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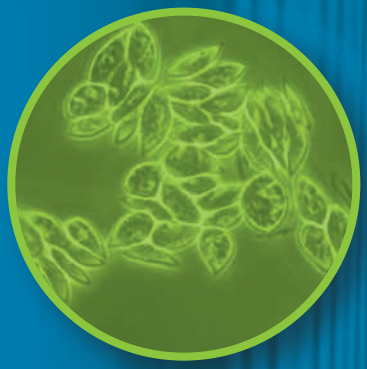

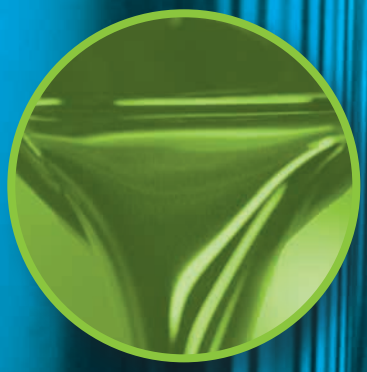

National Algal Biofuels Technology Review

Bioenergy Technologies Office

June 2016

U.S. DEPARTMENT OF ENERGY | Energy Efficiency & Renewable Energy

National Algal Biofuels Technology Review

U.S. Department of Energy Office of Energy Efficiency and Renewable Energy Bioenergy Technologies Office
June 2016

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2010 *National Algal Biofuels Technology Roadmap*:

eere.energy.gov/bioenergy/pdfs/algal_biofuels_roadmap.pdf

A complete list of roadmap and review contributors is available in the appendix.

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Preface

Thank you for your interest in the U.S. Department of Energy (DOE) Bioenergy Technologies Office's (BETO's) *National Algal Biofuels Technology Review*. This 2016 update to the 2010 *National Algal Biofuels Technology Roadmap* is a review of algal biofuels research at every step of the supply chain. It addresses several research areas highlighting advances, outlining unknowns, and discussing opportunities for advancement.

Domestic renewable energy provides potential solutions to priorities for the United States, such as decreasing dependence on foreign oil, revitalizing rural America by creating new jobs across many sectors of the economy, and reducing carbon emissions. Through strategic investments and close coordination with partners in industry, academia, national laboratories, and other agencies, DOE is committed to developing and demonstrating transformative and revolutionary bioenergy technologies for a sustainable nation.

Algae have significant potential to support an advanced biofuels industry. The goal of the BETO Advanced Algal Systems Program is to develop cost-effective algal biofuels production and logistics systems. The program focuses on supporting the growth of the emerging domestic algae industry and its interest in commercialization for fuels and products, specifically by reducing costs of production and ensuring the sustainability and availability of resources. DOE revived its investment in algal biofuels in 2009 in response to the increased urgency of lowering greenhouse gas emissions and producing affordable, reliable renewable energy, as well as the increasing recognition that we will not achieve these goals via any single technology pathway. Since then, BETO has invested in a variety of research, development, and demonstration (RD&D) projects that tackle the most impactful barriers associated with the scale-up of commercial algal biofuels. BETO is proud of the progress of our partners, and has the pleasure of highlighting many of their projects within this review, along with the work of the broader research community.

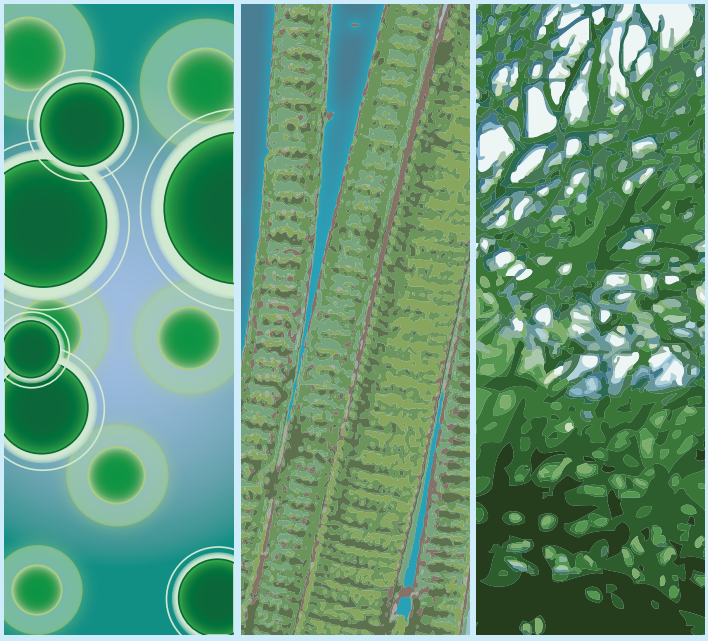
The *National Algal Biofuels Technology Review*, as a summary of algal biofuels research and development to-date, serves as one reference to inform the implementation of the BETO strategy to achieve the vision of a thriving and sustainable bioeconomy fueled by innovative technologies. This review is intended to be a resource for researchers, engineers, and decision-makers by providing a summary of algal biofuel research progress to date and the challenges that could be addressed by future RD&D activities. We hope this review fosters and informs participation from all stakeholders as the next steps are taken to advancing an algal biofuels industry together. DOE looks forward to continuing its work with diverse partners in the development of renewable energy options that provide the greatest benefits in the years to come.

Jonathan L. Male
Director, Bioenergy Technologies Office
U.S. Department of Energy

FROM ALGAE TO BIOFUELS

An Integrated Systems Approach to Renewable Energy that Is

ALGAE FEEDSTOCKS



MICROALGAE CYANOBACTERIA MACROALGAE

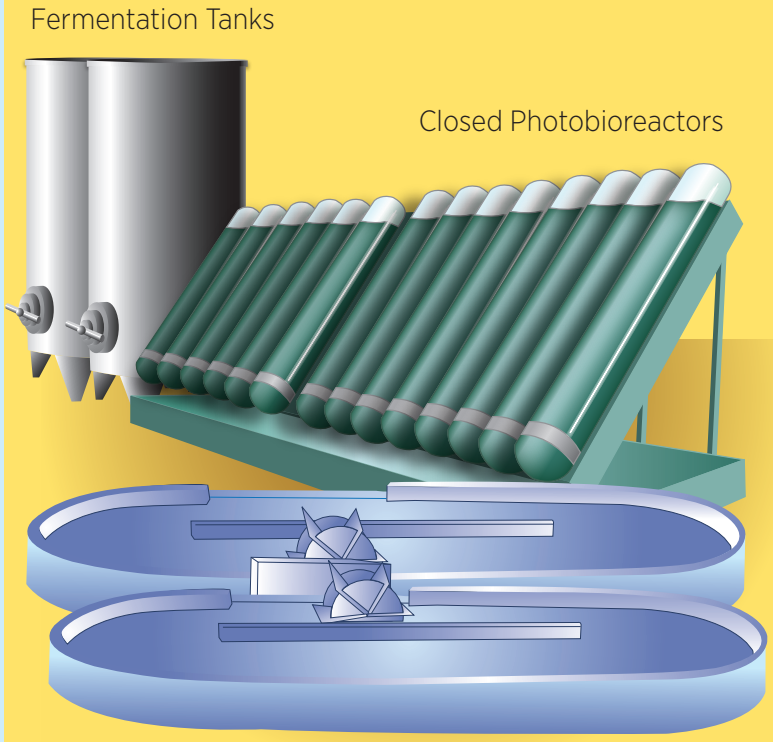
Algae as feedstocks for bioenergy refers to a diverse group of organisms that include microalgae, macroalgae (seaweed), and cyanobacteria (formerly called “blue-green algae”). Algae occur in a variety of natural aqueous and terrestial habitats ranging from freshwater, brackish waters, marine, and hyper-saline environments to soil and in symbiotic associations with other organisms.

Understanding, managing, and taking advantage of the biology of algal strains selected for use in production systems is the foundation for processing feedstocks into fuels and products.

CULTIVATION

Microalgae and cyanobacteria can be cultivated via photoautotrophic methods (where algae require light to grow and create new biomass) in open or closed ponds or via heterotrophic methods (where algae are grown without light and are fed a carbon source, such as sugars, to generate new biomass). Macroalgae (or seaweed) has different cultivation needs that typically require open off-shore or coastal facilities.

Designing an optimum cultivation system involves leveraging the biology of the algal strain used and inegrating it with the best suited downstream processing options. Choices made for the cultivation system are key to the affordability, scalability, and sustainability of algae to biofuel systems.



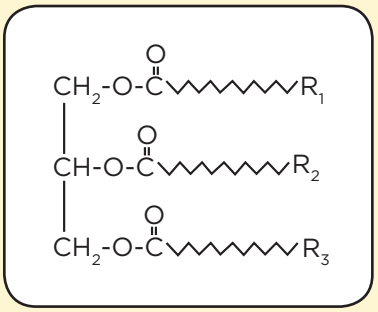
Example Cultivation Systems

Abundant, Affordable, and Sustainable

HARVESTING / DEWATERING

Some processes for the conversion of algae to liquid transportation fuels require pre-processing steps such as harvesting and dewatering. Algal cultures are mainly grown in water and can require process steps to concentrate harvested algal biomass prior to extraction and conversion. These steps can be energy-intensive and can entail siting issues.

EXTRACTION



Algal Lipid: Precursor to Biofuels

Three major components can be extracted from algal biomass: lipids (including triglycerides and fatty acids), carbohydrates, and proteins.

Most challenges in extraction are associated with the industrial scale up of integrated extraction systems. While many analytical techniques exist, optimizing extraction systems that consume less energy than contained in the algal products is a challenge due to the high energy needs associated with both handling and drying algal biomass as well as separating out desirable products. Some algal biomass production processes are investigating options to bypass extraction, though these are also subject to a number of unique scale-up challenges.

CONVERSION

Conversion to fuels and products is predicated on a basic process decision point:

1) Conversion of whole algal biomass;
2) Extraction of algal metabolites; or
3) Processing of direct algal secretions.

Conversion technology options include chemical, biochemical, and thermochemical processes, or a combination of these approaches.

The end products vary depending on the conversion technology utilized. Focusing on biofuels as the end-product poses challenges due to the high volumes and relative low values associated with bulk commodities like gasoline and diesel fuels.

End Uses:

- Biodiesel
- Renewable Hydrocarbons
- Alcohols
- Biogas
- Co-products (e.g., animal feed, fertilizers, industrial enzymes, bioplastics, and surfactants)

REGULATIONS AND STANDARDS

Contents

1. Overview of Algal Biofuels and Work from the U.S. Department of Energy

The Bioenergy Technologies Office (BETO) of the U.S. Department of Energy (DOE), Office of Energy Efficiency and Renewable Energy, is committed to advancing the vision of a viable, sustainable domestic biomass industry that produces renewable biofuels, bioproducts, and biopower; enhances U.S. energy security; reduces our dependence on fossil fuels; provides environmental benefits; and creates economic opportunities across the nation. BETO's goals are driven by various federal policies and laws, including the Energy Independence and Security Act of 2007 (EISA). To accomplish its goals, BETO has undertaken a diverse portfolio of research, development, and demonstration (RD&D) activities, in partnership with national laboratories, academia, and industry.

Algal biofuels and products offer great promise in contributing to BETO's vision, as well as helping to meet the Renewable Fuels Standard (RFS) mandate established within EISA. The RFS mandates blending of 36 billion gallons of renewable fuels by 2022, of which only 15 billion gallons can be produced from corn-based ethanol. Biofuels derived from algae can help to meet these longer-term needs of the RFS and represent a significant opportunity to impact the U.S. energy supply for transportation fuels. The state of technology for producing algal biofuels continues to mature with ongoing investment by DOE and the private sector, but additional RD&D is needed to achieve widespread deployment of affordable, scalable, and sustainable algae-based biofuels.

1.1 History of the Review

The original framework for the 2010 *National Algal Biofuels Technology Roadmap* was constructed at the Algal Biofuels Technology Roadmap Workshop, held December 9–10, 2008, at the University of Maryland, College Park. The workshop was organized by BETO (formerly known as the Biomass Program) to discuss and identify the critical challenges hindering the development of a domestic, commercial-scale algal biofuels industry. A major objective of the workshop was to gather the necessary information to produce an algal biofuels technology roadmap that both assesses the current state of technology and provides direction to BETO's RD&D efforts.

More than 200 stakeholders convened at the workshop, representing a diverse range of expertise from industry, academia, the national laboratories, government agencies, and non-governmental organizations. The workshop provided a stimulating environment to explore topics affecting the development of the algal biofuels industry. The workshop was able to capture the participants' experience and expertise during a series of technical breakout sessions that spanned critical aspects of the algal biomass supply chain and crosscutting issues. The outcomes from the workshop provided key inputs to the development of the original 2010 *National Algal Biofuels Technology Roadmap*.

Following the release of the initial draft of the roadmap, a 60-day public comment period was held to allow workshop participants to evaluate the roadmap for fidelity and incorporate new information, viewpoints, and criticisms not captured during the workshop. Every attempt was made to ensure that the roadmap development process was transparent and inclusive.

To assess progress since the publication of the 2010 roadmap, BETO hosted two strategy workshops (in November 2013 and March 2014). Stakeholders from industry, government, and academia discussed barriers and the RD&D needed to achieve affordable, scalable, and sustainable algae-based biofuels. The full proceedings of the two workshops can be found at energy.gov/eere/bioenergy/algal-biofuels-strategy-workshop.

In 2015, BETO began updating the roadmap to incorporate the output of these workshops and the progress made towards meeting the long-term needs of the RFS and the Office goals. Each chapter of the original roadmap was reviewed and revised to capture the progress made on the targets and milestones by projects within the BETO RD&D portfolio, as well as by the wider research and development (R&D) community. BETO enlisted external subject matter experts to review each chapter to ensure the state of technology is adequately represented. A list of the reviewers is included in appendix A.

The 2016 update to the 2010 *National Algal Biofuels Technology Roadmap* is a review of U.S. algal biofuels research at every step of the supply chain, and is titled the *2016 National Algal Biofuels Technology Review*. This document addresses areas of algal biofuels research in defined sections, highlighting advances, outlining unknowns, and discussing opportunities for advancement. As a summary of algal biofuels research, it serves as a reference for the development of a BETO strategy to sustainable and economical algal biofuels. It is not an outline of programmatic strategy, funding priorities, or policy recommendations. BETO programmatic strategy can be found in the *Bioenergy Technologies Office Multi-Year Program Plan* (DOE 2016a).

1.2 America's Energy Challenges

Energy independence and security has become a priority goal of the United States through increasing domestic energy production and reducing dependence on petroleum. The United States currently imports approximately 24% of total petroleum consumed domestically (EIA 2015a), and petroleum is the primary source of energy for the transportation sector. Petroleum fuels from crude oil provide approximately 92% of the total energy used for transportation, which includes gasoline, diesel, and kerosene (EIA 2015b).

In 2007, EISA set new standards for vehicle fuel economy, as well as made provisions to promote the use of renewable fuels, energy efficiency, and new energy technology research and development. The legislation established production requirements for domestic alternative fuels under the RFS that were intended to increase over time. Under EISA, the United States must produce at least 36 billion gallons of renewable transportation fuels by 2022, with 21 billion gallons of the target coming from advanced biofuels (Figure 1.1). As of 2014, 5% of the fuel used in the transportation sector came from biofuels (EIA 2015a).

The combustion of petroleum-based fuels has created serious concerns about climate change from greenhouse gas (GHG) emissions. Advanced biofuels are one of the few ways that GHG emissions from transportation can be effectively addressed in the near term. Advanced biofuels can increase domestic energy security, stimulate regional economic development, and address critical environmental issues. However, advanced biofuels face significant challenges in meeting the ambitious targets set by EISA. As required by EISA, advanced biofuels must demonstrate GHG emissions across their life cycle that are at least 50% less than GHG emissions produced by petroleum-based transportation fuels.

Many pathways are under consideration for production of biofuels and bioproducts from components of biomass. The most promising among these are routes to advanced biofuels such as high energy density, and fungible fuels for aviation and ground transport. Algal biomass may offer significant advantages that complement traditional feedstocks towards these fuels. For example, oleaginous microalgae have demonstrated potential oil yields that are significantly higher than the yields of oilseed crops (Table 1.1). Under EISA, four pathway assessments have been completed for algal biomass use for fuels (Table 1.2).

Table 1.1. Comparison of Oil Yield Feedstocks

Crop	Oil Yield (gal/acre/yr)
Soybean	48.0
Camelina	59.8
Sunflower	101.9
Jatropha	201.7
Oil palm	634.0
Algae*	1,500 (FY14) 2,500 (FY 18) 3,700 (FY20) 5,000 (FY22)

Source: Adapted from Darzins et al. (2010). *Note:* *Algae targets are set in the Bioenergy Technologies Office Multi-Year Program Plan (DOE 2016a) for intermediates.

Algal Feedstocks

The term "algae" refers to a vast range of organisms—from microscopic cyanobacteria to giant kelp. Algae are primarily aquatic organisms, and often are fast-growing and able to live in freshwater, seawater, or damp oils (DOE 2016b). Types of algae include microalgae, macroalgae (seaweeds), and cyanobacteria (also known as blue-green algae, or unicellular bacteria).

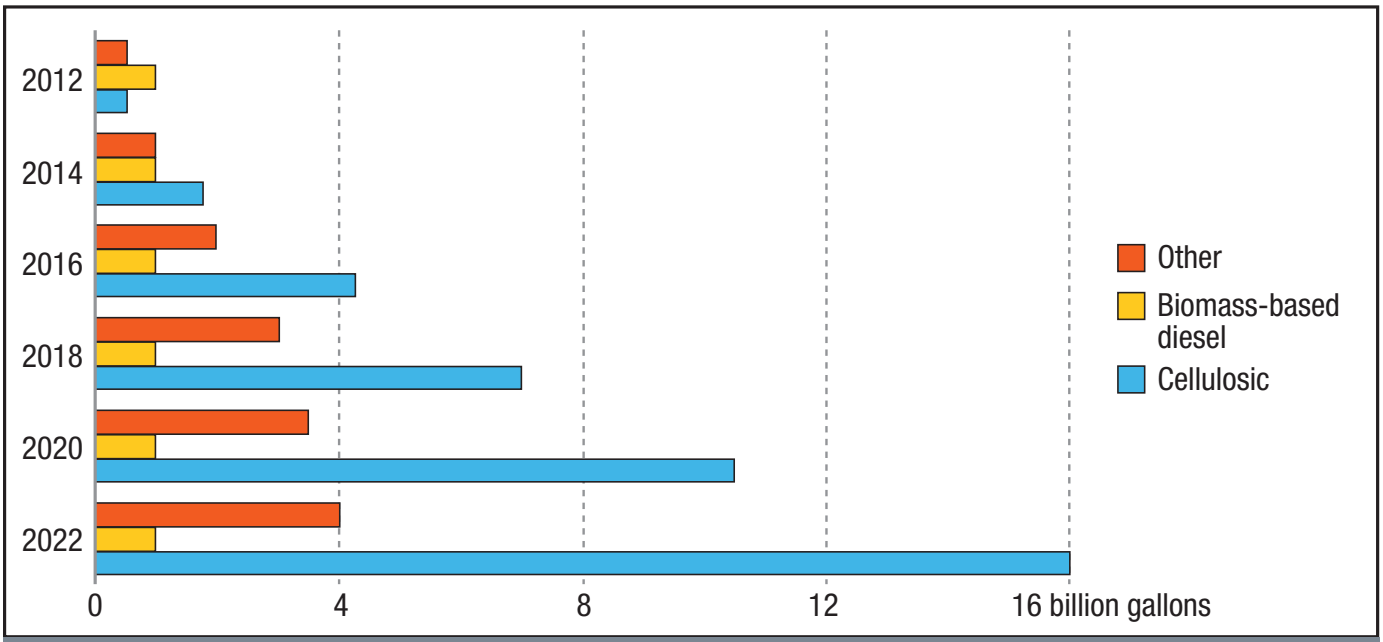


Figure 1.1. RFS2 advanced biofuel subcategory mandates (Source: Bracmort 2014)

Table 1.2. Generally Applicable Pathways under the RFS for Algal Biomass

Fuel Type	Production Requirement	Production Code	Completed Pathway Assessments
Biodiesel, renewable diesel, jet fuel and heating oil	One of the following: Trans-Esterifcation; hydrotreating; excluding processes that co-process renewable biomass and petroleum	4 (biomass-based diesel): must reduce lifecycle GHG emissions by at least 50%; compared to the diesel baseline; examples include biodiesel and renewable diesel	Viesel Fuel, LLC (2011); Endicott Biofuels, LLC (2011); Global Energy Resources (2011); Triton Energy, LLC (2010);
Biodiesel, renewable diesel, jet fuel and heating oil	One of the following: Trans-Esterifcation; hydrotreating; excluding processes that co-process renewable biomass and petroleum	5 (advanced): must reduce lifecycle GHG emissions by at least 50%; compared to the petroleum baseline; can be made from any type of renewable biomass except corn starch ethanol	Algenol Biotech LLC (2014)

Source: Data from EPA (2015a) and (2015b).

Algae are fast reproducers, requiring only a form of energy (such as sunlight or sugars), water, carbon dioxide, and a few nutrients to grow. Cultivation of algal biomass can be achieved in photoautotrophic, mixotrophic, or heterotrophic conditions. Most algae are autotrophic organisms, but the genetic diversity of the different types of algae gives researchers a wide variety of traits and characteristics that can be utilized to develop algal biofuel and bioproducts (DOE 2016a). For photoautotrophic cultivation, algae utilize light to grow and produce new biomass; heterotrophic cultivation processes grow algae without light, feeding carbon sources (sugars) as a source of energy. Mixotrophic environments provide the opportunity for algae to use light or a carbon source for growth and biomass production.

Algae can be a preferred feedstock for high energy density, fungible liquid transportation fuels. There are several aspects of algal biofuel production that have captured the interest of researchers and entrepreneurs around the world:

- Algal productivity can offer high biomass yields per acre of cultivation
- Algae cultivation strategies can minimize or avoid competition with arable land and nutrients used for conventional agriculture
- Algae can utilize wastewater, produced water, and saline water, thereby reducing competition for limited freshwater supplies
- Algae can recycle carbon from CO_2-rich flue gas emissions from stationary sources, including power plants and other industrial emitters
- Algal biomass is compatible with the integrated biorefinery vision of producing a variety of fuels and valuable co-products (Davis et al. 2012).

BETO funding opportunities and dedicated research programs are open to RD&D of microalgae, macroalgae, and cyanobacteria biomass. However, in the competitive selection process employed by the Office, microalgae and cyanobacteria have historically outperformed macroalgae systems and therefore, macroalgae technologies are not currently represented in a significant way in the BETO portfolio of work. For this reason, BETO does address macroalgae within this document, and acknowledges the potential of macroalgae systems to contribute to achieving program goals, but does not delve into the level of detail and rigor dedicated to microalgae and cyanobacteria systems. Chapters 2, 4, 8, and 10, in particular, address areas where macroalgae is unique and distinct from microalgae systems.

1.3 A History of Domestic Algal Biofuels Development

The advantages of algae as a feedstock for bioenergy have been apparent since the mid-twentieth century. Although a scalable, commercially viable system has not yet deployed at commercial scale, earlier studies have laid foundational approaches to the technologies being explored today.

Early Work to 1996

Proposals to use algae as a means of producing energy started in the late 1950s when Meier (1955) and Oswald and Golueke (1960) suggested the utilization of the carbohydrate fraction

of algal cells for the production of methane gas via anaerobic digestion. A detailed engineering analysis by Benemann et al. (1978) indicated that algal systems could produce methane gas at prices competitive with projected costs for fossil fuels. The discovery that many species of microalgae can produce large amounts of lipids as cellular oil droplets under certain growth conditions dates back to the 1940s. Various reports during the 1950s and 1960s indicated that starvation for key nutrients, such as nitrogen or silicon, could lead to this phenomenon. The concept of utilizing the lipid stores as a source of energy, however, gained serious attention only during the oil embargo of the early 1970s and the energy price surges throughout the decade; this idea ultimately became a major push of the DOE's Aquatic Species Program.

The Aquatic Species Program represents one of the most comprehensive research efforts to date on fuels from microalgae. The program lasted from 1978 until 1996 and supported research primarily at DOE's National Renewable Energy Laboratory (NREL; formerly the Solar Energy Research Institute). The Aquatic Species Program also funded research at many academic institutions through subcontracts. Approximately $25 million (Sheehan et al. 1998) was invested during the 18-year program. During the early years, the emphasis was on using algae to produce hydrogen, but the focus changed to liquid fuels (biodiesel) in the early 1980s. Advances were made through algal strain isolation and characterization, studies of algal physiology and biochemistry, genetic engineering, process development, and demonstration-scale algal mass culture. Techno-economic analyses and resource assessments were also important aspects of the program. In 1998, a comprehensive overview of the program was completed (Sheehan et al. 1998). Some of the highlights are described briefly:

The Aquatic Species Program researchers collected more than 3,000 strains of microalgae over a seven-year period from various sites in the western, northwestern, and southeastern United States, representing a diversity of aquatic environments and water types. Many of the strains were isolated from shallow, inland saline habitats that typically undergo substantial swings in temperature and salinity. The isolates were screened for their tolerance to variations in salinity, pH, and temperature, and also for their ability to produce neutral lipids. The collection was narrowed to the 300 most promising strains, primarily green algae (Chlorophyceae) and diatoms (Bacillariophyceae).

After promising microalgae were identified, further studies examined the ability of many strains to induce lipid accumulation under conditions of nutrient stress. Although nutrient deficiency actually reduces the overall rate of oil production in a culture (because of the concomitant decrease in the cell growth rate), studying this response led to valuable insights into the mechanisms of lipid biosynthesis. Under inducing unfavorable environmental or stress conditions, some species were shown to accumulate 20%–50% of their dry cell weight in the form of lipid, primarily triaglycerides (TAGs) (Hu et al. 2008).

Cyclotella cryptica, an oleaginous diatom, was the focus of many of the biochemical studies. In this species, growth under conditions of insufficient silicon (a component of the cell wall) is a trigger for increased oil production. A key enzyme is acetyl-CoA carboxylase (ACCase), which catalyzes the first step in the biosynthesis of fatty acids used for TAG synthesis. ACCase activity was found to increase under the nutrient stress conditions (Roessler 1988), suggesting that it may play a role as a "spigot" controlling lipid synthesis, and thus, the enzyme was extensively characterized (Roessler 1990). With the advent of the first successful transformation of microalgae (Dunahay et al. 1995), it became possible to manipulate the expression of ACCase in an attempt to increase oil yields. These initial attempts at metabolic engineering identified a pathway to modify the gene encoding in the ACCase enzyme; however, no effect was seen on lipid production in these preliminary experiments (Jarvis and Roessler 1999; Sheehan et al. 1998).

Additional studies focused on storage carbohydrate production, a biosynthesis of these compounds competes for fixed carbon units that might otherwise be used for lipid formation. For example, enzymes involved in the biosynthesis of the storage carbohydrate, chysolaminarin, in *C. cryptica* were characterized (Roessler 1987, 1988) with the hope of eventually turning down the flow of carbon through these pathways. The termination of the Aquatic Species Program in 1996 halted further development of these potentially promising paths to commercially viable strains for oil production.

During the course of the Aquatic Species Program research, it became clear that novel solutions would be needed for biological productivity and various problematic process steps. Cost-effective methods of harvesting and dewatering algal biomass and lipid extraction, purification, and conversion to fuel are critical to successful commercialization of the technology. Harvesting is a process step that is highly energy and capital intensive. Among various techniques, harvesting via flocculation was deemed particularly encouraging (Sheehan et al. 1998).

Extraction of oil droplets from the cells and purification of the oil are also cost-intensive steps. The Aquatic Species Program focused on solvent systems, but failed to fully address the scale, cost, and environmental issues associated with such methods. Conversion of algal oils to ethyl- or methyl-esters (biodiesel) was successfully demonstrated in the Aquatic Species Program and shown to be on the less challenging aspects of the technology. In addition, other biofuel process options (e.g., conversion of lipids to gasoline) were evaluated (Milne et al. 1990), but no further fuel characterization, scale-up, or engine testing was carried out.

Under Aquatic Species Program subcontracts, outdoor microalgal cultivation was conducted in California, Hawaii, and New Mexico (Sheehan et al. 1998). Of particular note was the Outdoor Test Facility in Roswell, New Mexico, operated by Microbial Products, Inc. (Weissman et al. 1989). This facility utilized two 1,000 m^2 outdoor, shallow (10–20 cm deep), paddlewheel-mixed raceway ponds, plus several smaller ponds for inoculum production. The raceway design was based on the "high rate pond" system developed at University of California, Berkeley. The systems were successful in that long-term, stable production of algal biomass was demonstrated, and efficiency of CO_2 utilization (bubbled through the algae culture) was shown to be more than 90% with careful pH control. Low nighttime and winter temperatures limited productivity in the Roswell area, but overall biomass productivity averaged around 10 g/m^2/day with occasional periods approaching 50 g/m^2/day. One serious problem encountered was that the desired starting strain was often outgrown by faster reproducing, but lower oil producing, strains from the wild.

Several resource assessments were conducted under the Aquatic Species Program. Studies focused on suitable land, saline water, and CO_2 resources (power plants), primarily in desert regions of the Southwest (Maxwell et al. 1985). Sufficient resources were identified for the production of many billions of gallons of fuel, suggesting that the technology could have the potential to have a significant impact on U.S. petroleum consumption. However, the costs of these resources can vary widely depending upon such factors as land leveling requirements, depth of aquifers, distance from CO_2 point sources, and other issues. Detailed techno-economic analyses underlined the necessity for very low-cost culture systems, such as unlined open ponds (Benemann and Oswald 1996). In addition, biological productivity was shown to have the single largest influence on fuel cost. Different cost analyses led to differing conclusions on fuel cost, but even with optimistic assumptions about CO_2 credits and productivity improvements, estimated costs for extracted algal oil were determined to range from $59–$186 per barrel (Sheehan et al. 1998). It was concluded that algal biofuels would not be cost-competitive with petroleum, which was trading at less than $20 per barrel in 1995.

Overall, the Aquatic Species Program was successful in demonstrating the feasibility of algal culture as a source of oil and resulted in important advances in the technology. However, it also became clear that significant barriers would need to be overcome in order to achieve an economically feasible process. In particular, the work highlighted the need to understand and optimize the biological mechanisms of algal lipid accumulation and to find creative, cost-effective solutions for the culture and process engineering challenges. Detailed results from the Aquatic Species Program research investment are available to the public in more than 100 electronic documents on the NREL website at nrel.gov/publications.

From 1968–1990, DOE also sponsored the Marine Biomass Program, a research initiative to determine the technical and economic feasibility of macroalgae cultivation and conversion to fuels, particularly to substitute natural gas via anaerobic digestion (Bird and Benson 1987). Primary efforts were focused on open ocean culture of California kelp. Similar to the findings of the Aquatic Species Program, researchers concluded that algal-derived substitute natural gas would not be cost-competitive with fossil fuel gas.

Table 1.3. Description of Some Federal Funding Initiatives for Algal Biofuels Research by U.S. Government Agencies/Organizations

Agency/Organization	Description of Funded Project
Defense Advanced Research Projects Agency	Funded $69 million in 2009 for the development of drop-in JP-8 jet fuel surrogate from algal and terrestrial feedstocks.
Air Force Office of Scientific Research	Partnered with NREL for Workshop on Algal Oil for Jet Fuel Production in 2008. Development of an algal bio-jet fuel program.
DOE Small Business Research	Awarded grant to Community Fuels on *Efficient Processing of Algal Bio-Oils for Biodiesel Production* in 2007.
DOE Advanced Research Projects Agency-Energy	Has awarded more than $25 million on research to convert macro- and microalgae into biofuels.
DOE Office of Science	Center for Advanced Biofuel Systems

Source: Data from Bracmort (2014).

Research from 1996 to 2008

Since the end of DOE's Aquatic Species Program in 1996, federal funding for algal biofuels research has come from DOE, the U.S. Department of Defense, the National Science Foundation, and the U.S. Department of Agriculture. Other initiatives, such as a major Defense Advanced Research Projects Agency solicitation, the Air Force Office of Scientific Research algal bio-jet program, and several DOE Small Business Innovation Research request for proposals, suggest that there has been increasing interest in algal biofuels and products. Additionally, DOE's Advanced Research Projects Agency-Energy, Office of Science, Office of Fossil Energy, and BETO have all funded research activities that include investigating macro- and microalgae, and cyanobacteria for biofuels and beneficial re-use of CO_2.

Many U.S. national laboratories also focused on algal biofuels and bioproducts research during this time. State funding programs and research support from private industry made up a significant proportion of research funding. Private investment in algal biofuels and products has been increasing at a dramatic rate over the last decade, significantly outpacing government funding.

In 2008, BETO (formerly known as the Office of Biomass Program) initiated the Advanced Biofuels Initiative, with algae considered as one of the primary research pathways. BETO held the National Algal Biofuels Technology Roadmap Stakeholder Workshop at the end of 2008 to discuss and identify the critical challenges currently hindering the development of a domestic, commercial-scale algal biofuels industry. The meeting resulted in the publishing of the roadmap in 2010, effectively kicking off the BETO Algae Program, also now known as the BETO Advanced Algal Systems Program.

Algae Program Research Consortia (2009–2014)

Since the 1980s, the United States has increasingly invoked public-private partnerships not only for large-scale infrastructure projects, but also for research and technology developments of national interest (Stiglitz and Wallsten 1999). Indeed, analyses of various federal agencies and government programs aimed at public-private partnerships are documented (Audretsch et al. 2002; Link et al. 2002), including specific studies on the impacts of DOE programs on the clean energy sector (Brown 2001; Brown et al. 2001; Gallagher et al. 2006). While benefiting both private and public entities from shared investment toward mutual objectives, public-private partnerships have the potential to accelerate commercialization of algal biofuel and products technology, leading to rapid industry growth and a stable market.

Since the kick-off of the Algae Program, public-private consortiums have been an integral part of the RD&D process. After publishing the original roadmap in 2010, the Algae Program selected four multidisciplinary research consortia through the Algal Biofuels Consortia Initiative, funded through the American Recovery and Reinvestment Act of 2009, to address the research needs identified in the roadmap across the algal biofuels supply chain. The four consortia included the National Alliance for Advanced Biofuels and Bioproducts (NAABB), the Sustainable Algal Biofuels Consortium (SABC), the Consortium for Algal Biofuels Commercialization (CAB-Comm), and the Cornell Consortium.

National Alliance for Advanced Biofuels and Bioproducts

The NAABB consortium was a three-year (2010–2013), $48.6 million project that brought together 39 institutions (as shown in Figure 1.2) to address many of the barriers specifically identified in the original roadmap. Led by the Donald Danforth Plant Science Center, NAABB focused on three main focus areas: feedstock supply (strain development and cultivation), feedstock logistics (harvesting and extraction), and conversion/production (accumulation of intermediates and synthesis of fuels and co-products) (NAABB 2014).

Specific outcomes range from basic advances in algal biology—such as the genetic sequencing of production strains, development of a new open pond cultivation system, and demonstration of the use of low-energy harvesting technology—to the development of hydrothermal liquefaction (HTL) as a conversion pathway for algae. The consortium successes include more than 100 scientific publications, 33 intellectual property disclosures, 2 new companies, 2,200 isolates screened and deposition of 30 highly productive strains into the UTEX Culture Collection of Algae at the University of Texas, and new outreach tools for the algal community (the journal *Algal Research* and the International Conference on Algal Biomass, Biofuels, and Bioproducts conference series) (NAABB 2014). Analysis completed showed that the combined innovations from the NAABB project can reduce the cost of algal biofuel to $7.50 gallons of gasoline equivalent (GGE) (NAABB 2014). The work of NAABB consortium has become the standard baseline for a large amount research currently being conducted in the algal biofuel and products field.

Cornell Marine Algal Biofuels Consortium

The Cornell Marine Algal Biofuels Consortium was a 5-year, $9 million dollar project led by Cornell University and Cellana, Inc. that focused on large-scale production of marine microalgae for fuel and products. Domestic partners included the University of Southern Mississippi, San Francisco State University, and the University of Hawaii, with international collaboration with Norland University, GIFAS, and the Sahara Forest Project. This consortium utilized the large-scale production facility operated by Cellana in Kona, Hawaii, to develop integrated design cases for the production of high-value products alongside advanced biofuel production. Highlighted technical accomplishments include the development of two new novel strains for large-scale production, an improved

Figure 1.2. NAABB consortium partner institutions (Source: Olivares 2015)

operating capacity of 350 days per year, demonstration of the economic feasibility of delivering a fuel price of $2.76–8.96 GGE, and demonstration of a sustained production of >3,800 gal/acre/yr algal oil for two strains. With the projected production yields, the Cornell Consortium exceeded the BETO Multi-Year Program Plan (MYPP) targets for algal oil productivity for 2014.

Consortium for Algal Biofuels Commercialization (CAB-Comm)

The Consortium for Algal Biofuels Commercialization (CAB-Comm) was a 4-year (2011–2015), $11 million project led by the University of California, San Diego, partnering with the University of Nebraska, Lincoln; Rutgers University; the University of California, Davis; Scripps Institution of Oceanography; Sapphire Energy; and Life Technologies. The objectives of the consortia were three-fold: crop protection, improved nutrient utilization and recycling, and improved genetic tools. The outcomes of the project include increase in biomass productivity, the creation of advanced biotechnology tools, and the commercialization of co-products with industrial partners. For example, research from the CAB-Comm project developed a number of genetic tools for green algae, cyanobacteria, and diatoms that are now available for public purchase through Life Technologies. Another important breakthrough of the project was the approval received from the U.S. Environmental Protection Agency (EPA) on the TSCA Environmental Release Application for outdoor testing of genetically modified species of algae. Overall, the consortium produced more than 82 publications, 13 patents, and 26 disclosures.

Sustainable Algal Biofuels Consortium (SABC)

The Sustainable Algal Biofuels Consortium was a 2-year (2010–2012), $6 million Arizona State University-led consortium of nine institutions that focused on the biochemical conversion of algae to fuel products. Partners in the Consortium included the NREL, Sandia National Laboratories, SRS Energy, Lyondell Basell, Georgia Institute of Technology, Colorado School of Mines, Novozymes, and Colorado Collaboratory. Objectives of the consortia included the development of a feedstock matrix of algal biomass based on species and growth/process conditions; determination and characterization of the biochemical composition of selected strains; exploration of multiple biochemical routes to hydrolyze and convert untreated or pretreated whole algal biomass,

oil extracts, and algal residues; and determination of the acceptability of algal biofuels as replacements for petroleum-based fuels. A key outcome was the development of a novel approach to the fractionation of the algae into simultaneous carbohydrate- and lipid-derived fuels after acid pretreatment of the biomass, and converting each fraction to high-value fuel products.

Integrated Biorefineries

In 2010, BETO funded three integrated biorefineries that focused on algal cultivation and processing, spending approximately $97 million from the Recovery Act.

Solazyme, Inc.

Solazyme Inc. was awarded $22 million from DOE for an integrated pilot project in Riverside, Pennsylvania, involving heterotrophic algae that can convert cellulosic sugars to diesel fuel. The plant has a capacity to take 13 metric tons of dry lignocellulosic feedstocks, including switchgrass, corn stover, wheat straw, and municipal green waste, and transform it through an industrial fermentation process into biodiesel and renewable diesel from purified algal oil. The biofuels produced by the project aimed to reduce life-cycle greenhouse gas emissions by 90%, with a capacity of producing 300 KGY of purified algal oil.

Starting in 2014, Solayzme commenced operations of two facilities in Iowa: the Archer Daniels Midland Company's facility and the downstream processing American Natural Products facility (Solazyme 2014a). The facilities focus on the production of oil products, including lubricants, metalworking, and home and personal care products. Solazyme has also constructed and subsequently operates a renewable oils plant in Brazil, as part of a joint venture with Bunge Global Innovation LLC. Since the awarding of funds, the company has also established partnerships with Mitsui & Co. Ltd. and Versalis with joint development agreements with AkzoNobel, Bunge Limited, Flotek Industries, and Unilever (Solazyme 2014b, 2015). Additionally, Solayzme has commercial supply agreements with Unilever, Goulston Technologies Inc., and Koda Distribution Group. In 2016, Solazyme changed the name of the company to TerraVia, and plans to focus on food, nutrition, and other specialty products; all industrial market products created by Solazyme are now managed by Solazyme Industrials (Solazyme 2016).

Sapphire Energy Inc.

Sapphire Energy Inc. was awarded $50 million from DOE for a demonstration-scale project involving the construction and operation of a 300-acre algae farm and conversion facility in Columbus, New Mexico, for the production of renewable bio-crude (jet fuel and diesel fuel). The target capacity of the plant was 1 million gallons per year of finished product, or 100 barrels of green crude oil per day. The biofuels produced aim to have a 60%–70% reduction of GHG versus traditional fossil fuels. Collaborators on this project included the Linde Group, Earthrise, the Harris Group, AMEC/Geomatrix, Brown and Caldwell, Sandia National Laboratory, and New Mexico State University.

Since 2010, Sapphire Energy has initiated the operation of the 300-acre farm in Columbus, as well as establishing partnerships with Monsanto Company (2011), Earthrise Nutritionals, LLC (2012), Institute for Systems Biology (2012), Linde Group (2013), and Tesoro Refining and Marketing Company (2013). In 2013, Sapphire Energy established a joint development agreement with Phillips 66 to collectively analyze data from the co-processing of algae and conventional crude oil into fuels, or "Green Crude" (Phillips 66 2013). Subsequently, the Green Crude has been upgraded into a diesel fuel that is ASTM 975 compliant.

Algenol Biotech LLC.

Algenol Biotech LLC. of Fort Meyers, Florida, was awarded $25 million from DOE for an integrated pilot project involving photosynthesis-driven conversion of solar energy to ethanol and the delivery of a photobioreactor system that can be scaled for commercial purposes. The project utilizes a hybrid cyanobacteria species to directly secrete ethanol within a closed bioreactor. The target capacity of the plant was to produce more than 100,000 gallons of ethanol per year, with a targeted GHG reduction of 80% versus conventional gasoline. Collaborative partners include NREL, Membrane Technology & Research, Inc., the Georgia Institute of Technology, and the University of Colorado.

Since the awarding of funds, Algenol has constructed an integrated biorefinery project on 36 acres in Fort Meyers, Florida, with thousands of photobioreactors on two "wetted" acres with the goal to produce 100,000 gallons of ethanol per year at full scale. In 2014, the Algenol Direct to Ethanol pathway received approval from the EPA as an advanced biofuels pathway, meeting the greenhouse gas emissions reduction requirement with a 69% reduction when compared to conventional gasoline (Algenol 2015).

Research Since 2012

In August 2012, the Advanced Algal Systems Program initiated research to address water and nutrient supply concerns via the Advancements in Sustainable Algal Production funding opportunity announcement (FOA). Selected projects supported the research and development of integrated algae cultivation and water and nutrient recycling technologies for algal biomass production, as well as demonstrated minimal water and external nutrient inputs and the use of wastewater and nutrients. Three projects were selected for up to $6.3 million over 3 years: California Polytechnic State University, Sandia National Laboratories, and University of Toledo.

The FOA included a second topic area, focused on developing long-term, synchronized cultivation trials and user-facilities

across the country to help scale lab work to production environments, reducing risk to start-up companies and smaller entities. The two consortia selected to fulfill this task are the Algae Testbed Public-Private Partnership (ATP3) and Regional Algal Feedstock Testbed Partnership (RAFT).

Algae Testbed Public-Private Partnership

ATP^3 is a 5 year (2012–2015), $15 million dollar partnership led by the Arizona Center for Algae Technology and Innovation at Arizona State University. The objectives of the partnership are to establish collaborative open testbeds that increase stakeholder access to outside testing facilities, as well as collect and publish high-impact data from long-term algal cultivation trials for analyses. The overall output will be to make high-impact data on algal cultivation and composition in relation to geographical and meteorological parameters openly available. Partners include NREL, Sandia National Laboratories, Cellana, California Polytechnic University (Cal Poly), Georgia Institute of Technology, the University of Texas, Florida Algae LLC, Commercial Algae Management, Valicor Renewables, and Open Algae.

There are five testbed sites throughout the United States that are incorporated in the ATP^3 Project (Cellana, Cal Poly, Georgia Institute of Technology, and Florida Algae), as shown in Figure 1.3. Education and training is a key component of the project, with weeklong educational workshops available for the public to receive training to lecture modules on algal cultivation and production, as well as hands-on field site and laboratory activities. Overall, the project has hosted 30 customers at the testbed facilities since its start in 2012, with steadily increasing project costs and total testbed revenue expected to be more than $250,000 for 2015.

Regional Algal Feedstock Testbed

The RAFT project is a 4-year (2013–2017), $5 million project led by University of Arizona with the goal to create long-term cultivation data necessary to understand and promote algae biomass production. Partners on the RAFT project include New Mexico State University, Pacific Northwest Laboratory (PNNL), and Texas A&M Agrilife Research. RAFT's objectives include obtaining long-term algal cultivation data in outdoor pond systems, improving and refining cultivation and techno-economic models, and increasing the sustainability of algae biomass production. Four testbeds in Texas, New Mexico, Washington, and Arizona are used to model long-term cultivation of multiple algae strains. The New Mexico State University algal testbed facility includes enclosed paddlewheel PBR's, a 4,000-L Solix PBR system, multiple open raceways (7,500–30,000 L) and greenhouses. Additionally the testbed includes extensive laboratory analytic capabilities for measurement of physiological algal parameters (e.g., high-resolution measures of algal photosynthetic rate, flow cytometry, PAM fluorescence) and extensive chemical analysis capability for complex fuel precursor mixtures and algal omics applications.

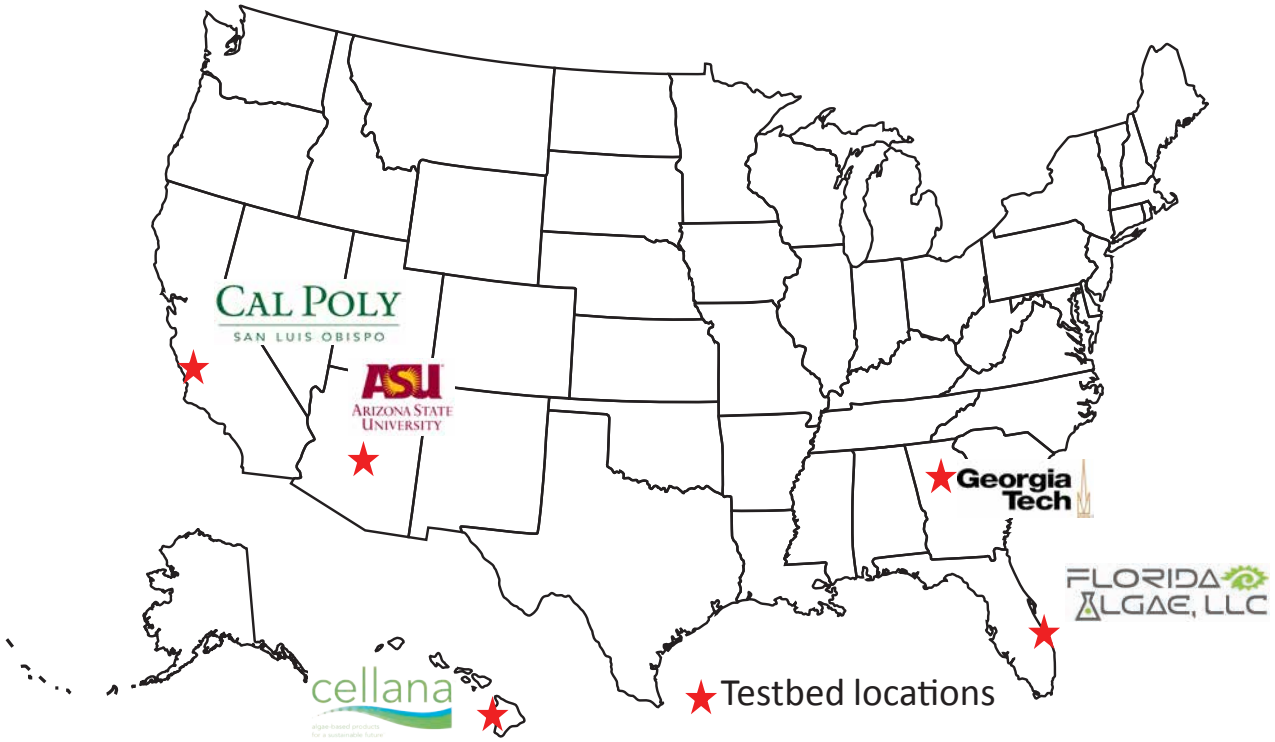


Figure 1.3. Algae Testbed Public-Private Partnership testbed locations (Source: Dirks 2015)

Up to 2015, the project has established a data management system and defined a system for monitoring growth, productivity, nutrients, and culture health for the testbeds.

Advancements in Algal Biomass Yield

In 2013, the Advanced Algal Systems Program supported the selection and award of five algae projects intended to expedite improvements in algal biomass yield for fuels through increased productivity and semi-integrated processes through the Advancements in Algal Biomass Yield (ABY) Phase 1 FOA. The goal of ABY Phase 1 is to demonstrate the potential for a biofuel intermediate yield of 2,500 gallons per acre, annual average, by 2018, though the advancement of integrated R&D on algal biology and downstream processing. Project partners funded under the ABY Phase 1 FOA include Hawaii Bioenergy ($5 million), Sapphire Energy ($5 million), Arizona State University (previously awarded to New Mexico State University) ($5 million), California Polytechnic University ($1.5 million), and Cellana, LLC ($3.5 million).

Innovative Pilot

Also in 2013, BETO's Demonstration and Market Transformation Program funded BioProcess Algae, LLC ($6.4 million), through the Innovative Pilot (iPilot) FOA to grow low-cost algae using renewable carbon dioxide, lignocellulosic sugars, and waste heat provided by a co-located ethanol plant in Shenandoah, Iowa. The BioProcess Algae goal is to produce hydrocarbon fuels meeting military specifications by integrating low-cost autotrophic algal production, accelerated lipid production, and lipid conversion. While the primary product from the proposed biorefinery will be military fuels, the facility will also co-produce additional products, including other hydrocarbons, glycerine, and animal feed.

Targeted Algal Bioproducts and Biofuels

The 2014–2015 Targeted Algal Bioproducts and Biofuels (TABB) FOA selected projects that seek to improve the value proposition for algal biofuels by employing multi-disciplinary consortia to produce algae bioproduct precursors (alongside fuel components), as well as single-investigator or small-team technology development projects focused on crop protection and CO_2 utilization technologies for improving biomass productivity. Projects funded in the TABB portfolio include two consortiums: Producing Algae and Co-Products for Energy (PACE), led by the Colorado School of Mines; and the Marine Algae Industrialization Consortium (MAGIC), led by Duke University. Four additional project partners funded through the FOA include Global Algae Innovations, Inc., Arizona State University, University of California, San Diego, and Lawrence Livermore National Laboratory.

National Laboratory Annual Operating Plans

In addition to these competitively awarded projects, BETO annually dedicates between $7 and $10 million (total) to national laboratory partners supporting a targeted portfolio of applied R&D across the algal biofuels supply chain. This core R&D portfolio focuses on advanced biology and feedstock production, conversion interfaces, and analyses of techno-economics and sustainability. For example, Pacific Northwest National Laboratory has a focus on advanced HTL technologies development and testing at laboratory and engineering scale. Los Alamos National Laboratory focuses on pursuing improved productivity and robustness via strain selection, genetic engineering, and integrated omics. NREL conducts work on techno-economic analyses of cultivation options, compositional analysis, and evaluation of high-value co-product options in the algal lipid upgrading process. Sandia National Laboratories works to demonstrate high and resilient biomass productivity through benthic algae turf assemblages.

1.4 Algae-to-Biofuels and Products: Opportunity and Challenges Ahead

Abundant, affordable, and sustainable feedstocks are essential to the burgeoning biofuels industry. Algae can play a significant role in providing biomass in areas not suitable to traditional agriculture or where unique resource utilization supports a mix of feedstocks. In contrast to the development of cellulosic biofuels, which benefit from direct agricultural and process engineering lineages, there are no parallel established foundations for cultivating algae at a similar scale. Therefore, strategic investments are required to support algal biofuels commercialization activities.

