011. *Renewable Fuel Standard. Potential Economic and Environmental Effects of U.S. Biofuel Policy*. Washington, DC: The National Academies Press.
- NREL (National Renewable Energy Laboratory). 2006. *Biodiesel and Other Renewable Diesel Fuels*. Golden, CO: National Renewable Energy Laboratory.
- Olusola, J.O., M.M. Adediran, A.K. Oluseyi, and U.L. Ajao. 2009. Processing of triglycerides to diesel range hydrocarbon fuels: easily practicable small scale approach. *Energy and Environment* 20-21:1325-1341.
- Photon8. 2011. Questionnaire reply from Photon8. Received by the NRC Committee on the Sustainable Development of Algal Biofuels on November 18.
- Pyter, R., E. Heaton, F. Dohleman, T. Voigt, and S. Long. 2009. Agronomic experiences with *Miscanthus x giganteus* in Illinois, USA. *Biofuels: Methods and Protocols* 581:41-52.
- Redfield, A.C. 1958. The biological control of chemical factors in the environment. *American Scientist* 46:205-221.
- Robertson, D.E., S.A. Jacobson, F. Morgan, D. Berry, G.M. Church, and N.B. Afeyan. 2011. A new dawn for industrial photosynthesis. *Photosynthesis Research* 107(3):269-277.
- Serrano-Ruiz, J.C., E.V. Ramos-Fernandez, and A. Sepulveda-Escribano. 2012. From biodiesel and bioethanol to liquid hydrocarbon fuels: New hydrotreating and advanced microbial technologies. *Energy and Environmental Science* 5(2):5638-5652.
- Sims, B. 2011. GTI signs licensing agreement with Shell Group subsidiary. Available online at <http://www.bio-refiningmagazine.com/articles/5239/gti-signs-licensing-agreement-with-shell-group-subsiary>. Accessed June 12, 2012.
- Szmant, H.H. 1989. *Organic building blocks of the chemical industry*. New York: Wiley.
- Thomas, H. 2011. Questionnaire reply from Open Algae. Received by the NRC Committee on the Sustainable Development of Algal Biofuels on July 5.
- Tilman, D., J. Hill, and C. Lehman. 2006. Carbon-negative biofuels from low-input high-diversity grassland biomass. *Science* 314(5805):1598-1600.
- Van Gerpen, J. 2005. Biodiesel processing and production. *Fuel Processing Technology* 86(10):1097-1107.
- Williams, P.J.L.B., and L.M.L. Laurens. 2010. Microalgae as biodiesel and biomass feedstocks: Review and analysis of the biochemistry, energetics and economics. *Energy and Environmental Science* 3(5):554-590.

4

Natural Resource Use



Fuel production from fossil and biological feedstocks is resource intensive, and algal biofuels require resource inputs in the form of water, energy, land, and nutrients. Algal biofuels have been produced at small scale, sufficient to prove that there are a number of possible production pathways. Although production of algal biofuels is technically feasible, they have to be shown to be economically, environmentally, and socially sustainable to become a practical substitution for petroleum-based fuels. The scaling of the pathways for algal biofuel production that are deemed practical for commercial production poses a new demand on natural resources. The levels of nutrients, water, land, and energy necessary to

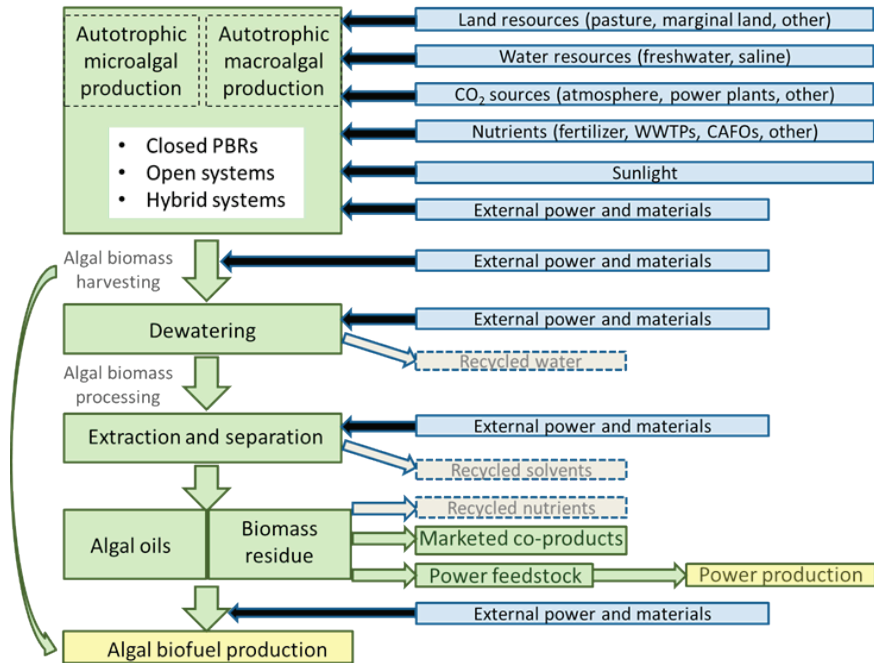


FIGURE 4-1 Resource requirements of algal biodiesel production.
 NOTE: WWTPs denote municipal wastewater treatment plants. CAFOs are concentrated animal feeding operations.

produce alternative biofuels in an economically, environmentally, and socially sustainable way would have to be carefully considered (McKone et al., 2011). This chapter focuses on the current sustainability knowledge of the water, nutrient, land, and energy requirements of algal biofuels at all steps in the photoautotrophic algae-based production process (Figure 4-1). Where relevant data are available, quantitative case studies for at least two potential pathways for algal biofuel production are presented in this Chapter. In addition, potential assessment indicators are provided when appropriate, and current knowledge and data gaps are identified for each of the four resource requirement categories.

Major new advancements in the current knowledge base will require multi-hectare scale demonstration facilities to be built and maintained in operation for a period of time sufficient to allow detailed real-time analyses of the key variables required for commercial success (Campbell et al., 2011). Moreover, commercial-scale demonstrations will be necessary to assess and to improve algal biofuel technologies and their integration with the existing energy infrastructure (Sagar and van der Zwaan, 2006; Katzer, 2010). Innovations that result in reduced resource use along the entire algal biofuel supply chain will remove some of the existing barriers to the development of large-scale, sustainable, and economically viable algal biofuel enterprises. In addition, improvements in algal productivity and biofuel yield will help to reduce resource requirements per unit of algal biofuel produced.

4.1 WATER

Water provides the essential physical environment in which cultivated algae grow and reproduce (Murphy and Allen, 2011). It also acts as a thermal regulator and provides a

medium for essential nutrient resources—carbon dioxide (CO₂), nitrogen (N), phosphorus (P), and other nutrients—for algal biomass production. Water has to be pumped to and contained and circulated in mass cultivation systems whether they involve either open ponds or closed photobioreactors. Closed photobioreactors also may use water spraying or submersion to maintain temperature of the culture. Given that the agricultural demand for water in the United States accounts for 85 percent of consumptive water use, large-scale production of biomass, including algae, has the potential for large regional strain on water systems unless nonfreshwater sources are used when possible.

Irrespective of the type of fuel produced, water is an integral element of fuel production, and thus an important nexus exists between fuel production and water supplies (Pate, 2007; Murphy and Allen, 2011). In the case of algal biofuel production, water is necessary for biomass feedstock production, and it can be lost during the processing of the algal biomass to fuels. This section discusses water requirement and consumptive use of fresh water along different steps of the algal biofuel supply chain and throughout the life cycle of algal biofuel production. In this report, water requirement refers to the quantity of water needed throughout the life cycle of algal biofuel production. Consumptive use of fresh water is the quantity of fresh water withdrawn from surface or groundwater sources that is lost to the immediate environment through evaporation or incorporation into products. In a sustainability assessment, the consumptive use of freshwater needs to be assessed in the context of regional water availability. For example, water withdrawn from a fossil aquifer that is declining quickly is less sustainable than the same amount of water withdrawn from an aquifer that replenishes more quickly. Where data are available, estimates for water use are compared to those for other biofuels and petroleum-based fuels.

4.1.1 Water Requirements in the Supply Chain

The water requirements of any algal cultivation system depend on the physical structure and configuration of the system, the local climate, and the ability to reclaim and reuse system water (Table 4-2; Murphy and Allen, 2011). Open ponds are subject to evaporative water losses (Yang et al., 2011) that are influenced by multiple factors including pond area, volume, and water level; water and air temperature; and wind velocity, humidity, and atmospheric pressure (Boyd and Gross, 2000). The average U.S. evaporation rate from a pond system is estimated to be 0.9 cubic meters of water per square meter per year (Murphy and Allen, 2011), but evaporative losses from open ponds vary by geographical region. Moreover, some operation regimes (for example, stirring and sparging) can increase the water loss to levels that are greater than would be predicted by evaporation and purging alone.

In outdoor open-pond algae cultivation, freshwater addition is necessary to compensate for evaporative water loss and to avoid salt buildup. Therefore, fresh water is necessary in any algal cultivation system, irrespective of the type of culture water used (Yang et al., 2011). The linkage between evaporative losses and purging, or blow-down, for an open-pond system is illustrated in Figure 4-2. A significant amount of water (F_{out}) is lost to evaporation in open ponds thereby concentrating total dissolved solids in the pond water. Whether they are fed with fresh water or with saline water, all algal cultivation systems have a control point for the maximum allowable concentration of dissolved solids that is maintained in the culture. If this set point is based on salinity, evaporation would raise the pond salinity so that steps would have to be taken to compensate for this increase. Addition of water with a lower salt concentration and flushing of water from the pond are two steps that can be taken to maintain salinity below the defined control point (x_{control}). Both steps can increase the water requirement and consumptive water use.

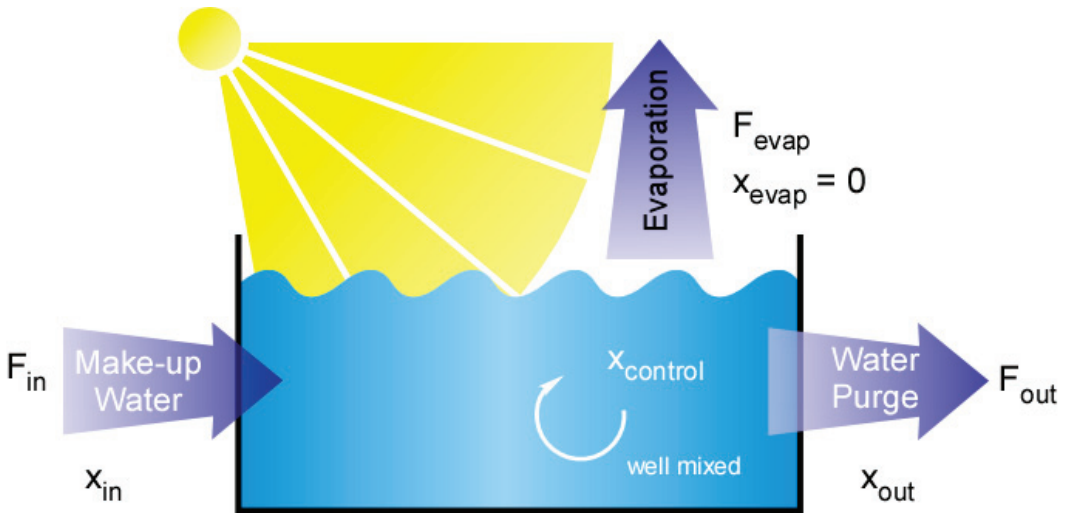


FIGURE 4-2 Dissolved solids control in a simplified open-pond cultivation system.

NOTE: Make-up water addition (F_{in}) and water purge (F_{out}) are used to control the critical concentration of total dissolved solids in the pond water (x_{control}). If the pond is well-mixed, the concentration of total dissolved solids in the purge (x_{out}) is equal to the concentration in the pond (x_{control}).

In addition to evaporative losses, water can seep into and out of open ponds, particularly if they are clay-lined or if liner failure occurs. Water percolation is strongly influenced by the composition and texture of the underlying soils (for example, clay versus sand). Seepage rates are typically on the order of 5 to 6 millimeters per day (Weissman et al., 1989; Boyd and Gross, 2000), which is low compared to rates of evaporative water loss in many regions of the United States. In contrast to open ponds, closed photobioreactors are not affected by surface evaporation and seepage, and the lowest reported values for estimated water use are associated with closed systems. However, the water requirements of a photobioreactor system depend on its actual configuration and operating conditions.

The reclamation and recycling of water are key determinants of the total water requirements of both open-pond and closed photobioreactor systems. Whether and how much of the harvest water can be reclaimed and reused depend on the efficiency of separation processes, the quality of the return water, and the sensitivity of the algal culture itself to changes and impurities in the return water, including any waste products produced by the resident algae (Murphy and Allen, 2011).

The water requirement for processing of algal biomass to biofuel is small relative to evaporative losses during cultivation in open-pond systems. Water use for processing algal biomass to fatty-acid methyl ester (FAME) was estimated to be 1 liter per liter of biodiesel produced. Water loss during the drying of algae to prepare the biomass for processing to fuel is unavoidable, and some water also is unavoidably lost during the extraction of oil from algae and esterification of algal oil. However, Pate et al. (2011) stressed that evaporative water loss under operating conditions involving the inland use of water with a high salt content will result in salinity increases unless fresh water is used to make up for the loss or steps are taken either to mitigate or adapt to salt build-up. The use of inland saline water in algal biofuel production also could have other potential environmental effects (see Chapter 5).

4.1.2 Life-Cycle Water Requirements

Quantification of life-cycle water requirements of algal biofuel production would support managing future impacts on water demand and enable comparison of water use for algal biofuel with other fuels. The estimation of life-cycle use of any requirement for algal biofuel production (for example, water, nutrients, and energy), however, is complicated by the developing nature of the technologies. In addition to uncertainty as to how algal biofuel will evolve on the path of commercialization, there is also a lack of data on material and energy requirements of the current technologies.

An additional complication in open-pond algae cultivation is that water use varies significantly with climatic differences in temperature, humidity, and rainfall. Other biofuels and agricultural crops show such variability in water use. For example, regional variability in irrigation results in estimates of life-cycle water requirements to make ethanol from corn varying from 5 to 2,140 liters of water per liter of fuel, depending on in which U.S. state the corn is grown (Chiu et al., 2009). If the national average of water demand for corn-grain production is used, Chiu et al. (2009) estimated the water use for corn-grain ethanol to be 142 liters of water per liter of fuel. However, the geographical distribution of additional corn grown to meet ethanol demand is uncertain so that whether their water demand matches the national average for all corn also is unclear. The water intensity of open-pond algae cultivation depends critically on the future geographic distribution of cultivation. This future distribution is difficult to forecast, however, being based on the conflux of uncertain future technological performance, policy, and industry response. In the absence of a reliable forecast, studies of water intensity can clarify relationships between location, climate, and water use.

4.1.2.1 Life-Cycle Water Use of Freshwater Open-Pond Systems

A number of studies have analyzed water requirements of biofuel produced from algae cultivated in open-pond systems and include different phases of the life cycle (Harto et al., 2010; Wigmosta et al., 2011; Yang et al., 2011). There are large differences in assumptions and results among studies, which is not surprising given the challenges mentioned above. Table 4-1 summarizes the assumptions and results of three studies on open-pond algae cultivation to highlight differences in results and the origins of these differences. The results span over two orders of magnitude, from 32 to 3,650 liters of water per liter of algal biofuel. As a comparison, 1.9-6.6 liters of water are consumed to produce 1 liter of petroleum-based gasoline from crude oil or oil sands (King and Webber, 2008; Wu et al., 2009; Harto et al., 2010). Resolving the variability and uncertainty in these results is beyond the scope of this report. Instead, the goal of this report is to identify and prioritize issues that could affect the long-term sustainability of algal biofuels. Prioritization of research and development (R&D) for issues of concern could contribute to developing algal biofuels as a sustainable part of the energy future.

Harto et al. (2010) analyze life-cycle water requirements of a number of alternative transportation fuels, including corn-grain and switchgrass ethanol, soybean biodiesel, solar and wind generated electricity, and algal biofuels with algae cultivation in open-pond systems and closed photobioreactors. The scope of processes analyzed includes embodied water in facilities and vehicles. In most scenarios, water use in evaporation and fuel production dominate the life cycles. Scenario analyses that combine pessimistic versus optimistic assumptions for productivities with evaporation yield variability from 32 to 656 liters of fresh water per liter of biodiesel. The low-end scenario of 32 liters per liter applies to algae

TABLE 4-1 Summary of Assumptions and Results of Studies for Life-Cycle Water Requirements for Open-Pond Algal Biodiesel

Study	Case	Assumptions			Estimated Water Loss or Use				
		Fuel Productivity (L/m ² /yr)	Net Evaporation (cm/d)	Cultivation (L/L)	Harvest (L/L)	Fuel Processing (L/L)	Coproducts (%)	Other	Life-Cycle Water Use (L/L)
Harto et al. (2010) ^a	Low case	5.5	0	0	01	31	46	1	32
	Base case	5.5	0.25	165	01	50	0	1	216
	High case	2.6	0.42	575	01	80	0	0.58	656
Wigmosta et al. (2011) ^b	79.5 gal/yr production	0.46	0.06	438	01	N/A ^b	0	0	438
	200 gal/yr production	0.46	0.20	1613	01	N/A ^b	0	0	1613
Yang et al. (2011)	100% harvest recycle	3.2	0.27	450	0	140	0	0	590
	50% harvest recycle	3.2	0.27	450	1550	140	0	0	2140
	No harvest recycle	3.2	0.27	450	3060	140	0	0	3650

^aHarto et al. (2010) and Wigmosta et al. (2011) assumed 100 percent recycling of harvest water.

^bWigmosta et al. (2011) did not estimate water use for processing algal biomass to fuels. They did not include water use upstream of algae cultivation (for example, water use for fertilizer production), but that amount is a small fraction of the total water requirement (Harto et al., 2010).

NOTES: L/L = liters water consumption per liter of biodiesel produced. As a comparison, life-cycle water use to produce gasoline from crude oil and oil sands has been estimated to be about 1.9-6.6 L/L.

TABLE 4-2 Life-Cycle Water Requirements to Produce Biofuel from Corn and Soybean

Crop	Study	Water Consumption (L/L)	Notes
Corn	Chiu et al., 2009	513-1,402	Average water use when irrigated. Range comes from different U.S. states.
	Dominguez-Faus et al., 2009	5-2,138	Average irrigated and nonirrigated. Range comes from state variability.
	Chiu et al., 2009	142	National average of irrigated and nonirrigated.
Soybean	Dominguez-Faus et al., 2009	1,400-2,900	Average water use when irrigated. Range comes from different U.S. states.
	Harto et al., 2010	133	National average irrigated and nonirrigated.

NOTE: L/L = water consumption per liter of ethanol or biodiesel produced. Ethanol has about two-thirds of the energy content of gasoline. Biodiesel has about the same energy content as gasoline.

cultivated in regions in which rainfall makes up for evaporative losses. Allocation of water use to coproducts in addition to fuel can significantly reduce water use associated with the biofuel product.

Wigmosta et al. (2011) developed a geographically resolved model of variability in water and land requirements in different areas in the United States. They estimated water requirements that range from 22 to 3,600 liters of water per liter of oil depending on location of cultivation. Their assumptions for productivity of algae are much lower than those of Harto et al. (2010) and Yang et al. (2011), highlighting the uncertainty associated with critical factors driving material requirements. Wigmosta et al. (2011) also constructed scenarios that build out the geographical distribution of algal biofuel production, starting with areas with lower evaporation and more rainfall. They found steep increases in water requirements as production moves to more water-intensive areas. Yang et al. (2011) explored water recycling, use of saline water instead of fresh water, performance of different algal strains, and geographic variability. They find that recycling harvest water is critical in managing water requirements.

The committee reviewed what is known about water requirements for other algal biofuel pathways. Sapphire Energy estimated that its proposed biorefinery in Columbus, New Mexico, would require 3,500 acre feet (4.32 billion liters) of fresh water to produce 30,000 barrels (4.77 million liters) of green crude each year, or 906 liters of water per liter of green crude. The green crude can be upgraded to drop-in fuels (USDA-RD, 2009). Therefore, Sapphire Energy's production pathway is comparable to either the reference pathway in Chapter 3 or the alternative pathway #1 depending on whether coproducts are included.

The estimates of life-cycle water use of algal biofuels (Table 4-1) were compared to those of other biofuels to explore whether algal biofuels are more or less water intensive than other biofuels. Table 4-2 shows results of studies of life-cycle water requirements of corn-grain ethanol and soybean biodiesel. For biofuels produced from corn grain, soybean, and algae cultivated in open ponds, water use depends more on the climate (rainfall in particular) where the biomass is grown rather than the type of biomass.

4.1.2.2 Life-Cycle Water Use in Closed Systems—Photobioreactors

Cultivating algae in photobioreactors has the potential to eliminate water consumption from evaporation, which could significantly reduce overall water demand. However, data for closed systems are even scarcer than for open systems. Harto et al. (2010) estimated the

life-cycle water requirements of a photobioreactor system at 30–63 liters fresh water per liter of biodiesel, though this result is based on expert opinion, not empirical measurement of a functioning system. The Algenol process is a closed photobioreactor using sea water and fresh water. In its environmental impact assessment for a proposed biorefinery in Fort Meyers, Florida, Algenol estimated that the facility would require 3.6 million gallons of seawater and 210,000 gallons of fresh water to produce 100,000 gallons of algal ethanol each year (or 36 liters of salt water per liter of ethanol and 2.1 liters of fresh water per liter of ethanol) (DOE, 2010a). The freshwater use equals 3.15 liters of fresh water to produce each liter of gasoline-equivalent fuel. The Algenol estimate does not include upstream water use for inputs to their facilities.

4.1.2.3 Algae Cultivation Using Salt or Brackish Water or Wastewater

Using salt-tolerant algal species would allow the use of alternative water sources such as seawater, saline, and brackish groundwater, or coproduced water derived from oil, natural gas, and coal-bed methane wells (DOE, 2010b). This physiological flexibility of algae implies that locating algae production to areas where alternative water sources are available could reduce consumption of fresh water in cultivation. Cultivating saline algae in inland ponds also could reduce the potential for invasion of the ponds by undesirable freshwater organisms.

Vasudevan et al. (2012) estimated the consumption of fresh water in a saline water, open-pond, algae cultivation facility for three cases that they formulated—a base case (nominal, in their language) with reasonable assumptions in technology and system performance, a case with pessimistic assumptions, and a case with optimistic assumptions. The estimated requirement for freshwater make-up was 1,000 liters of freshwater per liter of oil, with a range of 200–2,000 liters from optimistic to pessimistic cases (Vasudevan et al., 2012). This result suggests that the need for freshwater make-up is significant when saline water is used for algae cultivation. However, the make-up water use depends on productivity and salinity limits of algae used, climate, and other uncertainties and variabilities that have yet to be resolved.

Wastewater also can be used in cultivating algae, thereby reducing groundwater and surface water consumption and treating wastewater by reducing nitrogen and phosphorus content. Pittman et al. (2011) discussed the potential benefits and limitations of using wastewater to produce algae for biofuels cost effectively, and concluded that dual-use microalgae cultivation for wastewater treatment and biofuel production has the potential to use up nutrients in wastewater and reduce the amount of fresh water required for biofuel generation from algal biomass. The potential environmental benefits and concerns of algal biofuel production using wastewater as a water and nutrient feed will be discussed further in Chapter 5 of this report, but this concept has not yet been tested at scale.

4.1.3 Scale-up Considerations

The freshwater demands of algal biofuel production will be high if algal biofuels are used to substitute for a significant fraction of annual U.S. liquid transportation fuel consumption, particularly if open ponds are to be used for algae cultivation. If open ponds are used for algae production, then a significant amount of water will be required to replace evaporative losses from the pond surface and to prevent dissolved salt buildup in the biomass cultivation system (Yang et al., 2011). Recent estimates reported by the U.S. Department of Energy (DOE, 2010b) suggest that water losses on the order of several hundred liters of water per liter of algal oil or algal biodiesel produced would result from the operation of open ponds in arid, sunny regions of the continental United States. The most

optimistic production scenario presented in DOE (2010b) was for the southwestern United States. Those estimates were based on high rates of areal production (31 grams per square meter [g/m²] per day) and high average cellular oil contents (50 percent by dry weight). Taking meteorological conditions into account, Wigmosta et al. (2011) estimated the consumptive water use to compensate for evaporative loss from ponds to be 312 trillion liters per year if algae are grown to produce 220 billion liters of algal biofuels. That amount is about twice the quantity of water used for irrigated agriculture in the United States (177 trillion liters in 2005; USGS, 2012). If they limit the algae cultivation to areas with high rainfall, such as areas near the Gulf Coast, the Great Lakes, and most of the eastern seaboard, then consumptive use of fresh water per unit fuel produced can be reduced by 75 percent.

Pienkos (2007) estimated that between 16 and 120 trillion gallons (60 and 454 trillion liters) of water per year would be required to produce the algal oil needed to produce 60 billion gallons (227 billion liters) per year of biodiesel. The amount of petroleum-based fuels consumed by the U.S. transportation sector in 2010 was about 207 billion gallons (783 billion liters) per year (EIA, 2011).

Pate et al. (2011) estimated the consumptive use of fresh water necessary to achieve target biodiesel production levels of 10 billion, 20 billion, and 50 billion gallons (37.8 billion, 75.7 billion, and 189 billion liters, respectively) per year from freshwater algae for four regions of the United States. Their estimates of water use for algal biofuel (Figure 4-3)

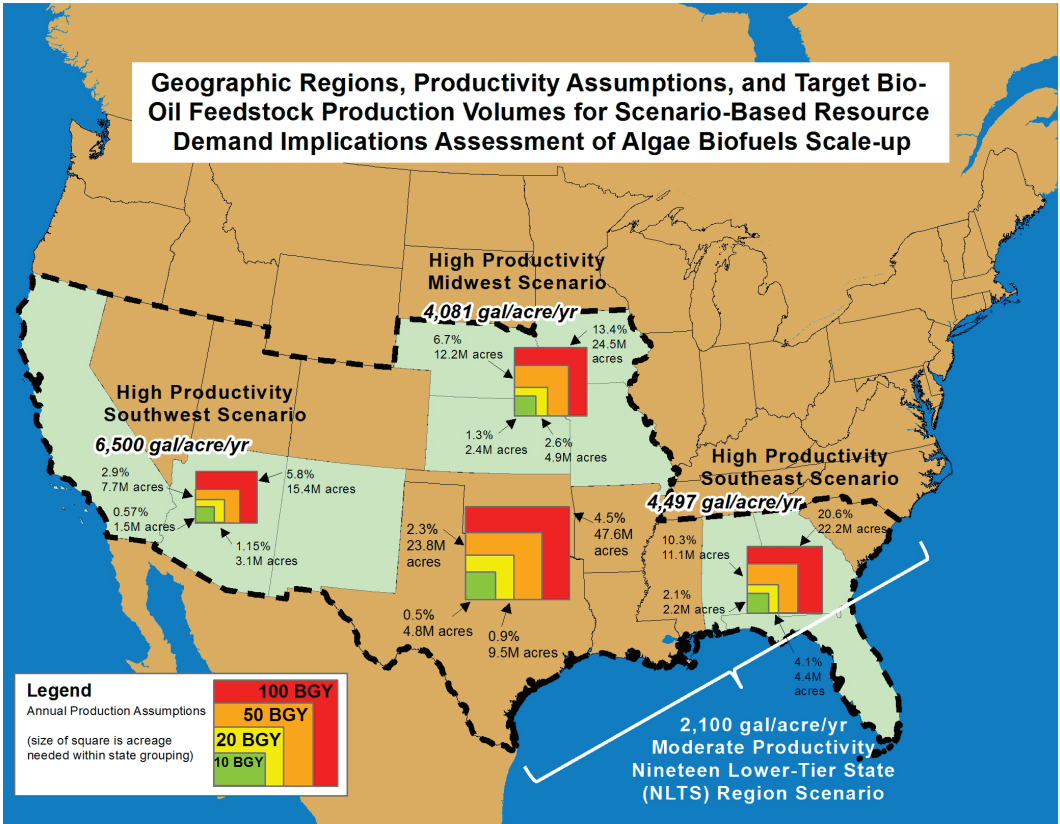


FIGURE 4-3 Algal biofuel scale-up scenarios for four different geographical regions of the United States: the Southwest, Midwest, Southeast, and nineteen lower-tier states.

SOURCE: Pate et al. (2011).

NOTE: 1 gallon = 3.78 liters; 1 acre = 0.40 hectare; 1 gallon per acre = 9.5 liters per hectare.

varied with the geographic region, the volume of feedstock production targeted, and the algal productivity assumed to be achieved. The projections of Pate et al. (2011) suggest that using fresh water only in open-pond algal production systems likely will be a significant sustainability issue if 10 billion gallons (37.8 billion liters) of biodiesel are to be produced each year, depending on the region. Cultivating freshwater algae to 10 billion gallons (37.8 billion liters) per year production appeared to be unattainable in the Midwest and Southeast regions due to water requirements, which represent more than 70 percent and more than 170 percent of the total water used for irrigation in the Midwest and in the Southeast, respectively.

Although water requirements for algal biofuels are estimated to be higher than those of petroleum-based fuels, sustainable use of freshwater needs to be considered in the context of regional availability and other competing uses (NRC, 2011). For example, a petroleum refinery located in a dry region, where groundwater recharge is low with water shortage could be more detrimental to its local supply than would open-pond algal cultivation systems located in a region with rising groundwater level, even though the petroleum refinery uses considerably less water than algae cultivation. The total demand on local water resources by algal biofuel production will depend on management practices for individual facilities (for example, the type and quantity of water used), the number of facilities located within a watershed, and both the existing volume and time trends in the volume of local aquifers, as influenced by competing water uses. Water use and freshwater and saline-water withdrawals in the United States have been estimated by the U.S. Geological Survey (Kenny et al., 2009).

Pate et al. (2011) suggested that the irrigation water from other agricultural applications will need to be diverted to algal biofuel production if 10 billion gallons of fuels are to be produced from algae cultivated in fresh water. Diverting irrigation water from agriculture to algae cultivation for fuels will raise the concern of water use for fuel versus food and feed. Large water withdrawals from surface waters or from groundwater that is connected to surface water systems can affect ecosystems. Many ecosystems require minimum seasonal flows to support life cycles of fish (Jager and Rose, 2003, Nagrodski et al., 2012) and riparian vegetation (Stromberg et al., 1996, Greet et al., 2011). Stream macroinvertebrate communities and diversity also are affected by stream flow (Dewson et al., 2007). In some regions, groundwater depth can affect terrestrial vegetation composition and nutrient cycling (Goedhart and Pataki, 2011). Effects of water withdrawals for algae cultivation on ecological populations and ecosystem processes would be important to consider in concert with effects of irrigation, hydropower, industrial water use, and municipal water use.

Pate et al. (2011) stressed that approaches are needed for algal biofuel production that use nonfreshwater such as coastal marine water; wastewater from agricultural, municipal, and industrial sources; brackish or saline groundwater; and produced water from oil, gas, and coal-bed-methane wells. Cost-effective approaches for reducing evaporative water loss and for dealing with salinity build-up need to be developed. Such approaches will be more important for inland sites where evaporation and salinity build-up are expected to be higher than in coastal marine operational settings that have high relative humidity.

4.1.4 Sustainability Indicators

The sustainability implications of water use are difficult to quantify. Many studies use consumptive water use as a measure. Consumption is withdrawal and subsequent "loss" of ground or surface water through evaporation or runoff. The link between water consumption and sustainability effects, such as ecosystem change or scarcity for human

needs, depends on local conditions. As water supplies are increasingly stressed, there is an increasing need for methods to connect different uses of water to sustainability impacts.

Indicators of sustainability of freshwater requirements for algal biofuel production include the following (Mulder et al., 2010; GBEP, 2011):

- Consumptive freshwater use expressed as kilograms of water per kilogram of fuel produced (biodiesel or ethanol) or liters of water per liter of fuel produced.
- Energy return on water invested (EROWI), megajoule per liter (MJ/L).

These indicators permit general comparisons among sites, feedstocks, and production technologies, but do not provide information about sustainability relative to local supplies. Additional project-specific and site-specific information—total consumptive water use by a facility relative to current supply at the site and relative to projected future demands for all purposes, including biofuel production—will be required for this purpose. For example, a facility estimated to require 1 percent of available supply in an area that is not expected to experience significant population growth or increased agricultural demand for water is likely to be more sustainable than a facility requiring 50 percent of available supply in an area with a rapidly growing population or agricultural demand.

Indicators in addition to water consumption also are used. Water withdrawal refers to the quantity of fresh or ground water withdrawn. The use of green, blue, and gray water footprints are gaining interest in some research communities (Gerbens-Leenes et al., 2009; Hoekstra, 2009). Green water is rainwater evaporated during production such as crop growth. Blue water is irrigation water evaporated during crop growth. Grey water is the quantity of water needed to dilute pollutants from a process to meet water-quality standards. The choice of which of these indicators to use is a matter of debate; it is important that researchers report raw data on water use in addition to processed results for indicators.

4.1.5 Information and Data Gaps

Evaporation during cultivation is a major contributor to life-cycle water requirements. Some studies use pan evaporation to approximate water use in algae cultivation (Harto et al., 2010; Yang et al., 2011). Evaporation from algal ponds could, however, behave differently from pan evaporation. Wigmosta et al. (2011) used mathematical models intended to improve upon the use of pan evaporation data. Empirical data from actual ponds in various operating conditions would enable validation and construction of improved models. The extent to which water can be recycled in harvesting and other process steps also is a critical factor. Empirical data on and actual experience with water recycling in cultivation systems are needed.

Water balance and management, along with issues associated with potential salt build-up and salt management, are essential areas for future research, modeling, and field assessment (NRC, 2008; Gerbens-Leenes et al., 2009). If nonfreshwater is to be used in algae cultivation to alleviate consumptive use of fresh water, then current knowledge of the extent, the water quality and chemistry, and the sustainable withdrawal capacity of those nonfreshwater resources needs to be expanded (DOE, 2010b). For example, Subhadra and Edwards (2011) described the principal fresh and saline aquifers located in the southwestern United States, but comprehensive information on the geography and availability of fresh and saline aquifers in other regions suitable for algal biofuel production is needed. Although saline aquifers in the United States were mapped in the 1960s (Feth, 1965), the depth of the aquifer and other factors, such as aquifer hydraulic conductivity and well

yield, largely are unknown. The distribution and physical and chemical characteristics of saline groundwater resources need to be defined to predict the effects of extracting saline groundwater on freshwater resources and on the environment (Alley, 2003). Without such information, coastal regions may be more suitable for large-scale saltwater algal production systems than inland regions (Darzins et al., 2010).

Data on the regional availability of fresh water, salt water, and other nonfreshwater (for example, wastewater) and on the regional demand of water for agriculture and other uses are needed to assess the potential availability of different water resources for algae cultivation.

4.1.6 Potential Effect on Social Acceptability

Water security is a pressing concern globally and an emerging concern in the United States. Algal biofuel production will, to a still unspecified extent, affect consumptive use of fresh water. Freshwater availability and quality are intricately related to agricultural productivity, human health, and safety. The security impacts to this system from large-scale algal biofuel production could be significant. As global weather patterns continue to become more and more extreme, resulting in harsh, prolonged drought in arid climates, uncertainty over water availability has begun to threaten geopolitical stability and represents a serious risk to human health. While the relative abundance of freshwater resources and advanced water transportation and irrigation infrastructure has insulated the United States from the immediate and severe public health and water security issues that many nations currently face, access to fresh water in multiple regions of the country is increasingly limited and likely will become a major national security concern in the future. The Ogallala Aquifer in the Midwestern United States, yielding approximately 30 percent of the U.S. groundwater used for irrigation and supplying 82 percent of the potable water for those living within the aquifer boundary, could be completely depleted in as little as two to three decades (Guru and Horne, 2001). The Southwest has been facing and will continue to face serious water shortages in the coming decades, as aquifers are drained and surface water resources become increasingly scarce.

In recent years, public concern over energy security generally has overshadowed those for water security as oil prices have fluctuated. However, in the coming years, public concerns over water availability and the associated food security and health risks could increase and override those for energy security. If the algal biofuel industry relies heavily on freshwater resources, it could face a considerable setback as the increased use of freshwater resources becomes less acceptable to the public. This will be particularly damaging if infrastructure is already in place and capital already has been deployed in facilities that are subsequently shut down over concerns for their consumptive use of fresh water. Therefore, water recycling and use of nonfreshwater resources are important to ensuring the social acceptability of the large water requirements for algal biofuel production.

4.2 NUTRIENTS

Algae require key elemental nutrients for metabolic maintenance and growth, as is true of terrestrial plants. The exact elemental stoichiometry of algal cells varies from one environment to another and among different algal species. However, photoautotrophic algae use photosynthesis to convert light energy into new algal biomass with an elemental stoichiometry that on average obeys the following equation (Stumm and Morgan, 1988):



Rearranging Eq. 4-1, the elemental content of algae can be expressed more simply as:



The carbon-to-nitrogen-to-phosphorus stoichiometry of algae can be considered to average $\text{C}_{106}:\text{N}_{16}:\text{P}_1$ by moles, a value that is commonly known as the Redfield ratio (Redfield, 1958). Although the Redfield ratio is not a universal biochemical optimum (see Sardans et al., 2011b), Eq. 4-2 allows quantitative predictions to be made about the carbon, nitrogen, and phosphorus demands of algal biomass production. As implied in Eq. 4-1, CO_2 is essential for the photosynthetic production of algal biomass, providing elemental carbon that is required for the cellular synthesis of organic biomolecules, including the carbohydrates and lipids that can be converted into liquid biofuels (Falkowski and Raven, 2007). Nitrogen-containing molecules are involved in energy capture and release, cell structure, and metabolism. Phosphorus-rich molecules are essential to energy transfer. Both nitrogen and phosphorus are essential components of the genetic polymers ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) (Sternner and Elser, 2002). Providing sufficient and temporally stable supplies of CO_2 , nitrogen, and phosphorus is essential if algal biofuel production is to be deployed at large scale. If the species being cultivated are dominated by silicon (Si)-requiring taxa, such as diatoms, then adequate Si would have to be provided. Unlike water requirements, the amount of nutrients needed to promote algal growth can be expected to be the same whether open ponds or closed photobioreactors are used. However, nutrients can be recycled more readily in closed photobioreactors than in open ponds. The extent and efficiency of nutrient recycling that is used in the post-cultivation processing of algal biomass into biofuels and coproducts will affect the net nitrogen and phosphorus requirements of biofuel production systems. Nutrients cannot be recycled 100 percent because of losses as a result of precipitation¹ and nutrients tied up in dead algal biomass. The dead algal biomass cannot be left in the pond to mineralize because of undesirable consequences to the culture medium. The formation of such suspended sludge and the accompanying dissolved organic matter is a sink for nutrients and reduces light availability for the growth of live algae. These practical problems of nutrient recycling have not been discussed in the literature.

Understanding the limitations and needs for CO_2 is critical in addressing productivity of feedstock formation (that is, biomass or lipid yield depending on the processing pathway used to produce fuel). Feedstock productivity, in turn, affects the economic viability of algal biofuels. If relatively pure streams of supplemental CO_2 are required for algae cultivation, siting algae cultivation facilities close to this resource could reduce transportation costs. However, the proximity to this resource could exacerbate the siting limitations imposed by flat land and sufficient water resources. (See section on Land in this chapter.) Most published studies on algal biofuels acknowledge that efficient algae production requires external sources of concentrated CO_2 (supplied in gaseous form or as bicarbonate), in part because the rate of resupply of CO_2 to algal cultivation systems from the atmosphere can be limited by diffusion across the air-water interface (DOE, 2010b; Williams and Laurens, 2010; Pate et al., 2011). As noted in Chapter 2, the provision of supplementary CO_2 can stimulate algal biomass yield. For example, Yue and Chen (2005) observed more than a doubling

¹Amount of nutrient loss due to precipitation depends on the chemical composition of the rainwater and the nutrient composition of culture water.

of *Chlorella* biomass when CO₂ concentrations were increased over ambient atmospheric levels (see Figure 2-11 in Chapter 2). From an engineering perspective, maximum biomass yields can be achieved only with aeration or supplementation. Companies responding to the committee's solicitation of input on resource requirements indicated that they rely on supplemental CO₂ to maximize algae production.

Because atmospheric CO₂ concentration has been increasing, aquatic scientists have begun to assess the potential effects of CO₂ concentration on algal productivity and stoichiometry. In most algae, the activity of the primary photosynthetic enzyme ribulose-1,5-bisphosphate carboxylase oxygenase (Rubisco) is less than half-saturated under CO₂ levels in equilibrium with the atmosphere, suggesting that CO₂ is a factor that limits the rate of photosynthetic carbon fixation (Urabe et al., 2003). For example, by using experimental manipulations of the CO₂ concentration in supersaturated boreal lakes, Jansson et al. (2012) demonstrated that rates of phytoplankton primary production were up to tenfold higher in CO₂-supersaturated lake water relative to water containing CO₂ at equilibrium concentrations. Moreover, the supplementation of algal production systems with CO₂ potentially can enhance algal biomass yields per unit nitrogen and phosphorus in algal cells, especially under low-light conditions, thereby increasing nitrogen and phosphorus use efficiency. Even a moderate increase in CO₂ could potentially yield increased C:N and C:P ratios in algae (Leonardos and Geider, 2005; Hessen and Anderson, 2008; Van De Waal et al., 2010). In a recent large-scale outdoor experiment, a threefold increase in CO₂ relative to current atmospheric concentrations resulted in algal C:N ratios (8.0 by moles) that exceeded Redfield stoichiometry (Riebesell et al., 2007).

4.2.1 Estimated Nutrient Requirements

4.2.1.1 Carbon Dioxide

The estimated CO₂ requirements for algal biofuel production are substantial. For example, Pienkos (2007) estimated that 14-21 kilograms of CO₂ is required to produce the algal biomass needed to create 1 gallon of biodiesel (3.69-5.54 kilograms of CO₂ per liter). Pate et al. (2011) estimated that 14-35 kilograms of CO₂ is required to produce 1 gallon of algal oil (3.69-9.23 kilograms of CO₂ per liter of algal oil). Both of these values can be compared with estimates made by algal biofuel companies for their demonstration facilities. For example, Algenol estimated that 734 tonnes of CO₂ would be required to produce 100,000 gallons (378,000 liters) of ethanol each year in all its photobioreactors (DOE, 2010a); this is equivalent to 1.94 kilograms of CO₂ per liter of ethanol or 2.91 kilograms of CO₂ per liter of gasoline equivalent. Sapphire Energy estimated that 15,000 to 30,000 tonnes of CO₂ would be used annually as an additive to promote algal growth for the production of 30,000 barrels (4.78 million liters) of green crude (USDA-RD, 2009); this is equivalent to 3.14-6.28 kilograms of CO₂ per liter of green crude or gasoline equivalent, assuming that 1 liter of green crude can be upgraded to 1 liter of gasoline-equivalent fuel product.

4.2.1.2 Nitrogen and Phosphorus

Pate et al. (2011) analyzed the nitrogen and phosphorus requirements of a production system that uses open ponds for algae cultivation to produce algal oil. (This is analogous to the reference pathway or alternative pathway #1 in Chapter 3). They assumed Redfield C:N:P stoichiometry (Eq. 4-2) in algal biomass production. For simplicity, they assumed 100-percent nutrient uptake efficiencies, and did not account for the potentially higher

nitrogen and phosphorus inputs necessary to compensate for inefficiencies and losses in the biofuel production system. Nutrient recycling was not assumed in their model projections. Calculating by these assumptions, they projected that each metric tonne of dry weight algal biomass produced by their system required 88 kg N and 12 kg P. Assuming an algal biomass with 50-percent oil content, these nitrogen and phosphorus requirements are equivalent to 0.61 kg N and 0.083 kg P per gallon of algal oil produced (or 0.16 kg N and 0.022 kg P per liter of algal oil produced). However, if a 20 percent algal oil content is assumed, then these nutrient requirements increase to 1.5 kilogram of nitrogen and 0.21 kilogram of phosphorus per gallon of oil produced (or 0.40 kg N and 0.055 kg P per liter of algal oil produced). Similar estimates of 1.1 kilogram of nitrogen and 0.24 kilograms of phosphorus per gallon of biodiesel produced (0.29 kg N and 0.063 kg P per liter of biodiesel) have been reported by Yang et al. (2011).

Luo et al. (2010) suggested that direct ethanol synthesis could have lower nitrogen and phosphorus requirements than the reference pathway because cyanobacterial biomass is not removed from the production system during biofuel harvesting. Therefore, nitrogen and phosphorus are needed only for the growth and maintenance of the standing biomass; nutrients incorporated into the algal biomass are not lost during ethanol capture. Luo et al. (2010) estimated that an ethanol production of 56,000 liters per hectare (ha) per year would correspond to a nitrogen and phosphorus requirement of 0.065 g N/MJ and 0.0024 g P/MJ (0.002 kg N and 0.0001 kg P per liter of gasoline equivalent).

4.2.2 Scale-up Considerations

4.2.2.1 Carbon Dioxide

Anthropogenically produced CO₂ can be used in algal biofuel production (DOE, 2010b). For example, flue gas from coal-fired power plants is a potential source of CO₂ (Benemann et al., 2003a,b). The potential advantages of collocating algal production facilities with stationary industrial CO₂ sources and potential barriers to their use are discussed in the report, *National Algal Biofuels Technology Roadmap* (DOE, 2010b). A map of power plant sources of CO₂ located within 20 miles (32 kilometers) of municipal wastewater facilities in the preferred climate regions identified in the Sandia National Laboratories' scoping assessment is also included in that report. If collocating with stationary sources of CO₂ is not feasible, then the cost of capturing and transporting CO₂ would have to be considered.

Under the scenario assumptions that Pate et al. (2011) used, about half of the stationary emission sources in the 19 lower-tier states would be needed to supply sufficient CO₂ during daylight hours to support the production of 10 billion gallons (37.8 billion liters) per year of algal oil. For all other scenario regions (Figure 4-3), 90 percent to 150 percent of all CO₂ emission sources would be needed to produce 10 billion gallons of algal oil. They concluded that only a small number of stationary emission sources would be within a reasonably affordable access range in regions of the United States that are best suited for large-scale algal biomass cultivation, unless a costly infrastructure for CO₂ capture and pipelining is in place. Pienkos (2007) estimated that 0.9 billion to 1.5 billion tonnes of CO₂ would be needed to produce algal oil for the much larger target of 60 billion gallons (227 billion liters) of biodiesel per year. This demand for CO₂ is equivalent to 36 to 56 percent of all current CO₂ emissions from all U.S. power plants.

If CO₂ supplementation is required for high-yield production of algae, the extent to which algal biomass can be produced affordably at a commercial scale in the United States would be constrained. Moreover, as noted by Campbell et al. (2011), future commitments to

reducing atmospheric CO₂ emissions under the Kyoto Protocol suggest that there could be a substantial move toward electricity generation with low CO₂ emissions, for example, using renewable electricity (NAS-NAE-NRC, 2010) or coal-generated electricity with carbon capture and storage (NAS-NAE-NRC, 2009). Decarbonizing electricity generation would reduce the number of locations in which flue gas could be provided economically for algae cultivation for biofuels. Other sources and forms of inorganic carbon, such as the provision of solid bicarbonate, might be developed affordably for large-scale autotrophic microalgal biomass production (Pate et al., 2011). However, if the solid bicarbonate is mined from fossil sources, its use to produce algae for fuels could increase fossil energy input and the life-cycle of greenhouse-gas (GHG) emissions of algal biofuels (see Chapter 6 for discussion on GHG emissions).