There is still a great deal of RD&D required to reduce the level of risk and uncertainty associated with the commercialization of the algae-to-biofuels process. By reviewing the progress made in developing algal biofuels and products and the current technology gaps and crosscutting needs, this document provides a review of the current state of technology and identifies where continued focus is needed to make the greatest impact in this industry.

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2. Algal Biomass, Genetics, and Development

The term "algae" commonly refers to a diverse mix of organisms from different kingdoms of life. Traditionally, algae have been unified based on the absence of vascular tissues and their ability to carry out photosynthesis and live in aquatic habitats. Algae can be single-celled or filamentous bacteria, or they can be single or multicellular eukaryotes. Although they typically live in aquatic environments and are capable of photosynthesis, this is not always the case. Types of algae include microalgae, macroalgae (seaweeds), and cyanobacteria (historically known as blue-green algae). Due to their diverse characteristics, the type and strain of algae cultivated will ultimately affect every step of the algal biofuels supply chain.

This chapter of the *Algae Review* includes a great deal of research that has been performed since the publication of the roadmap in 2010. Where applicable, updates and new information will be highlighted to demonstrate the progress that has been made and whether the new data impacts any of the R&D challenges for the industry.

2.1 Strain Isolation, Screening, and Selection

Isolation and Characterization of Naturally Occurring Algae

The goals of algae isolation and screening efforts are to identify and maintain promising algal specimens for cultivation and strain development. The Aquatic Species Program (ASP), from 1980–1996, focused its algal biology efforts on algae that could produce natural oils and grow under severe environmental conditions. The best performing candidates were found in two classes, the Chlorophyceae (green algae) and the Bacillariophyceae (diatoms). The ASP bioprospecting efforts resulted in a large culture collection containing more than 3,000 strains of organisms. After screening, isolation, and characterization efforts, the collection was winnowed down to around 300 species, housed at the University of Hawaii. The current status of this culture collection has been reported as mostly lost due to lack of support for ongoing preservation efforts.

Since 2010, a large number of bioprospecting studies for oleaginous algae have been completed, adding to the sum total of around 44,000 algae described (Guiry 2012; De Clerck et al. 2013) with many others remaining undocumented. From 2010–2013, NAABB had an aggressive bioprospecting component in which 2,000 independent algal isolates were collected across the United States, with identification of more than 60 strains that outperformed existing benchmark production algal strains. Like the ASP, NAABB strain prospecting and screening also found Chlorophyceae to be the most productive strains, with ash content low enough for downstream processing. Thirty of the best-performing strains were deposited within the UTEX Culture Collection of Algae (Neofotis et al. 2016). Under NAABB, a protocol for the rapid screening of new strains for biomass accumulation and lipid production was developed (NAABB 2014; Neofotis et al. 2016). Three well-performing strains were selected from the NAABB bioprospecting efforts and examined in cultivation trials: *Nannochloropsis salina*, *Auxenochlorella protothecoides*, and the top performer, *Chlorella sorokiniana* DOE1412. Similarly, in a screen of 600 marine microalgae, the two highest lipid producers were found to be *Nannochloropsis oceanica* CCAP 849/10 and a marine *Chlorella vulgaris* CCAP 211/21A strain (Slocombe et al. 2015).

Natural Habitats

Algae can be isolated from a variety of natural aqueous habitats ranging from freshwater to brackish water, marine and hyper-saline environments, and soil (Round, 1984). As in the case with the bioprospecting efforts led by NAABB, there are several guiding principles for large-scale sampling efforts (NAABB 2014). These include coordination to ensure the broadest coverage of environments while avoiding duplication of efforts and selection of specific locations by site selection criteria using dynamic maps, geographic information system (GIS) data and/or analysis tools. Ecosystems to be sampled could include aquatic environments (i.e., oceans, lakes, rivers, streams, ponds, and geothermal springs, which include fresh, brackish, hypersaline, acidic, and alkaline environments) and terrestrial environments in a variety of geographical locations to maximize genetic diversity. Collection sites can include public lands as well as various sites within our national and state park systems.

In all cases, questions of proprietary rights of isolated strains should be considered. Sampling strategies should not only account for spatial distribution but also for the temporal succession brought about by seasonal variations of algae in their habitats. Additionally, within an aqueous habitat, algae are typically found in planktonic (free-floating), attached (associated with specific substrated, such as vascular plants) and benthic (associated with soils/sediments) environments. Many species of algae may be capable of existing in multiple forms dependent on life-cycle and environmental conditions. Planktonic algae may be used in suspended mass cultures, whereas attached or benthic algae may find application in biofilm-based production facilities.

Isolation Techniques

For isolation of new strains from natural habitats, traditional cultivation techniques may be used, such as plating and growth in enrichment cultures, which are a medium with specific and known qualities that favors the growth of a particular microorganism while inhibiting the growth of others. However, some algal strains take weeks to months to be isolated by

traditional methods (for a comprehensive review of algal culturing techniques, see Andersen and Kawachi 2005). As a result, large-scale sampling and isolation efforts have been developed, such as high-throughput automated isolation techniques involving fluorescence-activated cell sorting (Sieracki et al. 2004). Flow cytometry for the counting and sorting of algae is widely utilized in R&D and production (for a summary of flow cytometry, see Peniuk et al. 2015 and Picot et al. 2012). High-throughput screening should also take into account the media composition (such as broad, multiple media recipes) and the standardization of screening conditions. Because of morphological similarities when comparing many algal species, actual strain identification should be based on molecular methods such as rRNA sequence comparison, the nuclear rDNA Internal Transcribed Spacer 2 Region sequence for discrimination at the genus or species level, or in the case of closely related strains, other gene markers.

Screening Criteria and Methods

An ideal screen would cover three major areas: (1) growth physiology, (2) metabolite production, (3) and strain robustness (such as sensitivity to pathogens and predators). The term "growth physiology" encompasses a number of parameters such as maximum specific growth rate, maximum cell density, tolerance to environmental variables (such as temperature, pH, salinity, oxygen levels, CO_2 levels, and light), photosynthetic productivity, and nutrient requirements. Because all of these parameters require significant experimental effort, the development of automated systems that provide information regarding all parameters simultaneously would be helpful (see chapter 3 for available tools). The standardization of screening methods, such as culture media, light intensity, and the time of day of sampling, should also be taken into consideration.

Screening for metabolite production may involve determining the cellular composition of proteins, lipids, carbohydrates, and other metabolites, and measuring the productivity of the organism regarding metabolites useful for biofuels generation. The exact screens employed would depend on the cultivation approaches and fuel precursor or other valuable products of interest. For example, a helpful screen for oil production would allow for distinction between neutral and polar lipids, and would provide fatty acid profiles (see chapter 3 for available methods, and see also nrel.gov/biomass/microalgal_procedures.html). Furthermore, many strains also secrete metabolites into the growth medium. Some of these could prove to be valuable co-products if protected from consumption by other organisms, and product-specific approaches are needed to develop screening methods for extracellular materials.

For mass culture of a given algal strain, it is also important to consider the strain's robustness, which includes parameters such as culture consistency, resilience to abiotic stress, community stability, and susceptibility to pathogens and predators present in a given environment. Previous studies revealed that algae strains tested in the laboratory do not always perform similarly in outdoor mass cultures (Sheehan et al. 1998). Therefore, to determine a strain's robustness, small-scale simulations of mass culture conditions should be performed using location-specific and large-scale cultivation-specific paramaters. The development of small-scale, high-throughput screening technologies that mimic outdoor culture or an understanding of how small-screen technologies translate to large-scale outdoor cultivation is an important step in enabling the testing of hundreds to thousands of different algal isolates.

The bottleneck in screening large numbers of algae stems from a lack of high-throughput methodologies that would allow simultaneous screening of multiple phenotypes, such as growth rate and metabolite productivity. To meet this need, several tools are being developed. A high-throughput methodology utilizing iodine staining was developed to screen algal strains with altered starch metabolism from a large pool of candidates (Black et al. 2013). To isolate single cells with high lipid contents out of large populations, flow cytometry in combination with lipid-staining dyes is emerging as a robust screening tool (Doan and Obbard 2011; Terashima et al. 2015; Manandhar-Shrestha and Hildebrand 2013; Traller and Hildebrand 2013; Xie et al. 2014).

In addition, the spectroscopic characterization of algal lipids by infrared spectroscopy (both near-infrared [NIR] and Fourier transform infrared [FTIR]) for the simultaneous determination of lipid, protein, and carbohydrate content is an accurate, rapid, and non-destructive method that is now being widely applied as a high-throughput lipid fingerprinting tool (Laurens and Wolfrum 2010; Laurens and Wolfrum 2013; Hirschmugl et al. 2006; Dean et al. 2010; Wagner et al. 2010; Mayers et al. 2013). Not only are rapid screening procedures necessary for the biofuels field, but they could prove extremely useful for the identification of species (particularly in mixed field samples) necessary for the future of algal ecology. They could also reduce the number of redundant screens of algal species.

Selecting Algal Model Systems for Study

Given the diversity of algae, a large number of model systems could be studied. However, in a practical sense, the number of algal systems that can be studied in depth has to be limited because a critical mass of researchers is required to make progress on a given species.

In relation to biofuels, there are two types of algal model systems to consider studying: species or strains amenable to providing information on basic cellular processes regarding growth physiology or the synthesis of fuel precursors, and species or strains with characteristics useful for large-scale growth. Species with sequenced genomes and transgenic capabilities are the most amenable to investigating cellular processes since the basic tools are in place. Given the general adaptability of strain improvement approaches (e.g.,

mutagenesis/selection or genetic manipulation) in particular classes of algae (but not necessarily across classes), a logical approach with current technology is to identify strains with predicted or demonstrated desirable large-scale outdoor cultivation characteristics, and then develop improvement approaches in the lab.

Useful Algal Characteristics

As mentioned, several characteristics are important for biofuel production, including growth physiology, metabolite production, and strain robustness. Culture stability over long periods will be a key to low-cost production of biofuels. Rapid growth or the ability to uptake and store nutrients efficiently is important both for overall productivity and the ability to compete with contaminating strains. Additionally, efficient nitrogen fixation and carbon concentrating mechanisms could result in reduced resource use, such as added nitrogen and CO_2. Other traits, such as the ability to grow to high cell density in continuous culture, may allow a strain to be maintained while at the same time reducing the amount of water to be processed daily. In addition, salt tolerance may be a useful characteristic for reduced freshwater usage. Resistance to predators, viruses, and abiotic stress is also a desirable phenotype (see chapter 4). Also, the ability to flocculate without addition of chemical flocculating agents could reduce the costs of harvest as long as it could be controlled to avoid settling in the cultivation system.

Targeting Desired Fuel Product or Intermediate

One consideration in choosing model systems is the type of fuel, intermediate, or co-product to be produced. Possible fuel types of interest could include ethylene, hydrogen gas, lipids, isoprenoids, carbohydrates, alcohols (either directly or through biomass conversion), or methane (via anaerobic digestion). Co-products could include pharmaceuticals (therapeutic proteins and secondary metabolites), food and feed supplements, materials for nanotechnology (in the case of the silica cell wall of diatoms), or petrochemical replacements (see chapter 8). A reasonable first approach to identify model species that are optimal for the production of a desired fuel is through a survey of the literature, or a screen of environmental isolates for species that naturally make abundant amounts of the desired product. In such a strain, cellular metabolism is already geared toward production, which simplifies characterization and possible strain development for production. In addition, as conversion processes are developed that are capable of producing biocrude from biomass (see chapter 7), general biomass production is also a targeted research focus.

Secretion of Products or Intermediates

The ability of an algal species to secrete fuel precursors may be attractive because it could reduce or skip the cell harvesting and biomass deconstruction/separation steps. However, there may be practical considerations, such as, if the desired product is volatile, collection of the head space above the culture will be necessary to isolate it, which will necessitate the use of closed bioreactors. The secreted product may also be toxic to growth, requiring immediate removal from the medium. Also to be considered is whether secretion actually makes the product more readily available. For example, although there are algae known to secrete long-chain hydrocarbons (e.g., *Botryococcus braunii*), they are still associated with the cells in a lipid biofilm matrix, and thus are not free to form an organic hydrocarbon phase in solution (Banerjee et al. 2002). Even if sustained secretion could be achieved, it is not clear what would be the effect of a lipid emulsion in an algal culture. For example, an abundance of exported lipids could unfavorably alter fluidics properties. Finally, secretion of either intermediates or products into the growth medium will make these compounds available to contaminating organisms for potential catabolism. Although its focus has recently shifted to carbon dioxide capture, pilot-scale experimentation of a secretion system was being explored at Algenol Biotech LLC in Fort Myers, Florida, using a proprietary cyanobacterial strain to produce ethanol, with a capacity of 10,000 gallons per year of ethanol at full scale. In addition to ethanol, Algenol produced diesel fuel, gasoline, and jet fuel (see chapter 7) from periodically collected algae biomass (www.algenol.com/about-algenol).

Capability for Heterotrophic or Mixotrophic Growth

Heterotrophic or mixotrophic growth capabilities may be attractive attributes of algal strains. In heterotrophic growth, algae are grown without light and are fed a carbon source, such as sugars, to generate new biomass. Mixotrophic growth utilizes both heterotrophic and photoautotrophic growth. In some species, addition of supplemental carbon results in increased lipid accumulation (Xu et al. 2006; Albrecht et al. 2016; Ren et al. 2016), even under mixotrophic conditions when photosynthetic efficiency may be limited (Ceron Garcia et al. 2006). Furthermore, species-variable night biomass losses can impact algal biomass net productivity of photosynthetic cultures (Edmundson and Huesemann 2015). If cells are grown mixotrophically with a carbon source utilized during the night, growth in both light and dark periods is possible, and high cell densities can be achieved. Potential disadvantages of the addition of external carbon sources is the cost of addition at large scales and the possibility of increased contamination by undesired microbes living off the carbon source. However, this is not generally a problem with well-established fully-heterotrophic fermentation technologies that are currently deployed worldwide at massive scale to manufacture everything from cancer drugs to high-volume/low-cost commodities such as lysine and ethanol. Currently, the BETO mission supports only the use of sustainable lignocellulosic sugars in heterotrophic or mixotrophic growth systems.

2.2 Algal Physiology and Biochemistry

Photosynthetic algae have evolved strategies to prevent photoinhibition (light-induced oxidative damage). A large

majority of absorbed incident light is dissipated as heat and could be considered "wasted." The processes of photoinhibition and the accumulation of organic macromolecules, such as carbohydrates and lipids, are integrated. Under stress conditions, such as high light or nutrient starvation, some microalgae preferentially accumulate lipids (such as triacylglycerols [TAGs]), some accumulate carbohydrates, and some accumulate both as their main storage compound. Certain microalgal species also naturally accumulate large amounts of TAG (30%–60% of dry weight), and exhibit photosynthetic efficiency and lipid production greater than terrestrial crop plants (Hu et al. 2008). Cyanobacteria, as a general rule, accumulate mostly carbohydrates, although concentrations of 14% lipid (typically from polar membrane glycerolipids) have been reported (Cuellar-Bermudez et al. 2015). Promising species of cyanobacteria, such as *Leptolyngbya* sp. BL0902, have been shown to accumulate 28.8% fatty acid methyl esters and large proportions of mono-unsaturated fatty acids, preferable for biodiesel production (Taton et al. 2012). Lipids and carbohydrates, along with biologically produced hydrogen and alcohols, are all potential biofuels or biofuel precursors. It is, therefore, important to understand the metabolic pathways and processes that generate them in order to advance biofuel production (Figure 2.1).

Photosynthesis, Light Utilization, and Carbon-Concentrating Mechanisms

When algae are cultivated photosynthetically, the efficiency of photosynthesis is a crucial determinant in their productivity, affecting growth rate, biomass production, and potentially, the percent of biomass that is the desired fuel precursor. Theoretical best case biomass productivity values in the range of 33–42 g/m^2/day with a range of 40,700–53,200 L.ha^{-1}.year^{-1} unrefined oil have been calculated (Weyer et al. 2010). These values represent what may be possible with optimization of both biological and production systems. Theoretical productivity is an important concept, because it can be used to set achievable goals for both cultivation process design and strain improvement projects. In one analysis, the maximum conversion efficiency of total solar energy into primary photosynthetic organic products is around 10%, with 30%–50% of the primary product mass lost on producing cell protein and lipid (Williams and Laurens, 2010).

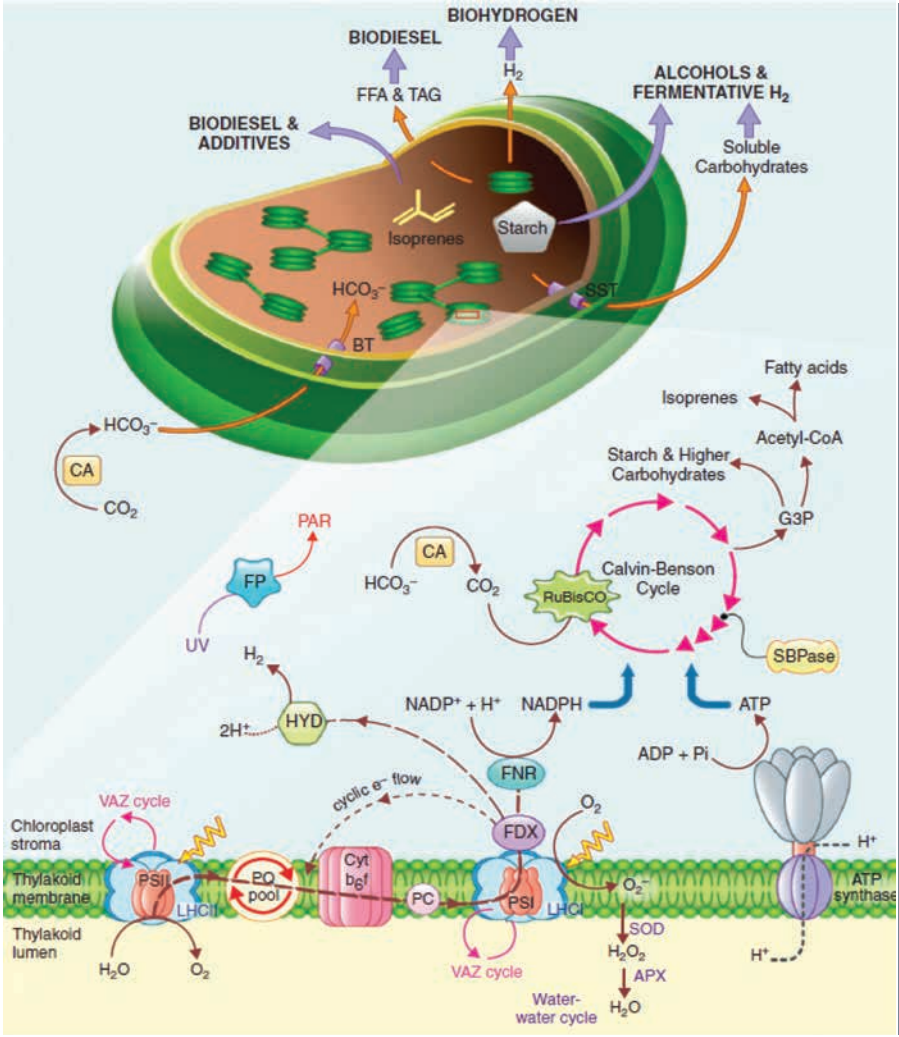

Figure 2.1. Generic chloroplast of a green alga showing placement of fuel-relevant primary metabolites and their integration into bioenergy production. Also depicted are the major components of photosynthesis and carbon fixation, including elements with the potential to be engineered for optimization of these pathways. APX = ascorbate peroxidase; BT = bicarbonate transporter; CA = carbonic anhydrase; Cyt b6f = cytochrome b6f; FDX = ferredoxin; FFA = free fatty acids; FNR = ferredoxin-NADP+ reductase; FP = fluorescent protein; G3P = glyceraldehyde 3-phosphate; HCO_3^- = bicarbonate; HYD = hydrogenase; LHC = light-harvesting complex; PAR = photosynthetically active radiation; PC = plastocyanin; PS = photosystem; PQ pool = plastoquinone pool; SBPase = sedoheptulose-1,7-bisphosphatase; SOD = superoxide dismutase; SST = soluble sugar transporter; TAG = triacylglycerol; UV = ultraviolet light; VAZ = xanthophyll cycle. (Source: Work et al. 2012)

There are many good reviews available that cover basic algal photosynthetic processes (Nelson et al. 1994; Eberhard et al. 2008; Nelson and Yocum 2006; Krause and Weis 1991; Johnson and Alric 2013; Hildebrand et al. 2013). Regardless of the cultivation practices used to maximize light exposure (see chapter 4), there remains limitations of algal photosystems regarding light utilization. The majority of light that falls on a photosynthetic algal culture is not utilized. In high cell density cultures, cells nearer to the light source tend to absorb all the incoming light, preventing it from reaching more distant cells (Perrine et al. 2012). Under certain light regimes, photoinhibition or the decrease of photosynthesis due to light damage can occur (Long et al. 1994; Foyer et al. 1994; and Niyogi 1999). In an effort to overcome this response, it was shown that reducing the size of the photosystem antenna can increase the efficiency of light utilization (Polle et al. 2000, 2002, 2003; Nakajima and Ueda 1997, 2000; Melis et al. 1999; Melis 2009; Perrine et al. 2012; Kirst et al. 2012), which has the potential to benefit large cultures as light penetration would potentially increase. Work on reducing photosystem antenna size in potential production strains of algae has begun; truncated photosystem antenna mutants of *Chlorella sorokiniana* created via UV-induced random mutagenesis show greater productivity than wildtype in both lab-scale and outdoor trials, illustrating the promise of photosystem antenna reduction in production strains (Cazzaniga et al. 2014). Similar strategies have been employed in generating *Nannochloropsis gaditana* mutants with improved photosynthetic activity (Perin et al. 2015). Targeted genetic engineering strategies in biofuel production strains to alter the photosystem antenna size in response to light intensity within the water column are in progress within DOE's current funding portfolio.

There is still much to learn about the dynamics and regulation of the photosynthetic apparatus (Eberhard et al. 2008). As shown in Figure 2.2, organization and composition of the photosynthetic apparatus varies between classes of algae, so particular strategies to reduce the photosystem antenna size

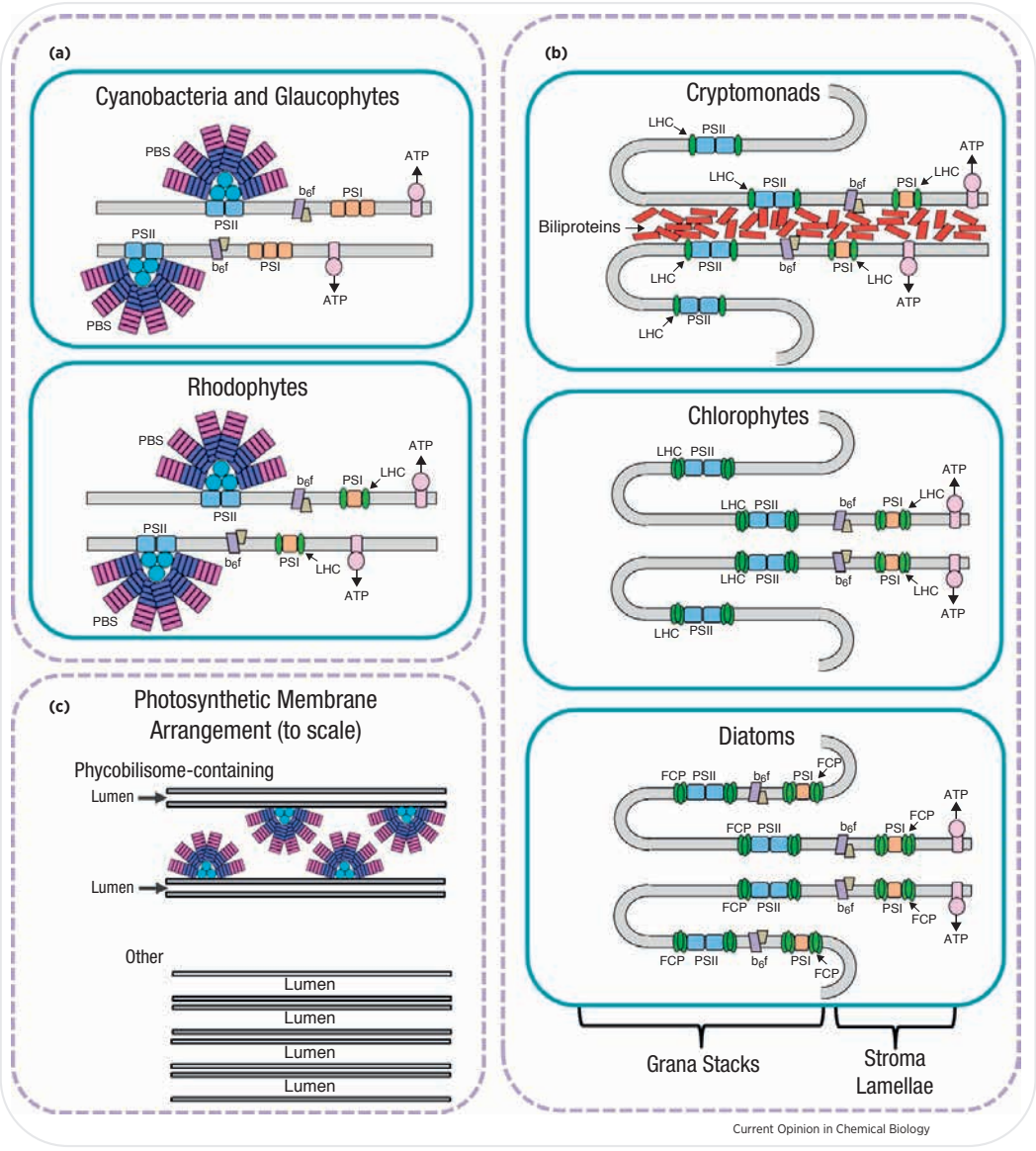

Figure 2.2. Organization schemes of the photosynthetic apparatus in evolutionary diverse classes of microalgae (Source: Hildebrand et al. 2013). PBS = phycobilisomes; PSII = photosystem II; b_6f =cytochrome b6f complex; PSI = photosystem I; ATP = adenosine triphosphate; LHC = light harvesting complex; FCP = fucoxanthin chlorophyll binding protein complex. (a) Clusters the phycobilisome-containing classes. (b) Clusters classes containing grana stacks and stroma lamellae. (c) Shows the relative spacing of photosynthetic membranes in phycobilisome-containing and other chloroplasts.

may be class-specific. More emphasis should be placed on understanding these processes if we are to better engineer the capture and utilization of light energy for biomass production. Understanding the effects of light intensity and frequency of light flashes on the photosynthetic efficiency and growth of algae has been an increasingly important focus of research (for review, see Work et al. 2012; Stephenson et al. 2011). Heterokonts utilize different accessory pigments (fucoxanthins) than green algae, which extends the wavelength range into the green portion of the spectrum. Notably, investigation into engineering the photosynthetic antenna pigment to extend the spectrum of light captured by algae has been proposed, influenced in part to the discovery of a red-shifted chlorophyll, chlorophyll *f*, in a cyanobacterium (Chen et al. 2010; Chen and Blankenship 2011; Gan et al. 2014). However, downstream rate limitations in photosynthetic electron transfer may limit the ability to utilize aditional captured photons, since light is thought to saturate at one quarter full sunlight intensity (Perrine et al. 2012).

Most eukaryotic algae and all cyanobacteria have inorganic carbon concentrating mechanisms (CCMs). A minority of algae do not have a CCM and rely on diffusive CO_2 entry into the cell, whereas some algae are intermediate between diffusive CO_2 entry and occurrence of a CCM. Expression of the CCM is also known to be facultative in some but not all species. The CCM raises the CO_2 concentration at the site of ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO), a strategy for carbon acquisition enabling algae to survive and grow when the CO_2 concentration is low and limiting photosynthesis. RubisCO can fix either CO_2 or O_2, and although fixing CO_2 is the dominant outcome, the inadvertent fixing of O_2 leads to an energetically wasteful cycle called photorespiration. Crop improvement approaches include targeting ways to increase the productivity of photosynthetic CO_2 fixation by boosting the steady-state CO_2 concentration around RubisCO, reducing photorespiration. This approach has the effect of making RubisCO more efficient, which can also improve nitrogen use efficiency by reducing the amount of RubisCO needed to maintain photosynthesis.

In cyanobacteria, RubisCO is encapsulated within carboxysomes (a bacterial microcompartment), and in algae, it is aggregated into a pyrenoid. The carboxysome and the pyrenoid allow CO_2 levels to be elevated around RubisCO, allowing enhanced CO_2 fixation. There are many good reviews on the CCMs in algae and cyanobacteria (such as Moroney et al. 2013; Raven and Beardall 2016; Giordano et al. 2005; Raven 2010; Rae et al. 2013; Hagemann et al. 2016; Price et al. 2012). Algal and cyanobacterial CCMs are generally thought to be based on active transport of an inorganic carbon species.

Recent efforts into elucidating the mechanism of CO_2 concentration and uptake into green algae in the model-strain *Chlamydomonas reinhardtii* have highlighted the proteins involved in assimilating carbon under limiting CO_2 conditions (Wang et al. 2015a). In *Chlamydomonas reinhardtii*, the CCM involves bicarbonate (HCO_3-) conversion to CO_2 in the thylakoid lumen where external inorganic carbon had to cross four membranes in series with a final CO_2 efflux from the lumen to the stroma for fixation by RubisCO. The carbon transporter HLA3 is involved in inorganic carbon uptake under very low CO_2 concentrations, and its constitutive expression results in an increased photosynthetic O_2 evolution rate (Gao et al. 2015). Based on analysis of the organic products of photosynthesis, green algal and cyanobacterial CCMs are generally thought to have C_3 biochemistry (three carbons in the product), whereas marine diatoms may have C_4-like metabolism. Other components of the CCM have also been examined; however, a complete elucidation of the roles of component proteins remains unclear with more known for cyanobacteria than for algae. Clarification of this pathway and the roles of component proteins in a wider range of algal species may provide future gene targets for increasing biomass productivity.

Carbon Partitioning and Metabolism

Knowing how and when carbon is partitioned into lipids and/or carbohydrates could be very useful for biofuels strain development and designing cultivation strategies. Understanding carbon partitioning will require extensive knowledge of metabolic pathways. Metabolic networks have been reconstructed in various microbes from genomic and transcriptomic data, pathway analysis, and predictive modeling (Vemuri and Aristidou 2005). Research has also been done in plant systems to understand carbon flux in biosynthetic and degradative pathways (Lytovchenko et al. 2007; Schwender et al. 2004; Allen et al. 2009; Sweetlove and Fernie 2005; Libourel and Shachar-Hill 2008). However, carbon partitioning in algae is less understood, and research on how algal cells control the flux and partitioning of photosynthetically fixed carbon into various groups of major macromolecules (i.e., carbohydrates, proteins, and lipids) is critically needed (Boyle and Morgan 2009; Yang et al. 2002). A fundamental understanding of "global" regulatory networks that control the partitioning of carbon between alternative storage products will be important for metabolic engineering of algae.

Furthermore, a link between carbon and energy storage molecules (such as starch or laminarin/chrysolaminarin in algae) and lipid metabolism has been established. Storage carbohydrate, such as starch, is a common carbon and energy storage compound in plants and algae, and shares the same precursors with the energy storage lipid TAG (Figure 2.1). It is, therefore, possible that TAG and carbon storage molecules could be inter-convertible, a potentially important implication for biofuel production.

In microalgae, an interaction between storage carbohydrate (chrysolaminarin) metabolism and lipid accumulation has been indicated by studies on the diatom *Cyclotella cryptica* (Roessler 1988). More recently, a stable mutation of the

STA6 locus encoding the small subunit of ADP-glucose pyrophosphorylase (AGPase) in *Chlamydomonas reinhardtii* abolished starch synthesis and a 10-fold increase in cellular TAG content under nitrogen deprivation (Li et al. 2010a and 2010b). In an examination of this *sta6* starchless mutant under nutrient replete conditions, disrupting starch synthesis did not result in higher lipid or protein, exhibiting greater sensitivity to photoinhibition and accumulating lower biomass (Krishnan et al. 2015); this indicates a critical role for starch biosynthesis in multiple functions. The *sta6* mutant also lacks a cell wall (another major carbon sink), which may increase its sensitivity to starch synthesis disruption. Under nitrogen depletion, starchless mutants of the oleaginous microalga *Scenedesmus obliquus* show not only higher TAG accumulation, but also equal photosynthetic efficiency when compared to wildtype (Breuer et al. 2014; de Jaeger et al. 2014). Examination of *Chlorella sorokiniana* showed starch to be the preferred carbon storage sink in nitrogen-replete conditions, with increased lipid levels in response to decreased starch (Li et al. 2015) or extended nitrogen deprivation (Negi et al. 2015), indicating promise for future targeted engineering in this production strain.

Recent thermodynamic and kinetic analyses of starch and lipid production in green algae indicate that greater energy can be captured from photons via carbohydrate synthesis than lipid synthesis (Subramanian et al. 2013). It could, therefore, be fruitful to further research *de novo* storage molecule synthesis, degradation, and interaction with lipid metabolism in algae. Newly developed screening tools that determine starch content, such as by NIR and FTIR (See "Screening Criteria and Methods" section of this chapter; Laurens and Wolfrum 2013) and in individual growing algal colonies (Black et al. 2013), will enable mutant screening in the future; however, the comprehensive characterization of polysaccharides is not well-developed and standardized across multiple organisms.

Since 2010, several papers examining carbon partitioning in several strains of algae have been published (Johnson and Alric 2013; Breuer et al. 2015; Smith et al. 2012; Jia et al. 2015; McNeely et al. 2014; Wu et al. 2015; Polle et al. 2014; Bittar et al. 2013). Notably, these studies indicate the variability in the organization of metabolic pathways, even within a single algal group (Smith et al. 2012). Although collecting transcriptomic and genomic data to analyze these pathways is relatively easy, analyzing and interpreting this data to select targets for metabolic engineering to improve fuel precursor production remains a challenge. Furthermore, the rates of carbohydrate and lipid synthesis in algae are not well characterized; to facilitate the design of improved biomass production systems, it is important to understand the kinetic constraints of starch and lipid synthesis, accumulation, and turnover, and the direct feedbacks on carbon fixation.

In cyanobacteria, central carbon metabolism is composed of interrelated pathways (the Calvin–Benson–Bassham cycle, glycolysis, the pentose phosphate pathway, and the tricarboxylic acid cycle), making targeted engineering of carbon metabolism for the production of biofuels challenging. Recently, in research supported in part by BETO, examination of phosphoketolase mutants in wildtype or xylose-catabolizing mutants of *Synechocystis* indicate a significant contribution of the phosphoketolase pathway to carbon metabolism in the light when supplemented with xylose and sole responsibility for the production of acetic acid in the dark (Xiong et al. 2015a). This pathway, which splits xylulose-5-phosphate (or fructose-6-phosphate) to acetate precursor acetyl phosphate, was previously uncharacterized in photosynthetic organisms. This pathway may be present in other organisms of interest and could potentially be exploited to increase the efficiency of carbon metabolism and photosynthetic productivity, although substantial energy used for CO_2 fixation is lost during the conversion of pyruvate (C_3) to acetate (C_2).

Algal Carbohydrates

Algae are incredibly diverse in the kind of simple and complex carbohydrates that they use for carbon storage and cell structure. If carbohydrates are to be used as fuel precursors, for example for fermentation to produce alcohols (see chapter 7), it is important to determine the predominant types that are present. Carbohydrate metabolism forms the basis of the cell's carbon energetic pathways and could be important to improving productivity and overall fuel yields from algal cell biomass (for a review, see Chen et al. 2013 and Markou et al. 2012).

Many green microalgae are plant-like, featuring rigid cellulose-based cell walls and accumulating starch as their main carbohydrate storage compound. Several algae commonly use starch for energy storage, including some red algae and dinoflagellates. Other algae, for example—many which are brown algae and diatoms—accumulate carbohydrates, such as laminaran, mannitol, or fucoidin as food reserves. Cyanobacteria often store large quantities of glycogen (Chao and Bowen 1971; Yoo et al. 2002). The wide range of storage carbohydrates are not fully characterized and reported on in the literature. A detailed characterization, not just of the isolated polymers, but also of the regulatory networks surrounding transitory carbohydrate metabolism, is necessary.

These major storage polysaccharides represent potential biochemical feedstocks for conversion to liquid fuels. Microorganisms capable of fermenting laminarin and mannitol from the macroalgae *Laminaria hyperborea* to ethanol have been identified and partially characterized (Horn et al. 2000a and 2000b). Other abundant polysaccharides (e.g., alginate found in many brown algae) are considered less suitable for ethanol fermentation because the redox balance favors formation of pyruvate as the end product (Bird and Benson 1987). However, these polysaccharides may still prove useful as intermediates to other types of conversion processes and final fuels.

Another important consideration in algal strains is the composition and structure of the polysaccharide cell wall (for a review, see Popper et al. 2011). These structures can be an important source of carbohydrates, but like those from plants, must typically be broken down into simpler sugars before conversion into biofuels. Cell walls can also be a technical barrier, for example, when trying to access DNA for genetic manipulations, or efficiently extracting biofuel precursors from cells in mass culture. As mentioned above, many algal cell walls from different groupings are cellulose-based, though their physical structure and the presence or absence of other structural polysaccharides varies greatly. There are also many algae that completely lack cellulose and have other polymers that provide structure to the cell (Raven et al. 1992), while some algae lack cell walls entirely. Diatoms are also unique among algae for the presence of silica in their cell walls. Some red algae also have a thick extracellular matrix composed of important products such as agar or carrageenan. In order to genetically transform or to enhance product extraction, the cell wall structures of production strains of microalgae have been examined. The composition of the *Nannochloropsis gaditana* cell wall was determined to be a bilayer structure with a cellulosic inner wall surrounded by an algaenan layer, an aliphatic, non-hydrolyzable polymer (Scholz et al. 2014).

Most cyanobacteria have a peptidoglycan layer and cell envelope similar to those of gram-negative bacteria, and are encased in a polysaccharide sheath (Hoiczyk and Hansel 2000). An important lesson is the recognition of the diversity of algal polysaccharides and cell walls, and the technical challenges these structures may present in strain manipulation, feedstock potential, and extraction processes.

Lipid Synthesis and Regulation

Primary Pathway for TAG Synthesis

Some algae, naturally or under stress conditions, accumulate significant quantities of neutral storage lipids such as triacylglycerols (TAGs), which are important potential fuel precursors. The major pathway for the formation of TAG in plants first involves *de novo* fatty acid synthesis in the stroma of plastids. The syntheses of cellular and organelle membranes, as well as of neutral storage lipids such as TAG, use 16 or 18 carbon fatty acids as precursors. In plants, TAG is formed by incorporation of the fatty acid into a glycerol backbone via three sequential acyl transfers (from acyl CoA) in the endoplasmic reticulum. A simplified overview of major pathways for fatty acid and TAG synthesis in algae in shown in Figure 2.3. In a recent study in *Chlamydomonas reinhardtii*, it was hypothesized that a large fraction of TAGs is assembled *de novo* by the chloroplast pathway following nitrogen deprivation (Fan et al. 2011). It

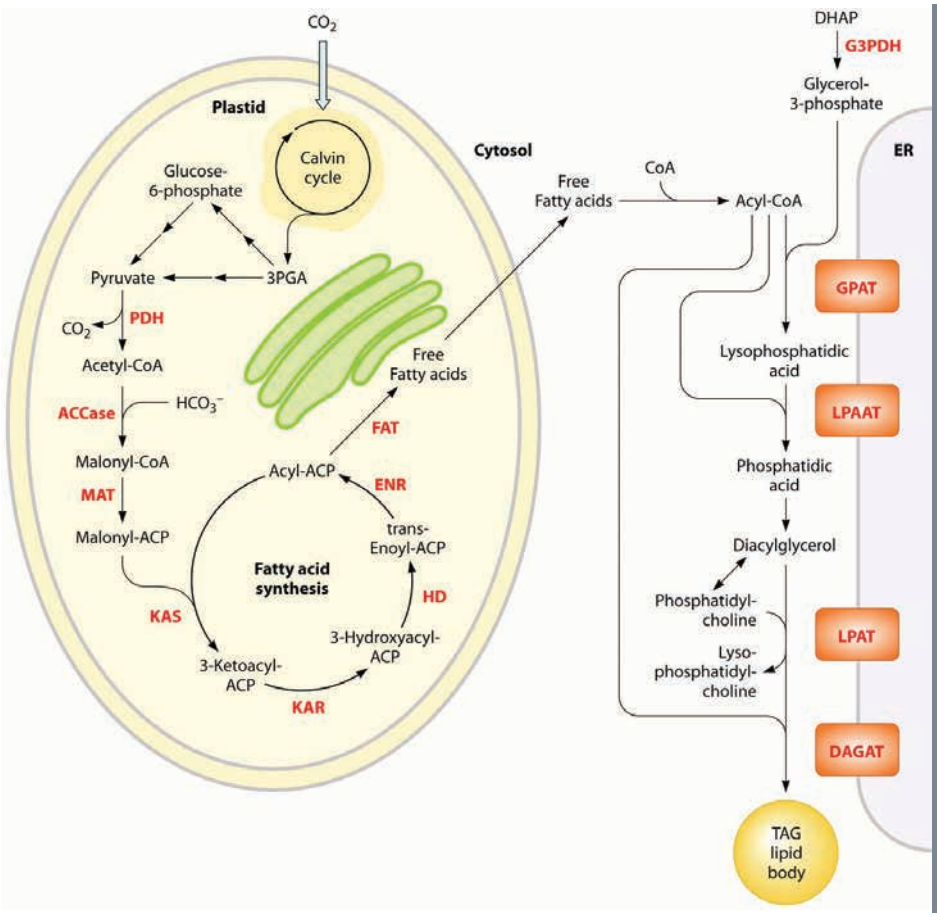

Figure 2.3. Simplified overview of the metabolites and major pathways in microalgal lipid biosynthesis shown in *black* and enzymes shown in *red*. Free fatty acids are synthesized in the chloroplast, while TAGs may be assembled at the ER. ACCase = acetyl-CoA carboxylase; ACP = acyl carrier protein; CoA = coenzyme A; DAGAT = diacylglycerol acyltransferase; DHAP = dihydroxyacetone phosphate; ENR = enoyl-ACP reductase; FAT = fatty acyl-ACP thioesterase; G3PDH = gycerol-3-phosphate dehydrogenase; GPAT = glycerol-3-phosphate acyltransferase; HD = 3-hydroxyacyl-ACP dehydratase; KAR = 3-ketoacyl-ACP reductase; KAS = 3-ketoacyl-ACP synthase; LPAAT = lyso-phosphatidic acid acyltransferase; LPAT = lyso-phosphatidylcholine acyltransferase; MAT = malonyl-CoA:ACP transacylase; PDH = pyruvate dehydrogenase complex; TAG = triacylglycerols. (Source: ec.asm.org/content/9/4/486/F2.expansion.html.)

has been proposed that TAG metabolism in *Chlamydomonas reinhardtii* involves the plastid in ways not observed in plants and that TAG molecules are assembled in the plastid envelopes exclusively or in parallel to assembly at the endoplasmic reticulum (Liu and Benning 2013).

TAG biosynthesis in algae has been proposed to occur via the Kennedy Pathway described in plants. Fatty acids produced in the chloroplast are sequentially transferred from CoA to positions 1 and 2 of glycerol-3-phosphate, resulting in the formation of the central metabolite phosphatidic acid (Ohlrogge and Browse 1995). Dephosphorylation of phosphatidic acid catalyzed by a specific phosphatase releases diacylglycerol (DAG). Since diglycerides are usually present in high amounts in rapidly growing cultures, it may be of interest to research these TAG intermediates. In the final step of TAG synthesis, a third fatty acid is transferred to the vacant position 3 of DAG by diacylglycerol acyltransferase (DGAT), an enzyme that is unique to TAG biosynthesis (Lung and Weselake, 2006; Athenstaedt and Daum 2006). The acyltransferases involved in TAG synthesis may exhibit preferences for specific acyl CoA molecules, and thus may play an important role in determining the final acyl composition of TAG (Hu et al. 2008). Three types of DGATs have been identified in eukaryotic cells: DGAT1 and DGAT2 are membrane proteins that play a direct role in the synthesis of TAG, whereas DGAT3 is cytosolic and not involved in oil production (Cao et al. 2013; Hernandez et al. 2012). Overexpression of a native DGAT2 in the diatom *Thalassiosira pseudonana* resulted in improved TAG accumulation with no effect on growth (Manandhar-Shrestha and Hildebrand 2015). However, in *Chlamydomonas reinhardtii*, the overexpression of potential DGAT2 candidate genes did not increase intracellular TAG under non-lipid accumulating conditions, highlighting the species-specific complexity of lipid biosynthesis and that generalizations of one algal species are not necessarily universal (La Russa et al. 2012).

Alternative pathways to convert membrane lipids and/or carbohydrates to TAG have been demonstrated in algae, bacteria, plants, and yeast in an acyl CoA-independent way (Yoon et al. 2012; Arabolaza et al. 2008; Dahlqvist et al. 2000; Stahl et al. 2004). There is evidence that lipid remodeling is responsible for TAG accumulation in several strains of microalgae (Negi et al. 2015; Goncalves et al. 2013; Urzica et al. 2013; Martin et al. 2014; Abida et al. 2015; Levitan et al. 2015a; and others); however, the mechanistic pathway of this conversion of membrane to lipids has not yet been elucidated. Moreover, phospatidic acid and DAG can also be used directly as substrates for synthesis of polar lipids, such as phosphatidylcholine and galactolipids. These pathways are worth investigating when developing strains for improved lipid production.

The regulation of the synthesis of fatty acids and TAG in algae is poorly understood. This lack of understanding, in conjunction with outdoor conditions (such as fluctuating temperature and light), may contribute to why the lipid yields obtained from algal mass culture efforts fall short of the high values (50% to 60%) observed in the laboratory (Hu et al. 2008; Sheehan et al. 1998). Algae can exhibit a large range in varibility in their relative protein, carbohydrate, and lipid contents, depending on growth conditions (such as nutrient content) and genetics. The storage carbohydrate (polysaccharides) or oil (lipid) content of algae can range anywhere from 6% to 64% of the total biomass (Subramanian et al. 2013). Many studies have been published on the effect of nutrient deprivation on TAG accumulation. In one such study of the marine diatom *Phaeodactylum tricornutum*, under nitrate deprivation, 60% of TAG was synthesized from *de novo* carbon fixation while the remaining 40% was obtained from the transformation of pigment, protein, carbohydrate, and other membrane components (Burrows et al. 2012). Understanding the mechanisms of lipid regulation can help to maximize scenarios for lipid production and strain improvement.

Because fatty acids are common precursors for the synthesis of both membrane lipids and TAG, how the algal cell coordinates the distribution of the precursors to distinct destinations, or how the inter-conversion between the two types of lipids occurs, needs to be elucidated. If the ability to control the fate of fatty acids varies among algal taxonomic groups or even between isolates or strains, the basal lipid and TAG content may represent an intrinsic property of individual species or strains. If this proves to be true, it could be a challenge to extrapolate information learned about lipid biosynthesis and regulation in laboratory strains to production strains. Similarly, it will be difficult to use information regarding lipid biosynthesis in plants to develop hypotheses for strain improvement in algae. As an example, the annotation of genes involved in lipid metabolism in the green alga *Chlamydomonas reinhardtii* has revealed that algal lipid metabolism may be different from that in plants, as indicated by the presence and/or absence of certain pathways and by the size of the gene families that relate to various activities (Riekhof et al. 2005). Thus, *de novo* fatty acid and lipid synthesis should be studied in order to identify key genes, enzymes, and new pathways, if any, involved in lipid metabolism in algae.

Alternative Pathways to Storage Lipids

Algae may possess multiple pathways for TAG synthesis, and the relative contribution of these individual pathways to overall TAG formation may depend on environmental or culture conditions. Analyzing different algae could help to elucidate the possible pathways of TAG synthesis: the *de novo* Kennedy Pathway, the potential pathway for lipid formation from starch reserves mentioned earlier, and other potential pathways to convert membrane phospholipids and glycolipids into TAG. The thylakoids of chloroplasts are the main intracellular membranes of eukaryotic algae, and their lipid composition dominates extracts obtained from cells under favorable growth conditions. Algal chloroplasts contain monogalactosyldiacylglycerol as their main lipid (~50%), with smaller amounts of

digalactosyldiacylglycerol (~20%), sulfoquinovosyldiacylglycerol (~15%), and phosphatidlyglycerol (~15%) (Harwood 1998). Under stress conditions as degradation of chloroplasts occurs, the fate of these abundant lipids remains unclear. It has been proposed that these alternative pathways that convert carbohydrate, excess membrane lipids, and other components into TAG play an important role for cell survival under stress.

Organelle Interactions

Chloroplast membranes control the exchange of metabolites between the plastid and the cytoplasm. As mentioned earlier, the chloroplast stroma is the primary location for fatty acid biosynthesis in plants. Fatty acids can then be either assembled into glycerolipids at chloroplast membranes or they can be exported to the endoplasmic reticulum and assembled into lipids for cellular membranes. Some glycerolipids assembled at the endoplasmic reticulum are then returned to the plastid where they are assimilated. Lipid trafficking is, therefore, an important aspect of membrane formation and lipid fate (Benning 2008). Current work in plants is focused on deciphering lipid transport across plastid envelopes. Such work is also important in algae to better understand the interaction among organelles as it relates to lipid formation and lipid trafficking.

Oxidative Stress and Storage Lipids

Under environmental stress conditions (such as nutrient starvation), some algal cells stop division and accumulate TAG as the main carbon storage compound. Synthesis of TAG and deposition of TAG into cytosolic lipid bodies may be, with exceptions, the default pathway in some algae under stress conditions (Hu et al. 2008). In addition to the obvious physiological role of TAG as a carbon and energy storage compound, the TAG synthesis pathway may also play a more active and diverse role in the stress response. The *de novo* TAG synthesis pathway can serve as an electron sink under photo-oxidative stress (discussed earlier). It is well-known that nutrient deprivation or limitation and environmental stress (such as high light) results in higher lipid production.

In addition to fluctuating weather, light and self-shading, as well as reactor translucence when in a close system, influence lipid accumulation (Pulz 2001 and Simionato et al. 2013). With increasing light intensity, the synthesis of lipid increases (Liu et al. 2012; Siaut et al. 2011; Ho et al. 2012). Under high light stress, excess electrons that accumulate in the photosynthetic electron transport chain induce over-production of reactive oxygen species, which may in turn cause inhibition of photosynthesis and damage to membrane lipids, proteins, and other macromolecules. However, the formation of fatty acids could help consume excess electrons, and thus relax the over-reduced electron transport chain under high light or other stress conditions.

The TAG synthesis pathway is also often coordinated with secondary carotenoid synthesis in algae (Rabbani et al. 1998; Zhekisheva et al. 2002). The molecules (e.g., β-carotene, lutein, or astaxanthin) produced in the carotenoid pathway are sequestered into cytosolic lipid bodies. Carotenoid-rich lipid bodies serve as a "sunscreen" to prevent or reduce excess light from striking the chloroplast under stress. Because of the potential importance of stress conditions on lipid production in algae, the exact relationship between oxidative stress, cell division, and storage lipid formation warrants further study.