4.2.2.2 Nitrogen and Phosphorus

The nitrogen and phosphorus demands that Pate et al. (2011) concluded would be necessary to support four levels of algal biodiesel produced from algae grown in four different geographical regions of the United States are shown in Table 4-3. The projected nitrogen requirements for the producing 10 billion gallons per year (37.8 billion liters per year; assuming a biomass oil content of 50-percent dry weight) of algal oil represents about half of

TABLE 4-3 Estimates of the Nitrogen and Phosphorus Resource Demands (in millions of metric tonnes/year) Required to Produce Different Levels of Algal Biodiesel Production in Different Geographical Regions of the United States

Scenario Region	Total biomass (BM) produced and projected nitrogen (N) and phosphorus (P) needed ^a in millions of metric tonnes per year (M mt/year)				Projected nitrogen and phosphorus demand for algae as percent of total U.S. use in 2006 ^b			
	10 BGY (37.8 BLY)	20 BGY (75.7 BLY)	50 BGY (189.3 BLY)	100 BGY (378.5 BLY)	10 BGY (37.8 BLY)	20 BGY (75.7 BLY)	50 BGY (189.3 BLY)	100 BGY (378.5 BLY)
Nutrient use								
SW, MW, and SE ^c with 50% lipid	BM:70 N:6.1 P:0.8	BM:140 N:12.3 P:1.7	BM:350 N:31 P:4.2	BM:700 N:61 P:8.3	N:44 P:20	N:88 P:41	N:221 P:102	N:436 P:202
NLTS ^d region with 20% lipid	BM:170 N:15 P:2.1	BM:350 N:31 P:4.2	BM:870 N:77 P:10	BM:1740 N:153 P:21	N:107 P:51	N:221 P:102	N:550 P:244	N:1093 P:512

^aAssuming elemental algal biomass composition C:N:P ratio of 106:16:1 (Redfield, 1934) and 100-percent nutrient-uptake efficiency independent of algal productivity and cultivation system area at 50-percent dry-weight biomass lipid content for SW (Southwest), MW (Midwest), and SE (Southeast) scenarios, and 20-percent lipid content for scenario region.

^bTotal U.S. consumption in 2006 estimated as 14.0 M mt elemental N consumed as ammonia and 4.1 M mt elemental P consumed as phosphate rock from Mineral Commodity Summaries in 2010 (USGS, 2010).

^cWith scenario lipid productivities of ~6,500 (SW), ~4,100 (MW), and ~4,500 (SE) gal/acre (~24,600 [SW], ~15,500 [MW], and ~17,000 [SE] L/0.40 hectares) per year at 50-percent lipid.

^dNLTS (nineteen lower-tier state region) scenario assumes moderate annual average algal lipid productivity of ~2,100 gal/acre (~7,950 L/0.40 hectares) per year at 20-percent lipid content over nineteen lower-tier states of AZ, AK, AL, CA, CO, FL, GA, IA, KS, LA, MO, MS, NE, NM, NV, OK, SC, TX, and UT.

Note: The table shows the four different levels of algal biofuel production (10, 20, 50, and 100 billion gallons per year; 37.8, 75.7, 189, and 379.5 billion liters per year (BLY), respectively) in four different geographical regions of the United States (Southwest, Midwest, Southeast, and nineteen lower-tier states). Bold values show problem levels for nutrient availability from commercial fertilizer.

SOURCE: Table 2 from Pate et al. (2011).

the total U.S. consumption of nitrogen from ammonia and about one-fifth of total U.S. consumption of phosphorus from phosphate rock in 2006. If the assumed average oil content decreases from 50 to 20 percent, these requirements are about 107 percent for nitrogen from ammonia and 51 percent for phosphorus of the total U.S. consumption in 2006. Assuming that nutrients in harvest water are not recycled, Pate et al. (2011) concluded that these algal biofuel-elevated demands for phosphorus likely would be unsustainable due to limited natural resource supplies (for example, Cordell et al., 2009; Vaccari, 2009).

4.2.3 Opportunities for Mitigation

Recycling of spent growth medium and of the residual nitrogen and phosphorus that remain in post-process algal biomass residuals will be essential for the sustainable production of algal biofuels. If anaerobic digestion is used to process waste algal biomass after lipid extraction, then cellular nitrogen and phosphorus can be recovered and recycled. The methane produced can be used to generate electricity and contribute to improving the energetic balance in the overall algae-to-biofuel process (Sialve et al., 2009). In addition, municipal and agricultural wastewater potentially can be used as nutrient feedstocks, thereby reducing external inputs of nitrogen and phosphorus fertilizers (see Chapter 5).

Sturm et al. (2012) performed a pilot-scale algal biomass production experiment using four outdoor bioreactors fed by effluent from a Lawrence, Kansas, wastewater treatment plant. Using wastewater for algae cultivation is expected to induce nitrogen-limited conditions for algal growth. These actively aerated bioreactors were run as continuous-flow systems at a hydraulic residence time of 10 days, without additional CO₂ supplementation. In contrast to natural freshwater and marine ecosystems, the algal biomass produced in these wastewater cultivation systems had a lower average biomass C:P ratio of 67:1 by moles (and thus a higher phosphorus demand per unit carbon sequestered) than would be predicted either from Redfield stoichiometry or from the analysis of Sterner et al. (2008). That ratio is also at the lower end of the range of C:P ratios observed in nutrient-replete phytoplankton cultures (C:P = 64-86; see Table A2 in Geider and La Roche, 2002). In contrast, the algae produced by these bioreactors contained a higher average C:N ratio of 17:1 by moles (and thus a *lower* nitrogen demand) than predicted by Redfield or by Sterner et al. (2008). In addition, this value is at the upper limit of the range of C:N ratios observed in nutrient-replete phytoplankton cultures (C:N = 4-17; see Table A2 in Geider and La Roche, 2002). The algal biomass produced in these wastewater cultivation systems (Sturm et al., 2012) was rich in phosphorus (low C:P ratios), and algal growth appeared to be limited by nitrogen (high C:N ratios and low N:P ratios). It is thus unclear what C:N:P stoichiometry should be assumed when calculating the potential nitrogen and phosphorus demands of large-scale algal biomass production efforts, which potentially may be supplied with nutrient feedstocks having widely varying N:P supply ratios and levels of inorganic carbon supplementation.

Colocation of biofuel production facilities with power plants could provide the facilities with a ready source of supplemental CO₂, but the number of sites suitable for colocation would constrain the use of these CO₂ sources. The use of fossil bicarbonate could mitigate the CO₂ constraint, but with consequent potential impacts on fossil energy input and life-cycle GHG emissions.

4.2.4 Sustainability Indicators

The key nutrients required for algae cultivation are CO₂, nitrogen, and phosphorus. Corresponding general indicators of sustainability of nutrient requirements include (GBEP, 2011):

- kg CO₂ required/L of fuel produced.
- kg CO₂ required/tonne dry biomass of algae.
- kg N required/L of fuel produced.
- kg N required/tonne dry biomass of algae.
- kg P required/L of fuel produced.
- kg P required/tonne dry biomass of algae.

Additional indicators for assessment of nutrient requirements for diatoms:

- kg Si required/L of fuel produced.
- kg Si required/tonne dry biomass of algae.

Additional indicators that could be developed, analogous to EROWI, are the energy return per amount of nutrient input (MJ/kg, or its inverse—nitrogen or phosphorus use, kg/MJ), and nutrients required to meet various production targets relative to existing national usage or supply.

4.2.5 Information and Data Gaps

Earlier sections discussed the use of wastewater and recycling nutrients from lipid-extracted algae as opportunities to reduce synthetic nitrogen and phosphorus inputs. Those opportunities are critical to meeting the sustainability objective of enhancing or maintaining (rather than depleting) natural resources. However, integrating wastewater treatment by algae with algae cultivation for fuels needs to undergo R&D and demonstration to optimize the systems and establish the feasibility of the concept. Similarly, R&D is needed to incorporate nutrient recycling into algal biofuel production systems. The potential for combining the use of wastewater in algae cultivation and the production of a fertilizer co-product is worth further investigation.

With respect to estimating nutrient requirements, additional calculations of the potential nitrogen and phosphorus demands of algal biofuel production need be performed to confirm or modify the values suggested by Pate et al. (2011). (See Box 4-1 for detailed discussion.) The C:N:P stoichiometry of algae is critical to estimating the quantities of nutrients required for large-scale deployment of algal biofuels.

4.2.6 Potential Effects on Social Acceptability

Large-scale deployment of algal biofuels requires large inputs of nitrogen and phosphorus fertilizers. If the nutrients are not recycled or supplied from waste sources, nutrient requirements of algae for fuels could incur indirect and unintentional impacts on food prices (Pate et al., 2011). The long-term supply of phosphorus is also cause for concern, as many believe that the world's supply of phosphate may have peaked or that a peak in supply is impending (Craswell et al., 2010). It will be detrimental to the algal biofuel industry if it is viewed as massive sink for nutrients that are in short supply, particularly if it is perceived that they are in direct competition with food producers. Technology development to use wastewater in algae cultivation and to recycle nutrients tied up in lipid-extracted algae could minimize potential competition for fertilizers between agricultural crops and algae cultivation.

BOX 4-1 Estimating Quantities of Nitrogen and Phosphorus Needed to Support an Algal Biofuel Industry

Pate et al. (2011) estimated the quantities of nitrogen and phosphorus needed to produce at least 38 billion liters of algal biofuel. Their analysis was based on the assumption of Redfield stoichiometry determined in marine systems ($C_{106}:N_{16}:P_1$). However, the canonical Redfield ratio recently has been called into question by Sterner et al. (2008), who reviewed more than 2,000 measurements of the chemical content of suspended particulate matter (seston) from freshwater and marine ecosystems worldwide and documented an enormous level of variability in nutrient use efficiency. They found that small freshwater lakes exhibited higher average ratios of C:P = 224 (standard deviation = 156) and C:N = 10.0 (standard deviation = 3.0) than the Redfield stoichiometry (C:P = 106 and C:N = 6.6 by moles). Across their entire dataset, a non-Redfield proportionality of $C_{166}:N_{20}:P_1$ best described the elemental composition of algae.

These trends potentially imply a higher nitrogen- and phosphorus-use efficiency (a higher yield of algal biomass per unit nitrogen and phosphorus consumed by algal cells) in artificial algal biomass production systems than was assumed in the study by Pate et al. (2011). Stoichiometric data provided in a recent algal biofuel study by Sturm et al. (2012) do not support this conclusion for phosphorus, however. Sterner et al. (2008) suggest that algal stoichiometry varies significantly with habitat type: freshwater seston tends to have a greater nutrient use efficiency (higher C:P and C:N ratios) than marine seston, implying that the future nitrogen and phosphorus demands of freshwater- versus marine-based algal biomass cultivation systems potentially could differ.

Another key question revolves around the potential effects of CO₂ enrichment on algal nutrient-use efficiency. The responses of both vascular plants and phytoplankton to enhanced CO₂ are variable and often species-dependent (Sardans et al., 2011a), and the consistency of CO₂ effects remains uncertain. Given the observed variation in algal C:N:P stoichiometry that has been reported in the literature, three key questions therefore arise:

- What expected values (or what ranges of expected values) of C:P and C:N would best be used to update the analyses of Pate et al. (2011)?
- At what CO₂ levels will consistent and predictable effects of CO₂ enrichment on algal nutrient-use efficiency occur?
- Will the net effects of CO₂ enrichment differ in single-strain algal cultivation systems versus systems that contain mixed-species assemblages?

These three unanswered questions represent research needs that can be filled only by field-based measurements of algal biomass yield and C:N:P stoichiometry, using pilot-scale or commercial-scale large outdoor photobioreactor systems operating under a wide range of environmental conditions.

4.3 LAND

A major constraint on the future expansion of biofuel production is likely to be the limited amount of land suitable for producing bioenergy crops and for expanding related refinery and transportation infrastructure (Cai et al., 2011). Much greater efforts will be needed to develop a comprehensive picture of the ideal siting locations for algae cultivation facilities (Darzins et al., 2010). Careful land-use planning to create specific locations where all-important resource demands can be met can help to build capacity and allow algae to make a vital, even if only modest, contribution to the U.S. biofuels industry (Lundquist et al., 2010).

The future development and scale-up of algal biofuels needs to be assessed from the multiple perspectives of site location, resource availability, and resource demands (DOE, 2010b). Key land considerations (Cai et al., 2011) can be expected to include:

- What total land area will be required for the proposed algal biofuel facility or facilities?
- What land and sites are potentially available?
- Where is this land located?
- What is this land currently being used for?
- What is the price of this land?
- What is the topography associated with this land?
- What are the climatic conditions associated with this land?
- Are the water resources required to support algal biofuel production available, either on or sufficiently near this land?
- Are the nutrient resources required to support algal biofuel production available, either on or sufficiently near this land?
- Which of the lands meeting all suitability criteria are available for actual facility siting and development?

4.3.1 Siting Requirements

The mass cultivation of algae is likely to be technically feasible in many regions of the United States (DOE, 2010b). However, the actual siting of future algal biofuel production facilities will be influenced by numerous economic, legal, political, physical, and social factors (Darzins et al., 2010). Complete detailed life-cycle assessments (LCA) and an environmental-impact assessment before any large-scale deployment are useful to and important for ensuring a smooth path to commercialization, particularly if the land being considered for siting has not ever been developed (Pienkos and Darzins, 2009). In addition, the sites where algal cultivation systems can be installed will be constrained by high land cost, agricultural activity, environmental value, and intrinsic cultural value of the land being considered (Darzins et al., 2010). Biofuel-driven land-use change (LUC) also potentially could create significant environmental impact and sustainability concerns (see Chapter 6). A diverse set of site-specific factors (Figures 4-4, 4-5, and 4-6; Table 4-4) would have to be matched carefully to the cultivation systems used for algal biofuel production if the essential requirements for successful large-scale algal biomass production (suitable land and climate, sustainable water supplies, and sustainable nutrient supplies) are to be aligned in terms of their geographical location (DOE, 2010b). For example, local variation in solar irradiance, day length, and air temperature determine the effective length of the growing season for high-yield algae cultivation, as is the case for natural lakes (Marshall and Peters, 1989). In addition, surface water evaporation rates (Figure 4-6), which affect water losses if open-pond systems are used for algae cultivation, vary geographically.

A preliminary high-level assessment of microalgal biomass production potential was performed by Sandia National Laboratories and reported in DOE (2010b). The climate criteria used in this spatially explicit analysis were: annual average cumulative sun hours of 2,800 hours or above, annual average daily temperature of 55°F (12.8°C) or higher, and annual average freeze-free days of 200 days or more. In terms of adequate sunlight and suitable climate, parts of Hawaii, California, Arizona, New Mexico, Texas, Louisiana, Georgia, and Florida appeared to be the most promising in the United States. Northern states such as Minnesota, Wisconsin, Michigan, and the New England states experienced strong seasonal variability in insolation and temperature (see Exhibit 9.4 in DOE, 2010b). The apparent lack

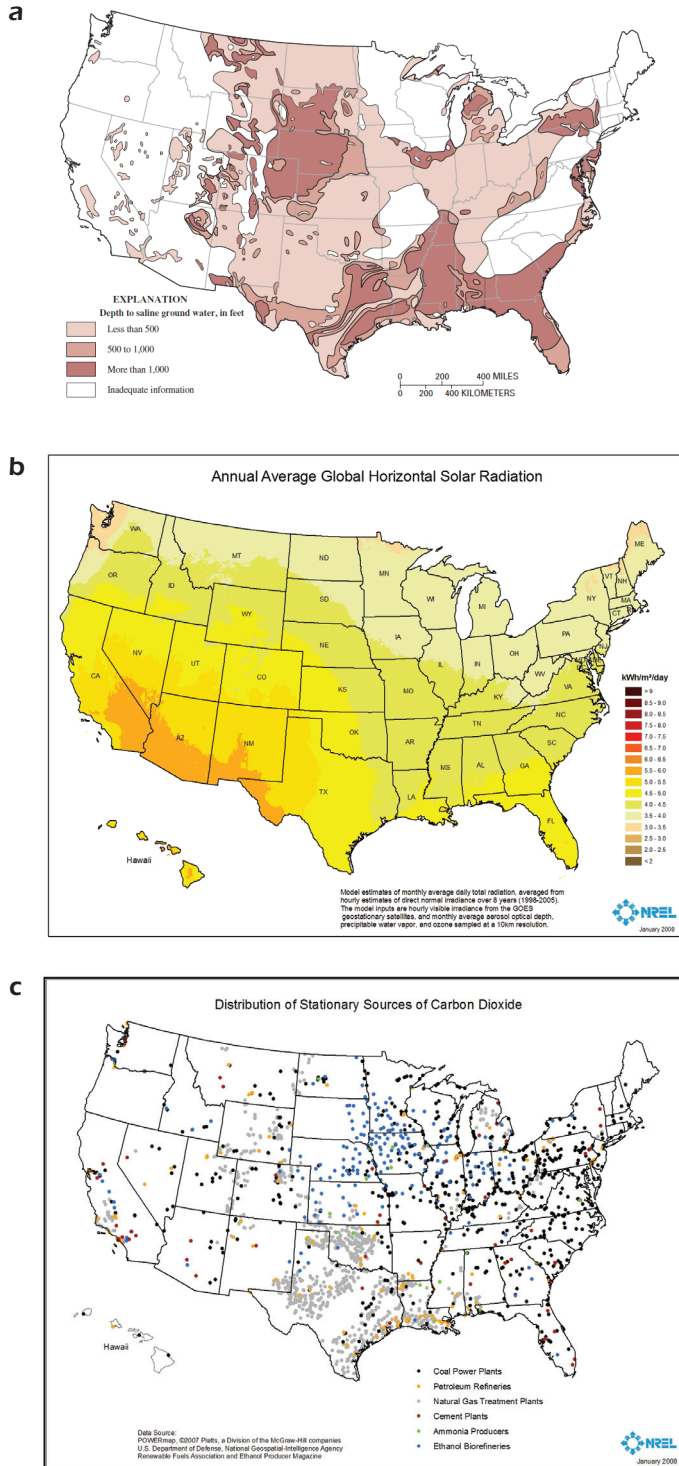


FIGURE 4-4 Resource availability for large-scale algae cultivation. NOTE: (a) Depth to saline groundwater (units); (b) Annual average solar radiation (units); (c) Large stationary sources of CO₂. SOURCE: Pienkos and Darzins (2009).

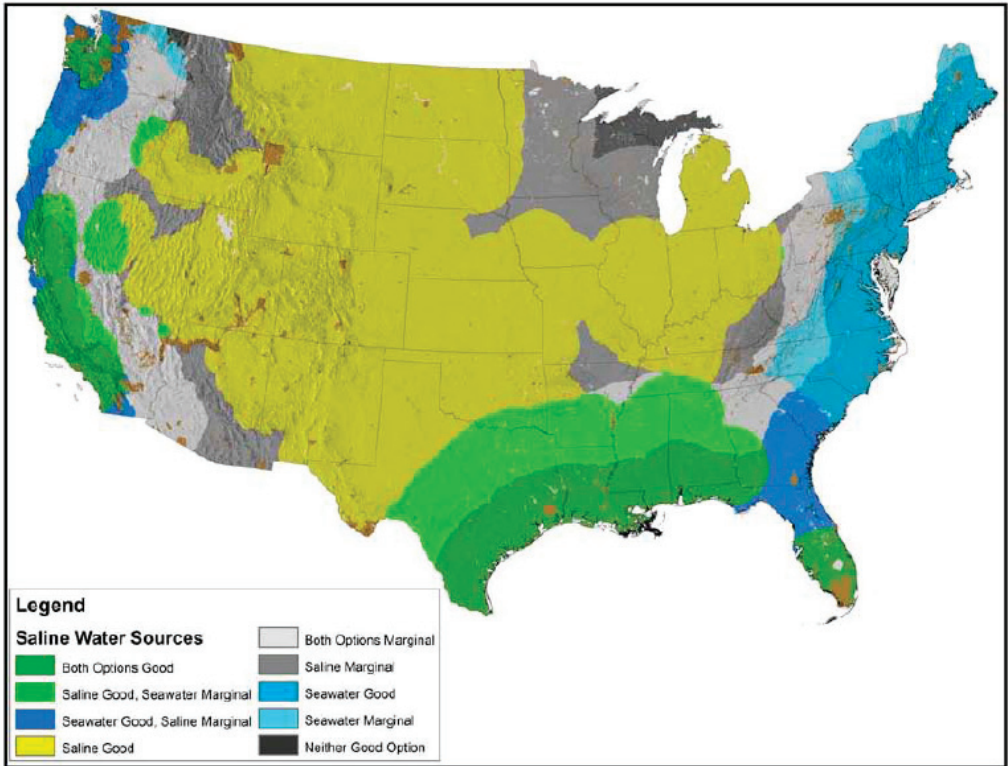


FIGURE 4-5 Map of seawater and saline groundwater resources in the continental United States, based on estimated potential energy production versus energy needed to transport water. SOURCE: Pate (2011); map created by the Pacific Northwest National Laboratory.

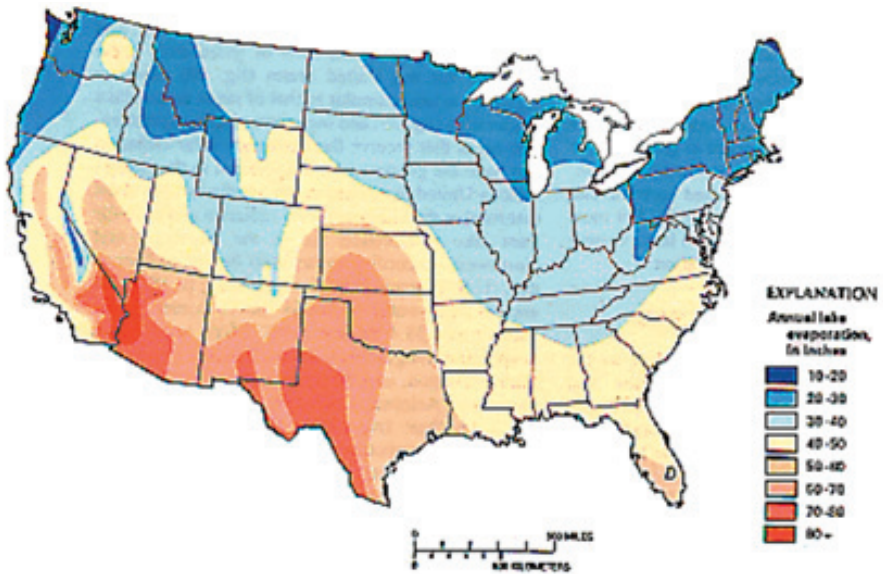


FIGURE 4-6 Mean annual lake evaporation in the continental United States. SOURCE: Hanson (1991). NOTE: 1 inch = 2.54 cm; 1 mile = 1.61 km.

TABLE 4-4 Key Land, Climate, and Water Resource Elements for Large-Scale Algal Biofuel Production

Land (Siting)	Local Climate	Water
Topography	Solar irradiance	Location
Land use and land cover	Temperature	Supply and demand
Land ownership	Evaporation rate	Salinity
Soil type and geology	Severe weather	Allocation

SOURCE: Adapted from Exhibit 9.2 in DOE (2006).

of attractiveness of the Gulf Coast region in the Sandia study was attributed to lower annual average solar insolation. In contrast, another study by the Pacific Northwest National Laboratory (Wigmosta et al., 2011) suggested that annual productivities in this region were higher than those estimated by the Sandia study. However, the predictions by Sandia National Laboratories are consistent with the map of potential algal oil production developed by Wigmosta et al. (2011) for open-pond production systems (discussed in the next section Estimated Land Requirement).

At the site level, local topography and soils also can potentially limit the availability and suitability of land for open-pond cultivation. The installation of large shallow ponds requires flat terrain that has a slope of no more than 5 percent because of the intrinsic needs of the technology and because of increased costs of site development and fluid pumping (Darzins et al., 2010). Local wind conditions affect mixing and temperature in ponds and the integrity of pond liners. High wind in a dusty or sandy location could contribute to sediment loading to open-pond cultivation systems, which may require frequent clean up.

Darzins et al. (2010) stressed that siting biofuel production systems close to water and nutrient resources would place additional limits on algal biofuels' contributions to future liquid transportation markets. They noted that even where land suitable for large-scale algal biofuel production exists, the local availability of essential resources could affect the economics of production, net energy return, and GHG emissions. The optimal sites for commercial-scale algal biofuel production would have either the required resources in close proximity or mechanisms in place to ensure adequate and uninterrupted supplies of these resources. In particular, access to large volumes of fresh water, saline water, or both will be essential for algae cultivation (see Figures 4-4a and 4-5). Darzins et al. (2010) expressed concern that the availability of sufficient supplies of supplemental CO₂ is uncertain (see Figure 4-4c) in the geographical regions that are best suited to year-round algae cultivation.

In addition to the photosynthetic surface area necessary for algae cultivation, estimates of the total land required for an algae cultivation facility would include the space required for inoculum cultivation; systems for delivering inoculum to cultivation vessels; harvesting systems; reservoirs for holding water; waste management, storage, and recycling facilities; and other support systems (Murphy and Allen, 2011). For example, basins may be needed to hold water releases from blowdown in an open-pond system. Similarly, additional land for berm formation is required if the raceway ponds are constructed with earthworks. Murphy and Allen (2011) accounted for the land required for infrastructure to support the primary cultivation facilities by using a scaling factor of 1.6:1 when estimating the total area burden for open raceway ponds. This scaling factor is the ratio of photosynthetic pond surface area to the area of associated land needed to support those ponds. Clarens et al. (2011) used a smaller scaling factor of 1.25:1.

Algae cultivation facilities could be sited on unprofitable cropland (typically land that is not suitable for commodity crops), in which case the potential for large-scale algal biofuel production to affect food production would have to be considered. Pre-existing surface

water resources (for example, aquaculture ponds and coastal zone waters) also could be considered. In addition, algal biofuel facilities could be sited on lands that are highly suitable for solar energy production, such as arid lands in the southwest or sunny coastal regions. However, insufficient information is currently available to assess the viability or production capacity of these potential pathways. Moreover, potential tradeoffs (for example, water availability) would have to be considered.

Nutrient removal from wastewater by algae can be coupled with biofuel production (Pittman et al., 2011). For example, more than 15,000 existing U.S. domestic wastewater treatment plants (WWTPs) collectively produce about 34 billion gallons (128.7 billion liters) of wastewater effluent per day (EPA, 2008). Several thousand small (less than 10 ha) and a few large-scale (more than 100 ha) algal pond systems currently are being operated in the United States for municipal wastewater treatment (Lundquist et al., 2010). Food processing facilities and agricultural dairy and feedlot operations are other sources of nutrient-rich liquid wastes. Operations such as these potentially provide desirable colocation sites in which wastewater could be used as a water and nutrient source for integrated algal biomass production systems. One problem is that sufficient land is rarely available in WWTP locations in urban areas. Two types of systems can be envisioned: dedicated algae cultivation facilities, whose primary purpose would be the production of algal biomass (such systems also would require wastewater treatment and nutrient recycling); and wastewater treatment facilities, whose primary goal would be to perform wastewater treatment, but would produce harvestable algal biomass as a consequence of the treatment process (see Exhibit 9.7 and associated text in DOE, 2010b). Such integrated systems would be expected to have multiple benefits, including providing an inexpensive nutrient source, reducing demands for other sources of fresh water, potentially reducing operating costs for wastewater facilities, improving the quality of treated effluents, and potentially reducing GHG emissions (Craggs et al., 2011; Wiley et al., 2011). However, the number of potential colocation sites with sufficient adjacent land area that would be suitable for large-scale algal biomass cultivation is unclear.

Other innovative siting efforts might be feasible if suitable supplies of light and other resources are available. For example, aquaculture is an extensive industry in the United States; many catfish production ponds are out of production and thus potentially are available for alternative use in algal biofuel production. More than 100,000 hectares of catfish production ponds potentially could be available in the state of Mississippi alone (Hanson, 2006) if these existing facilities were found to be suitable for algal biomass production and harvesting.

4.3.2 Estimated Land Requirements

Algal biomass production potentially requires much less land area than terrestrial biofuel feedstock cultivation. The land requirements for algal biofuel production are potentially 1-2 orders of magnitude lower than any crop-based biofuel, whether based on volumetric yield or energy yield per unit area (Table 4-5; see also Table 1 in Singh et al., 2011). Future improvements in algal oil content and areal productivity (kg/ha per year) of algal cultivation systems can be expected to further lessen the amount of land needed to produce a given quantity of liquid biofuel (Pienkos and Darzins, 2009).

Wigmosta et al. (2011) performed a national-scale assessment of potential algal biofuel production and its resource uses in open-pond systems. The assessment was based on a theoretical facility consisting of 100 30-centimeter deep, 4-hectare ponds that required about 400 hectares of land for the ponds themselves, and an additional 90 hectares to

TABLE 4-5 Ranked Comparison of the Oil Yield and Land-Use Requirements of Microalgae with Nine Agricultural Crop-Based Biodiesel Feedstocks

Plant Source	Seed Oil Content (% oil by weight in biomass)	Oil Yield (L oil/ha year)	Land Use (m ² year/kg biodiesel)	Biodiesel Productivity (kg biodiesel/ ha year)
Soybean (<i>Glycine max</i> L.)	18	636	18	562
Camelina (<i>Camelina sativa</i> L.)	42	915	12	809
Canola/rapeseed (<i>Brassica napus</i> L.)	41	974	12	862
Sunflower (<i>Helianthus annuus</i> L.)	40	1,070	11	946
Microalgae (low oil content)	30	58,700	0.2	51,927
Microalgae (medium oil content)	50	97,800	0.1	86,515
Microalgae (high oil content)	70	136,900	0.1	121,104

SOURCE: Adapted from Mata et al. (2010). Reprinted with permission from Elsevier.

accommodate operational infrastructure. Potential algal oil production and its associated land and water resource requirements were simulated on the basis of the dominant physical processes that affect algal growth. The supplies of water, nutrients, and CO₂ were not limited in the simulations to provide theoretical estimates for annual mean and annual maximum open-pond microalgae production if all the sites with suitable land and water requirements are developed.

The analysis by Wigmosta et al. (2011) identified 11,588 non-competitive areas totaling 430,830 km² that potentially could be used for large-scale, open-pond algae production. Strong geographical variation in their theoretical annual mean levels of biofuel production (L/ha per year) was projected (Figure 4-7)—the land and freshwater availability favors locations in the Gulf Coast region. The highest predicted rates of annual mean production occurred in South Central Texas and much of Florida, but land prices in those regions could affect the feasibility of their use for algae cultivation. Their study suggested that under the assumptions of their analysis and current technology, algae can potentially be cultivated at large scale in 5.5 percent of the land in the conterminous United States to generate 220 billion liters of oil per year (Wigmosta et al., 2011). That amount of algal oil is equivalent to 28 percent of total U.S. petroleum consumption for transportation.

Pate et al. (2011) also performed quantitative assessments of the land demands resulting from algal biofuel generation and concluded that land requirements were likely to be the most manageable among the resource demands (water, nutrients, and land) considered in their study. As is the case with water requirements, land demands vary with the geographic region, the quantity of feedstock to be produced, and the level of algal productivity assumed to be achieved. They concluded that the Southwestern and the Nineteen Lower-Tier State regions in the United States potentially were more likely to meet the siting requirements for algal biofuel production scale-up than the Midwestern and Southeastern regions. However, the total land area required to meet targeted biofuel production levels is expected to be inversely correlated with the annual biomass productivity and algal lipid contents that actually can be achieved in practice (see Figure 2 in Pate et al., 2011) (see also Figure 3a in Xu et al., 2011). Batan et al. (2010; 2011) used a detailed, industrial-scale engineering model for photobioreactor-grown *Nannochloropsis* to calculate a land requirement of 4.41 million hectares (10.9 million acres) to produce 40 billion gallons (151.4 billion liters) of microalgae-derived biodiesel per year. That land area is equivalent to 16 percent of the total land area in Colorado and 0.45 percent of U.S. land area.

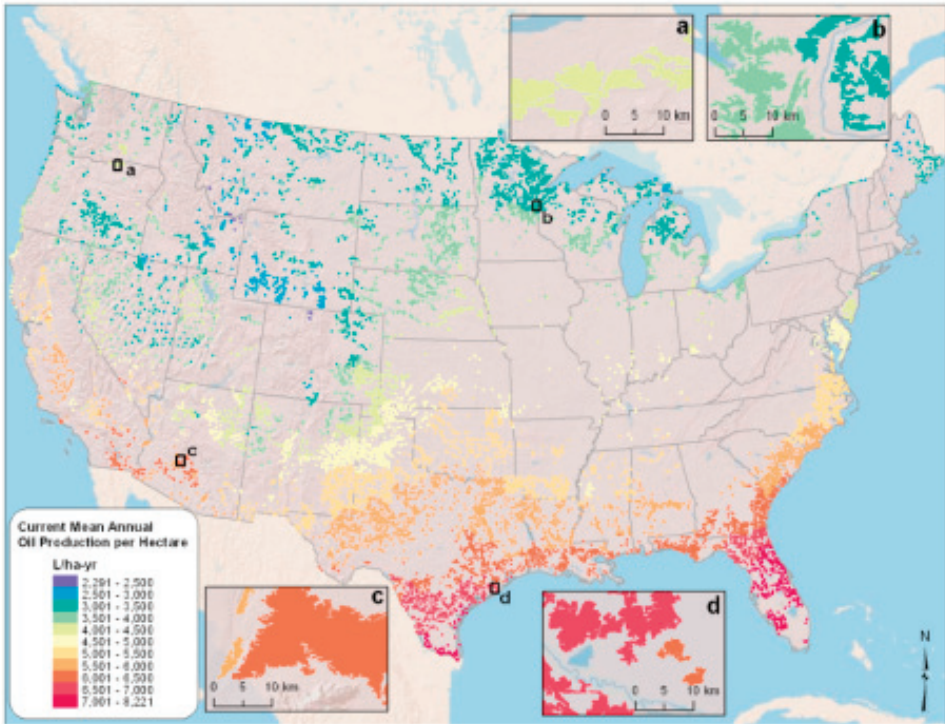


FIGURE 4-7 Estimated mean annual algal oil production (L/ha per year) under current technology in the continental United States, predicted by a GIS-based analysis. The insets illustrate finer-level detail at two northern and two southern locations.

SOURCE: Wigmosta et al. (2011). Reprinted with permission from the American Geophysical Union.

4.3.3 Opportunities for Mitigation

Opportunities for mitigating land requirements for algal biofuel systems lie in productivity—the only way to reduce the area required is to increase the productivity per unit area. Thus, developing more highly productive algal strains, engineering different pond designs, or using photobioreactors rather than ponds all would reduce land requirements. The related topics of impacts of land use and how to mitigate those effects are discussed in Chapter 5.

4.3.4 Sustainability Indicators

Indicators of sustainability of land requirements for algal biofuel production include the following:

- Liters of fuel produced per hectare.
- Kilograms of fuel produced per hectare.
- Energy return on land invested (MJ/ha), analogous to EROWI.
- Land required to meet various production targets relative to existing national usage or supply.

The definition of the indicators would need to specify whether energy needed to prepare land and to transport resources to the site is included. Land indicators are highly dependent on where the land is, though all the indicators have at least some site specificity. DOE could help address these indicators through more detailed studies of the potential biofuel production capacities of specific technologies in various regions of the country.

4.3.5 Information and Data Gaps

Land-use availability databases, land-suitability databases, and geo-spatial resource maps compiled specifically for the purpose of assessing the potential for algal biofuel production are incomplete (Darzins et al., 2010). A number of relevant resource maps for the United States and the world can be found in Lundquist et al. (2010), who also present a detailed geographic information systems (GIS)-based analysis of algal biofuel production in California. Continued efforts to perform meta-analyses of existing LCAs are desirable to comprehensively assess the land requirements and the most likely site locations for future algal biofuel production.

4.3.6 Effects on Social Acceptability

Part of the appeal of algae production as a renewable fuel source is the potential small land area required to produce a given quantity of energy relative to terrestrial crops. However, commercial-scale algal biofuel facilities, especially those relying on open-pond cultivation systems, still will require thousands of hectares of land to achieve sufficient economies of scale. Despite the algal industry's general focus on using marginal and degraded land for development of commercial-scale facilities, questions still may be raised as to the potential social and health-related risks of developing such large areas for the purpose of algae production.

Land in the desert Southwest is suitable for the scale-up of algae cultivation facilities because of favorable climatic conditions for algal growth. While much of the land in this region has been designated as marginal due to its inability to support food production, public support for large-scale land developments, even in the renewable-energy sector, is not guaranteed. The arid conditions and infertile soil in the desert Southwest support highly fragile ecosystems that take far longer to recover following major disturbances than ecosystems in wetter climates. These areas also contain a number of threatened or endangered species that are the focus of major conservation efforts and public awareness campaigns, such as the desert tortoise. Setbacks to the construction of commercial-scale photovoltaic facilities have resulted over concern for the vulnerability of these ecosystems, forcing solar developers to purchase additional land as conservation easements and to create wildlife rehabilitation and protection programs (BLM and DOE, 2010). The algal biofuel industry likely will face similar hurdles as it continues toward commercialization; actively engaging the public and conservation organizations during the site selection and permitting process could help overcome those barriers.

Even if algae developers have gained acceptance from the public, the definition of marginal or degraded land is fluid and depends on both the technology available for farming and the macroeconomic and geopolitical conditions at a given time. For example, land that is considered marginal and unsuitable for traditional freshwater farming today may be considered suitable in the future if a cultivar of a food crop is developed to tolerate dryer or less fertile soils. Pressure to use that land for food rather than energy production

may be intensified if food security concerns escalate. This pressure would be magnified significantly should breakthroughs in desalination technology make farming viable in areas where it was previously uneconomical to move fresh ground or surface water. In such a scenario, the acceptability of an algae development that has been permitted successfully and previously in good standing with local communities and the public at large could be called into question.

Land may have more value for development or recreational purposes than for massive open-pond systems. Local municipalities and communities may not be open to production facilities being located close to areas that might be developed to attract retirees, tourists, or other economic development projects. Wind turbines are perceived by some to be aesthetically displeasing, and so could these large open or closed algal production systems. The advantages and disadvantages of using prime recreational or production lands for algal biofuel production will have to be discussed, debated, and decided upon by the stakeholder community. If oil prices continue to rise, or if foreign oil supplies suddenly were no longer available, then the argument to use land for algae production for biofuels over any economic development or aesthetic might be more pro than con.

4.4 ENERGY

To exploit the high photosynthetic efficiency of algae, energy must be invested in cultivation systems and biorefineries to grow the algae, to manage water utilizations, and to process algae into the desired fuel. Given the considerable energy use in the supply chains of other biofuels, (for example, Farrell et al., 2006), analysis of the full fuel cycle of algal supply chains is critical to understanding energy implications. This section reviews the state of knowledge of the energy properties of algal biofuel production systems.

4.4.1 Life Cycle Energy Studies of Algal Biofuels

The method to assess energy and material flows of supply chains is LCA (see also Chapter 1). The primary challenge in applying LCAs to algal biofuel production is the early stage of development of the technology. It is not yet clear what technologies along the processing chain will emerge as the most commercially feasible. Also, many technologies are in the laboratory or pilot-scale stage. Their technical (and energy) characteristics when scaled up to the industrial level are not yet clear. Nonetheless, there is a great deal of recent research activity to assess life-cycle energy use of algal biofuel production. Table 4-6 shows the energy return on investment (EROI) from a selection of recent publicly available studies of raceway systems. EROI is defined as the ratio of total energy outputs (biofuel + coproducts) to energy inputs, where energy inputs are summed over the life cycle: cultivation, nutrient procurement, harvesting, extraction, processing, and associated supply chains (Eq. 4-3).

$$EROI = \frac{\text{Energy outputs of fuel and coproducts}}{\text{Energy inputs for growing and processing}} \quad (\text{Eq. 4-3})$$

Unless the EROI for an energy production system is greater than 1.0, energy needed to make a fuel is greater than energy contained in the fuel and coproducts. Thus, production pathways for algal biofuels that have EROI less than 1 clearly are unsustainable. Ideally, the EROI of an algal biofuel is at least comparable to the EROI of other alternative fuels.

TABLE 4-6 Energy Return on Investment (EROI) Values for Open-Pond Systems

Source	Nutrients	Harvesting	Extraction/ Processing	Products and Coproducts	EROI
Brentner et al. (2011) reference case	Flue gas	Centrifuge, drying, press	Hexane/esterification	BD ^b	0.13
Brentner et al. (2011) reference case with coproducts	Flue gas	Centrifuge, drying, press	Hexane/esterification	BD ^b , electricity, nutrients	0.28
Brentner (2011) best case swap raceways	Flue gas	Chitosan-flocculation	Supercritical methanol	BD ^b , electricity, nutrients	0.96
Clarens et al. (2010)	Industrial CO ₂	Alum flocculation, centrifuge	-	Algal biomass	1.06
Clarens et al. (2011) C-2	CC ^a coal plant	Auto-flocculation, gravity, homogenization	Hexane/esterification	BD ^b , electricity, nutrients	1.36
Clarens et al. (2011) C-3	Flue gas	Auto-flocculation, gravity, homogenization	Hexane/esterification	BD ^b , electricity, nutrients	1.99
Clarens et al. (2011) C-4	WW ^c , flue gas	Auto-flocculation, gravity, homogenization	Hexane/esterification	BD ^b , electricity, nutrients	2.32
GREET baseline pathway	Flue gas	Bio-flocculation, flotation, centrifuge, homogenization	Hexane/esterification	BD ^b , electricity, nutrients	0.39
Jorquera et al. (2010)	N/A	N/A	N/A	Algal biomass	7.01
Sander and Murthy (2010) filter press	N/A	Filter press, drying	Hexane/esterification	BD ^b , fermentation stock	3.33
Sander and Murthy (2010) centrifuge	N/A	Centrifuge, drying	Hexane/esterification	BD ^b , fermentation stock	1.77
Stephenson et al. (2010)	Flue gas	Alum flocculation, centrifuge, homogenization	Hexane/esterification	BD ^b , electricity, nutrients	1.60
Vasudevan et al. (2012) dry extraction, nominal	Flue gas	Dissolved air flotation, centrifuge	Belt dryer	BD, electricity	0.3
Vasudevan et al. (2012) wet extraction, nominal	Flue gas	Dissolved air flotation centrifuge	Stream lysing, centrifuge, wash	BD, electricity	2.51

^aCC = carbon capture.

^bBD = biodiesel.

^cWW = wastewater.

Notes: The GREET (Greenhouse Gases, Regulated Emissions, and Energy Use in Transportation) model is a spreadsheet model developed at the Argonne National Laboratory for evaluating energy and emission impacts of advanced vehicle technologies and new transportation fuels.

Results in EROI show tremendous variation. The values at the low end suggest that the technology in its current form is not feasible for net energy return. In contrast, values at the high end suggest algal biofuel could have EROI considerably higher than corn-grain ethanol (EROI = 1.2; see Farrell et al., 2006). One source of variation in EROI is differences in choices of processes and inputs in the supply chain. Table 4-6 summarizes some results from comparison of technology systems. The three scenarios from Clarens et al. (2011) show changes as a function of carbon source and use of wastewater as replacement of nutrient input. Brentner et al. (2011) and Sander and Murthy (2010) highlighted how changing harvesting (and extraction) technologies have major effects on energy use.

TABLE 4-7 Energy Use in Cultivation Stage in Closed Photobioreactors

Source	Bioreactor Type	Cultivation Energy (MJ Input/MJ Biodiesel)
Stephenson et al. (2010)	Air lift tubular	6
Jorquera et al. (2010)	Air lift tubular	14
Jorquera et al. (2010)	Flat plate	0.61
Brentner et al. (2011)	Annular	19
Brentner et al. (2011)	Tubular	57
Brentner et al. (2011)	Flat plate	1.4

Coproducts significantly affect the energy analysis. The typical scenario analyzed is anaerobic digestion of algae residuals to produce electricity and recover nutrients. One can see from Brentner et al. (2011) that changing from landfilling of algae residuals to anaerobic digestion nearly doubles EROI in their calculations. In Sander and Murthy (2010), the energy credit for using algae residuals is 10 times larger than the energy content of the produced biodiesel. This result can be interpreted as an assertion that the algal biorefinery becomes primarily a source of fermentation inputs and biodiesel is a secondary output.

There is considerable variability within similar technology or supply pathways—for example, Sander and Murphy's EROI of 1.77 versus Brentner et al.'s EROI of 0.28. This variability is related to the use of different data or assumptions for the same processes and different boundary choices for supply chains. Variability in data has two sources. One source of variability is generic in LCA. Different analysts choose different data sources, and there is not a systematic way to realize convergence (Williams et al., 2009). The second source of variability is tied to the emerging nature of the technology. LCA is a method designed to assess existing technologies through chaining process input-output tables. Many processes in algal biofuel production systems are still in the laboratory or pilot phase. There is much uncertainty in how these technologies will evolve and scale up in the future; actual energy use could be much higher or much lower than suggested by the current suite of initial LCA studies.

The results in Table 4-6 address open-pond cultivation. Given the potential for closed photobioreactors to mitigate other resource and environmental issues such as water consumption (Harto et al., 2010), the energy use of closed systems is important to consider. A few studies estimated the direct energy use for the feedstock cultivation step in algal biofuel production systems that use photobioreactors (Table 4-7; Jorquera et al., 2010; Stephenson et al., 2010; Brentner et al., 2011). Tubular and annular reactors are thought to require far more energy to operate than is contained in the biodiesel product. Flat-plate reactors are thought to require far less energy, though Brentner et al. (2011) reported a net energy input higher than contained in biodiesel output (1.4 megajoules per megajoule of biodiesel). While the caveats for other results apply here as well, these studies suggest that the energy use of photobioreactors could fundamentally affect the net energy balance of algal biofuels (Jorquera et al., 2010).

4.4.2 Energy Requirements in the Supply Chain and Credits for Coproducts

Analyses of prior studies provide insight into the current understanding of what production stages are important contributors to energy requirements despite the large uncertainties and variability associated with energy requirements of algal biofuel production. Table 4-8 abstracts results from a meta-analysis of LCA studies to summarize the range in energy requirements of different stages (Liu et al., 2011). This meta-analysis included data

TABLE 4-8 Range in Energy Requirements by Process Stage for Open-Pond Cultivation of Algae to Produce Biodiesel (Plus Coproducts)

Production Stage	Energy Requirement (MJ/MJ Biodiesel)
Nutrients (fertilizer + CO ₂)	0.2-1.6
Cultivation	0.04-3.14
Separation	0.01-0.26
Extraction	0.19-0.51
Conversion	0.03-0.22

SOURCE: Liu et al. (2011). Reprinted with permission from Elsevier.

from several studies (Lardon et al., 2009; Clarens et al., 2010; Jorquera et al., 2010; Sander and Murthy, 2010; Stephenson et al., 2010; Campbell et al., 2011).

The high end of the range of energy requirements for nutrients corresponds to the use of virgin fertilizers and industrial CO₂ in production. Cultivation consumes energy primarily for mixing and pumping water. Murphy and Allen (2011) suggested that energy needs for water management could be substantially higher than current LCA studies indicate. Extraction is generally assumed to be via hexane solvent and conversion to biodiesel via transesterification.

The treatment of the energy credits for coproducts is critical in the energy balance of algal biofuels. For production of bio-electricity, the energy credit per megajoule of biodiesel ranges from 0.3-1.3 megajoule/megajoules biodiesel (Lardon et al., 2009; Stephenson et al., 2010; Campbell et al., 2011). Sander and Murthy (2010) assume that residual biomass from algae production substitutes for corn used in ethanol production, yielding an energy credit for the coproduct of 10.7 megajoules/megajoule biodiesel.

4.4.3 LCA Issues Related to Algal-Lipid Processing

Pathways described in Chapter 3 describe two different ways of converting crude algal lipids to liquid fuels. Both yield fuels suitable for use in diesel applications. The majority of the published LCAs assumed the production of FAME diesels, which are less desirable than “drop-in” fuels because of FAME’s incompatibility with existing infrastructure for petroleum-based fuels. Given the lack of LCA work on green diesel products from algae, differences in energy use between FAME and green diesel are addressed by analogy with conventional diesel processing. A number of studies of conventional diesel processing that have been reviewed allow comparisons to be made (Kalnes et al., 2009).

Comparison between seed oil-derived diesel fuels treated by esterification and by hydrotreating show that there is little difference in either the energy return or carbon emissions. These studies start with the same raw oils, meaning that the differences only reflect the processing to finished fuel. Figure 4-8 shows that these are nearly identical and, therefore, life-cycle impacts will be similar between esterification processes and hydrotreating.

4.4.4 Opportunities for Mitigation

Keeping other factors constant, increasing the productivity of algal growth drives down energy use for cultivation and harvesting. This said, if achieving higher productivity involves major process changes, such as using photobioreactors instead of raceways, the

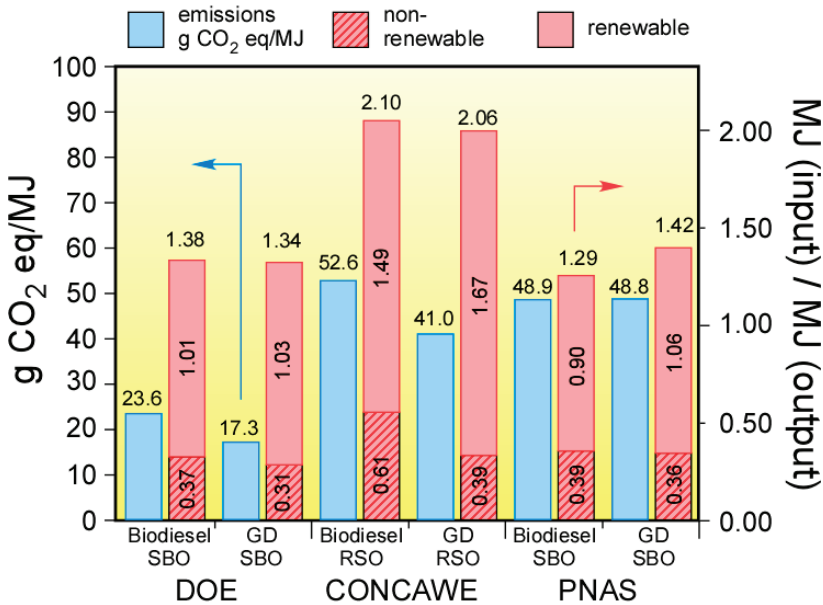


FIGURE 4-8 Comparison of conventional esterified biodiesel production with green diesel (GD) production based on soybean oil (SBO) and rapeseed oil (RSO).

NOTE: Each of the three completed studies shows little difference on energy use or carbon emissions between green diesel and biodiesel. By analogy, green diesel production from algal lipids relative to conventional biodiesel processing is likely to have similar life-cycle impacts. SOURCE: Adapted from Kalnes et al. (2009).

embodied energy of the process change needs to be assessed and compared to direct energy savings. Water management is clearly an important factor in energy use. Efficient pumps and gravity-driven designs, for example, could mitigate high energy use for water management. The embodied energy in providing nutrients, including CO₂, can be substantial. The extent to which waste products can be used as nutrients has the potential to substantially reduce energy use. Separating algal products from water is a major factor driving energy use in separation and extraction. Drying processes in particular are energy intensive. Brentner et al. (2011) called attention to the potential of supercritical processes to reduce energy use for processing algae. Given the importance of coproducts in the net energy balance, developing higher “energy value” coproducts could be an important mitigation strategy.

4.4.5 Sustainability Indicators

A number of different metrics are already in use to assess energy systems (Farrell et al., 2006; GBEP, 2011; Baral et al., 2012), including:

- EROI.
- Net Energy Value (NEV).
- Fossil Inputs.

Before discussing the different measures in more detail, it is important to first recapitulate the definition and quantification of energy use. The key issue is that energy inputs and outputs come in different forms having differing utility, in particular chemical (for example, heating value of fuel), electricity, and heat. While one could simply add different energy types by unit conversion, different forms of energy are not interchangeable. For example, it takes more than one unit of heat to make one unit of electricity. One approach to put different energy forms on a comparable basis is the idea of primary (or source) energy. The precise definition of primary energy varies, but in general it includes the heat or fossil inputs needed to make electricity. In some cases, it also includes indirect energy use associated with delivering fossil fuels. For the U.S. energy system, one common conversion used is 3.4 megajoules of upstream primary energy per kilowatt hour of electricity. Analysts often use different definitions of energy and do not always explicitly state which definition is being used. Care is needed when comparing energy results from different studies.

Energy outputs generally include only those utilized; that is, waste heat is not included. Energy output is estimated by direct unit conversion, conversion to source energy, or in some cases, using the energy needed to make products that coproducts replace. NEV forms the difference.

$$NEV = \text{Energy outputs of fuels and products} - \text{Energy inputs.}$$

Fossil inputs measure quantities of fossil fuels used in processing. EROI and NEV refer generically to energy, which could be supplied by fossil or renewable forms. Fossil inputs are thus specific to how heat and electricity are supplied and thus require definition of the enveloping energy system.

4.4.6 Information and Data Gaps

Much uncertainty remains as to the current and future energy properties of algal cultivation systems, pointing to critical gaps. Scarcity of data on material flows at existing scales of algal biofuel production presents a challenge in assessing EROI. LCA studies of algae use process modeling to estimate energy consumption. Additional empirical data can help validate these models.

Although there are gaps in data, data collection by itself will not resolve the uncertainties of life-cycle energy implications of algal biofuels. The true energy behavior is a result of scale-up and learning processes that bring algae from the laboratory and pilot scale to industrial scale. While future energy behavior is challenging to forecast, given the substantial investment and path dependence associated with bringing an energy technology to scale, due diligence demands that a serious forecasting effort be made. While there are a variety of cost forecasting methods available, such as learning curves and scaling factors, methods to forecast energy and material flows of developing technologies are undeveloped. Efforts need to be made to develop such methods. Increased data availability from laboratory and pilot scales is critical to calibrate and validate the forecasting methods that emerge.

4.5 CONCLUSIONS

A review of published literature suggests that the scale-up of algal biofuel production to yield 37.8 billion liters of algal oil (10 billion gallons) would place an unsustainable demand on energy, water, and nutrients with current technology and knowledge. Estimated

values for EROI range from 0.13 to 3.33. The estimated consumptive use of fresh water for producing 1 liter of gasoline equivalent of algal biofuel is 3.15–3,650 liters. The estimated requirement for nitrogen and phosphorus needed to produce 37.8 billion liters of algal biofuels ranges from 6 million to 15 million metric tons of nitrogen and from 1 million to 2 million metric tons of phosphorus if the nutrients are not recycled or included and used in coproducts.

Freshwater use for production of algal biofuel is inevitable because fresh water is necessary to compensate for water loss and to avoid salt buildup as a result of evaporation during cultivation. Two key drivers for freshwater requirement in algal biofuel production are evaporative loss in open-pond cultivation and discharge of harvest water in biofuel production systems that harvest the algae. Therefore, water use would be a serious concern in an algal biofuel production system that uses fresh water in open ponds without recycling harvest water. Freshwater use also could be reduced by using saltwater or brackish water in algae cultivation. However, information on the availability of inland saline water resources is sparse.

Recycling of harvest water also is important in reducing nitrogen and phosphorus input to algae cultivation. To promote high yield, algae are cultivated in nutrient-rich media. The nutrients will be lost if harvest water is not recycled. If algal biomass is harvested to process to fuels, there could be another opportunity to recover nitrogen and phosphorus after processing because the fuel products do not contain those two elements.

Recycling nutrients via reuse of harvest water or the use of wastewater from agricultural or municipal sources provides an opportunity to reduce energy use, as synthetic fertilizer input contributes to energy input over the life cycle of algal biofuels. Energy inputs for water management (for example, pumping groundwater, and moving water in cultivation systems), harvesting, and water extraction (for example, drying of algal biomass) are two key drivers in the overall energy balance of algal biofuel production systems.

A key aspect to sustainable development of algal biofuels is siting (for example, suitable climate and collocation of key resources) and recycling of key resources. Siting of algal biofuel production facilities needs to account for climate, topography, and proximity to water and nutrients. Siting algae cultivation far away from water and nutrient resources would incur additional costs and energy consumption for transporting those resources to the cultivation facilities. Although several studies assessed land, water, and nutrient requirements and energy balance of algal biofuel production, few studies considered all four requirements simultaneously.

A national assessment of land requirements for algae cultivation that takes into account climatic conditions; fresh water, inland and coastal saline water, and wastewater resources; sources of CO₂; and land prices is needed to inform the potential amount of algal biofuels that could be produced economically in the United States.

SUMMARY FINDINGS FROM THIS AND EARLIER CHAPTERS

Based on a review of literature published until the authoring of this report, the committee concluded that the scale-up of algal biofuel production sufficient to meet at least 5 percent of U.S. demand for transportation fuels^a would place unsustainable demands on energy, water, and nutrients with current technologies and knowledge. However, the potential to shift this dynamic through improvements in biological and engineering variables exists. (See also Chapters 2 and 3 on improvements in biological and engineering variables.)

Sustainable development of algal biofuels would require research, development, and demonstration of the following:

- **Algal strain selection and improvement to enhance desired characteristics and biofuel productivity.** (See Chapter 2.)
- **An EROI that is comparable to other transportation fuels, or at least improving and approaching the EROIs of other transportation fuels.**
- **The use of wastewater for cultivating algae for fuels or the recycling of harvest water, particularly if freshwater algae are used.**
- **Recycling of nutrients in algal biofuel pathways that require harvesting unless coproducts that meet an equivalent nutrient need are produced.**

A national assessment of land requirements for algae cultivation that takes into account climatic conditions; fresh water, inland and coastal saline water, and wastewater resources; sources of CO₂; and land prices is needed to inform the potential amount of algal biofuels that could be produced economically in the United States.

^a U.S. consumption of fuels for transportation was about 784 billion liters in 2010. Five percent of the annual U.S. consumption of transportation fuels, which would be about 39 billion liters, is mentioned to provide a quantitative illustration of the water and nutrients required to produce algal biofuels to meet a small portion of the U.S. fuel demand.

REFERENCES

- Alley, W.M. 2003. Desalination of Ground Water: Earth Science Perspectives. Reston: U.S. Geological Survey.
- Baral, A., B.R. Bakshi, and R.L. Smith. 2012. Assessing resource intensity and renewability of cellulosic ethanol technologies using Eco-LCA. *Environmental Science and Technology* 46 (4):2436-2444.
- Batan, L., J. Quinn, T. Bradley, and B. Willson. 2011. Erratum: Net energy and greenhouse gas emissions evaluation of biodiesel derived from microalgae (*Environmental Science and Technology* (2010) 44 (7975-7980)). *Environmental Science and Technology* 45(3):1160.
- Batan, L., J. Quinn, B. Willson, and T. Bradley. 2010. Net energy and greenhouse gas emission evaluation of biodiesel derived from microalgae. *Environmental Science and Technology* 44(20):7975-7980.
- Benemann, J., P.M. Pedroni, J. J. Davison, H. Beckert, and P. Bergman. 2003a. Technology roadmap for biofixation of CO₂ and greenhouse gas abatement with microalgae. Paper read at the Second Annual Conference on Carbon Sequestration, May 5-8, Alexandria, VA.
- Benemann, J.R., J.C. Van Olst, M.J. Massingill, J.C. Weissman, and D.E. Brune. 2003b. The controlled eutrophication process: Using microalgae for CO₂ utilization and agricultural fertilizer recycling. Paper read at the 6th Greenhouse Gas Control Technologies Conference (GHGT6), October, Kyoto Japan.
- BLM (Bureau of Land Management) and DOE (U.S. Department of Energy). 2010. Solar Energy Development Draft Programmatic Environmental Impact Statement (Draft Solar PEIS). Washington, D.C.: Bureau of Land Management and U.S. Department of Energy.
- Boyd, C.E., and A. Gross. 2000. Water use and conservation for inland aquaculture ponds. *Fisheries Management and Ecology* 7(1-2):55-63.
- Brentner, L.B., M.J. Eckelman, and J.B. Zimmerman. 2011. Combinatorial life cycle assessment to inform process design of industrial production of algal biodiesel. *Environmental Science and Technology* 45:7060-7067.
- Cai, X.M., X.A. Zhang, and D.B. Wang. 2011. Land availability for biofuel production. *Environmental Science and Technology* 45(1):334-339.

- Campbell, P.K., T. Beer, and D. Batten. 2011. Life cycle assessment of biodiesel production from microalgae in ponds. *Bioresource Technology* 102(1):50-56.
- Chiu, Y.-W., B. Walseth, and S. Suh. 2009. Water embodied in bioethanol in the United States. *Environmental Science and Technology* 43(8):2688-2692.
- Clarens, A.F., H. Nassau, E.P. Resurreccion, M.A. White, and L.M. Colosi. 2011. Environmental impacts of algae-derived biodiesel and bioelectricity for transportation. *Environmental Science and Technology* 45:7554-7560.
- Clarens, A.F., E.P. Resurreccion, M.A. White, and L.M. Colosi. 2010. Environmental life cycle comparison of algae to other bioenergy feedstocks. *Environmental Science and Technology* 44(5):1813-1819.
- Cordell, D., J.O. Drangert, and S. White. 2009. The story of phosphorus: Global food security and food for thought. *Global Environmental Change-Human and Policy Dimensions* 19(2):292-305.
- Craggs, R.J., S. Heubeck, T.J. Lundquist, and J.R. Benemann. 2011. Algal biofuels from wastewater treatment high rate algal ponds. *Water Science and Technology* 63(4):660-665.
- Craswell, E.T., P.L.G. Vlek, and H. Tiessen. 2010. Peak phosphorus—Implications for soil productivity and global food security. Paper read at the 19th World Congress of Soil Science, Soil Solutions for a Changing World, August 1-6, Brisbane, Australia.
- Darzins, A., P.T. Pienkos, and L. Edey. 2010. Current status and potential for algal biofuels production: A report to IEA Bioenergy Task 39. Available online at http://www.fao.org/uploads/media/1008_IEA_Bioenergy_-_Current_status_and_potential_for_algal_biofuels_production.pdf. Accessed September 14, 2012.
- Dewson, Z.S., A.B.W. James, and R.G. Death. 2007. A review of the consequences of decreased flow for instream habitat and macroinvertebrates. *Journal of the North American Benthological Society* 26(3):401-415.
- DOE (U.S. Department of Energy). 2006. Energy Demands on Water Resources: Report to Congress on the Interdependency of Energy and Water. Washington, DC: U.S. Department of Energy.
- . 2010a. Algenol Integrated Biorefinery for Producing Ethanol from Hybrid Algae. Washington, DC: U.S. Department of Energy.
- . 2010b. National Algal Biofuels Technology Roadmap. Washington, DC: U.S. Department of Energy, Energy Efficiency and Renewable Energy.
- Dominguez-Faus, R., S.E. Powers, J.G. Burken, and P.J. Alvarez. 2009. The water footprint of biofuels: A drink or drive issue? *Environmental Science and Technology* 43(9):3005-3010.
- EIA (Energy Information Administration). 2011. September 2011 Monthly Energy Review. Washington, DC: U.S. Energy Information Administration.
- EPA (U.S. Environmental Protection Agency). 2008. Clean Watersheds Needs Survey 2004 Report to Congress. Washington, DC: U.S. Environmental Protection Agency.
- Falkowski, P.G., and J.A. Raven. 2007. *Aquatic Photosynthesis*. 2nd ed. Princeton, NJ: Princeton University Press.
- Farrell, A.E., R.J. Plevin, B.T. Turner, A.D. Jones, M. O'Hare, and D.M. Kammen. 2006. Ethanol can contribute to energy and environmental goals. *Science* 311:506-508.
- Feth, J.H.F. 1965. Preliminary map of the Conterminous United States Showing Depth to and Quality of Shallowest Ground Water Containing More Than 1,000 Parts Per Million Dissolved Solids. Reston, VA: U.S. Geological Survey.
- GBEP (Global Bioenergy Partnership). 2011. GBEP Sustainability Indicators for Bioenergy. Rome: FAO (Food and Agriculture Organization of the United Nations).
- Geider, R.J., and J. La Roche. 2002. Redfield revisited: Variability of C:N:P in marine microalgae and its biochemical basis. *European Journal of Phycology* 37(1):1-17.
- Gerbens-Leenes, W., A.Y. Hoekstra, and T.H. van der Meer. 2009. The water footprint of bioenergy. *Proceedings of the National Academy of Sciences of the United States of America* 106(25):10219-10223.
- Goedhart, C.M., and D.E. Pataki. 2011. Ecosystem effects of groundwater depth in Owens Valley, California. *Ecology* 92(3):458-468.
- Greet, J., J.A. Webb, and R.D. Cousens. 2011. The importance of seasonal flow timing for riparian vegetation dynamics: a systematic review using causal criteria analysis. *Freshwater Biology* 56(7):1231-1247.
- Guru, M., and J. Horne. 2001. The Ogallala Aquifer. Pp. 321-328 in *Water Resource Management*, R.A. Falconer, W.R. Blain, C.A. Brebbia, P. Anagnastopoulos, K. Katsifarakis, and A.H.D. Cheng, eds. Haldiki, Greece: WIT Press.
- Hanson, R.L. 1991. Evapotranspiration and droughts. U.S. Geological Survey Water-Supply Paper 2375:99-104.
- Hanson, T.R. 2006. Catfish farming in Mississippi. *Mississippi History Now* (April). Available online at <http://mshistory.k12.ms.us/articles/217/catfish-farming-in-mississippi>. Accessed March 15, 2012.
- Harto, C., R. Meyers, and E. Williams. 2010. Life cycle water use of low-carbon transport fuels. *Energy Policy* 38(9):4933-4944.
- Hessen, D.O., and T.R. Anderson. 2008. Excess carbon in aquatic organisms and ecosystems: Physiological, ecological, and evolutionary implications. *Limnology and Oceanography* 53(4):1685-1696.

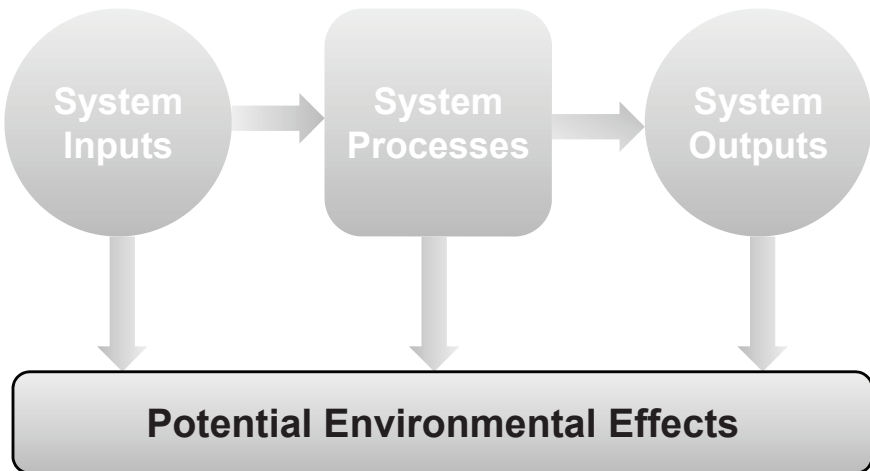
- Hoekstra, A.Y., A.K. Chapagain, M.M. Aldaya, and M.M. Mekonnen. 2009. *Water Footprint Manual: State of the Art 2009*. Enschede, The Netherlands: Water Footprint Network.
- Jager, H.L., and K.A. Rose. 2003. Designing optimal flow patterns for fall Chinook salmon in a central valley, California, river. *North American Journal of Fisheries Management* 23(1):1-21.
- Jansson, M., J. Karlsson, and A. Jonsson. 2012. Carbon dioxide supersaturation promotes primary production in lakes. *Ecology Letters* 15:527-532.
- Jorquera, O., A. Kiperstok, E.A. Sales, M. Embiruçu, and M.L. Ghirardi. 2010. Comparative energy life-cycle analyses of microalgal biomass production in open ponds and photobioreactors. *Bioresource Technology* 101(4):1406-1413.
- Kalnes, T.N., K.P. Koers, T. Marker, and D.R. Shonnard. 2009. A techno-economic and environmental life cycle comparison of green diesel to biodiesel and syndiesel. *Environmental Progress and Sustainable Energy* 28(1):111-120.
- Katzer, J.R. 2010. Sustainable research, development, and demonstration. *Industrial and Engineering Chemistry Research* 49(21):10154-10158.
- Kenny, J.F., N.L. Barber, S.S. Hutson, and K.S. Linsey. 2009. *Estimated Use of Water in the United States in 2005*. Reston, VA: U.S. Geological Survey.
- King, C.W., and M.E. Webber. 2008. Water intensity of transportation. *Environmental Science and Technology* 42(21):7866-7872.
- Lardon, L., A. Helias, B. Sialve, J.P. Stayer, and O. Bernard. 2009. Life-cycle assessment of biodiesel production from microalgae. *Environmental Science and Technology* 43(17):6475-6481.
- Leonardos, N., and R.J. Geider. 2005. Elevated atmospheric carbon dioxide increases organic carbon fixation by *Emiliania huxleyi* (Haptophyta), under nutrient-limited high-light conditions. *Journal of Phycology* 41(6):1196-1203.
- Liu, X., A.F. Clarens, and L.M. Colosi. 2011. Algae biodiesel has potential despite inconclusive results to date. *Bioresource Technology* 104:83-86.
- Lundquist, T.J., I.C. Woertz, N.W.T. Quinn, and J.R. Benemann. 2010. *A Realistic Technology and Engineering Assessment of Algae Biofuel Production*. Berkeley, CA: Energy Bioscience Institute.
- Luo, D.X., Z.S. Hu, D.G. Choi, V.M. Thomas, M.J. Realff, and R.R. Chance. 2010. Life cycle energy and greenhouse gas emissions for an ethanol production process based on blue-green algae. *Environmental Science and Technology* 44(22):8670-8677.
- Marshall, C.T., and R.H. Peters. 1989. General patterns in the seasonal development of chlorophyll a for temperate lakes. *Limnology & Oceanography* 34(5):856-867.
- Mata, T.M., A.A. Martins, and N.S. Caetano. 2010. Microalgae for biodiesel production and other applications: A review. *Renewable and Sustainable Energy Reviews* 14(1):217-232.
- McKone, T.E., W.W. Nazaroff, P. Berck, M. Auffhammer, T. Lipman, M.S. Torn, E. Masanet, A. Lobscheid, N. Santero, U. Mishra, A. Barrett, M. Bomberg, K. Fingerhahn, C. Scown, B. Strogon, and A. Horvath. 2011. Grand challenges for life-cycle assessment of biofuels. *Environmental Science and Technology* 45:1751-1756.
- Mulder, K., N. Hagens, and B. Fisher. 2010. Burning water: A comparative analysis of the energy return on water invested. *Ambio* 39(1):30-39.
- Murphy, C.F., and D.T. Allen. 2011. Energy-water nexus for mass cultivation of algae. *Environmental Science and Technology* 45(13):5861-5868.
- Nagrodski A., G.D. Raby, C.T. Hasler, M.K. Taylor, and S.J. Cooke. 2012. Fish stranding in freshwater ecosystems: Sources, consequences, and mitigation. *Journal of Environmental Management* 103:133-141.
- NAS-NAE-NRC (National Academy of Sciences, National Academy of Engineering, and National Research Council). 2009. *America's Energy Future: Technology and Transformation*. Washington, DC: The National Academies Press.
- . 2010. *Electricity from Renewable Resources*. Washington, DC: The National Academies Press.
- NRC (National Research Council). 2008. *Water Implications of Biofuels Production in the United States*. Washington, DC: The National Academies Press.
- . 2011. *Renewable Fuel Standard. Potential Economic and Environmental Effects of U.S. Biofuel Policy*. Washington, DC: The National Academies Press.
- Pate, R. 2007. Overview of energy-water interdependencies and the emerging energy demands on water resources. Albuquerque: Sandia National Laboratories.
- . 2011. U.S. Department of Energy, Office of Biomass Program (OBP) Sponsor Perspectives for NRC Study on Algae Biofuels Sustainability. Presentation to the NRC Committee on Sustainable Development of Algal Biofuels on March 17.
- Pate, R., G. Klise, and B. Wu. 2011. Resource demand implications for U.S. algae biofuels production scale-up. *Applied Energy* 88(10):3377-3388.

- Pienkos, P.T. 2007. The potential for biofuels from algae. Paper read at the Algal Biomass Summit, November 15, San Francisco, CA.
- Pienkos, P.T., and A. Darzins. 2009. The promise and challenges of microalgal-derived biofuels. *Biofuels Bioproducts and Biorefining-Biofpr* 3(4):431-440.
- Pittman, J.K., A.P. Dean, and O. Osundeko. 2011. The potential of sustainable algal biofuel production using wastewater resources. *Bioresource Technology* 102(1):17-25.
- Redfield, A.C. 1934. On the proportions of organic derivations in sea water and their relation to the composition of plankton. Pp. 177-192 in James Johnstone Memorial Volume, R.J. Daniel, ed. University Press of Liverpool.
- . 1958. The biological control of chemical factors in the environment. *American Scientist* 46:205-221.
- Riebesell, U., K.G. Schulz, R.G.J. Bellerby, M. Botros, P. Fritsche, M. Meyerhöfer, C. Neill, G. Nondal, A. Oschlies, J. Wohlers, and E. Zöllner. 2007. Enhanced biological carbon consumption in a high CO₂ ocean. *Nature* 450(7169):545-548.
- Sagar, A.D., and B. van der Zwaan. 2006. Technological innovation in the energy sector: Research and Development, deployment, and learning-by-doing. *Energy Policy* 34(17):2601-2608.
- Sander, K., and G.S. Murthy. 2010. Life cycle analysis of algae biodiesel. *International Journal of Life Cycle Assessment* 15(7):704-714.
- Sardans, J., A. Rivas-Ubach, and J. Peñuelas. 2011a. The C:N:P stoichiometry of organisms and ecosystems in a changing world: A review and perspectives. *Perspectives in Plant Ecology, Evolution and Systematics*. DOI: 10.106/j.ppees.
- . 2011b. The elemental stoichiometry of aquatic and terrestrial ecosystems and its relationships with organismic lifestyle and ecosystem structure and function: A review and perspectives. *Biogeochemistry*. DOI: 20.11.08.002 10.1007/s10533-011-9640-9
- Sialve, B., N. Bernet, and O. Bernard. 2009. Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. *Biotechnology Advances* 27(4):409-416.
- Singh, A., P.S. Nigam, and J.D. Murphy. 2011. Renewable fuels from algae: An answer to debatable land based fuels. *Bioresource Technology* 102(1):10-16.
- Stephenson, A.L., E. Kazamia, J.S. Dennis, C.J. Howe, S.A. Scott, and A.G. Smith. 2010. Life-cycle assessment of potential algal biodiesel production in the United Kingdom: A comparison of raceways and air-lift tubular bioreactors. *Energy and Fuels* 24:4062-4077.
- Sterner, R.W., T. Andersen, J.J. Elser, D.O. Hessen, J.M. Hood, E. McCauley, and J. Urabe. 2008. Scale-dependent carbon: Nitrogen: phosphorus seston stoichiometry in marine and freshwaters. *Limnology and Oceanography* 53(3):1169-1180.
- Sterner, R.W., and J.J. Elser. 2002. *Ecological Stoichiometry*. Princeton, NJ: Princeton University Press.
- Stromberg, J.C., R. Tiller, and B. Richter. 1996. Effects of groundwater decline on riparian vegetation of semiarid regions: The San Pedro, Arizona. *Ecological Applications* 6(1):113-131.
- Stumm, W., and J.J. Morgan. 1988. An introduction emphasizing chemical-equilibria in natural-waters. *Agriculture Biology and Environmental Sciences*(41):18.
- Sturm, B.S., E. Peltier, V. Smith, and F. De Noyelles. 2012. Controls of microalgal biomass and lipid production in municipal wastewater-fed bioreactors. *Environmental Progress and Sustainable Energy* 31:10-16.
- Subhadra, B.G., and M. Edwards. 2011. Coproduct market analysis and water footprint of simulated commercial algal biorefineries. *Applied Energy* 88(10):3515-3523.
- Urabe, J., J. Togari, and J.J. Elser. 2003. Stoichiometric impacts of increased carbon dioxide on a planktonic herbivore. *Global Change Biology* 9(6):818-825.
- USDA-RD (U.S. Department of Agriculture Rural Development). 2009. Environmental Assessment for Sapphire Energy Inc.'s Integrated Algal Biorefinery (IBR) Facility in Columbus, New Mexico. Washington, DC: U.S. Department of Agriculture.
- USGS (U.S. Geological Survey). 2010. Mineral Commodity Summaries 2010. Reston, VA: U.S. Geological Survey.
- Vaccari, D.A. 2009. Phosphorus: A looming crisis. *American Scientist* 300:54-59.
- Van De Waal, D.B., A.M. Verschoor, J.M.H. Verspagen, E. Van Donk, and J. Huisman. 2010. Climate-driven changes in the ecological stoichiometry of aquatic ecosystems. *Frontiers in Ecology and the Environment* 8(3):145-152.
- Vasudevan, V., R.W. Stratton, M.N. Pearlson, G.R. Jersey, A.G. Beyene, J.C. Weissman, M. Rubino, and J.I. Hileman. 2012. Environmental performance of algal biofuel technology options. *Environmental Science and Technology* 46(4):2451-2459.
- Weissman, J.C., D.T. Tillett, and R.P. Goebel. 1989. Design and Operation of an Outdoor Microalgae Test Facility. Golden, CO: Solar Energy Research Institute.
- Wigmosta, M.S., A.M. Coleman, R.J. Skaggs, M.H. Huesemann, and L.J. Lane. 2011. National microalgae biofuel production potential and resource demand. *Water Resources Research* 47(4):W00H04.

- Wiley, P.E., J.E. Campbell, and B. McKuin. 2011. Production of biodiesel and biogas from algae: A review of process train options. *Water Environment Research* 83(4):326-338.
- Williams, E., Weber, C., and Hawkins, T. 2009. Hybrid approach to managing uncertainty in life cycle inventories. *Journal of Industrial Ecology* 15(6):928-944.
- Williams, P.J.L.B., and L.M.L. Laurens. 2010. Microalgae as biodiesel and biomass feedstocks: Review and analysis of the biochemistry, energetics and economics. *Energy and Environmental Science* 3(5):554-590.
- Wu, M., M. Mintz, M. Wang, and S. Arora. 2009. Water consumption in the production of ethanol and petroleum gasoline. *Environmental Management* 44(5):981-997.
- Xu, L., D.W.F.W. Brilman, J.A.M. Withag, G. Brem, and S. Kersten. 2011. Assessment of a dry and a wet route for the production of biofuels from microalgae: Energy balance analysis. *Bioresource Technology* 201:5113-5122.
- Yang, J., M. Xu, X. Zhang, Q. Hu, M. Sommerfeld, and Y. Chen. 2011. Life-cycle analysis on biodiesel production from microalgae: Water footprint and nutrients balance. *Bioresource Technology* 102(1):159-165.
- Yue, L., and W. Chen. 2005. Isolation and determination of cultural characteristics of a new highly CO₂ tolerant fresh water microalgae. *Energy Conversion and Management* 46(11-12):1868-1876.

5

Environmental Effects



As with production and use of any fuels, aspects of biofuel production and use have benefits and adverse effects. This chapter discusses potential environmental effects from the production and use of algal biofuels, the potential influence of perceived or actual impacts on societal acceptance, and some of the health impacts potentially emanating from the specific environmental effects. Potential environmental effects discussed in this chapter include those resulting from land-use changes, water quality, net greenhouse-gas (GHG) emissions, air quality, biodiversity, waste generation, and effects from genetically engineered algae (with an emphasis on new or enhanced traits).

Where possible, this chapter discusses the potential for algal biofuels to improve aspects of sustainability compared to petroleum-based fuels and other biofuels and the potential for mitigating negative effects along the life cycle of algal biofuel. Environmental indicators of sustainability and data to be collected to assess sustainability are suggested. In some environments and biofuel management systems, metrics for assessing environmental performance are easy to measure and adequate baseline data are available, but that is not the case in all systems.

A number of potential environmental concerns are evident, and if the concerns are not addressed they could become significant risks under large-scale deployment. As in any other industrial or agricultural enterprise, once they are recognized, such risks can be managed by standards or regulations so that industry is required to reduce effects to acceptable levels. For the sake of comprehensiveness, a number of potential environmental risks are mentioned in this chapter, but some are less likely to occur than others. Some of the environmental risks might require exploratory assessment and subsequent monitoring to ensure that they do not become sustainability concerns if algal biofuel production is scaled up.

5.1 WATER QUALITY

Producing algal biofuels could improve or harm water quality depending on the resource input and management used in algae cultivation, weather events, integrity of infrastructure, and processing of spent water. Water-quality concerns associated with commercial-scale production of algal biofuels, if sufficient culture waters are released to natural environments, include eutrophication of waters, contamination of groundwater, and salinization of water sources. Potential water-quality benefits are reduced runoff of herbicides and insecticides compared to corn-grain ethanol or soybean-based biodiesel because of their reduced use, and reduced eutrophication if there are no releases of culture water or if algae are used as a means to remove nutrients from municipal waste, confined animal feeding operations, and other liquid wastes. Water-quality effects will depend on the nutrient content of the algal culture medium; whether feedstock production systems are sealed, artificially lined, or clay lined; and the likelihood of extreme precipitation events. Leakage of culture fluid to groundwater or surface water could occur if the integrity of the pond or trough system is compromised, if flooding occurs, or if spills occur during transfers of fluid during process stages or waste removal, but most of these events could be avoided with proper management.

5.1.1 Releases of Culture and Process Water

As discussed in Chapter 4, the water for algae cultivation is likely to be reclaimed and reused to reduce the water requirement and consumptive water use. The liquid effluent also can be recycled from anaerobic digestion of lipid-extracted algae to produce biogas (Davis et al., 2011). If harvest water is to be released instead of recycled, it or effluent from anaerobic digestion would contain nitrogen (N) and phosphorus (P), the concentrations of which depend on the nitrogen and phosphorus taken up by the harvested algal biomass (Sturm and Lamer, 2011). Released waters could be more saline than receiving waters, particularly if water from saline aquifers is used for algae cultivation. Such point-source discharge will be regulated by the Clean Water Act, and a National Pollutant Discharge Elimination System permit would have to be obtained to operate the algae cultivation facilities (EPA, 2011a). However, permit violation has been observed in some biofuel refineries

that use terrestrial crops as feedstock (Beeman, 2007; Smith, 2008; EPA, 2009b; Buntjer, 2010; Meersman, 2010; O'Sullivan, 2010). Regulation and compliance assurance would address concerns about release of harvest water.

The potential for accidental release of cultivation water exists; for example, clay or plastic liners could be breached through normal weathering or from extreme weather events, some of which are predictable. High precipitation or winds could lead to overtopping of ponds or above-grade raceways. In those cases, the entire contents of algal cultures could be lost to surface runoff and leaching to surface water or groundwater. Siting in areas prone to tornadoes, hurricanes, or earthquakes would increase the likelihood of accidental releases. However, producers are likely to take preventive measures when extreme weather events are forecasted, and they would put effort into preventing accidental releases of cultivation water because such events could adversely affect their profit margin.

5.1.2 Eutrophication

5.1.2.1 Potential Environmental Effects

Large-scale algae cultivation requires the provision of large quantities of nutrients, especially nitrogen and phosphorus, to ensure high yield (see section Nutrients in Chapter 4). Even where nitrogen and phosphorus are not in oversupply, the total nutrient concentrations in algal biomass will be high. Although accidental release of cultivation water into surface water and soil is unlikely, such an event could lead to eutrophication of downstream freshwater and marine ecosystems, depending on the proximity of algal ponds to surface and groundwater sources.

Eutrophication occurs when a body of water receives high concentrations of inorganic nutrients, particularly nitrogen and phosphorus, stimulating algal growth and resulting in excessive algal biomass. As the algae die off and decompose, high levels of organic matter and the decomposition processes deplete oxygen in the water and result in anoxic conditions (Smith, 2003; Breitburg et al., 2009; Rabalais et al., 2009; Smith and Schindler, 2009). In some cases, eutrophication-induced changes could be difficult or impossible to reverse if alternative stable states can occur in the affected ecosystem (Scheffer et al., 2001; Carpenter, 2005).

Eutrophication effects have been well studied, and they depend on the nutrient loadings to the receiving waters and the volume and residence time of water of these systems (Smith et al., 1999; Smith, 2003). High nutrient loading could lead to anoxia in the deep cool portion of lakes or in hypoxia in the receiving water bodies. Potential biotic effects of eutrophication include changes in algal density and in the structure and biomass of the broader ecological community (Scheffer et al., 1997; Reynolds et al., 2002; Smayda and Reynolds, 2003). Fish yield is affected by phytoplankton¹ biomass and by the nutrient ratios in the edibility of phytoplankton (Oglesby, 1977; Bachmann et al., 1996).

Nutrient levels play a key role in determining the productivity and structure of the primary producing community in estuaries and coastal marine waters (Deegan et al., 2002; Smith, 2006) and by extension, the productivity and structure of higher trophic levels. Nutrient-enriched shallow marine systems tend to have a reduced seagrass community (Burkholder et al., 1992; Hauxwell et al., 2003) because elevated nitrogen concentrations and loadings adversely affect seagrass (Efroymsen et al., 2007 and references cited therein).

¹A collection of microscopic photosynthetic organisms that float or drift in fresh water or sea water.

In high-nitrate environments, seagrasses can be shaded by epiphytic algae and macroalgae (Drake et al., 2003) or sometimes by phytoplankton blooms (Nixon et al., 2001). Seagrasses affect the entire estuarine food web because they stabilize sediments; serve as habitats and temporary nurseries for fish and shellfish; are sources of food for fish, waterfowl, benthic invertebrates, or manatees; and provide refuges from predation. Eutrophication and other nutrient-related effects could be a concern for cultivation of microalgae or macroalgae in large suspended offshore enclosures (for example, Honkanen and Helminen, 2000).

Eutrophication also has implications for social acceptability (Codd, 2000), for example, because of eutrophication-related aesthetic concerns (Grant, 2010), and aesthetics can affect the recreational value of water bodies. It is unknown whether rare releases of culture water or the physical appearance of open ponds for algae cultivation could have negative effects on the social acceptability of algal biofuels.

5.1.2.2 Opportunities for Mitigation

Quantifying water losses from raceways, ponds, or photobioreactors would indicate whether repairs of small leaks are necessary. These culture systems can be designed and tested to withstand natural disasters that are possible during the lifetime of the infrastructure. In coastal locations, for example, facility and infrastructure designs would need to consider the probabilities that hurricane winds and water surges could reach the algae cultivation site (Guikema, 2009). Mitigation plans for accidental releases would be desirable. Open-pond algae cultivation also can be sited in locations that are not prone to hurricanes or away from lakes and streams. With respect to harvest water, engineering solutions can maximize recycling.

5.1.3 Waterborne Toxicants

5.1.3.1 Potential Environmental Effects

Some compounds present in algal ponds or photobioreactors could be toxic to humans or other organisms depending on exposure levels. Herbicides often are added to open systems to prevent growth of macrophytes and for selective control of algae (NALMS, 2004), but their application likely would be regulated as in the case of agriculture. If wastewater or oil well-produced water (Shpiner et al., 2009) is used as a water source for algae cultivation, heavy metals could be present. Wastewater could include industrial effluent (Chinnasamy et al., 2010) and municipal wastewater that has undergone various levels of treatment (Wang et al., 2010). The composition and amount of toxicants vary by the type of wastewater. Produced water (water contained in oil and gas reservoirs that is produced in conjunction with the fossil fuel) may contain high levels of organic compounds, oil and grease, boron, and ammonia (NH_3) (Drewes et al., 2009). Many algal species including cyanobacteria, diatoms, and chlorophytes can bioconcentrate heavy metals (Watras and Bloom, 1992; Vymazal, 1995; Mathews and Fisher, 2008). Mercury could be introduced into feedstock production waters if unscrubbed flue gas from coal-fired power plants is used as a carbon dioxide (CO_2) source (O'Dowd et al., 2006). Therefore, potential risks from using each type of produced water need to be identified so that adequate containment and mitigation measures can be implemented in cultivation and processing.

Waterborne toxicants (toxic substances made or introduced into the environment anthropogenically, not including algal toxins) potentially pose risk to humans or other

animals if exposures occur. Occupational exposures could be significant, especially during the harvesting phase. Thus, monitoring of toxic compounds in the culture media is important. Potential toxicity exposure to animals through drinking is discussed in the section on terrestrial biodiversity. The release of culture waters to natural environments could pose other risks to animal consumers. Toxic concentrations and doses for various chemicals are available in the Environmental Protection Agency (EPA) Integrated Risk Information System database for humans (EPA, 2012), in Suter and Tsao (1996) for aquatic biota, in Sample et al. (1996) for terrestrial wildlife, and in other government and independent compilations. Cultivation of algae in wastewater may require special handling and means of containment. Monitoring for the presence of toxicants or pathogens might be necessary to ensure the quality of the culture water.

5.1.3.2 Opportunities for Mitigation

Monitoring of metals and other compounds in water sources, nutrient sources, and culture media in demonstration facilities would provide information about whether waterborne toxicants pose a significant concern. If so, technical solutions for removing waterborne toxicants would be needed to prevent occupational and ecological exposures. Mercury is removed from flue gas in some configurations of coal-fired electric-generating units (EPA, 2010). However, mercury removal is ineffective for certain types of coal and plant configurations (NETL, 2011). Contaminants in flue gas could place another constraint on the type of coal-fired electricity facilities that would be suitable for providing CO₂ for algae cultivation (see sections Estimated Land Requirements and Estimated Nutrient Requirements in Chapter 4).

5.1.4 Groundwater Pollution

5.1.4.1 Potential Environmental Effects

Open ponds may not be suitable for many soil types without using lining, and a thorough review of potential effects on surface water and groundwater quality would have to be conducted if clay-lined ponds are to be used. If outdoor ponds are poorly lined or the lining fails as a result of wear, then seepage of the pond water into the local groundwater system could occur. Clays that are compacted and graded have structural integrity that can be comparable to synthetic liners (Boyd, 1995). However, integrity can be compromised by poor construction. Nitrate leaching has been observed below structured clay soils (White et al., 1983), but the qualitative applicability of these results to clay-lined algal ponds is unknown. Local terrestrial vegetation might take up some of the culture media released through seepage. In some areas, if open ponds contain high concentrations of dissolved inorganic nitrate, seepage may contribute to concerns related to nitrate poisoning if the groundwater is used for drinking by livestock or humans.

Withdrawal of freshwater adjacent to briny aquifers or injection of saline wastewater into the ground could result in salinization of groundwater if fresh water and briny aquifers are not well separated. Salinization of groundwater is a potential problem for some agricultural lands where irrigation is prevalent (Schoups et al., 2005). However, one of the key advantages of algal biofuel is that the feedstock could be produced on nonarable land (Ryan, 2009; Assmann et al., 2011), so salinization of agricultural lands as a result of freshwater withdrawal for algae cultivation is not likely.

5.1.4.2 Opportunities for Mitigation

Using sealed algal cultivation systems would practically eliminate the potential for leakage, barring catastrophic breaches. Where open systems are used, technologies (such as the development of impermeable, long-lived liner systems) and regional solutions for minimizing nutrient leakage could be deployed, and regulations to minimize leakage could be developed. For example, Phyco BioSciences uses a trough system that has a lightweight, fabricated liner. The liner is expected to eliminate leakage or minimize percolation to less than 0.01 percent (Cloud, 2011). Potential preventive measures might include specifications for soil type, combined with defined values for the minimum depth from the pond bottom to groundwater. Moreover, local regulations likely require lined ponds, which would reduce the probability of leakage of waters but contribute to capital costs and lead to temporary system closures when the liners are replaced because of wear or failure. Measures to prevent inadvertent discharge of water (for example, overflow corridors or basins) during extreme weather events would be helpful in preventing water pollution.

5.1.5 Wastewater Treatment

Wastewaters derived from municipal, agricultural, and industrial activities potentially could be used for cultivating algal feedstocks either in open ponds or in photobioreactors for algal biofuels and could provide an environmental benefit. Microalgae have been used in wastewater treatment for a long time (Oswald et al., 1957), where they provide photosynthetically produced oxygen for the bacterial breakdown of organic compounds present in the waste (Benemann, 2008). Microalgae have been shown to be effective for wastewater treatment in diverse systems including oxidation (stabilization) ponds and shallow raceway systems and using both phytoplankton and periphyton (Green et al., 1995; Hoffmann, 1998; Pittman et al., 2011; Sandefur et al., 2011). High rate algal ponds (HRAPs), which are shallow, open raceway ponds used for treating municipal, industrial, and agricultural wastewater, combine heterotrophic bacterial and photosynthetic algal processes (Park et al., 2011). The ponds allow the growth of high-standing crops of algae, which remove nitrogen and phosphorus from the wastewater (Sturm et al., 2012). The concept of adapting HRAPs for the purpose of biofuel production was proposed more than five decades ago (Oswald and Golueke, 1960). Park et al. (2011) reviewed the potential benefits and opportunities of using HRAPs for wastewater treatment and harvesting the algae for energy or fuel production. The feasibility and scale of such systems will be determined by the amount of wastewater, the availability of land near the facilities generating the wastewater and produced water, and the climatic conditions of the region. (See also Chapter 4.) If wastewater is used, the wastewater treatment rate and the harvesting schedule would determine the maximum volume of ponds or photobioreactors.

A major goal of wastewater treatment is removal of nitrogen and phosphorus (Pittman et al., 2011). In conventional treatment systems, phosphorus is especially difficult to remove (Pittman et al., 2011). In advanced wastewater treatment, phosphorus typically is either chemically precipitated using aluminum- or iron-based coagulants to form an insoluble solid, or it is stripped from the water by microbial activity (EPA, 2007). The recovered phosphorus is then buried in a landfill or treated to create sludge fertilizer (Pittman et al., 2011). Given that readily available supplies of phosphorus may begin running out by the end of the 21st century (Vaccari, 2009), conservation and stewardship of U.S. phosphorus supplies are essential. Recycling nutrients from wastewater and using them for further algae production could be an attractive option for using otherwise discarded nutrients (Exhibit 9.7 and associated text in DOE, 2010b; see also section Nutrients in Chapter 4).

Algae-based treatments have been found to be as efficient as chemical treatment in removing phosphorus from wastewater (Hoffmann, 1998). Moreover, because harvested algal biomass contains the nutrients that were absorbed during cellular growth, wastewater-integrated systems can perform an important nutrient removal service. In laboratory-scale experiments, more than 90 percent of nitrogen and 80 percent of phosphorus were removed from primary treated sewage by the green alga *Chlorella vulgaris* (Lau et al., 1995). Similarly, laboratory cultures of *Chlorella* and *Scenedesmus* removed 80 to 100 percent of NH_3 , nitrate, and total phosphorus from wastewater that already had undergone secondary treatment (Martinez et al., 2000; Zhang et al., 2008; Ruiz-Marin et al., 2010). Sturm et al. (2012) performed a six-month, pilot-scale algal production experiment using large (10 cubic meters) outdoor bioreactors fed by effluent from the secondary clarifier of the wastewater treatment facility in Lawrence, KS. They reported only a 19 percent removal of dissolved nitrogen and a 43 percent removal of dissolved phosphorus from this treated effluent. These differences in nutrient removal observed may be related, in part, to the different scales of the studies. The ultimate level of nutrient removal benefit may depend on the level of wastewater treatment that occurs prior to nutrient uptake in the algal cultivation systems and on the chemical and ecological conditions that exist in the wastewater-fed production system.