Lipid Body Formation and Relationship to Other Organelles

Algae are an economically important source of a wide range of lipophilic products, including vitamins, hydrocarbons and very long-chain ω-3, ω-6, ω-7, and ω-9 fatty acids; however, the study of lipid bodies in algae is relatively recent compared to plants and fungi. Lipid body structural information and physiological data throughout lipid body formation are available for *Chlamydomonas reinhardtii* (Goodson et al. 2011; Wang et al. 2009), and lipid droplet-focused proteomic studies have indicated the presence of lipid metabolism-related proteins (for a review, see Liu and Benning 2013). The study of lipid-body biogenesis in plants has focused largely on the role of oleosins (Murphy 1993; Huang 1992). This is understandable in view of their exclusive localization on lipid-body surfaces, their apparently widespread distribution, and their great abundance in many lipid-storing seeds. Nevertheless, there are now doubts about the role of oleosins in the biogenesis of plant lipid bodies. It has been suggested that oleosins may be primarily associated with the stabilization of storage lipid bodies during the severe hydrodynamic stresses of dehydration and rehydration that occurs in many seeds (Murphy 2001; Deruyffelaere et al. 2015; for a review, see Jolivet et al. 2013).

Lipid bodies may dock with different regions of the endoplasmic reticulum and plasma membrane, or with other organelles, such as mitochondria and glyoxysomes/peroxisomes, in order to load or discharge their lipid cargo (Zehmer et al. 2009). In oil-producing microorganisms, as rapid lipid body accumulation occurs, a close relationship is often found between neutral lipids like TAG and the membrane phospho- and glyco- lipids (Alvarez and Steinbuchel 2002). This relationship may be both metabolic, with acyl and glycerol moieties exchanged between the different lipid classes, and spatial, with growing evidence of direct physical continuities between lipid bodies and bilayer membranes. In order to better understand lipid metabolism in algae, the structure and function of lipid bodies across species, and their interactions with other organelles related to storage lipid formation, requires further study.

Besides biochemical analysis to study algal lipids and carbohydrates, studies involving transcriptomic and proteomic studies, for example, help provide information about photosynthetic carbon partitioning and lipid/carbohydrate synthesis in algae. Based on such information, metabolic engineering through genetic manipulation represents yet another strategy

for the production of algal oils. While more is being understood about the regulation of lipid synthesis in the well-studied strain *Chlamydomonas reinhardtii* (Gargouri et al. 2015), since 2010, characterization of lipid synthesis pathways by transcriptomic and/or proteomic analysis in other algal species, such as *Dunaliella tertiolecta* (Rismani-Yazdi et al. 2011), *Nannochloropsis oceanica* (Dong et al. 2013; Li et al. 2014a; Jia et al. 2015), and *Chlorella vulgaris* (Guarnieri et al. 2011, 2013), and by lipid profiling (Allen et al. 2014, 2015) has begun. It is becoming clear that lipid synthesis activity differs between species (Allen et al. 2014).

Biohydrogen

Some microalgae and cyanobacteria can produce H_2, a potential fuel product, in the following reactions: $2H_2O$ + light energy $\rightarrow O_2 + 4H^+ + 4e^- \rightarrow O_2 + 2H_2$. Three pathways have been described in green algae: two light-driven H_2-photoproduction pathways, and a third, light-independent, fermentative H_2 pathway coupled to starch degradation (see Figure 2.4; Melis et al. 2000; Gfeller and Gibbs 1984). As a substrate, the light-driven pathways can either employ water (through photosystems II and I) or NADH from the glycolytic breakdown of stored carbohydrate (through photosystem I). In all pathways, ferredoxin is the primary electron donor to the hydrogenase enzyme. Hydrogenases are the enzymes responsible for releasing molecular H_2 (Ghirardi et al. 2007). There are two major types of hydrogenases: (1) those containing iron (which are generally H_2-evolving) and (2) those containing both nickel and iron (which are generally H_2-uptake enzymes). One of the most important characteristics of hydrogenases is that they are O_2 sensitive.

Four biological challenges limiting biohydrogen production in algae have been identified: (1) the O_2 sensitivity of hydrogenases, (2) competition for photosynthetic reductant at the level of ferredoxin, (3) regulatory issues associated with the over-production of adenosine triphosphate (ATP), and (4) inefficiencies in the utilization of solar light energy (Seibert et al. 2008).

These challenges could be addressed by (1) engineering hydrogenases with improved tolerance to O_2 (Cohen et al. 2005), (2) identifying metabolic pathways that compete with hydrogenases for photosynthetic reductant and engineering their down-regulation during H_2 production (Mathews and

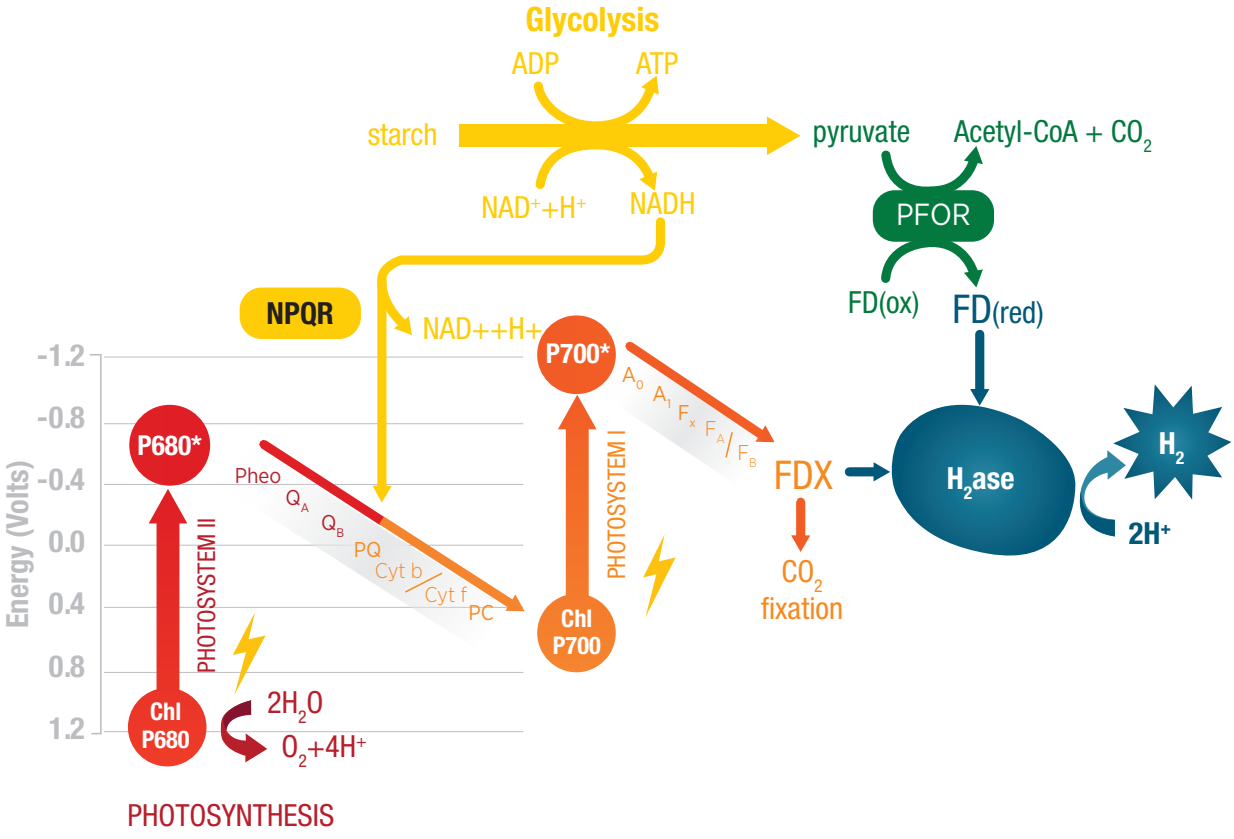

Figure 2.4. Three different pathways for H_2 production. Two are driven by light and the third occurs in the dark. Either water or starch can be the electron donor. Carbon is fixed under normal photosynthesis with water as the donor, but the electron acceptor is switched at the level of ferredoxin (FD) from CO_2 to protons under conditions that lead to H_2 production. (Drawing courtesy of Prof. M. Posewitz, Colorado School of Mines)

Wang, 2009), (3) engineering the photosynthetic membrane for decreased efficiency of photosynthetic-electron-transport-coupled ATP production (ATP is not required for H_2 production), and (4) engineering the photosynthetic antenna pigment content for increased efficiency of solar light utilization, (5) compartmentalization of hydrogenase in an anaerobic compartment (Polle 2003).

There has been a focus on using cyanobacteria to produce H_2 (Tamagnini et al. 2002; Prince and Kheshgi 2005). While many of the challenges described above exist in these organisms, they are typically more easily engineered than eukaryotic algae and have more O_2- tolerant hydrogenases (Ghirardi et al. 2007). A possibility to improve the efficiency of biological H_2 production includes developing biohybrid (those with biological and synthetic components) and synthetic photosynthetic systems that mimic the fuel-producing processes of photosynthetic organisms. In all cases, more knowledge of photosynthesis, hydrogen evolution pathways, and hydrogenase structure and function is needed.

To circumvent the inhibition of hydrogenase by O_2, another option for H_2 production is to take advantage of the fermentation pathways that exist in some algae for H_2 production at night, using the carbon reserves produced during the day. In cyanobacteria, fermentation is constitutive, accounting for their ability to adapt quickly to changing environmental conditions (Stal and Krumbein 1987). All cyanobacteria examined thus far employ the Embden-Meyerhof-Parnas Pathway for degradation of glucose to pyruvate. Several cyanobacteria were found to use pyruvate-ferredoxin oxidoreductase, which reduces ferredoxin for subsequent H_2 production via nitrogenase or hydrogenase (Stal and Moezelaar 1997). This temporal separation of H_2 production from photosynthesis has been demonstrated in the unicellular cyanobacteria *Cyanothece sp.* ATCC 51142 (Toepal et al. 2008) and *Oscillatoria* (Stal and Krumbein 1987), using nitrogenase as the catalyst. Using hydrogenase as the catalyst, the unicellular non-N_2-fixing cyanobacterium *Gloeacansa alpicola* can evolve H_2 from the fermentation of stored glycogen (Serebryakova et al. 1998). Similarly under non-N_2-fixing conditions, the hydrogenase from *Cyanothece* PCC 7822 produces H_2 in the dark and also excretes typical fermentation by-products including acetate, formate, and CO_2 (van der Oost et al. 1989).

It is well-established that dark fermentation suffers from low H_2 molar yield (less than 4 moles of H_2 per mole hexose) (Turner et al. 2008). This is due to the production of organic waste by-products described above along with ethanol. In order to fully realize the potential of H_2 production via indirect biophotolysis, several challenges must be addressed: (1) improve photosynthetic efficiency to increase the yield of carbohydrate accumulation, (2) remove or down-regulate competing fermentative pathways thus directing more of the cellular flux toward hydrogen production, and (3) learn to express multiple hydrogenases so that electrons from both ferredoxin and NAD(P)H can serve as electron donors to support H_2 production.

Towards understanding hydrogenase diversity within the green algae, the physiology of a photosynthetically coupled [FeFe]-hydrogenase, containing a unique FeS cluster-binding domain, in *Chlorella variabilis* NC64A was described for the first time (Meuser et al. 2011). Several genetically modified strains have led to improved hydrogen production (for review, see Dubini and Ghirardi 2015); however, the limited ability to do gene targeting and site-directed mutagenesis in most strains of algae hinders this effort. Under the umbrella of the Consortium for Algal Biofuels Commercialization (CAB-Comm), when a gene involved in glycolysis (GAPDH-1) was deleted in cyanobacteria, the contribution of glycolysis to fermentative metabolism was reduced while rerouting the carbon through the Oxidative Pentose Phosphate pathway, resulting in a 2.3-fold increase in hydrogen production (Kumaraswamy et al. 2013). This demonstrates the potential of metabolic engineering for redirecting carbon for hydrogen production.

2.3 Algal Biotechnology

The biotechnology industry grew from more than 100 years of basic biology and genetics R&D. Collectively, biological process engineering breakthroughs directly enabled new multi-billion dollar commercial enterprises for agriculture, human health, and the production of chemicals. Thus, the importance of being able to harness biotechnology approaches to generate algae with desirable properties for the production of biofuels and bioproducts cannot be overlooked. However, methods to manipulate diverse classes of algae, except cyanobacteria, genetically remain far behind those developed for commonly used bacteria, fungi (yeast), and land plants.

Efforts should continue to be undertaken to understand the fundamental genetic and cellular processes involved in the synthesis and regulation of potential fuel precursors from diverse species of algae. While a better understanding of the basic biology of algal growth and metabolite accumulation using modern analytical approaches will provide a wealth of hypotheses for strain improvements, the limited algal genetic toolbox that can be used to modify process-relevant strains remains a significant technical hurdle. Thus, this section seeks to (1) examine the genetic tools available to modify algal strains, (2) describe enabling technologies and analyses that can be applied for biofuels and bioproducts, and (3) highlight a few examples of how algal biotechnology has been applied to date. Methods to cultivate and process algae in commercial settings are no less important to biotechnology, and these are the subjects of chapters 4 and 5.

Enabling Technologies: Omics Approaches and Bioinformatics

Omics technologies have been developed to simultaneously measure all of the components a biological system: genes (genomics), transcripts (transcriptomics), proteins (proteomics), metabolites (metabolomics), and phenotypes (phenomics)—enabling a global investigation of the molecular

and biochemical mechanisms that constrain biological function. Together, these methods have revolutionized the study of organisms both in culture and in natural habitats. These biotechnological advancements have been complemented by developments in computer sciences, creating the new field of bioinformatics where powerful new databases and search algorithms are helping biologists share and build upon experimental results in ways and timescales that were never before possible.

Algal species are being analyzed using these analytical approaches to better understand the underlying cellular processes and regulation involved in defining the attributes of the strain. Undoubtedly, the characterization of these cellular processes will prove useful for applications, forming the foundation for applied research and technology development.

Algal Genomes

Sequenced genomes are essential for determining the physiological potential of production strains and for strain improvement. With the development of more powerful sequencing methods, in which costs have been substantially reduced and more coverage is obtained in a shorter period of time, obtaining a genome sequence should be strongly considered for any strains being developed for biofuels research or production. It must be noted though, that the genomic data are only as useful as the annotation (the assignment of gene functions or families), so it will be important to provide sufficient resources and time to allow for detailed analysis of the data.

Genome size in algae can vary substantially, even in closely related species (Connolly et al. 2008). One reason for this variation is likely to be the accumulation of repeated sequences in the larger genomes (Hawkins et al. 2006). The challenge of sequencing larger, repeat-laden genomes is becoming easier through new, long read sequencing technologies, like PacBio and Oxford Nanopore. Scaffolding technologies from BioNano, Opgen, and Dovetail Genomics have further improved contiguity, enabling the assembly of nearly complete chromosomes. Conversely, full application of these technologies requires acquiring high molecular weight DNA, which is challenging given the complexity and thickness of algal cell wall in many strains.

Eukaryotic algae constitute members from at least eight major phyla, all featuring a complex series of primary and secondary endosymbioses (Falkowski et al. 2004). Although plastid genomes are generally conserved, it is likely that the different symbioses have affected the distribution of DNA between the plastid and nucleus (Wilhelm et al. 2006), which could impact the regulation and processes of fuel precursor production and may result in differences in the targeting of proteins to different intracellular compartments. A genomic survey of representatives from all major algal classes is desirable, with a special focus on classes or individual species within classes that make abundant fuel precursors.

Except for cyanobacteria, for which many completed genome sequences are available, the nuclear genomes of only a handful of microalgal species have been fully or partially sequenced prior to 2010, including three unicellular green algae (*Chlamydomonas reinhardtii*, *Volvox carteri*, *Chlorella variabilis*), a red alga (*Cyanidioschizon merolae*), several picoeukaryotes (*Osteococcus lucimarinus*, *Osteococcus tauris*, *Micromonas pussilla*, *Bathycoccus sp.*), a pelagophyte (*Aureococcus annophageferrens*), a coccolithophore (*Emiliania huxleyi*), several diatoms (*Phaeodactylum tricornutum*, *Thalassiosira pseudonana*, *Fragilariopsis cylindrus*), and the organellar genomes of *Dunaliella salina*.

Since 2010, substantial progress has been made towards sequencing diverse strains of microalgae. As a part of the NAABB sequencing effort, the nuclear genomes of several potential biofuel-production strains were sequenced to varying degrees of completion (Table 2.1), including three strains of *Chlorella sorokiniana* that differ significantly in sequence homology (Barry et al. 2015), *Nannochloropsis salina* (Starkenburg et al. 2014), and *Chrysochromulina tobin* (Hovde et al. 2014). In addition, other microalgae have been sequenced including *Nannochloropsis gaditana* (Radakovits et al. 2012; Wang et al. 2014), *Nannochloropsis granulata* (Wang et al. 2014), *Nannochloropsis oculata* (Wang et al. 2014), *Nannochloropsis oceanica* (Vieler et al. 2012; Wang et al. 2014), *Dunaliella salina* (Smith et al. 2010), *Coccomyxa subellipsoidea* (Blanc et al. 2012), the plastid genome of the red alga *Porphyridium purpureum* (Tajima et al. 2014), the glaucophyte *Cyanophora paradoxa* (Price et al. 2012), *Klebsormidium flaccidum* (a charophyte closely related to the land plant ancestor) (Hori et al. 2014), the diatom *Phaeodactylum tricornutum* (Bowler et al. 2008), and *Auxenochlorella protothecoides* (Gao et al. 2014). The sequencing of *Scenedesmus obliquus* has recently been completed (greenhouse.lanl.gov/organisms). Gene annotation and comparative genomic analysis of the data collected in these studies continues.

Bioinformatics analysis of sequenced genomes, especially at the basic level of gene annotation, will be essential to make sequence data usable. If not properly done, bioinformatics can represent the largest stumbling block to achieving that goal. Quality standards and appropriate training should be established at the onset of activities to ensure consistent and useful annotation. This could include the standardization of using a particular sequencing approach that provides sufficient coverage of full-length transcripts to ensure accurate gene modeling. Comparative genomics approaches between related organisms and organisms that carry out similar functions can also help assign gene function and identify metabolic pathways of interest. Furthermore, metabolic network modeling that integrates

Table 2.1. Sequenced Genomes under NAABB

Organism	Genome size (Mbp)	Assembly quality	Annotation
Picochlorum sp. DOE101	15.2	Improved HQ draft	Yes
Auxenochlorella protothercoides UTEX25	21.4	Improved HQ draft	No
Chrysochromulina tobin	59	High quality draft	Yes
Nannochloropsis salina 1776	29.4	High quality draft	Yes
Tetroselmis sp. LANL1001	220*	Standard draft	No
Chlorococcum sp.	150*	Standard draft	No
Chlorococcum sp.	59.3	Standard draft	Yes
Chlorella sorokiniana DOE1412	59.7	Standard draft	Yes
Chlorella sorokiniana str. 1228	61.2	Standard draft	Yes
Scenedesmus obliquus str. 1228	120*	Standard draft	No
RESEQUENCING PROJECTS			
A. protothecoides adapted mutant	21.4	N/A	N/A
Picochlorum sp. adapted mutant	15.2	N/A	N/A

Table created by Shawn Starkenburg, Los Alamos National Laboratory.

the genome-annotated enzymatic reactions and computational approaches can facilitate the elucidation of metabolic properties and functions at a systematic level, such has been done to examine carbon flux in *Auxenochlorella protothecoides* (Wu et al. 2015) and in *Synechocystis* 6803 (Xiong et al. 2015a).

Algal Transcriptomes

While genome sequencing will be an important component of any algal biofuels technology development effort, quantitative transcriptome profiling using new, high-throughput sequencing technologies will also become increasingly important because it will not only help with genome annotation (e.g., identifying coding regions of DNA), but it is also emerging as a robust approach for genome-wide expression analysis in response to particular environmental conditions.

Since 2010, analysis of gene expression by transcriptome analysis has become a standard tool in assessing environmental response in potential biofuel-production strains of microalgae. After gene identification either by partial or complete nuclear genome sequencing, several transcriptomic profiling studies of potential production strains of microalgae have been completed to elucidate gene expression under nutrient (such as nitrogen, phosphorous, or silicon) deprivation (Jia et al. 2015; Guarnieri et al. 2011; Li et al. 2014; Corteggiani Carpinelli et al. 2014; Mansfeldt et al. 2016; NAABB 2014; Smith et al. 2016), at different growth phases (Zheng et al. 2013; NAABB 2014), under heterotrophic and autotrophic growth (Gao et al. 2014; NAABB 2014), and many other conditions such as under varying light treatments, growth phase, nutrition, and heavy metal stress (NAABB 2014).

Transcriptomic analysis of one species can also assist in the annotation of genes of other species. Under NAABB, extensive transcriptome sequences to analyze genes involved in lipid production in *Chlamydomonas reinhardtii* were collected and utilized to generate gene models and functional annotations for the nuclear genome sequence collected for *Nannochloropsis salina*, *Picochlorum sp.*, and *Auxenochlorella protothecoides*, enabling the construction of metabolic pathways (NAABB 2014).

New, high-throughput sequencing technologies enable comprehensive coverage of transcripts and quantification of their relative abundance. Most transcriptomics approaches evaluate mRNA levels; however, small RNAs also play major regulatory roles in algae, including gene silencing (Kim et al. 2015; Cerutti et al. 2011; Bartel 2004; Cerutti and Casas-Mollano 2006). Small RNAs have been identified in microalgae (Zhao et al. 2007; Lopez-Gomollon et al. 2014) and in the

cyanobacterium *Synechocystis* (Klähn et al. 2015), and should be considered in investigations of gene expression regulation, especially with regard to translational regulation.

Algal Proteomes

The cellular complement of proteins reflects its metabolic potential, and ultimately determines how a cell functions in response to the environment. Mass spectrometry approaches and other proteomics technologies allow for robust evaluation of soluble and membrane-associated proteins in the form of protein peptides (for review, see Guarnieri and Pienkos 2015). These approaches not only enable protein identification, but also allow for protein quantification and detection of post-translational modifications (Domon and Aebersold 2006; Tanner et al. 2007; Castellana et al. 2008). It should be noted that proteomics is not feasible without a genome or annotated transcriptome from the same or a closely related organism.

Metabolomics and Lipidomics

The metabolome is the collection of small molecular weight compounds in a cell that are involved in growth, maintenance, and function. Because the chemical nature of metabolites varies more than for mRNA and proteins, different metabolomic analysis tools are applied, including combination of liquid chromatography/mass spectrometry, gas chromatography/mass spectrometry, and nuclear magnetic resonance (Dunn et al. 2005; Jones et al. 2012).

Lipids are a subset of the molecular repertoire of the algae cell. As cellular components, lipids contribute high energy density to algal cells and knowledge of their composition and production is therefore widely sought. While gas chromatography provides quantitation of lipid acyl groups (measured as methyl esters of acyl lipid side chains), mass spectrometry-based approaches also provide a means to interrogate intact lipid molecules. Lipid mass spectrometry approaches (Kind et al. 2012; Han and Gross 2005; Dettmer et al. 2007; Vieler et al. 2007; Milne et al. 2006; Holguin and Schaub 2013; Lu et al. 2013; MacDougall et al. 2011; Murphy and Gaskell 2011; Jones et al. 2012; Yao et al. 2015) identify changes in global lipidomes for different cultivation regimes and species to inform process engineering and improve yields (Yu et al. 2009). For molecular identification of the collected elemental compounds without doing tandem mass spectrometry and/or accurate mass measurement, an assembled reference database is required. Although not algae or plant specific, reference databases have been derived from the Lipid Metabolites and Pathways Strategy (LipidMAPS) database, a multi-institutional effort to identify and quantitate all of the major and many minor lipid species in mammalian cells (lipidmaps.org). Quantitative comparison of lipid type and abundance are critical components of lipid-based biofuels approaches as lipid characteristics can determine the suitability of the final fuel produced. The assembly of a public database of algal and plant lipids would speed this effort.

Algal Genetic Engineering

Because biological productivity is the key driver for economic viability, the ability to improve on native strains is a potentially important element in the research effort toward algal biofuels. Genetic approaches are commonly used to introduce, to delete or disrupt, and to modify genes or gene expression in a particular organism. Some of these methods can also be used to study the localization of gene products (mRNAs and proteins) within cells. For algae that undergo sexual reproduction, traits can be recombined into a single individual by mating parental strains. For all of these approaches, the stability of the desirable trait through many generations and the possibility of unintended horizontal gene transfer to other organisms are important research questions to consider in the context of mass production.

Mutagenesis

The generation and characterization of mutants is a powerful approach to understand gene function and potentially generate strains with desirable characteristics. As long as an appropriate screening process is developed, spontaneous mutants arising from errors in DNA replication can be identified. However, this approach is limited by the low frequency of naturally occurring mutations, which necessitates a large amount of screening. Mutants are more readily generated by standard chemical or UV-based mutagenesis approaches. Drawbacks of these approaches include the introduction of multiple mutations in a genome and in mapping the locus or loci responsible for the phenotype. When using these approaches, by selecting for competitive growth as well as the trait of interest (such as high TAG accumulation), deleterious secondary mutations may be prevented (Manandhar-Shrestha and Hildebrand 2013). Also, mapping mutant loci has been simplified recently by the reduction in cost of whole genome sequencing and the development of single-nucleotide polymorphism identification algorithms.

Targeted or tagged mutagenesis offers the advantage of simplified identification of the mutated gene. Targeted approaches rely on homologous recombination (if the native gene is to be entirely replaced) or introduction of a modified copy of the gene that inserts elsewhere into the genome. Certain strategies can also enable changes in gene expression. Tagging can be accomplished by introducing a selectable marker randomly into the genome (Adams et al. 2005), or through the use of transposons (Miller and Kirk 1999).

Any mutagenesis approach requires an appropriate screening technique to enrich for and isolate mutants. This can include either a requirement for mutants to grow under certain conditions (e.g., in the presence of an antibiotic), or to exhibit a characteristic phenotypic change that is easily assayed. For the latter, changes in fluorescence properties (e.g., reduced chlorophyll fluorescence; Polle et al. 2002), chlorophyll fluorescence parameter Fv/Fm (reflecting the maximum quantum efficiency

of PSII photochemistry), or increased neutral lipid accumulation via Nile Red staining (Cooksey et al. 1987) accompanied by a biochemical compositional analysis, can be screening criteria.

Given a well-developed screening approach, iterative selection could be used to generate useful algal strains without the need to generate genetically engineered algae—something that could be desirable for large-scale algal production. For example, current DOE funding supports the iterative and selective identification of high lipid-producing isolates of production strains utilizing flow cytometry.

For a review on current technologies employed to probe and edit the Chlamydomonas reinhardtii nuclear genome, see Jinkerson and Jonikas 2015.

Selectable Markers

A powerful way to manipulate genomes is the ability to introduce DNA into the cell, and to select for cells in which the DNA is present. Typically, this is accomplished by introducing an antibiotic resistance gene as a selectable marker (Hasnain et al. 1985; Dunahay et al. 1995), along with the DNA of interest (transgene), into the organellar or nuclear genome. Since in most instances, antibiotic resistance is not directly linked to transgene expression, it may not be necessary to maintain antibiotics at large-scale if the transgene is stably integrated into the genome or if counter-selection methods have been used to remove the antibiotic resistance cassette (Cheah et al. 2013). Recent studies have demonstrated that the long-term stability of transgene expression in the absences of direct selection can be impacted by the copy number of the transgene. Transformants with only single gene inserts had transgene expression levels that were > 90% of the initial expression levels after more than a year without selection unlike transformants with multiple gene copies (Kumar et al. 2013). The use of antibiotics in large-scale production should be avoided due to two major drawbacks. The first concern is the cost of the antibiotic. The second concern is the environmental implications of widespread antibiotic use, which could exacerbate current problems with increased antibiotic-resistant microbes. Antibiotic resistance is a powerful tool for research; however, other methods will need to be considered for production scale if transgene expression is dependent on antibiotic resistance. Selective markers that confer resistance to herbicide have been established in algae (Brueggeman et al. 2014; Cui et al. 2014), and marker systems that take advantage of the ability to genetically complement auxotrophic and metabolism mutants have also been achieved (Kindle et al. 1989; Debuchy et al. 1989).

For research purposes, if no other selectable marker is available, the decision as to which antibiotic selection marker to use includes whether the antibiotic compound is sensitive to light and whether its potency is modulated by the salinity of the growth medium. Several antibiotic markers have been developed for microalgae, including resistance to neomycin, kanamycin (Hasnain et al. 1985; Dunahay et al. 1995), zeocin (Apt et al. 1996; Hallmann and Rappel 1999), and nourseothricin (Poulsen et al. 2006). Besides whether or not the organism of interest is naturally resistant to an antibiotic marker, the mechanism of antibiotic resistance can also be an important factor. For example, zeocin resistance requires stoichiometric binding of the antibiotic by the resistance protein, whereas nourseothricin is inactivated enzymatically. A direct comparison of the two has shown that the nourseothricin system generates larger numbers of transformants (Poulsen et al. 2006), presumably because requirements for expression levels of the gene are lower and less taxing to the cells. Furthermore, use of certain antibiotic resistance markers may have public health implications. Avoidance of resistance to clinically useful antibiotics must be considered.

Sophisticated metabolic engineering could require the introduction of multiple selectable or complementary markers. Most of the current selectable markers are derived from bacterial genes, but markers based on resistance generated by conserved ribosomal protein mutations have also been successful (Del Pozo et al. 1993; Nelson et al. 1994). Caveats are that the mutated selectable marker gene may need to be expressed at a higher level than the native gene (Nelson et al. 1994), or that the native gene may need to be replaced in order to generate the phenotypic effect. For complementation approaches, appropriate mutations must be generated in the species of interest, ideally in well-characterized genes that can be easily complemented.

Once an appropriate antibiotic resistance or complementing gene is identified, constructs must be made to place the gene under control of an expression element that functions in the species of interest. This typically involves using control elements from a highly expressed gene in that species. However, there are examples of control elements that work across evolutionarily diverse species (Dunahay et al. 1995).

Transformation Methods

Gene transfer systems have been established in many algal strains, including cyanobacteria (*Synechococcus*, *Synechocystis*, *Anabaena*, *Nostoc*, *Arthrospira*, *Leptolyngbya*), green algae (*Chlamydomonas*, *Dunaliella*, *Chlorella*, *Volvox*), diatoms (*Cyclotella*, *Navicula*, *Phaeodactylum*, *Thalassiosira*), dinoflagellates (*Amphidinium*, *Symbiodinium*), red algae (*Cyanidioschyzon*, *Porphyridium*, *Gracilaria, Porphyra*), brown algae (*Laminaria*, *Undaria*), stramenopiles (*Nannochloropsis*), and euglenoids (*Euglena*). Hallmann (2007) provides a comprehensive review of algal transgenics and implications for biotechnology.

A common method for introducing DNA into algal cells is the biolistic ("gene gun") approach (Armaleo et al. 1990), which is useful for both nuclear and chloroplast transformation (Boynton et al. 1988; Dunahay 1993; Xiong and Sayre 2004). Other successful methods include electroporation (Shimogawara et al. 1998), vortexing with glass beads (Kindle et al. 1991) or silicon carbide whiskers (Dunahay 1993), *Agrobacterium tumefaciens*-mediated transformation (Cheng et al. 2012), polyethylene glycol-mediated transformation (Ohnuma et al. 2008), and bacterial conjugation (Karas et al. 2015). For most of these approaches, a fundamental challenge to introducing DNA into a cell is the nature of the cell wall. If methods exist to remove or perforate the cell wall, then chemically based methods of transformation could be applied. Many transformation methods also exist for cyanobacteria, including conjugation, electroporation, and biolistic approaches (Matsunaga and Takeyama 1995).

Sexual Crossing

Breeding of desired characteristics from a number of phenotypic variants can allow for strain development without creating genetically engineered algae. Algal strains often contain multiple copies of their genome, and so recessive genotypes may not manifest unless that genotype is allowed to "breed true" through a series of sexual crosses. With the exception of *Chlamydomonas reinhardtii*, classical genetic approaches using sexual crossing are not well developed in microalgae, but this methodology could prove to be extremely important. Some diatoms can be propagated vegetatively only for a limited number of generations and must be crossed periodically to maintain culture viability. Heterosis, the phenomena of hybrid vigor where the progeny of a cross between strains of a species are often more robust than each parental strain, is extremely important for crops used in agriculture (Jiang et al. 2013). Improved understanding of sexual crossing in algae is an important step towards enabling selective breeding done in traditional agriculture.

Homologous Recombination

Homologous recombination-based gene integration approaches are common in many strains of cyanobacteria, but less so in microalgae. DNA introduced into the nucleus of microalgal cells generally integrates randomly into the genome (Dunahay et al. 1995). Gene replacement via homologous recombination is more desirable than random integration because it can overcome phenotypic dominance issues when more than one copy of the gene is present, and can be used to knockout genes. Successful recombination approaches have included the addition of long flanking regions to the gene of interest (Deng and Capecchi 1992), use of single stranded DNA (Zorin et al. 2005), or co-introduction of recombinase genes with the transforming DNA (Reiss et al. 1996). Homologous recombination has been shown in *Nannochloropsis* (Kilian et al. 2011) and may serve as a useful targeted transformation tool for this organism.

Gene Expression Control Elements

Gene expression control elements (also known as transcriptional regulators) can modulate the levels of mRNA, which can then subsequently affect algae traits. These include, but are not limited to, promoters, transcription factors, transcriptional activators and repressors, and small regulatory RNAs. Frequently, transgenes are overexpressed by using strong control elements, but considering the need for balance in cellular metabolism, intermediate, slightly elevated, or reduced levels of expression may be desirable. Control element strength can be evaluated by monitoring mRNA levels by quantitative polymerase chain reaction or high-throughput transcriptomics (i.e., microarrays and RNA-seq).

In addition, inducible and repressible promoters that allow for precise control over timing of gene expression and are actuated by simple manipulations are desirable. The nitrate reductase promoter has proven useful in this regard in microalgae, because it is induced with nitrate in the growth medium, and repressed with ammonium (Poulsen and Kroger, 2005). More recently, the NAB-1 light-inducible promoter was utilized in *Chlamydomonas reinhardtii* to alter antenna size in response to light (NAABB 2014), and a green-light inducible system was designed for the cyanobacterium, *Synechocystis* (Abe et al. 2014a; Miyake et al. 2014). Copper- and IPTG-induced promoters are also available for cyanobacteria. For commercial applications, the practical and economic feasibility of altering gene expression in a scaled process should be considered. An important regulator for protein synthesis is the ribosome binding site, which influences translation initiation rate. Synthetic versions of the ribosome binding site have been shown to be effective in modulating protein expression in cyanobacteria (Xiong et al 2015b). Identification of other inducible or repressible control elements would be useful for both research and commercial applications.

Expression of antibiotic resistance, a common lab-scale screening tool for successful transformation, is generally not dependent upon expression of the transgene. To overcome this limitation in lab-scale screenings, a strategy utilizing the foot-and-mouth-disease-virus 2A self-cleavage peptide was developed in *Chlamydomonas reinhardtii* (Rasala et al. 2012) to link the expression of antibiotic resistance to transgene expression; research is currently underway to exploit this gene-stacking method in other strains of algae.

Small regulatory RNAs have been designed for precisely controlling gene regulation and expression (Storz et al. 2011). Termed riboregulators, these RNAs increase translation by the interaction of a trans-activating riboregulator with a target mRNA. The designing, selection, and tuning of these synthetic

sequences has resulted in increased expression in cyanobacteria (Abe et al. 2014b). Riboregulators have been designed with a large dynamic range of gene activation with physiologically relevant concentrations in bacterial cells (Krishnamurthy et al. 2015). As these tools are applied to algal systems, improved metabolic engineering, which often involves controlled expression of genes within a pathway, will be possible.

RNA Interference (RNAi)

RNAi can be a useful tool to down-regulate gene expression, especially in the study of polyploid organisms or when dealing with redundant genes where traditional genetic manipulations are difficult. RNAi operates through double-stranded RNAs that are cut down to small sizes and used to target suppression of specific genes by base pairing. RNAi can inhibit transcription (Storz et al. 2005) and control translation by either cleaving specific mRNAs or sequestering them away from the ribosome (Valencia-Sanchez et al. 2006). Two general types of RNAi vectors can be constructed—one containing an inverted repeat sequence from the gene to be silenced, and another in which bidirectional transcription generates the double stranded RNA. RNAi has also been induced with artificial microRNA vectors and transiently with double stranded RNA (for a review of RNAi in algae, see Cerutti et al. 2011).

RNAi approaches have been investigated in green algae (such as *Chlamydomonas reinhardtii* [Perrine et al. 2012]), in red algae (Ohnuma et al. 2009), in diatoms (Trentacoste et al. 2013), and many others (for a review, see Cerutti et al. 2011 and Banerjee et al. 2016). In a practical sense, selecting for functional RNAi can be problematic (Fuhrmann et al. 2001). Even on vectors containing both a selectable marker and an RNAi construct, only a small percentage of selected transformants will have functional RNAi. One solution to this problem was developed in *Chlamydomonas reinhardtii*: the selection process was based on a high-throughput phenotypic screen for functional RNAi by co-targeting an amino acid synthesis pathway along with the desired gene of interest (Rohr et al. 2004).

Genome Editing: ZFNs, TALENs, and CRISPR/Cas-Based RNA-Guided DNA Endonucleases

Zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), honing endonucleases, and CRISPR/Cas-based RNA-guided DNA endonucleases are powerful molecular tools that are redefining the boundaries of genetic analysis and manipulation. Genome editing allows the precise and efficient introduction of genetic variations, including mutation of single nucleotides, gene insertion, and deletion of chromosomal regions. Both ZFNs and TALENs employ engineered nucleases composed of sequence-specific and programmable DNA-binding domains fused to a non-specific DNA cleavage module. In the Type II CRISPR/Cas system, pieces of foreign DNA, spacers, are integrated within the CRISPR genomic loci and transcribed into short RNAs, crRNAs. These crRNAs anneal to trans-activating crRNAs and direct sequence-specific cleavage and silencing of pathogenic DNA by Cas proteins. The target recognized by the Cas protein, Cas9, requires a specific sequence within the crRNA and a conserved sequence upstream of the crRNA-binding region, allowing the CRISPR/Cas system to be programmed to cleave virtually any DNA sequence by redesign of the crRNA.

To date, CRISPR/Cas9 genome editing has been applied to human cells grown in lab dishes and to monkeys, dogs, mice and pigs, yeast, fruit flies, the worm *Caenorhabditis elegans*, zebrafish, and plants (sciencenews.org/article/gene-drives-spread-their-wings). Notably, an attempt at using CRISPR/Cas9 in *Chlamydomonas reinhardtii* was successful at transient modification, reporting Cas9 toxicity when constitutively expressed (Jiang et al. 2014), whereas the technology was shown to generate stable targeted mutations with no evident toxicity in a marine diatom, *Phaeodactylum tricornutum* (Nymark et al. 2016); therefore, the broad application of CRISPR/Cas9 in algae may not be straightforward. The development of CRISPR/Cas systems in potential production strains of microalgae is currently being funded by BETO. In combination with CRISPR/Cas9, gene drives have been shown to be a powerful tool in converting heterozygous mutations to homozygosity in a mutagenic chain reaction (Gantz and Bier 2015). This tool could be utilized in the future in multi-chromosomal microalgae to propagate genetic mutations within a cell or across generations of a mating population.

TALEN-mediated genome editing has been demonstrated in several species, including rice, mosquitos, silk worm, cricket, fruit fly, zebrafish, frog, mouse, rat, and human cells. Transcription activator-like effectors (TALEs) can also be designed to induce expression of a gene. In *Chlamydomonas reinhardtii*, a designed TALE system was used to target genes involved in sulfur metabolism and show elevated expression and protein activity after engineering (Gao et al. 2014). Albeit at low frequency, the use of ZFNs for nuclear gene targeting has been demonstrated in *Chlamydomonas reinhardtii* (Sizova et al. 2013).

Directed Evolution of Enzymes/Proteins

Directed evolution mimics the process of natural selection to evolve proteins or nucleic acids toward a goal. In the directed evolution of enzymes, proteins are modified by genetic engineering in a targeted manner to function more efficiently or to have favorable characteristics, such as attempting to engineer RubisCO to make it a faster catalyst (Whitney et al. 2011). Regarding core cellular metabolic processes, a substantial amount of regulation occurs at the protein level, including allosteric activation and metabolic feedback. Indeed, this level of regulation integrates the proteome with the metabolome. Although time consuming, approaches to modify proteins by genetic engineering could be valuable for the development of algal biofuels technology.

Protein Targeting, Tagging, and Reporter Gene Technologies

Targeted protein expression to specific algal cell locations, such as the chloroplast or mitochondria, may be advantageous when engineering specific metabolic pathways or conferring desirable traits. Vectors have been developed for the model strain *Chlamydomonas reinhardtii* that enable protein targeting to the nucleus, mitochondria, endoplasmic reticulum, and chloroplast utilizing native and foreign localization signal sequences (Rasala et al. 2014); development of these systems in potential biofuel production strains of algae will be valuable to future genetic engineering efforts.

Tagging proteins with fluorescent markers is useful in determining their intracellular location and can provide at least semi-quantitative evaluation of their abundance in a simple measurement. This information could be useful in monitoring intracellular metabolic processes associated with biofuel precursor production. Green fluorescent protein and its derivatives are the most widely used and versatile protein tags, but others have demonstrated utility and some possible advantages (Regoes and Hehl 2005; Gaietta et al. 2002). One algae-related complication to using conventional fluorescent protein reporters or tags is interference from native photosynthetic pigments resulting in competitive absorbance of excitation light, reabsorbance of emitted fluorescence, and interfering fluorescence. In the model green algae *Chlamydomonas reinhardtii*, a rainbow of fluorescent tags have been optimized and expressed (Rasala et al. 2013; Figure 2.5). In addition, flourescence resonance energy transfer-based biosensors have been expressed in microalgae for the quantification of cytoplasmic metal cation concentrations (Rajamani et al. 2014).

Reporter genes are utilized to monitor gene expression and have been used successfully in many organisms. The greatest difficulty in the utility of these systems is due to their low protein expression and detection. In algal systems, reporter genes have been highly expressed in the *Chlamydomonas reinhardtii* chloroplast by optimizing codon usage (Mayfield and Schultz 2004) and in the nucleus (Noor-Mohammadi et al. 2014; Ruecker et al. 2008). These systems have yet to be applied to or developed for potential biofuel-production strains of algae.

Applications of Biotechnology to Algal Bioenergy

Cyanobacteria

Genetic manipulation of cyanobacteria is generally more advanced than that of eukaryotic algae because many of the tools developed for bacterial genetics are applicable in cyanobacteria, although often requiring optimization. For example, spontaneous transformation, double-homologous recombination, and protein tagging are routine in some cyanobacterial systems, and at least half a dozen selectable markers are available for *Synechocystis* (Vermaas 1998). Recent methods

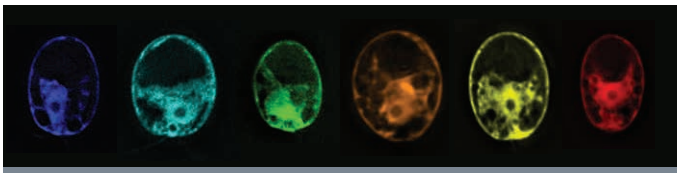

Figure 2.5. A rainbow of fluorescent tags expressed in *Chlamydomonas reinhardtii* (Image by Beth Rasala, University of California, San Diego, from Science Daily, sciencedaily.com/releases/2013/03/130307145109.htm)

have been developed to optimize high-efficiency integrative transformation; premethylation of DNA in *Escherichia coli* with a native *Synechocystis* methyltransferase C resulted in 11- to 161-fold-higher efficiency in the subsequent integrative transformation of *Synechocystis* 6803 (Wang et al. 2015b).

Cyanobacteria generally do not accumulate storage lipids, but they are prolific carbohydrate and secondary metabolite producers. Some strains can double quickly (a doubling time of 2 hours has been reported for *Synechococcus* sp. PCC 7002), and some strains can fix atmospheric nitrogen and produce hydrogen.

Synechocystis has been used extensively for the study of carbon metabolism toward production of hydrocarbon fuels and intermediates. The genome of this strain was sequenced more than a decade ago, the first among photosynthetic organisms (Kaneko et al. 1996). Many photosynthesis and carbon metabolism mutants have been generated, and high-throughput analytical techniques have been applied to the study of its transcriptome, proteome, and metabolome (Liu et al. 2011; Singh et al. 2008; Fulda et al. 2006; Koksharova et al. 2006; Eisenhut et al. 2008; Xiong et al. 2015a).

Transgenic approaches have enabled the production and secretion of cellulose, sucrose (Nobles and Brown 2008), ethanol (Deng and Coleman 1999), isobutanol (Atsumi et al. 2009), ethylene (Ungerer et al. 2012; Eckert et al. 2014), and free fatty acids (Ruffing 2014) in *Synechococcus*. Alkanes/alkenes (Wang et al. 2013) and isoprene, the basic unit of synthetic rubber, have been produced in engineered strains of *Synechocystis* (Lindberg et al. 2010). Members of Algoma Algal Biotechnology LLC have engineered fast-growing *Synechococcus* PCC 7002 to produce isoprene at ~80-fold the published rate for cyanobacteria (algomaalgal.com/about/about.html). See Angermayr et al. (2015) for a review of metabolic engineering in cyanobacteria. *Synechococcus* and *Anabaena* strains have been studied for their hydrogen production potential (Tamagnini et al. 2002). The latter is a filamentous strain that can form heterocysts, which are cells with specialized structure and metabolism that function anaerobically (important for the production of hydrogen). The engineering of an *Escherichia coli* xylose utilization pathway in *Synechocystis*, a trait not found in cyanobacteria, resulted in an increase in the excretion of keto acids, a carbon-excess

response that could be exploited to enhance biofuel production (Lee et al. 2015). Partly funded by BETO, ethylene was efficiently produced from the TCA cycle of *Synechocystis* with expression of a *Pseudomonas* ethylene-forming enzyme, and the engineered strain had an increased photosynthetic activity (Ungerer et al. 2012; Xiong et al. 2015b).

Despite all of the progress, a comprehensive understanding of carbon metabolism and regulation is not yet available in all cyanobacteria. In order to redirect carbon to a fuel production pathway, it will be necessary to further characterize the dominant carbon storage compounds (sinks) in cyanobacteria, including glycogen, glucosylglycerol, sucrose, and polyhydroxybutyrate (PHB), and the conditions that trigger carbohydrate accumulation. For example, it is known that glycogen accumulates under normal growth conditions in *Synechocystis*, whereas glucosylglycerol and sucrose can accumulate under salt stress (Yoo et al. 2007; Miao et al. 2003). Removal of glycogen synthesis results in increased production of target metabolites such as isobutanol (Li et al. 2014b) and lactic acid (van der Woude et al. 2014). In addition, under nitrogen starvation, glycogen synthesis mutants halt growth while converting CO_2 to excreted organic acids in "overflow metabolism" (Carrieri et al. 2012). Because these cells can transition between growth and overflow metabolism in response to nitrogen availability (Carrieri et al 2015), a catalysis/repair cycle may be possible for algal production of fuels and chemicals. It has not been determined how these pathways can be manipulated for the benefit of biofuels production. Furthermore, strain selection is important; for example, *Synechococcus elongatus* PCC 7942 does not contain the PHB pathway (Ruffing and Jones 2012). These studies can not only serve to advance the understanding of how the production of different carbon storage molecules are controlled in response to physiological conditions, but may also serve to guide the development of other types of algae for biofuels production.

Microalgae

Unicellular eukaryotic microalgae are the product of more than 3 billion years of evolution, and are highly diverse (Falkowski et al. 2004; Hildebrand et al. 2013). The plastids

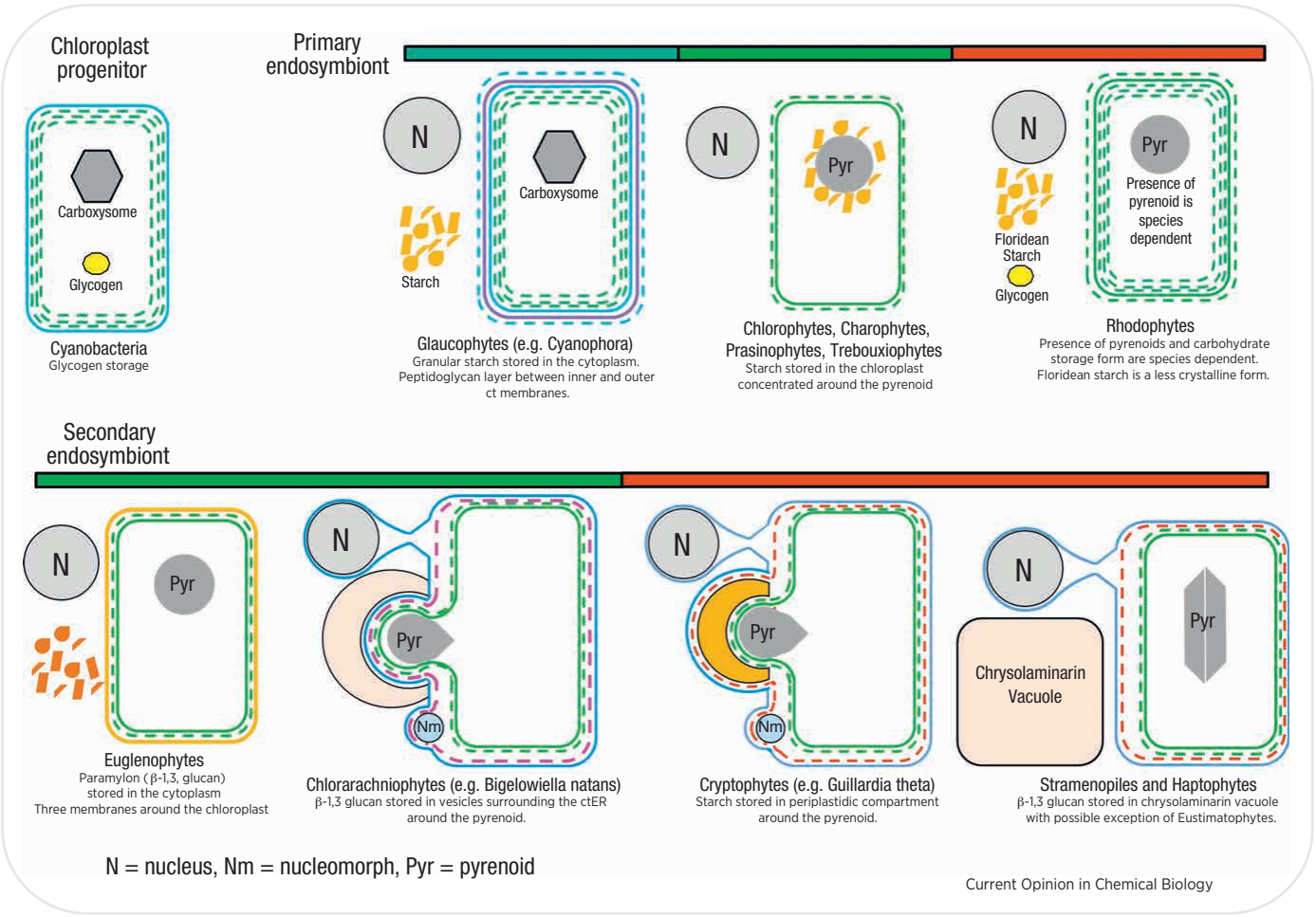

Figure 2.6. Organization of carbon fixation and carbohydrate storage in evolutionary-diverse classes of algae (Source: Hildebrand et al. 2013).

of photosynthetic microalgae are the results of endosymbiosis between a heterotrophic eukaryote and a cyanobacterium. Multiple endosymbiotic events occurred during the evolution of microalgae; for example, there is evidence that the chromalveolates (including the cryptophytes, the haptophytes, and the heterokonts or stramenopiles) acquired their plastids via endosymbioses that involved two eukaryotes: a non-photosynthetic host and a red algal endosymbiont (Green 2011). These events likely had significant effects on the metabolic pathways and regulation of fuel precursor synthesis (Hildebrand et al. 2013; Figure 2.6).