Algae have the potential to remove nutrients from agricultural or industrial wastewater. Some studies have found high efficiencies of removal of nitrogen and phosphorus from wastewater containing manure (Gonzalez et al., 1997; Wilkie and Mulbry, 2002; An et al., 2003), and this wastewater also could be used as input to algal biofuel systems. Algal biofuel systems have the potential to increase water quality and to promote municipal or agricultural wastewater treatment systems with improved sustainability. However, the maintenance of lipid-rich strains and the manipulation of growth conditions to promote high lipid production have yet to be demonstrated consistently for outdoor pond systems, including wastewater treatment ponds (DOE, 2010b). Industrial wastewaters have lower nutrient concentrations and higher toxicant concentrations, and thus are less likely to be used to generate the algal biomass necessary for commercial-scale production of biofuels (Pittman et al., 2011).

Integrated algal biofuel production systems can remove many other pollutants, such as metals and organic contaminants, including endocrine disruptors (Mallick, 2002; Munoz and Guieysse, 2006; Ahluwalia and Goyal, 2007; DOE, 2010b). Whether pollutant uptake by algae is desirable depends on whether coproducts are to be produced with algal biofuels or whether the lipid-extracted algae are to be used for nutrient recycling. Pollutant removal by these systems would improve water quality, but it also could pose a potential risk if organisms such as migrating waterfowl directly or incidentally consumed high metal content algae during the cultivation process, or if humans or wildlife were exposed chronically to the dried algae during biomass processing. Uptake of pollutants by algae is not desirable if residual biomass is to be used for human cosmetic products or animal feed.

5.1.6 Comparison of Pathways

The pathways described in Chapter 3 affect the types, probabilities, and magnitudes of water-quality effects (Table 5-1). For example, slow releases of nutrients to natural environments (and increased potential for eutrophication and groundwater pollution) are common for open systems but not for closed systems. Herbicides likely would be used only in open systems. The water quality benefit for wastewater treatment is achieved only if wastewaters are used as nutrient sources, but the scenarios in Chapter 3 do not specify this.

TABLE 5-1 An Illustration of Potential Benefits and Adverse Effects to Water Quality from Different Pathways for Algal Biofuel Production

Potential Effect	Pathway				
	Open-pond, salt water, producing biodiesel, recycling nutrients and water	Open-pond, salt water, producing biodiesel + coproducts	Open-pond, salt water, producing FAME ^a , recycling nutrients and water	Photobioreactor, salt water, direct synthesis, recycling water	Open-pond, salt water, producing biomass, pyrolysis, recycling some nutrients and water
Releases of Culture Water	Slow releases from seepage, overtopping likely, catastrophic breaches rare	Slow releases from seepage, overtopping likely, catastrophic breaches rare	Slow releases from seepage, overtopping likely, catastrophic breaches rare	No slow releases, catastrophic breaches rare	Slow releases from seepage, overtopping likely, catastrophic breaches rare
Eutrophication and Related Effects	Rare, only when large volume releases occur	Rare, only when large volume releases occur	Rare, only when large volume releases occur	Very rare, only when large volume releases occur	Rare, only when large volume releases occur
Waterborne Toxicants	Herbicides, heavy metals may be present and pose occupational or ecological exposures and risks	Herbicides, heavy metals may be present and pose occupational or ecological exposures and risks	Herbicides, heavy metals may be present and pose occupational or ecological exposures and risks	Heavy metals may be present and pose occupational exposures and risks	Herbicides, heavy metals may be present and pose occupational or ecological exposures and risks
Groundwater Pollution	Possible, depending on soil type, distance to groundwater, and frequency of release	Possible, depending on soil type, distance to groundwater, and frequency of release	Possible, depending on soil type, distance to groundwater, and frequency of release	Rare, only when catastrophic breaches occur	Possible, depending on soil type, distance to groundwater, and frequency of release
Wastewater Treatment	Algae may treat wastewater if wastewater is used as nutrient source	Algae may treat wastewater if wastewater is used as nutrient source	Algae may treat wastewater if wastewater is used as nutrient source	Algae may treat wastewater if wastewater is used as nutrient source	Algae may treat wastewater if wastewater is used as nutrient source

^aFatty-acid methyl esters.

5.1.7 Sustainability Indicators

Proposed sustainability indicators for water quality include aqueous concentrations and loadings of nutrients, herbicides, metals, and salinity of groundwater (GBEP, 2012). These indicators are standard measures for quality of water and wastewater (Eaton et al., 2005). Concentrations of nutrients are included because they relate to benefits or potentially adverse effects on water quality (for example, eutrophication). These usually are measured quantities, and baseline levels and natural variability also can be measured. Loadings are field measures or simulation results representing the contribution of released algal biofuel culture media to receiving waters. These may be compared to other loadings to those waters.

- Nitrate concentration in streams and groundwater.
- Total nitrogen concentration in streams, lakes, reservoirs, and estuaries.
- Total phosphorus concentration in streams, lakes, reservoirs, and estuaries.
- Nitrate loading to streams and groundwater.
- Total phosphorus loading to streams.
- Herbicide concentrations in streams.
- Herbicide loading to streams.
- Metal concentrations in streams.
- Metal concentrations in cultures.
- Salinity of groundwater.

5.1.8 Information and Data Gaps

Good design and engineering would minimize the potential for releases of water and nutrients from open-pond systems to surface water and to ground water. Toxicant concentrations (for example, metals) need to be characterized, particularly if wastewater or produced water is used as culture medium. Information on the nutrient removal efficiencies of commercial-scale facilities would be needed if algal biofuel production is to be combined with wastewater treatment.

5.2 LAND-USE CHANGE

5.2.1 Potential Environmental Effects

Land-use change is a change in anthropogenic activities on land, which often is characterized in part by a change in land cover, including the dominant vegetation. Land-use changes play a role in the sustainability of algal biofuel development because of associated environmental effects, such as net GHG emissions, changes in biodiversity, and changes in ecosystem services such as food production. Moreover, there is growing societal concern about the spatial and temporal scales of some types of conversions, such as deforestation and urbanization. The impacts of algal biofuel development will depend in part on the type of land conversion, the extent (area) of land use that has changed, the intensity of land disturbance and management, and the duration of the change (for example, whether it is reversible).

Commercial-scale production of algal biofuels will require substantial land area for each facility (see Chapter 4), and the large-scale deployment of algal biofuels will lead to conversion of lands from other existing uses. Land conversion for ponds, processing facilities, and refineries for most products will be localized, and potential land conversion for related infrastructure, such as roads and power lines to the facilities, will be more diffuse and will involve linear features. This section focuses on land-use change (LUC) associated with algae cultivation, because change associated with feedstock processing or refining facilities is not different in kind from that of other liquid fuel sources.

High-value lands used by agriculture, by other commodity industries, and for residential purposes are unlikely to be used for algae cultivation because algae cultivation does not require fertile soils and because capital and operating costs would have to be kept low for algal biofuel companies to operate close to the profit margin (Table 5-2). Similarly, the conversion of forestland is unlikely because of the high costs of clearing and site preparation and the high value for residential or recreational use. Land-use change for algal biofuels is

TABLE 5-2 A Summary of the Committee’s Judgment on the Likelihood of Land (or Water Surface) Conversion to Algae Cultivation Ponds and Facilities, Based on Value for Other Land (or Surface Water) Uses

Land Type	Possible or Likely	Unlikely
Productive agricultural land		X
Marginally or unproductive agricultural land	X	
Desert	X	
Brownfields	X	
High-value coastal land		X
Low-value coastal land	X	
Forest land		X
Rangeland, low-density grazing land	X	
Parks and conservation land		X
Wetlands		X
Residential land		X
Industrial parks	X	
Urban land other than brownfields		X
Former catfish pond lands	X	
Offshore	X	

NOTE: Low-value land is assumed to be used to cultivate algae for biofuels.

more likely to involve brownfields², rangelands, deserts, scrubland, abandoned farmland, or unproductive farmland, some of which may be on coasts or in near-shore marine waters. On coasts, dredge spoil islands might be additional options for use. For example, Phycal, an algal biofuel company, is using fallow land in Hawaii that was previously a pineapple plantation but is no longer economically viable for that use. Sapphire, another company operating in the Southwest, plans to develop nonagricultural land for algae cultivation. (Siting requirements are described in Chapter 4.) Competing land demands could change over time and may influence the landscape of algal biofuels. For example, some of the same lands that are attractive for algal biofuel development are also attractive for large-scale solar power development (BLM and DOE, 2010).

Direct land-use change generally is defined as a direct cause-and-effect link between biofuel development and land conversion in the absence of strong external mediating factors. Direct land-use change occurs within the biofuel production pathway when land for one use is dedicated for biofuel production. However, in practice, direct land-use change from biofuel production generally is assumed to include lands used for feedstock production, processing, storage, and refining areas. Indirect land-use change occurs when biofuel production causes new land-use changes elsewhere domestically or in another country through market-mediated effects (NRC, 2011).

Direct and indirect land-use changes could affect the net GHG emissions of biofuels (NRC, 2011). Direct land-use change can result in carbon sequestration or net GHG emissions, depending on the type of land conversion and prior land use. For example, converting from annual-crop production to perennial-crop production can enhance carbon storage on that piece of land (Fargione et al., 2008). Conversely, clearing native ecosystems to produce row-crops would result in a one-time release of a large quantity of GHGs into the atmosphere (Fargione et al., 2008; Gibbs et al., 2008; Ravindranath et al., 2009). In the

²Brownfields are “industrial or commercial propert[ies] that [remain] abandoned or underutilized because of environmental contamination or the fear of such contamination” (Environmental Law Institute, 2012).

context of algae cultivation, converting pastureland to algal ponds is likely to contribute to GHG emissions. Perennial pasture is effective in sequestering carbon in soil (Franzluebbers, 2010; Gurian-Sherman, 2011). Removal of such vegetation would result in a one-time loss of carbon and the elimination of any potential for further carbon sequestration if the land is to be left as a pasture. In contrast, if the algae cultivation ponds are installed on degraded land that is not storing much carbon, immediate emissions from the conversion will be minimal.

Indirect land-use change could occur if the use of land to cultivate biofuel feedstocks replaces and ultimately reduces the production levels of crops destined for a commodity market. The lowered production of those commodities could drive up market prices, which in turn could trigger agricultural growers to clear land elsewhere to grow the displaced crops in response to market signals (Babcock, 2009; Zilberman et al., 2010). However, as stated above, because algal feedstock cultivation does not require fertile cropland, arable land likely will not be used for algal biofuels (Sheehan et al., 1998; Gong and Jiang, 2011), and displacement of commodity crops by algae is unlikely. In addition, protein from lipid-extracted algae potentially can replace soybean or other terrestrial crops as feedstuff (Wijffels and Barbosa, 2010) and reduce the demand for land by terrestrial crops. The nutritional compatibility of algal feedstuff and the animal diet would have to be examined.

Pasture and rangeland could be converted to algae cultivation, and displacement of these land uses by algae also may or may not result in other indirect effects. If the pasture or rangeland is surplus and not in use, then repurposing the land will not incur indirect land-use change (ILUC). In contrast, if algae cultivation displaces grass-fed cattle production, producers might decide to change to corn-fed cattle production. Changing from grass-fed to corn-fed cattle production also would exert pressure on the corn-grain market. Alternatively, if existing pasture and rangeland is limiting beef production, such that removing some of this land would decrease production, then grass-fed cattle production might be replaced elsewhere. The indirect land-use changes not only affect ecosystem services, but result in changes in GHG emissions that have to be considered in life-cycle GHG assessments for algal biofuels.

If the indirect effects of algal biofuel production are to be quantified, then the potential biodiversity, water quality, and water balance impacts would include those associated with indirect land conversions. Previous quantification of indirect effects of biofuels generally has been limited to GHG effects and food security effects.

As in the case of terrestrial-crop biofuels, market-mediated indirect land-use changes are difficult to ascertain, and estimates of associated GHG emissions are highly uncertain (NRC, 2011). Although complex models have been used to project the extent of indirect land-use changes as a result of terrestrial-crop biofuels, the committee is not aware of similar projections for algal biofuels. Algae cultivation is less likely to incur indirect land-use changes because it does not require prime agricultural land. Converting crop lands to new crops (algal biofuels) also will require new ownership or a willingness on the part of farmers to grow a new commodity. Growing algal biofuels will require differing work schedules than row crop farming. Even if cropland is not to be converted to algal ponds, the above discussion of potential pasture conversion illustrates a potential for indirect land-use change.

5.2.2 Comparison of Pathways

With respect to land-use change, the primary relevant difference among the pathways in Chapter 3 is the difference between the land required for open-pond and photobioreactor systems (see Chapter 4). The spatial and temporal scales of land-use change would be commensurate with those of land use.

5.2.3 Potential Opportunities for Mitigation

In general, algal biofuel development will avoid forestland and land with agricultural value. Avoiding pastureland and areas of high biodiversity or recreational value also would eliminate some of the sustainability concerns associated with commercial development of algal biofuels.

5.2.4 Sustainability Indicators

Land-use change is not consistently proposed as a criterion for sustainability, even though it often is considered a factor influencing aspects of the sustainability of biofuel (for example, GHG emissions, biodiversity, water quality, and soil quality). Therefore, some compilations of sustainability indicators do not include indicators of sustainable land use (for example, McBride et al., 2011). However, there are aspects of land use, such as infrastructure, impervious surfaces, and some disturbances, that may be long lasting or irreversible and may not be adequately considered using indicators of other categories of sustainability. Potential indicators of sustainable land use include percent impervious surface (Sutton et al., 2009; Uphoff et al., 2011; Weiland et al., 2011) and land disturbance area. Changes in impervious surface area affect the water cycle and watershed dynamics, as well as terrestrial and aquatic habitats. The area of land disturbed can be considered a measure of sustainability. Land disturbance areas can be normalized based on a land-condition factor (Eq. 5-1) that captures the degree to which aspects of development, processing, infrastructure, potential accidents, and use of energy change the land from its natural state (Lenzen and Murray, 2001, 2003) and its ability to provide ecosystem services.

$$\frac{\text{Land Disturbance Area} \times \text{Land Condition Factor}}{\text{Energy Output (or Volume) of Liquid Fuel}} \tag{Eq. 5-1}$$

Table 5-3 shows examples of land condition factors that can be multiplied by disturbed area to give a currency of disturbance. This metric relates to ecological footprint methods that sometimes are applied to energy comparisons (Stoglehner, 2003), but it does not attempt to encompass effects on water, GHG emissions, and other ecological impacts that can be more controversially subsumed in ecological footprints (Fiala, 2008; Özdemir et al., 2011).

TABLE 5-3 Illustrative Land Condition Factors for Land-Cover Changes Relevant to Algal Biofuel Production

Land Use or Land Cover Type	Land Condition ^c
Built ^a (refineries, offices) or paved	1.0
Ground denuded of vegetation but no pond	0.8
Earthen pond or raceway containing algal culture	0.5
Lined pond or raceway containing algal culture	0.6
Partially disturbed grazing land ^b	0.2

^aValue taken from Lenzen and Murray (2003). Reprinted with permission from Elsevier.

^bValue taken from Lenzen and Murray (2003) and can represent areas between algal biofuel facilities, where grazing may occur. Reprinted with permission from Elsevier.

^cLand condition factors capture the proportion of disturbed land or relative intensity of disturbance, with land in a natural state having a land condition factor of 0 and paved land having a land condition factor of 1. Land condition factors are multiplied by disturbance area to allow comparison of disturbed areas of different intensities and scales.

Trends in land-use change related to algal biofuel production are important to quantify. However, until there is a history of commercial development of algal biofuel production facilities, probable land-use changes and trends will need to be projected based on economic and social drivers and environmental contributing factors.

Where important or rare ecosystem services are provided by the baseline land use, a measure of those services could serve as a sustainability indicator for algal biofuels. The services of pastures, rangelands, and coastal waters that might be displaced by feedstock production facilities would be important to quantify. Relevant metrics would be:

- National or regional area of grassland and shrubland devoted to livestock grazing; however, data are lacking on the acreage used for livestock grazing (The H. John Heinz III Center for Science and the Environment, 2008).
- Number of livestock fed on grasslands and shrublands (West, 2003; The H. John Heinz III Center for Science and the Environment, 2008).
- Pasture yield calculated on a per-area or per-forage biomass basis (methods described in Burns, 2008).

A less direct indicator of livestock numbers or biomass would be area covered by grassland and shrubland (West, 2003; The H. John Heinz III Center for Science and the Environment, 2008). Additional sustainability indicators have been suggested for brownfield redevelopment efforts. Some of these are summarized in Wedding and Crawford-Brown (2007) and would be appropriate where algal biofuel production is sited on brownfields.

5.3 GREENHOUSE-GAS EMISSIONS

The potential to mitigate GHG emissions is one of the motivations to develop biofuels. The basis of mitigation is that carbon emissions from combusting a biofuel are cancelled by the corresponding capture in photosynthesis. This said, the net GHG emissions of producing biofuels and coproducts are not zero because of carbon and other GHGs emitted in processing. In this section, the results of life-cycle assessment (LCA) studies of GHG emissions are reviewed critically.

5.3.1 Life-Cycle GHG Emissions of Algal Biofuels

Primary GHG emissions from algal biofuels are expected to be connected to the use of energy in the processing chain (see section Energy in Chapter 4). The translation of energy use to GHG emissions is complicated by variability in the carbon overhead of different forms of energy, in particular electricity. The average direct GHG emissions of electricity production in the United States is 606 grams of CO₂ equivalent per kilowatt hour (EIA, 2002). Depending on the mix of fossil fuels, hydropower, nuclear, wind, and other sources providing power to the grid, emissions vary by state from 13 to 1,017 grams CO₂ equivalent per kilowatt hour (EIA, 2002). The approach taken by many analysts is to use a national average emission factor (Liu et al., 2011).

LCA results for net GHG emissions for algae biofuel production vary from a net negative value (that is, a carbon sink) to positive values substantially higher than petroleum gasoline (Table 5-4). As with the case for energy use (see Chapter 4), drivers of variability in CO₂ emissions are nutrient source, productivity and process performance, and the credit associated with coproducts. For example, Sander and Murthy (2010) assumed that residual algal biomass substitutes for corn in ethanol plants. Corn is energy intensive to produce; the

TABLE 5-4 Results from Sample Studies of Life-Cycle CO₂ Emissions of Algal Biodiesel Production in Common Normalization

Source	Life-Cycle CO ₂ Emissions (kg CO ₂ equivalent per liter of biodiesel)
Clarens et al. (2010)	8.7
Lardon et al. (2009)	4.0
Stephenson et al. (2010)	0.6
Sander and Murthy (2010)	-0.8
Campbell et al. (2011)	-1.1

TABLE 5-5 Meta-Analysis Results for Ranges in Carbon Emissions Over the Life Cycle of Algal Biofuel Estimated by Various Studies

Scenario	GHG Emissions (kilograms CO ₂ eq per liter biodiesel)
Virgin CO ₂ , no coproduct	5.9-8.2
Virgin CO ₂ , w/ coproduct	2.0-4.2
Recycled CO ₂ , no coproduct	2.1-2.9
Recycled CO ₂ , w/ coproduct	(-1.2)-(-2.9)

NOTE: The direct carbon emissions of driving an average gasoline automobile is about 0.15 kg CO₂ eq per kilometer.

SOURCE: Liu et al. (2012). Reprinted with permission from Elsevier.

GHG credit from replacing corn with oil-extracted algae as a feedstock for ethanol results in a negative carbon balance. For reference, the direct carbon emission of combusting gasoline is about 2.7 kg CO₂ equivalent per liter of fuel (Farrell et al., 2006).

The vast differences in results in Table 5-4, ranging from a net carbon credit to emissions far larger than those from petroleum-based diesel, present a challenge for interpretation. Liu et al. (2012) performed a meta-analysis of these studies to analyze variability in processing energy by replacing differences in data and assumptions for nutrients and coproducts with common data (Lardon et al., 2009; Clarens et al., 2010; Jorquera et al., 2010; Sander and Murthy, 2010; Stephenson et al., 2010; Campbell et al., 2011). Differences in nutrient sourcing and coproducts are treated via four scenarios: virgin versus recycled CO₂ and no coproducts versus coproducts. The common coproduct system used is generation of bioelectricity from gas generated by anaerobic digestion with the electricity generated substituting for carbon emissions from the U.S. grid. Table 5-5 shows the ranges in results from the six treated studies, after normalization, for the four scenarios.

These meta-analysis results suggest that the CO₂ source and coproducts are critical factors in the GHG balance. It is, however, premature to conclude that algal biofuels based on recycling CO₂ and producing biogas has net negative GHG emissions. The variability in Table 5-5 is based on differences in energy data and assumptions in the six existing studies. It is not yet clear if current LCA analyses of algal biofuel production systems will accurately reflect the energy use of a real-world, scaled-up system.

None of the studies above addresses the potential issue of indirect land-use change from biofuels. As stated earlier, it is possible that conversion of pastureland to algae cultivation facilities would necessitate conversions to pastureland elsewhere. However, uncertainties are too great to quantify this probability or to calculate net GHG emissions under these assumptions. (See section Land-Use Change in this chapter.)

While many agricultural processes emit non-carbon GHGs such as nitrous oxide (N_2O) and methane (Weber and Matthews, 2008), these emissions have not been established empirically as significant for algae cultivation. N_2O could be emitted from cultivation systems, and these emissions would need to be quantified in the future for cultivation conditions that might promote N_2O or methane emission. One study of a single species quantified N_2O emissions from algal culture under laboratory conditions (Fagerstone et al., 2011). In this study of *Nannochloropsis salina* with nitrate as a nitrogen source, elevated N_2O emissions were observed under a nitrogen headspace (photobioreactor simulation) during dark periods, but N_2O emissions were low during light periods. In contrast, when the headspace consisted of air (open-pond simulation), N_2O emissions were negligible. Denitrifying bacteria were present.

Denitrification is the microbial reduction of nitrate and nitrite with generation of N_2O and, ultimately, gaseous nitrogen. Anaerobic environments are required for the transformation, but high rates of denitrification occur where oxygen is available alternately, then unavailable (Kleiner, 1974). In rivers, ponds, lakes, and estuaries, the production of N_2O is correlated with nitrate concentrations in the water (Stadmark and Leonardson, 2005). The denitrification rate depends on the underlying soil and the liner's permeability.

Whether anaerobic denitrification is the only potential pathway for N_2O generation in algal cultivation systems is unclear. Weathers (1984) has shown that certain Chlorophyceae in axenic culture evolve N_2O when using nitrite as a nitrogen source. Florez-Leiva et al. (2010) found that coastal open-pond systems containing *Nannochloris* emitted large quantities of N_2O during senescence. They speculated that oxidation of ammonium (NH_4) by bacteria was the likeliest N_2O -generation pathway under the observed aerobic conditions. Proper management of the algal cultivation systems, which would prevent senescence of algae and maintain aerobic conditions in ponds, likely would keep N_2O emissions to low levels.

Methanogenesis can occur in freshwater and marine sediments, waterlogged soils, marshes, and swamps where oxygen is low. These conditions might prevail in some ponds with substantial biomass or other organic matter in the sediment. Methane is released when organic acids, alcohols, celluloses, hemicelluloses, and proteins are degraded. Methane production is related to water temperature (Stadmark and Leonardson, 2005) and is maximized at neutral pH (Alexander, 1977). Methanogenesis is suppressed by nitrogen compounds that bacteria can use as electron acceptors, including nitrate and nitrite (Bollag and Czlonkowski, 1973), but these compounds may be reduced easily in oxygen-depleted environments. Methanogenesis and denitrification might be enhanced if the culture fails. During catastrophic failure of the culture, the dense algal cultures in algal biofuel ponds can become anaerobic and emit a variety of volatile nitrous or sulfur compounds as well as methane. However, culture failures would be expected to be short-term and rare occurrences if algal biofuel companies are to maintain a profit margin.

5.3.2 Opportunities for Mitigation

The opportunities for mitigating energy use discussed in the section Energy in Chapter 4 apply to reduction of GHG emissions. There is additional potential to mitigate GHGs by using low-carbon energy sources for processing and by substituting for carbon-intensive coproducts. For example, the carbon benefit of generating bioelectricity is larger in areas where the grid relies on fossil fuels. The yields for producing and properties of different coproduct options are poorly understood. The potential for N_2O and methane emissions could be reduced through thorough mixing and proper management of algae cultivation (Fagerstone et al., 2011).

5.3.3. Data and Method Gaps

The data gaps for estimating energy use and the method gaps in reducing energy use discussed in the section Energy (Chapter 4) apply to reduction of GHG emissions.

5.3.4 Sustainability Indicators

An appropriate sustainability indicator for GHG emissions is the amount of CO₂ equivalent emitted per unit energy produced, which has been selected as an indicator for GHG emissions of biodiesel and commonly has been used in discussing energy-related GHG emissions (GBEP, 2011; Mata et al., 2010).

5.4 LOCAL CLIMATE

5.4.1. Potential Environmental Effects

The introduction of large bodies of water in arid or semi-arid environments could alter the local climate of the area by increasing humidity and reducing temperature extremes. Similarly, the introduction of large-scale, open-pond algal cultivation systems in arid or semi-arid environments, where much of algae production in the United States is projected to take place (see Chapter 4), could affect local climate and ecosystems. The use of photobioreactors would not likely alter local climate.

Studies of reservoirs provide some useful ecological information. Reservoirs created by the damming of rivers could affect evaporation rates of the surrounding landscape, leading to changes in vegetation cover and terrestrial species diversity (Huntley et al., 1998). Large dams can affect surrounding climate and precipitation, particularly in Mediterranean and semi-arid climates (Degu et al., 2011).

5.4.2 Sustainability Indicators

The sustainability indicators for potential changes in local climate are trends in relative humidity and trends in temperature distribution statistics.

5.4.3 Information and Data Gaps

While parallels can be drawn from the introduction of large reservoirs in arid regions, the variability in size, geography, and production methods that will emerge as the algae industry grows will necessitate additional research to fully understand and address the impacts associated with local climate alteration.

5.5 AIR QUALITY

5.5.1 Potential Environmental Effects

The air quality impacts of algal biofuel production will depend on system design. Different air quality issues arise in conjunction with the different steps of the algal biofuel supply chain. Thus, this section is organized by the steps along the production pathways. The wide range of potential organisms for producing algal biofuels and the wide range of final fuel products result in a broad range of possible air emissions.

This section focuses on the air quality emissions unique to algal biofuel production and does not consider emissions of fossil fuels used to power processing equipment or emissions of fossil fuels that may be used in manufacturing fertilizer or pesticides. The purpose of the chapter is to consider emissions unique to algal biofuel production so that appropriate indicators are identified. However, emissions from fossil fuels used along the production pathway of algal biofuel would need to be considered in any LCA of the air-quality impacts of different algal biofuel designs. Further, how algal biofuels will be scaled up and how air quality might change with increasing scale is uncertain.

5.5.1.1 Open-Pond Cultivation

The committee is not aware of any measured emissions of atmospheric pollutants from algal biofuel feedstock ponds published in the literature. Under normal running conditions in open ponds, the cultures are aerobic, and low emissions of volatile organic compounds (VOCs) are expected (Rasmussen, 1974; A. Ben-Amotz, Israel Oceanographic and Limnological Research Ltd., personal communication on November 1, 2011; Zuo et al., 2012). However, macroalgae and microalgae growing in natural marine environments are known to be important sources of VOCs, including isoprene and monoterpenes (Giese et al., 1999; Shaw et al., 2010). Researchers from Texas A&M University currently are screening and quantifying VOCs from a wide variety of marine and freshwater algal cultures and algal paste. Three of the species tested are being grown for biofuels in open raceways, open ponds, and closed photobioreactors, with test samples derived from cultures being grown in treated wastewater with CO₂ enrichment. In preliminary findings, 45 VOCs have been identified (P. Zimba, Texas A&M University, unpublished data).

Other emissions expected are aerosols that may be emitted directly or created in the atmosphere through reactions of gaseous emissions of precursor gases of sulfur dioxide (SO₂), nitrogen oxides (NO_x), NH₃, and VOCs. Aerosols could include algae and nutrients, as well as a wide range of compounds that are produced by microalgae, including toxins. (See section Pathogens and Toxins later in this chapter.) Microalgae in the natural marine environment are known sources of sulfate aerosols (for example, Liss et al., 1997).

A large number of algae produce odorous secondary metabolites (reviewed in Smith et al., 2008), but those algae are not likely to be selected for large-scale production. The odors are produced during aerobic growth as secondary metabolites. Other odorous compounds are associated with the decay of algae under anaerobic conditions where bacteria break down the organic material and produce hydrogen sulfide and NH₃, both of which have a strong odor. In open ponds intended for algae cultivation, anaerobic conditions are minimized. Emissions from photobioreactors would be lower than those from open ponds if undesirable gaseous products and odorous chemicals are scrubbed before gas exchange with the outside environment is permitted.

5.5.1.2 Drying

Drying processes may produce coarse and fine particulates, including algae and lysed algae. The concentrations of particulates in air will depend on the technologies used; for example, belt dryers and convective systems will lead to greater local emissions than passive solar drying. Whether emissions move beyond the facility will depend on the level of containment. Particulates could be an occupational hazard even in closed facilities. In confined areas, dust could be an explosion hazard. Poor drying methods also can give rise to decomposition of biomass and release of VOCs, amines, methane, and other compounds.

5.5.1.3 Extraction

Most proposed algal biofuel processing methods involve extraction of lipids or other compounds from cells using organic solvents. Extraction with organic chemicals, by necessity, results in some solvent emissions, and the quantities emitted depend on the technology applied. The most common solvent that is openly discussed by manufacturers is hexane (Demirbas, 2009; Lardon et al., 2009; Gong and Jiang, 2011). In an environmental assessment, Sapphire Energy, Inc., an algal biofuel company, noted that “less than 50 ppm of hexane will remain in the algal solids after the hexane recovery process. This residual hexane will be emitted fugitively from the algal solids to the atmosphere during conveyance to the IABR [Integrated Algal Biorefinery] oil purification process” (USDA-RD, 2009). Desirable properties of these solvents are low cost, recoverability, low toxicity, nonpolar structure, and poor extractor of non-lipid cell components (Rawat et al., 2011). Hexane is used as an extractant of vegetable oils in biodiesel production with fugitive hexane emissions (Hess et al., 2009). Compliance with regulatory standards likely would minimize release of solvents.

5.5.1.4 Pyrolysis

Technologies to convert total biomass to drop-in liquid fuels are being tested. These processes may have additional feed inputs and will have different air emissions from those from production of esterified or green diesels. Pyrolysis of biomass yields three energy products—solids (char), liquids (bio-oils), and gases—in various proportions depending on the temperature, pressure, residence time, and other factors. The gases are recycled to provide energy for the system and thus do not contribute directly to air emissions except for any fugitive emissions that might escape the system. The heating of the pyrolysis units might contribute a small amount of NO_x and carbon monoxide (CO). Additional energy, likely supplied by natural gas may be required to sufficiently dry the algal biomass prior to pyrolysis. Particulate emissions, acid gases, and hydrocarbon emissions from pyrolysis are not characterized in the literature. The bio-oil produced from whole-cell pyrolysis will require additional upgrading to produce transportation fuels. The upgrading can be done with a separate hydrotreating step or a process similar to the Integrated Hydrolysis and Hydroconversion process. In either case, input of hydrogen is required. The production of hydrogen produces low levels of NO_x (Spath and Mann, 2001) and makes a CO₂ stream that could be used to supply the algae cultivation.

5.5.1.5 Anaerobic Digestion

Anaerobic digestion for processing wastewater from algal biofuel production facilities is described in Chapter 2. NH₃ has been observed to be present in biogas from anaerobic digestion at concentrations up to 450 ppm (Schomaker, 2000). The concentration of NH₃ in biogas would depend on the nitrogen content of the particular feed material. Early work by Golueke et al. (1957) found that anaerobic digestion of algae yielded N₂ and NH₃ concentrations of the order of 4 to 5 percent (volumetric average) of the total gas production. NH₃ would not be released to air around the facility because of the desire to recycle nutrients required for cultivation.

5.5.1.6 Transportation Emissions

The primary categories of environmental effects associated with the end use of biofuels in vehicles are evaporative emissions and tailpipe emissions from fuel combustion.

TABLE 5-6 Comparison of Typical Tailpipe Emissions from Biofuels to Conventional Gasoline or Diesel

BIOETHANOL (E10)	BIOETHANOL (E85)	BIODIESEL (B20 and B100)	FISCHER-TROPSCH
<ul style="list-style-type: none"> • 16% reductions in CO. • Reduction in particulate emissions. • No significant reduction in NO_x emissions. • Higher acetaldehyde emissions. 	<ul style="list-style-type: none"> • 15% reductions in ozone-forming volatile organic compounds. • 40% reductions in CO. • 20% reductions in particulate emissions. • 10% reductions in NO_x emissions. • 80% reductions in sulfate emissions. • Lower reactivity of hydrocarbon emissions. • Higher ethanol, acetaldehyde emissions. 	<ul style="list-style-type: none"> • 10% (B20) and 50% (B100) reductions in CO emissions. • 15% (B20) and 70% (B100) reductions in particulate emissions. • 10% (B20) and 40% (B100) reductions in total hydrocarbon emissions. • 20% (B20) and 100% (B100) reductions in sulfate emissions. • 2% (B20) and 9% (B100) increases in NO_x emissions. • No change in methane emissions (either B20 or B100). 	<ul style="list-style-type: none"> • NO_x reductions due to the higher cetane number and even further reductions with the addition of catalysts. • Little or no particulate emissions due to low sulfur and aromatic content. • Expected reductions in hydrocarbon and CO emissions.

NOTES: E10 is a fuel blend of up to 10% denatured ethanol. E85 is a fuel blend of up to 85% denatured ethanol. B20 is a blend of 20% biodiesel and 80% petroleum diesel and is the most common biodiesel blend in the United States. B100 is 100% biodiesel. Fischer-Tropsch (F-T) synthesis converts a mixture of CO and hydrogen (which may be derived from biomass) into liquid hydrocarbons.

SOURCES: Dufey (2006), EPA (2002a,b,c), and Graham et al. (2008).

Generally, the type and quantities of emissions vary depending on fuel characteristics (for example, chemical properties and blends), age of the vehicle or other equipment, power output of engine, operating condition of engine, how the vehicle or other equipment is operated, and ambient temperature (Graham et al., 2008; Yanowitz and McCormick, 2009; Ginnebaugh et al., 2010). Using biofuels in place of petroleum-based fuels decreases emissions of some air pollutants while increasing others (Table 5-6; NRC, 2011). EPA established emission standards for tailpipe emissions of CO, hydrocarbons, NO_x, and particulate matter to which vehicle manufacturers and refiners have to comply (EPA, 2009a).

5.5.1.7 Life-Cycle Assessment

Emissions of air pollutants need to be assessed over the life cycle of algal biofuels and compared to petroleum-based fuels and other alternatives. The Hill et al. study (2009) and data therein (NRC, 2011) illustrate the importance of such assessment. They found that although the uses of gasoline and terrestrial-plant biofuels (corn-grain ethanol and cellulosic ethanol) release similar amounts of VOC, PM, NO_x, SO_x, and NH₃, emissions from the production stages are significantly different between petroleum-based fuels and biofuels. Biofuels emit higher quantities of VOCs, NO_x, NH₃, and PM_{2.5} than petroleum-based fuels (Hill et al., 2009). The committee is not aware of any LCA of such air pollutants for algal biofuels. Such analysis is critical in assessing whether biofuel production and use result in air quality improvement compared to fossil fuel and it provides information on stages in the supply chain that are key contributors to air pollutants.

TABLE 5-7 An Illustration of Potential Contributions from Different Stages of the Algal Biofuel Supply Chain to Air Pollutants

Potential Effect	Pathway				
	Open-pond, salt water, producing biodiesel, recycling nutrients and water	Open-pond, salt water, producing biodiesel + coproducts	Open-pond, salt water, producing FAME, recycling nutrients and water	Photobioreactor, salt water, direct synthesis, recycling water	Open-pond, salt water, producing biomass, pyrolysis, recycling some nutrients and water
Emissions from Culture	VOCs, nitrous oxides, methane, aerosols possible	VOCs, nitrous oxides, methane, aerosols possible	VOCs, nitrous oxides, methane, aerosols possible	None	VOCs, nitrous oxides, methane,
Odors from Culture	Some odors	Some odors	Some odors	No odors	Some odors
Drying	Particulates generated, amount depending on drying process	Particulates generated, amount depending on drying process	Particulates generated, amount depending on drying process	No drying	Particulates generated, amount depending on drying process
Extraction	Some solvent vaporization	Some solvent vaporization	Some solvent vaporization	No extraction so no solvent vaporization	No extraction so no solvent vaporization
Pyrolysis	Not applicable	Not applicable	Not applicable	Not applicable	Particulate emissions, hydrocarbon slip, and acid gases all possible from combustion of off-gas
Anaerobic Digestion	Possibility of low or negligible NH ₃ release	Not applicable	Possibility of low or negligible NH ₃ release	Not applicable	Not used

5.5.2 Comparison of Pathways

With respect to air quality, the differences in expected effects among the pathways in Chapter 3 depend on the type of culture system (open versus closed), the drying process, and whether or not extraction and pyrolysis steps are present in the pathway (Table 5-7).

5.5.3 Potential Social Acceptability Effects

Algae produce a number of aerosols and secondary metabolites, some of which may be noxious (for example, malodorous) or harmful to humans. Similarly, some supply-chain processes, such as extraction and drying, may emit solvents or particulates that could affect local air quality if not contained. If an algal biofuel facility is located near human populations, measures likely will be taken to contain or limit the release of any products that negatively affect local air quality or are perceived to be a risk to public health. The health costs of some types of air emissions were discussed in Hill et al. (2009). Depending on the quantity of these outputs, and the proximity of population centers to a production facility, the reduction in air quality and perceived health and quality-of-life risks may impact the

siting and permitting processes, making it more difficult for developers to secure land and obtain permits. If the public is not made aware of these potential effects prior to the siting and permitting of a facility, there is a risk that the production of undesirable compounds will be viewed as unacceptable after the construction of the facility has been completed. If this is the case, litigation or protests may slow or shut down operations, resulting in financial losses for the developer and negative attention for the industry at large.

5.5.4 Opportunities for Mitigation

The more contained a process is, whether it is the biomass cultivation process, drying, solvent extraction, pyrolysis, or digestion, the lower the emissions to air will be. Therefore, photobioreactors could have reduced air-quality impacts compared to open-pond systems. However, full LCA of the air pollutant emissions associated with the production of the bioreactor materials and system operation also would be needed to assess whether photobioreactors represent a small or negligible impact on air quality. Although passive processes (for example, solar drying) reduce air quality impacts compared to active processes that generate dust or increase volatilization rates, they are not practical solutions at large scale. Siting facilities at a distance from human population centers and ecological species of concern would mitigate potential adverse effects of air pollution on humans.

5.5.5 Sustainability Indicators

Appropriate sustainability metrics for air quality would depend on the processes used in algal biofuel production. Concentrations would have to be measured or modeled at scales appropriate to bound regulatory levels or potential human health or annoyance effects. These may include:

- For open pond systems, concentrations of VOCs and odorous secondary metabolites.
- For active drying processes, concentrations of particulates in air.
- For extraction processes, air concentrations of the solvent used.
- For pyrolysis, particulates, hydrocarbons, and acid gases.

5.5.6 Information and Data Gaps

Measuring air emissions from large open ponds can provide information for occupational and other environmental exposure estimates that can be compared to thresholds for human health or environmental effects. Information and data gaps include the relationship between particular drying technologies and the types and concentrations of particulates released, releases of solvents during extraction, likely concentrations of NH_3 in air during anaerobic digestion, and chemicals potentially released during pyrolysis. That information would be submitted when the biorefineries seek air-quality permits.

5.6 SPECIES INVASIVENESS AND AQUATIC BIODIVERSITY

Species invasiveness is a concern unique to biofuels produced from algae and vascular plants. In addition, changing land use or altering landscapes to produce algal biofuel feedstocks can affect biodiversity. Effects of many biofuel feedstocks on biodiversity and mechanisms leading to those effects are beginning to be understood. However, existing studies (Fargione et al., 2009; Fletcher et al., 2011; Wiens et al., 2011) focus primarily on

terrestrial ecosystems and terrestrial plant biofuel feedstocks, rather than on aquatic systems and algal feedstocks.

5.6.1 Distributions of Algae in Natural Aquatic Environments

Many cyanobacteria and eukaryotic microalgae are cosmopolitan in their spatial (biogeographical) distributions and therefore could not be invasive if released in regions included in their broad habitat range. However, they are not necessarily found in every location where their habitat requirements (for example, pH, salinity, temperature, moisture, and climate) are met, so their distribution is often mosaic-like (Hoffmann, 1996). Other algae may be endemic to particular regions, for example, some cyanobacteria in Swedish lakes (Rott and Hernandez-Marine, 1994) and particular marine species (Hoffman, 1994). Endemic species could become invasive if transported elsewhere, but these species could also exist in low numbers in other locations even though they have not been recorded there. Algae may have broader distributions than what has been recorded because of the lack of sampling on some continents (especially of benthic habitats) and because of the lack of detection of organisms at low densities (Hoffmann, 1996). Coastal marine macroalgae tend to be less cosmopolitan in their spatial distribution than phytoplanktonic cyanobacteria and microalgae. Macroalgae have narrower temperature, light, substratum, and nutrient preferences.

The wide range of processes that could transport microalgae away from open water also could contribute to their dispersal and consequentially to a broad distribution. Many algal species can be transported by air (Grönblad, 1933). Vectors of algae include aquatic insects (Stewart et al., 1970), dragonflies, wasps (Maguire, 1963), fish (digestive tracts, Velasquez, 1940), beetles (references in Kristiansen 1996), water-living mammals such as raccoons (Maguire, 1963), minks (Irene-Marie, 1938), and muskrats (Roscher, 1967). The most important vectors of algae are birds (Atkinson, 1972; Kristiansen, 1996). In one study of 16 species of waterfowl, 86 species of algae were found on the feet, 25 species on the feathers, and 25 species on the bills. Most algae survived out of surface waters for four hours, but most did not survive for more than eight hours (Schlichting, 1960).

Some species of algae may appear to be rare. Whitford (1983) explains that species of freshwater algae may appear to be rare for several reasons (for example, infrequent historical collections, species with long-lived spores that do not easily germinate, and species that are highly specific in their habitat requirements), but that very few freshwater species are actually rare. This suggests that few rare species of algae could be displaced by invasive algae used to produce biofuel feedstocks.

5.6.2 Releases of Algae to Natural Environments

Releases of improved nongenetically engineered or genetically engineered strains of algae from biofuel production cultures to natural environments can be expected to be common, especially from open ponds. Releases may occur during the feedstock production stage or possibly during the harvesting or drying stages. Releases probably will occur most often through aerosolization, although leakages from ponds or weather-related spillage (for example, high tides and heavy storms) also are possible.

The probability of release from an open pond would be related to pond area and freeboard space (that is, the distance between normal water level and the top of the cultivation pond), the direction and speed of prevailing winds, the frequency and quantity of

precipitation (for example, rain splash), distance to water bodies, the probability and intensity of visitation by potential vectors such as aquatic birds and mammals, and the absolute abundance (in cells per mL) of the species that might be released into the environment. Humidity affects the survival of unicellular algae (Ehresmann and Hatch, 1975). Survival rates differ among algal groups. In one study climatic characteristics such as temperature, relative humidity, rainfall, wind velocity, and hours of sunshine affected the release and vertical transport of algae (Sharma and Singh, 2010).

Atmospheric density of algae is affected by aerosolization rate (Sharma and Singh, 2010), wind speed, and rainfall, as well as survival rate. The abundance of algae in the atmosphere also depends on taxonomy of the algae. In one study, cyanobacteria had the highest density, whereas chlorophytes and diatoms were much less common (Sharma and Singh, 2010; Wilkinson et al., 2011).

Dissemination to distant sites can occur through the air, through water, and by boats (Alexander, 1971) or animal vectors. The wide range of vectors that could remove algae from open ponds include aquatic insects (Stewart et al., 1970), dragonflies (Maguire, 1963), birds (Atkinson, 1972), and raccoons (Maguire, 1963), among others. Closed photobioreactor systems would have a much lower risk of release and transport of algae. Harvesting operations from open or closed systems could be a major potential route for loss of microalgae to the surrounding environment.

If algae require culture media with characteristics substantially different from the surrounding natural environment (especially if the algae have narrow tolerance limits to nutrients concentrations, pH, or salinity), then releases to the local landscape likely would result in low survival rates. Survival rate would be further reduced if the cultured species is not tolerant of desiccation (Hoffmann, 1996).

5.6.3 Potential Environmental Effects

Environmental concerns associated with releasing algae from biofuel facilities into natural waters include the potential for species invasiveness, alteration of nutrient recycling and trophic relationships, and the displacement of rare algal species. Although some researchers and producers are considering the use of regionally native species that are adapted to the local climate (Odlare et al., 2011), other algal production facilities may use nonnative species or species that have been selected and bred or genetically modified for desirable characteristics for algal biofuel production. Some of the nonnative or improved species may be invasive in some environments. Invasive algae can compete with native species for light, space, or nutrients, and have different tolerances for stressors, compared to native species (White and Shurin, 2011). Thus, invasive species can affect community composition and ecosystem processes (Strayer et al., 2006). Successful invasions are characterized by the invasive potential of the invader and the invasibility of the native community (Lonsdale, 1999). Species that are not invasive in one environment may be invasive when introduced to a different habitat (Raghu et al., 2006). For example, an algal species that thrives in saline waters may not survive or may invade freshwater ecosystems, even if released in a large quantity. Whether the ecological niches of invaders and the invaded community overlap is a predictor of success as well (Mehner et al., 2010). Whether a particular cultured algal species poses a threat as an invasive species to the surrounding aquatic environments needs to be considered. Some of the same characteristics that can make a species desirable as a biofuel feedstock, for example, rapid growth, vegetative propagation, pest resistance, and robustness in culture, also are those associated with invasiveness.

TABLE 5-8 An Illustration of Potential Environmental Effects Resulting from Species Invasion by Algae Cultivated for Fuels

Potential Effect	Pathway				
	Open-pond, salt water, producing biodiesel, recycling nutrients and water	Open-pond, salt water, producing biodiesel + coproducts	Open-pond, salt water, producing FAME, recycling nutrients and water	Photobioreactor, salt water, direct synthesis, recycling water	Open-pond, salt water, producing biomass, pyrolysis, recycling some nutrients and water
Transport of Invasive Algae into New Environments	Possible but unlikely with appropriate controls	Possible but unlikely with appropriate controls	Possible but unlikely with appropriate controls	Impossible unless accidental breach of photobioreactor	Possible but unlikely with appropriate controls
Algal Blooms	Possible for nontarget species in cultures; low likelihood of blooms of nontarget species released to natural environments	Possible for nontarget species in cultures; low likelihood of blooms of nontarget species released to natural environments	Possible for nontarget species in cultures; low likelihood of blooms of nontarget species released to natural environments	Impossible unless accidental breach of photobioreactor	Possible for nontarget species in cultures; low likelihood of blooms of nontarget species released to natural environments

Releases of some exotic algal species, particularly from open-pond cultures, could threaten the integrity of local and regional ecosystems (Ryan, 2009). Blooms of exotic species could displace native species, with adverse impacts on organisms that feed on those species propagating through aquatic food webs. An example is the diatom *Didymosphenia geminata* (also known as *Didymo* or *Rock Snot*) that can cause dense algal blooms. The blooms block sunlight and cause a local decline in native plant and animal life. Historically, *D. geminata* occurred mostly in northern latitudes in nutrient-poor waters, but it now has been observed in nutrient-rich water at lower latitudes—possibly a genetic variant that has broader tolerances than the original genotype (see Global Invasive Species Database; ISSG, 2012).

5.6.4 Comparisons of Pathways

The primary variable that is different among the pathways in Chapter 3 and would influence the likelihood of species invasions and changes in biodiversity is whether the pond system is open or closed (Table 5-8).