The different compartmental and metabolic arrangements in different classes of microalgae are likely to have resulted in different efficiencies in carbon processing; thus, a comparative analysis of different classes should result in a better understanding of optimized processes. For example, fatty acid synthesis, which occurs in the chloroplast, is at least partly regulated by nuclear-encoded gene products, and there are fundamental differences in the interaction between the nucleus and chloroplast in algae with different numbers of endosymbiosis events (Wilhelm et al. 2006). Continued exploration of the evolutionary diversity of algae is important to identify species that are adept at making fuel precursors with high productivity under various environmental conditions.

Chlamydomonas reinhardtii is the most studied eukaryotic algae. In addition to having a sequenced nuclear genome (Merchant et al. 2007) and well-developed transgenic capabilities (Jinkerson and Jonikas 2015; Rasala et al. 2014), it can be sexually crossed. It is not an abundant lipid producer, but nevertheless, *Chlamydomonas reinhardtii* can serve as a model system for understanding the fundamentals of lipid synthesis and regulation. Lipid production, like the production of other carbohydrate-based storage compounds, is also often dependent on environmental conditions, some of which await elucidation and development.

Transgenic efforts to improve biofuel production in other strains of microalgae are currently underway. Since 2010, targeted metabolic engineering of production strains in order to increase the production of lipids in microalgae has begun, including in model-strain *Chlamydomonas reinhardtii* (Blatti et al. 2012), diatoms *Thalassiosira pseudonana* and *Phaeodactylum tricornutum* (Trentacoste et al. 2013; Manandhar-Shrestha and Hildebrand 2015; Levitan et al. 2015b), *Haematococcus pluvialis* (Lei et al. 2012), and *Nannochloropsis oceanica* (Kaye et al. 2015). These studies have identified several protein targets for potential improvement of lipid production in the cell, including overexpression of acyl carrier protein, 3-ketoacyl-ACP-synthase, acyl-ACP thioesterase, and Δ12 desaturase, DGAT, and the knockdown of lipases involved in lipid catabolism or nitrate reductase involved in the assimilation of inorganic nitrogen.

As discussed throughout this chapter, microalgal engineering efforts are focused on several pathways, such as enhancing photosynthesis, enhancing or altering lipid biosynthesis, metabolic engineering to enhance existing pathways, or the introduction of new pathways for desired products. With the ongoing sequencing, transcriptomic, and proteomic efforts, an understanding of metabolic pathways, carbon partitioning, and lipid synthesis should be close at hand. However, significant knowledge gaps need to be filled between omics data, gene annotation, transgenic approaches, and an understanding of each unique microalgal system. Furthermore, the efficacy of each genetically engineered organism needs to be explored; for example, variation in gene expression throughout the cell culture cycle and eventual gene silencing in genetically modified *Nannochloropsis salina* has been observed (Beacham and Ali 2016). Potential epigenetic gene silencing mechanisms are widespread in algae, and the role that these mechanisms may play in transgene expression throughtout algal life cycles and under various environmental conditions remains virtually unexplored (Kim et al. 2015; Barahimipour et al. 2015).

Considerations of Genetic Modifications

Despite the great promise of genetically engineered algae, there is nevertheless a great deal of uncertainty regarding the need for or the appropriateness of deploying these strains. For the purpose of this review, genetically engineered algae are defined as strains carrying coding sequences obtained from a foreign species. In the United States, there has long been a framework for the oversight of biotechnology (CF 51 Fed. Reg. 23301, 1986; and 57 Fed. Reg. 6753, 1992). Since the beginning of the deployment of genetically engineered organisms, there have usually been built-in safeguards to prevent the release of genetically engineered organisms to avoid potential disruption of ecosystems. However, even with these safeguards, there have been several unintended releases of genetically engineered crops over the past 20+ years (GAO report 2008). Understanding the basic biology that will inform such aspects as lateral gene transfer, potential for toxin production, potential for large-scale blooms and subsequent anoxic zone formation, and choice of cultivation methods in terms of organism containment, are very important. Despite the uncertainty regarding the development of genetically engineered algae as production strains, development of genetic tools is still imperative from a research standpoint.

The United States has established pathways to obtain regulatory approvals for the use of genetically engineered algae in biofuel or biobased chemical production (see Glass 2015 for an extensive description; CF 51 Fed. Reg. 23301, 1986; and 57 Fed. Reg. 6753, 1992; EPA regulations [40 CFR Parts 700, 720, 721, 723, and 725 Microbial Products of Biotechnology; Final Regulation Under the Toxic Substances Control Act; Final Rule]). Regulations adopted by EPA under the Toxic Substances Control Act require notifications to the agency before commercial use of genetically engineered microorganisms

and agency review of proposed R&D activities of genetically engineered microbes, such as open-pond growth of genetically engineered algae; and other regulations under the USDA, FDA, and local agencies also need to be considered. The first Toxic Substances Control Act Experimental Release Application for the experimental outdoor use of a genetically engineered alga, *Scenedesmus dimorphus*, was approved in 2013 under a series of applications submitted by Sapphire Energy, Inc. (San Diego, California).

Several genetic engineering approaches and their predicted ecological risks have begun to be assessed (Table 2.2; Henley et al. 2013). Experiments exploring the possibility of algal drift are ongoing and will inform on the spread and proliferation of genetically engineered algae in the environment.

2.4 Macroalgae

Macroalgae, or seaweeds, represent a broad group of eukaryotic photosynthetic marine organisms. They are evolutionarily diverse and abundant in the world's oceans and coastal waters. They have low lipid content as a general rule but are high in carbohydrates that can be converted to various fuels. Macroalgae are multicellular and possess plant-like structural features. They are typically comprised of a blade or lamina, the stipe, and holdfast for anchoring the entire structure to hard substrates in marine environments. The life cycles of macroalgae are complex and diverse, with different species displaying variations of annual and perennial life histories, combinations of sexual and asexual reproductive strategies, and alternation of generations. Lipid accumulation in macroalgae typically being less than 5% of total dry weight (McDermid and Stuercke 2003), although concentrations approaching 20% lipid have been reported in some species (Chu et al. 2003; McDermid and Stuercke 2003)

Macroalgae are historically classified as Phaeophyta (brown algae), Chlorophyta (green algae), and Rhodophyta (red algae) on the basis of their predominant pigments. Currently, taxonomic affinities are under re-examination with the use of molecular tools and phylogenetic markers. As such, the status of macroalgal systematics is in a state of flux (Ben Ali et al. 2001; Baldauf 2003). The brown macroalgae such as the kelps are classified as Heterokonta within the Chromalveolata, which includes diatoms. Green macroalga such as *Ulva* (also known as sea lettuce) are classified together with common green microalgae such as *Chlamydomonas* and *Chlorella* as Chlorophyta. Red macroalgae such as *Porphyra* spp. also have microalgal counterparts, such as the unicellular alga *Porphyridium cruentum*.

Many macroalgae species are capable of sexual reproduction, and traditional mutagenesis and breeding have been used to improve commercial varieties of seaweed since the 1950s (Bird and Benson, 1987). Advances in seaweed cell and molecular biology are currently being applied toward a better understanding of seaweed genetics and cell function. For example, restriction fragment length polymorphisms and random amplified polymorphic DNA analysis are used in seaweed population genetics (Alberto et al. 1999; Bouza et al. 2006; Dutcher and Kapraun 1994; Ho et al. 1995; Niwa et al. 2005b), and strain selection and characterization (Jin et al. 1997; Meneses and Santelices 1999; Niwa et al. 2005a). Use of gene-specific probes (Jacobsen et al. 2003; Moulin et al. 1999; Roeder et al. 2005), and expression profiling (Collen et al. 2006) are being applied to understand cell function in representatives of red, brown, and green seaweeds. Recombination of existing genes through selection and procedures such as protoplast fusion will be the basis for new strain creation where outplanting of individuals for growth in natural environments is a goal. Genome sequencing projects will facilitate efforts such as global genomic and proteomic profiling, constructing detailed pathways for secondary metabolite production, and metabolic engineering of seaweed genes to create valuable products. Genome sequencing projects are underway for the macroalga *Porphyra purpurea* at the Joint Genome Institute (U.S. Department of Energy) and completed for *Ectocarpus siliculosus* (Cock et al. 2010). Plastid genomes sequences have been completed for red macroalga *Grateloupia taiwanensis* (DePriest et al. 2013), *Pyropia haitanensis* and *Porphyra yezoensis* (Wang et al. 2013).

Table 2.2. Genetic Engineering Approaches and Their Predicted Ecological Risks

Potential approach	Target genetically modified (GM) trait	Pros	Practical consequences of the approach	Selective advantage for GM algal dominance *in situ*	Potential harm if released GM alga thrives in nature	Predicted risk—should be tested experimentally in mesocosms, and tied to regulatory protection goals
1. Physiological enhancements						
Select for or GM-based high TAG content	High TAG accumulation	Higher yields of biodiesel	None	None—high TAG content would slow growth in situ	None unless constitutive high TAGs deter grazing	Very low—biotic, chemical and physical factors would like control GM alga in situ.
GM-enhanced photosynthesis	Smaller light harvesting antenna size	Higher cell density, C update in optimally dense cultures	None	None	None	Very low—unlikely to be competitive under in sit light conditions.
Select for or GM in optimal nutrient eficiency	Nutrient use efficiency	None	Low probability of success	None—natural algal assemblages already have diverse NUE strategies	None	Very low
2. Counteract contaminating algae						
GM in herbicide resistance and apply herbicide to cultures	Reduced competition	Reduced broad spectrum competitors	Small metabolic cost to detoxify herbicide; must contain herbicide	Would be favored by ambient residual nerbicides in surface waters	Reduced ecosystem services if ambient; herbicides favor GM alga. Higher risk if HGT to EDAB algal species	Low if little herbicide release to surface waters and avoid environmetally common herbicides such as atrazine. Low probability of HGT to EDAB algal species or algae toxic to humans.

Source: Henley et al. (2013). *Note:* EDAB = ecosystem-disruptive algal bloom; GM = genetically modified; HGT = horizontal gene transfer; NUE = nutrient use efficiency; TAG = triacyglycerol.

Table 2.2. *(continued)*

Potential approach	Target **genetically modified (GM)** trait	Pros	Practical consequences of the approach	Selective advantage for GM algal dominance *in situ*	Potential harm if released GM alga thrives in nature	Predicted risk—should be tested experimentally in mesocosms, and tied to regulatory protection goals
Select for natural of GM in allelopathy	Allelopathy	Reduced broad spectrum competitors	Metabolic cost reduces biomass production	Reduced competition	Reduced ecosystem services	Low—if an existing algal allelopathic compound is used. Moderate if a novel allelopathic compound is introduced or upon HGT to EDAB algal species.
3. Reduce grazing						
Select for preexisting toxin production (section 4)	N/A	Reduced macro- and miro-plankton grazers	Metabolic cost reduces biomass production	Reduced grazing pressure	Reduced ecosystem services	Low—some native algae are toxic and it is unlikely that GM alga more competitive than native toxic algae. Health risk to workers?
GM in a novel toxin	Reduced grazing	Reduced macro- and miro-plank-ton grazers	Metabolic cost reduces biomass production	Reduced grazing pressure	Reduced ecosystem services	Moderate—nov-el toxin(s) more likely to rovide a competitve advantage for released GM algae. Possible HGT to native EDAB algae or other organ-isms. Health risk to workers?

Source: Henley et al. (2013).

Table 2.2. *(continued)*

Potential approach	Target **genetically modified (GM)** trait	**Pros**	**Practical consequences of the approach**	**Selective advantage for GM algal dominance *in situ***	**Potential harm if released GM alga thrives in nature**	**Predicted risk—should be tested experimentally in mesocosms, and tied to regulatory protection goals**
3. Reduce grazing						
Select for or GM in poor food quality	Reduced grazing	Reduced macro- and micro-plankton grazers	None if no effect on biofuel production	Reduced grazing pressure	Reduced ecosystem services	Low—escaped algae GM for biofuel production are unlikely to have other competitive advantages *in situ*.
Apply pesticides to mass cultures	N/A	Reduce macro- but not micro-grazers (physiology similar to algae)	Toxic waste issue when effluent released	Selective advactage only if pesticide pollution persists	None unless effluent creates local environments favorable for the GM algae	Low—if the waste effluent is treated properly.
Biological control; fish trophic cascade (section 5.4)	None	Reduce macro- but not micro-grazers	Partial control of grazing. Attract birds as vectors for microbes	None	None unless an invasive species is used as the control agent and it escapes	Very low
Physical separation (size-cutoff filtration)	None	Exclude macrograzers without pesticides	Economically and energetically impractical?	N/A	N/A	N/A
4. Enhance pathogen resistance						
Select for natural pathogen resistance (Section 5.4)	None	Help reduce losses due to pathogens	Must rotate or add new genotypes of GM algae to resist new pathogens	N/A	N/A	None—resistance is selected from natural variation already present in wild type algae

Source: Henley et al. (2013).

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3. Resources for Algal Research

The original roadmap discussed the tools and resources currently available to the algae community. In the past 6 years, an immense amount of work has been done to develop additional molecular tools, databases, and other resources that can aid algal research. This chapter describes the resources available for research scientists today to grow, characterize, assess, and genetically modify algae (defined here as microalgae and cyanobacteria) at R&D-scale. Standardized methods to describe algal lipid content, maintain algae in culture collections, and characterize algal growth are vital for progressing algal biofuel research. Databases that collect genome, transcriptome, and proteome sequences of a suite of algal strains provide crucial, cataloged, open-access information to scientists. As molecular toolboxes are developed for these strains, their availability will become important in the examination of potential biofuel-production strains by genetic manipulation.

3.1 Algae Testbed Services and Real-Time Data Collection and Sharing

In addition to establishing and maintaining collaborative, open testbeds, ATP^3 provides academic and industry clients with services such as strain identification and isolation; biomass production and culture maintenance; analytical services to measure algal biomass and composition; equipment testing; and education and training in algal taxonomy, biology, and analytical techniques. (See chapter 1 for an introduction to the ATP^3 and RAFT testbeds.)

Although data is continuously collected across the global algae industry and by academic groups, the data may not be publicly available, and/or data is not comparable across different sites and production systems because of procedural inconsistencies. As part of the efforts to provide openly available, real-time data to the algae community, the ATP^3 and RAFT testbeds have created online databases for public access and data management. In its efforts to execute long-term, unified field studies at all its member sites, ATP^3 collects data on the effect of environmental and process conditions on algal growth rates and algal composition. This data is reported soon after collection at en.openei.org/wiki/ATP3. RAFT will provide data to users at knowyouralgae.com. Linking these databases to the Bioenergy Knowledge Discovery Framework (bioenergykdf.net) is a future goal of the program. Furthermore, a comparison between the actively collected empirical data on productivity with the theoretically predicted Biomass Assessment Tool (BAT) (see chapter 11 for more information on the BAT) could inform a next-generation predictive model to assess the potential contribution of algae as a biofuel feedstock of the future.

3.2 Role of Culture Collections as National Algae Data Resource Centers

Culture collections are necessary to preserve the diversity of natural habitats, protect genetic material, and provide basic research resources. At present, only a few major algal collection centers exist in the United States and other countries. They currently maintain thousands of different algal strains and support the research and industrial community with their expertise in algae biology. The function of a culture collection often transcends simple depository functions. Culture collections may also support research on strain characterization, cryopreservation, and phylogeny either by themselves or in connection with outside collaborators. Currently, no central database exists that provides global information on the characteristics of currently available algal strains. Protection of intellectual property in private industry has further restricted the flow of relevant strain data. Some minimal growth information is available from existing culture collections, but it is very difficult to obtain more detailed information on growth, metabolites, and robustness of particular existing strains. The establishment of a central, open-access strain repository could accelerate R&D of algae-based biofuels-production systems.

A number of algal strains are currently available from culture collections, such as the following:

- UTEX Culture Collection of Algae at the University of Texas at Austin, with more than 3,000 different strains of living algae
- The collection of microalgae at the Department of Life Sciences, University of Coimbra, with about 3,000 cultures identified to species level
- The Provasoli-Guillard National Center for Marine Algae and Microbiota, formerly the Center for the Culture of Marine Phytoplankton, at the Bigelow Laboratory for Ocean Sciences in West Boothbay Harbor, Maine, with more than 2,500 strains and the self-proclaimed largest collection of cryopreserved marine algae in the world
- The Culture Collection of Algae and Protozoa at the Scottish Association for Marine Science near Oban, Scotland, with more than 2,500 strains of algae and protozoa
- The Culture Collection of Algae at Goettingen (SAG) University, with about 1,600 species of microscopic algae (about 2,400 strains)
- The Belgian Co-Ordinated Collections of Micro-Organisms collection hosted by the Laboratory for Protistology & Aquatic Ecology (Gent University, Belgium) that specializes in diatoms

- The Pasteur Culture Collection of Cyanobacteria, and the Australian National Algae Culture Collection, part of the Commonwealth Scientific and Industrial Research Organisation with microalgal classes sourced from tropical Australia to Antarctica.

As part of the NAABB effort, 30 strains of the 1,595 strains screened were deposited at the UTEX Culture Collection of Algae (NAABB 2014). For both direct-breeding and metabolic-engineering approaches to improve biofuels production, these culture collections serve as a bioresource for further biofuels research.

As the major culture collections already collect and document data on strains, they could potentially serve as nuclei for the development of national algal resource centers. For example, the fatty acid profiles of 2,076 microalgal strains from the culture collection of algae at SAG were determined in the stationary phase, and the obtained fatty acid profiles were added into a database providing information about fatty acid composition (Lang et al. 2011). A similar effort could be supported in the United States as a first step to characterize the strains available in culture collections to provide a basis for lipid-productivity boundary conditions for different strains. Culture collection organizations could be responsible for the gathering and dissemination of detailed information about potentially valuable strains. Information could include the following items:

1. Strain name (species, subspecies name, taxonomy, reference)
2. Strain administration (number in collection, preservation, cryotechniques)
3. Environment and strain history (specific habitat, collector)
4. Strain properties (cytological, biochemical, molecular, pigment profiling and screening results)
5. Mutants
6. Plasmids and phages
7. Growth conditions (media, temperature, pH, optima) and germination conditions
8. Biological interaction (symbiosis, pathogenicity, toxicity)
9. Practical applications (general and industrial)
10. Omics data (genomics, transcriptomics, proteomics, or metabolomics).

3.3 Omics Databases

Open and easy access to genomic and proteomic information and sequence is an important priority for molecular biologists and molecular geneticists alike. The National Center for Biotechnology Information GenBank database is designed to provide and encourage access to the most up-to-date and comprehensive DNA sequence information for all organisms to members of the scientific community. Protein sequences are archived in another international consortium, Universal Protein Resource, which is a central repository of protein sequence and function. However, these databases are not algae-specific and do not provide species-specific gene annotation or integration with other data of interest to algal researchers. Phytozome (phytozome.net), a comparative hub for plant genome and gene family data and analysis, provides sequence and functional annotations for several strains of algae sequenced at the Joint Genome Institute, as well as selected species sequenced elsewhere. Although these databases provide valuable information for some species, there is no database that compiles all of the sequenced strains of algae into one searchable resource with comparative gene annotation. In order to meet these needs, under past NAABB- and BETO-funded national laboratory work, two publicly available omics-based databases were developed: the Algal Functional Annotation Tool and the Greenhouse. Further development of these tools will become vital as new species are sequenced, proteomics data are accumulated, strains are environmentally selected or genetically modified, and performance data is analyzed.

In order to aid with identifying algal proteins of unknown function, researchers at the University of California, Los Angeles developed the Algal Functional Annotation Tool (pathways.mcdb.ucla.edu/algal/index.html). The Algal Functional Annotation Tool is a bioinformatics resource to visualize pathway maps, determine biological search terms, or identify genes to elucidate biological function *in silico*. These types of analyses have been designed to support lists of gene identifiers, such as those coming from transcriptome gene-expression analysis. By analyzing the functional annotation of an interesting set of genes, common biological motifs may be elucidated and a first-pass analysis can direct further searches. Currently, the following databases have been parsed, processed, and added to the tool:

- Kyoto Encyclopedia of Genes and Genomes Pathways Database
- MetaCyc Encyclopedia of Metabolic Pathways
- Panther Pathways Database, Reactome Pathways Database
- Gene Ontology
- MapMan Ontology
- Eukaryotic Clusters of Orthologous Groups
- Pfam
- InterPro.

Currently the Algal Functional Annotation Tool only contains data for *Chlamydomonas reinhardtii* and *Chlorella* NC64A.

The metabolome coverage for less-investigated species of algae in pathway databases is currently nonexistent (Kind et al. 2012). As mentioned in chapter 2, although not algae- or

plant-specific, reference databases have been derived from the LipidMAPS (Lipid Metabolites and Pathways Strategy) database (Holguin and Schaub 2013). Even for the best-studied algae, *Chlamydomonas reinhardtii*, the Kyoto Encyclopedia of Genes and Genomes Pathways Database has incorporated the LipidMAPS categories, but it lacks the majority of molecular lipids. To allow collection and later queries, molecules, taxonomy, and metadata must be directly submitted to electronic databases (Kind et al. 2009), but these public repositories do not yet exist.

Developed by Los Alamos National Laboratory scientists, the goal of the Greenhouse (greenhouse.lanl.gov/) is to provide a centralized website to display and share sequence-based data relevant to the improvement and advancement of algal biofuel feedstocks. Still in the beginning stages of development, this database seeks to provide consistent annotations across all algal species and strains; searchable data based on taxonomy, gene name, locus tag, protein function/families, pathways, Enzyme Commission numbers, and others; BLAST (Basic Local Alignment Search Tool)-searchable algal genomic databases; numerous comparative analyses and interactive visualizations; exportable sequences and information; data in a variety of formats; and user workspaces to allow manipulation of data within customized groups. Importantly, the Greenhouse plans to provide an integration of data (such as lipid accumulation and productivity), phenomics, and metabolomics.

3.4 Genetic Toolboxes

A wide assortment of genetic tools has been developed for the lab-strain *Chlamydomonas reinhardtii*, including fluorescent tags and reporter genes for both nuclear and chloroplast expression (Rasala et al. 2013; Mayfield and Schultz 2004; Kumar et al. 2013), targeted expression constructs that link expression of the gene-of-interest to a selection gene (Rasala et al. 2012; Rasala et al. 2014), and nuclear gene editing and activation (Gao et al. 2015 Jinkerson and Jonikas 2015). Transformation and expression in cyanobacteria is also well-established (Koksharova and Wolk 2002). In the genetic engineering of production strains of algae, few robust molecular toolboxes have been developed, mainly due to the difficulty in selecting native promoter and terminator sequences for expression and successful transformation methods that are often species-specific (Kilian et al. 2011; Georgianna et al. 2013 Vieler et al. 2012; Radakovits et al. 2012). Although many transformation procedures are published, not all vectors or their sequence are publicly available. See chapter 2 for more on gene transformation and expression in algae.

3.5 Growth Prediction Tools

The modeling of algal growth is especially important when predicting algal performance under a variety of fluctuating environmental and production conditions. In 2010, the biomass growth model was developed to calculate the growth of specific algal strains specific for raceway pond conditions (Wigmosta et al. 2011; Huesemann et al. 2013). The biomass growth model was further enhanced to predict biomass productivity in nutrient-replete outdoor ponds subjected to typical diurnal fluctuations in light intensities and water temperatures; the model was validated for three difference species (*Chlorella sorokiniana*, *Nannochloropsis salina*, *Picochlorum* sp.) (Huesemann et al. 2016a). This tool relies on experimental growth data collected in the lab for each strain of interest under varying conditions, and it is an important component of the BAT. (See chapter 11 for more background on BAT.) The model takes into account a strain's maximum specific-growth rate as a function of temperature, light intensity, pH, and salinity. The model also considers light attenuation by biomass and the strain's biomass-loss rate in the dark as a function of temperature and the average light intensity during the preceding light period. To date, only a small number of strains have been examined, due to the monumental task of experimentally determining the strain-specific model-input parameters.

Other algae growth models have been developed. In contrast to the raceway-specific biomass growth model, a model predicting algae growth in photobioreactors under various conditions was introduced and validated with *Chlorella sorokiniana* and *Chlamydomonas reinhardtii* (Blanken et al. 2016). Recently, a physics-based computational algae growth model that could model multiple types of growth systems across varying scales was developed (Gharagozloo et al. 2014). Using this model, light and temperature were determined to be the most limiting environmental parameters to the growth of *Nannochloropsis salina*. If the predictive capability of this model is expanded to the growth of algae in production-scale systems, there may be less of a need for large-scale growth experiments. A concerted effort to expand these tools to a more diverse set of fresh and salt water strains under production conditions would be of considerable benefit to future algal performance-modeling efforts. Furthermore, alternative or complementary methods of growth prediction may be developed from improved understanding of the relationship between growth phases and specific metabolite levels.

3.6 Standardization and Biomass Analysis Resources

Biochemical composition of algal biomass is variable, dependent on the strain and nutrient makeup of the culture medium. Algal cells change their metabolic composition throughout growth and in response to environmental and physiological stimuli. Thus, in the production of algae for biofuels, the time of harvest can greatly affect fuel yields and downstream processing characteristics. Analytical tools developed for algal biomass characterization need to be accurate, precise, and strain agnostic. In order to standardize these analyses, work by BETO-funded scientists at NREL has led to the development of standardized analytical procedures (Laurens et al.

2012; Laurens et al. 2014; Laurens et al. 2015; Templeton and Laurens 2015). A current set of procedures is available as open-source laboratory analytical procedures online and are updated periodically based on community feedback (nrel.gov/bioenergy/microalgal-biofuels-analysis.html). These procedures have been implemented in a multi-institutional testbed project, where aligning procedures across different sites was imperative to compare geographical influences on productivity. This was the first demonstration and implementation of standardization of procedures for algal biomass characterization and could form the basis for further development of voluntary consensus standardization of composition metrics in algae. Toward developing and advocating algal industry standards and best practices, the Algae Biomass Organization released in October 2015 a set of minimum descriptive parameters and metrics required to fully characterize the economic, sustainability, and environmental inputs and outputs of an aquatic biomass processing operation in *Industrial Algal Measurements*, Version 7.0 (ABO Technical Standards Committee 2015). These are voluntary procedures, and the development team is open to feedback from the community.

3.7 Lab-Scale Performance Tools

In order to predict production-scale performance of algal strains, cultures are often studied in laboratory-scale experiments. Although small volumes allow rapid screening and testing of algae under a variety of conditions, these small-scale experiments often do not mimic the behavior of cultures outdoors. Prior to 2010, laboratory-scale photobioreactors that imitate the dynamic environmental conditions found under biofuel production conditions were unavailable. Work performed under NAABB produced a new type of environmental photobioreactor (ePBR) that simulated key environmental parameters that influence algal photosynthesis and growth the most such as light intensity and quality, temperature, gas exchange, and mixing (Figure 3.1; NAABB 2014; Lucker et al. 2014). The ePBR consists of a columnar vessel to mimic a water column in an algal production pond by using collimated white light from a high-power, light-emitting diode (LED) to reasonably reproduce both the intensity of sunlight and the light gradient throughout the water column. The ePBR provides programmable computer control over light, mixing, temperature, and gas flow while autonomously measuring optical density and pH. Additionally, the system is scalable for parallel and matrix experiments. The total volume of the ePBR is relatively small, ~550 mL, which allows for growth-comparative assessments; however, in most cases, this volume is unable to provide enough biomass for compositional assessment of the biomass. This has led to some groups developing their own programmable system (e.g., NREL's Simulated Algal Growth Environment reactor).

The company Phenometrics was formed to produce and sell the ePBR and began selling an updated version, the PBR101,

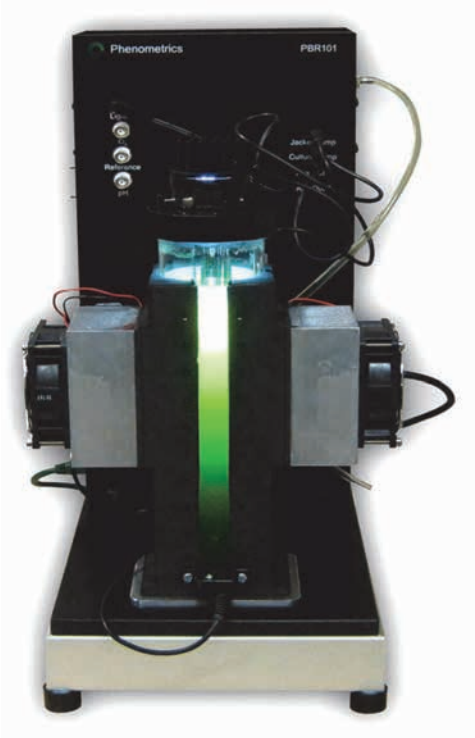

Figure 3.1. An ePBR (Photo courtesy of Phenometrics)

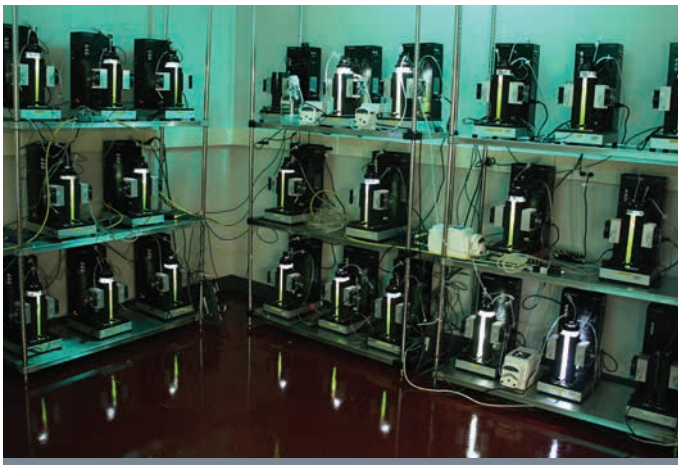

Figure 3.2. The Los Alamos National Laboratory/New Mexico Consortium ePBR matrix

in 2013. Phenometrics has sold to more than 100 companies, research institutes, and universities for both research as well as commercial purposes (scale-up and production optimization), with most sales consisting of multiple units. Currently, large matrices of ePBRs are operated at Los Alamos National Laboratory (37; see Figure 3.2), Michigan State University (soon to be 50), and the University of Technology in Sydney, Australia (20). The ePBRs have been utilized to examine the effects of variable temperature and light on algal growth (Lucker et al. 2014; Tamburic et al. 2014), the characteristics of *Chlorella sorokiniana* under nitrogen limitation (Negi et al.

2015), the selection of algal strains with desirable traits under simulated outdoor conditions, and other ongoing projects.

While the Phenometrics ePBRs are excellent lab-scale tools to evaluate the growth of microalgae under varying environmental conditions, there is uncertainty as to whether they accurately simulate the biomass growth in outdoor ponds. In an effort to determine the performance of new strains in outdoor ponds at any geographic location and season of choice, Pacific Northwest National Laboratory (PNNL) utilizes four indoor, 800-L, temperature-controlled raceway ponds, illuminated each with a panel containing 4,500 multi-colored computer-dimmable LEDs (Figure 3.3), for climate-simulated culturing studies. The required sunlight and temperature scripts for a selected geographic location, season, and pond depth are generated by the BAT (Wigmosta et al. 2011). Validation studies with *Chlorella sorokiniana*, DOE1412, demonstrated that the biomass productivities in the indoor, climate-simulation ponds were comparable to those observed in outdoor ponds (Huesemann et al. 2016b). Consequently, PNNL's indoor testbeds have been used to determine the seasonal biomass productivity of *Chlorella sorokiniana* under conditions simulating southern Florida and other geographic locations in the United States.

PNNL has also built and validated a system of six 1.8-L Laboratory Environmental Algae Pond Simulator (LEAPS) photobioreactors with LED lighting and temperature control (Figure 3.4). The PNNL indoor ponds and LEAPS photobioreactors are important tools for quantifying the biomass productivity of new strains under conditions simulating outdoor ponds at any geographic location and season of choice.

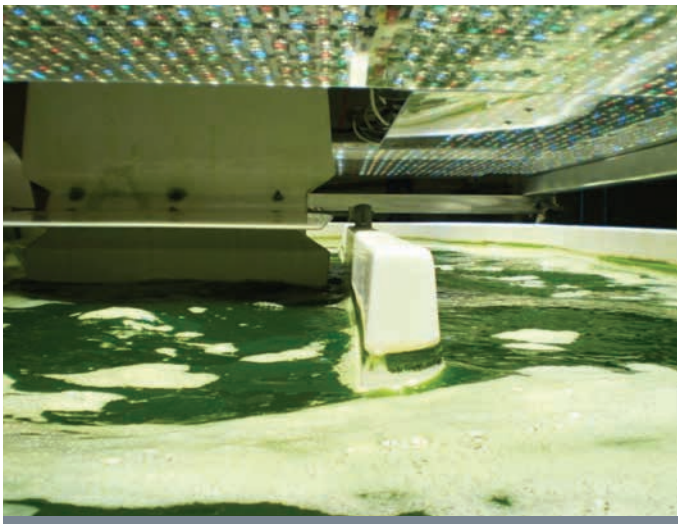

Figure 3.3. PNNL's indoor, LED-lighted, and temperature-controlled climate-simulation raceway pond (Photo courtesy of Michael Huesemann, PNNL)

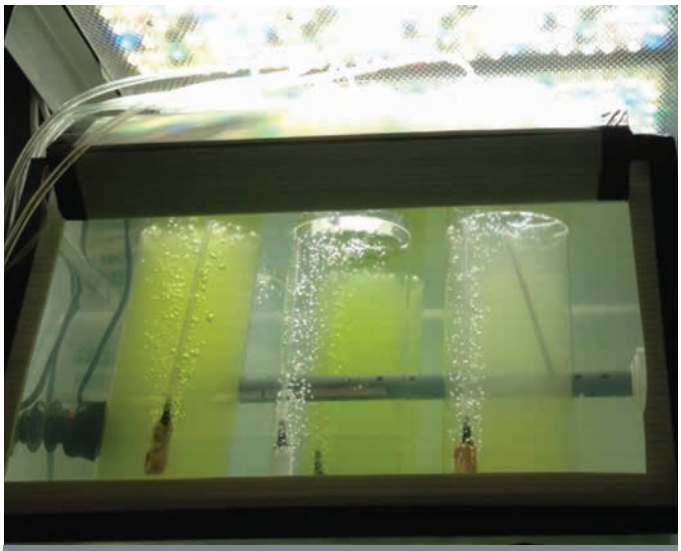

Figure 3.4. PNNL's LEAPS photobioreactors (Photo courtesy of Michael Huesemann, PNNL)

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4. Algal Cultivation

4.1 Cultivation Pathways

There are a number of engineering solutions for the cultivation of microalgae and cyanobacteria: closed systems (e.g., photobioreactors), open systems (e.g., open ponds), hybrid systems, and attached-growth systems. Each of these systems has advantages and disadvantages from both technical and economic perspectives, and therefore, selection of system type is largely dependent on the product(s) being produced. A brief description of these cultivation methods, the advantages and disadvantages, as well as potential applications is provided below. (Macroalgal cultivation approaches are discussed in section 4.3). Data from DOE-funded work over the last 6 years has demonstrated the importance of techno-economic analysis (TEA) and systems analysis to evaluate cultivation systems within context of integration requirements for upstream and downstream processing. Systems analysis is described further in chapter 11.

Photoautotrophic vs. Heterotrophic

Cultivation of algae can be achieved via photoautotrophic, heterotrophic, or mixotrophic methods, which also vary in their challenges and advantages (Table 4.1). In photoautotrophic cultivation, algae require light to grow and create new biomass. In heterotrophic cultivation, algae are grown without light and are fed a carbon source, such as sugars, to generate new biomass. Mixotrophic cultivation harnesses both the photoautotrophic and heterotrophic ability of algae. Heterotrophic and mixotrophic cultivation strategies present a different set of advantages and challenges compared with photoautotrophic methods. Optimal conditions for production and contamination prevention are often easier to maintain, and there is the potential to utilize lignocellulosic sugars (the only sugars allowable for BETO funding) or carbon-rich wastewater for algal growth. Growth on a carbon source also achieves high biomass concentrations that can reduce the extent and cost of the infrastructure required to grow the algae (Xu 2006). However, the primary challenges with these approaches are the cost and availability of suitable feedstocks such as lignocellulosic sugars. Because these systems rely on primary productivity from other sources, they could compete for feedstocks with other biofuel technologies.

Open vs. Closed Systems

For photoautotrophic cultivation strategies, where algae require light to grow and generate new biomass, capital costs for closed photobioreactor construction are currently higher than for open ponds. However, it is important to acknowledge the operational advantages and disadvantages of both cultivation approaches (Table 4.1). Environmental and socioeconomic sustainability considerations for both approaches are discussed in chapter 10.

In theory, closed photobioreactor systems can provide improved environmental control (temperature, evaporative water loss, monoculture maintenance, predator and pathogen control) compared to open ponds (Wang et al. 2012). Depending on design, deployment location, and local environmental conditions, photobioreactors may lose as much water from evaporation as open ponds due to aeration and lack of condensation capability, whereas others may lose less water, but may not receive the benefit of evaporative cooling. This results in a requirement for more-active temperature management (such as incorporating heat exchangers with active cooling subsystems that add capital and operating expense). The need for cooling in photobioreactors can be avoided by growing thermotolerant or extremophilic algae strains (Selvaratnam et al. 2014) or by cultivating in cooler climates. Photobioreactors are unlikely to be sterilizable and may require periodic cleaning due to biofilm formation or other contamination issues; but, long-term culture maintenance is likely to be superior to that in open ponds where contamination and "foreign" algae are more readily introduced. Photobioreactors can also provide a higher surface-to-volume ratio and so can support higher-volumetric cell densities, reducing the amount of water that must be processed—and thus, the cost of harvest (Chisti 2007).

Traditionally, photobioreactors have suffered from problems of scalability. Mixing and gas exchange (both CO_2 and O_2) at larger unit sizes can pose challenges. Due to their modular nature, scale-up can be achieved by increasing the number of photobioreactor units, as demonstrated by Algenol Biotech, LLC. However, to achieve similar volumetric scales of biomass production to an open-pond approach, a significant number of photobioreactors are likely to be needed, which could pose commercial challenges of higher capital cost (greater amounts of material for individual photobioreactors and tubing to deliver nutrients and CO_2) and operational complexity (monitoring and maintaining culture conditions as well as harvesting from a greater number of units). If photobioreactors are oriented vertically, they could reduce land footprint and deliver an even greater areal productivity per unit area. Both types of cultivation systems must contend with optimizing light exposure, as opposed to heterotrophic cultivation, which does not depend on light abundance.

Many of these issues are being addressed through improved material usage and enhanced engineering designs. As reviewed by Quinn and Davis (2015), all of the studies that directly compare the costs associated with open raceway ponds and photobioreactors conclude that the open raceway ponds are economically advantageous (Davis et al. 2011; Richardson et al. 2012). However, these studies assume similar productivities and culture stability, qualities that are expected to improve in large-scale photobioreactors compared to open systems (Quinn et al. 2012). For a summary of TEA studies, see chapter 11.

Pond designs vary, and so they have variable energy and nutrient requirements, as well as capital and operating

Table 4.1. Comparative Features of Microalgal and Cyanobacterial Cultivation Approaches

Growth Mode	System Approach	Advantages	Challenges
Photoautotrophic Cultivation	Closed Photobioreactors	• Less loss of water than open systems • Superior long-term culture maintenance and stability • Higher surface-to-volume ratio can support higher volumetric cell densities	• Capital intensive • Scalability problems • Temperature maintenance required if they do not have evaporative cooling • Decontamination and biofilm cleaning • Optimal light exposure is necessary
	Open Ponds	• Evaporative cooling maintains temperature • Lower capital costs than closed systems • More easily scalable	• Subject to daily and seasonal changes in temperature, solar insolation, humidity, wind, etc. • Inherently difficult to maintain monocultures • Optimal light exposure is necessary
	Open Attached Systems	• Growth of polyculture that can be relatively stable and robust • Pulsed, shallow flow provides higher surface-to-volume ratio that can support higher volumetric cell densities • Evaporative cooling maintains temperature • Unnecessary to supplement growth with CO_2 and nutrients • Ability to clean contaminated surfaced waters of excess N and P • Lower capital costs than closed systems • More easily scalable	• Subject to daily and seasonal changes in temperature, solar insolation, humidity, wind, etc. • Maximum light exposure is necessary • Subject to higher ash content in harvested algal material • Polyculture algae biomass is generally low in neutral lipids and higher in carbohydrate and protein content, requiring thermochemical or biochemical processing for fuels • Without supplementation, productivity subject to variations in N, P, and C content of source waters
Heterotrophic Cultivation	Closed Industrial Bioreactors	• Easier to maintain optimal conditions for production and contamination prevention • Opportunity to utilize inexpensive lignocellulosic sugars for growth • High biomass concentrations	• Cost and availability of suitable feedstocks such as lignocellulosic sugars • Competition for feedstocks with other biofuel technologies • Conversion process rather than a primary source of biomass

expenditures. Raceway ponds, often referred to as "Oswald" ponds, typically use paddle wheels to circulate the culture; they are 30–60 cm deep, operate at a hydraulic residence time of 3–6 days, and channel velocities of 15 cm per second (ABO 2015). Some novel pond-construction designs include serpentine gravity-flow ponds (the channels are continuously sloped to induce circulation by gravity and pumping) and hypothetical 50-acre ponds that do not require paddle wheel mixing. See Davis 2016 for a comparison of pond-engineering designs. As in the photobioreactor facility design, commercial-scale, open-pond cultivation facilities are also expected to scale via modular-unit design.

In hybrid systems, photobioreactors could play a critical role as breeder/feeder systems linked to open ponds, providing high cell density algal inocula for production ponds (Ben-Amotz 1995), or a series of linked turbidostats or chemostats (Benson

et al. 2007). At the 2.5-ha Cellana, LLC Kona Demonstration Facility, large photobioreactors (25 m^3 culture volume) continuously supply cost-efficient microalgae inocula for open ponds (Huntley et al. 2015).

In addition to ponds, open, attached cultivation systems, such as the Algae Turf Scrubber® (ATS™), are an alternative cultivation approach that has the potential for large-scale production. The ATS is an ecologically engineered system that uses shallow, pulsed, turbulent flow in sloped floways naturally seeded with a diverse polyculture assemblage of benthic and planktonic algae and other organisms (Laughinghouse 2012). Originally designed in the early 1980s for the simulation of aquatic ecosystems (Adey et al. 2007) and later adapted for nutrient removal from contaminated surface waters (Adey et al. 1993), ATS systems have been used in the treatment of agricultural drainage, municipal wastewater, and non-point source contamination of lakes, rivers, estuararies, and coastal bays. Various pilot-scale ATS systems have been demonstrated (Adey et al. 2011; Adey et al. 2013; Craggs et al. 1996a; and Craggs et al. 1996b; Kangas et al. 2014; Mulbry et al. 2008; Lundquist et al. 2004; and Sandefur et al. 2011), as well as larger multi-acre, commercial-scale systems (HydroMentia 2005). ATS offers an interesting approach for possible biofuel feedstock production because of its dual-use capability of cleaning surface waters of excess nutrients while also producing robust polyculture algal biomass without the cost and logistics of supplying additional nutrients and supplemental CO_2.

For a discussion of current, open and closed algal-cultivation systems and characteristic system parameters, see chapter 7 of the ABO *Industrial Algal Measurements* (ABO 2015).

4.2 Cultivation Scale-Up Challenges

Scaling up algal technologies continues to be one of the largest challenges facing the industry. The inherent difficulties of scaling up from laboratory to commercial operations present biological, technical, economic, and sustainability barriers to success. Nutrient sources (including inorganic carbon in the form of carbonate or CO_2) and water treatment/recycling seem trivial and inexpensive at small scales and, yet, represent major technical and economic problems at commercial scales. Tapping into existing agricultural or municipal waste streams will lower nutrient costs but could introduce pathogens, chemical compounds, or heavy metals into the biomass stream (Hoffman et al. 2008; Wilson et al. 2009). Additionally, little is known about artificial algae-pond ecology or pathology, and investigation into these areas will be important for the development of large-scale cultivation, risk–mitigation, and remediation strategies. For example, the effects of standard operating procedures, such as inoculation and harvesting protocols, on pond ecology or pathology at large-scale are not well-understood.

As a result of the pervasiveness of issues related to outdoor cultivation, an investment in "open source" testbed facilities for public sector RD&D is thought to foster more cultivation research. To this end, the ATP^3, with five locations in the United States, was developed in 2013 with funding from BETO and cost-sharing from partners. ATP^3 provides open testbed facilities for collaborative research, development, and deployment of algal technologies, production, analysis, and commercialization processes (atp3.org). Although the ATP^3 testbeds address some of the issues with outdoor cultivation, such as strain performance, they are still considered not large enough to address many issues related to scale.

Process-Development-Scale and Integrated Biorefinery "Lessons Learned"

Process-development demonstration facilities, including Sapphire Energy in New Mexico, Algenol Biotech LLC in Florida, Cellana LLC and Global Algae Innovations in Hawaii, provide important data on large-scale algal cultivation. Research conducted by Sapphire Energy concluded that the most significant barriers to algal biomass production at large scale is the lack of understanding of microalgal biology for optimal biomass production and the difficulty in the translation of high-yielding lab-scale experimental results to large-scale production (White and Ryan 2015).

At Cellana's Kona Demonstration Facility, cultures of *Staurosira* and *Desmodesmus* had higher biomass and lipid yields when grown with nitrogen, and CO_2 delivery was highlighted as a potential constraint (Huntley et al. 2015). Beal et al. (2015) performed TEA and life-cycle analysis of 10 case studies that sought to assess the barriers of large-scale cultivation at the Kona Demonstration Facility. These case studies included: unreliable cultivation methods, large nutrient requirements (of carbon, nitrogen, and phosphorus), low energy return on investment (EROI), high capital costs, and competition from existing commodity products. The analysis determined that most cases have an inhibitory EROI with high capital costs and large nutrient requirements (carbon, nitrogen, and phosphorus) (Beal et al. 2015).

Integrated biorefineries apply research and development to scale designs to a degree relevant to commercial facilities. BETO funding of integrated biorefinery projects helps to advance the industry by minimizing the risk of these technologies for private investors. Integrated biorefineries use novel technologies and diverse biomass feedstocks—requiring significant investments in RD&D to reduce costs, improve performance, and achieve competitiveness with fossil fuels. Algae integrated biorefineries are unique from the rest of the demonstration projects in the BETO portfolio in that they must incorporate the cultivation of their feedstock in addition to refining the biomass into biofuels.

The process of developing an algae integrated biorefinery is challenging because of the integration of novel technologies or novel technology applications. A detailed and realistic project scope and expectations are critical to the successful deployment of an integrated biorefinery.

BETO has, so far, funded four integrated biorefineries focused on algal cultivation and processing:

- Solazyme, Inc., an integrated pilot project involving heterotrophic algae that can convert cellulosic sugars to diesel fuel
- Sapphire Energy, Inc., a demonstration-scale project involving the construction and operation of a 100-acre algae farm and conversion facility for the production of renewable bio-crude
- Algenol Biotech, LLC, an integrated, pilot project involving the photosynthetic production of ethanol and the delivery of a photobioreactor system that can be scaled for commercial operation
- BioProcess Algae, LLC, a pilot project growing low-cost algae using renewable CO_2, lignocellulosic sugars, and waste heat provided by a co-located ethanol plant.

Lessons learned from these BETO-funded projects have identified four broad cultivation challenges important to address for economically viable, commercial-scale algal cultivation:

- Culture stability and management
- Scalable system designs, including effective cultivation system management and operations
- Nutrient source scaling, sustainability, and management
- Water conservation, management, and sustainability.

Stability of Large-Scale Cultures

In open-cultivation systems, it will be challenging to maintain algal monocultures at large scales, and therefore, it may become necessary to understand and manage the communities that will be present. In addition to the crop algae, some members of the underlying microbiome will be of positive value, such as those that can scavenge and recycle nutrients or synthesize essential vitamins (Natrah et al. 2014; Helliwell et al. 2014; Kazamia et al. 2012a; Cole, 1982). Others will compete for shared resources, and, still, others will cause culture disruption. In these instances, opportunities may exist to use beneficial microbiome members in co-culture or polyculture to strengthen pond resilience through an ecological approach (Kazamia et al. 2012b; Smith and Crews 2014).

One of the more worrisome components of large-scale algae cultivation is that algal predators and pathogens are both pervasive and little understood (Becker 1994; Honda et al. 1999; Cheng et al. 2004; Brussaard 2004). Although there may be between 40,000 and several million phytoplankton species, as of 2009, there were only 150 formal descriptions of phycoviruses (Wilson et al. 2009). However, from the study of *Paramecium bursaria* chlorella virus 1 (PBCV-1) and other viruses, it is becoming apparent that all viral factories appear to share fundamental, structural features that are essential for their function (Milrot et al. 2015). Chytrid fungi have also been known to cause the collapse of industrial algal cultivation ponds (Hoffman et al. 2008) and in a wastewater photobiorector (Carney et al. 2014), but very little is known about host specificity, and even less is known about host resistance mechanisms (Carney and Lane 2014). In production ponds at Sapphire Energy, Inc., a parasite of the microalgae *Scenedesmus dimorphus* was recently characterized, revealing it as a new species in the phylum Aphelida (Letcher et al. 2013; Letcher et al. 2015). A host-resistance mechanism against amoeba grazing of cyanobacteria has been identified by screening a mutagenesis library of *Synechococcus elongatus* (Simkovsky et al. 2012). Further studies to classify and characterize algal predators and pathogens will enable the development of culture-management strategies.

Research from Sapphire Energy, Inc. reported that rotifers, ciliates, and amoebas were commonly found grazers at the Columbus, New Mexico, farm, and that pests were often seasonal (White and Ryan 2015). Invasion of unwanted alga taxa, described as weed contamination, was reported as the most-harmful invasive issue. Weed contamination appeared in ponds at predictable times of year, as well as seasonally or during certain types of weather events. These findings stressed the importance of developing and employing integrated pest-management practices (McBride et al. 2014; White and Ryan 2015). The predatory bacterium, *Vampirovibrio chlorellavorus*, has emerged in pond crashes of *Chlorella* sp. (Soo et al. 2015). Recently, Heliae Development, LLC obtained a patent for the control of *Vampirovibrio chlorellavorus* infection of mixotrophic *Chlorella* cultures through a pH shock treatment (Ganuza and Tonkovick 2015).

Important questions concerning the threat of contamination of large-scale algal cultures include the following:

- Are agricultural or municipal waste streams—a potentially significant source of nutrients for algal cultivation—actually a liability because of significant reservoirs of algal pathogens and predators?
- To what extent will local "weedy" algae invade and take over photobioreactors and open ponds that are focused on cultivating specific, intended monocultures?
- What prevention or treatment measures might limit such takeovers?

- What are the regulations to allow treatments to be used at scale (e.g., regulatory approval for relabeling of pesticides for use on algae ponds, or regulations on concentrations of non-pesticide chemicals)?

Methods for rapid, automated, or semi-automated biological and chemical monitoring in production settings that are also sensitive, selective, and inexpensive will be essential for assessing the health and compositional dynamics of algal cultures. "Environmental" DNA-sequence analysis can contribute to the development of polymerase chain reaction-based (Zhu et al. 2005; Boutte et al. 2006; Viprey et al. 2008) or flow-cytometry-based taxonomic assays (Marie et al. 2005; Day et al. 2012). To screen algal health, quantitative polymerase chain reaction has been developed to screen for weedy algal strains (Fulbright et al. 2014; McBride et al., 2014) and for detection of fungal disease at a large-scale facility (White and Ryan 2015). Utilizing microscopic techniques and flow cytometry to monitor algal cell morphology and pest presence, and examining photosynthetic potential of the culture via chlorophyll fluorescence, assist in early pest detection (White and Ryan 2015; Fulbright et al. 2014; Carney and Lane 2014; Collins et al. 2014). However, the cost of reagents and the labor involved in physically sampling algal culture at large scales has led to the development of alternative pond-assessment tools. Spectroradiometric monitoring, the measurement of hyperspectral reflectance, is emerging as a promising analytical tool to rapidly monitor open ponds without physically sampling the culture to quantify algal growth rates, assess algal stress, detect the presence of invading species, and determine the optimum time for harvesting (Reichardt et al. 2012; Reichardt et al. 2014). Continuous monitoring will be necessary in open systems since seasonal variation in competitors, predators, and pathogens is expected (Hoffman et al. 2008; Rittmann et al. 2008; Wilson et al. 2009).