5.6.5 Opportunities for Mitigation

Algal species known to be noninvasive or unlikely to cause harmful blooms could be selected for large-scale cultivation for fuels. Invasiveness varies in different natural environments, and site-specific assessments might be necessary to reduce risks of invasion. Moreover, species that are intolerant of conditions in natural waters (for example, salinity) in the vicinity of the biofuel facility may be selected to minimize the risk of invasion if released.

Landscape design also may be considered to limit any potential impacts of releases of algae from pond systems. Placing systems well away from waterways and wetlands where pond algae may thrive could reduce or minimize the likelihood of blooms of released species. When considering the factors that affect the probability of release and the abundance of released organisms above, then mitigation measures might include shields from wind and mechanisms to discourage vectors.

5.6.6 Sustainability Indicators

Indicators of sustainable ecological communities include metrics of aquatic diversity and invasiveness of algae. One category of such metrics would be diagnostic traits for invasiveness. Qualitative metrics that are related to invasiveness, but not necessarily diagnostic, include:

- Fast growth in natural environments.
- Wide habitat tolerances, for example, tolerances for temperature, light, and nutrients.
- Pest and herbivore resistance.
- Aggressive competition for resources, for example, light, nutrients, or space.

More direct metrics of aquatic biodiversity that relate to the sustainability of biofuels are recommended by McBride et al. (2011) and are pertinent here:

- Presence of taxa of special concern. These may include rare fish, aquatic invertebrates, or macrophytes.
- Habitat area of taxa of special concern, which for aquatic organisms might translate to stream reach length for taxa of concern.

Additional sustainability indicators for aquatic biodiversity might include the types of metrics found in recovery plans for species protected under the Endangered Species Act (Table 5-9).

TABLE 5-9 Recovery Goals for Endangered and Threatened Species in U.S. Fish and Wildlife Service Recovery Plans

Type of Recovery Goal	Metric for Recovery
Population	Total population size Number of subpopulations Number of individuals in each subpopulation Trends in total population size Trends in number of subpopulations Trends in number of individuals in each subpopulation
Demography	Age structure of population Productivity and net recruitment
Habitat	Total range (presence/absence) Quality of habitat Quantity of habitat

SOURCE: Adapted from Efromyson et al. (2009), whose sources were Campbell et al. (2002) and Gerber and Hatch (2002). Reprinted with permission from Elsevier.

5.7 TERRESTRIAL BIODIVERSITY

5.7.1 Landscape Pattern of Development

5.7.1.1 Potential Environmental Effects

The pattern of landscape conversion for any new infrastructure could affect terrestrial species and community diversity through at least three distinct mechanisms that also apply to algal biofuel production or other energy production (McCabe, 1994; DOE, 2009, 2010a; Garvin et al., 2011):

- Displacement of terrestrial vegetation and wildlife habitat from the facility area and replacement with a pond or photobioreactor containing a monoculture or a few species of algae.
- Reduction in local wildlife habitat area below the threshold needed for the species.
- Fragmentation of wildlife habitat such that mates are more difficult to find or migration corridors are disrupted. The magnitude of land requirements (discussed in Chapter 4) and the types of conversions (discussed in section Land-Use Change in this chapter) influence the magnitude of potential effects on ecological populations and communities.

Displacement of native vegetation and individual vertebrates usually is limited to the area of the facility, but some species are sensitive to human infrastructure and tend to be displaced to distances beyond the boundaries of the facility, for example, female sage grouse avoiding nesting within 950 meters of infrastructure associated with natural gas fields (Holloran et al., 2010).

Extensive infrastructure, especially from multiple facilities, could fragment habitat for some wide-ranging vertebrates. Fragmentation of habitat is determined less by the area of a facility than by the dimensions compared to significant habitat types or corridors. One measure of fragmentation is the ratio of the perimeter (patch edge length) to the area of a habitat patch (Dale and Pearson, 1997). Thus, a linear facility would tend to be fragmenting in more environments than one that is closer to square. However, the latter configuration is more practical for system maintenance, so extensive linear facilities are not considered. Other potential measures of fragmentation include the percent of the landscape occupied by a given habitat, the number or density of habitat patches within a given area (more patches means greater fragmentation), and the degree of connectedness or isolation among habitats (McGarigal et al., 2005).

Even where habitat is not fragmented, human infrastructure and associated disturbance could reduce the habitat area beyond minimum levels required by certain species. Carlsen et al. (2004) review critical patch sizes (contiguous habitat area necessary to conserve a population) required by many species, such as the minimum patch size that can sustain a viable population. They found that few studies examined behavioral or population dynamics associated with large areas of contiguous habitat, which also contained smaller patches of unsuitable or disturbed lands (as in algal biofuel development or oil and gas development). An exception is a theoretical study of American badger at an oil production site that investigated the effects of increasing areas of patches of disturbance on an otherwise highly suitable matrix of tallgrass prairie in Oklahoma (Jager et al., 2006). Critical disturbance areas would depend on the species of concern, the habitat type, habitat suitability, and type of infrastructure.

Impacts on terrestrial vegetation and wildlife could vary widely, depending on the specific sites chosen and the land-use baseline and dynamics prevailing in the absence of algae cultivation and algal biofuel refineries. According to Wigmosta et al. (2011), within the land area potentially suitable for biofuels, land cover types consisted of 42 percent shrub or scrub, 19 percent herbaceous, 14 percent evergreen forest, 10 percent pastureland, 8 percent deciduous forest, and 7 percent other lands including mixed forest, barren, and low-intensity developed. As discussed in Chapter 4, the most favorable conditions in terms of land and water requirements were in the Gulf Coast region. Shrub-scrub habitat in the United States is widely distributed but is threatened by changes in land-use patterns; numerous bird species dependent on this habitat type are in decline (NRCS and WHC, 2007). Development of large areas of shrub-scrub for ponds, up to 181,000 square kilometers (using figures from Wigmosta et al., 2011), could accelerate this decline.

5.7.1.2. Opportunities for Mitigation

The presence and abundance of wildlife need to be assessed prior to construction, as is done for facilities that are subject to environmental assessment (DOE, 2010a). Landscape design could minimize potential effects on biodiversity. Dale et al. (2011) suggest that incorporating design considerations recommended for bioenergy could prevent or minimize adverse effects on terrestrial biodiversity, for example by maintaining corridors for movement of terrestrial wildlife. In planning the size of individual ponds, their density on the landscape, and associated production facilities, managers would have to consider potential environmental impacts on biodiversity.

5.7.2 Wildlife Drinking

5.7.2.1 Potential Environmental Effects

Open algal ponds may be sources of water to wildlife that may prove beneficial in arid conditions or harmful if toxic to certain species. The risks of animals being exposed to salinity or chemicals in water from algae cultivation ponds and having adverse effects from drinking or dermal exposures are unknown.

Toxicity from salt exposure is possible. This occurs when salt or chloride are accumulated in blood at toxic levels and, in the case of birds, at rates too high to be excreted by salt glands. For example, mortality from sodium toxicity has been observed at hypersaline playa lakes of southeast New Mexico (Meteyer et al., 1997). However, the water for algae cultivation is not likely to be hypersaline. Coastal bird species have specialized organs to accommodate high salt levels (Hughes, 2003). Lethal and sublethal salinity concentrations for some species are summarized in a U.S. Department of the Interior report (1998), with toxicity threshold values for ducks ranging from 9 to 20 parts per thousand (compared to the salinity of most seawater at 35 parts per thousand).

Many chemical and behavioral factors could influence exposure of wildlife to salt and other chemicals in open-pond systems. For example, artificial water developments in desert environments are sometimes an important water source for local bird populations (Lynn et al., 2006), but can be less important for some migratory species (Lynn et al., 2006) or animals that may have a strong fidelity for specific water sources (Dickens et al., 2009). If ponds are sited near wastewater treatment facilities and CO₂ sources (that is, near population centers), then water is unlikely to be rare in the landscape and wildlife will have many options for water sources. Ponds with dense algae might not be as attractive to wildlife as more pristine

water, but this hypothesis is untested. Similarly, the effect of dense algae on the attractiveness of ponds for wildlife drinking is unknown. For oil-field wastewater evaporation ponds, bird exposures appear to be episodic, coinciding with migration behavior (Ramirez, 2010). To consider potential exposures of wildlife to toxicants in culture water from algal biofuel facilities and their potential effects, analogies may be made to agricultural evaporation ponds and oil-field wastewater evaporation ponds.

In the western part of the San Joaquin Valley, California, agricultural evaporation ponds have been developed where other options for disposal of drainage water are limited. Birds use evaporation ponds for resting, foraging, and nesting (Evaporation Ponds Technical Committee, 1999). One study of shorebird use in California's Central Valley found that agricultural evaporation ponds are very attractive to these birds (Shuford et al., 1998). In another study, northern pintails (*Anas acuta*) wintering in Tulare Basin, CA, were found not to use or select agricultural drain-water evaporation ponds or sewage treatment ponds (which might appear similar to some algal biofuel ponds) and to prefer flooded fields and marshes (Fleskes et al., 2003).

For agricultural evaporation ponds, the primary wildlife concern has been the concentration of selenium (Evaporation Ponds Technical Committee, 1999); its environmental transformations and accumulation have been studied (Gao et al., 2007). Whether selenium might represent a significant exposure in algal ponds depends on the availability of selenium in source water and in underlying soils if pond water seeps out. Some investigators suggest that waterfowl exposed to waters from agricultural evaporation ponds might be at risk from uranium toxicity (Duff et al., 1997). Uranium accumulation in pond sediments was attributed in part to decaying algae. Arsenic dynamics also have been studied as a potential concern (Ryu et al., 2010).

In another potentially analogous example, birds (Ramirez, 2010), as well as bats, amphibians, reptiles, small mammals, game species, and insects (Ramirez, 2005), have been observed to be attracted to large (0.4 to 2 hectares) evaporation ponds from oilfield wastewater disposal facilities in the western United States. Bird fatalities from those ponds generally are attributed to oil, but sodium toxicity and surfactants have been implicated in some cases (Ramirez, 2010).

Attraction to algal ponds could be a major problem if they contain toxic chemicals or pathogens at harmful concentrations. Fish injuries (Čada, 1998) and bird fatalities (Osborn et al., 2000) have affected the social acceptability (and therefore the sustainable development) of hydropower and wind energy, respectively. Adaptive management can play a role in mitigating any adverse effects on wildlife through exposure via drinking.

5.7.2.2 Opportunities for Mitigation

The committee is not aware of any reports of wildlife drinking being a concern in existing open-pond algae cultivation facilities. As the number and size of facilities increase, concentrations of potential toxicants in water and wildlife drinking exposure needs to be monitored to ensure that the latter is not a concern. The fail-safe mitigation for wildlife exposure to salinity or any toxicants in culture waters is to use closed photobioreactor systems. Moreover, salinity concerns would be eliminated through the use of fresh water, though Chapter 4 discusses resource constraints for fresh water at commercial scales of development. Mitigations for open-pond systems might include netting to prevent exposure (as in the oilfield wastewater evaporation ponds), but this would be expensive and only necessary if wildlife exposure proves to be a problem. Rapid stirring could make ponds less suitable as wildlife drinking habitat than still water. Other wildlife deterrents may be

TABLE 5-10 An Illustration of Potential Environmental Effects on Wildlife from Different Pathways of Algal Biofuel Production

Potential Effect	Pathway				
	Open-pond, salt water, producing biodiesel, recycling nutrients and water	Open-pond, salt water, producing biodiesel + coproducts	Open-pond, salt water, producing FAME, recycling nutrients and water	Photobioreactor, salt water, direct synthesis, recycling water	Open-pond, salt water, producing biomass, pyrolysis, recycling some nutrients and water
Alteration of Terrestrial Habitat	Displacement of vegetation and vertebrates (habitat loss), possible fragmentation of habitat	Displacement of vegetation and vertebrates (habitat loss), possible fragmentation of habitat	Displacement of vegetation and vertebrates (habitat loss), possible fragmentation of habitat	Displacement of vegetation and vertebrates (habitat loss), possible fragmentation of habitat, probably at a smaller spatial scale than for other pathways	Displacement of vegetation and vertebrates (habitat loss), possible fragmentation of habitat
Wildlife Exposed to Saline Water or Toxicants	Exposure and adverse effects are possible	Exposure and adverse effects are possible	Exposure and adverse effects are possible	Exposure to water in a closed system is not possible	Exposure and adverse effects are possible

possible. Some of the mitigations used for oilfield wastewater evaporation ponds, such as covering the surface with plastic balls to make the ponds less attractive to birds (Ramirez, 2010), are not options for photosynthetic fuel sources. Similarly, mitigation strategies used in agricultural evaporation ponds, such as steepening pond slopes or maintaining deep water levels that reduce suitability of bird feeding habitat, are not practical for algae cultivation that requires shallow ponds (Evaporation Ponds Technical Committee, 1999). As open ponds are monitored for chemical contaminants, toxicity thresholds for these chemicals will help determine when culture waters need to be disposed and renewed.

5.7.3 Comparisons of Pathways

As with land-use change, regarding the landscape pattern of development, the primary relevant difference among the pathways in Chapter 3 is the difference between the land required for open-pond and photobioreactor systems (see Chapter 4). For wildlife drinking, the primary variable of interest is closed versus open systems (Table 5-10).

5.7.4 Sustainability Indicators

Metrics of terrestrial biodiversity for the sustainability of biofuels that are recommended by McBride et al. (2011) and pertinent to issues related to the landscape pattern of development include:

- Presence of taxa of special concern (presence).
- Habitat area of taxa of special concern (hectare).

Habitat area can be a proxy for population size (Turlure et al., 2010). As with aquatic diversity metrics, additional sustainability indicators for terrestrial biodiversity might be obtained from recovery plans for species listed under the Endangered Species Act (Table 5-9).

For wildlife exposures to salinity and contaminants in drinking water, sustainability indicators would include:

- Dosage received by wildlife (direct measure).
- Number of vertebrate fatalities from drinking from algal ponds per year (direct measure).
- Concentrations of toxicants, toxins, or salinity in culture medium (less direct measure).
- Abundance of vertebrates drinking from open ponds per year (less direct measure).

5.7.5 Information and Data Gaps

Patterns of development of algal biofuel facilities in relation to wildlife corridors have not been studied because locations for future development are uncertain. The spatial scale and landscape pattern of these developments needs to be understood to simulate the effects on wildlife populations. As algae cultivation expands in number and scale, the potential for wildlife drinking needs to be assessed at sites. If wildlife drinking is observed, then concentrations of toxicants in source waters and culture waters need to be measured to ensure that there is no threat to wildlife health. Alternatively, measures to deter wildlife drinking can be implemented.

5.8 ENVIRONMENTAL EFFECTS OF GENETICALLY ENGINEERED ORGANISMS

5.8.1 Potential Environmental Effects

The environmental sustainability of genetically engineered feedstocks for bioenergy (Wolt, 2009; Moon et al., 2010) and the potential implications of regulations on sustainable development of the industry (Moon et al., 2010; Strauss et al., 2010) have been considered previously, but the emphasis has been on engineered terrestrial crops (Moon et al., 2010) rather than algae. Some algal biofuel companies, such as Algenol and Synthetic Genomics, are conducting research on genetically engineered organisms for algal biofuel production (Gressel, 2008). In a hypothetical, worst-case scenario, genetically engineered algae that have been introduced to natural environments might persist and become so abundant that they create harmful algal blooms (Snow and Smith, 2012). Clearly, any adverse effects of released genetically engineered algae, if observed, would affect the sustainable development of algal biofuel technologies. The evaluation of potential effects of genetically engineered algae will be a complex undertaking, given the diversity of organisms, range of engineered functions, and range of environments potentially receiving the engineered organisms (Tiedje et al., 1989). This section of the report addresses the novel traits and genetic structure of genetically engineered cyanobacteria and microalgae for biofuels and whether they have unique or more uncertain risks. (Potential genetic manipulation methods are discussed in Chapter 2.)

Past broad assessments of the risks of genetically engineered organisms have concluded that the product (novel traits) is more important than the process (genetic engineering techniques) for evaluating risk (NRC, 1987; Tiedje et al., 1989; Snow et al., 2005). However, novel traits may be more common when the process for creating new algae involves direct genetic manipulation than when horizontal gene transfer occurs in evolutionary time.

Several traits of algae for biofuels may be modified through genetic engineering methods. Most are intended to increase biomass or oil productivity, though some could be designed to minimize survival or reproduction following release. Increasing productivity could involve objectives such as enhancing lipid content as a precursor to biodiesel (which could involve growing cells in nitrogen-deficient or silicon-deficient media), introducing biological pathways that permit direct production of fuels that need minimal processing prior to distribution and use, modifying cells to secrete feedstock or fuel directly into the culture medium, modifying carbohydrate metabolism in cells (increasing glucan storage, decreasing starch degradation), increasing tolerance to stressors (such as salt, light, pH, temperature, glyphosate) (Radakovits et al., 2010), and improving resistance to disinfectants. Some of these engineered traits and intended or unintended accompanying traits could affect either the suitability of algae for biofuel production purposes or their survival and physiology when released into natural systems. Phenotypic changes that could lead to potential major ecological effects of released organisms include those that result in increases in physiological tolerance or altered substrate use or that change the species' geographic range (Tiedje et al., 1989).

Predictors of potential adverse effects of genetically engineered algae include probability of release, abundance of organisms released (predictor of establishment), survival rate and fitness, reproduction rate, probability of dissemination to distant sites, interactions with other organisms, probability of genetic exchange, and probability of an adverse effect (Alexander, 1985). New traits potentially can influence these factors, but few of these relationships are understood. Cell density in the culture medium could be affected by engineered traits. The scale and frequency of releases might determine whether the release leads to a self-sustaining (established) population (Tiedje et al., 1989). The survival rate of a genetically engineered microalga or cyanobacterium will be determined by a combination of the species identity, the genetic modification(s), and the environment to which it is released. Algae with high lipid content probably will be more attractive to predators. Some researchers suggest that most genetically engineered organisms will have lower fitness in receiving environments than unmodified organisms (Tiedje et al., 1989). Algae could be cross-bred or engineered to have high growth rates under specific culture conditions, and some of these might have high growth rates under specific natural conditions. New traits conferred on algae by genetic modifications would determine whether and how community interactions might be altered. Radakovits et al. (2010) pointed out that it is uncertain how genetically engineered strains will perform in scaled-up production systems with varying conditions and with wild-type competitive strains.

Genetic exchange might lead to unexpected effects. Snow et al. (2005) asserted that genetic exchange between recombinant microbes and indigenous microbes is probable. The three types of horizontal transfer are transformation of free deoxyribonucleic acid (DNA), conjugation, and transduction. The transfer of genes between microorganisms is common in some species (Snow et al., 2005). About 1 to 20 percent of the genomes of bacteria consist of DNA acquired recently (in an evolutionary context), predominantly from other prokaryotes but also from eukaryotes, for example, metazoa (Ochman et al., 2000; Koonin et al., 2002; Snow et al., 2005). Genetic exchange between prokaryotes and eukaryotes is not well studied (Rogers et al., 2007) beyond specific pairwise interactions such as T-DNA transfer from *Agrobacterium* species to plant cells (Gelvin, 2010). Some ancient, evolutionary scale transfers have been recorded. For example, a gene for plastid-targeted fructose biphosphate aldolase was transferred from red algae to some *Prochlorococcus* and *Synechococcus* species (Rogers et al., 2007). Another study, for example, showed evidence for the horizontal transfer of a self-splicing, homing intron from a cyanobacterium

(*Calothrix*) to the chloroplast genome of *Euglena myxocylindracea* (Sheveleva and Hallick, 2004). Gene transfer between dissimilar organisms is possible, though rare. There is evidence that nuclear genes encoding chloroplast proteins have been transferred from an alga to an ascoglossan sea slug that consumes the algae (Pierce et al., 2003). Horizontally transferred genes can code for selectable traits, such as antibiotic resistance, pathogenicity, and metabolic enzymes (Snow et al., 2005). Horizontal gene transfer depends on the density of organisms with which exchange is possible.

The principal adverse effects of any algae, whether genetically engineered or not, could include health and ecological effects from toxin production, ecological effects from blooms, and species replacement. These potential effects are discussed elsewhere in this report. The potential propagation of antibiotic resistance markers also would be a concern, but species containing these markers would unlikely be used for commercial-scale production of biofuels. Toxin production by genetically engineered algae is unlikely because toxin-producing strains would be avoided, or strains probably would be engineered to remove toxin genes. A genetically engineered strain might have a lower risk of adverse impact than a natural strain that has not had such modifications. Categories of potential ecological risks from genetically engineered organisms that were highlighted by Tiedje et al. (1989) and Snow et al. (2005) would need to be considered in assessments of released algae. These include creating new or more effective pathogens, affecting non-target species, disrupting biotic communities and ecosystems, reducing biodiversity or species-genetic diversity, or degrading valuable biological resources, many of which are discussed in the section on invasive species. Little evidence is available to evaluate the potential for any of these effects. Species that are genetically engineered to become more tolerant of environmental stressors, such as salt or temperature, could bloom in habitat conditions where blooms previously have not occurred. Species replacement is a potentially delayed effect (Tiedje et al., 1989). Whether exposure to genetically engineered organisms or genetic exchange with these organisms poses any potential hazards depends on the particular traits of the organism (Snow et al., 2005).

Most approaches to risk assessment suggest that familiarity with genetically engineered organisms is an important predictor of risk (Efroymson, 1999). That is, genetically engineered organisms that have a history of safe use in applications similar to proposed uses (for example, at similar densities in similar ecosystems) would not be likely to threaten environmental sustainability of algal biofuels. Similarly, microorganisms that are not developed from dissimilar source organisms but rather are created from closely related organisms (see EPA, 1990) are less likely to have new traits and to cause adverse effects.

5.8.2 Social Acceptability of Genetically Engineered Algae

If algal biofuel companies are moving toward the use of genetically engineered algae, popular and political resistance could be anticipated. Some concerns over genetically engineered algae depend on the capability of these algae to survive and invade natural environments (as in the case of invasive algae) outside a production environment where temperature, nutrient loads, salinity, and pH all can be optimized. People have expressed concerns regarding the release of genetically engineered microorganisms, ranging from impacts of large-scale releases (and failure of control mechanisms) on biodiversity to ecosystem and evolutionary processes (Hagedorn and Allender-Hagedorn, 1997). Other concerns regarding genetic technologies relate to the unnaturalness of organisms (Tenbult et al., 2005; Connor and Siegrist, 2011); these cannot be abated through technical mitigation. It is the public

perception of risk, and not necessarily the scientific basis for risk, that will be preeminent in determining acceptability of genetically engineered algae to communities. Concerns over genetically engineered algae and perceptions of risks associated with introducing nonnative species into new geographies will need to be addressed.

While concerns surrounding the use of genetically engineered algae for energy production are likely to be raised as the industry continues to develop, the United States remains one of the most accepting countries in the developed world in terms of the adoption rates of genetically engineered crops (USDA-ERS, 2011). In 2008, U.S. farmers planted more than 32 million acres of Monsanto's "triple-stack" genetically engineered corn, and it is estimated that this number will increase to approximately 56 million acres by 2015 (Kaskey, 2009).

Nonetheless, social acceptability of gene technology depends on the type of application. In one study, medical applications were perceived to be more beneficial, less hazardous, and more ethical than food applications (Frewer et al., 1995). In a Swiss study, lay people distinguished between acceptability of medical and non-medical applications of gene technology but not among agricultural, nutritional, and industrial applications (Connor and Siegrist, 2011). Further, prevailing concerns over the use of genetically engineered crops in the United States are related to human health and food safety rather than potential ecological risks (Kamaldeen and Powell, 2000). However, concerns about genetically engineered microorganisms in surveys and in the popular press have related more to environmental effects than to health or ethical issues (Hagedorn and Allender-Hagedorn, 1997), and these concerns might be expected to dominate for microalgae. Coproduct markets such as health supplements, food additives, and cosmetics could attract additional scrutiny from consumers. It is unknown whether the U.S. public may be more tolerant of the use of biofuels from genetically engineered algae as an energy source than if the crops were grown for food.

Social acceptability of a new technology also depends on how a decision is framed. For example, Wolfe and Bjornstad (2003) suggested that options regarding the use of genetically engineered organisms for hazardous waste remediation likely would be presented in the context of multiple technology options. It is less likely that stakeholders evaluating the use of genetically engineered algae in their regions would be explicitly weighing the relative benefits and risks of different liquid fuels produced elsewhere.

Social acceptability of gene technology depends on trust (Siegrist, 2000). Whether the public is more willing to accept the use of naturally occurring algal strains than those that have been genetically altered for maximum fuel production might depend on the engagement of managers of the facility, other stakeholders, and the public.

5.8.3 Opportunities for Mitigation

Containment of genetically engineered algae might be desirable as a precaution against unknown effects and societal concerns. Physical containment of released algae will be difficult or impossible. Physical containment solutions, such as those proposed for vascular plants (for example, fences and border plants; Moon et al., 2010), are ineffective against released algae. Containment options might include using species that require saline water in freshwater environments or those that have a nutrient requirement that is not met outside of the photobioreactor or pond. The use of environment-dependent "molecular switches" has been proposed to increase the likelihood of community acceptance of genetically engineered crops (Chapotin and Wolt, 2007). Similarly, some modified traits could reduce fitness in natural environments. For example, reduced light harvesting antennae not only would increase growth in ponds but also would reduce the ability to compete with native algal

species for light in natural waters (Sayre, 2011). Moreover, removing bicarbonate pumps, which increases fitness in the cultivation environment with high CO₂, could reduce the competitive ability to take up inorganic carbon in natural waters (Sayre, 2011). If algicides are used, they would be effective against non-target organisms. Terminator genes that cause released cells to die could be developed so that those genes would be suppressed in a photobioreactor but derepressed in natural environments (Sayre, 2011). If algal strains that cannot produce toxins are used, potential risk is minimized. If genetically engineered organisms are released, monitoring should be undertaken so that effects of particular organism-environment combinations can be better understood (Snow et al., 2005).

5.8.4 Sustainability Indicators

Sustainability indicators for genetically engineered algae generally would be the same as indicators for native algae. That is, if effects of concern include biodiversity or water quality, appropriate metrics are described in those chapters. However, the sustainability goals for genetically engineered algae likely would include two other issues:

- Minimizing dissemination of genetically engineered algae.
- Establishing methods to determine whether an observed effect was caused by a genetically engineered alga.

Abundance of genetically engineered algae released to water could be measured through species-specific tests if the species were not native. Moreover, some modified traits, such as altered antennae, might be detectable microscopically and thus quantifiable in water. Particular DNA sequences also might be detectable. Moreover, markers could be added to algae to allow easy measurement in specific media.

5.8.5 Information and Data Gaps

The ecological risks of a release of genetically engineered microorganisms have to be carefully assessed before they are used in commercial-scale algal biofuel production. Whether there are plausible scenarios under which genetically engineered algae, or organisms that acquire genes from the genetically engineered algae, could proliferate to levels that might harm humans or the environment in some way needs to be examined. More information is needed on potential relationships between traits that are targets for modification and behavior of cyanobacteria or microalgae that could alter rates of release, survival, growth, transport, genetic exchange, and ecological or human health effects. Little research to date has been conducted in the United States on behavior of genetically engineered algae in open ponds, in part because EPA notification guidelines can lead to delays for researchers. Information is needed on the social acceptability of the use of genetically engineered algae for biofuels, particularly in open systems.

5.9 WASTE PRODUCTS

5.9.1 Potential Environmental Effects

Sustainability of a production process is enhanced by recycling raw materials and minimizing of waste. If the oil-extracted biomass is recycled or made into coproducts, a

source of waste would be reduced or eliminated. Anaerobic digestion is another method of waste disposal and can generate electricity as a coproduct (Chapter 2). For the disposal of waste biomass, blow down of solids from production and recycling ponds, and saltwater, companies are considering landfilling waste, underground injection, and diverting processed water to sewage systems.

Solid waste from algal biofuel manufacturing processes is most likely to be generated as sludge from an anaerobic digester from which the volatile organic acids have been converted to methane and CO₂; the methane is useful as a fuel supplement for the process. Anaerobic digestion in many cases is followed by aerobic digestion to convert dissolved solids to sludge, concentrated by settling in the large aerobic settlers. Such systems have been operated commercially for decades and most likely will be incorporated into algal biofuel production processes. Golueke et al. (1957) reported average NH₄⁺ concentrations of digested sludge in the range of 1600 to 1850 milligrams per liter for anaerobic digestion of algae, which is comparable to some of the high values reported for piggery waste (Sukias and Tanner, 2005; Sukias and Craggs, 2011). Another source of solid waste is the spent synthetic plastic liner from open ponds or closed bioreactors that will need to be disposed periodically.

According to Jim Sears (J. Sears, A2BE Carbon Capture, personal communication on September 22, 2011), who chaired the "Committee on Technical Standards" for the Algal Biomass Organization, "there are as many proposed processes for producing algal biofuels as there are companies." Thus, whether generation of waste products would be a concern cannot be known until operations at commercial scale are in place and compositions can be ascertained. Maximizing recycling would reduce the need for waste product disposal.

5.9.2 Opportunities for Mitigation

Recycling of nutrients is the obvious mitigation for waste generation. Algenol, an algal biofuel company, plans to recycle seawater waste for cultivation (less than 6 liters of seawater waste per liter of ethanol is produced if photobioreactors last greater than 6 years).

If digested sludge is produced, municipal waste treatment plants usually spread the nutrient-rich waste on designated land, with the benefit of conditioning and nourishing the soil. Algal fuel process sludge from wastewater treatment is not expected to be significantly different. The National Pollutant Discharge Elimination System permitting process governs discharge of sludge; most states have a permitting process under this federal program. The composition of the sludge is monitored to ensure compliance with the permit.

5.9.3 Sustainability Indicators

Many sustainability indicators relevant to waste are described elsewhere, for example, quantifying recycling of nutrients and salinity of ground water. If saline wastewater is injected to groundwater, then sustainability indicators also could include annual volume injected per volume of reservoir per year.

5.9.4 Information and Data Gaps

Information is needed about the types and rate of waste generation for most algal biofuel production processes. When and if processes move toward commercialization, state and local regulations will govern the acceptable disposal of waste, which will necessarily be well characterized by then.

5.10 PATHOGENS AND TOXINS

5.10.1 Potential Environmental Effects

5.10.1.1 Algal Toxins

Known toxin-producing strains are not likely to be used in algal biofuel production systems. Indeed, many species have food grade status or are being used as feed in aquaculture. However, some species regarded as benign may in fact produce toxins previously unknown. Examples of these include newly discovered euglenoid toxins (Zimba et al., 2010) and free radical toxins (Moeller et al., 2007). In addition, contaminating toxin-producing algae and cyanobacteria could potentially colonize production systems, especially open ponds.

Human toxins that are produced by cyanobacteria have been found in freshwater, marine, and estuarine organisms and include hepatotoxins, cytotoxins, dermatotoxins, and neurotoxins among others (Smith et al., 2008). Ecotoxicity from algal toxins is observed in fish (Zimba et al., 2001b), shellfish (Lance et al., 2011), or invertebrate herbivores such as *Daphnia* and shrimp (Zimba et al., 2006; Sarnelle, 2010). Toxins can affect viability, growth, and fecundity of many organisms (Plumley, 1997).

The chemical structures of freshwater toxins are probably more diverse than those of marine toxins, including alkaloids, phosphate esters, macrolides, chlorinated diaryllactones, and penta- and hepta-peptides (Rouhiainen et al., 1995; Smith et al., 2008). Species of toxin-producing algae in the divisions Euglenophyceae, Bacillariophyceae, Dinophyceae, Haptophyceae, and Raphidophyceae have been documented. Some cyanobacteria also are producers of toxins and probably are responsible for the production of most freshwater algal toxins from harmful algal blooms (Plumley, 1997). Toxicity of some compounds can exceed that of curare (Zimba et al., 2001a). Irritants and allergens also are produced by certain algae. Toxin production in some cyanobacteria is influenced by environmental variables and competition (Moeller et al., 2007; Briand et al., 2008), though the physiological and ecological causes of toxin production are largely unknown (Paerl and Millie, 1996; Carrick, 2011). Harmful blooms of toxin-producing algae are not the sole source of algal toxins, nor are algal toxins always associated with blooms (Plumley, 1997). Moreover, blooms cannot be predicted with accuracy in natural environments (Carrick, 2011), and this likely applies to open biofuel cultivation systems as well.

Both freshwater and marine forms of toxin-producing algae could colonize production systems. The current state of knowledge about phytoplankton community composition is not sufficient to predict whether toxin-producing strains could invade and bloom in algal biofuel production systems, even if these systems are seeded either initially or continuously with non-toxigenic algal strains.

Compounds presently not known to be harmful because of their presence in low concentrations in small-scale, low-intensity algal biomass production may have harmful impacts when concentrated 100,000 times during the harvesting and drying phases. Concentrated cultivation methods may lead to the identification of previously unknown toxic material (Moeller et al., 2007; Zimba et al., 2010). If the lipid-extracted algae are to be used in value-added coproducts, the quality of those products would have to be monitored.

The outdoor open-pond production systems likely will develop diverse algal populations (Smith et al., 2010). Monitoring algal composition is critical to maintaining desired

characteristics for processing biomass to fuels, ensuring that coproducts from lipid-extracted algae are safe for use, and minimizing downstream effects of water-soluble toxins.

5.10.1.2 Human and Animal Pathogens in Algal Cultivation Systems

Cultivated *Spirulina*, *Chlorella*, and *Haematococcus* have been used to produce food-grade products, and the presence of pathogens has not been a concern. However, algal production systems are diverse communities that may contain pathogens particularly if municipal wastewater, wastewater from concentrated animal feeding operations (CAFOs), biosolids (sewage sludge), or manures are used as water or nutrient supplies. Although the algal cultivation systems using wastewater are similar to the thousands of algal wastewater ponds in the United States, different occupational exposures might arise because the algal biomass being handled in algal biofuel production is larger in quantity (that is, higher sludge mass in algae cultivation for fuels than in algal wastewater pond) and higher in concentration (from harvesting and drying before processing to fuels). The density and probability of particular pathogens in wastewater is related to the level of treatment, with greater pathogen numbers and diversity in primary treated sewage than secondary treatment. Primary treatment is the sedimentation of solids, secondary treatment is the removal of suspended and dissolved organic materials, and tertiary treatment is the removal of inorganic constituents such as nitrogen and phosphorus. Biosolids (sewage sludge), for example, may include bacterial, viral, protozoan, or helminth pathogens (EPA, 2011b). In a study of mesophilic anaerobic digested biosolids from 18 locations in the United States, *Clostridium perfringens*, *Shigella*, *Campylobacter*, *Salmonella*, enteric viruses, and adenoviruses were detected, but *Ascaris* and *Escherichia coli* 0157:H7 were not. The original wastewater would be expected to contain at least these species. In another study of treated wastewater and biosolids in Michigan, adenovirus, enterovirus, and norovirus were detected in 100, 70, and 10 percent of samples, respectively (Simmons and Xagorarakis, 2011). The taxonomic identities and abundances of pathogens in biosolids (and by extension, wastewater) are determined by the incidence of infection within the wastewater-generating community and the particular wastewater treatment process used (Straub, 1993; EPA, 2011b). Survival of some pathogens from biosolids in soil has been studied (Zerzghi et al., 2009), but the survival of human and animal pathogens in algal biofuel cultures is only beginning to be investigated.

Where pathogens are present in algal cultures, there could be occupational health effects or environmental effects (if release occurs). The presence of fecal coliforms or other pathogens would limit the options for coproducts.

5.10.2 Comparison of Pathways

The key difference in pathways is that photobioreactors are less likely to be colonized by toxin-producing strains than open ponds (Table 5-11).

5.10.3 Opportunities for Mitigation

Selecting strains known not to produce toxins will mitigate toxin concerns in closed systems and aid in mitigating toxin concerns for open systems, though toxins could be

TABLE 5-11 An Illustration of Potential Effects from Toxins and Pathogens from Different Pathways of Algal Biofuel Production

Potential Effect	Pathway				
	Open-pond, salt water, producing biodiesel, recycling nutrients and water	Open-pond, salt water, producing biodiesel + coproducts	Open-pond, salt water, producing FAME, recycling nutrients and water	Photobioreactor, salt water, direct synthesis, recycling water	Open-pond, salt water, producing biomass, pyrolysis, recycling some nutrients and water
Toxins in Algal Culture	Possible if toxin-producing strains colonize production systems	Possible if toxin-producing strains colonize production systems	Possible if toxin-producing strains colonize production systems	Very unlikely. Strain is selected that does not produce toxins, and contamination with toxin-producing strain very unlikely.	Possible if toxin-producing strains colonize production systems
Pathogens in Algal Culture	Possible if wastewater is used as nutrient source and possible colonization by environmental pathogens	Possible if wastewater is used as nutrient source and possible colonization by environmental pathogens; possible movement of spores into coproducts	Possible if wastewater is used as nutrient source and possible colonization by environmental pathogens	Possible if wastewater is used as nutrient source	Possible if wastewater is used as nutrient source, very unlikely colonization by environmental pathogens because of low residence time

produced by non-target algae in the open-pond community. Genomic approaches could be used to screen for genes required for toxin synthesis in candidate algal strains for biofuel production (La Claire, 2006; Ianora et al., 2011). Periodic monitoring could ensure that well-known toxins are not produced.

Minimizing sources of human and animal pathogens in algal culture could include:

- A high level of treatment or sterilization of wastewater. For example, Phycal passes wastewater through a 0.2 micrometer filter prior to use and uses ultraviolet sterilization for initial treatment as well as treatment of recycled water. Reuse of wastewater for algal biofuel production could follow established wastewater reuse regulations.
- Using agricultural grade fertilizers (for example, Sapphire Energy, Inc.).
- Use of high-pH brines to reduce survival of pathogen competitors of cyanobacteria (for example, Phycobio Sciences).

5.10.4 Sustainability Indicators for Algal Toxins and Pathogens

Indicators of sustainable development of algal biofuels include metrics of algal toxins and pathogens in water, which consist of concentrations of toxins in water, measures of toxic effects (for example, in animal models) that are diagnostic of particular toxins, or

genetic markers of toxin production. Methods to distinguish some toxin-producing strains from other strains are available. For example, an oligonucleotide probe can distinguish hepatotoxic from neurotoxic *Anabaena* and these strains from *Nostoc* spp. (Rouhiainen et al., 1995). Information supporting genetic markers of toxin production is increasingly available, for example, a PCR-based test to assess the potential for microcystin occurrence (Nonneman and Zimba, 2002). However, these tests cannot identify unknown toxins, and they can give false-positive results where toxins are not expressed, for example, where multigene families are needed for arrangement (Zimba et al., 2010).

Directly measuring all pathogens in algal culture media is not generally practical. Indicator species are often microorganisms that are nonpathogenic, abundant, and associated with the presence of a suite of pathogens (EPA, 2011b). For example, densities of fecal coliform and *Salmonella* can be used as indicators for assessing the efficiency of wastewater treatment (40 CFR 136). These can be measured via culture methods or through quantitative polymerase chain reaction tests for fecal indicator bacteria that provide same-day information (Dorevitch et al., 2011). The abundance of fecal indicator bacteria can be related to disparate pathogens, such as protozoa (Dorevitch et al., 2011). However, typical indicator organisms are not useful in all media or for all pathogens. For example, Pepper et al. (2010) found that indicator organisms in Class B biosolids were not correlated with the numbers of pathogenic organisms. Criteria for selecting non-pathogen indicator organisms of pathogens in waters have been summarized by EPA (2011b), based on information in Gerba (2009) and NRC (2004). These include attributes of organisms and testing methods. Analytical methods for detecting low densities of pathogens have not been sufficiently developed and tested to be recommended (EPA, 2011b). Any potential indicators would have to be tested in algal ponds or photobioreactors for potential relationships with pathogen levels. Attributes of non-pathogen indicator organisms of pathogens in waters and attributes of methods for detecting pathogen indicator organisms are described in an EPA report *Problem Formulation for Human Health Risk Assessment of Pathogens in Land-applied Biosolids* (EPA, 2011b).

5.11 MOSQUITO-BORNE DISEASES

5.11.1 Potential Environmental, Health, and Social Acceptability Effects

Health effects from and social acceptability of algal biofuels could be affected if open ponds are poorly managed and provide habitats for mosquito larvae. Photobioreactors and raceways would not represent mosquito habitat unless there is substantial leakage of culture fluid, and puddles are formed. Mosquitoes lay their eggs opportunistically in standing water, which can vary from large lakes to small puddles or buckets. The full area of algae cultivation ponds would not be optimal habitat because of the required stirring and agitation for adequate mixing of nutrients and light exposure. Females of most mosquito species only infrequently lay eggs in flowing or agitated water (Lothrop and Mulla, 1996; Mogi and Motomura, 1996). Moreover, waters that are in motion can interfere with the surface tension required for mosquitoes' respiratory siphons to function (Schober, 1966). However, any relatively still edges of open ponds (analogous to stream banks and floodplains along moving streams) and outlying puddles or open-water storage vessels would be suitable for the growth of mosquito larvae. Because algae constitute food for mosquito larvae, the high nutrient and carbon content of algal cultivation systems (when and where the water is relatively still) can be prime habitat (Rydzanicz and Lone, 2003). The turbidity

of some cultivation systems would provide refuges from visual predators (Jacob et al., 2008; Jackson et al., 2009). Fewer mosquito species can tolerate saline conditions than fresh water (Patrick and Bradley, 2000), but some species can tolerate salinities of 100 percent sea water (Grueber and Bradley, 1994).

Providing habitat for mosquitoes could be a concern for human health and the acceptability of algal biofuel production for several reasons including:

- Mosquitoes are considered a pest and a nuisance that may not be tolerated by people living near a cultivation facility. Communities near proposed constructed wetland sites sometimes object to siting based on the anticipation of a mosquito problem (Anderson et al., 2007).
- Mosquitoes are vectors for numerous human infectious diseases in the United States, such as Eastern equine encephalitis, La Crosse encephalitis, St. Louis encephalitis, West Nile virus, Western equine encephalitis, and Dengue fever (recently reported in Florida). West Nile virus is also hypothesized to be a factor in the decline of sage grouse (Naugle et al., 2004).
- If the ponds for algae cultivation become breeding grounds for mosquitoes, there is a risk that the larvae will become a pest, reducing algal population densities below economically productive levels through predation.

5.11.2 Opportunities for Mitigation

Measures could be taken to control mosquito and other pest populations in and around algae cultivation ponds. The extensive use of agitators, aerators, and fountains decrease the suitability of open ponds for mosquito habitat (Jackson et al., 2009) and distribute nutrients and algae in the system. If standing water at cultivation facilities is minimized, mosquitoes and associated health effects should not be a problem.

Other mitigation options include site-specific surveys that can inform mosquito management agencies regarding the timing, species, and abundance of mosquitoes to develop disease-reduction plans (Anderson et al., 2007). Control options include chemical treatments like insecticides and biological methods such as the introduction of natural predators such as mosquitofish that consume mosquito larvae. If some of these measures are used without prior consultation and acceptance by the public, or if it is perceived that a population control method poses a threat to the human health or well-being, local communities might not accept algae production as a viable source of energy.

5.11.3 Sustainability Indicators

The sustainability indicators for mosquito-borne diseases are density of mosquito larvae in ponds and changes in incidence of mosquito-borne diseases attributable to cultivation ponds.

5.12 CONCLUSIONS

Reducing GHG emissions from the transportation sector has been one of the primary motivations for using alternative liquid transportation fuels. Therefore, the life-cycle GHG emissions are key factors in considering the sustainable development of algal biofuels. Published estimates of GHG emissions span a wide range, with some studies suggesting that algal biofuel production has high GHG emissions. The utility of these LCAs is that they point out key drivers of CO₂ emissions in the algal biofuel supply chains and indicate

the aspects or processes that could benefit from research and development for improving GHG emissions.

Some concerns of medium importance to consider include:

- The presence of waterborne toxicants or pathogens in algal cultivation systems if waste streams (flue gas or wastewater) are to be used as sources of nutrients or water. Their presence would affect occupational safety and the safety of coproducts if the residual algal biomass is used to produce certain coproducts to maximize recycling and to improve process economics.
- Effects from land-use changes if pasture and rangeland are to be converted to algae cultivation. Displacing pasture and rangeland could incur direct and indirect land-use changes that would affect the net GHG emissions of algal biofuels.
- Air quality emissions over the life cycle of algal biofuels. Emissions from the processing facilities and tailpipe emissions will be regulated, but emissions from other parts of the supply chain also need to be considered. The committee is not aware of any published studies that include measured emissions of air pollutants from open-pond cultivation.
- Potential effects on local climate. The introduction of large-scale algal cultivation systems in arid or semi-arid environments could alter the local climate of the area by increasing humidity and altering temperature extremes.
- Releases of cultivated algae to natural environments and potential alteration of species composition in receiving waters.
- Effects on terrestrial biodiversity from changing landscape pattern as a result of infrastructure development for algal biofuels.
- Potential adverse effects and unintended consequences of introduction of genetically engineered algae for biofuel production.
- Waste products from processing algae to fuels.

This chapter discussed the potential environmental effects of algal biofuel production. Some of those effects require assessment and monitoring to ensure that they do not pose serious sustainability concerns (for example, potential land conversion, air emissions, effects on biodiversity, waste products from algal biofuel production systems, and potential presence of pathogens and unknown or unidentified toxins). Other environmental effects discussed could be avoided with proper management and good engineering designs (for example, release of culture water leading to eutrophication, seepage of culture water into local ground water, and habitats for mosquito larvae).

SUMMARY FINDINGS FROM THIS AND EARLIER CHAPTERS

Algal biofuels have the potential to contribute to improving the sustainability of the transportation sector, but the potential is not yet realized. Additional innovations that require research and development are needed to realize the full potential of algal biofuels. (See Chapters 2 and 3 for biological and engineering innovations needed and Chapter 4 for resource recycling.)

Engineering solutions to enhance algae cultivation, to facilitate biomass or product collection, and to improve processing of algae-derived fuels can increase the EROI and reduce the GHG emissions of algal biofuel production. (See Chapters 2 and 3 for engineering solutions and Chapter 4 for a discussion on EROI.)