Furthermore, developing an understanding of pond speciation, predator-prey relationships, and ecology dynamics will be important. Early detection schemes for invasive species, predators, and pathogens will be a key to the success of remedial actions and determining when decontamination and subsequent restart procedures represent the only alternative (McBride et al. 2014). This information will also inform efforts to develop robust, competitive production strains. The frequency of contamination events that require decontamination/restarts will be an important parameter in the cost of production because of productivity lost during down time and the potential need to either discard or treat the contaminated culture prior to water recycle. The development of chemical treatments or physiological adaptations and genetic modifications of production strains may become necessary. Dynamic pond monitoring will be important for both wild-type and genetically modified algae, whose competitiveness in the field cannot be accurately predicted. Thus, an investment toward basic research in multi-trophic, molecular-level algal ecology can be important for developing the potential of algae and increasing annual biomass yields. BETO's 2014 competitive award solicitation, "Targeted Algal Biofuels and Bioproducts," dedicated a topic area to crop-protection R&D.

Scalable System Designs: Maintaining Productivity

Closing the lab-to-field yield gap is crucial to the development of large-scale algae production (White and Ryan 2015). Bench-scale systems that can be shown to directly mimic conditions and outcomes of a large-scale production pond or photobioreactor are important to this endeavor (see chapter 3 for current, available tools). Research at the interface between basic algal biology and cultivation science and engineering will yield significant improvements in productivity while at the same time lower the cost of production. Utilization of existing and new knowledge related to the physiological regulation of lipid or carbohydrate accumulation, coupled with scalable cultivation schemes, should lead to enhancements in productivity. For example, nitrogen nutrition has long been known to affect lipid accumulation in phytoplankton, and nitrogen deprivation has been examined in production strains with varying effects on lipid production (Ketchum and Redfield 1938; Shifrin and Chisholm 1981; Benemann and Oswald 1996; Sheehan et al. 1998; Huntley et al. 2015; Negi et al. 2015; Pal et al. 2011). Data also suggest that high salt and high light stress in some marine phytoplankton also affect lipid content (Azachi et al. 2002; Pal et al. 2011).

From a productivity standpoint, supplemental CO_2 has long been known to increase algal growth rate, and this area is receiving increasing attention from the search for renewable, sustainable fuels. New approaches include improving algae utilization of CO_2 from emission gasses (Rosenberg et al. 2008; Douskova et al. 2009), increasing mass transfer of CO_2 to the culture, and developing a greater understanding of the mechanisms of biological CO_2 concentration (Lapointe et al. 2008; Spalding 2007. There is justification to carry out research development and demonstration in both areas, as siting requirements for efficient algal cultivation will not always co-locate with high-volume point sources of CO_2 (see chapter 10). In addition to controlling nutrient supply, control of CO_2 flux within the system enables pH management, which can deter contamination and may improve metabolic flux (Selvaratnam et al. 2014; Wang and Curtis 2015). Co-locating algae facilities with CO_2 point sources is discussed further in chapter 10.

Better methods to detect the amount of desired fuel precursor produced will be required to assess the productivity of potential strains. Fluorescent and nuclear magnetic resonance-based methods for rapid lipid content screening in algae have been developed and applied to many different types of phytoplankton (Cooksey et al. 1987; Reed et al. 1999; Eltgroth et al. 2005; Gao et al. 2008; Doan and Obbard 2011; Terashima et al. 2015; Traller and Hildebrand 2013; Xie et al. 2014). These

tools, as well as others such as near infrared spectroscopy, need to be more rigorously studied, automated, and adapted for rapid, inexpensive, high-throughput monitoring. The synthesis of new non-toxic, permeable, fluorescent indicators other than Nile Red are also important. For example, derivatives of the boron-dipyrromethene (BODIPY) molecule with higher lipophilicity or lower quantum yields in aqueous solvent are being utilized and may prove to be more reliable indicators of algal lipid content and lipid oxidation (Gocze and Freeman 1994; Hallenbeck et al. 2015; Cheloni et al. 2013).

There is an immediate need to standardize productivity models and establish protocols for measurement of yields, rates, densities, metabolites, and normalization across varying large-scale growth platforms. To encourage data harmonization, ABO collected a set of cultivation and biomass test methods by standard development agencies (ABO 2015). Along with standards, coordinated research among analytical chemists, physiologists, biochemists, and genetic, chemical, civil and mechanical engineers is needed for rapid progress. National and international efforts toward generating quality assurance policy standards early on in the development of an algal biofuel industry could facilitate the deployment of algal-based biofuels by ensuring consistent, fit-for-purpose fuels and products.

Nutrient Sources, Sustainability, and Management

Nutrient supplies for algal cultivation have a sizeable impact on cost, sustainability, and production siting. The primary focus is the major nutrients—nitrogen, phosphorous, and iron (and silicon, in the case of diatoms). Nitrogen, phosphorous, and iron additions account for an operating cost of 6–8 cents-per-gallon of algal fuel in 1987 U.S. dollars (Benemann and Oswald 1996). This calculation takes into account a 50% rate of nutrient recycle. Phosphorous appears to be an especially important issue as there have been calculations that the world's supply of easily-accessible phosphate is in danger of running out (Cordell et al. 2009). Requirements for additional nutrients, such as sulfur, trace metals, vitamins, etc. must also be considered, but vary depending upon the specific strain and water source chosen. The use and availability of carbon-based nutrients for heterotrophic growth will also affect the economics and sustainability of such systems. Strain selection should take nutrient requirements into account. Nitrogen is typically supplied in one of three forms: ammonia, nitrate, or urea. The ideal form of nitrogen is a function of relative costs and the specific strain's biology. Because synthetic nitrogen fixation processes utilize fossil fuels (particularly natural gas), costs are tied to fossil fuel prices.

It is possible to consider the use of nitrogen-fixing cyanobacteria as a way to provide nitrogen biologically, perhaps in co-culture with eukaryotic algae. However, such a scheme will certainly have some impact on overall productivity levels as photosynthetic energy could be diverted from carbon fixation to nitrogen fixation, which may or may not be compensated for by the "free" nitrogen. It is important to note that flue gas fed to algal cultures may provide some of the nitrogen and sulfur needed from nitrogen oxide and sulfur oxide (Douskova et al. 2009). Careful control of nutrient levels is also critical. While limitation of a key nutrient will have serious impacts on biomass productivity, it may also be desirable to use nutrient limitation (e.g., nitrogen, phosphorous, or silicon) as a means to induce oil accumulation in the cells (Sheehan et al. 1998). On the other hand, too much of a particular nutrient may prove toxic. Also, unused nutrients in the culture medium may pose a problem for wastewater discharge. Although economics dictate that the bulk of water derived from the harvesting step must be returned to the cultivation system (where remaining nutrients can feed subsequent algal growth), a certain amount of "blowdown" water may need to be removed to prevent salt buildup. If this blowdown water contains substantial nitrogen and phosphorous, disposal will become a problem due to concerns of eutrophication of surface waters.

Finding inexpensive or underutilized sources of nutrients will be an important factor in algal cultivation. Agricultural- or commodity-grade nutrients are generally applicable, but reagent-grade sources of nutrients could make the price of a gallon of algal fuel cost-prohibitive. Utilizing the nutrient content of municipal, agricultural, or industrial waste streams is a very attractive alternative. Options for algal growth with nutrients recovered from waste streams include use of nutrients from industrial and agricultural wastewaters and anaerobic digestor effluent (Cai et al. 2013). Struvite ($MgNH_4PO_4$), a major precipitate in wastewater streams, has been shown to provide increased nutrient utilization efficiency and satisfies algal trace metal requirements (Davis et al. 2015).

Currently, algae are used in some wastewater treatment facilities because of their ability to provide oxygen for the bacterial breakdown of organic materials and to sequester nitrogen and phosphorous into biomass for water cleanup. Utilizing agricultural runoff also poses economic benefits by preventing eutrophication. A potential problem with this approach, however, is the lack of enough water for large-scale algal biofuel production and the impact on facility siting (see chapters 10 and 11). Wastewater treatment facilities, for example, tend to be near metropolitan areas with high land prices and limited land availability, and it is not practical to transport wastewater over long distances. Further research into the availability and compatibility of wastewater resources is warranted.

Another approach to reduce nutrient costs is to pursue a diligent recycle. The final fuel product from algal oil is generally deplete of nitrogen, phosphorous, or iron as these nutrients end up primarily in the spent algal biomass. From a cultivation-sustainability perspective, nutrient recycle may prove to be more valuable than using the spent biomass for products such as animal feed. However, from an overall operational perspective, if the protein component of algal biomass is

used to substitute terrestrial sources of feed, it could result in a more economical and environmentally sustainable use of nutrient resources. For a discussion of nutrient recycling in relation to specific conversion processes, see chapter 7. As a general overview, if the biomass residues are, for example, treated by anaerobic digestion to produce biogas, then most of the nutrients will remain in the digestor sludge and can be returned to the growth system (Benemann and Oswald 1996; Lundquist et al. 2010). Likewise, the first stage of sequential HTL has been utilized for nutrient recycle to the cultivation system (Selvaratnam et al. 2015). The processes through which these nutrients are re-mobilized and made available for algal growth are not well understood. This may be particularly problematic for the recycling of silicon, which is a component of diatom cell walls. In the future, it may also become necessary to expand the limits of analysis to include recycling of nutrients from animal waste. Nutrient sourcing and the control of nutrient levels are important factors for cultivation economics, productivity, and sustainability. Important research areas therefore include the following:

- TEA and life-cycle analysis to understand the cost, energy, and environmental sustainability implications of various scenarios of nutrient and water use and recycling
- Studies to explore the mechanisms of nutrient recycling, (e.g, from anaerobic digestion sludges)
- GIS analyses of wastewater resources to understand availability, compatibility with cultivation sites, and potential impact of such sources on algal biofuels production.

Water Management, Conservation, and Sustainability

One of the main advantages of using algae for biofuels production is algae's ability to thrive in water unsuitable for land crops, such as saline water from aquifers and seawater. At the same time, however, water management poses some of the largest issues for algal biofuels. If not addressed adequately, water can easily become a "showstopper," either because of real or perceived socioeconomic sustainability challenges (e.g., public concern over freshwater supply) or environmental sustainability issues (see chapter 10). For a discussion of the use of wastewater in algal cultivation and the regulations and permitting required, see chapter 4 of the Algae Biomass Organization's Industrial Algae Measurements (ABO 2015).

At installation, a scaled algae cultivation system will require a large, upfront volume of water. For example, a hypothetical 1 ha, 20 cm deep open pond will require 530,000 gallons to fill. In desert areas, evaporative losses can exceed 0.5 cm per day (182.5 cm/yr) (Weissman and Tillet, 1989), which is a loss of 13,000 gallons per day from the 1-ha pond. Sapphire Energy's Columbus Algal Biomass Farm measured an evaporation rate around 203 cm/yr, greater than the local evaporation rate of 152–178 cm/yr in Columbus, New Mexico (White and Ryan 2015). Though the water used to initially fill the pond can be saline, brackish, produced water from oil wells, municipal wastewater, or other low- quality water stream, the water being lost to evaporation is freshwater, and continually making up the volume with low-quality water will concentrate salts, toxins, and other materials in the culture. This can be prevented by adding fresh water, which depending on resource siting and without monitoring could be costly or unsustainable, or by disposing of a portion of the pond volume each day as "blowdown." The amount of blowdown required for salinity control is dependent upon the acceptable salt level in the culture and the salinity of the replacement water.

Water recycling is essential, but the amount that can be recycled depends on the algal strain, water, process, and location. Some actively growing algal cultures can double their biomass on a daily basis, meaning that half the culture volume must be processed daily. This is an enormous amount of water (260,000 gallons per day in the 1-ha example above). To contain costs, it is desirable to recycle most of that water back to the culture. However, accumulated salts, chemical flocculants used in harvesting, or biological inhibitors produced by the strains themselves could impair growth if recycled to the culture. Furthermore, moving around such large volumes of water is very energy intensive and could impose a significant cost.

Treatment may be essential for water entering and exiting the process. Incoming water (surface water, groundwater, wastewater, or seawater) may be suitable as is, or may require decontamination, disinfection, or other remediation before use. Disposal of the spent water, which could contain salts, residual nitrogen and phosphorous fertilizer, accumulated toxics, heavy metals (e.g., from flue gas), flocculants, and residual live algal cells, could pose a serious problem, and treatment (e.g., desalination, activated charcoal filtration, etc.) of the recycled stream could be cost-prohibitive. These constituents are examples of water quality indicators and could be monitored to evaluate the environmental sustainability of algae cultivation. Surface disposal and reinjection of spent water into wells may be an option, as regulated by EPA and is already practiced by the oil industry, but live cells could adversely affect biodiversity of neighboring ecosystems or result in the dissemination of genetically modified organisms. Sterilization of blowdown water, however, would be a very costly and energy-intensive proposition. The development of inexpensive methods for reducing viable cell counts of culture fluids prior to discharge would be helpful, such as major shifts in pH or temperature.

Because of the importance of issues surrounding the use of water, research in the following areas is warranted:

- GIS analysis of water resources, including saline aquifers, and their proximity to utilizable cultivation sites that may have lower-pan evaporation rates

- Understanding the long-term effects of drawing down saline aquifers, including the geology of these aquifers and associations with freshwater systems
- Analysis and definition of the regulatory landscape surrounding discharge of water containing various levels of salt, flocculants, toxins (including heavy metals), and live cells
- Developing cultivation systems with minimal water consumption. This could include reducing evaporative cooling loads through such strategies as selecting thermotolerant strains of algae
- Studying water recycle and methods to maximize recycle (and minimize blowdown), while effectively managing the accumulation of salt and other inhibitors
- Investigating ways to reduce the cost of water treatment, makeup water/recycle, and water movement (pumping costs).

4.3 Macroalgae

Modern macroalgal cultivation technology that is based on the use of artificially produced seed as a source of propagules has been in practice since the 1950s. Typically, seeds grown in greenhouses are attached to substrates (usually rope structures), then reared to plantlet size and transplanted to coastal farms for grow-out to harvestable size. Modern tools developed in the terrestrial plant breeding community are now available to macroalgae biologists and culturalists to advance the vegetative propagation of macroalgae through cell and tissue culture techniques. Although the field is still at an early stage of development in the United States, the micropropagation of plants is a concept that has been adopted by macroalgae biologists (Garcia-Reina et al. 1991). Demonstrations of successful callus formation and plantlet regeneration have been reported in commercially important seaweeds such as *Undaria* (Kawashima and Tokuda 1993) and the phycocolloid-producing seaweeds *Gracilaria, Hypnea, Sargassum, Turbinaria, and Gelidiella* (Collantes et al. 2004; Kumar et al. 2004; Kumar et al. 2007). Growth of plantlets regenerated from protoplasts is possible in both the laboratory (Dipakkore et al. 2005) and field (Dai et al. 2004; Dai et al. 1993). Recent studies have shown that *Porphyra*, in particular, appears especially promising for growing plants from protoplasts (Dai et al. 2004; Dai et al. 1993; Dipakkore et al. 2005).

Macroalgae can be cultivated in offshore, near-shore, or in open-pond facilities. The operation of large offshore seaweed farms was initially tested by the Marine Biomass Program through several deployments of kelp on growth structures in deep waters off the coast of Southern California; using artificially upwelled water as a nutrient source. While it was determined that such structures would support growth of kelp, difficulties were encountered with the stability of either the structures themselves or the stability of the attachment of kelp to the structures. However, modern prototypes for offshore growth of the kelp, *Laminaria hyperborean*, have been successfully tested in the North Sea (Buck and Buchholz 2004; Buck and Buchholz 2005), thus, providing optimism for future efforts. Near-shore coastal environments are already being exploited by countries like China, Japan, and Chile, which have viable seaweed aquaculture industries. Globally, about 24 million tons of aquatic plants are produced via aquaculture every year (FAO 2014). In the United States, environmental regulations and popular resistance against use of coastal regions for large scale aquaculture needed to support a biofuel industry could represent challenges to the industry.

Land-based pond systems have also been considered for macroalgal cultivation (Friedlander 2008; Hanisak 1987; Yun et al. 2015), both as free-standing algal farms and in an integrated aquaculture scenario in co-culture with finfish and mollusks. In the latter, wastes from the other species represent a nutrient supply for the macroalgae. *Porphyra* spp., *Saccharina latissima* and *Nereocystis luetkeana* have been successfully co-cultured with salmonid fish species (Bruton et al. 2009). The capital cost of building a pond could be offset through co-culturing with finfish and mollusks as well as producing higher value co-products from the macroalgae.

Recently, the ATS system was proposed for the mass cultivation of freshwater macroalgae. ATS is naturally seeded with multiple filamentous macroalgae taxa, including common freshwater genera *Oedogonium*, *Rhizoclonium*, *Ulothrix*, and *Microspora* (for a review, see Yun et al. 2015). ATS has also been demonstrated to grow a highly diverse and dynamic profile of benthic and planktonic polyculture in waters ranging from fresh to marine (Adey et al. 2013; Laughinghouse 2012).

The advantages of the land-based systems over water-based ones have been listed as follows (Chynoweth 2002):

1. Ease of plant management
2. Use of plants with or without holdfast structures
3. Ease of nutrient application without dilution
4. Avoidance of open-sea problems such as bad weather, disease, and predation
5. Possibility of farm operations located in close proximity to conversion operations.

For contribution to a biofuels marketplace, considerable scale-up from current activities, improvement in strain selection, and major technological improvements in efficiency of water movements and pond-construction costs are needed (Friedlander 2008). Other options for algal cultivation are being investigated; these include the cultivation of polyculture algae in open raceway ponds and open ATS systems, and the harvesting of naturally occurring marine algal blooms. It should be noted that, especially in open systems, monocultures are inherently difficult to maintain and require significant

investment in methods for detection and management of competitors, predators, and pathogens. One possible approach to contend with this is to cultivate an intentionally mixed or natural assemblage of organisms, such as in ATS systems, in an attempt to maximize total harvested biomass. This model would require a downstream biorefinery capable of processing simple and complex carbohydrates, proteins, and lipids into a variety of useful products. Nutrients, including CO_2, must also be managed in a way that balances productivity and pathogen sensitivity with the plasticity of algal physiological adaptation. CO_2 supplementation in the growth of *Oedogonium* for bioenergy resulted in a 2.5 times higher biomass productivity, indicating the potential of integrating the large-scale culture of freshwater macroalgae with existing carbon-waste streams (Cole et al. 2014).

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5. Harvesting and Dewatering

The conversion of algae that has been cultivated in ponds, photobioreactors, or offshore systems into liquid transportation fuels requires processing steps such as harvesting (Dodd and Anderson 1977; Butterfi and Jones 1969; McGarry and Tongkasa 1971), dewatering, and, potentially, extraction of fuel precursors (e.g., lipids and carbohydrates, see chapter 6). Cultures with as low as 0.5 g/L algae must be concentrated to slurries containing at least 20% dewatered algae given the known processing strategies (see chapter 7). The final slurry concentration will depend on the extraction methods employed and will impact the required energy input. As the desired percentage of dry biomass increases, energy costs climb steeply. Composition and physiological attributes of the strain or strains will also impact the energy costs and efficiency of the process. Some strains will lend themselves well to settling-based harvesting, whereas others will require a much more active harvesting-management strategy. Final slurry concentration also impacts plant location because of transportation, water quality, and recycling issues. A feasible algae-to-fuel strategy must, therefore, consider the energy costs and siting issues associated with harvesting and dewatering. Drying processes have a significant impact on TEA, GHG accounting, and life-cycle analyses. Addressing these issues requires careful analysis of engineering designs, combined with RD&D, to develop specific processing technologies to support those designs and a fundamental understanding of how algal biology can impact harvesting and dewatering strategies. Processing technologies depend on the algal feedstocks being considered. Processes that pertain to unicellular algae are quite different from the approaches applicable to macroalgae.

5.1 Harvesting and Dewatering

DOE has funded multiple projects to develop harvesting and dewatering technologies for microalgae. The most comprehensive comparison of harvesting technologies was completed by the NAABB. In the NAABB program, researchers selected five technologies for investigation based on ease of technology integration, level of environmental impact, high-volume processing capability, and demonstration potential (NAABB 2014). NAABB researchers collected data at lab scale on harvesting-technology performance, energy balance, and factors to cost in order to conduct a TEA of the harvesting strategies compared to baseline technologies. Harvesting technologies were compared on the basis of energy input, chemical costs, electricity cost, operating expenses, and parasitic energy loss. Based on this analysis, NAABB researchers selected three of the harvesting projects—electrolytic harvesting, cross-flow membrane filtration, and ultrasonic harvesting—for further characterization at larger scale; all three showed promise as primary harvesting technologies with large energy savings and significant GHG-emission reduction at the demonstrated scales compared to centrifugation. Since the consortia effort, BETO has funded harvesting logistics work through the Small Business Innovation Research program (energy.gov/sites/prod/files/2014/10/f18/eere_fy15_phase_1_release_2_topics_10-24-14.pdf). A discussion of these and other harvesting technologies follows below.

Ultrasonic Harvesting

Ultrasonic harvesting is a process that applies standing acoustic waves in a flow-through system to gently aggregate algal cells, facilitating sedimentation out of the cultivation media. Under the NAABB, a pilot-scale ultrasonic harvester was assembled and tested outdoors with *Nannochloropsis oculata* feedstock obtained from Solix Algredients, Inc.'s algae cultivation facility. Using this system, NAABB researchers demonstrated the ability to concentrate algae, sometimes as high as 18 times above initial feedstock concentrations, though they faced challenges in scaling the ultrasonic harvesting technology (NAABB 2014). While ultrasonic technology shows promise to significantly reduce microalgae harvesting costs (Coons et al. 2014), it has not yet demonstrated suitable performance with energy inputs less than 1 kWh/m^3. Additional research is needed to identify the practical limits of microalgae properties that lead to aggregation in an acoustic field, as well as the maximum energy efficiencies that can be obtained through improved ultrasonic harvester design.

Filtration

Solid/liquid filtration technologies are well studied, and filtration without prior flocculation can be used to harvest and dewater algae (Ferguson et al. 1995; Downing et al. 2002; Saidam and Butler 1996). Microalgae and cyanobacteria present unique filtration challenges because most strains considered for energy feedstocks have cell diameters less than 10 µm. Filtration is conceptually simple but potentially very expensive, and can be optimized through further understanding of several issues:

- The filter's pore size is critically important as it is defined by the size of the algae species and algae-aggregation rate. Small algae pass through larger pores decreasing filter efficiency. Decreasing pore size, however, leads to blinding, the blocking of filter pores, and the reduction of filtering rates. Culture purity becomes important as a distribution of microorganism size will affect filtration efficiency and blinding rates.
- Filter material also influences filtration and recovery efficiency. Materials can be used that optimize filtration and have the ability to remove the algae later. For instance, filter materials with controlled hydrophobicity and/or algae affinity can be developed. Durability and blinding are also issues.
- Filtration design is an important variable with both static and dynamic filtering operations. Moving filters

have been used in drum and cylinder press designs (Oswald 1991). Power costs will certainly influence design.

- An important step is recovering the algal biomass from the filter. Washing the filter is one practice, but doing so leads to re-dilution of the product. Filtration designs should consider minimal or zero washing requirements.

Cross-flow membrane filtration utilizes novel ceramic-coated membrane sheets with engineered pore structures and surface properties for algal harvesting. NAABB researchers developed a thin, porous, nickel-alloy metal-sheet membrane and assembled a cross-flow module on a mobile unit tested at the Texas Agrilife Research Station using active cultures of *Nannochloropsis salina* and *Chlorella sorokiniana* (NAABB 2014). Cross-flow membrane filtration was also shown to dewater to 24% solids.

On their DOE-sponsored Advancements in ABY Phase 1 project, Global Algae Innovations developed an advanced membrane-filtration system for combined harvest/dewatering filtration, without use of flocculants or coagulants, which has demonstrated harvest of millions of liters from open pond systems at 20,000 L/hr with an energy use of ~0.04 kWh/m^3 and 100% harvest efficiency. The system does not utilize any flocculants or coagulants, and algal slurry of 15%–20% solids is attained. The permeate is clear and free of algae or bacteria. Global Algae Innovations has commercialized the technology, which is scalable to throughputs of hundreds of millions of gallons per day and has been demonstrated for multiple strains of green algae, diatom algae, cyanobacteria, and red algae.

In Cornell University's Marine Algal Biofuels Consortium (Cornell Consortium), researchers utilized gravitational settling with a filter press for the diatom *Staurosira* sp. and the chlorophyte *Desmodesmus* sp., delivering the biomass of these marine microalgae as viscous slurry in large-scale production at Cellana's Kona Demonstration Facility in Hawaii. The employed harvesting technology relies on selecting ideal strains that are negatively buoyant upon nutrient exhaustion with preferably low ash content. Cornell Consortium researchers examined the capital expenses at two locations and found that the harvesting system accounted for 4.2% and 3.1% of the capital expenses for a base case microalgae and processing facility of 111 ha in Hawaii and the Gulf Coast, respectively (Huntley et al. 2015; Beal et al. 2015). For a review on membrane filtration technologies, see Mo et al. (2015).

Flocculation and Sedimentation

Microalgae and cyanobacteria remain in suspension in well-managed, high-growth-rate cultures due to their small size (~1 to 30 µm). This facilitates the transport of cells to the photoactive zone through pond or bioreactor circulation. Their small sizes, however, make harvesting more difficult. Flocculation leading to sedimentation occurs naturally in many older cultures. In managed cultures, some form of forced flocculation, usually involving chemical additives, is required to promote sedimentation at harvest.

A number of different forms of forced flocculation have been employed. Chemical additives that bind algae or otherwise affect the physiochemical interaction between algae are known to promote flocculation (Lee et al. 1998; Knuckey et al. 2006; Pan et al. 2001). Alum, lime, cellulose, salts, polyacrylamide polymers, surfactants, chitosan, and other man-made fibers are some chemical additives that have been studied. Manipulating suspension pH with and without additives is also effective, and autoflocculation in the form of photosynthetically driven CO_2 depletion for pH control has been studied (Sukenik and Shelaf 1984). Bioflocculation where algae are co-cultured with another organism that promotes sedimentation has also been considered (Lavoie and de la Noüe 1987). For example, Lee et al. (2013) reported that microalgae-associated bacteria could promote the flocculation of *Chlorella vulgaris*.

Electrolytic aggregation is a process whereby the surface charge on algal cells is neutralized allowing them to spontaneously aggregate and sediment. The NAABB team conducted field tests of the electrolytic process using a commercial electrocoagulation unit traditionally used for wastewater treatment, achieving a 50x concentration factor and 95% recovery of *Nannochloropsis salina* while using only 25% of the energy used by a baseline centrifugation strategy (NAABB 2014).

Optimizing flocculation methods, type, mixtures, concentrations, and chemistry to maximize algae recovery will very likely depend on strain selection, the mechanism of algae-flocculant interactions, and on empirical determinations in particular processes. It is possible to imagine selecting or designing strains to aggregate on cue or designed with a particular flocculant interaction in mind. Culture-manipulation techniques, therefore, may be useful for promoting flocculation. Future research in flocculation chemistry must take into account the following:

- Chemical flocculant recovery techniques are required to minimize cost and control water effluent purity.
- Metal ion contaminants introduced by corroding electrode materials from electrolytic harvesting technologies present challenges for algal biofuel production and may require the development of inert electrodes.
- The effect of residual flocculant or pH manipulation in recycled water on culture health and stability and lipid production must be understood and controlled. Likewise, the presence of flocculant in further downstream extraction and fuel conversion processes must be understood and controlled.
- The environmental impact of flocculant or pH manipulation in released water effluent, and fuel conversion and use must be considered.

- Bioflocculation, electroflocculation, and electrocoagulation must be scaled up with cost and energy analysis.
- Optimized sedimentation tank designs with integration into further downstream dewatering techniques, water recycling, and flocculate recovery are required.

Flocculation and Dissolved Air Flotation

Flocculation and dissolved air flotation (DAF) were established for sewage treatment and later studied in algae harvesting (Sim et al. 1988; Botes and Vanvuuren 1991; Edzwald 1993; Phoochinda and White 2003; Kwak et al. 2005; Bare et al. 1975; Koopman and Lincoln 1983; Sharma et al. 2013). Flocculation is used to increase the size of the algae aggregates, and then, air is bubbled through the suspension causing the algal clusters to float to the surface. The algae-rich top layer is scraped off to a slurry tank for further processing.

All of the issues arising from the use of flocculants for sedimentation (e.g., floc optimization, water and algae purity, and flocculant reclamation) are also encountered in flocculation and dissolved air flotation. In addition to flocculant efficiency, recovery is largely dependent on bubble size and distribution through the suspension. Dissolved air flotation facilities require optimized integration with any engineered design for further downstream processing.

Centrifugation

Centrifugation is widely used in industrial suspension separations and has been investigated in algal harvesting (Molina et al. 2003). The efficiency is dependent on the selected species as related to cell size and density excess with the media. Energy requirements are dependent on the bowl diameter, rotational speed, and density of the algal water. Centrifugation technologies must consider large, initial-capital equipment investments; operating energy and costs; and high throughput processing of large quantities of water and algae. Centrifugation is cost and energy prohibitive for first-stage harvesting in large-scale algae biorefineries; it is considered more appropriate as a final dewatering technology (Davis et al. 2012).

Other Harvesting Techniques

A number of other techniques at various stages of R&D have been proposed to harvest and dewater microalgae. These include, but are not limited to, the use of organisms growing on immobilized substrates where the amount of initial water is controlled and the growth substrate can be easily removed; manipulation of electric fields; and bioharvesting, where fuel precursors are harvested from higher organisms (e.g., shrimp and tilapia) grown with algae (Johnson and Wen 2009).

5.2 Drying

Drying is required to achieve high biomass concentrations. Because drying generally requires heat, methane drum dryers and other oven-type dryers have been used. However, the costs and energy usage and, therefore, GHG emissions climb steeply with incremental temperature and/or time increases. Air drying is possible in low-humidity climates but will require extra space and considerable time. Solutions involving either solar or wind energy are also possible.

Drying prevents microbial spoilage and reduces the costs of handling, transporting, packaging, and storing microalgae (Bennamoun et al. 2013). In thermochemical conversion processes, drying improves the efficiency of solvent-based oil extraction and prevents the formation of water-oil emulsions during extraction (Viswanathan et al. 2011; Sathish et al. 2014). Jones et al. (2014) stipulate that the fluctuations in microalgae-production rates between the summer and winter (on average 5:1) may provide an opportunity for a portion of wet biomass to be dried during high season for use in low season.

Microalgae Drying Methods

Solar drying is characterized by a lack of control during the drying process, possible degradation because of biochemical and microbiological reactions, and weather dependency. Long drying times at low temperatures increase the bacterial count. Vairappan et al. (2014) compared direct-sun drying and shade drying of red algae *Kappaphycus alvarezii* Doty and found that direct sunlight caused depolymerization of carrageenan molecules, which pose health hazards to humans. Color pigments of dried algae were also damaged in direct-sun drying.

Spray drying is a widely used method for a broad range of microalgae—especially for human food (Show et al. 2013). Spray dryers may not be able to remove internal moisture. To overcome this problem, fluidized bed dryers are incorporated as a second stage to remove internal moisture (Law and Mujumdar 2007). Spray dryers are harsh to the quality of microalge and cause a significant decrease in the carotenoid content of spray-dried microalgae *Phaeodactylum tricornutum* (Ryckebosch et al. 2011). For spray dried spirulina, Morist et al. (2001) reported a drop in carbohydrate and protein contents, and Sarada et al. (1999) observed a 55% loss of phycocyanin in comparison to fresh biomass. In spray drying of *Dunaliella salina*, lower outlet temperature resulted in higher beta-carotene recovery (Leach et al. 1998).

Freeze drying is used for pharmaceuticals and hormones that cannot tolerate even moderate temperatures. Freeze-dried microalgae maintain their chemical, physical, and biological characteristics for a long time (Vairappan et al. 2014). Ryckebosch et al. (2011) assessed the effect of storage on fresh biomass and freeze-dried microalgae *Phaeodactylum tricornutum*. Total lipid content in freeze-dried microalgae was significantly higher than fresh microalgae after a period of storage. Freeze drying is very expensive because providing vacuum conditions requires too much energy (Liapis and Bruttini 2007).

Spouted bed dryers force a gas, usually inert, through moist microalgae causing mixing and a high rate of drying (Law and Mujumdar 2007). Oliveria et al. (2008) studied *Spirulina platensis* drying in two different configurations of spouted beds and found that protein solubility decreased from 100% in fresh microalgae to 37%. Phycocyanin content decreased from 16.3% in fresh biomass to 14.7%.

Conveyor belt dryers dry the material on a moving belt while the thin layer of material passes through a series of drying tunnels. The application of heat can be through a convective flow of gas over or through the product. In a variation of the belt dryer, heat is applied to the product by conduction, through the belt's thickness. The belt is in contact with circulating hot water (refractance drying—as sold through G3 Enterprises Inc., gwdryers.com) The lower operating temperature has the benefit of a lower fire hazard and lower volatile organic compound emissions (Poirier 2007; Li et al. 2012).

Algae drying kinetics are measured in thin-layer drying experiments in which the wet sample is placed on a balance in a controlled chamber. The temperature, moisture content, and air velocity as drying media are controlled. The time-varying mass of the sample to be dried is measured and recorded. Based on this data, moisture content of the sample as a function of time is plotted. The more informative plot is the drying rate as a function of the material's moisture content. Various conditions like air temperature, air velocity, and sample load affect the kinetics of microalgae drying (Molnar 2007). Research has been conducted to observe the effect of different drying conditions on drying characteristics of microalagae.

Viswanathan et al. (2012) analyzed thin-layer drying characteristics of a consortium of green algae consisting of *Scenedesmus bijuga*, *Chlamydomonas globose*, and *Chlorella minutissima* as a possible biofuel source. The initial moisture content was 88.93% (wet basis). Drying was performed in a convective oven with a constant parallel airflow velocity of 0.3 ms^{-1} at drying temperatures of 30˚C, 50˚C, 70˚C, and 90˚C. The material thickness was 2.85 mm. The page model was the best to fit experimental data. For all temperatures, moisture content decreased exponentially with passing time. The drying-rate curve showed that, at all temperatures, drying occurred at a falling rate with no constrant drying rate, meaning that drying was limited by the diffusion mechanism, and due to resistance of individual cell walls against diffusion of moisture to the top surface. Extracellular polysaccharides in the cell wall may also form a thin membrane on the drying surface, which inhibits moisture diffusion to the surface.

5.3 Systems Engineering

While specific process technologies have been studied, breakthroughs are still needed in each, given the importance, as well as current cost and achievable scale, of harvesting and dewatering. Moreover, new strategies should continue to be developed to combine and integrate these processes in order to take an algae culture and convert it into slurry of a specific concentration. Current processing technologies (see chapter 7) utilize feedstock slurries with approximately 20% algae by mass in water. From the technologies investigated by the NAABB, ultrasonic harvesters and extractors may be capable of meeting DOE/BETO 2022 cost metrics, but ultrasonic technologies require further development at industrially relevant scales (Coons et al. 2014). Continued refinement and expanded demonstration of processing strategies at scale and at consistent, high performance over long durations of operation are needed. Importantly, the variety of algal feedstocks utilized by the current industry will need to be tested under processing technologies in order to examine the applicability of approaches over a broad spectrum of species. The quality of feedstock in the performance of these logistics technologies needs to be determined, as well as the potential impact to downstream conversion processes. Even when combining promising harvesting and extraction technologies, significant improvements are still needed for economic and financial success of the industry (Richardson et al. 2014).

The least amount of energy that is needed for the most innovative harvesting and dewatering technologies is largely unknown and represents a critical gap. This has important implications for plant designers trying to answer simple questions like "What percentage of the plant's total energy requirements or what percentage of that made available by algae must be directed toward harvesting and dewatering?" Using the harvest and dewatering technology developed and demonstrated by Global Algae Innovations in ABY Phase 1, and assuming cultivation at an average of 1 g/L, harvesting to 20% solids would consume 0.04 W-h/g, which is less than 1% of the energy content of the algae.

This type of unit-operations analysis of energy input for a range of dry-weight content based on extraction needs requires consideration of capital equipment investments, operations, maintenance, and depreciation. The cost of harvesting and dewatering will depend on the final algae concentration needed for the chosen extraction and conversion method. This will likely be a significant fraction of the total energy cost of any algae-to-fuel process and a significant fraction of the total amount of energy available from algae. A quick and preliminary energy balance example shown below provides some food for thought regarding harvesting and dewatering technologies.

Preliminary Look at Energy Balance

The energy content of most algae cells is of the order of 5 kW-h/kg if the energy content of lipids, carbohydrates, and proteins and the typical percentage of each in algae are considered (Illman et al. 2000; Coons et al 2014). It is possible to estimate the energy requirements in kW-h/kg of algae for harvesting, dewatering, and drying as a function of the mass

percentage of algae in harvested biomass. The energy requirements for flocculation, sedimentation, and the belt filter press are expected to be minimal. However, based on the latent heat of vaporization of water at 0.64 kW-h/kg, energy balance can become an issue in systems that propose to concentrate the algal biomass to around 10 wt% or 20 wt%, and then dry it to enable downstream processing and extraction because of the high-mass fraction of water that must be vaporized. In spite of gaps in data precluding more detailed analyses, algal biofuel production schemes at scale will likely need to implement innovative technologies and integrated systems in order to overcome this challenge.

Possible approaches may include developing strains of algae with much higher energy content than available today, along with innovative solutions to lower the energy intensity of harvesting and drying algae.

5.4 Approaches for Macroalgae

Harvesting

Currently, of the roughly 1.6 million dry metric tons of total seaweed harvested worldwide, about 90% is derived from cultivated sources (Roesijadi et al. 2008). Manual harvesting is common for both cultivated and natural systems, and mechanized harvesting methods, which can involve mowing with rotating blades, suction, or dredging with cutters, have also been developed. Invariably, such mechanized harvesters require boats or ships for operation. Modern seaweed harvesting vessels can be equipped with pumps to move harvested seaweeds directly into nets or other containment structures (Ugarte and Sharp 2001). Application of mechanical harvesters in European seaweed operations have been described in a recent feasibility analysis for seaweeds as a biofuels feedstock in Ireland (Bruton et al. 2009).

The concept of large, offshore macroalgae farms and associated biorefineries has, from the outset, included mechanized harvesting techniques. The exact nature of such mechanization will obviously depend on the form of cultivation and type of algae being cultured. For example, attached forms that tend to stand upright, such as *Macrocystis*, may be amenable to mowing. Floating seaweeds such as *Sargassum* spp. could be cultivated in floating pens, and low-growing, attached forms such as *Gracilaria* will require different approaches that are compatible with their growth characteristics. In forms such as *Laminaria*, grown on offshore rings (Buck and Buchholz 2005), harvesting may require retrieval and transport to shore. Similarly, cultivation in land-based pond systems will require technology appropriate for that mode of culture.

As a result of growing concern about the potential environmental consequences of harvesting natural populations of seaweed near-shore, strict regulations have been put in place in some countries (Pringle and Tseng 1989). To manage seaweed harvests, laws stipulate the percentages of harvestable stock allowed to be harvested and the length of intervals between harvests to allow for the growth and recovery of biomass (Ugarte and Sharp 2001). The establishment of large, offshore seaweeds may alleviate pressure from near-shore environments and create market opportunities for products apart from fuels, although issues related to sustainability and potential environmental consequences will need to be carefully evaluated.

Preprocessing

The general preprocessing requirements for macroalgal biomass prior to extraction or direct conversion have been categorized as follows (Bruton et al. 2009):

- Removal of foreign objects and debris (e.g., by washing)
- Milling
- Dewatering.

Seaweeds immediately following harvest can have stones, sand, litter, adhering epifauna, and other forms of debris that should be removed before further processing. Screening for debris is considered mandatory, with the degree of screening dependent on the mode of culture and end-use. Algae that are grown in suspension culture, as opposed to attached-to-the-bottom culture, will likely have less debris, and the amount of debris will likely have less impact in procedures that can utilize whole seaweeds (Bruton et al. 2009).

Milling is used to reduce seaweeds to particle sizes that are more efficiently processed. Smaller particles, with higher surface-area-to-volume ratios, will have higher reaction efficiency during anaerobic digestion for biogas, fermentation for alcohols, and HTL for bio-oils.

Macroalgae have less demand for dewatering as part of the pretreatment process. Anaerobic digestion, fermentation, and HTL have either a high tolerance or requirement for water. Dewatering may be more important as a method to increase shelf life and reduce weight and associated transportation costs if algae are to be transported from sites of harvest to distant processing plants (Bruton et al. 2009). Dewatering to about 20%–30% water content is noted to have a stabilizing influence, which is beneficial for transportation and other processes requiring further drying (Bruton et al. 2009). In anaerobic digestion and fermentation, shredded or milled macroalgal biomass can go directly into either reactions or extractions. Hydrothermal conversions are suited for wet biomass and become efficient at 15%–20% solids or 80%–85% water content (Peterson et al. 2008). Although some dewatering may be necessary for some seaweeds with water content approaching 90%, the exact ratio of water to solids for marine biomass remains to be determined.

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6. Extraction of Algae

Though industrial scale extraction processes have been developed for terrestrial feedstocks, the algal biofuel industry suffers from a limited number of well-defined and demonstrated industrial-scale methods for extracting and separating components in the algae that are of interest. Existing extraction techniques are mainly suitable for analytical- and laboratory-scale procedures, or for the recovery/removal of high-value products. To produce algal biofuels as a competitive bulk commodity, extraction techniques employed must be efficient and scalable. In addition, for many processes to be economically viable, these methods may also need to be compatible with wet and freshly harvested biomass, thereby avoiding the costly drying step. This section describes the techniques that are under investigation to determine if they have the potential to be efficient methods for extraction of oils from algal biomass.

Technologies that make a biofuel or a bioproduct from dewatered algae fall under two main approaches: through a whole algae pathway or through extraction and separation of oils and lipids from algal biomass. The approach of processing the whole algae will be covered in chapter 7, "Algal Biofuel Conversion Technologies." The second approach, which is to extract and separate the components in the algae that are of interest, will be covered in this chapter. It should be noted that the majority of this chapter will focus on the extraction and separation of lipids from algae, as that is the simplest and most researched fraction that is converted to fuels. However, there is value in other components in the algal cell (carbohydrates and proteins) that are likely to play a significant role in supporting the commercialization of algal biofuels (Foley et al. 2011; Davis et al. 2014; Laurens et al. 2015; Dong et al. 2016). The economic implications of moving from a lipid-centric approach to a more holistic approach are documented and discussed in the report *Process Design and Economics for the Conversion of Algal Biomass to Biofuels* (Davis et al. 2014).

6.1 Lipid Separations and Extractions from Algae

One of the biggest challenges when extracting and separating lipids from algal biomass is the limited number of scalable, cost-effective, efficient, and demonstrated methods. Existing extraction and separation techniques that are used for the recovery/removal of high-value, low-volume products (solvent extraction, distillation) may require little cost improvement to make new, high-value compounds in algae economically viable. However, in order to adapt these methods to extract components from algae for use as a competitive bulk commodity (biofuel), improvements to these extraction techniques are needed to improve efficiency and cost-effectiveness at large scale.

When examining the various extraction methods that could be used with an algal feedstock, there are several considerations that determine which method will be most effective for a particular process. Some of these factors include, but are not limited to the following:

1. The composition of the biological component that is targeted for extraction
2. Algal species and growth conditions
3. Harvest process operations (discussed in the chapter 5)
4. Favorable life-cycle analysis (LCA) and TEA (discussed in chapter 11)
5. Required degree of dewatering necessary for lipid extraction (discussed in chapter 5).

It is important to note that two of the factors listed above, #1 (composition of the biological component that is targeted for extraction) and #2 (algal species and growth conditions), have an enormous influence on the extraction efficiency and susceptibility of the cell biomass to pretreatment and cell rupture. Specifically, the impact of the biomass nutritional status (e.g., nutrient-deplete or nutrient-replete growth of the cells) on extraction efficiency and susceptibility to different processes is an area that needs to be researched in more detail. Under nutrient-deplete conditions, the macromolecular rearrangement of the cell biochemistry leads to an accumulation of lipids, but it often also leads to a change in the cell wall properties (Gerken et al. 2013), which can make the lipids less accessible to solvents due to mass-transfer inhibition. Because the mass-transfer properties of different lipids are based on the compatibility of solvents and lipid polarity (Ryckebosch et al. 2014), the choice of solvent ideally should match the polarity of the lipid fraction. For example, due to the cellular rearrangement of lipids over the course of nutrient starvation resulting in higher concentrations of neutral triglyceride lipids, a more non-polar solvent system would be more appropriate for nutrient-stressed cell biomass. As such, the lipid composition (polar, neutral, unsaponifiable fractions) will be a large determinant of the processing options selected. Ultimately, the separations and lipid mass-transfer rates will be defined by the compositional and polarity differentials between the lipids and the solvents used.

Plant seed oil extractions are tailored mainly to triglyceride-rich lipids; thus, a non-polar, hexane-based extraction process is ideal. However, not all technologies demonstrated in plant seed oil-extraction processes are applicable to algae due to the high levels of emulsifiers present in algal cells, such as polar lipids, sterols, etc., which can cause significant complications in the overall extraction kinetics (Halim et al. 2014). In the interpretation of the methods presented in this chapter, it is necessary to recognize that there are likely a number of extraction methods that are used in the oilseed industry that have not been included in this discussion because they have not been demonstrated in algae. Thus, all of the methods presented

below have some preliminary data that have been obtained using algae.

It is also important to highlight that while many terrestrial feedstocks can be removed from their environment at total solids >40%, microalgae and cyanobacteria are normally cultivated as single cells suspended in water at concentrations <1% solids. Many effective extraction techniques require concentrated substrates; thus, a high degree of concentration may be necessary before some types of extraction can be performed. For this reason, some algae-to-biofuels processes attempt to bypass the extraction step by either converting whole algal biomass or by inducing the secretion of the desired product directly (see chapter 7).

Another major difference between microalgae and terrestrial crops and methods used by the oilseed industry is that algal oil is typically much more complex than oil from plants, such as soybean oil. The complexity of algal oil and the many fractions involved is depicted in Figure 6.1 where a sample of "algal oil" is being subjected to conventional column chromatography. Crude soybean oil comprises roughly 96% triglycerides, 3.7% phospholipids (which is removed in the degumming process and sold as lecithin) and trace amounts of sterols, etc. (Hammond 2005). By contrast, algal oils are typically highly complex, comprising fatty acids, (not only in the form of triglycerides but also glycolipids and sphingolipids) as well as phospholipids, carotenoids, chlorophyll, and other components. For this reason, especially on a commercial scale, a full understanding of the algal oil within the chosen strain is essential, and the product specifications are required.

Figure 6.1. Conventional column chromatography of algae oil. (Photo courtesy of Origin Oil.)

A shortfall of relevant and publicly available information on efficient extraction of lipids and oils at larger-scale continues to limit algal-based biofuel development. In addition, the cost (TEA) and sustainability (LCA) parameters of extraction technologies play an important part in the feasibility comparisons and predictions of scale-up potential (see chapters 10 and 11 for more details). Laboratory-scale comparisons of lipid extractions from microalgae (Lee et al. 2010) have been carried out, but these techniques often rely on freeze-dried, pulverized biomass. While considerable knowledge exists for the separation of plant-biomass lipid extracts and the preparation for conversion to biodiesel (Zhang et al. 2003), little is known about the scale-up separation challenges for extracted algal lipids. An exception to this is described in U.S. patent 8,591,912, which was issued to Valicor (Kadam and Goodall 2013) and describes the technology designed to extract lipids from *Nannochloropsis oculata* grown by Qualitas Health in a large algae farm in Imperial, Texas. The technology uses ethanol to efficiently extract all the lipids intact, without a cell-disruption step. Because the bulk of these lipids are polar lipids (glycolipids, phospholipids, etc.) it results in an eicospentanoic acid product with very high bioavailability (almegapl.com).

A number of the techniques used to extract lipids and other products from algae are summarized in the sections below. The lipid-extraction methods have been divided into three main classes: physical methods of extraction and/or cellular biomass pretreatment, catalytic methods of extraction and/or cellular biomass pretreatment, and solvent extraction methods. It should be noted that a combination of these technologies may be required to get sufficient and efficient extraction of lipids. Thus, one technology may be used as a "pre-treatment" to improve yields.

6.2 Physical Methods of Extraction and/or Cellular Biomass Pretreatment

This section describes some of the physical methods that can be used to assist in the extraction of lipids from algae. Most of the processes can also be considered a "pre-treatment" of the biomass as all methods, at least at this point in the technology development process, require an additional step to extract lipids (i.e., use of a solvent). However, these techniques may prove to be important and have the potential to positively impact the economics of an algal biofuels process. For example, effective physical disruption can help offset the need to use elevated temperature and pressure in processes that force the solvent into contact with desired biopolymers. In addition, different methods can be used to disrupt the cell membrane prior to the application of the extraction solvents. For the purposes of this review, technologies that facilitate physical disruption can include cell homogenizers, bead mills (or bead-beating), microwave-assisted extraction techniques, pulsed electric field (PEF)-assisted extraction techniques, and

ultrasound-assisted extraction techniques (Mata et al. 2010). Some of these techniques have been used for years at the laboratory scale, such as cell homogenizers and bead mills, but the ability of these technologies to scale economically is limited. Thus, this review will not include a discussion of cell homogenizers or bead mills. Instead, it will focus on the more novel and potentially scalable technologies.

Microwave Assisted

The use of microwaves to disrupt cells and increase the efficiencies of algal lipid- and oil-extraction is a promising development (Lee et al. 2010; Lohman et al. 2013), though applications outside of the laboratory are unclear. Microwaves are defined as electromagnetic radiation of frequencies that normally range between 0.3 to 300 GHz and are used to assist in the heating of algal biomass to facilitate the breakdown of the material in a more uniform manner. One of the major hurdles with microwave radiation technology is the limited penetration depth of the microwaves into the absorbing medium (Vyas et al. 2010). This can have an impact on reactor design and energy consumption and will need to be studied further if the technology is determined to have scale-up potential.

The use of microwave-assisted extraction (MAE) to extract lipids from microalgae is a relatively new method, and others are still being developed that are crucial for assessing scalability. Literature has demonstrated the use of MAE to extract lipids from algae in conjunction with a solvent system (Lee et al. 2010; Balasubramanian et al. 2011; Iqbal and Theegala 2013; Lohman et al. 2013). Different processes were used in these studies, and it is worth discussing these differences as the results are not exactly comparable. Iqbal and Theegala, as well as Lee et al., describe a batch method, with solvent added prior to heating in the microwave field (Iqbal and Theegala) or after microwave exposure (Lee et al.). Balasubramanian's team, on the other hand, used a continuous flow system for microwave-based cell disruption, followed by solvent addition after exposure.

Iqbal and Theegala tested two co-solvent systems using 20% and 40% biodiesel (BD20 and BD40) at 80°C, 100°C, and 120°C in MAE. The results were compared to those of the MAE using chloroform and ethanol (1:2) as well as conventional 8-hour Soxhlet extraction. Results indicated that approximately 66% and 78% of the oil within the cells were extracted with BD40 at 80°C and 100°C, respectively. Increasing the temperature to 120°C increased the efficiency of BD40 extraction to 115.5% relative to conventional Soxhlet extraction. The BD20 co-solvent was less efficient and only extracted 27%, 34%, and 24% of oil at 80°C, 100°C, and 120°C temperatures, respectively. MAE using chloroform and ethanol showed 32%, 93%, and 108% of oil compared to Soxhlet. While these results demonstrate that less-toxic solvents can be used to extract algae, the TEA and LCA implications for the use of solvents such as biodiesel (Iqbal and Theegala 2013) and hexane (Balasubramanian et al. 2011), in conjunction with MAE, have not yet been explored. However, it is worth noting that these studies were performed either in a wet-paste environment (Iqbal and Theegala) or an even more dilute solution (Balasubramanian et al. 2011; Lee et al. 2010), which is important as it saves a large amount of energy otherwise required for drying.

Pulsed Electric Field

PEF-assisted extraction techniques have recently been investigated to determine whether their use can be applied as a pre-treatment step to enhance the extraction of valuable components from microalgae. PEF techniques were developed from well-known electroporation techniques that have been used to transform cells of genes for genetic engineering. PEF is based on the theory that when cell membranes are subjected to short electric pulses under high electric-field strength, pores are formed in the cell membrane. The formation of these pores can cause an increase in the lipid recovery from microalgae when used in combination with solvent extraction (Parniakov et al. 2015). The applicability of this technology is likely limited to a pre-treatment step in combination with other extraction technologies. While there are a number of questions that remain with respect to the applicability of this technology in a process-relevant application, this technology is beginning to be tested and further developed by industry, as described below.

OpenAlgae has developed a technology platform that breaks algal cells while wet to liberate oil by utilizing an electromagnetic field that acts on the charged algal cell membranes to rupture the cells (Kipp et al. 2013 and Siebert et al. 2015). Electromagnetic lysing has been tested at the laboratory-, pilot-, and prototype-scale on live, concentrated, and wet algae, and it has been found in some cases to be more cost-effective than mechanical methods. OpenAlgae's cell-lysis process exploits the electrochemical gradient across the cell membranes of live cells to create physical torsion and disrupt cells. However, OpenAlgae has observed that if the cell viability of a culture is low, the electromechanical approach will rupture the cells in the culture less efficiently, requiring other disruption methods. After lysing, the biomass stream contains oil, including sub-micron-sized oil drops. Even in the absence of solids, the recovery of such drops would be difficult; however, with algal cell solids, this separation becomes even more difficult because the oil drops are wet and interact with algae solids. Thus, the recovery of algal oil produced using this technology requires another technology to complete the full extraction process.

OpenAlgae has developed a patented coalescence contactor and membrane technology for extracting neutral lipids from lysed algal biomass post-EM. The technology achieves lipid removal at ambient conditions without using a solvent. Only neutral oils are recovered; the yield is in large part a function

of the amount and type of oil accumulated in the cells. The contactor itself is commercially available (although it is used for different applications). This process has been tested at pilot scale for multiple algae strains and oil mixtures, and in non-algae oil applications. The output of the OpenAlgae process is a wet, de-oiled biomass stream and algal oil that does not remove phospholipids and other polar lipids. Thus, the nitrogen and phosphorus of the cell is retained in the de-oiled biomass, allowing these nutrients to be removed by subsequent operations. Other cited advantages of this approach include the lack of moving parts and the potential to be robust and scalable.

Ultrasonic

While the use of ultrasound technology has been established for other applications such as biomass pretreatment for bioethanol (Esfahani and Azin 2012; Methrath Liyakathali et al. 2016), its use for extraction from lipids from microalgae has been limited. In general, ultrasonic disruption of cells improves the lipid extraction 1.5–2.0 times compared to conventional extraction methods. The improved extraction by ultrasound technology is due to the sonochemical and mechano-acoustic effects produced by ultrasounds, which enhance the inter-particle collisions between molecules, which, in turn, affect the chemical and physical structures of the microalgae (Bussemaker and Zhang 2013; Methrath Liyakathali et al. 2016). In a liquid media, ultrasonic waves generate rapidly expanding and collapsing microbubbles, leading to cavitation that has the potential to disrupt physical structures such as membranes of cell walls that tend to aggregate at the interface of the cavitating bubble.

Araujo et al. studied the lipid extraction from microalgae using four different ultrasound-assisted techniques and compared the results with Soxhlet extraction. They noted that the Bligh and Dyer method, assisted by ultrasound, resulted in the highest lipid extraction (Araujo et al. 2013) with the yield being approximately 3.0 times higher compared to other methods. The highest yield obtained by this method from *Chlorella vulgaris* was 52.5% w/w. Keris-Sen et al. investigated the effect of different ultrasound-power intensities as well as the effect of different solvents (n-hexane and chloroform/methanol mixture) and found that lipid extraction increased 1.5–2.0 times with the application of ultrasound at 0.4 $kWhL^{-1}$ in the presence of solvent. They also noted that the ultrasonic effect was reduced at higher energy intensities (Keris-Sen et al. 2014). Adam et al. applied the ultrasound method for 'solvent-free' extraction of lipids from fresh *Nannochloropsis oculata* microalgae cells. The optimum conditions for oil extraction obtained were 1000-W ultrasound power with 30-minute extraction time for biomass with a dry weight of 5% resulting in a 0.21% oil recovery (Adam et al. 2012).

Ma et al. compared the effect of ultrasound and microwave pretreatment on lipid extraction from microalgae and concluded that microwave heating extracts lipids at a higher rate compared to ultrasound technology (Ma et al. 2014). Gerde et al. investigated microalgae cell disruption using ultrasonic treatment from *Shizochytrium limacinum* and *Chlamydomonas reinhardtii* and noted that increasing the sonication time increased the production of free radicals and that careful control of the sonication conditions is needed to optimize the extraction (Gerde et al. 2012). Greenly et al. studied the cell disruption for various microalgae species and noted that the first few seconds of sonication leads to most cell disruption; moreover, at longer exposure times, different species showed different response to sonication (Greenly and Tester 2015). While various studies evaluated the effect of sonication and cell disruption in an ultrasonic system, (Gerde et al. 2012; Greenly and Tester 2015; Wang and Yuan 2015), more studies are needed to understand the exact mechanism and optimum conditions for using ultrasonic technology to extract lipids from microalgae. Other limitations, such as design simplicity, scale-up of the process, and LCA, also need to be further investigated.

6.3 Catalytic Methods of Extraction and/or Cellular Biomass Pretreatment

Acid/Base Hydrolysis

Solvent-based lipid extraction and direct transesterification techniques are inhibited when performed in the presence of water, which is a requirement when working with algae. Thus, the use of acid/base hydrolysis has been a focus of research over the past several years as a "pre-treatment" or "conditioning" step to facilitate these extraction techniques (Griffiths et al. 2010). Acid hydrolysis has been used as a pre-treatment or conditioning step in several processes that are described in more detail below, as well in conversion processes described in chapter 7 (such as transesterification).

Dilute acid pretreatment has been demonstrated to effectively hydrolyze algal structural and storage polysaccharides to release monomeric sugars (primarily glucose and mannose) into an aqueous stream (Davis et al. 2014; Laurens et al. 2014; Dong et al. 2016). In the context of a larger process called the Parallel Algal Processing (PAP), the aqueous stream can be separated from solid residue (rich in lipids and protein) by a solid/liquid separation, which allows the sugars released in the liquor phase to be fermented to ethanol (or higher-value co-products) (Davis et al. 2014; Dong et al. 2016). The aqueous phase generated by the hydrolysis is suitable for microbial growth, as demonstrated in the PAP process (Davis et al. 2014; Dong et al. 2016). However, due to sugar losses in the solid residue, resulting in a lower overall fuel yield, another process, termed Combined Algal Processing (CAP), was developed to avoid these losses. In this process, the acid hydrolysis step is immediately followed by a fermentation step. In both processes, the acid hydrolysis is used as a pre-treatment step to improve extractability downstream (Dong et al. 2016). Please see chapter 7 for more details on the CAP process.

The use of acid hydrolysis in an algal process is also being investigated by industry. For example, this is the first step of the Valicor AlgaFrac™ extraction technology that has been researched extensively in DOE-funded consortia (NAABB and SABC). Valicor operates a 10-kg dry weight (DW)/batch pilot facility in Dexter, Michigan, that these projects, as well as many others, have used to test acid hydrolysis of algae. The acid hydrolysis process, which is usually performed at ~pH 2 using sulfuric acid and elevated temperature, results in lysis of virtually all algae (Manganaro et al. 2015). In addition to hydrolyzing cell walls, the strong acid step also hydrolyzes all the polar lipids in the algal oil—this serves to make them soluble in hexane and also results in the removal of the bulk of the phosphorus, nitrogen, and metals (catalyst poisons) in the aqueous phase. This produces oil that can be readily hydrotreated and has been used in various DOE-funded projects. This technology was also utilized as part of an integrated algal biorefinery funded by DOE for about 5 years starting in late 2009 (CEHMM 2013).

Another example of a wet lipid-extraction procedure that was developed using acid/base hydrolysis demonstrated 79% of transesterifiable lipids that were able to be extracted from wet algal biomass (84% moisture) (Sathish and Sims 2012). Of the extracted lipids, 76% were isolated by further processing and converted to fatty acid methyl esters (Sathish and Sims 2012). This process was further optimized to enable the extraction of 77% of the total transesterifiable lipids, while reducing the amount of materials and temperature required in the procedure (Sathish et al. 2015). In the process, they were also able to determine that the solid-precipitate phase was composed of up to 11.2 wt% nitrogen (70% protein). The authors also commented on the importance of the process being amenable to produce other bioproducts to enable a biorefinery model.

6.4 Solvent-Based Extraction of Lipids

The most commonly used methods that have been investigated to extract lipids from algae include solvent extraction, accelerated solvent extraction, mixed solvents, supercritical CO_2, and switchable solvents.

Solvent Extraction

Many processes designed to produce biofuels from algae rely on the accumulation of intra-cellular lipids. The extraction of lipids by solvents is one of the primary methods that have been used with microalgal strains. A number of solvents have been studied to determine their ability to successfully extract lipids that include but are not limited to hexane; ethanol; 1-butanol; DBU (1,8-diazabicyclo-[5.4.0]-undec-7-ene); dimethyl ether; and the mixtures chloroform/methanol; *n*-hexane/ethanol; *n*-hexane/isopropanol; *n*-hexane/2-propanol; methanol/1-ethyl-3-methylimidazolium methyl sulfate; DBU/ethanol; DBU/octanol; methylene chloride/methanol; dichloroethane/methanol; dichloroethane/ethanol; acetone/dichloromethane (for reviews, please see Halim et al. 2012 and Lam et al. 2012). The main characteristics of an extracting solvent include the ability to: (1) penetrate through the matrix enclosing the lipid material, (2) physically contact the lipid material, and 3) solvate the lipid. As such, the development of any extraction process must also account for the fact that the tissue structure and cell walls may present formidable barriers to solvent access. This generally requires that the native structure of the biomass be disrupted prior to extraction.

The most commonly used method to extract lipids from algae is based on the concept of "like dissolves like," which is the basis behind the earliest and well-known co-solvent extraction procedure (Bligh and Dyer 1959). After the extraction reaction is complete, water (which is not miscible with chloroform) is added to the co-solvent mixture until a two-phase system develops in which water and chloroform separate into two immiscible layers. The lipids mainly separate to the chloroform layer and can then be recovered for analysis. It is worth noting that the Bligh Dyer method is very useful for pulling out all hydrophobic components of algae including hydrophobic proteins. As a result, it can provide the highest yields but the lowest purity; thus, much of the material extracted by the Bligh and Dyer method cannot be directly converted to biofuels.

Chloroform will extract more than just the saponifiable lipids (i.e., the unsaponifiable lipids such as pigments, lipoproteins, and other lipid and non-lipid contaminants) (Fajardo et al. 2007). Consequently, other combinations of co-solvents have been proposed for the extraction of lipids: hexane/isopropanol for tissue (Hara and Radin 1978); dimethyl sulfoxide/petroleum ether for yeast (Park et al. 2007); hexane/ethanol and hexane/isopropanol for microalgae (Cartens et al. 1996; Nagle and Lemke 1990). The hexane system has been promoted because hexane and alcohol will readily separate into two separate phases when water is added, thereby improving downstream separations. However, a more recent publication, which looked at the extraction of algal lipids using 13 solvents, spanning a range of polarities and solubilities, confirmed that ethanol, chloroform, and hexane were more efficient in the extraction of lipids than the other solvents studied (Ramluckan et al. 2013).

Similarly, less volatile and toxic alcohols (e.g., ethanol and isopropanol) have been suggested in place of methanol. One example is the hexane/ethanol extraction co-solvent system that has been used as an alternative to the hexane/methanol co-solvent system (Grima et al. 1994). In other cases, single alcohol (e.g., 1-butanol and ethanol) solvents have been tried (Nagle and Lemke 1990). In these applications, the alcohol is first added as the extracting solvent. Separation is then achieved by adding both hexane and water in proportions that create a two-phase system (hexane and an aqueous hydroalcoholic) that partition the extracted lipids into the nonpolar

hexane (Fajardo et al. 2007). In general, applications using pure alcohol (ethanol and 1-butanol) performed similarly, if not slightly better, than alcohol/hexane mixtures, but never more than 90% of the Bligh and Dyer co-solvent method. Moreover, pure alcohol solutions of greater carbon length (such as butanol) have not compared well against the hexane/ethanol co-solvent system. These results suggest that the two important criteria when selecting a co-solvent system to extract lipids are: (1) The ability of a more-polar co-solvent to disrupt the cell membrane and thus make it sufficiently porous and (2) The ability of a second less-polar co-solvent to better match the polarity of the lipids being extracted, as discussed earlier in this section. However, to avoid the use of elevated temperature and pressure to push the solvent into contact with the analyte (at the cost of a very high input of energy), disruption of the cell membrane may also be necessary.

It is also worth noting that Iverson et al. (2001) found that while the Bligh and Dyer method worked well for samples that contained less than 2% lipids, it grossly underestimated the lipid content in samples of marine tissue that contained more than 2% lipids. The sequence of solvent addition can also affect extraction (Lewis et al. 2000). Starting from freeze-dried biomass, it has been demonstrated that the extraction of lipids was significantly more efficient when solvents were added in order of increasing polarity (i.e., chloroform, methanol, and then water) (Lewis 2000). They explained their results in terms of initial contact of the biomass with nonpolar solvents weakening the association between the lipids and cell structure, prior to their dissolution in the monophasic system of water, chloroform, and methanol. These important results have a key impact on liquid-phase extraction systems applied to "wet" biomass because they suggest that the water will form a solvent shell around the lipids, making it more difficult for less-polar solvents such as chloroform to contact, solubilize, and extract the lipids. It is also noteworthy that the extraction efficiency was not improved (when water was added first), despite the added agitation in the form of sonication or additional methanol.

Accelerated Solvent Extraction

Accelerated solvent extraction (ASE) was first proposed in the mid-1990s (Richter et al. 1996), using the technique on 1–30-g samples of dried biomass. ASE uses organic solvents at high-pressure and temperatures above their boiling point. In general, a solid sample is enclosed in a sample cartridge that is filled with an extraction fluid and used to statically extract the sample under elevated temperature (50°C–200°C) and pressure (500– 3000 psi) conditions for short time periods (5–10 minutes). Compressed gas is used to purge the sample extract from the cell into a collection vessel.

ASE is applicable to solid and semi-solid samples that can be retained in the cell during the extraction phase (using a solvent front-pumped through the sample at the appropriate temperature and pressure). It has been proposed for the extraction of liquid extracts (Richter et al. 1996; Denery et al. 2004) and lipids from microalgae (Schäfer 1998). In addition to improving yields and reducing extraction time, ASE can also be applied to remove co-extractable material from various processes, to selectively extract polar compounds from lipid-rich samples, and to fractionate lipids from biological samples (Hayes 2012).

ASE is most efficient if the extracting solvent, sample-solvent ratio, extraction temperature, and time have been optimized (Denery et al. 2004). Denery and coworkers optimized the extraction of carotenoids from *Dunaliella salina* and showed that higher or equal extraction efficiencies (compared to traditional solvent technology) could be achieved with the use of less solvent and shorter extraction times. The performance of ASE extraction was compared to that of the traditional Folch method for microalgae (Mulbry et al. 2009). The ASE, depending on the solvent, extracted 85%–95% of the fatty acid content in the harvested microalgae compared to 44%–55% of the fatty acids extracted by the Folch method in the first solvent-extraction cycle.

What remains unclear is the effectiveness of such an approach at large-scale in terms of how to handle large amounts of biomass, separate out desirable lipids, and optimize the energy cost. The latter is also noteworthy in the context that ASE, by definition, uses non-aqueous solvents and therefore, must use dried biomass, a step that also requires energy input.

Mixed Solvent Extraction

Hejazi et al. (2002) proposed the two-phase system of aqueous and organic phases for the selective extraction of carotenoids from the microalgae *Dunaliella salina*. Their observations were that solvents with lower hydrophobicity reach critical concentrations more easily and in the process, break down the cell membrane. By using solvents of higher hydrophobicity, the effect of the solvent on the membrane decreased and the extraction efficiency for both chlorophyll and β-carotene decreased as well. By applying a measurement of solvent hydrophobicity based on the partition coefficient of the solvent in a two-phase system of octanol and water, screening viability and activity tests of *Dunaliella salina* in the presence of different organic phases indicated that cells remained viable and active in the presence of organic solvents with a log P (octanol) > 6 and that β-carotene can be extracted more easily than chlorophyll by biocompatible solvents.

This work has served as the basis for the development of a technology that proposes to use solvents such as decane and dodecane in the presence of live microalgal cells, concentrated for the extraction of triglycerides without loss of cell viability and extraction of membrane-bound, free fatty acids. Conceptually, the cells can be returned to their original bioreactor for continued growth and production of triglycerides

for biofuels production. For example, some have proposed a modified technique to "milk" oils or neutral lipids from algae using biocompatible solvents and applied sonication. If this process can be applied to microalgae slurries with suspended solid concentrations as low as 1 wt%, this method may provide a unique avenue for the selective extraction of lipids suitable for biofuels (e.g., triglycerides) that excludes the extraction of lipids that cannot be transesterified, as well as pigments (such as chlorophyll), which can be difficult to separate from the desired lipids.

Ionic liquids are another type of solvent that has been used to extract lipids from a variety of feedstocks and sources. Ionic liquids have been shown to extract lipids from wet primary sludge for biodiesel production (Olkiewicz et al. 2015). In addition, there is the potential that these liquids may be a promising technology for the extraction of lipids from microalgae because they offer benefits such as non-volatility, thermal stability, and synthetic flexibility. Ionic liquids are categorized here as a mixed solvent system because the literature shows that a mixture is often used to obtain the maximum lipid yield. For example, the ionic liquid [Bmim][$MeSO_4$] was used to extract lipids from *Chlorella vulgaris*, and yields were compared to the lipids extracted using the Soxhlet method and the Bligh and Dyer method. The yields for total lipids from the Soxhlet and Bligh and Dyer methods were 21 and 29 mg/g dry cell weight whereas the ionic liquid yielded 47 mg/g dry cell weight (Kim et al. 2013). There is limited data and analysis on the potential of these solvents to scale up, and further research is warranted to investigate the TEA and LCA impacts.

Supercritical Fluid Extraction

Supercritical fluid extraction utilizes the enhanced solvating power of fluids above their critical point (Luque de Castro et al. 1999). It can be processed using solid and liquid feeds (Reverchon et al. 2006). Supercritical fluid extraction techniques have been used in the commercial extraction of substances from solid substrates (e.g., caffeine from coffee beans) for more than two decades (Brunner 2005). The majority of applications have used CO_2 because of its preferred critical properties (i.e., moderate critical temperature of 31.1°C and pressure of 73.9 bar), low toxicity, and chemical inertness (Luque de Castro et al. 1999), but other fluids used have included ethane, water, methanol, ethane, nitrous oxide, sulfur hexafluoride, as well as n-butane and pentane (Herrero et al. 2006). The temperature and pressure above the critical point can be adjusted as can the time of the extraction.

Supercritical extraction is often employed in batch mode, but the process can also be operated continuously (Brunner 2005). One of the more attractive points to supercritical fluid extraction is that after the extraction reaction has been completed and the extracted material dissolved into the supercritical fluid, the solvent and product can be easily separated downstream once the temperature and pressure are lowered to atmospheric conditions. In this case, the fluid returns to its original gaseous state while the extracted product remains as a liquid or solid. However, it is important to note that the used solvent returns to the gaseous phase only when a gas (at ambient) conditions is used, such as carbon dioxide. In addition, it is important to highlight that this method is considered to be flexible due to the highly tunable nature of the solvent based on process conditions, which allows for extraction of pure or higher quality triglycerides (Halim et al. 2012).

Supercritical fluid extraction has been applied for the extraction of essential oils from plants (Reverchon et al. 2006), as well as functional ingredients and lipids from microalgae (Mendes et al. 1994; Metzger and Largeau 2005; Najafabadi et al. 2015). However, economical production of biofuels from oleaginous microalgae via supercritical processing is challenged by the same issues of energy-intensive processing and scaling up the process that is developed mainly for analytical usage. Use of methanol as the solvating fluid has the effect of converting lipids, via transesterification, to biodiesel (Najafabadi et al. 2015; see chapter 5 for more detail).

Recent results from the literature indicate that, not surprisingly, conditions to obtain maximum lipid yield for supercritical fluid extraction are highly dependent on the strain. For example, the maximum concentration of lipids were extracted from *Shizochytrium limacinum* at conditions of 35 MPa, 40°C with 95% volume ethanol as co-solvent (Tang et al. 2011) whereas it was reported that the optimal conditions obtained for extracting lipids from *Botryococcus braunii* was 22–25 MPa and 50°C (Santana et al. 2012). Thus, it appears that this area of research that will continue to develop and will need to be explored in a process and strain-specific context.

Switchable Solvents

The use of switchable solvents is another solvent-based approach that may be used for the extraction of algal components that may have potential advantages compared to traditional solvents (Jessop et al. 2005; Lam et al. 2008). Switchable solvent systems use liquids that can be reversibly switched between two different sets of properties based on the surrounding conditions. These systems have the potential to eliminate the need for costly separation of multiple solvent systems and/or drying of the biomass prior to extraction. Two types of switchable solvent systems have been described: a single- or two-component switchable solvent system. A two-component system (SSS) consists of a non-ionic liquid (an alcohol and an amine base) that is converted to an ionic liquid (a salt in liquid form) upon exposure to CO_2. This approach may be applied to extract lipids from microalgae because the non-polar compounds have a high affinity for the solvent, which allows the oil to be extracted while the polar form of the SSS (after contacting the CO_2) can be used to recover the oil.

In a single-component switchable system (SHS), switchable hydrophilic solvents change their polarity in addition to changing their miscibility in water (Du et al. 2015). SHS, such as amidines, can be used for the extraction of low-polarity organic products and carbonated water can be used to remove the solvent. The SHS can be recycled by switching the solvent back to the hydrophobic state. Secondary amines are another class of compounds that have been identified as being able to function as an SHS using CO_2 as a trigger (Du et al. 2015). A few secondary amines that have been identified as potentially SHS include: N-ethyl-B-butyl amine, N-methyl-N-propylamine, di-propyl amine, and benzyl methyl amine (Jessop et al. 2007). Both amidines and secondary amines have been tested for lipid extraction from wet or dry algae (Du et al. 2013; Samori et al. 2010; Boyd et al. 2012, Samori et al. 2013). One process concept for extraction of oil from algae using switchable solvents is described in Figure 6.2. As this technology is further developed, a full TEA and LCA will be needed to assess the energy for heating and CO_2/N_2 requirements.

A variety of algae species have been used to test the extraction of lipids with switchable solvents that include *Desmodesumus*, *Nannochloropsis gaditana*, *Tetraselmis suecia*, and *Botrycoccus braunii* (Du et al. 2013; Samori et al. 2010; Boyd et al. 2012; Samori et al. 2013). Maximum, final total-lipid yield (wt%) from these studies range from 8.2% to 57.9%. A summary of these results was provided by Du et al. (2013). In many cases, the yields using these solvents are the same regardless of whether a wet or dry process was used. In addition, results with secondary amines as SHS indicate that lipid material can be extracted from slurries of fresh, unbroken microalgae, which could have large impacts on cost reduction associated with extraction of lipids from algae (Du et al. 2013).

6.5 Comparison of Extraction Methods

A major hurdle in this area of the field is the difficulty in comparing various lipid-extraction methods. Many different protocols are used to provide a comparison as a control value for the amount of lipids extracted from algal biomass, and there is no consistency in the underlying assumptions for the economical and sustainability comparisons. Often, the reported processes in the literature are only demonstrated on a laboratory scale, without clear measurements of the resulting product or the energy or cost implication of the equipment if scaled. In many cases, these control methods extract lipids that are not usable as a biofuel or cannot be converted to a biofuel (non-saponifiable lipids), but they are reported as "lipids" that are extracted. In other cases, methods are used that leave behind significant amounts of saponifiable lipids that could be converted to biofuels (Laurens et al. 2012; Griffiths et al. 2010; Slocombe et al. 2013). This is an area in the field that needs to be addressed in order to determine effective and efficient techniques of lipid extraction within the various methods that are being developed.

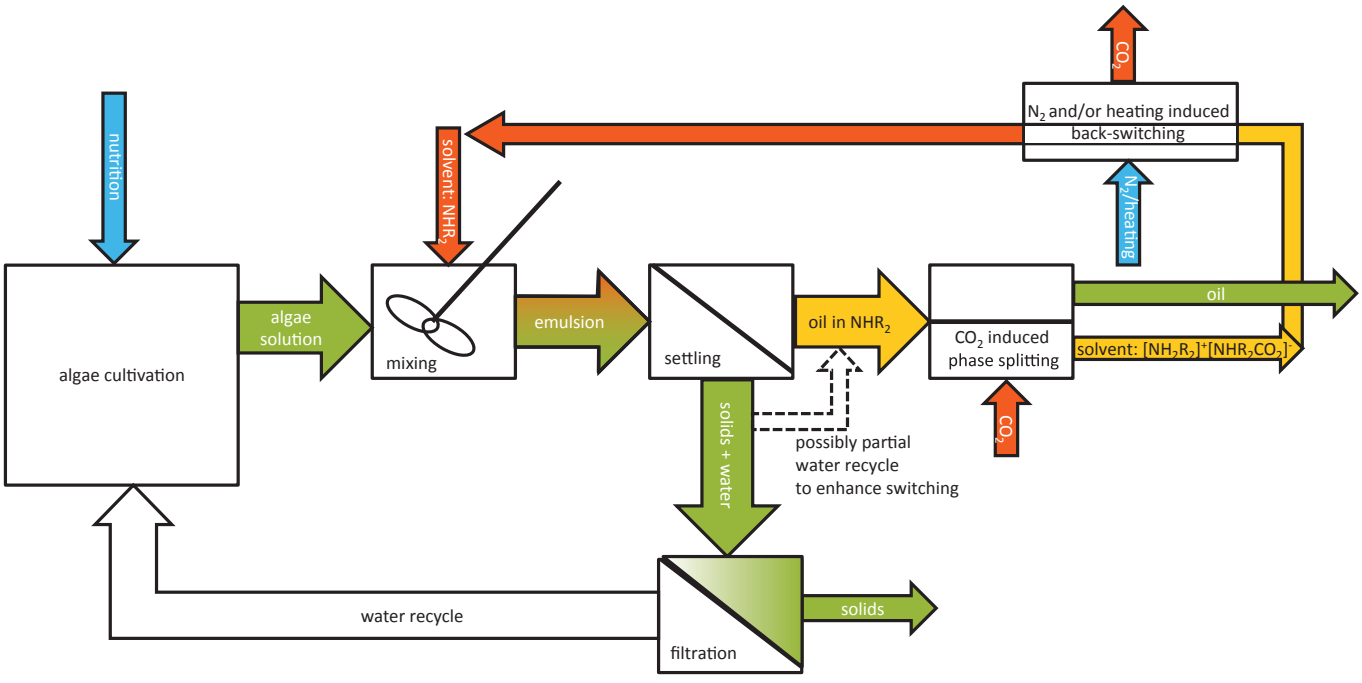

Figure 6.2. Process concept for extraction of oil from algae with a secondary amine solvent. This is followed by CO_2-induced phase splitting for solvent recovery and N_2-induced backswitching (Source: Du et al. 2013).

Few direct comparisons have been made of the technologies described in this section to determine which methods are better than others. What has been published has indicated that, based on data from the extraction of lipids of three different types of oleaginous microalgae, a comparison of bead beating, sonication, autoclaving, osmotic shock, and microwave-assisted extraction suggested that microwave-assisted extraction prior to solvent extraction is the most efficient method (Lee et al. 2010). However, the economics associated with these technologies was not addressed and it is not clear what the TEA and LCA impacts are of these technologies at scale. In addition, PEF-assisted extraction and ultrasound-assisted technologies were not compared.

6.6 Lipid Extraction Challenges

Presence of Water Associated with the Biomass

Several extraction methods, such as solvent-based (Kim et al. 2013) or supercritical extractions (Najafabadi et al. 2015) followed by transesterification or direct transesterification, suffer from lower efficiency when performed in the presence of water. Thus, the extraction method selected for a particular process is affected by the choice of upstream and downstream unit operations and vice versa. The presence of water can cause problems at both ends at larger scales and is one of the main motivations to move towards whole-cell processing (see chapter 7) due to the tremendous amount of energy required to dry algal biomass (if a dry extraction is required). When present in the bulk solution, water can either promote the formation of emulsions in the presence of ruptured cells or participate in side reactions. At the cellular level, intracellular water can prove to be a barrier between the solvent and the solute. In this context, the issue of solvent access to the material being extracted is as important as the miscibility of the analyte in the solvent. This is a principal motivation behind the application of extraction techniques at elevated temperatures and pressures (see the section on hydrothermal processing in chapter 7).

Separation of Desired Extracts from Solvent Stream

Extraction processes can yield undesirable components, such as chlorophyll and non- transesterifiable lipids. Very little information is available on this critical step that is necessary before converting the algal biocrude into finished fuels and products. What little has been published has focused on quantification of chlorophyll contamination of lipid extracts using various solvent systems (Ramluckan et al. 2014).

Process Integration

Research in the field has slowly moved from a very lipid-centric approach to a more whole-biomass, holistic approach in which all of the components of algae are being considered in terms of potential for fuels or products. The selective fractionation approach (Davis 2014) that replaced the lipid-only model (ANL, NREL, and PNNL 2012) is one example of this movement. The ability of the extraction-based approach to produce both fuels and products from one process is one advantage in comparison to the thermochemical-based routes that use the whole algae (see chapter 7). However, there are still challenges that exist in terms of determining the best combination of technologies to produce a blend of products and fuels in an economically feasible, extraction-based process. Some of these challenges include the following:

1. Gaining a more detailed understanding of the composition required (mix of proteins, lipids, and carbohydrates) that may produce an economically viable fuel and co-product
2. Understanding the potential interactions between the major biochemical constituents of the biomass undergoing pretreatment—these interactions may, themselves, limit solvent accessibility or lipid mass transfer (e.g., encapsulation of lipids in a solubilized starch-gel matrix)
3. More research on the methods described above that determines whether the technology can be scaled economically
4. Development of novel extraction technologies that take advantage of traits inherent to algae
5. Further development of wet extraction technologies to avoid drying requirements and associated capital costs
6. Applying potential lessons learned from the use of oleaginous yeast, which is an important platform for hydrocarbon biofuels based on terrestrial feedstocks
7. Lack of standards in the literature that describe yields from solvent extraction. It is important that researchers in the field are aware that different methods provide lipids of different levels of purity (relative to the fuel grade lipid components such as fatty acids) with the exception being that fatty acid methyl ester (FAME) analysis can give a reproducible metric. Therefore, more work is needed to enable a comparison of the work from different publications.

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7. Algal Biofuel Conversion Technologies

Possible fuels that can be produced from algae range from gaseous compounds like hydrogen and methane, to liquid alcohols and hydrocarbons, to viscous oils and high-carbon-content solids like coke. Liquid hydrocarbons that can be substituted for or blended with gasoline, jet, or diesel fuel are attractive products because: (1) they are the primary products derived from crude oil used for transportation in the United States, (2) they are potentially more compatible with existing fuel-distribution infrastructure in the United States than other biomass-derived fuels, and (3) specifications and standards for handling these fuels already exist.

The primary objective of this chapter is to summarize a number of potential strategies for converting algal biomass into suitable replacements for petroleum gasoline, diesel, and jet fuel. When a fuel meets all customer requirements, it is referred to as "fit for purpose." While a successful fuel-conversion strategy will address the full range of desired fit-for-purpose properties (e.g., distillation range, ignition characteristics, energy density, safety for storage and transport, etc.), these desired fuel characteristics are driven primarily by customer requirements and are discussed later in chapter 9. This chapter focuses on fuel-conversion strategies from a variety of perspectives to establish the current state of the art, as well as identify critical challenges and roadblocks.

Several themes emerged from the 2008 Algal Roadmap Workshop in relation to conversion of algal feedstocks to fuels:

1. The feedstock, conversion process, and final fuel specifications are highly interdependent and must be considered together if an optimal process is to be identified. As a result, accurate and detailed feedstock characterization (including composition, moisture content, and variability) is essential, since this is an upstream boundary condition for the entire downstream fuel-conversion process.
2. LCA of energy and carbon will be a key tool in selecting the preferred fuel conversion technologies from those discussed in this chapter. (See chapter 11 for a discussion of LCA and TEA.)
3. Alongside how to convert lipid, carbohydrates, or whole algae to fuels most efficiently, one of the greatest challenges in algal fuel conversion is how best to use any algal remnants or waste streams after desirable fuel precursors have been extracted. All of the petroleum feedstock that enters a conventional petroleum refinery must leave as marketable products or be used within the production system, and this must also hold true for the algae biorefineries of the future if they are to achieve significant market penetration. All algal carbon must be recovered to the maximum extent possible into fuels and products.

A large number of potential pathways exist for the conversion from algal biomass to fuels. These pathways can be classified into the following three general categories:

1. Those that focus on the biofuel production (e.g., ethanol, hydrogen, methane, and alkanes) from algae, such as through heterotrophic fermentation or direct secretion
2. Those that convert whole algal biomass to yield fuel molecules
3. Those that convert components from extracted algae (e.g., lipids, carbohydrates, proteins) to yield fuel molecules and other co-products.

These technologies are primarily based on similar methods developed for the conversion of terrestrial, plant-based oils and products into biofuels, although the compositional complexities of the output streams from algae must be dealt with before these methods can be applied effectively. For example, all conversion methods will employ dewatering at some point within the process. Pros and cons of these pathways within each of these categories are discussed below, and a summary of each fuel-conversion technology is given. Inputs, complexity, cost, and yields are provided (where known), and key barriers and RD&D opportunities are listed.

7.1 Production of Biofuels from Algae through Heterotrophic Fermentation or by Direct Secretion

The production of biofuels through heterotrophic fermentation or through photosynthetic growth coupled with direct alcohol or other product secretion have certain advantages in terms of process cost because they can eliminate several process steps (e.g., oil extraction) and the associated costs in the overall fuel-production process. Enclosed systems utilized for both of these processes also allow for maintaining highly controlled conditions, which first could be oriented toward biomass production and then oil production. Such a system can generate extremely high biomass concentrations. In heterotrophic growth, there is an enormous potential to use various, fixed carbon feedstocks (which would bring down the cost of production). In direct secretion, algal biomass can be grown phototrophically with alcohol or other products collected after secretion while maintaining the original culture until senescence. These approaches are quite different from the algal biofuel processes that use algae to produce biological oils, which are subsequently extracted and used as a feedstock for liquid fuel production, typically renewable diesel. There are several biofuels that can be produced directly from algae, including alcohols, alkanes, and hydrogen.

Alcohols

Algae, such as *Chlorella vulgaris* and *Chlamydomonas perigranulata*, are capable of producing ethanol and other alcohols through heterotrophic fermentation of starch (Hon-Nami 2006; Hirayama et al. 1998). This can be accomplished through the production and storage of starch via photosynthesis within the algae, or by feeding sugar to the algae directly, and subsequent dark, anaerobic fermentation of these carbon sources to produce ethanol. If these alcohols can be extracted directly from the algal culture media, the process may be less capital- and energy-intensive than competitive algal biofuel processes. Despite dark fermentation being low-energy, the alcohol titres obtained are often very low (such as 1% w/w for *Chlamydomonas reinhardtii* [Hirano et al. 1997]).

Genetic engineering of algae to enhance the secretion of alcohols is a promising alternative. This direct-secretion process typically consists of closed photobioreactors utilizing seawater with metabolically enhanced cyanobacteria that produce ethanol or other alcohols while being resistant to high temperature, high salinity, and high ethanol levels—previous barriers to commercial-scale volumes (Hirano et al. 1997; as in processes at Joule and Algenol). One key aspect of the system is that a source of cheap CO_2, such as a power plant, can be used to supply CO_2 to the bioreactors to accelerate the algae growth (Algenol Biotech LLC). An example of this process technology links sugar production to algal photosynthesis. There are claims that this process may consume more than 90% of the system's CO_2 through photosynthesis, wherein a portion of the carbon in these sugars is converted into ethanol. The ethanol is secreted into the culture media and is collected in the headspace of the reactor, purified, and stored. Algenol Biotech LLC claims that their direct-secretion process produces around 6,800 gallons of ethanol per year on one wet acre of algal cultivation (algenol.com).

Further breakthroughs that enable more efficient production systems and the development of new process technologies may be critical in terms of long-term commercial viability. Scaling of these systems to large-scale commercial biorefineries will also require significant advances in process engineering and systems engineering, such as employing modular units. Metabolic pathway engineering within these algae, enabled by metabolic flux analysis and modern genomics tools, may further help in producing a commercially viable organism. In addition to ethanol, it is possible to use algae to produce other alcohols, such as methanol and butanol, using a similar process technology, although the recovery of heavier alcohols may prove problematic and will need further R&D. The larger alcohols have energy densities closer to that of gasoline but are not typically produced at the yields that are necessary for commercial viability.

Alkanes

In addition to alcohols, alkanes may be produced directly by engineered algae phototrophically or by algae through heterotrophic metabolic pathways. Joule Unlimited, Inc. has genetically modified algae to produce and secrete alkanes when grown phototrophically (jouleunlimited.com). Alternatively, rather than growing algae in ponds or enclosed in plastic tubes that utilize sunlight and photosynthesis, algae can be grown inside closed reactors without sunlight. The algae are fed sugars, the cheap availability of which is a key consideration for cost-effective production of biofuels; these sugars are available from renewable feedstocks, such as lignocellulosic biomass, in a pressure- and heat-controlled environment. This process can use different strains of algae to produce different types of alkanes; some algae produce a mix of hydrocarbons similar to light crude petroleum. These alkanes can theoretically be secreted and recovered directly without the need for dewatering and extraction. With further processing, a wide variety of fuels can be made.

Using algae to convert cellulosic materials, such as switchgrass or wood chips, to oil may have an advantage over many other microorganisms under development for advanced biofuel production. When lignocellulosic biomass is pretreated to allow for enzymatic hydrolysis for production of sugars, many toxic byproducts are released including acetate, furans, and lignin monomers. In most other processes, these toxic compounds can add process costs by requiring additional conditioning steps or the concentration of biomass hydrolysate in the conversion step. Algae may prove to be more resistant to these compounds and sugar conversion. Lignocellulosic biomass could also be utilized by algae in mixotrophic cultivation (see chapter 4).

7.2 Processing of Whole Algae

In addition to the direct production of biofuels from algae, whole algae (regardless of how it was grown—phototrophically or heterotrophically) can be processed into fuels instead of first extracting oils and post-processing. These methods benefit from reduced costs associated with the extraction process, and the added benefit of being amenable to processing a diverse range of algae, though at least some level of dewatering is still required. There are five major categories of conversion technologies that are capable of processing whole algae: pyrolysis, gasification, anaerobic digestion, supercritical fluids, and subcritical hydrothermal liquefaction (Figure 7.1).

Pyrolysis

Pyrolysis is the chemical decomposition of a condensed substance by heating; for algae, this is generally at 400°C–600°C (López Barreiro 2013). Pyrolysis never takes place in the presence of oxygen. The thermochemical treatment of the algae, or other biomass, can result in a wide range of products, depending on the reaction parameters. Liquid product yield tends to

favor short residence times, fast heating rates, and moderate temperatures (Huber et al. 2006). Pyrolysis is extremely fast, in comparison to other conversion methods, with reaction times of the order of seconds to minutes. Slow pyrolysis produces lower biocrude yields than fast/flash pyrolysis, but the energy density is significantly higher than the biocrude produced from the latter.

Pyrolysis is being investigated for producing fuel from biomass sources other than algae. Although synthetic diesel fuel cannot yet be produced directly by pyrolysis of algae as they are unstable and therefore difficult to transport to a refinery, a degradable alternative liquid called bio-oil can be produced. Following hydrotreatment, the bio-oil can enter directly into the refinery stream and produce a suitable feedstock for generating standard diesel fuel (direct raw bio oil insertion is currently limited to 10%). Also, higher efficiency can be achieved by the so-called "flash pyrolysis" (or "fast pyrolysis") technology, where finely ground feedstock is quickly heated to 350°C–500°C for less than 2 seconds. For flash pyrolysis, typical biomass feedstocks must be ground into fine particles and dried to <10% moisture.

This is one area where algae have a major advantage over other biomass sources because it exists fundamentally in small units, has no fiber tissue to deal with, and is less heterogeneous than most biomass or coal. Several commercial plants for fast pyrolysis of biomass have been built in recent years in Finland, Canada, and Netherlands, producing fuel oil for heating. Fast pyrolysis bio-oil is currently being marketed and sold by Ensyn for food flavorings and as fuel oil (ensyn.com/products/fuel-products/pyrolysis-heating-oil). For a review of converting algae via pyrolysis under multiple operating condition regimes, see López Barreiro (2013).

A significant roadblock in using pyrolysis for algae conversion is moisture content. The energetic cost of drying the algae has been reported to cause the overall process to be energy negative (Jena et al. 2011). Furthermore, in a comparison of bio-oil from pyrolysis of algae and biocrude from hydrothermal liquefaction of algae, it was demonstrated that pyrolysis bio-oil had lower energy content and was less stable (Jena et al. 2011). Vardon (2012) provides comparison of yields and properties of hydrothermal liquefaction (HTL)-processed biocrude versus pyrolysis bio-oil generated from *Spirulina* and *Scenedesmus*, and compares net-energy yields for each process as a function of algal feedstock moisture content. Biocrude produced from HTL and slow pyrolysis of the same algal species are similar in energy density, but the net-energy yield was more favorable for HTL (Vardon et al. 2012). It appears that pyrolysis will not be cost-competitive over the short-term unless an inexpensive dewatering or extraction process is also developed and the bio-oil is demonstrated to be superior compared to oil produced from other conversion processes. Additionally, since pyrolysis is already a relatively mature process technology, it is expected that only incremental improvements will occur and a breakthrough in conversion efficiency appears unlikely.

Gasification

Gasification is the partial oxidative heating at temperatures greater than 700°C to produce different liquid fuels from biomass, for example through Fischer-Tropsch Synthesis (FTS)

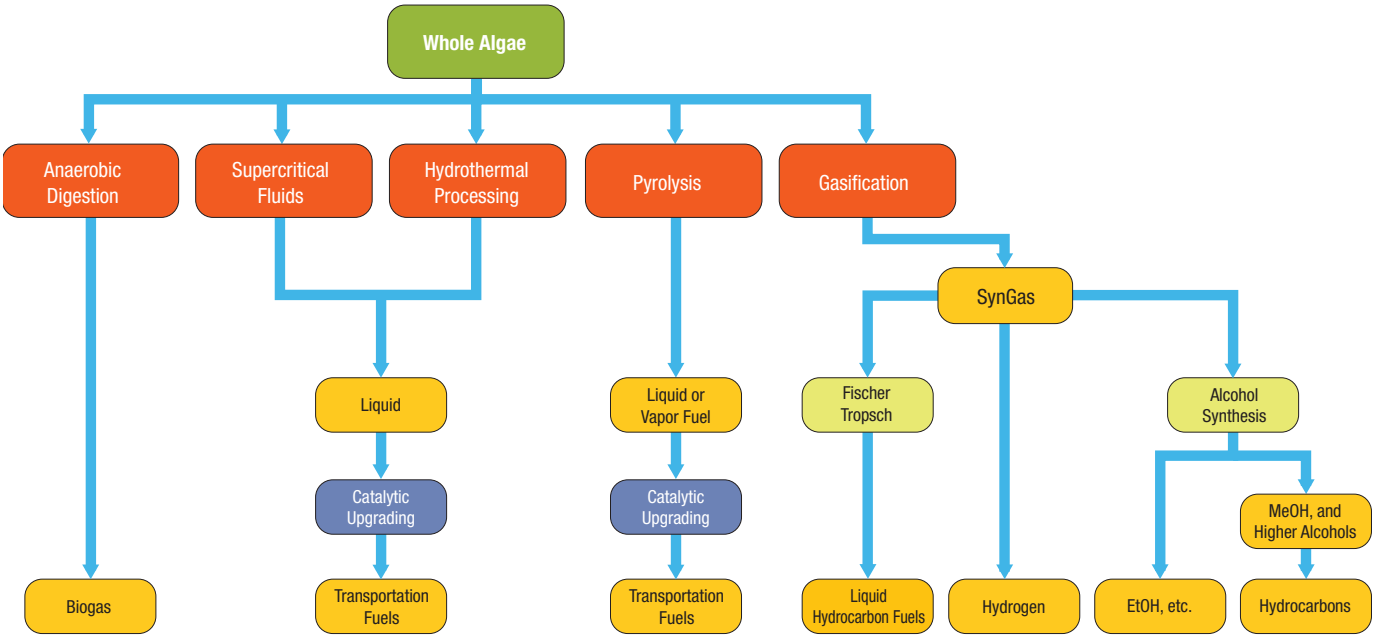

Figure 7.1. Schematic of the potential conversion routes for whole algae into biofuels (Source: Adapted from the 2010 *National Algal Biofuels Technology Roadmap.*)

or mixed alcohol synthesis of the resulting syngas. While there have been issues with scale-up, the synthesis of mixed alcohols using gasification of lignocellulose is relatively mature (Phillips 2007; Yung et al. 2009), and it is reasonable to expect that once water content is adjusted for, the gasification of algae to these biofuels would be comparatively straightforward. FTS is also a relatively mature technology where the syngas components (CO, CO_2, H_2O, H_2, and impurities) are cleaned and upgraded to usable liquid fuels through a water-gas shift and CO coupling (Okabe et al. 2009; Srinivas et al. 2007; Balat 2006).

Aside from the high-capital costs of FTS, conversion of bio-syngas has several advantages over other methods. First and foremost, it is possible to create a wide variety of fuels with acceptable and known properties. Additionally, bio-syngas is a versatile feedstock, and it can be used to produce a number of products, making the process more flexible. Another advantage is the possibility to integrate an algal feedstock into an existing thermochemical infrastructure. Additionally, since FTS is an exothermic process, it should be possible to use some of the heat for drying the algae during a harvesting/dewatering process with a recuperative heat exchanger.

The key roadblocks to using FTS for algae are thought to be similar to those for coal (Yang et al. 2005), with the exception of any upstream process steps that may be a source of contaminants, which will need to be removed prior to reaching the Fischer-Tropsch catalyst. FTS tends to require production at a very large scale to make the process efficient overall. However, the most significant problem with FTS of biomass is the high-capital cost of the gasifier, as well as the cleanup and tar reforming. Tars have high molecular weight and can develop during the gasification process. The tars cause coking of the synthesis catalyst and any other catalysts used in the syngas cleanup process and must be removed. The four basic mechanisms to deal with tar-related problems are as follows:

1. Catalytic reforming
2. Solvent tar removal
3. Oxidative reduction
4. High temperatures (approximately 1,100°C).

Tar formation can be minimized or avoided via entrained-flow gasification at high temperatures (Hallgren et al. 1993). While this technology requires sub-millimeter-sized particles, algae may have a unique advantage in this process. Typically, it is difficult to reach such a small size with other biomass sources, and doing so usually requires pretreatment, but certain species of algae may not require pretreatment due to their inherent small size. Another approach for tar-free syngas was demonstrated in a pilot plant in Freiberg, Germany, built by Choren Industries GmbH. The pilot plant used two successive reactors. The first reactor was a low-temperature gasifier that broke down the biomass into volatiles and solid char. The tar-rich gas was then passed through an entrained-flow gasifier where it was reacted with oxygen at high temperature (Raffelt et al. 2006). Another advantage of algae (relative to biomass) is the absence of lignin, which is known to generate high molecular-weight aromatics during gasification (Milne et al. 1998).

Even though FTS is a mature technology, there are still several areas that should be investigated and require R&D. First, it is necessary to determine the optimum conditions for indirect gasification of algae. It would be desirable to determine the feasibility of using the oxygen generated by algae for use in the gasifier to reduce or eliminate the need for a tar reformer. Oxygen generated by algae will be at atmospheric pressure, and the cost to collect and compress to gasification pressures (even low-pressure gasifiers) is likely to be cost prohibitive. Also, it would be useful to leverage ongoing biomass-to-liquid fuels research using cellulosic feedstocks.

Anaerobic Digestion of Whole Algae

The production of biogas from the anaerobic digestion of microalgae or macroalgae eliminates several of the key obstacles that are responsible for the current high costs associated with algal biofuels, including drying, extraction, and fuel conversion, and as such, may be a cost-effective methodology, though compared to the high cost of growing algae, methane production is a low-value product. This technology benefits from significant, full-scale experience at domestic wastewater-treatment facilities. Furthermore, it can be utilized to generate power from wastewater-grown algae, and it allows nutrient recovery (such as nitrogen and phosphorous) from lipid-extracted biomass. In addition, the methane produced readily separates from water without energy or chemical input. Recent work has demonstrated methane produced from anaerobic digestion can be converted to bioplastics poly(hydroxybutyrate) blends to increase the value of biogas (Criddle et al. 2015). Co-digestion of other feedstocks with algal biomass can be considered to raise the carbon-to-nitrogen ratio, or proteins may be pre-extracted to use as co-products prior to digesting. However, anaerobic digestion also has several technical challenges due to low concentrations of digestible, algal-biodegradable substrate, recalcitrant-substrate constituents, algal cell-wall degradability, algal low carbon-to-nitrogen ratio, ammonia toxicity, and effects from salinity and associated metal ions (for review, see Ward et al. 2014). Pretreatment of algal species may be necessary for maximum methane production (also see 7.4, "Processing of Algal Remnants after Extraction"). Several studies have been carried out that demonstrate the potential of this approach. A 2008 study indicated that biogas-production levels of 180.4 ml/g-d of biogas can be realized using a two-stage anaerobic digestion process with different strains of algae, with a methane concentration of 65% (Vergara-Fernández et al. 2008). Since then, anaerobic digestion of microalgae and macroalgae has been examined under varying conditions at several scales (see Ward et al. 2014 for

review). Scaling up of this technology to industrially relevant scales and optimizing the species-specific effects on methane production are two key challenges. Managing fugitive methane emissions may present a challenge as well. While it might be possible to manage these emissions via engineering or process flow, if attention is not paid to the issue, emissions could be large (Frank et al. 2012).

Supercritical Processing

Supercritical processing is capable of simultaneously extracting and converting oils into biofuels (Demirbas 2006). Supercritical fluid extraction of algal oil is far more efficient than traditional solvent-separation methods, and this technique has been demonstrated to be extremely powerful in the extraction of other components within algae (Mendes 2007). This supercritical transesterification approach can also be applied to algal oil extracts. Supercritical fluids are selective, thus providing high purity and product concentrations. Additionally, there are no organic solvent residues in the extract or spent biomass (Demirbas 2009a). Extraction is efficient at modest operating temperatures, for example, at less than 50°C, ensuring maximum product stability and quality. Additionally, supercritical fluids can be used on whole algae without dewatering, thereby increasing the efficiency of the process.

The supercritical extraction process can be coupled with a transesterification reaction scheme to enable a "one-pot" approach to biofuel production (Anitescu et al. 2008). In this process variant, supercritical methanol or ethanol is employed as both the oil-extraction medium and the reagent for transesterification (Warabi et al. 2004). In the case of catalyst-free, supercritical ethanol transesterification, it has been demonstrated that this process is capable of tolerating water, with a conversion yield similar to that of the anhydrous process in the conversion of vegetable oils. While the occurrence of water in the reaction medium appears as a factor in process efficiency, the decomposition of fatty acids is the main factor that limited the attainable ester content (Vieitez et al. 2008; Vieitez et al. 2009).