REFERENCES

- 40 CFR 136 (Code of Federal Regulations). TITLE 40—Protection of Environment; PART 136—Guidelines Establishing Test Procedures for the Analysis of Pollutants, U.S. Government. No. 40. Available online at http://ecfr.gpoaccess.gov/cgi/t/text/text-idx?c=ecfr&tpl=/ecfrbrowse/Title40/40cfr136_main_02.tpl. Accessed July 20, 2012.
- Ahluwalia, S.S., and D. Goyal. 2007. Microbial and plant derived biomass for removal of heavy metals from wastewater. *Bioresource Technology* 98(12):2243-2257.
- Alexander, M. 1971. Biochemical ecology of microorganisms. *Annual Review of Microbiology* 25:361-392.
- . 1977. *Soil Microbiology*. 2nd Edition. New York: John Wiley and Sons.
- . 1985. Ecological consequences—Reducing the uncertainties. *Issues in Science and Technology* 1(3):57-68.
- An, J.Y., S.J. Sim, J.S. Lee, and B.W. Kim. 2003. Hydrocarbon production from secondarily treated piggery wastewater by the green alga *Botryococcus braunii*. *Journal of Applied Phycology* 15(2-3):185-191.
- Anderson, A.L., K. O'Brien, and M. Hartwell. 2007. Comparisons of mosquito populations before and after construction of a wetland for water quality improvement in Pitt County, North Carolina, and data-reliant vectorborne disease management. *Journal of Environmental Health* 69(8):26-33.
- Assmann, A., A. Braun, S. John, A. Lei, and S. Southard. 2011. The Potential for Micro-Algae and Other "Micro-Crops" to Produce Sustainable Biofuels. Master of Science, University of Michigan, Ann Arbor.
- Atkinson, K.M. 1972. Birds as transporters of algae. *British Phycological Journal* 7:319-321.
- Babcock, B.A. 2009. Measuring unmeasurable land-use changes from biofuels. *Iowa Agriculture Review* Summer:4-6.
- Bachmann, R.W., B.L. Jones, D.D. Fox, M. Hoyer, L.A. Bull, and D.E. Canfield. 1996. Relations between trophic state indicators and fish in Florida (USA) lakes. *Canadian Journal of Fisheries and Aquatic Sciences* 53(4):842-855.
- Beeman, P. 2007. Biofuel plants generate new air, water, soil problems for Iowa. Available online at <http://www.desmoinesregister.com/article/20070603/BUSINESS01/706030325/Biofuel-plants-generate-new-air-water-soil-problems-for-Iowa>. Accessed January 21, 2012.
- Benemann, J.R. 2008. Opportunities and challenges in algae biofuel production. Available online at http://www.fao.org/uploads/media/algae_positionpaper.pdf. Accessed October 21, 2011.
- BLM (Bureau of Land Management) and DOE (U.S. Department of Energy). 2010. Solar Energy Development Draft Programmatic Environmental Impact Statement (Draft Solar PEIS). Washington, DC: Bureau of Land Management and U.S. Department of Energy.
- Bollag, J.M., and S.T. Czlonkowski. 1973. Inhibition of methane formation in soil by various nitrogen-containing compounds. *Soil Biology and Biochemistry* 5(5):673-678.
- Boyd, C.E. 1995. *Bottom soils, sediment, and pond aquaculture*. New York: Chapman and Hall.
- Breitburg, D.L., D.W. Hondorp, L.A. Davias, and R.J. Diaz. 2009. Hypoxia, nitrogen, and fisheries: Integrating effects across local and global landscapes. Pp. 329-349 in *Annual Review of Marine Science*.
- Briand, E., C. Yepremian, J.F. Humbert, and C. Quiblier. 2008. Competition between microcystin- and non-microcystin-producing *Planktothrix aqardhii* (cyanobacteria) strains under different environmental conditions. *Environmental Microbiology* 10(12):3337-3348.
- Buntjer, J. 2010. Heron Lake BioEnergy fined. *Daily Globe*, December 27. State and Regional News section.
- Burkholder, J.M., K.M. Mason, and H.B. Glasgow. 1992. Water-column nitrate enrichment promotes decline of eelgrass *Zostera-Marina*—Evidence from seasonal mesocosm experiments. *Marine Ecology-Progress Series* 81(2):163-178.
- Burns, J.C. 2008. ASAS centennial paper: Utilization of pasture and forages by ruminants: A historical perspective. *Journal of Animal Science* 86(12):3647-3663.
- Čada, G.F. 1998. Efforts to reduce the impacts of hydroelectric power production on reservoir fisheries in the United States. *International Review of Hydrobiology* 83(Special Issue):43-50.
- Campbell, P.K., T. Beer, and D. Batten. 2011. Life cycle assessment of biodiesel production from microalgae in ponds. *Bioresource Technology* 102(1):50-56.
- Campbell, S.P., J.A. Clark, L.H. Crampton, A.D. Guerry, L.T. Hatch, P.R. Hosseini, J.J. Lawler, and R.J. O'Connor. 2002. An assessment of monitoring efforts in endangered species recovery plans. *Ecological Applications* 12(3):674-681.
- CARB (California Air Resource Board) 2010. *Low Carbon Fuel Standard—Indirect Effects*. Sacramento: California Environmental Protection Agency.
- Carlsen, T.M., J.D. Coty, and J.R. Kercher. 2004. The spatial extent of contaminants and the landscape scale: An analysis of the wildlife, conservation biology, and population modeling literature. *Environmental Toxicology and Chemistry* 23(3):798-811.
- Carpenter, S.R. 2005. Eutrophication of aquatic ecosystems: Bistability and soil phosphorus. *Proceedings of the National Academy of Sciences of the United States of America* 102(29):10002-10005.

- Carrick, H.J. 2011. Niche modeling and predictions of algal blooms in aquatic ecosystems. *Journal of Phycology* 47(4):709-713.
- Chapotin, S.M., and J.D. Wolt. 2007. Genetically modified crops for the bioeconomy: Meeting public and regulatory expectations. *Transgenic Research* 16(6):675-688.
- Chinnasamy, S., A. Bhatnagar, R.W. Hunt, and K.C. Das. 2010. Microalgae cultivation in a wastewater dominated by carpet mill effluents for biofuel applications. *Bioresource Technology* 101(9):3097-3105.
- Clarens, A.F., E.P. Resurreccion, M.A. White, and L.M. Colosi. 2010. Environmental life cycle comparison of algae to other bioenergy feedstocks. *Environmental Science and Technology* 44(5):1813-1819.
- Cloud, B. 2011. Questionnaire reply from Phyco BioSciences Inc. Received by the NRC Committee on Sustainable Development of Algal Biofuels on July 25.
- Codd, G.A. 2000. Cyanobacterial toxins, the perception of water quality, and the prioritisation of eutrophication control. *Ecological Engineering* 16(1):51-60.
- Connor, M., and M. Siegrist. 2011. The power of association: Its impact on willingness to buy GM food. *Human and Ecological Risk Assessment* 17(5):1142-1155.
- Dale, V.H., K.L. Kline, L.L. Wright, R.D. Perlack, M. Downing, and R.L. Graham. 2011. Interactions among bioenergy feedstock choices, landscape dynamics, and land use. *Ecological Applications* 21(4):1039-1054.
- Dale, V.H., and S. Pearson. 1997. Quantifying habitat fragmentation due to land use change in Amazonia. Pp. 400-410 in *Tropical Forest Remnants: Ecology, Management, and Conservation of Fragmented Communities*, W.F. Laurance and R.O. Bierregaard, eds. Chicago, IL: University of Chicago Press.
- Davis, R., A. Aden, and P.T. Pienkos. 2011. Techno-economic analysis of autotrophic microalgae for fuel production. *Applied Energy* 88(10):3524-3531.
- Deegan, L.A., A. Wright, S.G. Ayvazian, J.T. Finn, H. Golden, R.R. Merson, and J. Harrison. 2002. Nitrogen loading alters seagrass ecosystem structure and support of higher trophic levels. *Aquatic Conservation-Marine and Freshwater Ecosystems* 12(2):193-212.
- Degu, A.M., F. Hossain, D. Niyogi, R. Pielke, J.M. Shepherd, N. Voisin, and T. Chronis. 2011. The influence of large dams on surrounding climate and precipitation patterns. *Geophysical Research Letters* 38. DOI: 10.1029/2010GL046482.
- Demirbas, A. 2009. Production of biodiesel from algae oils. *Energy Sources Part A—Recovery Utilization and Environmental Effects* 31(2):163-168.
- Dickens, M.J., D.J. Delehanty, J.M. Reed, and L.M. Romero. 2009. What happens to translocated game birds that “disappear”? *Animal Conservation* 12(5):418-425.
- DOE (U.S. Department of Energy). 2009. Construction and Operation of a Proposed Cellulosic Ethanol Plant, Range Fuels Soperton Plant, LLC (formerly Range Fuels Inc.) Treutlen County, Georgia. Washington, DC: U.S. Department of Energy.
- . 2010a. Algenol Integrated Biorefinery for Producing Ethanol from Hybrid Algae. Washington, DC: U.S. Department of Energy.
- . 2010b. National Algal Biofuels Technology Roadmap. Washington, DC: U.S. Department of Energy, Energy Efficiency and Renewable Energy.
- Dorevitch, S., M. Doi, F.C. Hsu, K.T. Lin, J.D. Roberts, L.C. Liu, R. Gladding, E. Vannoy, H. Li, M. Javor, and P.A. Scheff. 2011. A comparison of rapid and conventional measures of indicator bacteria as predictors of waterborne protozoan pathogen presence and density. *Journal of Environmental Monitoring* 13(9):2427-2435.
- Drake, L.A., F.C. Dobbs, and R.C. Zimmerman. 2003. Effects of epiphyte load on optical properties and photosynthetic potential of the seagrasses *Thalassia testudinum* Banks ex König and *Zostera marina* L. *Limnology and Oceanography* 48(1):456-463.
- Drewes, J., P. Xue, D. Heil, and G. Wang. 2009. *Multibeneficial Use of Produced Water Through High-Pressure Membrane Treatment and Capacitive Deionization Technology*. Denver, CO: U.S. Department of the Interior Bureau of Reclamation.
- Dufey, A. 2006. *Biofuels production, trade and sustainable development: Emerging issues*. London: International Institute for Environment and Development (IIED).
- Duff, M.C., C. Amrhein, and G. Bradford. 1997. Nature of uranium contamination in the agricultural drainage water evaporation ponds of the San Joaquin Valley, California, USA. *Canadian Journal of Soil Science* 77(3):459-467.
- Eaton, A.D., L.S. Clesceri, E.W. Rice, A.E. Greenberg, and M.A.H. Franson, eds. 2005. *Standard Methods for the Examination of Water and Wastewater*, 21st ed. Washington, DC: American Public Health Association, American Water Works Association, and Water Environment Federation.
- Efroymson, R., H. Jager, V. Dale, and J. Westervelt. 2009. A framework for developing management goals for species at risk with examples from military installations in the United States. *Environmental Management* 44(6):1163-1179.

- Efroymson, R.A. 1999. Regulating risk: Oversight of microbial products of biotechnology under the Toxic Substances Control Act. *Journal of Environmental Assessment Policy and Management* 1:329-347.
- Efroymson, R.A., D.S. Jones, and A.J. Gold. 2007. An ecological risk assessment framework for effects of onsite wastewater treatment systems and other localized sources of nutrients on aquatic ecosystems. *Human and Ecological Risk Assessment* 13(3):574-614.
- Ehresmann, D.W., and M.T. Hatch. 1975. Effect of relative humidity on survival of airborne unicellular algae. *Applied Microbiology* 29(3):352-357.
- EIA (U.S. Energy Information Administration). 2002. Updated State-Level Greenhouse Gas Emission Coefficients for Electricity Generation 1998-2000. Washington, DC: U.S. Energy Information Administration.
- Environment Law Institute. 2012. Glossary of brownfields terms. Available online at <http://www.brownfieldscenter.org/big/glossary.shtml>. Accessed August 17, 2012.
- EPA (U.S. Environmental Protection Agency). 1990. Points to consider in the preparation and submission of TSCA Premanufacture Notices (PMNs) for microorganisms. Washington, DC: U.S. Environmental Protection Agency.
- . 2002a. Clean alternative fuels: Biodiesel. Available online at http://www.afdc.energy.gov/afdc/pdfs/epa_biodiesel.pdf. Accessed February 1, 2012.
- . 2002b. Clean alternative fuels: Ethanol. Available online at http://www.afdc.energy.gov/afdc/pdfs/epa_ethanol.pdf. Accessed February 1, 2012.
- . 2002c. Clean alternative fuels: Fischer Tropsch. Available online at http://www.afdc.energy.gov/afdc/pdfs/epa_fischer.pdf. Accessed February 1, 2012.
- . 2007. Advanced wastewater treatment to achieve low concentration of phosphorus. Seattle, WA: U.S. Environmental Protection Agency, Region 10, Office of Water and Watersheds.
- . 2009a. Emissions standard reference guide. Available online at <http://www.epa.gov/otaq/standards/basicinfo.htm#3>. Accessed January 13, 2012.
- . 2009b. Ethanol Plant Clean Air Act Enforcement Initiative. Available online at <http://www.epa.gov/oecaerth/resources/cases/civil/caa/ethanol/index.html>. Accessed February 14, 2011.
- . 2010. Available and Emerging Technologies for Reducing Greenhouse Gas Emissions from Coal-Fired Electric Generating Units. Washington, DC: U.S. Environmental Protection Agency.
- . 2011a. Clean Water Act. Available online at http://cfpub.epa.gov/npdes/cwa.cfm?program_id=45. Accessed April 18, 2012.
- . 2011b. Problem Formulation for Human Health Risk Assessments of Pathogens in Land-applied Biosolids. Cincinnati, OH: National Center for Environmental Assessment.
- . 2012. Integrated Risk Information System. Available online at <http://www.epa.gov/IRIS/>. Accessed February 17, 2012.
- Evaporation Ponds Technical Committee. 1999. Evaporation Ponds. Available online at http://www.water.ca.gov/pubs/groundwater/evaporation_ponds_final_report_san_joaquin_valley_drainage_implementation_program/06-evapponds.pdf. Accessed October 10, 2011.
- Fagerstone, K.D., J.C. Quinn, T.H. Bradley, S.K. De Long, and A.J. Marchese. 2011. Quantitative measurement of direct nitrous oxide emissions from microalgae cultivation. *Environmental Science and Technology* 45(21):9449-9456.
- Fargione, J., J. Hill, D. Tilman, S. Polasky, and P. Hawthorne. 2008. Land clearing and the biofuel carbon debt. *Science* 319(5867):1235-1238.
- Fargione, J.E., T.R. Cooper, D.J. Flaspohler, J. Hill, C. Lehman, T. McCoy, S. McLeod, E.J. Nelson, K.S. Oberhauser, and D. Tilman. 2009. Bioenergy and wildlife: Threats and opportunities for grassland conservation. *BioScience* 59(9):767-777.
- Farrell, A.E. 2006. Ethanol can contribute to energy and environmental goals. *Science* 312(5781):1748-1748.
- Fiala, N. 2008. Measuring sustainability: Why the ecological footprint is bad economics and bad environmental science. *Ecological Economics* 67(4):519-525.
- Fleskes, J.P., R.L. Jarvis, and D.S. Gilmer. 2003. Selection of flooded agricultural fields and other landscapes by female northern pintails wintering in Tulare Basin, California. *Wildlife Society Bulletin* 31(3):793-803.
- Fletcher, R.J., B.A. Robertson, J. Evans, P.J. Doran, J.R.R. Alavalapati, and D.W. Schemske. 2011. Biodiversity conservation in the era of biofuels: Risks and opportunities. *Frontiers in Ecology and the Environment* 9(3):161-168.
- Florez-Leiva, L., E. Tarifeño, M. Cornejo, R. Kiene, and L. Farías. 2010. High production of nitrous oxide (N₂O), methane (CH₄) and dimethylsulphoniopropionate (DMSP) in a massive marine phytoplankton culture. *Biogeosciences Discussions* 7:6705-6723.
- Franzluebbers, A.J. 2010. Achieving soil organic carbon sequestration with conservation agricultural systems in the southeastern United States. *Soil Science Society of America Journal* 74(2):347-357.

- Frewer, L.J., C. Howard, and R. Shepherd. 1995. Genetic engineering and food: What determines consumer acceptance? *British Food Journal* 97(8):31-36.
- Gao, S., K.K. Tanji, R.A. Dahlgren, J. Ryu, M.J. Herbel, and R.M. Higashi. 2007. Chemical status of selenium in evaporation basins for disposal of agricultural drainage. *Chemosphere* 69(4):585-594.
- Garvin, J.C., C.S. Jennelle, D. Drake, and S.M. Grodsky. 2011. Response of raptors to a windfarm. *Journal of Applied Ecology* 48(1):199-209.
- Gelvin, S.B. 2010. Plant proteins involved in *Agrobacterium*-mediated genetic transformation. *Annual Review of Phytopathology* 48:45-68.
- Gerba, C.P. 2009. Environmental indicators. in *Environmental Microbiology*, 2nd edition, R.M. Maier, I.L. Pepper and C.P. Gerba, eds. NY: Academic Press. Pp. 485-498.
- Gerber, L.R., and L.T. Hatch. 2002. Are we recovering? An evaluation of recovery criteria under the U.S. Endangered Species Act. *Ecological Applications* 12(3):668-673.
- Gibbs, H.K., M. Johnston, J.A. Foley, T. Holloway, C. Monfreda, N. Ramankutty, and D. Zaks. 2008. Carbon payback times for crop-based biofuel expansion in the tropics: The effects of changing yield and technology. *Environmental Research Letters* 3(3):034001.
- Giese, B., F. Laturus, F.C. Adams, and C. Wiencke. 1999. Release of volatile iodinated C1-C4 hydrocarbons by marine macroalgae from various climate zones. *Environmental Science and Technology* 33(14):2432-2439.
- Gunnebaugh, D.L., J. Liang, and M.Z. Jacobson. 2010. Examining the temperature dependence of ethanol (E85) versus gasoline emissions on air pollution with a largely-explicit chemical mechanism. *Atmospheric Environment* 44(9):1192-1199.
- Golueke, C.G., W.J. Oswald, and H.B. Gotass. 1957. Anaerobic digestion of algae. *Applied Microbiology and Biotechnology* 5:47-55.
- Gong, Y.M., and M.L. Jiang. 2011. Biodiesel production with microalgae as feedstock: From strains to biodiesel. *Biotechnology Letters* 33(7):1269-1284.
- Gonzalez, L.E., R.O. Canizares, and S. Baena. 1997. Efficiency of ammonia and phosphorus removal from a Colombian agroindustrial wastewater by the microalgae *Chlorella vulgaris* and *Scenedesmus dimorphus*. *Bioresource Technology* 60(3):259-262.
- Graham, L.A., S.L. Belisle, and C.L. Baas. 2008. Emissions from light duty gasoline vehicles operating on low blend ethanol gasoline and E85. *Atmospheric Environment* 42(19):4498-4516.
- Grant, J. 2010. Coastal communities, participatory research, and far-field effects of aquaculture. *Aquaculture Environment Interactions* 1(2):85-93.
- Green, F.B., T.J. Lundquist, and W.J. Oswald. 1995. Energetics of advanced integrated waste-water pond systems. *Water Science and Technology* 31(12):9-20.
- Gressel, J. 2008. Transgenics are imperative for biofuel crops. *Plant Science* 174(3):246-263.
- Grönblad, R. 1933. Contribution to the knowledge of sub-aërial Desmids. *Societas Scientiarum Fennica. Commentationes Biologicae* 4:1-10.
- Grueber, W.B., and T.J. Bradley. 1994. The evolution of increased salinity tolerance in larvae of aedes mosquitoes—A phylogenetic analysis. *Physiological Zoology* 67(3):566-579.
- Guikema, S.D. 2009. Infrastructure design issues in disaster-prone regions. *Science* 323(5919):1302-1303.
- Gurian-Sherman, D. 2011. *Global Warming and Pasture-Raised Beef Production in the United States*. Cambridge, MA: Union of Concerned Scientists.
- Hagedorn, C., and S. Allender-Hagedorn. 1997. Issues in agricultural and environmental biotechnology: Identifying and comparing biotechnology issues from public opinion surveys, the popular press and technical/regulatory sources. *Public Understanding of Science* 6(3):233-245.
- Hauxwell, J., J. Cebrian, and I. Valiela. 2003. Eelgrass *Zostera marina* loss in temperate estuaries: Relationship to land-derived nitrogen loads and effect of light limitation imposed by algae. *Marine Ecology-Progress Series* 247:59-73.
- The H. John Heinz III Center for Science and the Environment. 2008. *The State of the Nation's Ecosystems 2008: Measuring the Lands, Waters, and Living Resources of the United States*. Washington, DC: The Heinz Center.
- Hess, P., M. Johnston, B. Brown-Steiner, T. Holloway, J. de Andrade, and P. Artaxo. 2009. Air quality issues associated with biofuel production and use. Pp. 169-194 in *Biofuels: Environmental Consequences and Interactions with Changing Land Use*, R.W. Howarth and S. Bringezu, eds. Ithaca: Cornell University.
- Hill, J., S. Polasky, E. Nelson, D. Tilman, H. Huo, L. Ludwig, J. Neumann, H.C. Zheng, and D. Bonta. 2009. Climate change and health costs of air emissions from biofuels and gasoline. *Proceedings of the National Academy of Sciences of the United States of America* 106(6):2077-2082.
- Hoffman, L. 1994. Biogeography of marine blue-green algae. *Archiv für Hydrobiologie Supplement* 75:137-148.
- Hoffmann, J.P. 1998. Wastewater treatment with suspended and nonsuspended algae. *Journal of Phycology* 34(5):757-763.

- Hoffmann, L. 1996. Geographic distribution of freshwater blue-green algae. *Hydrobiologia* 336(1-3):33-40.
- Holloran, M.J., R.C. Kaiser, and W.A. Hubert. 2010. Yearling greater sage-grouse response to energy development in Wyoming. *Journal of Wildlife Management* 74(1):65-72.
- Honkanen, T., and H. Helminen. 2000. Impacts of fish farming on eutrophication: Comparisons among different characteristics of ecosystem. *International Review of Hydrobiology* 85(5-6):673-686.
- Hughes, M.R. 2003. Regulation of salt gland, gut and kidney interactions. *Comparative Biochemistry and Physiology—A Molecular and Integrative Physiology* 136(3):507-524.
- Huntley, B., R. Baxter, K.J. Lewthwaite, S.G. Willis, and J.K. Adamson. 1998. Vegetation responses to local climatic changes induced by a water-storage reservoir. *Global Ecology and Biogeography Letters* 7(4):241-257.
- Ianora, A., M.G. Bentley, G.S. Caldwell, R. Casotti, A.D. Cembella, J. Engstrom-Ost, C. Halsband, E. Sonnenschein, C. Legrand, C.A. Llewellyn, A. Paldaviciene, R. Pilkaityte, G. Pohnert, A. Razinkovas, G. Romano, U. Tillmann, and D. Vaicute. 2011. The relevance of marine chemical ecology to plankton and ecosystem function: An emerging field. *Marine Drugs* 9(9):1625-1648.
- Irene-Marie, F. 1938. *Flora Desmidiale de la Region de Montreal*. LaPrairie, Canada.
- ISSG (Invasive Species Specialist Group). 2012. Global Invasive Species Database. Available online at <http://www.issg.org/database/species/ecology.asp?si=775>.
- Jackson, M.J., J.L. Gow, M.J. Evelyn, N.E. Meikleham, T.J.S. McMahon, E. Koga, T.J. Howay, L. Wang, and E. Yan. 2009. *Culex* mosquitoes, West Nile virus, and the application of innovative management in the design and management of stormwater retention ponds in Canada. *Water Quality Research Journal of Canada* 44(1):103-110.
- Jacob, B.G., E.J. Muturi, E.X. Caamano, J.T. Gunter, E. Mpanga, R. Ayine, J. Okelloonen, J.P.M. Nyeko, J.I. Shililu, J.I. Githure, J.L. Regens, R.J. Novak, and I. Kakoma. 2008. Hydrological modeling of geophysical parameters of arboviral and protozoan disease vectors in Internally Displaced People camps in Gulu, Uganda. *International Journal of Health Geographics* 7.
- Jager, H.I., E.A. Carr, and R.A. Efroymson. 2006. Simulated effects of habitat loss and fragmentation on a solitary mustelid predator. *Ecological Modelling* 191(3-4):416-430.
- Jorquera, O., A. Kiperstok, E.A. Sales, M. Embiruçu, and M.L. Ghirardi. 2010. Comparative energy life-cycle analyses of microalgal biomass production in open ponds and photobioreactors. *Bioresource Technology* 101(4):1406-1413.
- Kamaldeen, S., and D.A. Powell. 2000. *Public Perceptions of Biotechnology*. Guelph, Ontario: University of Guelph.
- Kaskey, J. 2009. Monsanto, Dow Chemical win approval for modified corn (update3). Available online at <http://www.bloomberg.com/apps/news?pid=newsarchive&sid=a57J5HHLMog4>. Accessed June 19, 2012.
- Kleiner, D. 1974. Quantitative relations for the repression of nitrogenase synthesis in *Azotobacter vinelandii* by ammonia. *Archives of Microbiology* 101:153-159.
- Koonin, E.V., Y.I. Wolf, and G.P. Karev. 2002. The structure of the protein universe and genome evolution. *Nature* 420(6912):218-223.
- Kristiansen, J. 1996. Dispersal of freshwater algae—A review. *Hydrobiologia* 336(1-3):151-157.
- La Claire, J.W. 2006. Analysis of expressed sequence tags from the harmful alga, *Prymnesium parvum* (Prymnesiophyceae, Haptophyta). *Marine Biotechnology* 8(5):534-546.
- Lance, E., F. Alonzo, M. Tanguy, C. Gerard, and M. Bormans. 2011. Impact of microcystin-producing cyanobacteria on reproductive success of *Lymnaea stagnalis* (Gastropoda, Pulmonata) and predicted consequences at the population level. *Ecotoxicology* 20(4):719-730.
- Lardon, L., A. Helias, B. Sialve, J.P. Stayer, and O. Bernard. 2009. Life-cycle assessment of biodiesel production from microalgae. *Environmental Science and Technology* 43(17):6475-6481.
- Lau, P.S., N.F.Y. Tam, and Y.S. Wong. 1995. Effect of algal density on nutrient removal from primary settled wastewater. *Environmental Pollution* 89:58-66.
- Lenzen, M., and S.A. Murray. 2001. A modified ecological footprint method and its application to Australia. *Ecological Economics* 37(2):229-255.
- Lenzen, M., and S.A. Murray. 2003. *The Ecological Footprint—Issues and Trends*. Sydney: The University of Sydney.
- Liss, P.S., A.D. Hatton, G. Malin, P.D. Nightingale, and S.M. Turner. 1997. Marine sulphur emissions. *Philosophical Transactions of the Royal Society B-Biological Sciences* 352(1350):159-168.
- Liu, X., A.F. Clarens, and L.M. Colosi. 2011. Algae biodiesel has potential despite inconclusive results to date. *Bioresource Technology* 104:83-86.
- Lonsdale, W.M. 1999. Global patterns of plant invasions and the concept of invasibility. *Ecology* 80(5):1522-1536.
- Lothrop, B.B., and M.S. Mulla. 1996. Diel patterns of oviposition and influence of agitated water surface in *Chironomus anonymus* (Diptera: Chironomidae). *Journal of the American Mosquito Control Association* 12(2 Part 1):215-219.

- Lynn, J.C., C.L. Chambers, and S.S. Rosenstock. 2006. Use of wildlife water developments by birds in Southwest Arizona during migration. *Wildlife Society Bulletin* 34(3):592-601.
- Maguire, B. 1963. Passive dispersal of small aquatic organisms and their colonization of isolated bodies of water. *Ecological Monographs* 33(2):161-185.
- Mallick, N. 2002. Biotechnological potential of immobilized algae for wastewater N, P and metal removal: A review. *Biometals* 15(4):377-390.
- Martinez, M.E., S. Sanchez, J.M. Jimenez, F. El Yousfi, and L. Munoz. 2000. Nitrogen and phosphorus removal from urban wastewater by the microalga *Scenedesmus obliquus*. *Bioresource Technology* 73(3):263-272.
- Mata, T.M., A.A. Martins, and N.S. Caetano. 2010. Microalgae for biodiesel production and other applications: A review. *Renewable and Sustainable Energy Reviews* 14(1):217-232.
- Mathews, T., and N.S. Fisher. 2008. Evaluating the trophic transfer of cadmium, polonium, and methylmercury in an estuarine food chain. *Environmental Toxicology and Chemistry* 27(5):1093-1101.
- McBride, A.C., V.H. Dale, L.M. Baskaran, M.E. Downing, L.M. Eaton, R.A. Efroymson, C.T. Garten Jr, K.L. Kline, H.I. Jager, P.J. Mulholland, E.S. Parish, P.E. Schweizer, and J.M. Storey. 2011. Indicators to support environmental sustainability of bioenergy systems. *Ecological Indicators* 11(5):1277-1289.
- McCabe, T.R. 1994. Assessing values of Arctic wildlife and habitat subject to potential petroleum development. *Landscape and Urban Planning* 28(1):33-45.
- McGarigal, K., S. Cushman, and C. Regan. 2005. Quantifying terrestrial habitat loss and fragmentation: A protocol. Available online at http://www.umass.edu/landeco/teaching/landscape_ecology/labs/fragprotocol.pdf. Accessed July 25, 2012.
- Meersman, T. 2010. Minnesota ethanol plants' price is pollution. *Star Tribune*, October 11. Available online at http://www.startribune.com/local/104746614.html?elr=KArks:DCiU1PciUiD3aPc:_Yyc:aUoaEYY_1Pc_bDaEP7U. Accessed January 21, 2011.
- Mehner, G., F. Leunert, S. Cir n, K.D. J hnk, J. R cker, B. Nixdorf, and C. Wiedner. 2010. Competitiveness of invasive and native cyanobacteria from temperate freshwaters under various light and temperature conditions. *Journal of Plankton Research* 32(7):1009-1021.
- Meteyer, C.U., R.R. Dubielzig, F.J. Dein, L.A. Baeten, M.K. Moore, J.R. Jehl, and K. Wesenberg. 1997. Sodium toxicity and pathology associated with exposure of waterfowl to hypersaline playa lakes of southeast New Mexico. *Journal of Veterinary Diagnostic Investigation* 9(3):269-280.
- Moeller, P.D.R., K.R. Beauchesne, K.M. Huncik, W.C. Davis, S.J. Christopher, P. Riggs-Gelasco, and A.K. Gelasco. 2007. Metal complexes and free radical toxins produced by *Pfiesteria piscicida*. *Environmental Science and Technology* 41(4):1166-1172.
- Mogi, M., and M. Motomura. 1996. Possible *Culex pipiens pallens* control by improvement of flow rates in water channels of Saga City, southwest Japan. *Journal of the American Mosquito Control Association* 12(4):647-650.
- Moon, H.S., J.M. Abercrombie, A.P. Kausch, and C.N. Stewart. 2010. Sustainable use of biotechnology for bioenergy feedstocks. *Environmental Management* 46(4):531-538.
- Munoz, R., and B. Guieysse. 2006. Algal-bacterial processes for the treatment of hazardous contaminants: A review. *Water Research* 40(15):2799-2815.
- NALMS, (North American Lake Management Society). 2004. Position statement 4. Use of herbicides in lakes. Available online at <http://www.nalms.org/media.acux/adbd6227-81e9-411a-be86-cc2691380d0f>. Accessed January 31, 2012.
- Naugle, D.E., C.L. Aldridge, B.L. Walker, T.E. Cornish, B.J. Moynahan, M.J. Holloran, K. Brown, G.D. Johnson, E.T. Schmidtman, R.T. Mayer, C.Y. Kato, M.R. Matchett, T.J. Christiansen, W.E. Cook, T. Creekmore, R.D. Falise, E.T. Rinkes, and M.S. Boyce. 2004. West Nile virus: Pending crisis for greater sage-grouse. *Ecology Letters* 7(8):704-713.
- NETL (National Energy Technology Laboratory). 2011. Secure and reliable energy supplies—Coal becomes a “future fuel.” Available online at http://www.netl.doe.gov/KeyIssues/future_fuel.html. Accessed January 12, 2011.
- Nixon, S., B. Buckley, S. Granger, and J. Bintz. 2001. Responses of very shallow marine ecosystems to nutrient enrichment. *Human and Ecological Risk Assessment* 7(5):1457-1481.
- Nonneman, D., and P.V. Zimba. 2002. A PCR-based test to assess the potential for microcystin occurrence in channel catfish production ponds. *Journal of Phycology* 38(1):230-233.
- NRC (National Research Council). 2004. *Indicators for Waterborne Pathogens*. Washington, DC: National Academies Press.
- . 2011. *Renewable Fuel Standard. Potential Economic and Environmental Effects of U.S. Biofuel Policy*. Washington, DC: The National Academies Press.
- . 1987. *Field testing genetically modified organisms: framework for decisions*. Washington, DC: National Research Council.

- NRCS (Natural Resources Conservation Service) and WHC (Wildlife Habitat Council). 2007. Biology - 62 - Fish and Wildlife Habitat Management Leaflet No. 42, Scrub-Shrub Bird. Available online at <http://policy.nrcs.usda.gov/viewerFS.aspx?hid=21225>. Accessed April 4, 2012.
- O'Dowd, W.J., H.W. Pennline, M.C. Freeman, E.J. Granite, R.A. Hargis, C.J. Lacher, and A. Karash. 2006. A technique to control mercury from flue gas: The thief process. *Fuel Processing Technology* 87(12):1071-1084.
- O'Sullivan, J. 2010. SD ethanol plants fined \$225k for water violations. Available online at <http://thepostsd.com/2010/12/12/sd-ethanol-plants-fined-225k-for-water-violations/>. Accessed February 14, 2011.
- Ochman, H., J.G. Lawrence, and E.A. Grolsman. 2000. Lateral gene transfer and the nature of bacterial innovation. *Nature* 405(6784):299-304.
- Odlare, M., E. Nehrenheim, V. Ribé, E. Thorin, M. Gavare, and M. Grube. 2011. Cultivation of algae with indigenous species—Potentials for regional biofuel production. *Applied Energy* 88(10):3280-3285.
- Oglesby, R.T. 1977. Relationships of fish yield to lake phytoplankton standing crop, production, and morphoedaphic factors. *Journal of the Fisheries Research Board of Canada* 34(12):2271-2279.
- Osborn, R.G., K.F. Higgins, R.E. Usgaard, C.D. Dieter, and R.D. Neiger. 2000. Bird mortality associated with wind turbines at the Buffalo Ridge wind resource area, Minnesota. *American Midland Naturalist* 143(1):41-52.
- Oswald, W.J., and C.G. Golueke. 1960. Biological transformation of solar energy. *Advances in Applied Microbiology* 2:223-262.
- Oswald, W.J., H.B. Gotaas, C.G. Golueke, and W.R. Kellen. 1957. Algae in waste treatment. *Sewage and Industrial Wastes* 29:437-455.
- Özdemir, E.D., M. Härdtlein, T. Jossen, D. Zech, and L. Eltrop. 2011. A confusion of tongues or the art of aggregating indicators—Reflections on four projective methodologies on sustainability measurement. *Renewable and Sustainable Energy Reviews* 15:2385-2396.
- Paerl, H.W., and D.F. Millie. 1996. Physiological ecology of toxic aquatic cyanobacteria. *Phycologia* 35(Supplement):160-167.
- Park, J.B.K., R.J. Craggs, and A.N. Shilton. 2011. Wastewater treatment high rate algal ponds for biofuel production. *Bioresource Technology* 102(1):35-42.
- Patrick, M.L., and T.J. Bradley. 2000. The physiology of salinity tolerance in larvae of two species of *Culex* mosquitoes: The role of compatible solutes. *Journal of Experimental Biology* 203(4):821-830.
- Pepper, I.L., J.P. Brooks, R.G. Sinclair, P.L. Gurian, and C.P. Gerba. 2010. Pathogens and indicators in United States class B biosolids: National and historic distributions. *Journal of Environmental Quality* 39(6):2185-2190.
- Pierce, S.K., S.E. Massey, J.J. Hanten, and N.E. Curtis. 2003. Horizontal transfer of functional nuclear genes between multicellular organisms. *Biological Bulletin* 204(3):237-240.
- Pittman, J.K., A.P. Dean, and O. Osundeko. 2011. The potential of sustainable algal biofuel production using wastewater resources. *Bioresource Technology* 102(1):17-25.
- Plumley, F.G. 1997. Marine algal toxins: Biochemistry, genetics, and molecular biology. *Limnology and Oceanography* 42(5):1252-1264.
- Rabalais, N.N., R.E. Turner, R.J. Díaz, and D. Justí. 2009. Global change and eutrophication of coastal waters. *ICES Journal of Marine Science* 66(7):1528-1537.
- Radakovits, R., R.E. Jinkerson, A. Darzins, and M.C. Posewitz. 2010. Genetic engineering of algae for enhanced biofuel production. *Eukaryotic Cell* 9(4):486-501.
- Raghu, S., R.C. Anderson, C.C. Daehler, A.S. Davis, R.N. Wiedenmann, D. Simberloff, and R.N. Mack. 2006. Adding biofuels to the invasive species fire? *Science* 313(5794):1742.
- Ramirez, P. 2010. Bird mortality in oil field wastewater disposal facilities. *Environmental Management* 46(5):820-826.
- Ramirez, P.J. 2005. Oilfield-produced wastewater discharges into wetlands: Benefits and risks to wildlife. *Environmental Geoscience* 12:65-72.
- Ravindranath, N.H., R. Mauvie, J. Fargione, J.G. Canadell, G. Berndes, J. Woods, H. Watson, and J. Sathaye. 2009. Greenhouse gas implications of land use change and land conversion to biofuel crops. Pp. 111-125 in *Biofuels: Environmental Consequences and Interactions with Changing Land Use*, R.W. Howarth and S. Bringezu, eds. Ithaca, NY: Cornell University.
- Rasmussen, R.A. 1974. Emissions of biogenic hydrogen sulfide. *Tellus XXVI* (1-2):254-260.
- Rawat, I., R. Ranjith Kumar, T. Mutanda, and F. Bux. 2011. Dual role of microalgae: Phycoremediation of domestic wastewater and biomass production for sustainable biofuels production. *Applied Energy* 88(10):3411-3424.
- Reynolds, C.S., V. Huszar, C. Kruk, L. Naselli-Flores, and S. Melo. 2002. Towards a functional classification of the freshwater phytoplankton. *Journal of Plankton Research* 24(5):417-428.
- Rogers, M.B., N.J. Patron, and P.J. Keeling. 2007. Horizontal transfer of a eukaryotic plastid-targeted protein gene to cyanobacteria. *BMC Biology* 5:26.
- Roscher, J.P. 1967. Algal dispersal by muskrat intestinal contents. *Transactions of the American Microscopical Society* 86:497-498.

- Rott, E., and M.C. Hernandez-Marine. 1994. *Pulvinularia suecica*, a rare stigonematalean cyanophyte. *Algal Studies* 75:313-322.
- Rouhiainen, L., K. Sivonen, W.J. Buikema, and R. Haselkorn. 1995. Characterization of toxin-producing cyanobacteria by using an oligonucleotide probe containing a tandemly repeated heptamer. *Journal of Bacteriology* 177(20):6021-6026.
- Ruiz-Marin, A., L.G. Mendoza-Espinosa, and T. Stephenson. 2010. Growth and nutrient removal in free and immobilized green algae in batch and semi-continuous cultures treating real wastewater. *Bioresource Technology* 101(1):58-64.
- Ryan, C. 2009. *Cultivating Clean Energy: The Promise of Algae Biofuels*. New York: Natural Resources Defense Council.
- Rydzanicz, K., and E. Lone. 2003. Species composition and seasonal dynamics of mosquito larvae in the Wrocław, Poland area. *Journal of Vector Ecology* 28(2):255-266.
- Ryu, J.H., S.D. Gao, and K.K. Tanji. 2010. Speciation and behavior of arsenic in evaporation basins, California, USA. *Environmental Earth Sciences* 61(8):1599-1612.
- Sample, B.E., D.M. Opresko, and G.W. Suter, II. 1996. *Toxicological Benchmarks for Wildlife: 1996 Revision*. Oak Ridge, TN: Oak Ridge National Laboratory.
- Sandefur, H.N., M.D. Matlock, and T.A. Costello. 2011. Seasonal productivity of a periphytic algal community for biofuel feedstock generation and nutrient treatment. *Ecological Engineering* 37(10):1476-1480.
- Sander, K., and G.S. Murthy. 2010. Life cycle analysis of algae biodiesel. *International Journal of Life Cycle Assessment* 15(7):704-714.
- Sarnelle, O. 2010. Effects of cyanobacteria on fitness components of the herbivore *Daphnia*. *Journal of Plankton Research* 32:471-477.
- Sayre, R. 2011. Sustainable Algal Biofuels. Presentation to the NRC Committee on Sustainable Development of Algal Biofuels on June 13.
- Scheffer, M., S. Carpenter, J.A. Foley, C. Folke, and B. Walker. 2001. Catastrophic shifts in ecosystems. *Nature* 413(6856):591-596.
- Scheffer, M., S. Rinaldi, A. Gagnani, L.R. Mur, and E.H. vanNes. 1997. On the dominance of filamentous cyanobacteria in shallow, turbid lakes. *Ecology* 78(1):272-282.
- Schlichting, H.E., Jr. 1960. The role of waterfowl in the dispersal of algae. *Transactions of the American Microscopical Society* 74:160-166.
- Schober, H. 1966. Agitation of water surfaces by sprinkling to prevent mosquito breeding. *Mosquito News* 26:144-149.
- Schomaker, A. 2000. Anaerobic digestion of agro-industrial wastes: Information networks technical summary on gas treatment. Available online at http://agrienvarchive.ca/bioenergy/download/AD_techsum_biogas_AD-NETT.pdf. Accessed February 1, 2012.
- Schoups, G., J.W. Hopmans, C.A. Young, J.A. Vrugt, W.W. Wallender, K.K. Tanji, and S. Panday. 2005. Sustainability of irrigated agriculture in the San Joaquin Valley, California. *Proceedings of the National Academy of Sciences of the United States of America* 102(43):15352-15356.
- Sharma, N.K., and S. Singh. 2010. Differential aerosolization of algal and cyanobacterial particles in the atmosphere. *Indian Journal of Microbiology* 50(4):468-473.
- Shaw, S.L., B. Gantt, and N. Meskhidze. 2010. Production and emissions of marine isoprene and monoterpenes: A review. *Advances in Meteorology* 2010(Article ID 408696).
- Sheehan, J., T. Dunahay, J. Benemann, and P. Roessler. 1998. *A Look Back at the U.S. Department of Energy's Aquatic Species Program: Biodiesel from Algae*. Golden, CO: National Renewable Energy Laboratory.
- Sheveleva, E.V., and R.B. Hallick. 2004. Recent horizontal intron transfer to a chloroplast genome. *Nucleic Acids Research* 32(2):803-810.
- Shpiner, R., S. Vathi, and D.C. Stuckey. 2009. Treatment of oil well "produced water" by waste stabilization ponds: Removal of heavy metals. *Water Research* 43(17):4258-4268.
- Shuford, W.D., G.W. Page, and J.E. Kjelmlyr. 1998. Patterns and dynamics of shorebird use of California's Central Valley. *Condor* 100(2):227-244.
- Siegrist, M. 2000. The influence of trust and perceptions of risks and benefits on the acceptance of gene technology. *Risk Analysis* 20(2):195-203.
- Simmons, F.J., and I. Xagorarakis. 2011. Release of infectious human enteric viruses by full-scale wastewater utilities. *Water Research* 45(12):3590-3598.
- Smayda, T.J., and C.S. Reynolds. 2003. Strategies of marine dinoflagellate survival and some rules of assembly. *Journal of Sea Research* 49(2):95-106.
- Smith, J.L., G.L. Boyer, and P.V. Zimba. 2008. A review of cyanobacterial odorous and bioactive metabolites: Impacts and management alternatives in aquaculture. *Aquaculture* 280(1-4):5-20.

- Smith, S. 2008. Pollution violations may test public support for biodiesel. Available online at <http://www.biodieselmagazine.com/articles/2383/pollution-violations-may-test-public-support-for-biodiesel>. Accessed January 21, 2011.
- Smith, V.H. 2003. Eutrophication of freshwater and coastal marine ecosystems—A global problem. *Environmental Science and Pollution Research* 10(2):126-139.
- . 2006. Responses of estuarine and coastal marine phytoplankton to nitrogen and phosphorus enrichment. *Limnology and Oceanography* 51(1):377-384.
- Smith, V.H., and D.W. Schindler. 2009. Eutrophication science: Where do we go from here? *Trends in Ecology and Evolution* 24(4):201-207.
- Smith, V.H., B.S.M. Sturm, F.J. deNoyelles, and S.A. Billings. 2010. The ecology of algal biodiesel production. *Trends in Ecology and Evolution* 25(5):301-309.
- Smith, V.H., G.D. Tilman, and J.C. Nekola. 1999. Eutrophication: Impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. *Environmental Pollution* 100(1-3):179-196.
- Snow, A.A., D.A. Andow, P. Gepts, E.M. Hallerman, A. Power, J.M. Tiedje, and L.L. Wolfenbarger. 2005. Genetically engineered organisms and the environment: Current status and recommendations. *Ecological Applications* 15(2):377-404.
- Snow, A.A., and V. Smith. 2012. Genetically engineered algae for biofuels: A key role for ecologists. *Bioscience* 62(8):765-768.
- Spath, P.L., and M.K. Mann. 2001. Technical Report: Life Cycle Assessment of Hydrogen Production via Natural Gas Steam Reforming. Golden, CO: National Renewable Energy Laboratory.
- Stadmark, J., and L. Leonardson. 2005. Emissions of greenhouse gases from ponds constructed for nitrogen removal. *Ecological Engineering* 25(5):542-551.
- Stephenson, A.L., E. Kazamia, J.S. Dennis, C.J. Howe, S.A. Scott, and A.G. Smith. 2010. Life-cycle assessment of potential algal biodiesel production in the United Kingdom: A comparison of raceways and air-lift tubular bioreactors. *Energy and Fuels* 24:4062-4077.
- Stewart, K.W., L.E. Milliger, and B.M. Solon. 1970. Dispersal of algae, protozoans, and fungi by aquatic hemiptera, trichoptera, and other aquatic insects. *Annals of the Entomological Society of America* 63(1):139-147.
- Stoglehner, G. 2003. Ecological footprint—A tool for assessing sustainable energy supplies. *Journal of Cleaner Production* 11(3):267-277.
- Straub, T.M. 1993. Hazards from pathogenic microorganisms in land-disposed sewage sludge. *Review of Environmental Contamination and Toxicology* 132:55-91.
- Strauss, S.H., D.L. Kershen, J.H. Bouton, T.P. Redick, H. Tan, and R.A. Sedjo. 2010. Far-reaching deleterious impacts of regulations on research and environmental studies of recombinant DNA-modified perennial biofuel crops in the United States. *Bioscience* 60(9):729-741.
- Strayer, D.L., V.T. Eviner, J.M. Jeschke, and M.L. Pace. 2006. Understanding the long-term effects of species invasions. *Trends in Ecology and Evolution* 21:645-651.
- Sturm, B.S., E. Peltier, V. Smith, and F. De Noyelles. 2012. Controls of microalgal biomass and lipid production in municipal wastewater-fed bioreactors. *Environmental Progress and Sustainable Energy* 31:10-16.
- Sturm, B.S.M., and S.L. Lamer. 2011. An energy evaluation of coupling nutrient removal from wastewater with algal biomass production. *Applied Energy* 88(10):3499-3506.
- Sukias, J.P.S., and R.J. Craggs. 2011. Digestion of wastewater pond microalgae and potential inhibition by alum and ammoniacal-N. *Water Science and Technology* 63(5):835-840.
- Sukias, J.P.S., and C.C. Tanner. 2005. Ponds for livestock wastes. Pp. 408-432 in *Pond Treatment Technology*, A. Shilton, ed. London: IWA Publishing.
- Suter, G.W., II, and C.L. Tsao. 1996. Toxicological Benchmarks for Screening of Potential Contaminants of Concern for Effects on Aquatic Biota on Oak Ridge Reservation: 1996 Revision. Oak Ridge, TN: Oak Ridge National Laboratory.
- Sutton, P.C., S.J. Anderson, C.D. Elvidge, B.T. Tuttle, and T. Ghosh. 2009. Paving the planet: impervious surface as proxy measure of the human ecological footprint. *Progress in Physical Geography* 33:510-527.
- Tenbult, P., N.K. de Vries, E. Dreezens, and C. Martijn. 2005. Perceived naturalness and acceptance of genetically modified food. *Appetite* 45(1):47-50.
- Tiedje, J.M., R.K. Colwell, Y.L. Grossman, R.E. Hodson, R.E. Lenski, R.N. Mack, and P.J. Regal. 1989. The planned introduction of genetically engineered organisms—Ecological considerations and recommendations. *Ecology* 70(2):298-315.
- Turlure, C., J. Choutt, H. Van Dyck, M. Baguette, and N. Schtickzelle. 2010. Functional habitat area as a reliable proxy for population size: Case study using two butterfly species of conservation concern. *Journal of Insect Conservation* 14(4):379-388.