Similar results have been observed for supercritical methanol processing of vegetable oils (Hawash et al. 2009). Because decomposition was a consequence of temperature and pressure conditions used in this study, further work should be focused on the effect of milder process conditions, in particular, lower reaction temperatures. In the case of combined extraction and transesterification of algae, further study will also be needed to avoid saponification. It also remains to be seen whether the processing of whole algae in this fashion is superior, in terms of yield, cost, and efficiency, to the transesterification of the algal oil extracts.

The economics of supercritical transesterification process, at least in the case of vegetable oil processing, have been shown to be very favorable for large-scale deployment. One economic analysis has been conducted based on a supercritical process to produce biodiesel from vegetable oils in one step using alcohols (Anitescu et al. 2008). It was found that the processing cost of the proposed supercritical technology could be near half of that of the actual conventional transesterification methods (i.e., $0.26/gal vs. $0.51/gal). It is, therefore, theoretically possible that if the other upstream algal-processing costs could be mitigated through the addition of a transesterification conversion process, the overall algal biorefinery could become cost-competitive with fossil fuels.

The clear, immediate priority, however, is to demonstrate that these supercritical process technologies can be applied in the processing of algae, either whole or just its oil extract, with similar yields and efficiencies at a level that can be scaled to commercial production. In particular, it must be demonstrated that this process can tolerate the complex compositions that are found with raw, unprocessed algae and that there is no negative impact due to the presence of other small metabolites.

Hydrothermal Processing

Hydrothermal processing can be subdivided into different techniques based on the temperature employed during treatment. HTL of biomass converts wet algal slurry to a range of liquid fuels by processing in a hot (523 K–647 K) and pressurized (4–22 MPa) water environment at residence times of 3–5 minutes in order to disassemble cells to liquid components (Patil et al. 2008; Garcia Alba et al. 2012; for review, see Elliott et al. 2015 and Elliott 2016). When processed at lower temperatures (<275°C) and pressure (<2 MPa), hydrochar is produced (though this may be a consequence of batch processing versus continuous throughput). At higher temperatures (>374°C) and pressures (>22.1 MPa), syngas is produced. This technology is a representation of the natural geological processes known to be involved in the formation of petroleum-based fossil fuels. These technologies harness the high activity of water in subcritical environments that is capable of decomposing the algal biomass into smaller molecules of higher energy density or more valuable chemicals. The main product of the liquefaction process is a liquid fuel known as biocrude that typically accounts for 45% wt. of the feedstock on a ash-free dry weight (AFDW) basis for fast-growing, low total-lipid algae; yields are much higher for high total lipid algae. Leow et al. (2015) showed that HTL biocrude yield varied from 33 wt% –68 wt% for *Nannochloropsis* sp. cultivated to varying composition before processing. Biocrude can be upgraded to the entire distillate range of petroleum-derived fuels.

The past 5 years has seen a tremendous expansion in HTL of whole algae R&D (for review, see Elliott 2016). The HTL processing of algae requires some dewatering to a slurry of typically 10%–20% dry solids usually accomplished by mechanical means. Continuous-flow reactor HTL of microalgae yields between 38%–64% (AFDW) with an energy recovery of 60%–78% (Jazrawi et al. 2013; Elliott et al. 2013).

Algal lipid, protein, and carbohydrate composition influences HTL-conversion efficiency, with higher total-lipid content and lower protein yielding higher energy recovery (Li et al. 2014). Furthermore, high-protein content results in higher nitrogen concentration in the biocrude, requiring significant denitrification during upgrading to limit nitrogen-oxide emissions (Sudasinghe et al. 2014). Potential production strains of algae have higher nitrogen contents (4%–10%) compared to lignocellulosic feedstocks (< 1%) (Vardon et al. 2012). A benefit of HTL as applied to algae is the potential for nutrient recycling through the precipitation of solids, allowing phosphorous recovery and the transformation of nitrogen into ammonium that can be fed back into the algae pond (Figure 7.2 from Elliott et al. 2015). Hydrothermal processing is also advantageous in that much of the energy for heat-up can be recovered and used elsewhere within the algal biorefinery.

As a part of NAABB, a unique algae HTL system that combines extraction and conversion (HTL-catalytic hydrothermal gasification [CHG]) to provide high-biocrude yield without the need for extraction solvents was developed (NAABB 2014). Wet algal biomass (15%–20% solids) is fed directly to the HTL system, which produces biocrude and an effluent water stream that phase separates without the need of solvent extraction. The biocrude stream is readily upgraded via hydrotreating to hydrocarbon fuel. The hydrotreated oil can then be fractionated into jet, diesel, and naphtha fractions. The effluent water stream is then processed with CHG to recover additional fuel in the form of a methane gas/CO_2 mixture, and the water stream is recycled to a pond. Advantages of the HTL-CHG processing pathway include

- Capture of 85% of the carbon in algae as fuel-grade components (biocrude that can be upgraded to diesel, jet, and gasoline)
- Production of a biocrude that can be readily converted to meet diesel and Jet A fuel standards
- Effective wastewater treatment to reduce the organic content and provide methane for process energy
- Recycle of water and nutrients (nitrogen, phosphorous, and other trace minerals for algal cultivation)
- Significant decrease in capital and operating costs compared to processes requiring high lipid-yielding algal biomass and extraction of the lipid from the biomass.

As part of the NAABB effort, a pilot-scale system that can be used for both HTL- and CHG-process development was designed and built by NAABB industrial partners.

Two-step sequential HTL (SEQHTL) was recently developed to isolate polysaccharides or other high-value products (Chakraborty et al. 2012), to produce biocrude with low nitrogen content (Prapaiwatcharapan et al. 2015), or to collect an aqueous phase rich in organic carbon and nutrients to enhance biomass productivity (Selvaratnam et al. 2015). As the name implies, a low temperature step (160°C, but depending on algal species and desired product) to remove polysaccharides precedes processing by HTL conversion at a higher temperature (300°C). Biocrude yields after SEQHTL have varying results, with some studies finding a trade-off between nitrogen removal and overall biocrude yield (Jazrawi et al. 2015) and others discovering a higher-percentage biocrude yield and predicting a higher energy recovery (Prapaiwatcharapan et al. 2015; Miao et al. 2012) with SEQHTL compared to direct HTL.

The commercial application of algal HTL processing to fuels is currently unrealized but scaled-up pilot-plant operations have begun, and several small algae processing companies center around HTL of whole algae (Elliott 2016). Pilot plants

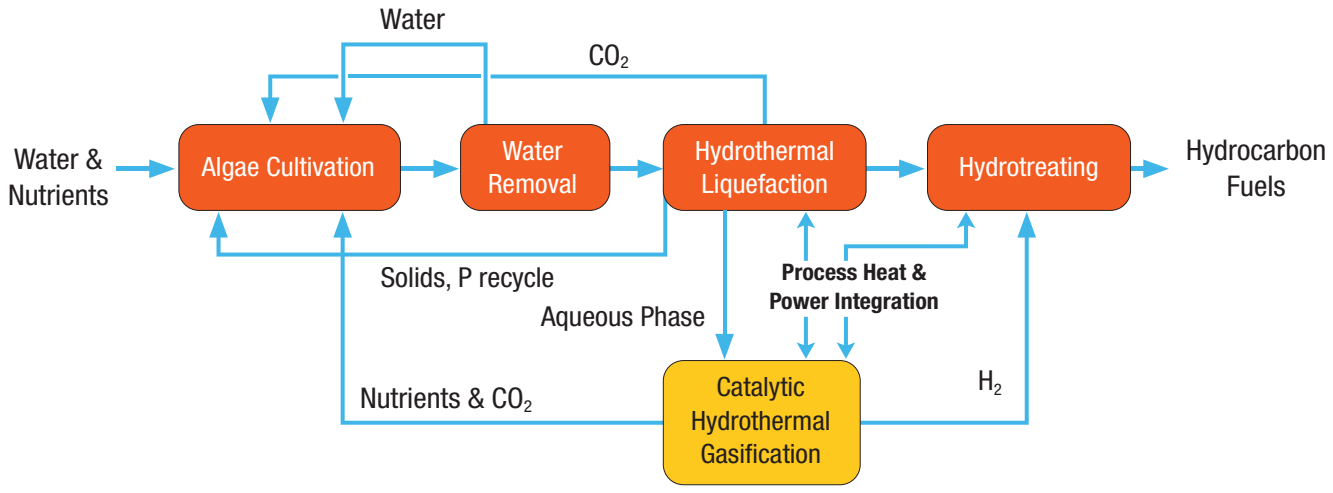

Figure 7.2. Hydrothermal processing of algae with nutrient recycle (Source: Elliott et al. 2015)

employing the HTL of algae have been tested or are being initiated at several locations, including Sapphire Energy Inc. in Columbus, New Mexico; Algenol Biotech LLC in Fort Myers, Florida; Reliance (contracted with Genifuel) in India; Muradel in Whyalla, Australia; and the University of Sydney in Australia (Elliott 2016). The HTL algae-processing technology licensed by Genifuel from PNNL was funded by BETO.

7.3 Conversion of Extracted Algae

There is an obvious and critical link between the type of extraction process used and the product composition, and as such, a fundamental and exhaustive understanding of the different types of inputs to the conversion technologies must be in place. Historically, a substantial focus within the algal biofuel community has been on the production and conversion of lipids from algae, as discussed in the 2012 "Harmonization Report" (Davis et al. 2012) (e.g., triacylglycerides, which can be converted into biodiesel). This appeared to be the most relevant process, or lowest-hanging fruit, where research should be focused due to the direct link between lipids and oil production for biofuels. Over the last several years, further research has demonstrated that there are a number of challenges associated with a lipid-only approach (Quinn et al. 2011; Davis et al. 2011). For example, the impact of timing the harvest can have a profound impact on lipid yields as well as other downstream processes (Laurens et al. 2015). In addition, it was also recognized that a lipid-centric approach underutilized other components (carbohydrates and proteins) within the algal cell. In a conceptual algal-biofuel process, it was assumed that these components, which are part of the spent biomass after the lipids are extracted, would be sent to an anaerobic digester (Davis et al. 2012). More-recent research suggests that obtaining value from all of these components (lipids, carbohydrates, and proteins) has the potential to be a more economical process (Laurens et al. 2015). This section discusses chemical, biochemical, and catalytic processes that can be employed to convert algal extracts. (Figure 7.3). For discussion of the technologies that can be used to prepare the

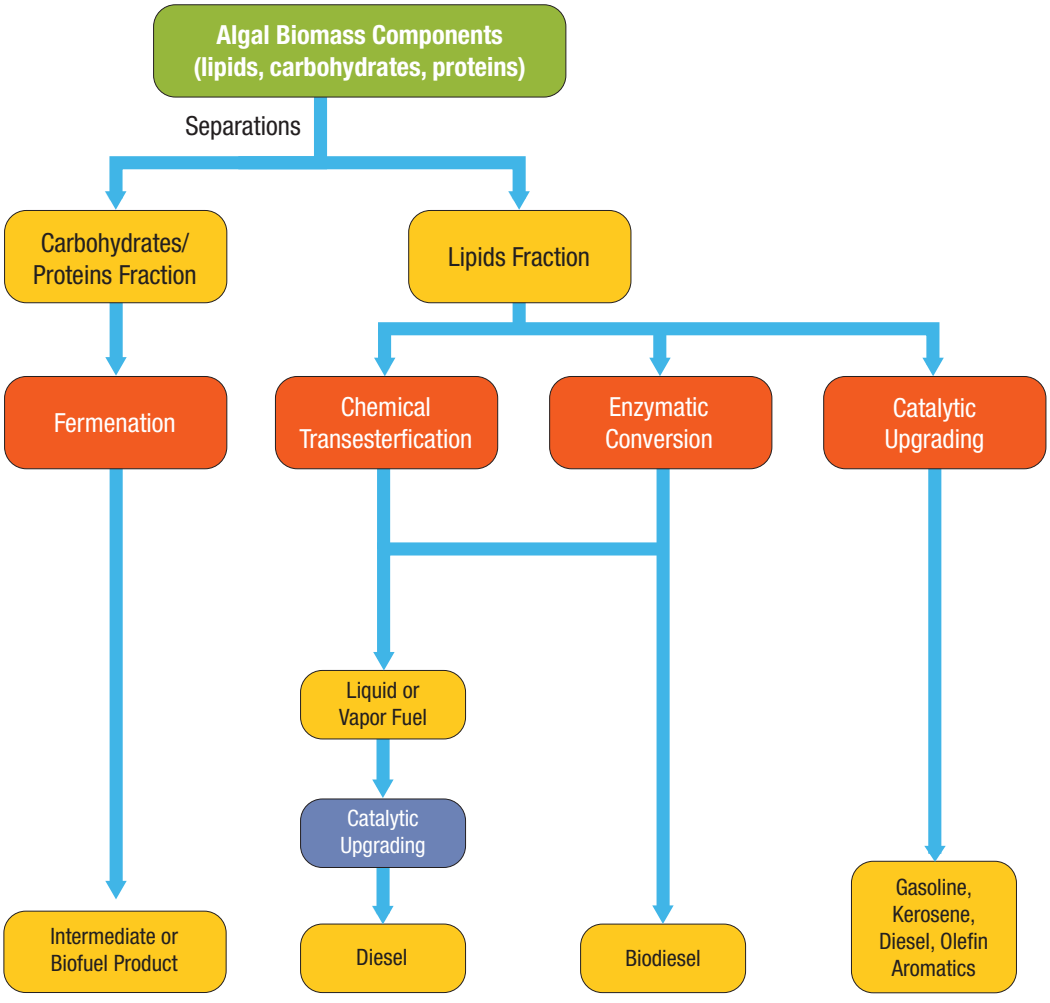

Figure 7.3. Schematic of the various conversion routes for algal biomass components into biofuels (Source: Adapted from the 2010 *National Algal Biofuels Technology Roadmap*.)

extracted algae for conversion (pre-treatment or extraction technologies), please see chapter 6.

Chemical Transesterification

The transesterification reaction is used to convert TAGs extracted from algae biomass to fatty acid methyl esters (FAMEs), which is simply a process of displacing an alcohol group from an ester by another alcohol (Demirbas 2009b). This process differs from direct transesterification (see section below) in that the oil is extracted prior to the transesterification reaction. A comparison of these methods is provided in Figure 7.4. Transesterification can be performed via catalytic or non-catalytic reaction systems using different heating systems that are required to initiate the reaction. This technology is relatively mature and has been demonstrated to be the "gold standard" in the conversion of vegetable oils into biodiesel (Hossain et al. 2008). In addition to the classic base-catalyzed methanol approach, it has been shown that transesterification of algal oil can be achieved with ethanol and sodium ethanolate serving as the catalyst (Zhou and Boocock 2006). The products of these reactions are typically separated by adding ether and salt water to the solution and mixing well. Biodiesel is then separated from the ether by a vaporizer under a high vacuum.

Acid-catalyzed transesterification (Wahlen et al. 2008) is another route that can be used to produce biodiesel from algae. The replacement of soluble bases by liquid-acid catalysts, such as H_2SO_4, HCl, or H_2PO_4, is also considered an attractive alternative as the acidic catalysts are less sensitive to the presence of water and free acids, and therefore, mitigate saponification and emulsification, enhancing the product recovery (Ataya et al. 2007). Though acid catalysts have these advantages, they are not currently preferred due to their lower activity than the conventional transesterification alkaline catalysts. However, acid catalysts are more applicable to the transesterification of different lipid types.

Base-catalysed transesterification is faster, but would leave free fatty acids behind and un-transesterified; this saponification can result in significant losses due to the water solubility of the fatty acid soaps (Laurens et al. 2012a). Higher temperatures and longer reaction times are, therefore, generally required as a result. In order to compensate for this, heteropolyacids (HPAs), such as $H_3PW_{12}O_{40}$, have been shown to lower the required temperatures and decrease the reaction times (Alsalme et al. 2008; Cao et al. 2008). Recently, it was shown that HPA-catalyzed transesterification of vegetable oil achieves higher reaction rates than conventional mineral acids due to their higher acid strength (Xu et al. 2008). The apparent

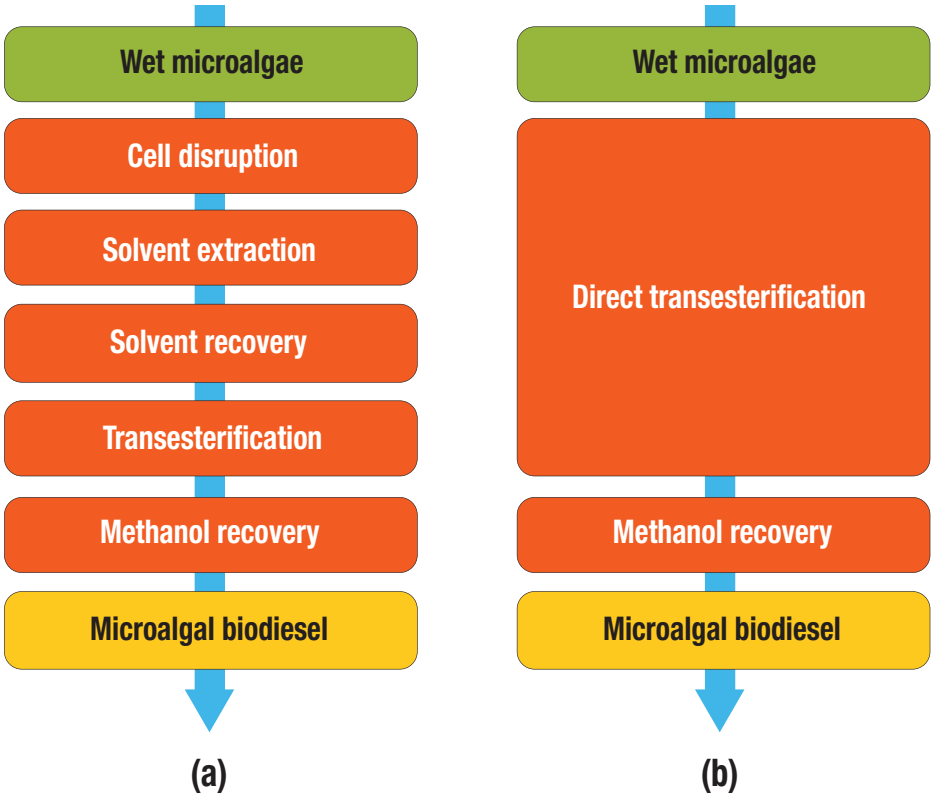

Figure 7.4. Chemical transesterification (a) and direct transesterification process (b) for production of biodiesel from algae (Source: Park et al. 2015)

higher activity of certain HPAs with respect to polyoxometallates of higher strength resulted in lower pretreatment temperatures. One recommended research focus would be to further develop these homogeneous catalysts to tolerate the contaminants expected to be present in algal extracts.

In addition to alternative catalysts, there are other processing variants that appear promising. An alternative heating system that can be used to enhance the kinetics of transesterification involves the use of microwaves (Refaat and El Sheltawy 2008). When the transesterification reaction is carried out in the presence of microwaves, the reaction is accelerated and requires shorter reaction times. As a result, a drastic reduction in the quantity of co-products and a short separation time are obtained (Lertsathapornsuk et al. 2008). These preliminary results indicate that microwave processing may be cost-competitive with the more-mature conversion processes currently available. In addition, catalysts may be used to enhance the impact of microwave irradiation (Yuan et al. 2008).

In the ultrasonic-reactor method, ultrasonic waves cause the reaction mixture to produce and collapse bubbles constantly. This cavitation simultaneously provides the mixing and heating required to carry out the transesterification process (Armenta et al. 2007). Thus, using an ultrasonic reactor for biodiesel production drastically reduces the reaction time, reaction temperatures, and energy input (Kalva et al. 2008). Hence, the process of transesterification can run inline rather than using the time-consuming batch process used in traditional base-catalyzed transesterification (Stavarache et al. 2007). It is estimated that industrial-scale ultrasonic devices can allow for the processing of several thousand barrels per day but will require further innovation to reach production levels sufficient for massive and scalable biofuel production.

Direct Transesterification of Lipids into Fatty Acid Methyl Esters

Direct transesterification refers to the catalytical conversion of lipids in algal biomass to FAMEs or biodiesel (Figure 7.5). This process was initially proposed by Lepage and Roy (1984) to increase the recovery of fatty acids from human milk and adipose tissue without prior extraction or purification. Conventional approaches are characterized as a one-step reaction where an alcohol (e.g., methanol) and an acid, alkaline, or enzymatic catalyst (e.g., acetyl chloride) are added directly to the biomass sample. However, more advanced methods are now available that include co-solvents, microwave, ultrasound, and supercritical methods; please see chapter 6 for details of these technologies. The theoretical advantages to these approaches are to increase fatty acid concentrations measured (as compared to Bligh and Dyer co-solvent system), give relatively high recoveries of volatile, medium-chain triglycerides, and eliminate the need to use antioxidants to protect unsaturated lipids. This method was applied to dried microalgal biomass in a modified approach to include hexane in the reaction phase in order to avoid a final purification step (Rodriguez-Ruiz et al. 1998). It was found that the entire reaction could be completed in 10 minutes.

Continuing efforts along this path, it was found that when applying direct transesterification using an acid catalyst (i.e., acetyl chloride), the efficiency of the reaction increased when a second, "less-polar" solvent such as diethyl ether or toluene was mixed with the methanol to modify the polarity of the reaction medium (Carvalho and Malcata 2005). In general, these findings suggest that the effectiveness of the second co-solvent (i.e., reaction medium) depends upon its ability to solubilize the target lipids coupled with its miscibility with methanol.

All the preceding co-solvent systems, however, remain largely bench-scale methods that are difficult to scale up to industrial processes due to the actual solvent toxicity and the low carrying capacity of the solvents (i.e., it is only efficient on biomass samples containing less than 2% w/w lipids). Accordingly, single-solvent systems at elevated temperature and pressure have gained favor for two principal reasons:

1. The elevated temperature and pressure increase the rate of mass transfer and degree of solvent access to all pores within the biomass matrix.

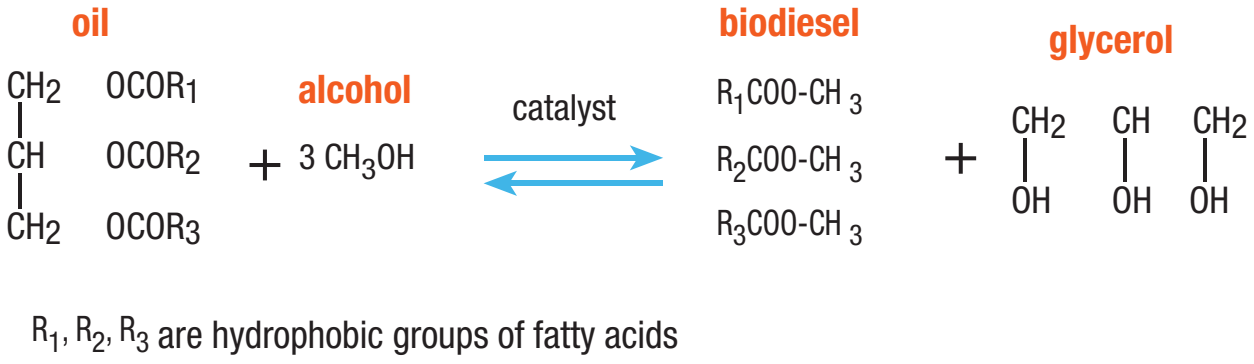

Figure 7.5. Transesterification reaction

2. The elevated pressures can reduce the dielectric constant of an otherwise immiscible solvent (and by analogy, the polarity) to values that match the polarity of the lipids (Herrero et al. 2006).

Consequently, the issue of solvent access to the material being extracted is as important as the miscibility of the analyte in the solvent. This observation is a key driving force behind the consideration of solvent extraction systems at elevated temperature and pressure.

Carbohydrate and Protein Fermentation

Work at NREL has proposed a pathway termed the CAP-conversion pathway (Figure 7.6, which represents several options for converting carbohydrates and lipids to fuel and blendstock products (Dong et al. 2015). This approach utilizes the fermentation of sugars and extraction/upgrading of lipids to hydrocarbon products (e.g., renewable diesel) supplemented by additional energy yield to ethanol as a representative fermentative product from sugars. Priority areas, technical targets, and accompanying cost projections for conversion of algal biomass to fuels and co-products are documented in the 2014 Combined Algal Processing Design Case (see chapter 11).

In another process, carbohydrate or sugar fermentation can be coupled with protein fermentation (Figure 7.7). Proteins are the dominant fraction in fast-growing photosynthetic

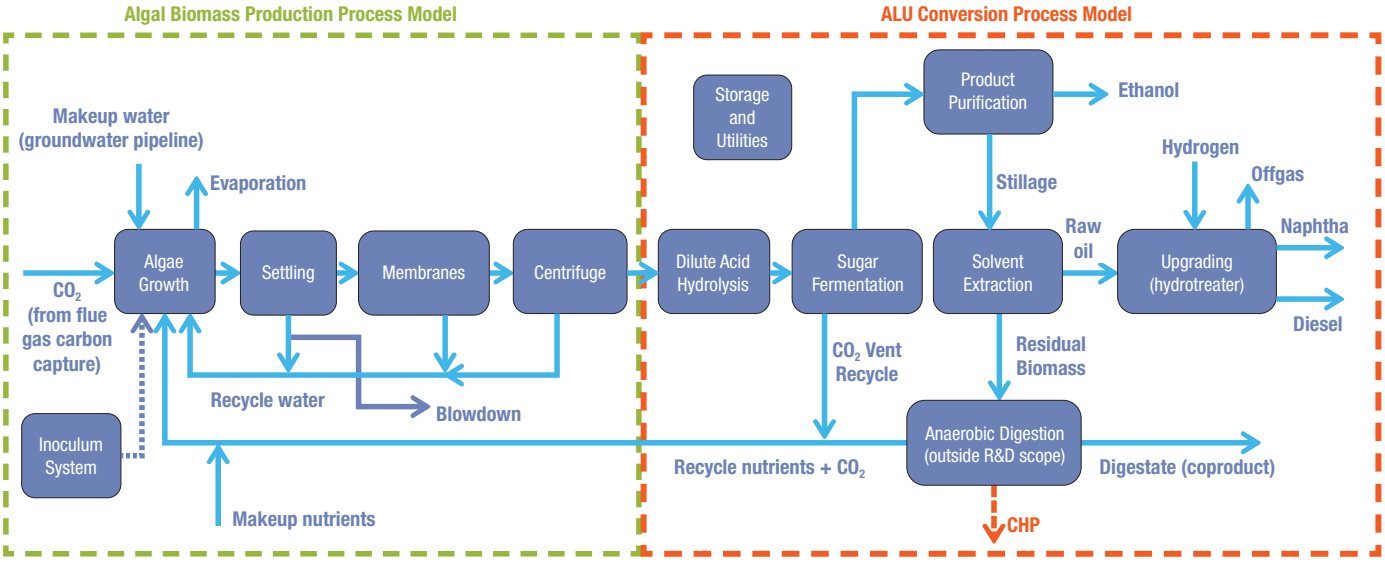

Figure 7.6. NREL combined algal processing pathway (Courtesy of Philip Pienkos, NREL)

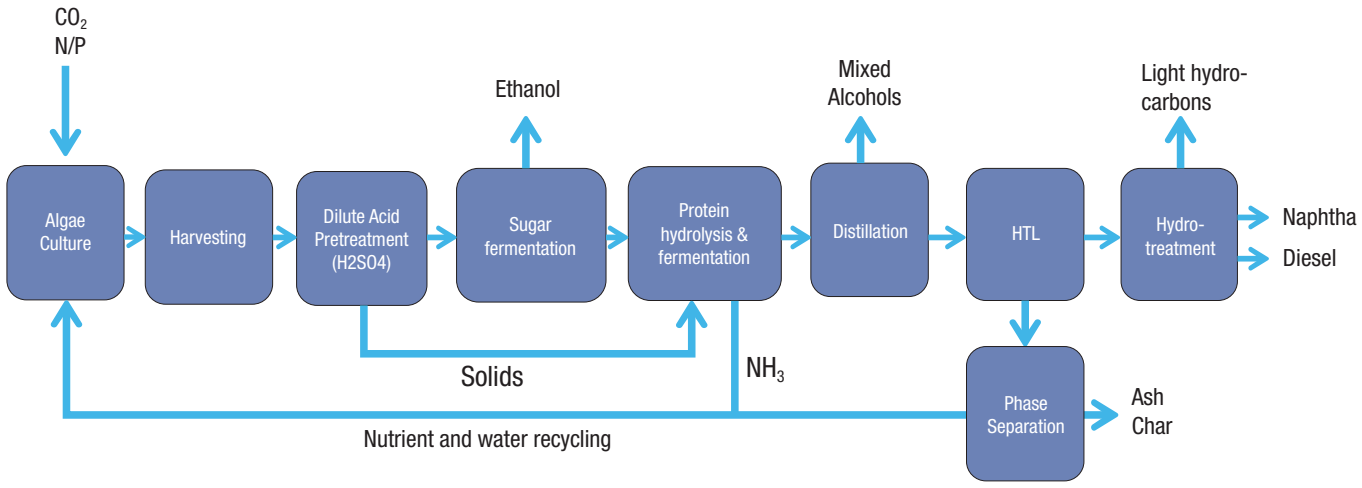

Figure 7.7. Process flow diagram for CAP coupled with protein fermentation (Courtesy of Ryan Davis, Sandia National Laboratories)

microorganisms, comprising a major component of algal biosynthetic pathways, the photosynthetic apparatus, CO_2-fixation pathways, and cell-growth machinery (Sheehan et al. 1998; Becker 2007). Protein content decreases with culture age and is inversely related to growth rate (Piorreck 1983; Becker 1994). The process of protein fermentation is the deamination of these proteins for conversion to fuel or chemicals, with recycling of the reduced nitrogen. *Escherichia coli* have been metabolically engineered to deaminate protein hydrolysates (YH83 *Escherichia coli*), converting cell proteins to C4 and C5 alcohols, producing up to 4,035 mg/L of alcohols from biomass containing ~22 g/L of amino acids (Huo et al. 2011). DOE/BETO currently funds the optimization of the bioconversion of microalgal proteins to mixed alcohol liquid fuels in an integrated process (Figure 7.7) to convert all the major algal biochemical pools (Davis 2014). Based on biomass collected from ATP[3], a mass-balance calculation gives a hypothetical yield of ~1500 gal/acre/yr as lipids and mixed alcohols from ~2000 gal/acre/yr biomass productivity.

Biochemical (Enzymatic) Conversion

Chemical processes give high conversion of TAGs to their corresponding esters but have drawbacks, such as being energy-intensive, having difficulty in removing the glycerol, and requiring removal of alkaline catalysts from the product and treatment of alkaline wastewater. Use of biocatalysts (lipases) in transesterification of TAGs for biodiesel production addresses these problems and offers an environmentally more-attractive option to the conventional processes (Svensson and Adlercreutz 2008). Although enzymatic approaches have become increasingly attractive, they have not been demonstrated at large scale mainly due to the relatively high price of lipase and its short operational life caused by the negative effects of excessive methanol and co-product glycerol. These factors must be addressed before a commercially viable, biochemical-conversion process can be realized.

One critical area that needs to be addressed is the solvent and temperature tolerance of the enzymes in order to enable efficient biocatalytic processing. The presence of solvents is sometimes necessary to enhance the solubility of the TAGs during the extraction process, and the enzymes used in the downstream conversion process must be able to function in the presence of these solvents to varying degrees to enable cost-effective biofuel production (Fang et al. 2006). There have been reports of using a solvent engineering method to enhance the lipase-catalyzed methanolysis of TAGs for biodiesel production (Su and Wei 2008; Liao et al. 2003). In particular, it has been noted that a co-solvent mixture may be critical in defining the optimal reaction medium for the lipases. This work indicates that the use of this co-solvent mixture in the enzymatic biodiesel production has several advantages: (a) both the negative effects caused by excessive methanol and co-product glycerol can be eliminated completely; (b) high reaction rates and conversion can be obtained; (c) no catalyst regeneration steps are needed for lipase reuse; and (d) the operational stability of the catalyst is high. Again, as with other approaches, one of the most significant roadblocks to demonstrating the validity of this approach lies in the conversion of algal oil extracts at a commercial scale and at competitive prices.

To that end, much R&D is needed in the discovery, engineering, and optimization of enzymes that are capable of producing these reactions in a variety of environments and on different types of oil feedstocks (Lopez-Hernandez et al. 2005). Bioprospecting for the enzymes in extreme environments may produce novel enzymes with desired characteristics that are more suitable for industrial applications (Guncheva et al. 2008). Enzyme immobilization may also play a key role in developing an economic method of biocatalytic transesterification (Yamane et al. 1998). Furthermore, research is needed into developing enzymes that can lyse algal cell walls; optimizing specific enzyme activity to function using heterogeneous feedstocks; defining necessary enzyme reactions (cell-wall deconstruction and autolysin); converting carbohydrates into sugars; catalyzing nucleic acid hydrolysis; and converting lipids into a suitable diesel surrogate. See Gerken et al. (2013), for a comprehensive comparison of enzyme activity on cell-wall deconstruction.

In order to explore these issues, a systematic algae-biomass, biochemical-conversion program consisting of established, leading-edge processes and those tailored specifically for algae, was carried out by the Sustainable Algal Biofuels Consortium funded by BETO (Figure 7.8). The program achieved several objectives that included the following:

- Integrated biomass production of a series of both freshwater and marine algal strains to yield up to 10's of kg of biomass of known and controlled composition and up to liter quantities of extracted crude algal oil
- Developed and refined characterization methods to quantify the various major constituents with the algal biomass and extracted oil samples (see chapter 3; Laurens et al. 2012b)
- Evaluated a series of pretreatment steps to fractionate whole biomass and residuals and isolate carbohydrates
- Showed that algal lipids could be upgraded to hydrocarbons with no cleanup steps and accomplished hydroisomerization of the n-alkanes
- Established baseline results for enzymatic hydrolysis using available cocktails for the release of fermentable sugars
- Explored the development of pretreatment protocols through identification of novel enzyme cocktails specific to algae
- Tested the conversion of algal hydrolysates

- Explored opportunities for alternate uses of the protein fraction
- Down-selected and demonstrated the best available integrated process to date for biochemical conversion at the kg scale. The Sustainable Algal Biofuels Consortium team screened 11 enzymes for their ability to degrade algal cell walls, but these did not end up being effective for the biofuel-relevant strains tested. The data indicates that for *Chlamydomonas reinhardtii* most of the enzymes evaluated demonstrated some level of cell-wall degradation, whereas none were effective in degrading the cell walls of *Chlorella zofingiensis* or *Scenedesmus* sp., clearly illustrating the variation in cell-wall composition of microalgae.

Catalytic Transesterification

The transesterification catalysts presented above are relatively mature in the field of biofuel production. Although very effective, these catalysts still require purification and removal from the product stream, which increases the overall costs. The development of immobilized heterogeneous and/or homogeneous catalysts that are very efficient and inexpensive is needed (McNeff et al. 2008). Acid and base catalysts could be classified as Brönsted or Lewis catalysts. However, in many cases, both types of sites could be present, and it is not easy to evaluate the relative importance of the two types of sites in the overall reaction in terms of efficiency and cost. Lewis acid catalysts, such as $AlCl_3$ or $ZnCl_2$, have been proven as a viable means of converting TAGs into fatty acid methyl esters. The presence of a co-solvent, such as tehtrahydrofuran, can play a vital role in achieving high conversion efficiencies of up to 98% (Soriano et al. 2009).

In another example, catalysts derived from the titanium compound possessing the general formula ATixMO, in which A represents a hydrogen atom or an alkaline metal atom, M a niobium atom or a tantalum atom, and x is an integer not greater than 7, were employed in vegetable oil transesterification. The catalysts obtained are stable and give high glycerol yield with high activities. A typical FAME yield of 91% and glycerol yield of 91% were obtained in a fixed-bed reactor at 200°C and 35 bar, using $HTiNbO_3$ as the catalyst. Vanadate metal compounds are stable, active catalysts during transesterification, with $TiVO_4$ being the most active (Cozzolino et al. 2006). This catalyst is also more active than $HTiNbO_3$, producing the same yields with lower residence times. Double-metal cyanide iron-zinc proved to be promising catalysts resulting in active transesterification of oil. These catalysts are Lewis acids, hydrophobic (at reaction temperatures of about 170°C), and insoluble. Moreover, they can be used even with

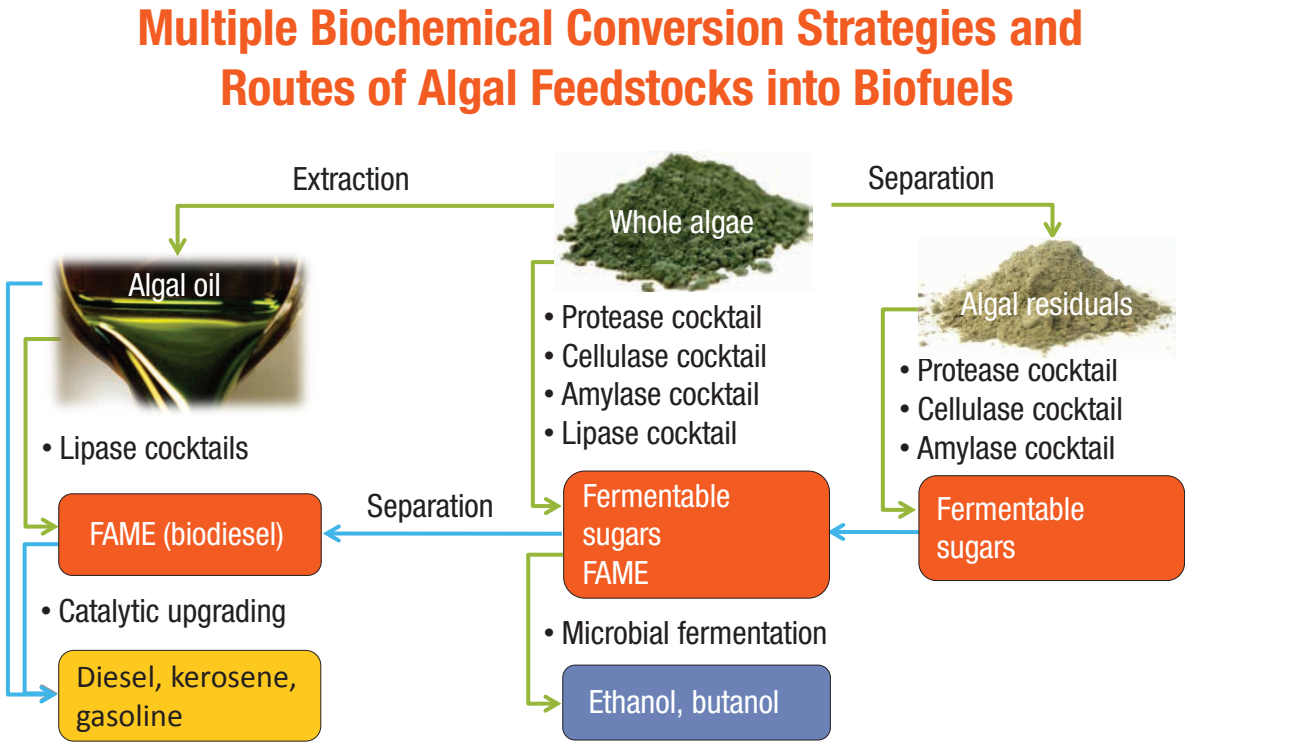

Figure 7.8. Biochemical conversion strategies and routes of algal feedstocks into biofuels from the Sustainable Algal Biofuels Consortium (Source: "SABC Project: Biochemical Conversion of Algal Biomass and Fuel Testing," azcati.com/sites/default/files/sabc.pdf)

oils containing significant amounts of free fatty acids and water, probably due to the hydrophobicity of their surface. The catalysts are active in the esterification reaction, reducing the concentration of free fatty acids in non-refined oil or in used oil. Other catalyst examples include MgO, CaO, and Al_2O_3.

One of the most difficult challenges is finding an ideal heterogeneous catalyst that has comparable activity in comparison to the homogenous catalyst at lower temperatures than the ones currently used (~220°C–240°C). At these temperatures, the process pressure is high (40–60 bar), which translates to very costly plant design and construction requirements. Many of the catalysts presented above seem to be good candidates for industrial process development but must resist poisoning and the leaching of active components. There remain significant, fundamental studies and unanswered questions that must be completed before these catalysts are fully understood. One particular concern is the stability and longevity of the catalysts in a representative reaction environment.

Conversion to Renewable Diesel, Gasoline, and Jet Fuel

All of the processes that take place in a modern petroleum refinery can be divided into two categories, separation and modification of the components in crude oil to yield an assortment of end products. The fuel products are a mixture of components that vary based on input stream and process steps, and they are better defined by their performance specifications than by the sum of specific molecules. As noted in chapter 9, gasoline, jet fuel, and diesel must meet a multitude of performance specifications that include volatility, initial and final boiling point, autoignition characteristics (as measured by octane number or cetane number), flash point, and cloud point. Although the predominant feedstock for the industry is crude oil, the oil industry has begun to cast a wider net and has spent a great deal of resources developing additional inputs such as oil shale and tar sands.

Gasoline, jet fuel, and diesel are generally described as "renewable" or "green" if they are derived from a biological feedstock, such as biomass or plant oil, but have essentially the same performance specifications as the petroleum-based analog. A major characteristic of petroleum-derived fuels is high energy content, which is a function of a near-zero oxygen content. Typical biological molecules have very high oxygen contents as compared to crude oil. Conversion of biological feedstocks to renewable fuels, therefore, is largely a process of eliminating oxygen and maximizing the final energy content. From a refinery's perspective, the ideal conversion process would make use of those operations already in place: thermal or catalytic cracking, catalytic hydrocracking and hydrotreating, and catalytic structural isomerization. In this way, the feedstock is considered fungible with petroleum and can be used for the production of typical fuels without disruptive changes in processes or infrastructure.

Various refiners and catalyst developers (such as UOP, Chevron, Eni, Statoil, Total, Neste, etc.) have already begun to explore the conversion of vegetable oils and waste animal fats into renewable fuels. Neste, UOP, Syntroleum, Eni, Sinopec, AltAir, and Valero/Diamond Green Diesel have all built large-scale commercial refineries to produce green diesel. More than 1.2 BGY of production capacity is in place. Fatty acids are well suited to conversion to diesel and jet fuel with few processing steps. This process has already provided the renewable jet fuel blends (derived from oils obtained from jatropha, camelina, used cooking oil, tallow, and algae) used in recent commercial-jet test flights and more than 2,000 commercial demonstration flights on regularly scheduled passenger-carrying operations. On the other hand, straight chain alkanes are poor starting materials for gasoline because they provide low-octane numbers, demanding additional isomerization steps or high-octane blendstocks. Algal lipids can be processed by hydrodeoxygenation (basically, a chemical reductive process). Referred to as hydrotreating, this process will convert the carboxylic acid moiety to a mixture of water, carbon dioxide, (or carbon monoxide) and n-alkane, and reduce double bonds to yield hydrocarbons. Glycerin can be converted to propane, which can be used for liquefied petroleum gas. Recent work shows that many of the hydrotreating processes can be performed under hydrothermal conditions (which may be advantageous for processing lipids derived from wet algal biomass). In particular, supported metal catalysts can be used to convert fatty acid feedstocks to linear alkane products (e.g., heptadecane) under hydrothermal conditions (Fu et al. 2010 and 2011; Vardon et al. 2015), and the glycerol product can be used to generate H_2(g) *in situ*, which is needed for the conversion of unsaturated fatty acids (Vardon et al. 2014). This contrasts with conventional refinery hydroprocessing of algal lipids, where the glycerol is actually a significant sink for H_2 gas increasing H_2 process demands.

The major technological barrier to the conversion of algal oils into biofuels is getting the input algal oil to meet the feed specs for the hydrotreating catalysts. Catalysts in current use have been optimized for existing petroleum feedstocks and have the appropriate specificity and activity to carry out the expected reactions in a cost-effective manner. It will be desirable to tune catalysts such that the attack on the oxygen-bearing carbon atoms will minimize the amount of carbon lost to gases, as well as the amount of H_2 used. Refinery catalysts have also been developed to function within a certain range of chemical components found within the petroleum stream (e.g., metals, and sulfur and nitrogen heteroatoms) without becoming poisoned.

Crude algal oil may contain high levels of phosphorous from phospholipids, nitrogen from extracted proteins, and metals (especially magnesium) from chlorophyll. It will be necessary to optimize both the level of purification of algal lipids as well as the tolerance of the catalyst for the contaminants to arrive at the most cost-effective process.

7.4 Processing of Algal Residuals after Extraction

One other critical aspect in developing a conversion technology that derives benefit from every potential input is the conversion of algal residuals after conversion of algal feedstock into fuel. This includes the anaerobic digestion of algal residuals to produce biogas, hydrothermal processing (Vardon et al. 2012), chemical co-product production through additional conversion, as well as the fermentation of any recoverable polysaccharides into biofuels. There are a number of options for processing algal residuals, which presumably consist of high protein levels and polymeric carbohydrates. In the context of fuel application, anaerobic digestion is a proven technology with proven installation at large scales for wastewater treatment.

Anaerobic digestion can be effectively used as a means of producing biogas from algae and algal remnants after extraction (Ashare and Wilson 1979; see Zhao et al. 2014 and Ward et al. 2014 for review). In particular, the organic fractions of the algae remaining after oil extraction are amenable to anaerobic digestion. The biogas product typically contains 60% methane and 40% CO_2 by volume. The liquid effluent contains soluble nitrogen from the original algal proteins; the nitrogen can be recovered in the form of ammonia for recycle to the culture. Phosphorous can also be recovered. There will also likely be a high amount of polysaccharides and other oligosaccharides present in the algal remnants that are well suited for traditional fermentation into ethanol and other biofuels.

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8. Commercial Products

The concept of a biorefinery for utilization of every component of the biomass raw material must be considered as a means to enhance the economics of the process. A near-term strategy of the algae industry is to focus on supporting bioproduct development that increases the value of algae biomass, enabling economic viability. This chapter will address options for producing bioproducts and discuss how some of them are better opportunities as they will not readily saturate corresponding markets in the long term. Chapter 10 will address within the context of the biorefinery the possibility of coupling biomass cultivation with CO_2 mitigation and wastewater treatment (for nutrient removal) to provide additional benefits to the technology, without invoking competing co-products.

Using appropriate technologies, all primary components of algal biomass—carbohydrates, fats (lipids or oils), proteins, and a variety of inorganic and complex organic molecules—can be converted into different products, either through chemical, enzymatic, or microbial conversion (see chapter 7). The potential for genetic modification to expand the capacity and scope of these technologies is vast. The nature of the end products and of the technologies to be employed will be determined, primarily by the economics of the system, and may vary from region to region according to land-use cost and productivity (Willke and Vorlop 2004). Moreover, novel technologies with increased efficiencies and reduced environmental impacts may have to be developed to handle the large amount of waste that is predicted to be generated by the process. The topic of conversion of algal biomass to other biofuels has already been discussed (see chapter 7); this chapter will focus on the non-fuel co-products. Under the biorefinery concept (Figure 8.1), the production of industrial, high-value, and high-volume chemicals from amino acids, glycerol, and nitrogen-containing components of algal biomass becomes feasible (Mooibroek et al. 2007) and must be considered in determining the economics of the process.

The use of terms such as "high volume" or "high value" can be extremely subjective, as a "high-value" product to a fine-chemical producer might be well over several dollars/lb but be considerably under a dollar for a commodity producer. For a discussion of the regulatory and process considerations for marketing algal-based food, feed, and supplements, see chapter 5 of the ABO's Industrial Algal Measurements (ABO 2015).

8.1 Commercial Products from Microalgae and Cyanobacteria

A large number of different commercial products have been derived from microalgae and cyanobacteria. As summarized in Table 8.1, these include products for human and animal nutrition, poly-unsaturated fatty acids, anti-oxidants, coloring substances, fertilizers and soil conditioners, and a variety of specialty products such as bioflocculants, biodegradable polymers, cosmetics, pharmaceuticals, polysaccharides, and stable isotopes for research purposes. Table 8.1 lists a summary of existing and potential high-value products from microalgae (Borowitzka 2013). By definition, these existing markets (and associated production plants and distribution channels) are for high-value products or co-products from algae, not commodity products.

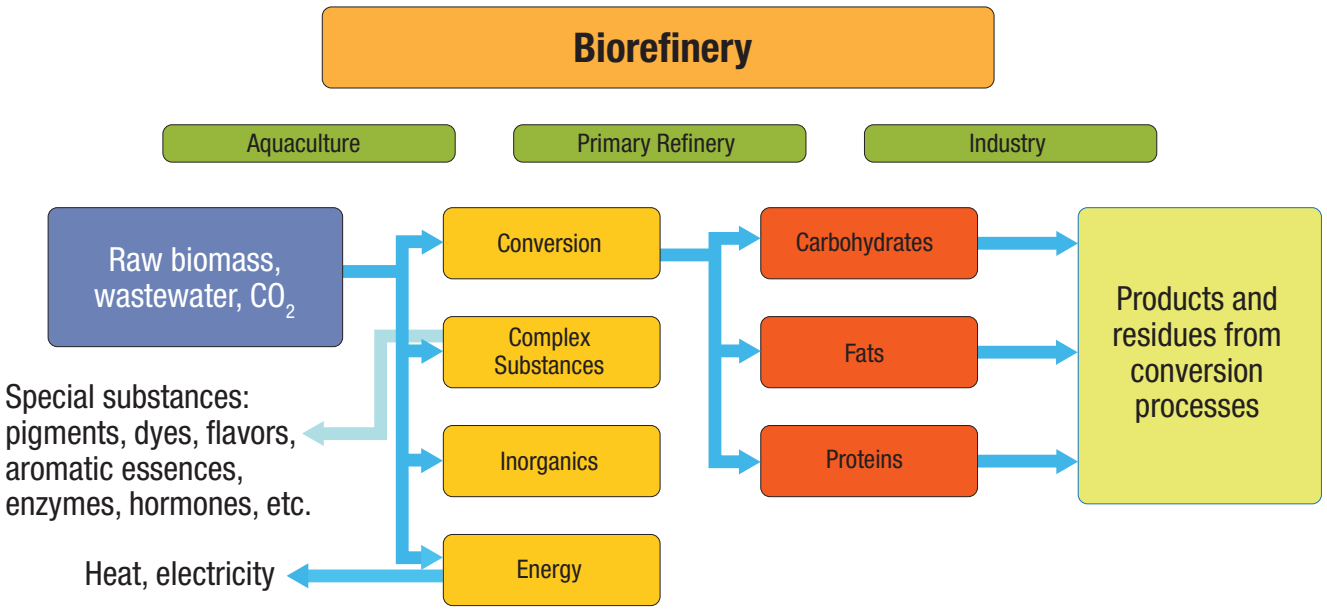

Figure 8.1. An overview of the biorefinery concept (Source: Modified from Kamm and Kamm 2007)

Like the existing fossil fuels market, the future algal-based biofuels market must also be commodities-based to meet required volumes at price points acceptable to the consumer. Acceptable prices may be higher than their alternative if a significant performance benefit can be assigned to the algae-derived product. With the possible exception of the existing market for microalgal biomass for animal and human nutrition and soil fertilizer, the biofuels markets will involve volumes (of biomass, product, etc.) and scales (sizes and numbers of commercial plants) that are significantly more than those associated with the existing high-value algae-derived products. An analysis on potential algal co-products and their respective markets is summarized in Table 8.2. Some of the price/ton values are missing from this table, because they are either not well-understood or not known due to the potential for a multitude of 'fuel additive' products with their associated market values. For products such as emulsifiers and nutraceuticals (such as phosphatidylcholine, phosphoinositol, and lecithin), the value and market size will be dominated by their specific application. For example, although each market segment (e.g., catering products, diapers, and packaging) is associated with an individual market value, the price of polysaccharide-derived bioplastics is assumed to be competitive with petroleum-derived plastic.