- Uphoff, J.H., M. McGinty, R. Lukacovic, J. Mowrer, and B. Pyle. 2011. Impervious surface, summer dissolved oxygen, and fish distribution in Chesapeake Bay subestuaries: Linking watershed development, habitat conditions, and fisheries management. *North American Journal of Fisheries Management* 31:554-566.
- USDA-ERS (U.S. Department of Agriculture Economic Research Service). 2011. Adoption of Genetically Engineered Crops in the United States. Available online at <http://www.ers.usda.gov/data/biotechcrops/>. Accessed June 18, 2012.
- USDA-RD (U.S. Department of Agriculture Rural Development). 2009. Environmental Assessment for Sapphire Energy Inc.'s Integrated Algal Biorefinery (IBR) Facility in Columbus, New Mexico. Washington, DC: U.S. Department of Agriculture.
- USDI (U.S. Department of the Interior). 1998. National Irrigation Water Quality Program Information Report No. 3: Guidelines for Interpretation of the Biological Effects of Selected Constituents in Biota, Water, and Sediment. Denver, CO.
- Vaccari, D. 2009. Phosphorus: A looming crisis. *Scientific American* 300(6):54-49.
- Velasquez, G.T. 1940. On the viability of algae obtained from the digestive tract of the Gizzard Shad, *Dorosotnu cepedianum*. *American Midland Naturalist* 22:376-412.
- Vymazal, J. 1995. *Algae and Element Cycling in Wetlands*. Boca Raton, FL: Lewis Publishing.
- Wang, L., M. Min, Y. Li, P. Chen, Y. Chen, Y. Liu, Y. Wang, and R. Ruan. 2010. Cultivation of green algae *Chlorella* sp in different wastewaters from municipal wastewater treatment plant. *Applied Biochemistry and Biotechnology* 162(4):1174-1186.
- Watras, C.J., and N.S. Bloom. 1992. Mercury and methylmercury in individual zooplankton—implications for bioaccumulation. *Limnology and Oceanography* 37(6):1313-1318.
- Weathers, P.J. 1984. N₂O evolution by green algae. *Applied and Environmental Microbiology* 48(6):1251-1253.
- Weber, C.L., and H.S. Matthews. 2008. Food-miles and the relative climate impacts of food choices in the United States. *Environmental Science and Technology* 42(10):3508-3513.
- Wedding, G.C., and D. Crawford-Brown. 2007. Measuring site-level success in brownfield redevelopments: A focus on sustainability and green building. *Journal of Environmental Management* 85(2):483-495.
- Weiland, U., A. Kindler, E. Banzhaf, A. Ebert, and S. Reyes-Paecke. 2011. Indicators for sustainable land use management in Santiago de Chile. *Ecological Indicators* 11(5):1074-1083.
- West, N.E. 2003. History of rangeland monitoring in the U.S.A. *Arid Land Research and Management* 17(4):495-545.
- White, L.F., and J.B. Shurin. 2011. Density dependent effects of an exotic marine macroalga on native community diversity. *Journal of Experimental Marine Biology and Ecology* 405(1-2):111-119.
- White, R.E., S.R. Wellings, and J.P. Bell. 1983. Seasonal variations in nitrate leaching in structured clay soils under mixed land-use. *Agricultural Water Management* 7(4):391-410.
- Whitford, L.A. 1983. On rare freshwater algae. *Transactions of the American Microscopical Society* 102(4):401-403.
- Wiens, J., J. Fargione, and J. Hill. 2011. Biofuels and biodiversity. *Ecological Applications* 21(4):1085-1095.
- Wigmosta, M.S., A.M. Coleman, R.J. Skaggs, M.H. Huesemann, and L.J. Lane. 2011. National microalgae biofuel production potential and resource demand. *Water Resources Research* 47: WH00H04.
- Wijffels, R.H., and M.J. Barbosa. 2010. An outlook on microalgal biofuels. *Science* 329(5993):796-799.
- Wilkie, A.C., and W.W. Mulbry. 2002. Recovery of dairy manure nutrients by benthic freshwater algae. *Bioresource Technology* 84(1):81-91.
- Wilkinson, D.M., S. Koumoutsaris, E.A.D. Mitchell, and I. Bey. 2011. Modelling the effect of size on the aerial dispersal of microorganisms. *Journal of Biogeography* 39:89-97.
- Wolfe, A.K., and D.J. Bjornstad. 2003. Making decisions about hazardous waste remediation when even considering a remediation technology is controversial. *Environmental Science and Technology* 37(8):1485-1492.
- Wolt, J.D. 2009. Advancing environmental risk assessment for transgenic biofeedstock crops. *Biotechnology for Biofuels* 2(1):27.
- Yanowitz, J., and R.L. McCormick. 2009. Effect of E85 on tailpipe emissions from light-duty vehicles. *Journal of the Air and Waste Management Association* 59(2):172-182.
- Zerzghi, H., C.P. Gerba, J.P. Brooks, I.L. Pepper. 2009. Long-term effects of land application of Class B biosolids on the soil microbial populations, pathogens, and activity. *Journal of Residual Science Technology* 7:51-61.
- Zhang, E.D., B. Wang, Q.H. Wang, S.B. Zhang, and B.D. Zhao. 2008. Ammonia-nitrogen and orthophosphate removal by immobilized *Scenedesmus* sp isolated from municipal wastewater for potential use in tertiary treatment. *Bioresource Technology* 99(9):3787-3793.
- Zilberman, D., G. Hochman, and D. Rajagopal. 2010. On the inclusion of indirect land use in biofuel regulations. *University of Illinois Law Review* 2011:413-434.
- Zimba, P.V., A. Camus, E.H. Allen, and J.M. Burkholder. 2006. Co-occurrence of white shrimp, *Litopenaeus vannamei*, mortalities and microcystin toxin in a southeastern USA shrimp facility. *Aquaculture* 261(3):1048-1055.

- Zimba, P.V., C.P. Dionigi, and S.S. Brashear. 2001a. Selective toxicity of exogenous L-lysine to cyanobacteria, relative to a chlorophyte and a diatom. *Phycologia* 40(5):483-486.
- Zimba, P.V., L. Khoo, P.S. Gaunt, S. Brittain, and W.W. Carmichael. 2001b. Confirmation of catfish, *Ictalurus punctatus* (Rafinesque), mortality from *Microcystis* toxins. *Journal of Fish Diseases* 24(1):41-47.
- Zimba, P.V., P.D. Moeller, K. Beauchesne, H.E. Lane, and R.E. Triemer. 2010. Identification of euglenophycin—A toxin found in certain euglenoids. *Toxicon* 55(1):100-104.
- Zuo, Z.J., Y.R. Zhu, Y.L. Bai, and Y. Wang. 2012. Acetic acid-induced programmed cell death and release of volatile organic compounds in *Chlamydomonas reinhardtii*. *Plant Physiology and Biochemistry* 51:175-184.

6

A Framework to Assess Sustainable Development of Algal Biofuels

Although each process in the production pathway could present sustainability challenges or opportunities to reduce resource use or mitigate environmental effects (as discussed in Chapters 4 and 5), the effect from one part of the supply chain could be offset by another part of the supply chain. Therefore, all the sustainability challenges and opportunities have to be assessed from a systems perspective. Thus, the committee reviewed life-cycle assessments (LCAs) performed to estimate resource use and environmental effects from cradle to grave for those parameters where published studies were available—for example, water use, net energy return, and net greenhouse-gas (GHG) emissions. Each pathway for producing algal biofuels combines cultivation, harvesting or product recovery, dewatering, and processing into a system. The abilities of different pathways to meet different aspects of sustainability vary, but in all cases, improvement in productivity, for example, cell density in algae cultivation, algal product (oil or alcohol), or biomass yield, and processing yield of biomass to fuels, helps reduce resource use and environmental effects.

Given the multiple resource requirements and potential environmental effects, specific sustainability concerns cannot be viewed in isolation from others. Any one LCA for a single resource use or environmental effect is insufficient to determine the overall sustainability of an algal biofuel production system. Issues arise as to how to assess the overall environmental sustainability of algal biofuels and how to balance the environmental objectives against economic and social objectives of sustainable development. In that regard, the committee was asked to discuss whether there are preferred cost-benefit analyses that best aid in the decision-making process.

This chapter first summarizes the sustainability concerns that might arise in each of the pathways for algal biofuel production discussed in Chapter 3. The summary illustrates how various pathways differ in their ability to meet different and sometimes competing sustainability objectives. Then, the chapter discusses tools that could aid in decision-making processes and proposes a framework for assessing sustainability of algal biofuel as a developing industry.

6.1 SUMMARY OF RESOURCE USE AND ENVIRONMENTAL EFFECTS OF DIFFERENT ALGAL BIOFUEL PRODUCTION PATHWAYS

6.1.1 Reference Pathway–Raceway Pond Producing Drop-in Hydrocarbon

Most algae for commercial products have been cultivated in open-pond systems because of their low costs compared to photobioreactors (Earthrise Nutritional, 2009; Milledge, 2011). Ensuring a high level of productivity of the desired algal species also could improve economic viability and reduce resource use and environmental effects per unit of fuel produced. Some of the key concerns for resource use and environmental sustainability include: Availability of suitable land for installing large ponds for algae cultivation.

- Evaporative loss of water from ponds, particularly in arid regions with low rainfall.
- Social perception and acceptance. They could be a key barrier if genetically modified organisms are to be cultivated in open ponds.

In the reference pathway, the nitrogen (N) and phosphorus (P) requirements are not a key sustainability concern because the lipid-extracted algae undergo anaerobic digestion to produce energy and these nutrients are returned to the algal culture. Energy generation from anaerobic digestion contributes to reducing energy input and hence GHG emissions. Other potential concerns that could be avoided if care is taken to maintain the algal cultures and the cultivation ponds include:

- Ground and surface water pollution.
- Presence of waterborne toxicants from contaminants.
- Potential for increasing mosquito-breeding grounds if ponds are not properly managed.

Some of the unknowns with respect to environmental sustainability include:

- Emissions of air pollutants from open ponds, which could be monitored to determine the extent of such emissions.
- Effects on terrestrial and aquatic biodiversity, but such effects could not be assessed unless the site of deployment for the algal biofuel production system and the cultivation system to be used are known.
- Site-dependent effect of open ponds on local climate.

The air quality emissions associated with drying, extraction, and processing to fuels could be mitigated by engineering solutions, particularly if most steps are performed indoors. Technology improvements in those steps and in harvesting could reduce energy use and hence reduce GHG emissions. The reference pathway produces a drop-in biofuel that can be used in the existing fuel distribution and vehicle infrastructure.

6.1.2 Alternative Pathway #1–Raceway Pond Producing Drop-in Hydrocarbon and Coproducts

The ability to meet various sustainability goals and the potential concerns for this pathway are similar to the reference case. The only difference lies in the production of coproducts other than energy from anaerobic digestion. That change could affect energy

requirements, GHG emissions, and nutrient requirements depending on what the high-quality coproduct is. Coproducts also affect economic viability.

If the coproduct is an animal feedstuff, then a coproduct credit could be assigned to the LCA of nutrient and energy requirements, and to GHG emissions for the animal feedstuff that is substituted by the coproduct of algal biofuel. Safety would have to be considered if the coproduct is to be fed to animals or used to fertilize food crops. Algae potentially can accumulate toxic compounds (for example, mercury can accumulate in cultivated algal cells if unscrubbed flue gas is used as a source of supplemental carbon dioxide [CO₂]). Toxicants accumulated in cultivated algae can be bioaccumulated if fed to animals or taken up by crop plants from fertilizers, or can inhibit anaerobic digestion if lipid-extracted algae are to be used for electricity generation. Other than safety, the nutritional quality of the feedstuff and the effect of the feedstuff on the quality of food animal (for example, meat quality) would have to be assessed to determine its suitability as a primary feedstuff or a supplement. A feedstuff coproduct can contribute to offsetting costs of algal biofuel production if there is a large enough market for the sale of the coproduct. If the feedstuff is only suitable for certain animals and has a limited market, then saturating the market with large quantities of the coproduct could lower its market price and utilization options.

If the coproduct is electricity, then market saturation will not be a concern. The energy requirement and GHG emissions could be lower compared to the reference pathway, and the cost of energy input into the algal biofuel production pathway could be reduced.

6.1.3 Alternative Pathway #2—Raceway Pond Producing FAME

The key difference between this and the reference pathway is the fuel produced, with this scenario assuming the fuel product to be fatty-acid methyl esters (FAME). With most processes along the supply chain being equal, the ability to meet various sustainability goals and the potential concerns for this pathway are similar to the reference case. However, FAME's poor cold-flow properties could affect their marketability and hence their economic viability. In northern-tier states, FAME might have to be stored in heated tanks in winter to keep the fuel fluid. In fact, many of the biodiesel refineries producing FAME from soybean in the United States are idle. In 2011, the production capacity of biodiesel in the United States was about 2 billion gallons per year, but only 1 million gallons were produced (EIA, 2010).

6.1.4 Alternative Pathway #3—Photobioreactors with Direct Synthesis of Ethanol

Growing microalgae in photobioreactors can avoid a number of the sustainability concerns associated with open-pond cultivation but may require substantial energy input for pumping and mixing water and for temperature control. Incidents of contamination by algae and other microorganisms and evaporative loss of water likely would be reduced. Other than using a different cultivation system from the other pathways discussed above, this pathway does not require harvesting, drying, and rupturing the algal cells to extract algal oil because the cyanobacteria secrete alcohol into the medium continuously. The direct synthesis of ethanol reduces downstream processing and could result in substantial energy savings and associated cost savings. In addition, some members of the public might find cultivation of genetically modified algae in enclosed reactors more acceptable than in open ponds.

A key barrier to sustainable development of algal biofuels using such systems is the potentially high capital cost (Tredici, 2007; Davis et al., 2011). Another disadvantage of this

pathway is that the fuel product, ethanol, is not compatible with the fuel distribution infrastructure for petroleum-based fuels. Although ethanol can be used in flex-fuel vehicles (FFV) that accommodate a blend of 85 percent ethanol and 15 percent gasoline (E85), most vehicles in the United States have internal combustion engines that use E10, which contains 90 percent gasoline and 10 percent ethanol. As of January 2011, the U.S. Environmental Protection Agency (EPA) allows the use of E15 in vehicle models of 2001 or newer. If every drop of the 520 billion liters of gasoline consumed in the United States in 2010 was blended with ethanol for E10, the maximum ethanol that could be used is 52 billion liters. The United States produced 50 billion liters of corn-grain ethanol that year. Therefore, the U.S. transportation sector would not be able to incorporate much more ethanol into the fuel system unless the market for flex-fuel vehicles expands.

6.1.5 Comparing the Sustainability of Different Pathways

The summaries of resource use and environmental effects of different pathways illustrate that each pathway has its strengths and weaknesses in meeting different sustainability goals. For example, the use of open ponds and closed photobioreactors illustrate tradeoffs between aspects of economic and environmental sustainability. Open-pond systems could raise more environmental concerns than closed-photobioreactor systems, but the cost differential between the two systems could be a key determinant of economic viability. The direct synthesis and secretion of ethanol by cyanobacteria without cell destruction would reduce nitrogen and phosphorus input during cultivation (particularly if nitrogen and phosphorus recycling are not fully implemented in algal biofuel production systems that require biomass harvesting) and energy use from downstream processing and could result in synergistic cost savings for a closed photobioreactor system. The question arises as to how to make a holistic assessment of the relative sustainability of different algal biofuel production systems, given the multiple indicators and LCAs that represent various sustainability goals and objectives. As discussed in Chapter 2, indicators and LCAs are tools that can be used to assess a particular aspect of sustainability. Other tools are needed to integrate across disciplines to assess overall sustainability, which includes energy security, and environmental, social, and economic sustainability. As outlined in the statement of task, the committee was not asked to perform any technoeconomic analyses. Environmental sustainability has been considered more extensively than social sustainability in the literature because some aspects of social sustainability will be local and social acceptability in part depends on public opinion, transparency, stakeholder participation, and risk of catastrophe, all of which are largely unexplored for algal biofuels. Therefore, this chapter focuses on environmental sustainability.

6.2 TOOLS FOR ASSESSING OVERALL SUSTAINABILITY

The holistic assessment of sustainability is complicated by the fact that some sustainability objectives can be assessed and compared across systems while others are region-specific and cannot be compared across systems. For example, resource use and environmental effects such as nutrient budgets, energy balances, and GHG emissions can be compared directly across systems. Methods for assessing these variables are strictly quantitative. Other environmental effects such as land-use change and biodiversity are region specific and scale specific.

Some resource use and environmental effects can be assessed quantitatively, but whether they contribute to moving toward or away from the sustainability objectives could

be region dependent. For example, consumptive water use and emissions of air pollutants can be quantified and compared across alternative algal biofuel production systems. However, a comparison without considering the regional context might not indicate whether the systems contribute to improved sustainability. One algal biofuel production system could be more sustainable with respect to consumptive water use than another even if both use the same quantity of fresh water over their life cycles because one system is situated in an area with high rainfall and near an aquifer that replenishes sufficiently every year, and another is situated in an arid area with a fossil aquifer. Similarly, two identical open-pond systems for algae cultivation in different locations could have different effects on biodiversity depending on the species present at each location. Systematically assessing the sustainability of algal biofuel production systems and comparing them to each other or with other transportation fuel systems presents distinct challenges to researchers and policy makers. As noted by Gasparatos et al. (2011), there is not a consistent language for putting “biofuels’ diverse trade-offs into perspective,” nor are there appropriate tools “for assessing the sustainability of different biofuel practices during their full life cycle.” Despite these challenges for assessing overall sustainability, different approaches have been proposed.

6.2.1 Ecosystem Service Analysis

Analysis of ecosystem services provides a means to assess the overall effects and trade-offs of algal biofuel production and use (Gasparatos et al., 2011). Ecosystem services are goods and services generated by ecosystem processes that benefit human well-being (NRC, 2011). Thus, the analysis of ecosystem services is a way to link environmental sustainability to social and economic sustainability.

Ecosystem services can be categorized as provisioning, regulating, and cultural services (MEA, 2003). Algal biofuel production is a provisioning ecosystem service. It provides liquid fuels to improve energy security, wastewater treatment if wastewater is to be used as a culture medium, animal feed if it is produced as a coproduct, and energy if lipid-extracted algal biomass is used to generate electricity via anaerobic digestion. Conversely, algal biofuel production systems could compete for resources with other systems that provide ecosystem services—for example fresh water, or land that could be used for food production or other human benefits. Biofuel production also could affect cultural services by changing relatively unmanaged landscapes to highly managed ponds and processing facilities. An adaptation of the Gasparatos et al. (2011) table “Key sustainability issues associated with biofuel production from an ecosystem services perspective” is summarized in Table 6-1.

Although Table 6-1 is focused on land-crop biofuels, the sustainability issues listed are not fundamentally different from those associated with algal biofuels. Energy security and climate regulation are among the primary factors in sustainability of biofuel production irrespective of the feedstock type. The availability of sufficient nutrients and land for production could be added to this list, although these are implicit in the listing for food production and ecosystem conservation. Each of the major resource use issues discussed in Chapter 4—land, water, and nutrients—thus can be viewed as elements in an ecosystem services framework. The analysis by Gasparatos et al. (2011) also makes clear the utility of ecosystem services for addressing social and economic considerations, which are not addressed in this report. Similarly, Tilman et al. (2009) highlight the importance of placing decisions about biofuels in the context of energy security, GHG emissions, biodiversity, and food supply sustainability.

The Ecological Society of America (2008) advocates conservation of ecosystem services as one of three principles for assessing the ecological sustainability of biofuels, and

TABLE 6-1 Key Sustainability Issues Associated with Biofuel Production, from an Ecosystem Services Perspective

Sustainability Issue	Main Ecosystem Services	Main Constituents of Well-Being
Energy security	Fuel (provisioning service)	Access to fuel Basic materials for good life Energy security
Climate change	Climate change regulation (regulatory service)	Access to basic materials, e.g., sufficient nutritious food Basic materials for a good life Security of resource access and security from disasters
Economic development (rural development)	Fuel (provisioning service)	Basic materials for a good life Health Security
Food production	Erosion regulation (regulatory service) Food (provisioning service)	Basic materials for a good life Good social relations Health Sufficient and accessible nutritious food
Ecosystem conservation	Services from conserved ecosystems: <ul style="list-style-type: none"> • aesthetic value (cultural service) • climate change regulation (regulatory service) • pollination of crops and other vegetation (regulatory service) • timber and forest nontimber products (provisioning services) • recreation and cultural service 	Basic materials for a good life Good social relations
Water provision	Steady and clean water supply (provisioning)	Basic materials for a good life Good social relations Health
Health	Clean air (regulatory service) Food (provisioning services) Water (provisioning services)	Health
Social cohesion	Sufficient and equitable supply of ecosystem services (provisioning, regulatory, supporting, and cultural services)	Good social relations
Maintenance of biodiversity	Biodiversity is not an ecosystem service per se but “the foundation of ecosystem services to which human well-being is intimately linked” (MA, 2005, p. 18).	Basic materials for good life Good social relations Health Security

SOURCE: Adapted from Gasparatos et al., 2011. Reprinted with permission from Elsevier.

Robertson et al. (2008, p. 50) recommend focused research on ecosystem services “to provide the information necessary for the development and implementation of land-management approaches that meet multiple needs.” Translation of ecosystem services analyses into tools that can help make informed decisions is thus a key need (Daily et al., 2009; Gasparatos et al., 2011) and has great promise for contributing to the understanding of the sustainability of algal biofuel production. Although analyses of ecosystem services integrate the various aspects of resource use and environmental effects, their application to a developing industry such as algal biofuels could be difficult because some aspects of ecosystem services

(for example, potential effects on biodiversity) cannot be analyzed until the actual site of deployment is known.

6.2.2 Cost-Benefit Analyses

Cost-benefit analysis is the comparison of the monetized costs of a proposed action compared to the benefits, usually with costs and benefits expressed in monetary terms and from a particular perspective (for example, those investing in the project, those regulating it, or society as a whole). An economic cost-benefit approach relies on an ideological framework that depends on the economic theory applied. An example of an economic cost-benefit analysis is the technical analysis of projects such as federal spending for flood control in which the determination of whether the overall benefits exceed the estimated costs is used to evaluate proposed systems. Cost-benefit analyses can incorporate factors that are noneconomic. For example, Simpson and Walker (1987) proposed to include environmental, technical, and risk analyses, in addition to economic analyses in cost-benefit analyses for energy investments. Because many environmental benefits and effects or ecosystem goods and services lack markets or market prices, methods have been developed to estimate their valuation by individuals or society. For example, stated preference methods use carefully designed questionnaires to estimate how much individuals are willing to pay for an increase in quantity of a particular ecosystem service or environmental benefits and how much compensation individuals are willing to accept for the loss of an ecosystem service or a negative effect they endure. Those values form the bases of monetization of ecosystem goods and services and environmental benefits and effects (Hanley and Barbier, 2009).

Cost-benefit analysis is a useful tool for assessing sustainability for the following reasons:

- It can express most relevant benefits and effects in monetary values that can be aggregated into one value (Hanley and Barbier, 2009) and allows direct comparison across algal biofuel production systems if it is applied consistently across systems.
- It aids decision making by showing the tradeoffs among nonmonetized variables that express societal values.
- If the key parameters of cost-benefit analyses are standardized, the analyses allow comparison of sustainability of different biofuels and ensure consistency in decision making (Hanley and Barbier, 2009).

There are some challenges to applying a cost-benefit analysis to environmental sustainability. Some ecosystem goods and services are not readily quantifiable so they cannot be valued (NRC, 2005). Although there are approaches to nonmarket valuation of ecosystem services, those approaches rely on a great deal of professional judgment and depend on the ideological orientation of the individual or group conducting the valuation (NRC, 2005; Bebbington et al., 2007). A key challenge to applying a cost-benefit analysis to algal biofuel production relates to nonmarket valuation. Because algal biofuel production is developing with multiple pathways being pursued, the actual effects of algal biofuel production on ecosystems and the environment are largely uncertain (as discussed in Chapters 4 and 5). Because the changes to the environment or provision of ecosystem goods and services that people care about cannot be described in precise ways, it is difficult for surveyed individuals to place a value on potential changes (Hanley and Barbier, 2009). Different individuals and groups are likely to value different sustainability goals differently. Therefore, Bebbington et al. (2007) cautioned against the over-reliance on cost-benefit analysis and proposed

the use of “sustainability assessment models” that recognize the need for accountings and also include a participatory approach to decision making. Prioritization of the sustainability goals and decisions on the appropriate tradeoffs to be made that meet the core societal needs requires the development of a collective vision of the desired attributes of a sustainable fuel industry (NRC, 2010).

6.2.3 Cumulative Impacts

In addition to assessing the sustainability goals quantitatively when possible, balancing the sustainability objectives, and minimizing tradeoffs, developing algal biofuels sustainably also would require consideration of the cumulative impacts to the environment. Cumulative effects are defined as “the impact on the environment which results from the incremental impact of the action when added to other past, present, and reasonably foreseeable future actions regardless of what agency (federal or non-federal) or person undertakes such other actions” (43 CFR 1508.7). Environmental assessments or environmental impact statements for proposed biofuel refineries include an assessment of cumulative effects, though some of them are limited in scale. For example, the environmental assessments for Algenol’s facility in Fort Meyer (DOE, 2010) and Sapphire Energy’s facility in New Mexico (USDA-RD, 2009) include a section on cumulative impacts on land use, air quality, soil, ground and surface water, and socioeconomic factors specific to the site. However, the cumulative impacts of future large-scale deployments of multiple algal biofuel production systems across the country have not been assessed.

Parallel lessons can be drawn from environmental impact assessment for solar energy development (BLM and DOE, 2010). Many of the locations considered desirable for algal biofuel production may overlap with potential areas for development of other renewable energy projects such as solar- or wind-powered electricity generation as well as a broad range of other activities. For example, according to the Draft Solar Energy Programmatic Environmental Impact Statement (BLM and DOE, 2010), the BLM-administered land area considered potentially available for solar development in six western states is about 87,000 square kilometers, with approximately one percent (866 square kilometers) needed to produce the 24,000 megawatts of power that would be generated over the 20-year period of the study. Another 8,000 megawatts could be produced on approximately 287 square kilometers in these same six states. NREL (2004) estimated that approximately 40,470 square kilometers of land would be required to meet all U.S. electricity demand using photovoltaic solar technology.

These values can be compared with the 430,830 square kilometers that Wigmosta et al. (2011) estimated would be needed nationwide to produce 220 billion liters per year of algal biofuels. Some of the general characteristics that make land desirable for solar development (for example, slope less than 5 percent and high insolation) are similar to the characteristics that make land desirable for algal biofuel production systems. Although the total land requirements are different, the similarities in desirable site characteristics suggest the importance of considering the possibility of competing solar-power development when evaluating the potential cumulative effects of algal biofuel production.

The Solar PEIS also notes ongoing and reasonably foreseeable future activities in its six-state study area (Arizona, California, Colorado, Nevada, New Mexico, and Utah) that include energy production and distribution, recreation, mineral production, military operations, grazing and rangeland management, fire management, forestry, transportation, and industrial development. A similarly broad range of activities is likely in areas that could be developed for algal biofuel production in these and other states. Further, the Solar PEIS

describes renewable energy development as “by far the largest potential new future use of rural lands” in the six-state area analyzed. Given the increasing demands for production of biofuel feedstocks in other areas of the United States, this is likely at least partly true nationally, in addition to the conversion of rural lands to suburban developments near metropolitan areas.

Large-scale production of algal biofuels in areas adjacent to land already developed for other energy sources could contribute to cumulative effects on land use, water supply, and biodiversity. Solar technologies in particular could place site-specific demands on these three factors that are similar in scale to those of algal biofuels: extensive land areas would be cleared of vegetation and maintained as such, with consequent impacts on biodiversity; solar thermal facilities require water for cooling; and all solar facilities require water for mirror or panel washing (BLM and DOE, 2010).

6.3 FRAMEWORK FOR INTEGRATED ASSESSMENT

LCAs and cumulative-impact, ecosystem-service, and cost-benefit analyses each assess sustainability on a somewhat different scale and each has a role in assessing the overall sustainability of algal biofuel production systems (Figure 6-1). Therefore, the committee is not suggesting a specific cost-benefit analysis to aid decision-making processes. Instead, the committee proposes a stepwise framework that encompasses these tools at different stages of algal biofuel development (Figure 6-2) to aid the Department of Energy (DOE) in its decision-making process on sustainable development of algal biofuels. The framework

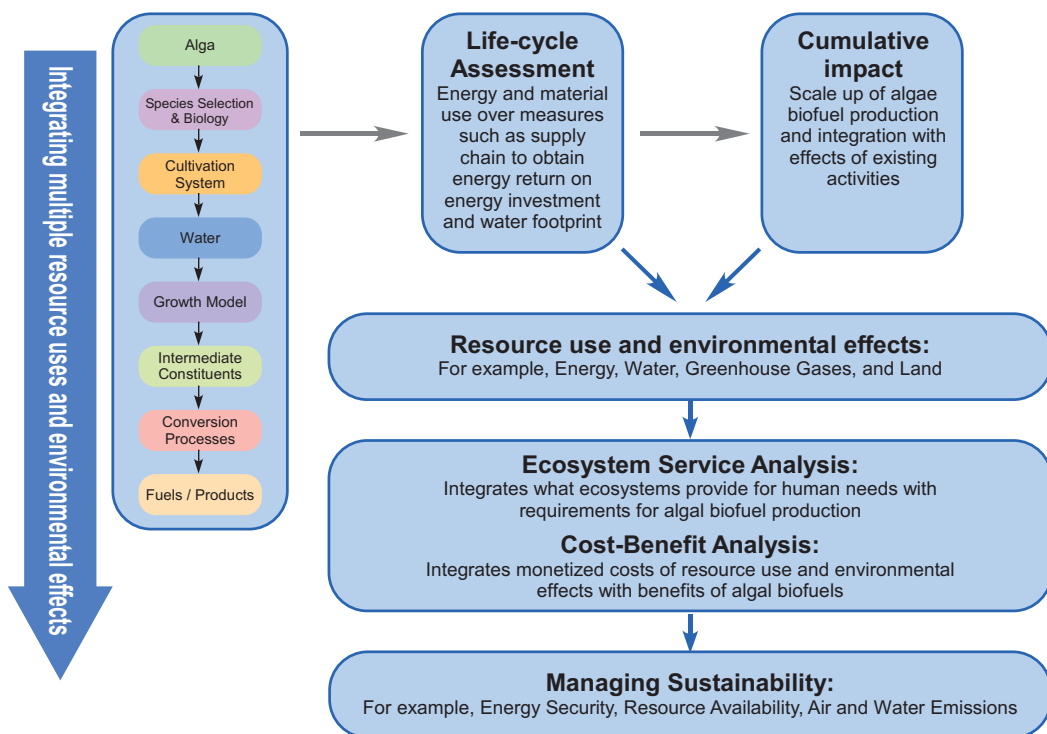


FIGURE 6-1 A diagram illustrating various tools for assessing sustainability at different scales.

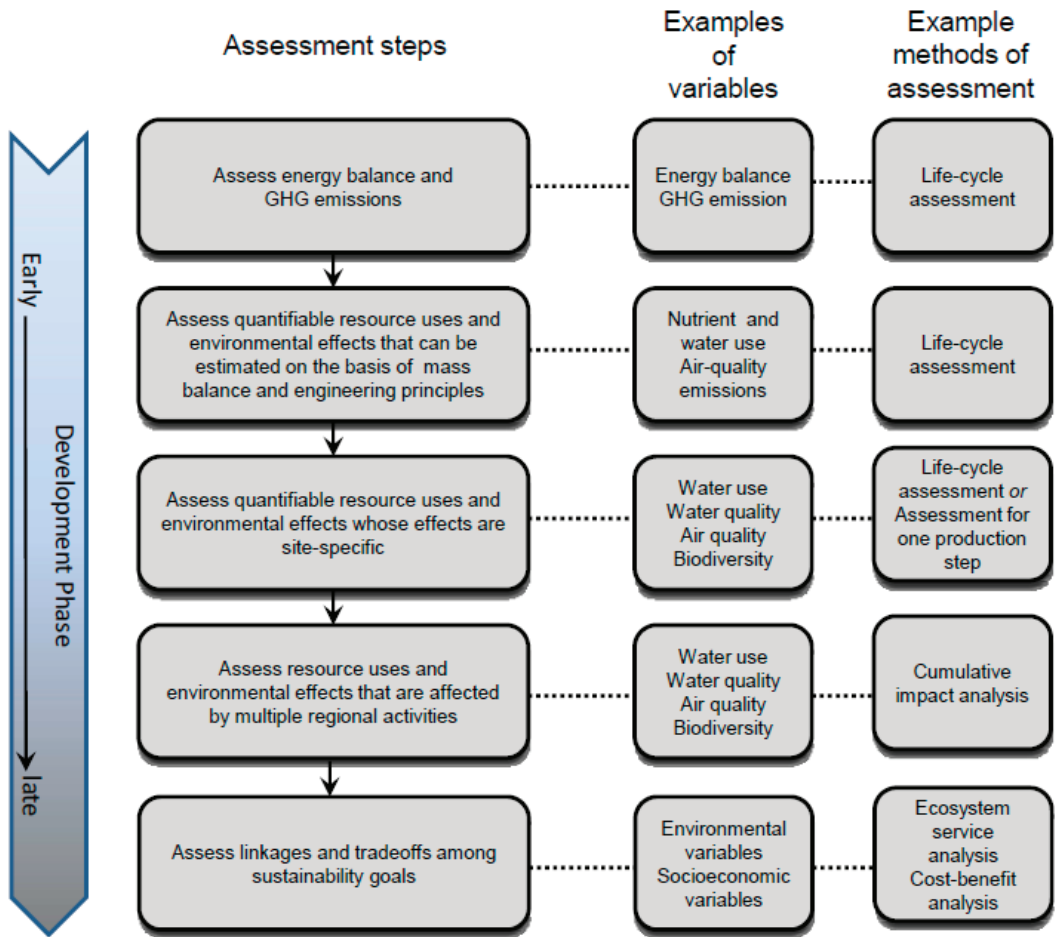


FIGURE 6-2 A potential framework for assessing sustainability of algal biofuels during different stages of development.

for assessment starts with assessing two of the primary goals for developing alternative liquid fuels—improving energy security and reducing GHG emissions. Then, a few variables that reflect commonly agreed-upon sustainability objectives and that can be estimated from mass balance and engineering principles are assessed. When the industry is further along in its development, progressively comprehensive and regional assessments can be made. Data also could be collected to verify assumptions and estimates made earlier in the decision framework when the algal biofuel production systems are operating. The indicators for assessing each variable were discussed in Chapters 4 and 5.

First, the energy return on investment (EROI) of less than 1 is definitely unsustainable; therefore, it is a logical first step for assessment. Specifically, a given algal biofuel production system would have to have or at least show progress toward EROI within the range of EROIs of other transportation fuels (Figure 6-3) because algal biofuels will be compared with other petroleum-based fuels and nonpetroleum-based alternatives. One of the most contentious issues associated with biofuels produced from land crops has been the level

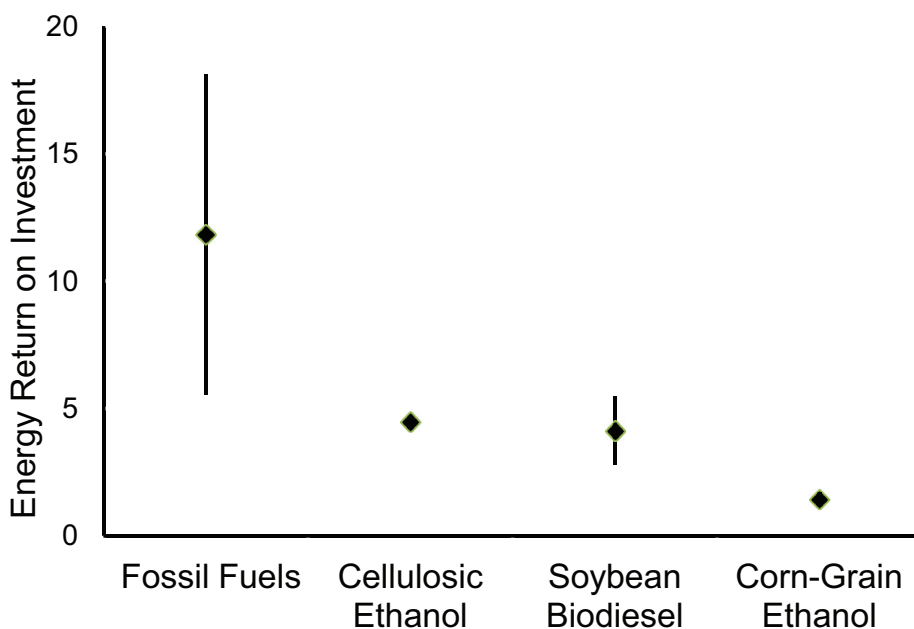


FIGURE 6-3 Estimates of EROI for different fuels reported in the literature.

NOTE: Symbol denotes average of values reported in the literature. Line represents one standard deviation.

SOURCES: Herweyer and Gupta (2008), Grandell et al. (2009), Hall and Day (2009), and Batan (2010) for petroleum-based fuels; Lynd and Wang (2004), Sheehan et al. (2004), and Farrell (2006) for cellulosic ethanol; Herweyer and Gupta (2008), Grandell et al. (2009), Hall and Day (2009), Batan et al. (2010), and Freise (2011) for biodiesel; Kim and Dale (2005), Farrell (2006), and Hill et al. (2006) for corn-grain ethanol; Clarens et al. (2010), Jorquera et al. (2010), Sander and Murthy (2010), Stephenson et al. (2010), Brentner et al. (2011), and Vasudevan et al. (2012) for algal biodiesel.

of EROI required for sustainable production of any fuel (Pimentel and Patzek, 2005). Algal biofuels would have to return more energy in use than was required in their production to be a sustainable source of transportation. Microalgal fuels use high-value energy inputs in the form of electricity and natural gas. If these high-quality energy sources are downgraded in the production of algal fuels, it is certainly a sustainability concern that can only be truly understood through careful life-cycle analysis. (See section Energy in Chapter 4.) EROI of 1, the breakeven point, is insufficient to be considered sustainable. However, the exact threshold for sustainability is not well defined. Hall (2011) proposed that EROI greater than 3 is needed for any fuels to be considered a sustainable source. EROI can be estimated with an LCA that tracks energy and material flow (Chapter 4).

Reducing GHG emissions is another key goal in developing alternative liquid transportation fuels, and GHG emissions are closely related to energy input and output of algal biofuel production systems (Chapter 5). GHG emissions have the same effect on global climate regardless of where the GHGs are emitted. The U.S. Congress enacted the Energy Independence and Security Act of 2007 (110 P.L. 140) to improve “energy independence and security” and “to increase the production of clean renewable fuels.” If reducing GHG emissions from the transportation sector is an important goal, then the fuels displacing

petroleum-based fuels would need to have lower net GHG emissions than the fuel that they are displacing.

In addition to energy balance and GHG emissions, nitrogen and phosphorus inputs are sustainability objectives that can be assessed using LCAs based on mass balance and engineering principles. Nitrogen and phosphorus consumption by algae cultivation could compete with food production. There are opportunities to mitigate the potential competition for nutrients with agriculture, including recycling nutrients from the lipid-extracted algae and using wastewater for algae cultivation. The feasibility of using wastewater for algae cultivation has to be assessed in at least a few dimensions:

- The number of locations that could accommodate the colocation of wastewater treatment and algae cultivation facilities.
- The potential for such systems to achieve both goals of wastewater treatment and algae cultivation for fuels without much compromise.
- The feasibility beyond the laboratory scale.

The estimated EROI, GHG emissions, and nutrient requirements would have to be reassessed once the likely locations of deployment are narrowed down. Then, the productivities and any potential land-use changes can be estimated with increased certainties, and the precision of the estimated resource requirements and GHG emissions can be improved. Those variables also can be measured after deployment to verify modeled estimates and to help improve future modeling efforts.

Though some resource uses or emissions can be estimated quantitatively, their effects on the environment are location specific. Requirements for land and water are two examples. Quantitative estimates of land requirements, though necessary, have to be considered in the context of the local climatic conditions, proximity to other resources, and land prices so as to achieve economically viable production of algal biofuels. Similarly, water use (saline, brackish, or fresh water) has to be assessed over the life cycle of fuel and in the context of regional availability. Thus, a national assessment of land requirements for algae cultivation that takes into account climatic conditions; brackish, fresh water, and wastewater resources; and sources of concentrated CO₂, and land prices could inform the potential amount of algal biofuels that could be produced economically in the United States. Such assessment could be done at a county-by-county resolution as in the case of the *U.S. Billion-Ton Update* (DOE, 2011) for biofuel feedstock. The committee cautions that the realized amount of algal biofuels produced likely will be lower than the potential amount (as in the case with other biofuels) because of many other factors associated with deployment. However, algal strain development to enhance algae's ability to scavenge CO₂ could reduce the need for concentrated CO₂ as a resource constraint. Once the potential locations for algal biofuel production are identified, existing uses of land and water, including neighboring and regional activities, have to be considered to assess the cumulative impacts.

Some environmental effects cannot be assessed unless the specific location of deployment is known. Some of these effects might be easily quantifiable. Others might require research and data collection before their effects can be understood and quantified. The resource and environmental effects also have to be assessed in the context of existing activities in the sites where algal biofuel production systems are to be developed (that is, a cumulative impact analysis). As the algal biofuel industry matures, the ability of different pathways for algal biofuel production to meet and balance yield with the other environmental, economic, and social sustainability goals has to be assessed in a holistic manner.

Ecosystem service analysis and cost-benefit analysis provide methods to examine tradeoffs among sustainability goals and an integrative perspective of sustainability.

Any given tool or framework for assessing sustainability for a given fuel does not determine whether the fuel contributes to improving sustainability of the transportation sector. In fact, the report *Toward Sustainable Agricultural Systems in the 21st Century* (NRC, 2010) suggests that sustainability is not a particular end state, but a trajectory toward achieving a set of environmental, economic, and social goals. In the context of this report, the question is whether substituting a portion of petroleum-based fuels with algal biofuels could move the transportation sector along a trajectory toward greater sustainability with respect to each of the four goals: contributing to energy security, maintaining and enhancing the natural resource base and environmental quality, producing fuels that are economically viable, and enhancing the quality of life for society as a whole. The environmental, economic, and social effects of algal biofuel production and use have to be compared with those of petroleum-based fuels and other fuel alternatives to determine whether algal biofuels contribute to improving sustainability.

Given the four aspects of sustainability and the multiple goals within each aspect, a participatory approach is necessary to develop a collective vision of the importance of various sustainability objectives relative to each other. Stakeholders would be involved from the beginning of a sustainability assessment. Such an approach that involves different stakeholders would help ensure that tradeoffs among sustainability goals would be acceptable to the various parties.

6.4 OPPORTUNITIES FOR ALGAL BIOFUELS TO IMPROVE SUSTAINABILITY

Algal biofuels have the potential to contribute to improving the sustainability of the transportation sector, but innovations and research and development (R&D) are needed to realize their full potential. Preliminary assessments in the literature suggest that several resource use and environmental challenges likely would have to be overcome for algal biofuel production to be scaled up in a sustainable way. Suitable locations for algal biofuels could be limited by the number and area of sites that are close to a source of CO₂, fresh water, brackish water, wastewater, or combination thereof. Innovations and R&D in various aspects of the supply chain will help realize much of the potential for algal biofuels to improve energy security, reduce GHG emissions, and enhance environmental quality. Algal strain development to improve biomass or lipid productivity would clearly increase fuel production per unit resource use and improve the economics of fuel production. Engineering designs to enhance algae cultivation, facilitate biomass or product collection (for example, algal lipid), and reduce processing requirements have the potential to greatly improve the energy balance, reduce GHG emissions, and enhance the overall sustainability of algal biofuels.

SUMMARY FINDING FROM THIS CHAPTER

The environmental, economic, and social effects of algal biofuel production and use have to be compared with those of petroleum-based fuels and other fuel alternatives to determine whether algal biofuels contribute to improving sustainability. Such comparison will be possible only if thorough assessments of each step in the various pathways for algal biofuel production are conducted.

REFERENCES

- Batan, L., J. Quinn, B. Willson, and T. Bradley. 2010. Net energy and greenhouse gas emission evaluation of biodiesel derived from microalgae. *Environmental Science and Technology* 44(20):7975-7980.
- Bebbington, J., J. Brown, and B. Frame. 2007. Accounting technologies and sustainability assessment models. *Ecological Economics* 61(2-3):224-236.
- BLM (Bureau of Land Management) and DOE (U.S. Department of Energy). 2010. Solar Energy Development Draft Programmatic Environmental Impact Statement (Draft Solar PEIS). Washington, DC: Bureau of Land Management and U.S. Department of Energy.
- Brentner, L.B., M.J. Eckelman, and J.B. Zimmerman. 2011. Combinatorial life cycle assessment to inform process design of industrial production of algal biodiesel. *Environmental Science and Technology* 45(16):7060-7067.
- Clarens, A.F., E.P. Resurreccion, M.A. White, and L.M. Colosi. 2010. Environmental life cycle comparison of algae to other bioenergy feedstocks. *Environmental Science and Technology* 44(5):1813-1819.
- Daily, G.C., S. Polasky, J. Goldstein, P.M. Kareiva, H.A. Mooney, L. Pejchar, T.H. Ricketts, J. Salzman, and R. Shallenberger. 2009. Ecosystem services in decision making: Time to deliver. *Frontiers in Ecology and the Environment* 7(1):21-28.
- Davis, R., A. Aden, and P.T. Pienkos. 2011. Techno-economic analysis of autotrophic microalgae for fuel production. *Applied Energy* 88(10):3524-3531.
- DOE (U.S. Department of Energy). 2010. Algenol Integrated Biorefinery for Producing Ethanol from Hybrid Algae. Washington, DC: U.S. Department of Energy.
- . 2011. U.S. Billion-Ton Update. Biomass Supply for a Bioenergy and Bioproducts Industry. Oak Ridge, TN: Oak Ridge National Laboratory.
- Earthrise Nutritional. 2009. Earthrise. Available online at <http://www.earthrise.com/>. Accessed February 9, 2012.
- EIA (Energy Information Administration). 2010. Monthly biodiesel production report. Available online at <http://www.eia.gov/biofuels/biodiesel/production/>. Accessed June 15, 2012.
- ESA (Ecological Society of America). 2008. Biofuel sustainability. Available online at <http://www.esa.org/pao/policyStatements/Statements/biofuel.php>. Accessed February 27, 2012.
- Farrell, A.E., R.J. Plevin, B.T. Turner, A.D. Jones, M. O'Hare, and D.M. Kammen. 2006. Ethanol can contribute to energy and environmental goals. *Science* 311:506-508.
- Freise, J. 2011. The ROI of conventional Canadian natural gas production. *Sustainability* 3(11):2080-2104.
- Gasparatos, A., P. Stromberg, and K. Takeuchi. 2011. Biofuels, ecosystem services and human wellbeing: Putting biofuels in the ecosystem services narrative. *Agriculture, Ecosystems and Environment* 142(3-4):111-128.
- Grandell, L., C. Hall, and M. Hook. 2009. Energy return on investment for Norwegian oil and gas in 1991-2008. Presentation to the 9th Annual ASPO conference, Brussels, Belgium on April 27-29, 2009.
- Hall, C.A.S. 2011. Sustainability: Special Issue on New Studies in EROI (Energy Return on Investment). *Sustainability* 3:1773-1777.
- Hall, C.A.S., and J.W. Day. 2009. Revisiting the limits to growth after peak oil. *American Scientist* 97(3):230-237.
- Hanley, N., and E.B. Barbier. 2009. Pricing Nature: Cost-Benefit Analysis and Environmental Policy. Northampton, MA: Edward Elgar Publishing Inc.
- Herweyer, M.C., and A. Gupta. 2008. Unconventional Oil: Tar Sands and Shale Oil—EROI on the Web, Part 3 of 6: Appendix D Tar sands/oil sands. The Oil Drum: Net Energy. Available online at http://www.theoil drum.com/pdf/theoil drum_3839.pdf. Accessed February 15, 2012.
- Hill, J., E. Nelson, D. Tilman, S. Polasky, and D. Tiffany. 2006. Environmental, economic, and energetic costs and benefits of biodiesel and ethanol biofuels. *Proceedings of the National Academy of Sciences of the United States of America* 103(30):11206-11210.
- Jorquera, O., A. Kiperstok, E.A. Sales, M. Embiruçu, and M.L. Ghirardi. 2010. Comparative energy life-cycle analyses of microalgal biomass production in open ponds and photobioreactors. *Bioresource Technology* 101(4):1406-1413.
- Kim, S., and B.E. Dale. 2005. Environmental aspects of ethanol derived from no-tilled corn grain: Nonrenewable energy consumption and greenhouse gas emissions. *Biomass and Bioenergy* 28(5):475-489.
- Lynd, L.R., and M.Q. Wang. 2004. A product-nonspecific framework for evaluating the potential of biomass-based products to displace fossil fuels. *Journal of Industrial Ecology* 7(3-4):17-32.
- MEA (Millennium Ecosystem Assessment). 2003. Ecosystems and Human Well-being. A Framework for Assessment. Washington, DC: World Resources Institute.
- Milledge, J.J. 2011. Commercial application of microalgae other than as biofuels: A brief review. *Reviews in Environmental Science and Biotechnology* 10(1):31-41.
- NRC (National Research Council). 2005. Valuing Ecosystem Services. Toward Better Environmental Decision-Making. Washington, DC: The National Academies Press.

- . 2010. *Toward Sustainable Agricultural Systems in the 21st Century*. Washington, DC: The National Academies Press.
- . 2011. *Sustainability and the U.S. EPA*. Washington, DC: The National Academies Press.
- NREL (National Renewable Energy Laboratory). 2004. *PV FAQs*. Washington, DC: U.S. Department of Energy.
- Pimentel, D., and T.W. Patzek. 2005. Ethanol production using corn, switchgrass, and wood; Biodiesel production using soybean and sunflower. *Natural Resources Research* 14(1):65-76.
- Robertson, G.P., V.H. Dale, O.C. Doering, S.P. Hamburg, J.M. Melillo, M.M. Wander, W.J. Parton, P.R. Adler, J.N. Barney, R.M. Cruse, C.S. Duke, P.M. Fearnside, R.F. Follett, H.K. Gibbs, J. Goldemberg, D.J. Mladenoff, D. Ojima, M.W. Palmer, A. Sharples, L. Wallace, K.C. Weathers, J.A. Wiens, and W.W. Wilhelm. 2008. Agriculture: Sustainable biofuels redux. *Science* 322(5898):49-50.
- Sander, K., and G.S. Murthy. 2010. Life cycle analysis of algae biodiesel. *International Journal of Life Cycle Assessment* 15(7):704-714.
- Sheehan, J., A. Aden, K. Paustian, K. Killian, J. Brenner, M. Walsh, and R. Nelson. 2004. Energy and environmental aspects of using corn stover for fuel ethanol. *Journal of Industrial Ecology* 7(3-4):117-146.
- Simpson, D., and J. Walker. 1987. Extending cost-benefit analysis for energy investment choices. *Energy Policy* 15(3):217-227.
- Stephenson, A.L., E. Kazamia, J.S. Dennis, C.J. Howe, S.A. Scott, and A.G. Smith. 2010. Life-cycle assessment of potential algal biodiesel production in the United Kingdom: A comparison of raceways and air-lift tubular bioreactors. *Energy and Fuels* 24(7):4062-4077.
- Tilman, D., R. Socolow, J.A. Foley, J. Hill, E. Larson, L. Lynd, S. Pacala, J. Reilly, T. Searchinger, C. Somerville, and R. Williams. 2009. Beneficial biofuels—The food, energy, and environment trilemma. *Science* 325(5938):270-271.
- Tredici, M.R. 2007. Mass production of microalgae: Photoreactors. Pp. 178-214 in *Handbook of Microalgal Culture: Biotechnology and Applied Phycology*, A. Richmond, ed. Ames, IA: Blackwell.
- USDA-RD (U.S. Department of Agriculture Rural Development). 2009. *Environmental Assessment for Sapphire Energy Inc.'s Integrated Algal Biorefinery (IBR) Facility in Columbus, New Mexico*. Washington, DC: U.S. Department of Agriculture.
- Vasudevan, V., R.W. Stratton, M.N. Pearlson, G.R. Jersey, A.G. Beyene, J.C. Weissman, M. Rubino, and J.I. Hileman. 2012. Environmental Performance of Algal Biofuel Technology Options. *Environmental Science and Technology* 46(4):2451-2459.
- Wigmosta, M.S., A.M. Coleman, R.J. Skaggs, M.H. Huesemann, and L.J. Lane. 2011. National microalgae biofuel production potential and resource demand. *Water Resources Research* 47(4):W00H04.

Appendixes

A

Biographical Sketches of Committee Members

Dr. Jennie C. Hunter-Cevera (chair) is an independent consultant specializing in integrating science toward applications in energy, environment, agriculture, and medicine. She has more than 22 years of experience in the biotechnology and pharmaceutical industry. She was executive vice president of discovery and analytical sciences at RTI International. Before joining RTI, she was president of the University of Maryland Biotechnology Institute and head of the Center for Environmental Biotechnology at the Lawrence Berkeley National Laboratory. She was elected to the American Academy of Microbiology in 1995, received the Society of Industrial Microbiology (SIM) Charles Porter Award in 1996, was elected a SIM Fellow in 1997, and was named the Nath Lecturer at West Virginia University in 1999. She was the 2004 recipient of the American Society for Microbiology Porter Award for achievement in biodiversity research and was an elected fellow of the American Association for the Advancement of Science (AAAS) fellow in 2007. Dr. Hunter-Cevera holds five patents, has 15 pending patents, and currently serves on the editorial board for *International Microbiology* and the advisory board for the *International Journal of Environmental Research and Public Health*.

Dr. Sammy Boussiba received his Ph.D. in physiology and biochemistry of cyanobacteria in 1981 from the Ben-Gurion University of the Negev, Israel. After 2 years of postdoctoral studies at Cornell University, he rejoined the Microalgal Biotechnology Laboratory (MBL) at the Jacob Blaustein Institute for the Desert Research (BIDR), Ben-Gurion University, Israel and has served as the head of this group since 1995. Professor Boussiba's current research interests concern the utilization of microalgae for human health and environmental protection. Among his achievements are the unique development of the biotechnology for the production of astaxanthin-rich *Haematococcus*, and the successful expression of BTI toxin genes into nitrogen-fixing cyanobacteria for combating tropical diseases. As an outcome of this research, commercial enterprises have been set up, one of which is a plant located in the Arava, for the production of the valuable carotenoid astaxanthin produced from the green alga *Haematococcus pluvialis*, and the second-establishment of a start-up

company, BioSan, which is involved in the commercialization of engineered cyanobacteria for mosquito biocontrol of pest diseases. Professor Boussiba is the author of more than 70 publications, and has supervised about 40 research students (M.Sc., Ph.D., and postdocs). In 2003, Professor Boussiba was awarded "Doctor Honoris Causa" by the University of West Hungary. As a reward for his achievements in microalgal biotechnology, he was awarded in 2004 a Chair in Economic Botany by the Senate of the Ben-Gurion University. In 2008, he was nominated as the Director of the French Associates Institute for Agriculture and Biotechnology of Drylands at Ben-Gurion University of the Negev. He currently serves as the vice president of the International Society of Applied Phycology (ISAP) and the chief scientist of the newly established European Algal Biomass Association (EABA). All of the above has positioned Professor Boussiba among the world's leading scientists in the field of microalgal biotechnology.

Dr. Joel L. Cuello is a professor in the Department of Agricultural and Biosystems Engineering at The University of Arizona, Tucson. He has been teaching and conducting research there since 1995. His research expertise focuses on algal biodiesel and hydrogen production, algal photobioreactor design and scale up, algal CO₂ capture, algal lighting strategies, algae alternative nutrient media, wastewater use for algal production, chemical production from plant cell cultures, and design of hybrid solar and electric lighting systems for plant applications. Dr. Cuello's research studies have received funding from the National Aeronautics and Space Administration (NASA), U.S. Department of Energy (DOE), Defense Advanced Research Projects Agency (DARPA), National Science Foundation (NSF), and U.S. Department of Agriculture (USDA), among others. He has written more than 40 refereed journal publications and 9 book chapters, and has been invited numerous times as keynote or guest speaker at institutions and conferences worldwide. On the industry side, Dr. Cuello served on the scientific advisory boards of three biofuel companies in Norway, India, and Africa, and served as a technical consultant for a number of domestic and international biofuel companies. Dr. Cuello obtained his Ph.D. in agricultural and biological engineering (with minor in chemical engineering), M.S. in agricultural and biological engineering, and M.S. in plant physiology from the Pennsylvania State University. He obtained his B.S. in agricultural engineering from the University of the Philippines, and he spent his U.S. National Research Council Postdoctoral Research Associateship at NASA John F. Kennedy Space Center in Cape Canaveral, Florida, conducting research on advanced, biologically based, space life support systems using algal and cyanobacterial culture systems. Dr. Cuello is co-inventor on four patent applications on algal photobioreactor and raceway designs.

Dr. Clifford S. Duke is the director of science programs for the Ecological Society of America (ESA), which promotes the continued development of ecological science and its integration into decision making and education, since 2003. The ESA Science Office, which originated with ESA's Sustainable Biosphere Initiative in 1992, focuses on the application of ecological science to environmental problem solving. The office works with ESA members, other professional societies, and public agencies to develop workshops and publications on a variety of topics related to ecosystem sustainability, global change, and biodiversity. Current projects include a series of reports on biofuels and sustainability, data sharing and archiving initiatives, and support for ESA's Emerging Issues Conference Series. Before joining the ESA staff, Dr. Duke worked for 14 years in environmental consulting, managing preparation of environmental impact statements and ecological risk assessments for Department of Defense and Department of Energy facilities. He previously held postdoctoral positions at Northeastern University, Wellesley College, and Harvard University. He

currently serves on the steering committee of the Sustainable Rangelands Roundtable and the Service to the Scientific Community Working Group of the AAAS Science and Human Rights Coalition. Dr. Duke received his B.A. in biology and environmental studies from the University of Vermont in 1977, a Ph.D. in botany (1985) and an M.A. in public policy science from Duke University (1986).

Dr. Rebecca A. Efroymsen is a senior scientist in the Environmental Sciences Division at the Oak Ridge National Laboratory. Her work relates to the interdisciplinary, applied field of ecological risk assessment. She has performed risk assessments for contaminated burial grounds, ponds, streams, and watersheds, with an emphasis on risks to plants, soil invertebrates, and microbial processes from metals and organic chemicals. Her accomplishments include a net environmental benefit analysis framework for remediating contaminated sites. Dr. Efroymsen has investigated effects of the application of biosolids to ecosystems. She has developed conceptual ecological risk assessment frameworks for agencies such as the Department of Energy, the Environmental Protection Agency, the Department of Defense, and the Bureau of Land Management, with topics ranging from managing rare species and their habitats to petroleum exploration and production to wastewater treatment systems to wind energy development. She has worked in the fields of ecosystem restoration and ecosystem valuation. Lately, she is developing environmental sustainability indicators for biofuels and developing a causal analysis framework to support the modeling of land-use change impacts of bioenergy. Her education includes a B.A. in biology from LaSalle University, and her M.S. and Ph.D. from Cornell University in environmental toxicology.

Dr. Susan S. Golden is a distinguished professor in the Division of Biological Sciences at the University of California, San Diego. She is currently working on a project consisting of the metabolic engineering of cyanobacteria for the production of biofuels and other molecules of interest. In summary, because cyanobacteria grow photosynthetically using water and CO₂, and are easy to manipulate genetically, they are attractive organisms for the production of molecules that have industrial applications. One such application is the production of biofuels as a supplementation of, or eventual replacement of, petroleum-based fuels. The project is using the powerful genetic tools that have been developed for *Synechococcus elongatus* to explore the production of biofuels in cyanobacteria. Dr. Golden is a member of the National Academy of Sciences. She received her B.A. in biology from Mississippi University for Women and her Ph.D. in genetics from the University of Missouri, Columbia.

Dr. Jennifer Holmgren is the chief executive officer of LanzaTech. She has more than 20 years of experience in the energy sector including a proven track record in the development and commercialization of fuels and chemicals technologies. Prior to joining LanzaTech, she was vice president and general manager of the Renewable Energy and Chemicals Business Unit at UOP LLC, a Honeywell Company. In that role, she led UOP's renewable business from its inception through to the achievement of significant revenues from the commercialization of multiple novel biofuels technologies. Dr. Holmgren holds a B.Sc. from Harvey Mudd College, a Ph.D. from the University of Illinois, Urbana-Champaign, and an MBA from the University of Chicago. She currently serves on multiple external advisory boards. She is the author or co-author of 50 U.S. patents and 20 scientific publications and is the 2003 recipient of the Council for Chemical Research's (CCR) Malcolm E. Pruitt Award.

Dr. Donald L. Johnson is a retired vice president of product and process technology at Grain Processing Corporation. He has also been senior development engineer and manager

of product development groups, and director of chemicals research and development departments at A.E. Staley Manufacturing Company, now at Tate & Lyle. He was a member of the advisory council at the College of Applied Science at Miami University, and member of the Departmental Visiting Committee of the Botany Department at the University of Texas at Austin. His primary interests and expertise are in the utilization and processing of renewable resources for food, ingredients, fuels, and industrial chemicals. Dr. Johnson is a member of the National Academy of Engineering. He has a Sc.D in chemical engineering from Washington University and a B.S. in chemical engineering from the University of Illinois.

Dr. Mark E. Jones is an executive external strategy and communications fellow for Dow Chemical. Since assuming this role in September 2011, Mark assists the chief technology officer with technical assessments and development of external communications and provides technical support for Dow's Renewable Chemistries Expertise Center (RCEC). Dr. Jones joined Dow in 1990 following a graduate career studying gas-phase ion molecule chemistry, which was not an area of great industrial interest. He was introduced to catalysis during his postdoctoral studies at the Cooperative Institute for Research in Environmental Science in Boulder. He spent his early career at Dow in heterogeneous catalysis within what would become core research and development. He participated in a number of catalyst scale-ups, process improvements, and commercializations. Much of his work was in alkane activation and partial oxidation, including the production of vinyl chloride directly from ethane, ethylene from methane, and oxidative carbonylation. From 2006 to 2009, Dr. Jones was technology strategy development scientist for basic plastics and chemical/hydrocarbons and energy R&D. In this role, he was working on a variety of alternative feedstock and sustainability issues. He then spent 2 years focusing on lithium ion batteries, developing processes for the production of battery materials, prior to assuming his current role. Dr. Jones authored over 16 issued U.S. patents and numerous publications. He holds a B.S. in chemistry from Randolph-Macon College and a Ph.D. in physical chemistry from the University of Colorado, Boulder.

Dr. Val H. Smith is a professor in the Department of Ecology and Evolutionary Biology at the University of Kansas, Lawrence. His research program focuses on the relationships between resource supplies and the structure and function of biological systems. His primary area of expertise is in the area of phytoplankton ecology, and he has worked extensively on the relationships between nutrient loading and the occurrence of bloom-forming blue-green algae in lakes and estuaries worldwide. He has extensive experience in the quantitative comparative analysis of both aquatic and terrestrial ecosystems, and has strong interests in the mechanisms that generate and maintain biological diversity, in addition to the mechanisms that regulate the biogeochemical cycles of carbon, nitrogen, and phosphorus. Recently, he has expanded his research into the area of disease ecology and is involved in both empirical and experimental investigations of the relationships between host nutrition and the outcome of infectious disease in plants and animals. In addition, his team seeks to produce renewable biofuels from algae produced in wastewater-fed, outdoor bioreactors. Dr. Smith received his Ph.D. from the University of Minnesota.

Mr. Cai Steger is an Energy Policy Analyst at Natural Resources Defense Council's (NRDC) new Center for Market Innovation, focusing on federal and state policies that drive clean technology innovation, investment and deployment, with a concentration on renewable energy—especially solar and algal biofuels. His recent projects include developing a federal

deployment mechanism to encourage large-scale penetration of distributed generation, analyzing impacts of climate legislation on investment in renewables, and managing a year-long project to understand the sustainability of algal biofuels production. He joined NRDC in May 2008. He has an MBA from Columbia Business School, a B.A. from University of California, Santa Barbara, and 8 years of strategy, research, and business development experience in multiple industries.

Dr. Gregory N. Stephanopoulos is Willard Dow Professor of Biotechnology and Chemical Engineering at the Massachusetts Institute of Technology. The central focus of his research is metabolic engineering, the improvement of cellular properties using modern genetic tools, aiming at the overproduction of fuels and chemicals, and biomedical research aimed at the elucidation of key physiological differences that characterize disease states and can guide drug and therapy development. He has received numerous awards, including the American Institute of Chemical Engineers (AIChE) Wilhelm Award in Chemical Reaction Engineering (2001), Founders Award (2007), the Marvin Johnson Award of the Biotechnology Division of the American Chemical Society (2000), the E.V. Murphee Award in Industrial and Engineering Chemistry (2010), the AIChE Food, Pharmaceutical and Bioengineering Division Award (1997), the Technical Achievement Award of the AIChE Southern California section (1984), the Charles Thom Award of the Society for Industrial Microbiology (2007), the Amgen Award in Biochemical Engineering (2009), and the George Washington Cover Award of the Biotechnology Industry Organization. Dr. Stephanopoulos is a member of the National Academy of Engineering. He received his Ph.D. in chemical engineering from the University of Minnesota, Minneapolis.

Dr. Larry P. Walker is a professor in the Department of Biological and Environmental Engineering at Cornell University. He has been involved in a number of biomass to energy and chemical projects in the past 25 years. These include an assessment of New York State biomass resources available for ethanol production, farm-scale methane production and co-generation, the application of nanotechnology to characterizing and studying important biocatalysts for industrial biotechnology, and optimization of solid-state fermentation for the production of biocontrol products. He is the director of the Northeast Sun Grant Initiative, director of Cornell Biofuels Research Laboratory, a member of the National Nanobiotechnology Center Executive Committee that oversees the research activities of the center, and the coordinator of a Cornell faculty cluster that is interested in the development of sustainable bio-based industries. He is a member of the American Council on Renewable Energy, American Institute of Medical and Biological Engineering, Higher Education Committee Steering Committee, and the Kavli Institute at Cornell for Nanoscale Science. Some of Dr. Walker's extramural activities include serving as co-editor in chief for the journal *Industrial Biotechnology*, adviser for the Renewable Fuels Roadmap and Sustainable Biomass Feedstock Assessment for New York, member of the New York State Climate Action Plan Advisory Panel, and former membership on the National Biomass Research and Development Technical Advisory Committee. In addition, Dr. Walker is a recipient of a New York State Technology and Advanced Research Faculty Development Program Award for Industrial Biotechnology Research. He also received the Outstanding Alumnus Award from CANR, Michigan State University, and the Outstanding Faculty Award from Cornell College of Agriculture and the Life Sciences. He is a graduate of Michigan State University with a B.S. in physics. His interest in renewable resources and environmental research led him to complete M.S. and Ph.D. degrees at Michigan State University in agricultural engineering.

Dr. Eric Williams is an associate professor in the Golisano Institute of Sustainability at the Rochester Institute of Technology. His research interests include industrial ecology and life cycle assessment, in particular applied to analyzing information technology (IT) and energy systems. IT-related work includes life-cycle assessment of semiconductors and computers and macro-analysis on relationships between energy consumption, telecommuting, and e-commerce. In the energy domain, he is working on systems assessment of energy supply technologies, using thermodynamics-based measures to characterize long-term trends in energy efficiency, and the effects of development and urbanization on energy demand in industrializing nations. He received his Ph.D. from the State University of New York in physics and his expertise includes industrial ecology, life-cycle assessment, and macro-assessment of energy supply and demand.

Dr. Paul V. Zimba is the director of the Center for Coastal Studies at Texas A&M University, Corpus Christi. He joined Texas A&M from U.S. Department of Agriculture (USDA), Agricultural Research Service, where he served as a research microbiologist in Stoneville, Mississippi, since 1999. Dr. Zimba's work at the USDA assisted in the analysis of off-flavor metabolites and secondary products being produced by algae in aquaculture systems. Prior to that Dr. Zimba worked as a research assistant professor in the Department of Fisheries at the University of Florida. He is an adjunct at the University of Mississippi and the State University of New York and has also served as an adjunct faculty member at Loyola University of New Orleans. His research interests include aquatic ecosystem ecology, algal toxin assessment, harmful algae, wetlands, aquaculture, microalgal taxonomy and physiology, carbon fixation assessment, remote sensing, aquatic ecosystem stressors, and cyanobacteria secondary metabolites. Dr. Zimba received his B.A. in biological sciences from Virginia's Wesleyan College in 1979. He received his M.S. in biology from Old Dominion University in 1985 and his Ph.D. from Mississippi State University in 1990.

B

Statement of Task

The committee is tasked to examine the promise of sustainable development of algal biofuels, identify potential concerns and unforeseen sustainability challenges and unintended consequences for a range of approaches to algal biofuel production, explore ways to address those challenges, and suggest appropriate indicators and metrics that can inform future assessments of environmental performance and social acceptance associated with sustainability. Although economics is an important aspect of sustainability, the study will not assess costs of algal biofuels. Algal biofuel production approaches and technical systems are still emerging, and facilities have not reached commercial scale. Public data on the economics of algal biofuel production are sparse. Therefore, it is premature for the committee to conduct generalized economic analyses of algal biofuels.

The study will:

- Identify the potential sustainability concerns for commercial production (including larger centralized and smaller distributed facilities) of algal biofuels associated with a selected number of different pathways of biomass production and conversion. Potential concerns to be addressed could include the availability and use of land, water, and nutrient resources; human health and safety associated with feedstock cultivation and processing; potential toxicity associated with algal metabolites and their adverse impacts on downstream coproducts; use of genetically modified organisms; and other impacts that are of social and environmental concern.
- Identify information or data gaps related to the impacts of algal biofuel production.
- Suggest indicators and metrics to be used to assess sustainability concerns across the algal biofuel supply chain and data to be collected now to establish baseline and to assess sustainability. Identify indicators that are most critical to address or have the greatest potential for improvement through DOE intervention. This input will inform DOE EERE-OBP's broader analysis of biofuels and bioenergy sustainability.

- Using selected approaches as illustrations, discuss whether any, or combinations of, the identified challenges could present major sustainability concerns. Identify preferred cost-and-benefit analyses that could best aid in the decision-making process, and discuss whether those decisions could be performance based and technology neutral.

The committee will conduct a review of published literature on assessing environmental sustainability of algal biofuel production. If available published literature is insufficient to satisfy the study requirements, the committee will solicit information from federal and state agencies, environmental groups, companies, and other organizations involved in research and development and implementation of science and technology, systems, and processes for production of algal biofuels and feedstocks to get an idea of ongoing and planned research on related environmental sustainability. The committee will write a report addressing its statement of task and supporting its conclusions and recommendations.

C

Presentations to the Committee

MARCH 17, 2011

Sponsor Perspectives for NRC Study on Algae Biofuels Sustainability
Ron Pate, U.S. Department of Energy

A National Resource Availability Assessment for Microalgae Biofuel Production
Ron Pate, U.S. Department of Energy

Sustainable Development of Algal Biofuels Meeting
Richard Greene, U.S. Department of Energy, Office of Science

ARS Research on Algal Biomass for Environmental Remediation that Is Relevant to Algal
Biofuels
Walter Mulbry, U.S. Department of Agriculture Agricultural Research Service

Algae Biofuels and EPA
Mark Segal, U.S. Environmental Protection Agency, Office of Pollution

Algal Biomass as an Animal Feed Ingredient: Opportunities and Challenges
Terry Proescholdt, U.S. Food and Drug Administration/Center for Veterinary Medicine

Presentation to the National Academy of Sciences—Committee on Sustainable Develop-
ment of Algal Biofuels
Jim Sears and Mary Rosenthal, Algae Biomass Organization

Opportunities for Improving Environmental Quality and Enhancing Natural Resource Base
Provided by Algal Biofuels
Greg Mitchell, Scripps Institution of Oceanography

JUNE 13, 2011

Sustainable Algal Biofuels

Richard Sayre, Danforth Center and Phycal, LLC

Microalgal Strains for Fuel: Starting Points and Cultivation Specifications

Peter Lammers, New Mexico State University

Oil From Algae—Greens, Diatoms, and Dinoflagellates Accumulate High Oil Levels

Art Grossman, Carnegie Institute of Washington

Genetic Engineering and Sustainable Algal Biofuels

Gary Sayler, University of Tennessee

Strain Selection for Algal Biofuels in Open Ponds: Ecological and Evolutionary Considerations

Allison Snow, The Ohio State University

Promises to Keep?

Phil Pienkos, National Renewable Energy Laboratory

Scaling Algal Biofuels Resource and Sustainability Challenges End-User and Investor Perspective

Evan Smith, Verno Systems Incorporated

A Cyanobacteria-Based Photosynthetic Process for the Production of Ethanol

Ron Chance, Algenol Biofuels

Algal Production and Processing

Gail Busch, Algepower

Near-Term Commercialization of Algal Biofuel

Alex Aravanis, Sapphire Energy

The Algae to Fuels Value Chain

Jim Rekoske, UOP Honeywell

Life Cycle Assessment of Algae-to-Energy Technologies

Andres Clarens, University of Virginia

Life Cycle Analysis of Algae-Based Fuels with the GREET Model

Ed Frank, Argonne National Laboratory

JUNE 14, 2011

Promise and Challenges in Sustainable Development of Algal Biofuels: Questions and Answers

John Benemann, Benemann Associations

Challenges in Sustainable Development of Algal Biofuels—Looking Back and Forward

John Sheehan, University of Minnesota

Resource Constraints on Sustainable Development of Algal Biofuels

Mark Wigmosta, Pacific Northwest National Laboratory

Bioenergy: The Need for Additional Carbon

Tim Searchinger, Princeton University

Environmental Effects of Large-Scale Algal Fuel Production

Virginia Dale, Oak Ridge National Laboratory

Environmental Sustainability Concerns for Algal-Based Biofuel Production

Gary Schafran, Old Dominion University

SEPTEMBER 8, 2011

Genome-Scale Network Analysis: Illuminating Photosynthetic Metabolism in the Alga *Chlamydomonas*

Jason Papin, University of Virginia

Algal Strain Selection and Development for Biofuel Production

Paul Roessler, Synthetic Genomics

An Assessment of the Environmental Performance of Algal Biofuels

David Marler, ExxonMobil

Algae and Fish: Food, Fuel, and More

Lissa Morgenthaler-Jones, LiveFuels

End User's Perspective of Sustainability of Algal Biofuels

Michael Lakeman, Boeing Commercial Airplanes

Species Invasion and Large-Scale Algae Cultivation

Jonathan Shurin, University of California, San Diego

Offshore Membrane Enclosure for Growing Algae

Jonathan Trent, National Aeronautics and Space Administration Ames Research Center

Social Acceptability of Large-Scale Algal Biofuel Production

Robert Beach, RTI International

D

Glossary

Alcohol fuels	Fuels that are organic compounds that contain one or more hydroxyl groups (-OH) attached to one or more of the carbon atoms in a hydrocarbon chain. Common alcohol fuels include ethanol, methanol, and butanol.
Algae	A group of aquatic eukaryotic organisms that contain chlorophyll. Algae can be microscopic in size (microalgae) or observable to the eye (macroalgae).
Aliphatic alcohol	An alcohol that contains a hydrocarbon fragment derived from a fully saturated, nonaromatic hydrocarbon.
Anoxia	The absence of dissolved oxygen.
Biodiesel	Diesel fuel consisting of long-chain alkyl esters derived from biological material such as vegetable oils, animal fats, and algal oils.
Biofuel	Fuel derived from biological sources.
Biomass	Any organic matter that is available on a renewable or recurring basis, including agricultural crops and trees, wood and wood residues, plants (including aquatic plants), grasses, algae, animal residues, municipal residues, and other residue materials.
Biorefinery	A commercial-scale processing facility that successfully integrates all processes for extracting and converting biomass feedstocks into a spectrum of saleable products.
Carbon sequestration	Net transfer of atmospheric carbon dioxide into long-lived carbon pools.

Cellulose	A polymer of glucose, $(C_6H_{10}O_5)_n$, that forms cell walls of most plants.
Commercial demonstration	The National Renewable Energy Laboratory defines a commercial demonstration for biofuel refinery as a facility that has the capacity to process 700 dry tons of feedstock per day. In addition, a commercial demonstration facility is a fully integrated facility that includes all processing steps at a scale sufficient to identify potential operational problems.
Cyanobacteria	Historically known as blue-green algae, cyanobacteria are prokaryotes that contain chlorophyll.
Demonstration facility	The National Renewable Energy Laboratory defines a demonstration facility for biofuel refinery as one that has the capacity to process 70 dry tons of feedstock per day. A true demonstration facility is a fully integrated facility that includes all of the processing steps that a commercial-scale facility would have.
Drop-in fuel	Non-petroleum fuel that is compatible with existing infrastructure for petroleum-based fuels.
Green diesel	Product of hydrotreated triacylglycerols.
Hemicellulose	A matrix of polysaccharides present in almost all plant cell walls with cellulose.
Hydrocarbon fuels	Organic compounds that contains primarily carbon and hydrogen and only trace amounts of other atoms such as sulfur, nitrogen, and oxygen. Hydrocarbon fuels include petroleum-based materials such as alkanes, olefins, and aromatics.
Hypoxia	Low dissolved oxygen concentrations, generally less than 2 milligrams per liter.
Land use	Defined by anthropogenic activities, such as agriculture, forestry and urban development, that alter land-surface processes including biogeochemistry, hydrology, and biodiversity.
Lignin	A complex polymer that occurs in certain plant cell walls. Lignin binds to cellulose fibers and hardens and strengthens the cell walls of plants.
Lignocellulosic biomass	Plant biomass composed of cellulose, hemicellulose, and lignin.
Pilot demonstration	The National Renewable Energy Laboratory defines a pilot demonstration for biofuel refinery as a facility that has the capacity to process 1-10 dry tons of feedstock per day. These facilities typically do not include fully integrated processes.

E

Select Acronyms and Abbreviations

Acetyl-CoA	Acetyl-coenzyme A
B20	A blend of 20 percent biodiesel and 80 percent petroleum diesel and is the most common biodiesel blend in the United States
B100	100 percent biodiesel
CAFOs	Concentrated animal feeding operations
CO	Carbon monoxide
CO ₂	Carbon dioxide
DAF	Dissolved air flotation
DAP	diammonium phosphate
DGAT	Diacylglycerol acyltransferase
DNA	Deoxyribonucleic acid
DOE	U.S. Department of Energy
DOE-EERE	U.S. Department of Energy, Energy Efficiency and Renewable Energy
DW	Dry weight
E10	A blend of up to 10 percent ethanol and the balance petroleum-based gasoline
E15	A blend of up to 15 percent ethanol and the balance petroleum-based gasoline
E85	A blend of up to 85 percent ethanol and the balance petroleum-based gasoline. For the past several years, E85 sold in the United States has averaged about 75 percent ethanol.
EIOLCA	Economic input-output approach to life-cycle assessment
EPA	U.S. Environmental Protection Agency
EROI	Energy return on investment

EROWI	Energy return on water invested
FAME	Fatty-acid methyl ester
FFV	Flex fuel vehicle
F-T	Fischer-Tropsch
GBEP	Global Bioenergy Partnership
GHG	Greenhouse gas
GIS	Geographic information systems
GREET	Greenhouse gases, regulated emissions, and energy use in transportation
IH2	Integrated hydropyrolysis and hydroconversion
ILUC	Indirect land-use change
KOH	Potassium hydroxide
LCA	Life-cycle assessment
LUC	Land-use change
N	Nitrogen
NASA	National Aeronautic and Space Administration
NER	Net energy ratio
NEV	Net energy value
NH ₃	Ammonia
NO _x	Nitrogen oxides
NRC	National Research Council
OBP	Office of the Biomass Program
OMEGA	Offshore Membrane Enclosure for Growing Algae
P	Phosphorus
PAR	Photosynthetically active radiation
R&D	Research and development
RNA	Ribonucleic acid
RSB	Roundtable on Sustainable Biofuels
Rubisco	Ribulose-1,5-bisphosphate carboxylase oxygenase
Si	Silicon
SO ₂	sulfur dioxide
SVR	Surface to volume ratio
TAGs	Triacylglycerols
TSCA	Toxic Substances Control Act
VCSS	Vapor compression steam stripping
VOCs	Volatile organic compounds
WWTP	Wastewater treatment plant

F

Conversion Factors

Mass			
1	ounce (oz)	≡ 28.3495231	g
1	pound	≡ 0.453592	kg
1	(short) ton	≡ 0.907185	(metric) tonne
Length			
1	foot (ft)	≡ 0.3048	meter (m)
1	mile	≡ 1.609344	kilometer (km)
Area			
1	mi ²	≡ 2.589988	km ²
1	acre	≡ 0.404685642	hectare (ha)
Volume			
1	ft ³	≡ 0.028317	m ³
1	gallon	≡ 3.785412	liter (L)
1	barrel	≡ 158.987295	L
Energy			
1	British Thermal Unit (BTU)	≡ 0.001055	megajoule (MJ)
Pressure			
1	pounds per square inch (psi)	≡ 6,894.76	Pascal (Pa)
Compound units			
1	pound per acre	≡ 1.120851	kg/ha
1	ton per acre	≡ 2.241702	tonne/ha

1 ounce (oz) per gallon	≡	7.489152	g/L
1 ounce per BTU	≡	26,870.16	g/MJ
1 ft ³ /acre	≡	0.028317	m ³ /ac
1 ft ³ /ton	≡	0.031214	m ³ /Mg
1 ft ³ /BTU	≡	26,839.19	m ³ /GJ
1 BTU per gallon	≡	0.000279	MJ/L

G

Economics of Coproduct Production from Large-Scale Algal Biofuels Systems

Coproducing algal biofuels and high-value products has been suggested as a strategy to address the challenge of making algal biofuels economically viable. The strategy has proven to be contentious at several levels. Coproducts are strongly linked to the economics and life-cycle assessments (LCAs) of algal biofuel production. The economics of algal biofuel production are outside the scope of this analysis, but are a key reason for the importance of coproducts. Coproducts are proposed as a means to improve the economics of algal biofuel production. Economic benefit comes at a cost, however, and a simple analysis is presented to explain the impacts and potential concerns.

Markets tend to correlate scale and price of sale, which is the cost of production plus return on capital (Figure G-1). This is frequently overlooked as coproducts are touted as a significant source of additional revenues for an economically suspect fuel production process. The correlation is somewhat poor across different products, but for a single product, scale and price are related by a power law. This means that doubling scale reduces price more than double. The value of coproduct is unlikely to drop indefinitely with increase in scale and could have an asymptotic function. For materials intended to sell into the massive fuels market, coproduct volumes swell rapidly with the scaling of fuel production unless a wide variety of coproducts for different markets are produced.

The Davis et al. (2011) work resulted in the development of a model for the economics of algal biofuel production.¹ This model allows scenarios around coproduct value to be explored. In particular, setting up the functional form to estimate the cost for sale of biodiesel as a function of coproduct fraction was completed. Figure G-2 shows the expected power law form (Eq. G-1).

$$\frac{\$}{L_{fuel}} = 0.8546f^{-0.89} \quad (\text{Eq. G-1})$$

¹ Aden, Andy; personal communication. National Renewable Energy Laboratory (NREL) model provided September 2011.

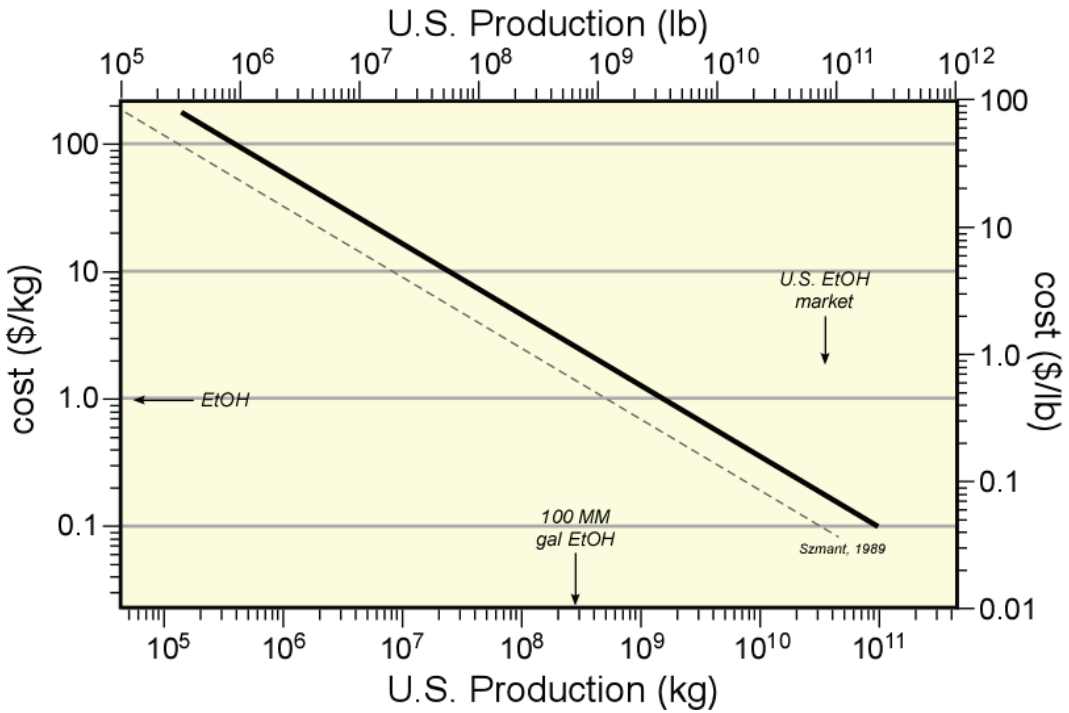


FIGURE G-1 Chart showing the general power law dependence of a materials cost with production scale.

NOTES: As scale increases, price generally decreases. This is true both for fuel components and coproducts. The dotted line shows Szmant’s original curve and the solid line is inflation corrected to 2010.

SOURCE: Adapted from Szmant (1989). Reprinted with permission from Elsevier.

Coproduct production is also a function of the lipid fraction (Eq. G-2):

$$\frac{kg_{coproduct}}{L_{fuel}} = \frac{\rho(1-f)}{f} \tag{Eq. G-2}$$

where ρ is the density of the lipid fuel and f is the lipid fraction, by weight, on a dry basis.

Extraction of the residual biomass has two major costs: one from electrical power and one from nutrient loss. Biogas is commonly quoted as having a heating value of 650 BTU per cubic foot (DOE-EERE, 2011) or approximately 20 megajoule per kilogram. Electrical energy production is assumed to be 33 percent efficient (Davis et al., 2011) and 85 percent of the potential is captured. This means that the energy potential in the residual biomass from anaerobic digestion may be approximated as (Eq. G-3):

$$\frac{kwh}{L_{fuel}} = \frac{\alpha\beta\rho(1-f)}{f} \times \frac{20MJ}{kg_{biogas}} \times \frac{1kwh}{3.6MJ} \tag{Eq. G-3}$$

where α is the efficiency of electricity generation from biomass and β is a loss term, entered as 0.85. This is a coarse approximation and neglects the effect of excess power generation for sale. It assumes that all the power produced by the anaerobic digester system needs to

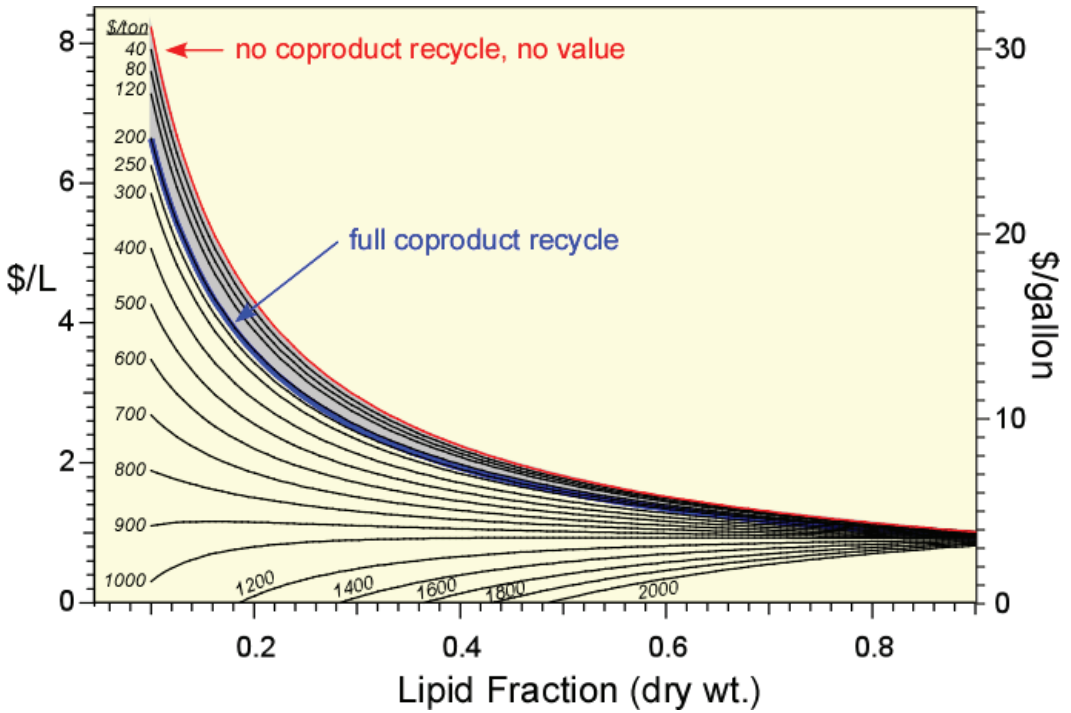


FIGURE G-2 Lipid fraction in the microalgae figures prominently in the amount of coproduct, and, therefore, the total value of the coproduct stream.

NOTES: Fuel costs are estimated using data from (Davis et al., 2011) as a function of the per pound sales price of the coproduct and the lipid fraction. The coproduct is assumed to be the total remaining biomass and the alternative value of the nutrients, and power lost through its sale is accounted for. Coproduct sales price is in dollars per tonne. Curves with a positive slope indicate that the coproduct is providing more of the total value than the fuel component. Negative slopes indicate that fuel is still the most valuable component. Potentially difficult to see, the \$200/ton line falls on top of the blue line representing the base case with all nutrients and power production.

be replaced with purchased power and all residual biomass is sold as coproduct. The approximation intends to show trends.

The nutrient requirements can be estimated based on the Redfield molar ratio for algae of 106C:16N:1P (Redfield, 1958). Fertilizer is assumed to be ammonia and diammonium phosphate (DAP). Assuming that all nitrogen (N) and phosphorus (P) remain in the residual biomass, the nitrogen content is approximately 8.6 percent by weight and the phosphorus 1.2 percent, in good agreement with estimates in detailed studies of algal fuel production (Davis et al., 2011). Therefore, the amount of diammonium phosphate (DAP) equivalent in the residual biomass can be approximated as (Eq. G-4):

$$\frac{kg_{DAP}}{L_{fuel}} = \frac{moles_P}{L_{fuel}} \times \frac{kg_{DAP}}{moles_P} = \frac{0.012kgP}{0.031kg_P / mole} \times \frac{0.132kg}{mole_{DAP}} \times \frac{\rho}{f} \quad (\text{Eq. G-4})$$

The nitrogen present in DAP has to be accounted for, yielding an approximate amount of ammonia equivalent in the residual biomass of (Eq. G-5):

$$\begin{aligned} \frac{kg_{NH_3}}{L_{fuel}} &= \left[\frac{moles_N}{L_{fuel}} - \left(\frac{2moles_N}{moles_{DAP}} \times \frac{moles_P}{L_{fuel}} \right) \right] \times \frac{0.017kg_{NH_3}}{mol_{NH_3}} \\ &= \rho \left(\frac{0.086kg_N}{0.014kg_N / mole} \times \frac{2 \times 0.012kg_P}{0.031kg_P / mole} \right) \times \frac{0.017kg_{NH_3}}{mole_{NH_3}} \end{aligned} \quad (\text{Eq. G-5})$$

What is left is a simplified approximation of the impact of coproduct sales on the price for sale of an algal biofuel. The power law relationship between the lipid fraction and the price of fuel is used as the starting economic estimate. Coproduct value reduces the price of the fuel, showing the benefit of coproduct sales. Power would have to be purchased to account for the loss of power from the anaerobic digestion, raising the price of the fuel. Fertilizers would have to be purchased to account for the loss of nutrients that are no longer being recycled to the algae cultivation.

Ammonia and DAP prices are nearly equivalent and are assumed to be identical at \$450/tonne. Power prices are selected to match published studies at \$0.081 per kilowatt hour (Davis et al., 2011).

$$\begin{aligned} \frac{\$}{L_{fuel}} &= 0.8546f^{-0.89} - \left(P_{coproduct} \times \frac{\rho(1-f)}{f} \right) + \left(P_{coproduct} \times \frac{\alpha\beta\rho(1-f)}{f} \right) \\ &\quad + \left(P_{fertilizer} \times \frac{\delta\rho}{f} \right) \end{aligned} \quad (\text{Eq. G-6})$$

where δ is 0.146 and is the collection of terms from both the nitrogen and phosphorus requirements (Eq. G-6).

Calculations can then be done to estimate the impact of coproduct sales as deviation from the economics of the reference pathway presented earlier, represented by the blue line in Figure G-2. Discontinuation of the recycle of nutrients from the algal biomass remaining after lipid extraction and elimination of power generation, as would be the case if this was put to other use without returning a revenue stream, requires that additional power and nutrients be purchased, effectively raising the price for sale of the algal biofuel. This is shown as the red line in Figure G-2 and represents an unrealistic, but instructive, case where the value in the residual biomass is not captured. The grey areas between the red and blue lines illustrate that the approximate value of the biomass is \$200 per tonne. One commonly suggested outlet for the residual biomass is as animal feedstuff. This would place its value in the \$400-500 per tonne range as a bulk feedstuff. At 30 percent lipid fraction, the impact on fuel price is on the order of \$0.40 per liter. If algal species begin to express lipids at higher levels, the benefit of the coproduct sales diminishes on a per liter basis because of the reduction in production of the non-lipid biomass.

At points where the coproduct has a value below that of fuel, the shape of the price curve largely follows the curve for no coproduct sales. However, when the coproduct value exceeds the value of fuel, there can be a dramatic drop in fuel price. The interplay of scale and reasonable price of sale, to first approximation, limit the price that the coproducts can reasonably garner. Coproducts certainly can improve the economics of fuel production only modestly because reasonable values for large-volume applications such as animal feedstuffs have established prices that are near the alternative values for the residual biomass

as a nutrient and power source. Clearly, high-value products are made using algae today with success. These products are small-volume applications where the value of the product, for example nutraceuticals, can be thousands or tens of thousands of dollars per tonne. Any fuel produced in addition to the high-value product could, indeed, be sold into the fuels market profitably. The available fuel is limited to the market accessible to the high-value product. Once the market for the high-value product is saturated, the economic benefit for fuel production decreases substantially. Coproduction of fuel and other products has limited potential and is not a solution to improving economics of widespread and large-scale deployment of algal biofuels.

REFERENCES

- Davis, R., A. Aden, and P.T. Pienkos. 2011. Techno-economic analysis of autotrophic microalgae for fuel production. *Applied Energy* 88(10):3524-3531.
- DOE-EERE. (U.S. Department of Energy, Energy Efficiency and Renewable Energy). 2011. Anaerobic digestion. Available online at http://www.eere.energy.gov/basics/renewable_energy/anaerobic_digestion.html. Accessed June 8, 2012.
- Redfield, A.C. 1958. The biological control of chemical factors in the environment. *American Scientist* 46:205-221.
- Szmant, H.H. 1989. *Organic building blocks of the chemical industry*. New York: Wiley.