Algae companies are faced with a trade-off: in the long term, massive lipid production will be required to meet targets for commodity yields; yet, in the short term, bioproducts of higher value in the marketplace must be pursued in order to offset the costs of production. Although it is clear that co-products may improve the economic viability of some algae processes in the short-term, the goal of the industry is to produce transportation fuels below their market price, thereby increasing fuel supplies without drastically increasing price. This situation is anticipated to continue until (1) a sufficient number of the challenges outlined earlier in the report for biofuel production have been overcome and associated life-cycle costs are reduced to realize sustainable biofuel production at volumes and price points that meet consumer demands or (2) new co-products that are low-cost and have very large potential markets are developed.

Food and Feed

The consumption of microalgal biomass as a human health-food supplement is currently restricted to only a few species, e.g., *Spirulina* (*Arthrospira*), *Chlorella*, *Dunaliella*, and to a lesser extent, *Nostoc* and *Aphanizomenon* (Radmer 1996; Pulz and Gross 2004; Spolaore et al. 2006). Global production includes ca. 3,000 t/yr *Spirulina*; ca. 2,000 t/yr *Chlorella*; ca. 1,200 t/yr *Dunaliella*; ca. 600 t/yr *Nostoc*; and ca. 500 t/yr *Aphanizomenon*. The market, at about $2.5 billion, is expected to grow in the future, and many other strains of algae have been screened for their potential use in the food industry (Batista et al. 2013). A whole algalin flour produced from *Chlorella protothecoides* (Solazyme, South San Francisco, California) was shown to have little potential for food allergy (Szabo et al. 2012) and may be produced for sale in the future.

Microalgae are also used as feed in the aquaculture of mollusks, crustaceans (shrimp), and fish (Benemann 1990). The most frequently used species are *Chaetoceros*, *Chlorella*, *Dunaliella*, *Isochrysis*, *Nannochloropsis*, *Nitzschia*, *Pavlova*, *Phaeodactylum*, *Scenedesmus*, *Skeletonema*, *Spirulina*, *Tetraselmis*, and *Thalassiosira*. Both the protein content and the level of unsaturated fatty acids determine the nutritional value of microalgal aquaculture feeds. The protein content and amino acid composition of the feed, in addition the digestibility of the feed, will determine its value in the market place. The market size, currently at ~$700 million, is expected to expand significantly. Under NAABB, economic analyses valued lipid-extracted algae as a feed supplement for mariculture at $200/ton, whereas whole algae for mariculture was valued at closer to $400/ton (NAABB 2014).

Microalgal biomass has also been used with good results (i.e., better immune response, fertility, appearance, weight gain, etc.) as a feed additive for cows, horses, pigs, poultry, and even dogs and cats. In poultry rations, microalgal biomass up to a level of 5 %wt–10 %wt. can be safely used as a partial replacement for conventional proteins (Spolaore et al. 2006). The main species used in animal feed are *Spirulina*, *Chlorella*, and *Scenedesmus*. De-fatted algae biomass has also recently shown feasibility in replacing corn, soybean, or cottonseed meal in diets for poultry, swine, and cattle (Lum et al. 2013; Austic et al. 2013; Isaacs et al. 2011; Lum et al. 2012; Lopez et al. 2013; Drewery et al. 2014; Lodge-Ivey et al. 2014), allowing the removal of biofuel products prior to animal feedstock utilization. In steers, post-extraction algal-residue supplementation increased forage utilization (McCann et al. 2014). Further research is required to understand strain-specificity on lipid-extracted algae quality as a protein feedstuff.

The market for microalgal animal feeds, estimated to be about $300 million, is quickly growing. Economic analyses based on the research performed under NAABB valued lipid-extracted algae as a feed supplement for animals at $160/ton (NAABB 2014). In a TEA/LCA based on actual production by the Cornell Marine Algal Biofuels Consortium, the minimum animal feed selling price (representing the price animal feed has to be sold for a facility to break even after a certain period assuming other products are sold at certain prices) ranged from $1,384/MT–$5,066/MT for all 10 cases[1] in the study, based in Texas and Hawaii (Beal et al. 2015), much higher than the NAABB-determined value of $160/ton.

Polyunsaturated Fatty Acids

Microalgae can also be cultured for their high content in polyunsaturated fatty acids (PUFAs), which may be added to human food and animal feed for their health-promoting properties (Benemann 1990; Radmer and Parker 1994; Radmer

Table 8.1. Summary of Existing and Potential High-Value Products from Microalgae

Product	Potential or existing algal source	Some alternate source(s)	Applications	Selected references
Carotenoids β-carotene	*Dunaliella salina*	*Blakeslea trispora*, synthetic	Pigmenter (food), pro-vitamin A, antioxidant	Borowitzka and Borowitzka 1989; Choudhari et al. 2008; Borowitzka 2010
Astaxanthin	*Haematococcus pluvialis, Chlorella zofingiensis*	*Xanthophyllomyces dendrorhous, synthetic*	Pigmenter (aquaculture), antioxidant	Cysewski and Lorenz 2004; Lemoine and Schoefs 2010; Rodríguez-Sáiz et al. 2010; Schmidt et al. 2011
Canthaxanthin	*Chlorella* spp., other green algae	*Dietzia natronolimnaea,* synthetic	Pigmenter (aquaculture, poultry and food)	Arad et al. 1993; Hanagata 1999; Nasrabadi and Razvai 2010
Zeaxanthin	*Chlorella ellipsoidea*; *Dunaliella salina* (mutant)	Paprika (*Capsicu annuum*); *Tagetes erecta*, synthetic	Anti-oxidant, food pigmenter	Jin et al. 2003; Koo et al. 2012
Lutein	*Scenedesmus* spp., *Muriellopsis* sp., other green algae	*Tagetes* sp., *Blakeslea trispora*	Antioxidant	Piccaglia et al. 1998; Blanco et al. 2007; Chouhari et al. 2008; Sánchez et al. 2008; Fernández-Sevilla et al. 2010
Phytoene, phytofluene	*Dunaliella*	Tomato (*Solanum lycopersicum*)	Antioxidant, cosmetics	von Oppen-Bezalel and Shaish 2009
Echinenone	*Botryococcus braunii*, cyanobacteria		Antioxidant	Jäger et al. 2002; Matsuura et al. 2012
Fucoxanthin	*Phaeodactylum tricornutum*	Brown algae	Antioxidant	Kim et al. 2012
Phycobilins (phycocyanin, phycoerythrin, allophycocyanin) Fatty acids	Cyanobacteria, Rhodophyta, Cryptophyta, Glaucophyta		Natural pigment (e.g. cosmetics and food products), fluorescent conjugates, antioxidant, etc.	Oi et al. 1982; Glazer and Streyer 1984; Arad et al. 1996; Eriksen 2008
Arachidonic acid	*Parietochloris incisa*	*Mortirella* spp.	Nutritional supplement	Bigogno et al. 2002; Solovchenko et al. 2008; Streekstra 2010
Eicosapentaenoic acid	*Nannochloropsis* spp., *Phaeodactylum tricornutum*, *Monodus subterraneus*, etc.	Fish oil	Nutritional supplement	Hu et al. 1997; Molina Grima et al. 1999; Sukenik 1999; Lu et al. 2001

Source: Borowitzka (2013).

Table 8.1. *(continued)*

Product	Potential or existing algal source	Some alternate source(s)	Applications	Selected references
Docosahexaenoic acid	*Crypthecodinium cohnii*, *Schizochytrium* spp., *Ulkenia* spp.	Fish oil	Nutritional supplement	Barclay 1994; Mendes et al. 2009; Barclay et al. 2010; Wynn et al. 2010
Sterols	Many species	Various plants	Nutraceutical	Fabregas et al. 1997; Volkman 2003; Francavilla et al. 2010
Squalene	*Aurantiochytrium* sp.	Shark liver	Cosmetics	Kaya et al. 2011
Polyhydroxyalkanoates	*Nostoc* spp, *Synechocystis* and other cyanobacteria	*Ralstonia* sp; GM *Escherichia coli*	Biodegradable plastics	Vincenzini and De Philippis 1999; Philip et al. 2007; Haase et al. 2012
Polysaccharides	*Porphyridium* spp., *Rhodella* spp., various cyanobacteria	Guar gum, xanthan	Thickeners, gelling agents etc., cosmaceuticals	De Philippis et al. 2001; Pereira et al. 2009; Arad and Levy-Ontman 2010
Mycosporine-like amino acids	Cyanobacteria, Dinophyta and other algal phyta		Sunscreens	Garcia-Pichel and Castenholz 1993; Llewellyn and Airs 2010

Source: Borowitzka (2013).

1996). Also, PUFAs form a feedstock for the production of polyurethane and polyol products, such as polyurethane foams (Pawar et al. 2016). The most commonly considered PUFAs are arachidonic acid, docosohexaenoic acid (DHA), γ-linolenic acid (GLA), and eicosapentaenoic acid. Arachidonic acid has been shown to be synthesized by *Porphyridium*, DHA by *Crypthecodinium* and *Schizochytrium*, GLA by *Arthrospira* (*Spirulina*), and eicosapentaenoic acid by *Nannochloropsis*, *Phaeodactylum* and *Nitzschia* (Spolaore et al. 2006). The potential of other microalgae and macroalgae species in producing long-chain PUFAs has also been examined (Ryckebosch et al. 2014; Pereira et al. 2012). However, only DHA has been produced thus far on a commercial scale by microalgae. All other PUFAs are more cost-effectively produced from non-algal sources (e.g., GLA from evening primrose oil). Although small, the DHA oil market is quickly growing, having presently a retail value of greater than U.S. $1.7 billion (GOED 2015). Although they are only a small volume of the total eicosapentaenoic acid and DHA oils consumed globally, algae oils accounted for ~10% of total sales in 2013 at $171.2 million. The relationship between triacylglycerol (TAG) yield for biofuels and PUFA co-production needs to be examined, as in some species, such as *Nannochloropsis oceanica*, TAG accumulation increases while the eicosapentaenoic acid amount declines by 30% during nitrogen deprivation (Pal et al. 2011).

Antioxidants

A number of antioxidants, sold for the health food market, have also been produced by microalgae (Borowtizka 1986; Benemann 1990; Radmer 1996). The most prominent is β–carotene from *Dunaliella salina*, which is sold either as an extract or as a whole-cell powder ranging in price from US$300–3,000 per kg (Spolaore et al. 2006).

The global carotenoid market value was $1.5 billion in 2014. This market is expected to reach nearly $1.8 billion in 2019,

[1] "The 10 cases include two algal species and various growth and processing configurations. All 10 were evaluated in Texas and Hawaii. In all cases, carbon is provided from an industrial point source as a 94% pure CO_2 waste stream located 15 km from the facility. The nutrients in Case 1 are sodium nitrate and sodium phosphate (the same as those used at the Kona Demonstration Facility), while the nutrients for Cases 2–10 are ammonia and diammonium phosphate (DAP). Productivity (in g/m^2/day) is normalized to the total facility area." (Beal et al. 2015).

Table 8.2. Illustration of Biomass Composition (as wt% of dry biomass) Ranges (based on typically observed or literature-reported or measured in our lab values)

Feedstock	Wt %	Product	Market size (T)	Price ($/T)
Fatty acids	10%–45%	Hydrocarbon fuel products	5,000,000	920
Omega-3-fatty acids	3%–6%	Polyols	11,000,000	~5,000–11,000
	3%–6%	Polyurethane	11,000,000	~5,000–11,000
	3%–6%	Nutraceuticals	22,000	~5,000–11,000
Hydroxy fatty acids	~1%	Surfactants, fuel additives	3,500,000	30,000–100,000
Branched chain fatty acids	~1%	Surfactants, fuel additives	3,500,000	
Fatty alcohols	~1%	Surfactants, fuel additives	3,500,000	
Sterols	2%–4%	Surfactants	2,000,000	1,500
	2%–4%	Hydrocarbon fuel products		920
	2%–4%	Phytosterol nutra/pharmaceuticals	25,000	4,000
	2%–4%	Emulsifiers		
Phytol	3%–4%	Raw material for vitamin E, fragrance, soaps, etc.	1	150,000
	3%–4%	Surfactants, fuel additives	3,500,000	
Polar lipids	10%–35%	Ethanolamine	600,000	~1,500
	10%–35%	Phosphatidylcholine, phosphoinositol and phosphatidyl ethanolamine (lecithin)		
Glycerol	2%–6%	Di-acids for nylon production	2,500,000	2,250
Fermentable sugars (glucose, mannose)	10%–45%	Polylactic acid polymers	300,000	
	10%–45%	Di-acids (e.g., adipic acid)	2,500,000	2,250
	10%–45%	Ethanol	60,000,000	
Mannitol	3%–6%	Polyether polyols	2,300,000*	
Alginate	~3%–5%	Alginate additives		
Starch	5%–40%	Polysaccharide-derived bioplastics	2,000,000	
Protein	19%–40%	Thermoplastics	5,000,000	
Amino acids/peptides	19%–20%	Polyurethane	11,000,000	
Amino acids/peptides	19%–20%	Biobutanol, mixed alcohol fuels	40,000,000	

*Market size and price based on IHS Technology report on sorbitol (https://technology.ihs.com).
Source: Table created by Lieve Laurens and Ryan Davis, NREL.

with a compound annual growth rate of 3.9% (BCC Research 2015). β-carotene, lutein, and astaxanthin account for 60% of the total market value (Borowitzka 2013), and β-carotene represents the largest product segment with 2010 sales estimated at $392 million.

Coloring Agents

Three major groups of pigments are found in algae: Carotenoids (such as β-carotene discussed previously, which makes an orange color), phycobilins (red or blue color), and chlorophylls (green color). Microalgae-produced coloring agents are used as natural dyes for food, cosmetics, and research, or as pigments in animal feed (Borowitzka 1986; Benemann 1990). Astaxanthin, a carotenoid produced by *Haematococcus pluvialis* and *Chlorella zofingiensis*, has been successfully used as an additive to salmon feed to give the fish meat a pink color preferred by the consumers (Olaizola 2003; Spolarore et al. 2006). Astaxanthin, and the related carotenoids lutein and zeaxanthin, have also been used in the feed of carp and even chicken (Pulz and Gross 2004; Spolaore et al. 2006). Phycobiliproteins (i.e., phycoerythrin and phycocyanin), produced by the cyanobacterium *Arthrospira* (*Spirulina*) and the rhodophyta *Porphyridium*, are used as food dyes, pigments in cosmetics, and as fluorescent reagents in clinical or research laboratories (Spolaore et al. 2006). Phycocyanin from *Spirulina*, produced in several strains of cyanobacteria and rhodophyta, gives a blue color and is widely used in confectionary and dairy products, such as candies, chewing gum, ice creams, and yogurt. For a list of algal pigments and their potential fields of application, see Koller et al. 2014.

The North America natural food colors market is expected to expand at a compound annual growth rate of 7.1% during the forecast period 2014–2020, and reach a valuation of $441.4 million by 2020 (Future Market Insights 2015). The volume of *Spirulina* used in the end-use industries is expected to increase five times from 2014 to 2020.

Much is still unknown about carotenoid metabolism and its regulation in algae (Varela et al. 2015), and further examination of these valuable pathways may lead to a better understanding of how to exploit its production.

Fertilizers

Currently, macroalgae (i.e., seaweeds) are used to fertilize plants and to improve the water-binding capacity and mineral composition of depleted soils (Metting et al. 1990). Microalgal biomass could in principle serve the same purpose. Recent studies examining the use of microalgae as a fertilizer showed improvement of both the soil organic matter and its water-holding capacity (Uysal et al. 2015). The Accelergy Corporation offers a biofertilizer of local, native algae strains that are isolated, cultivated, and incorporated into proprietary blends of bio-fertilizer (accelergy.com). Furthermore, plant-growth regulators could be derived from microalgae (Metting and Pyne 1986).

Other Specialty Products

There are a number of specialty products and chemicals that can be obtained from microalgae. These include bioflocculants (Borowitzka 1986), biopolymers, and biodegradable plastics (Philip et al. 2007; Wu et al. 2001; Koller et al. 2011; Khosravi-Darani et al. 2013; UCSD News 2015), cosmetics (Spolaore et al. 2006; Yaakob et al. 2014), pharmaceuticals and bioactive compounds (Burja et al. 2001; Metting and Pyne 1986; Olaizola 2003; Singh et al. 2005; Pulz and Gross 2004; Rasala and Mayfield 2015), polysaccharides (Benemann 1990; Borowitzka 1986; Pulz and Gross 2004; Borowitzka 2013), and stable isotopes for research (Benemann 1990; Radmer 1994; Pulz and Gross 2004). The market for these specialty products, aside from algae-based plastics, is likely to be very small due to their specialized applications.

8.2 Commercial Products from Macroalgae

Macroalgae possess high levels of structural polysaccharides that are extracted for their commercial value (Table 8.3). They include alginate from brown algae and agar and carrageenan from red algae. Alginate, which occurs in high concentrations in brown seaweeds, is considered recalcitrant to ethanol fermentation since the redox balance favors formation of pyruvate as the end product (Forro 1987). In addition to the commercial uses listed, alginate can be utilized in the making of polyurethane products with many potential applications (Zia et al. 2015).

8.3 Potential Options for the Recovery of Co-Products

Co-products from algal refineries should address one of these three criteria to be commercially viable and acceptable:

1. **Identical to an existing chemical, fuel, or other product.** In this instance, the only issue is price. The production cost of the new product must be equivalent to the material it replaces, and to be competitive, typically, it must be produced at a cost 30% lower than the existing material. Production/price stability is also an important factor. This sets a high bar, but it has been achieved for some chemicals and proteins/nutritional products.
2. **Identical in functional performance to an existing chemical, fuel, or other product.** Here price is a major factor, but the source of the material can often provide some advantage. This occurs with natural oils, which manufacturers, in many cases, would prefer if the costs were comparable, or with replacements such as algal proteins for distillers dry grains (DDGs) from corn for dry-grind ethanol processing. Price becomes less of an issue if the product can be labeled "organic" and, thus, saleable at a premium.

Table 8.3. Global Value of Seaweed Products per Annum

Product	Value
Human food (nori, aonori, kombu, wakame, etc.)	$5 billion
Algal hydrocolloids	
Agar (Food ingredient, pharmaceutical, biological/microbiological)	$132 million
Alginate (Textile printing, food additive, pharmaceutical, medical)	$213 million
Carrageenan (Food additive, pet food, toothpaste)	$240 million
Other uses of seaweeds	
Fertilizers and conditioners	$5 million
Animal feed	$5 million
Macroalgal biofuels	Negligible
Total	**$5.5–$6 billion**

Source: McHugh (2003).

3. **New material with unique and useful, functional performance characteristics.** In this case, the issues are less related to costs and more to the functional performance and potentially enhanced performance of the new product.

There are at least five different options for economic value from recovered biomass constituents (Figure 8.2):

- Option 1 – Maximum energy recovery from the lipid-extracted biomass, with potential use of residuals
- Option 2 – Recovery of protein from the lipid-extracted biomass for use in food and feed
- Option 3 – Recovery and utilization of non-fuel lipids
- Option 4 – Recovery and utilization of carbohydrates from lipid-extracted biomass and the glycerol from the transesterification of lipids to biodiesel
- Option 5 – Recovery of secreted alcohols and/or extraction of fuel lipids only, with use of the residual biomass as soil fertilizer and conditioner.

Each option and its associated technologies and future research needs are discussed below.

Option 1 – Maximum Energy Recovery from the Lipid-Extracted Biomass, with Potential Use of Residuals

Given the large amounts of lipid-extracted biomass residues that may be generated in future microalgal biofuels production systems, it may be difficult to identify large enough markets for potential co-products. Therefore, one option would be to convert as much of the lipid-extracted biomass into energy, which could then be either sold on the open market or used on-site in the various biorefinery operations.

Anaerobic digestion of whole (i.e., non-extracted) micro and macroalgal biomass has been successfully demonstrated, with reported methane yields of about 0.3 L/gram volatile solids (Huesemann and Benemann 2009). The economic value of the produced methane is equivalent to about $100 per ton of digested biomass, which is significant in terms of reducing the overall cost of liquid biofuels production. The residuals remaining after anaerobic digestion could either be recycled as nutrients for algal cultivation or could be sold as soil fertilizers and conditioners. For a review of anaerobic digestion of algal biomass, see Ward et al. (2014).

More recently, HTL of lipid-extracted algae was demonstrated, producing methane yields of around 0.4 L per gram volatile solids, with direct conversion to liquid fuel (Elliott et al. 2013; Zhu et al. 2013). The work was performed as part of NAABB as an outgrowth of work on hydrothermal gasification (see chapter 7). This technology is able to convert a much larger fraction of biomass into fuels compared to anaerobic digestion. The HTL conversion of lipid-extracted biomass has the potential advantage that

the resulting water stream could be recycled for nutrients such as ammonia into the microalgal culture ponds, thereby reducing the expense for nitrogen fertilizers. Furthermore, the mineral-rich ash generated by these thermochemical processes could possibly be used for nutrient recycle or as a soil amendment. BETO-funded projects to examine the use of HTL to separate lipids and co-products from production strains of algae are underway.

In addition, the yield of algal biofuel intermediates could be increased by the integrated conversion of all the biochemical pools. In order to optimize this process, proteins from microalgae could be fermented to mixed alcohol liquid fuels. By optimizing bacterial strains that convert protein to isobutanol, greater yields of biofuels could be achieved (see chapter 7 for a discussion of protein fermentation).

Option 2 – Recovery of Protein from the Lipid-Extracted Biomass for Use in Food and Feed

Following the extraction of lipids from the microalgal biomass for liquid-biofuel production, the protein fraction from the residual biomass could be extracted and used as a food and feed supplement. However, this would depend on the extraction procedures. For example, if the biomass is pretreated with acid, then the residual protein will not be in a form that will allow for easy digestion or as an addition to an animal feed diet (this is also true for the carbohydrate fraction of the biomass residue, which will no longer be accessible for conversion after the process). As was pointed out above, the market for animal feed (cattle, pigs, poultry, fish, and pets) is already very large and growing (estimated to rise to approximately 60 million tons per year for DDGs plus soluble [DDGS]) (Berger and Good 2007). The current price for DDGS ranges from $110– $150 per ton (USDA-AMS 2016). The market for oilseed meal is much larger than DDG, at more than 300 million metric tons/year and is expected to double in the next few decades (USDA 2016). Soybeans comprise about 90% of U.S. oilseed production, while other oilseeds—including peanuts, sunflower seed, canola, and flax—make up the remainder. The price of soybean meal is higher than DDG (currently $300/ton) (USDA 2016). Since protein is generally the key and often limiting ingredient in animal feed, supplementation with microalgal proteins could be advantageous. Furthermore, human nutrition may also benefit from supplementation with microalgal proteins.

Another market on par with these is protein-based polymers. Polymers are another large market in the 100 million

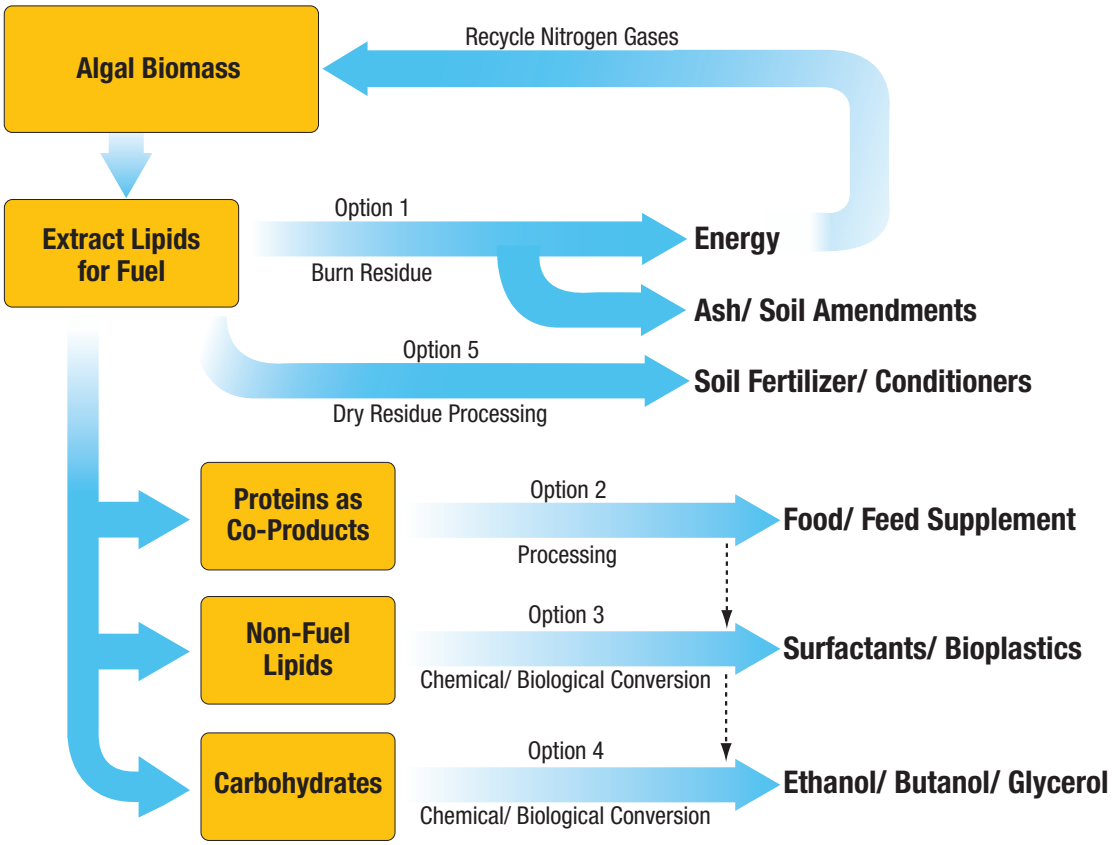

Figure 8.2. Overview of the five potential options for the recovery and use of co-products

metric ton range and the co-product value would be similar to soybean meal, ~$500/ton.

In addition, it may be possible to recover important enzymes such as cellulases or other industrial enzymes from the lipid-extracted biomass. However, this option would require the use of specially selected or engineered microalgal strains capable of producing these enzymes. The market for industrial enzymes, specifically cellulases for pretreating lignocellulosic feedstocks prior to fermentation to fuel ethanol, is potentially very large. Assuming that (a) microalgal cellulases could be provided at a cost of less than $0.20 per gallon ethanol; (b) approximately 100 grams of cellulase are needed per gallon of ethanol; and (c) at least 10.5 billion gallons of lignocellulosic ethanol will be produced by 2020, the projected market for cellulases is potentially very large (i.e., 1 billion kg).

Option 3 – Recovery and Utilization of Non-Fuel Lipids

It is well known that microalgae can synthesize a variety of fatty acids with carbon numbers ranging from C10 to C24, depending on the algal species and culturing conditions (Hu et al. 2008). Since the generation of gasoline, jet fuel, and diesel substitutes will require specific ranges of carbon-chain length, it will be necessary to either separate the product into the appropriate range or rearrange the carbon chains through catalytic cracking and catalytic reforming. It may be worthwhile, however, to separate specific lipids present in the algal oil that have utility as chemical feedstocks for the manufacture of surfactants, bioplastics, and specialty products such as urethanes, epoxies, lubricants, etc.

Option 4 – Recovery and Utilization of Carbohydrates from Lipid-Extracted Biomass, and the Glycerol from the Transesterification of Lipids to Biodiesel

After the extraction of lipids, the residual microalgal biomass may contain sufficient levels of carbohydrates that could be converted through anaerobic dark fermentations to hydrogen, solvents (acetone, ethanol, and butanol), and organic acids (formic, acetic, propionic, butyric, succinic, and lactic) (Huesemann and Benemann 2009; Kamm and Kamm 2007; Kawaguchi et al. 2001). Hydrogen and ethanol could be used as biofuel, while butanol and organic acids could serve as renewable feedstocks for the chemicals industry. For example, butanol is a valuable carbon compound for chemical synthesis of a variety of products, including polymers that are currently produced from fossil oil-derived ethylene and propylene; thus, butanol could serve as a renewable substitute (Zerlov et al. 2006). Similarly, succinate is an intermediate in the production of a variety of industrial surfactants, detergents, green solvents, and biodegradable plastics (Kamm and Kamm 2007). Lactic acid, which can be converted into polypropylene oxide, is the starting material for the production of polyester, polycarbonates, and polyurethanes; it is also used in the industrial production of green solvents, and its applications include the pharmaceutical and agrochemical industries (Datta et al. 2006).

Glycerol, a byproduct of the transesterification of microalgal lipids to biodiesel, could also be anaerobically fermented to the above mentioned and other end products (Yazdani and Gonzalez 2007). Furthermore, glycerol could be converted by certain bacteria to 1,3-propanediol, which is used in the formulation of a variety of industrial products such as polymers, adhesives, aliphatic polyesters, solvents, antifreeze, and paint (Yazdani and Gonzalez 2007; Choi 2008). Finally, glycerol could be used to generate electricity directly in biofuel cells (Yildiz and Kadirgan 1994). Once again, the issue of scale enters in. Production of 1 billion gallons of biodiesel will result in the formation of more than 400,000 tons of glycerol (biodieselmagazine.com/article.jsp?article_id=377). As the production levels for biodiesel (1.24 billion gallons in 2014) already has the market for glycerol saturated, additional capacity from algal lipids may find it exceedingly difficult to find uses.

It may also be possible to extract microalgal polysaccharides for use as emulsifiers in food and industrial applications (Mooibroek et al. 2007). Finally, microalgal carbohydrates could be recycled into pulp and paper streams, substituting for lignocellulosic materials derived from forestry resources.

As was the case with Option 3, this option will also require R&D efforts as discussed in chapter 2, Algal Biology; specifically, these are the development of high throughput technologies for the quantitative characterization of microalgal metabolites, including sugars and complex carbohydrates; and the development of genetic engineering tools to improve yields of products, including carbohydrates, if desired.

Option 5 – Recovery (Extraction or Secretion) of Fuel Lipids Only, with Use of the Residual Biomass as Soil Fertilizer and Conditioner

In case none of the above-mentioned four options are economical (i.e., the recovery and use of energy, proteins, non-fuel lipids, and carbohydrates is not cost-effective), it is possible to revert to the most simple option (Option 5), which involves the extraction of only fuel lipids and the subsequent use of the biomass residues rich in nitrogen and organic matter as soil fertilizer and conditioners. Also, algal biomass utilized for the secretion of alcohols, such as cyanobacteria, can be collected after senescence and used for the same process. As was mentioned above, the market for organic fertilizer is large and potentially growing.

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9. Distribution and Utilization

Distribution and utilization are challenges associated with virtually all biofuels. Although the biofuel product(s) from algal biomass would ideally be energy-dense and completely compatible with the existing liquid-transportation fuel infrastructure, few studies exist that address outstanding issues of storing, transporting, pipelining, blending, combusting, and dispensing algal biomass, fuels intermediates, and biofuels. Being later steps in the supply chain, distribution and utilization need to be discussed in the context of earlier decision points, such as cultivation and harvesting. In turn, these logistics through end-use issues influence siting, scalability, and the ultimate economics and operations of an integrated algal biofuels refinery. As a variety of fuel products—ethanol, biodiesel, higher alcohols, pyrolysis oil, syngas, and hydroreformed biofuels—are being considered from algal biomass resources, the specific distribution and utilization challenges associated with each of these possible opportunities is discussed.

9.1 Distribution

Lowering costs associated with the delivery of raw biomass, fuel intermediates, and final fuels from the feedstock production center to the ultimate consumer are common goals for all biofuels. In all cases, biofuels infrastructure costs can be lowered in four ways:

- Minimizing transport distance between process units
- Maximizing material energy density and stability
- Maximizing compatibility with existing infrastructure (e.g., high capacity storage tanks, delivery vehicles, pipelines, dispensing equipment, and end-use vehicles)
- Optimizing the scale of operations to the parameters stated above.

Discussions around distribution are complicated by the fact that several different fuels from algae are being considered, as discussed in detail in chapter 7. Ethanol, biodiesel, biogas, renewable gasoline, diesel, and jet fuels are all possible products from algal biomass. Each of these fuels has different implications for distribution. Some of these fuels appear to be more compatible with the existing petroleum infrastructure (Figure 9.1). Specifically, jet-fuel blends from a variety of oil-rich feedstocks, including algae, have been shown to be compatible for use in select demonstration flights (Buckman 2009; Efstathiou and Credeur 2009).

It is also anticipated that gasoline and diesel range fuels from algae will not require significant distribution system modifications during or after processing in the refinery. However, it

Figure 9.1. U.S. Energy Information Administration map of petroleum refineries, crude oil pipelines, and crude oil rail terminals (Source: EIA 2015)

remains unclear if crude produced from HTL can be blended into the existing crude oil pipeline.

With more than 10 billion gallons per year produced and consumed domestically, distribution-related issues for ethanol have been studied for some time, and algal ethanol can benefit from these analyses. While not as energy-dense as purely petroleum-derived fuels, ethanol is an important fuel oxygenate that can be used in regular passenger vehicles and special flex-fuel vehicles at up to 10% and 85% gasohol blends, respectively. However, considerable infrastructure investments need to be made for higher ethanol blends to become even more attractive and widespread. One issue is that ethanol is not considered a fungible fuel; it can pick up excessive water associated with petroleum products in the pipeline and during storage, which causes a phase separation when blended with gasoline (Wakeley et al. 2008). One possible way to address this is to build dedicated ethanol pipelines; however, at an estimated cost of $1 million/mile of pipeline, this approach is not generally considered to be economically viable (Reyold 2000). Another possibility is to distribute ethanol blends by rail, barge, and/or trucks. Trucking is currently the primary mode to transport ethanol blends at an estimated rate of $0.15/ton/kilometer (Morrow et al. 2006). This amount is a static number for low levels of ethanol in the blends (5%–15%); as the ethanol content in the blend increases, the transport costs will also increase due to the lower energy density of the fuel.

While the demonstration flights mitigate some infrastructure concerns, other distribution aspects concerning algal biomass, fuel intermediates, and final fuels remain poorly studied:

- The stability of the algal biomass under different production, storage, and transport scenarios is poorly characterized, with some evidence suggesting that natural bacterial communities increase the rate of algae decomposition (Rieper-Kirchner 1990). In the context of a variety of culturing and harvesting conditions differing in salinity, pH, and dewatering levels, it is difficult to predict how these factors will influence biomass storage and transport, as well as the quality of the final fuel product.
- An issue impacting oleaginous microalgae feedstocks is that the transport and storage mechanisms of algal lipid intermediates have not yet been established. It is conceivable that these "bio-crudes" will be compatible with current pipeline and tanker systems. However, it is known that the presence of unsaturated fatty acids causes auto-oxidation of oils (Miyashita and Takagi 1986), which carries implications for the producers of algae and selection for ideal lipid compositions. It is also known that temperature and storage material have important implications for biodiesel stability (Bondioli et al. 1995). Thus, materials and temperature considerations similar to plant lipids may be possibly taken into account for the storage of algae lipids (Hu et al. 2008).
- A significant factor restricting distribution of algal biomass as an intermediate is the amount of water it contains. Even for dewatered algal biomass, this will increase the mass to be transported by an order of magnitude and present corrosion issues. Drying presents concerns in terms of greenhouse gas emissions (see chapter 5); therefore, more research is needed, for example, on small-scale hydrotreatment.
- Depending on whether it will be dewatered/densified biomass and/or fuel intermediates that are to be transported to the refinery, conforming to existing standards (e.g., container dimensions, hazardous materials and associated human health impacts, and corrosivity) for trucks, rails, and barges is critical to minimizing infrastructure impacts. Because of the variability and instability of algal biomass, the distribution system will require novel monitoring and control. The optimal transport method(s) should be analyzed and optimized for energy inputs and costs, within the context of where the algae production and biorefinery facilities are to be sited. These have been challenging issues for lignocellulosic feedstocks (Hess et al. 2009) and can be expected to influence the economics of algal biofuels as well.

9.2 Utilization

The last remaining hurdle to create a marketable new fuel after it has been successfully delivered to the refueling location is that the fuel must meet regulatory and customer requirements. As mentioned in chapter 7, such a fuel must be "fit-for-purpose." Many physical and chemical properties are important in determining whether a fuel is fit-for-purpose; some of these are energy density, oxidative and biological stability, lubricity, cold-weather performance, elastomer compatibility, corrosivity, emissions (regulated and unregulated), viscosity, distillation curve, ignition quality, flash point, low-temperature heat release, metal content, odor/taste thresholds, water tolerance, specific heat, latent heat, toxicity, environmental fate, and sulfur and phosphorus content. Petroleum refiners have shown remarkable flexibility in producing fit-for-purpose fuels from feedstocks ranging from light crude to heavy crude, oil shales, tar sands, gasified coal, and chicken fat, and are, thus, key stakeholders in reducing the uncertainty about the suitability of algal feedstocks for fuel production.

Typically, compliance with specifications promulgated by organizations such as ASTM International ensures that a fuel is fit-for-purpose (ASTM International 2015a, 2015b, 2015c, 2015d, and 2015e). The failure of a fuel to comply with even one of the many allowable property ranges within the prevailing specifications can lead to severe problems in the field.

Table 9.1. Comparison of Typical Properties of Petroleum Oil and Oil from Fast Pyrolysis of Wood and Microalgae.

Properties	Typical values		
	Bio-oils		Petroleum oil
	Wood	Microalgae	
Carbon (%)	56.4	62.07	83.0–87.0
Hydrogen (%)	6.2	8.76	10.0–14.0
Oxygen (%)	37.3	11.24	0.05–1.5
Nitrogen (%)	0.1	9.74	0.01–0.7
Density (kg l^{-1})	1.2	1.06	0.75–1.0
Viscosity (Pa s)	0.04–0.20 (at 40°C)	0.10 (at 40°C)	2–1,000
Higher heating value (MJ kg^{-1})	21	29–45.9	42

Source: Brennan and Owende (2010).

Some notable examples have included elastomer-compatibility issues that led to fuel-system leaks when the blending of ethanol with gasoline was initiated; cold-weather performance problems that crippled fleets when blending biodiesel with diesel was initiated in Minnesota in the winter; and prohibiting or limiting the use of the oxygenated gasoline additive MTBE (methyl tert butyl ether) in 25 states because it has contaminated drinking-water supplies (McCarthy and Tiemann 2000).

In addition to meeting fuel standard specifications, algal biofuels, as with all transportation fuels, must meet EPA regulations on combustion-engine emissions. In 2012, in its final rule implementing the RFS program, EPA certified that commercial production of biodiesel and renewable diesel from algal oils that comply with the 50% threshold will qualify as advanced biofuels (EPA 2012). In 2014, EPA certified Algenol's DIRECT TO ETHANOL® fuel as an advanced biofuel with a life-cycle GHG reduction of 69% compared to gasoline (epa.gov/otaq/fuels/renewablefuels/new-pathways/documents/algenol-determination-ltr-2014-12-4.pdfh; ABO 2015). For a discussion of regulatory considerations and standards for algal biofuels, see chapter 6 of the ABO's *Industrial Algae Measurements* (ABO 2015).

For jet fuels, there can be no market penetration without a new specification. The existing ASTM D1655 standard for traditional jet fuel requires that the fuel be produced "from petroleum," so even if an algae-derived fuel meets all chemical and performance measures, it still will not meet that ASTM standard. There are new ASTM specifications for alternative jet fuel contained within ASTM D7566 (both Fischer-Tropsch synthetic paraffinic kerosene and hydro-processed esters and fatty acids synthetic paraffin kerosene [SPK]) that are relevant to algae, and algae-derived fuels can be produced to comply with these standards. However, algae-derived fuel produced by HTL or pyrolysis-type pathways, or through co-processing of algae-derived inputs in a petroleum refinery, will all require new technical standards to be developed and accepted by ASTM. Research and technology advancements may one day yield optimized conversion processes, which can deliver algae-derived compounds with improved performance, handling, and environmental characteristics relative to their petroleum-derived hydrocarbon counterparts. If significant benefits can be demonstrated, new specifications can be developed (e.g., ASTM D6751 and D7467). Additionally, some customers (such as airlines who face intensive scrutiny for their environmental impacts) may require the assurance of an objective, third-party, sustainability standard, such as that provided by the Roundtable on Sustainable Biomaterials. In addition to carbon LCA, elements for such an algae sustainability standard may include water quality, water consumptive use, soil impacts, local air quality, and food security.

The discussion below is divided into separate sections that deal with algal blendstocks to replace gasoline-boiling-range and middle-distillate-range petroleum products, respectively. These classifications were selected because the compounds comprising them are largely distinct and non-overlapping. Within each of these classifications, hydrocarbon compounds and oxygenated compounds are treated separately, since their production processes and in-use characteristics are generally different.

Algal Blendstocks to Replace Middle-Distillate Petroleum Products

Petroleum "middle distillates" are typically used to create diesel and jet fuels. The primary algae-derived blendstocks that are suitable for use in this product range are biodiesel (oxygenated molecules) and renewable diesel (hydrocarbon molecules). The known and anticipated end-use problem areas for each are briefly surveyed below.

Oxygenates: Biodiesel

Biodiesel is defined as "mono-alkyl esters of long-chain fatty acids derived from vegetable oils or animal fats" (ASTM International 2015b). Biodiesel has been demonstrated to be a viable fuel for compression-ignition engines, both when used as a blend with petroleum-derived diesel and when used in its neat form (i.e., 100% esters) (Graboski and McCormick 1998). However, many auto manufacturers have restricted the blend level of FAME allowable under warranty due to technical concerns with emission-control units, though this concern is not due to any problem with emissions from burning FAME, but rather the manner in which the catalytic converters are periodically cleaned by auto-combustion of fuel on the hot surface. The primary end-use issues for plant-derived biodiesel are: lower oxidative stability than petroleum diesel, higher emissions of nitrogen oxides (NOx), and cold-weather performance problems (Knothe 2007). The oxidative-stability and cold-weather performance issues of biodiesel preclude it from use as a jet fuel. The anticipated issues with algae-derived biodiesel are similar, with added potential difficulties including: 1) contamination of the esters with chlorophyll, metals, toxins, or catalyst poisons (e.g., sulfur and phosphorus) from the algal biomass and/or growth medium; 2) undesired performance effects due to different chemical compositions; and 3) end-product variability.

Hydrocarbons: Renewable Diesel and Synthetic Paraffinic Kerosene

The hydrocarbon analog to biodiesel is renewable diesel, which is a non-oxygenated, paraffinic fuel produced by hydrotreating bio-derived fats or oils in a refinery (Aatola et al. 2009). Algal lipids can be used to produce renewable diesel or SPK, a blendstock for jet fuel. These blendstocks do not have oxidative-stability problems as severe as those of biodiesel, and renewable diesel actually tends to decrease engine-out NOx emissions (Yamane et al. 2015). Nevertheless, unless they are heavily isomerized (i.e., transformed from straight- to branched- chain paraffins), renewable diesel and SPK will have cold-weather performance problems comparable to those experienced with biodiesel. However, hydrocarbons derived from algae are likely to be blended into petroleum fuels. The straight-chain hydrocarbons will provide a significant cetane enhancement, which can be exploited in refinery blending systems, which will also improve the cold-flow properties. The degree of saturation of the algal lipid is an important factor in the economics of hydrotreatment and also in the transportation stability of intermediates. Research is needed regarding how to optimize algal lipids intended for hydrotreatment. Also, as was the case with algal biodiesel, contaminants and end-product variability are concerns.

Algal Blendstocks for Alcohol and Gasoline-Range Petroleum Products

While much of the attention paid to algae is focused on producing lipids and the subsequent conversion of the lipids to diesel-range blending components (discussed above), algae are already capable of producing alcohol (ethanol) directly, and there are several other potential gasoline-range products that could be produced by algae-based technologies and biorefineries. Petroleum products in the alcohols and gasoline range provide the major volume of fuels used by transportation vehicles and small combustion engines in the United States. Ethanol or butanol is the most common biofuel currently used in gasoline, and these alcohols can be produced from fermentation of starches and other carbohydrates contained in algae.

Additionally, the hydrotreating of bio-derived fats or oils in a refinery will typically yield a modest amount of gasoline-boiling-range hydrocarbon molecules. Refiners refer to this material as "hydro-cracked naphtha." This naphtha tends to have a very low blending octane and would normally be "reformed" in a catalytic reformer within the refinery to increase its blending octane value prior to use in a gasoline blend.

9.3 Fuel and Engine Co-optimization

Future work may include the co-optimization of algae fuels with engines. In a DOE multi-year initiative, a team of national laboratories will, with guidance from industry and other important stakeholders, work to accelerate the concurrent development of advanced fuels and engines that are economically viable, environmentally sustainable, and commercially scalable.

The fuel and engine co-optimization effort includes two thrusts:

Thrust I – Improve near-term conventional spark ignition engine efficiency. High research octane number (RON) fuels enable more-efficient, higher-performance operation via engine downsizing and boosting. Many biofuel blending components exhibit high RON and can be introduced into the market in the near- to medium-term for engines optimized to operate on those fuels. Fuel properties beyond RON, such as heat of vaporization, burn rate, viscosity, volatility, and energy density will also be characterized, and the complexity of their interactions mapped to evaluate the full value opportunity. This thrust has lower risk relative to Thrust II because spark ignition engines are in use today—although not tuned to take advantage of the potential new fuels.

Thrust II – Enable full-operability advanced compression ignition engines. Thrust II will provide the science and technology underpinnings needed to make new fuels compatible with commercially viable, new advanced compression ignition engine technologies. This engine platform, which includes kinetically controlled and low-temperature combustion approaches, offers the promise of significantly greater thermal efficiencies with lower criteria-pollutant emissions, and presents attractive options for both light- and heavy-duty vehicles. Fuel research will focus on low-GHG advanced biofuel/petroleum blends. In addition, already-efficient conventional compression ignition engines can realize fuel-economy increases enabled by improved, low-GHG intensity fuels. Thrust II, to be conducted in parallel with Thrust I, presents a more complex technical challenge with higher potential risk and reward.

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10. Resources and Sustainability

The development and scale-up of algal biofuels production, as with any biomass-based technology and industry, needs to be analyzed from a resource availability and sustainability perspective. An algal biofuels production system requires that resource factors—such as suitable land and climate, management of water resources, supplemental CO_2 supply, and other nutrients—meet certain conditions for sustainable use and cost effectiveness. To achieve success regarding both technical and economic performance without adverse environmental impacts, resource factors must be appropriately matched to the required growth conditions of the algal species being cultivated and the engineered growth systems. The sustainability of algal production systems can be evaluated using a system of social, environmental, and economic indicators. Evaluation of these indicators for sustainability assessments of algal biofuel production will be affected by local siting and resources considerations. Resource assessment modeling, as well as techno-economic and life-cycle analyses, are discussed in chapter 11. This chapter provides an overview of the key resources and sustainability requirements for microalgae production and the progress that has been made in addressing these needs.

Figure 10.1 provides a simple, high-level overview of the major resource and environmental factors that pertain to the algal biofuels production inputs of climate, water, CO_2, energy, nutrients, and land. These factors and parameters are of greatest importance to siting, facilities design, production efficiency, and costs. For each parameter, a variety of conditions may be more or less cost-effective for the siting and operation of algal biomass production. Additional resources include materials, capital, labor, and other inputs associated with facilities infrastructure and conducting operations and maintenance.

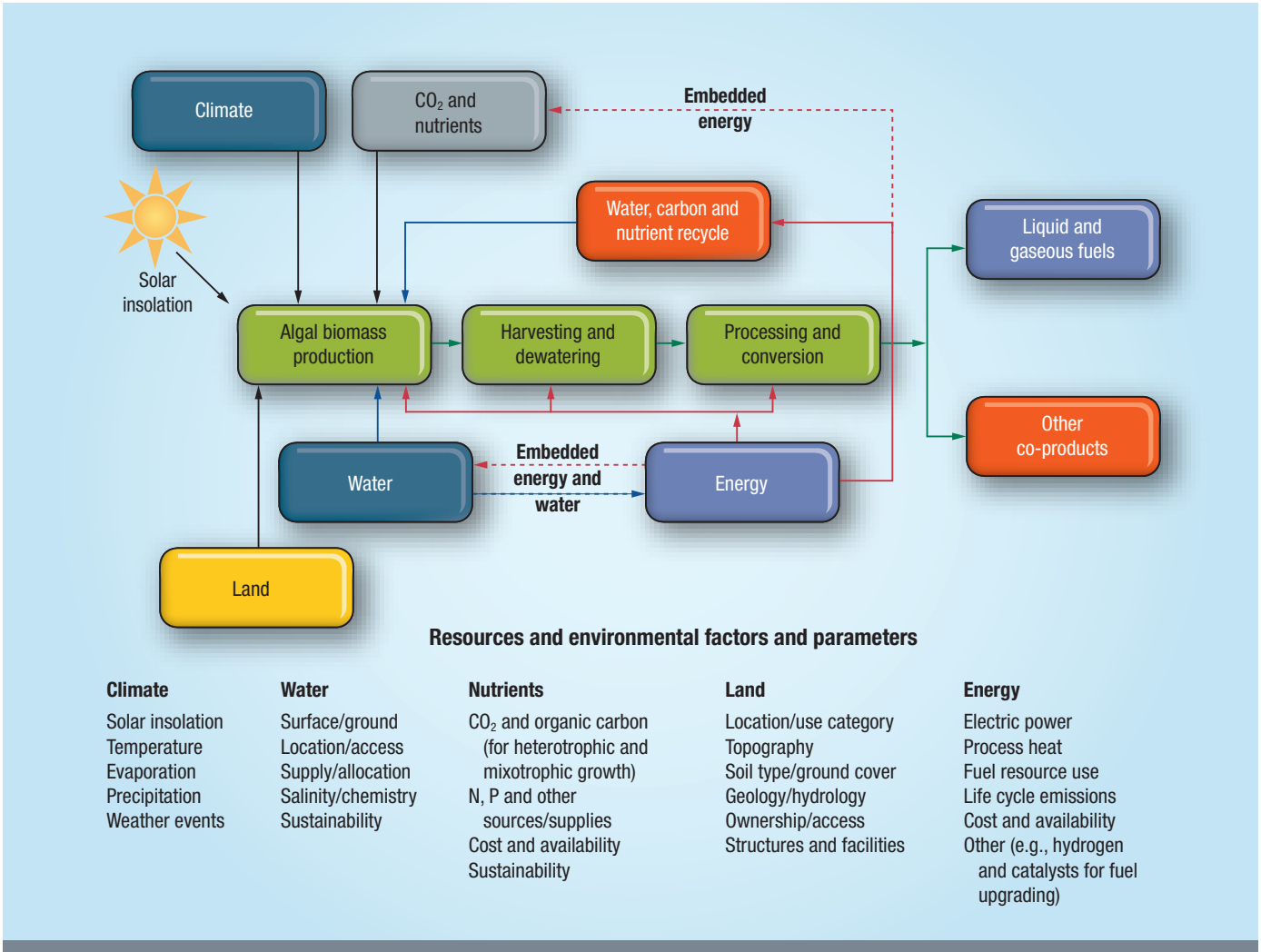

Figure 10.1. High-level overview of the algal biofuel supply chain, illustrating key resource inputs and related environmental issues spanning the operations of biomass production through downstream processing and conversion to fuels and co-products. (Source: From Pate 2013)

10.1 Resource Requirements for Different Cultivation Approaches

Photoautotrophic Microalgae Approach

Assessments of resource requirements and availability for large-scale, land-based photoautotrophic microalgal cultivation were first conducted during the Aquatic Species Program (Sheehan et al. 1998), focusing primarily on the Southwest and southern tier of the United States (e.g., Maxwell et al. 1985; Feinberg et al., 1990). Sufficient land, water, and CO_2 resources were identified at the time to suggest that the production of billions of gallons of algal biofuel could be supported if sufficiently high algae productivities could be achieved affordably at scale. Many of the findings of these earlier assessments still apply today, and the potential remains for biofuels and other co-products derived from photoautotrophic microalgae to significantly contribute to meeting U.S. transportation fuel needs and displacing petroleum use. An in-depth summary of the resource assessment modeling work completed for algal biofuels production can be found in chapter 11. Table 10.1 summarizes the key resource constraints for photoautotrophic cultivation, while Figure 10.2 provides an overview of the photoautotrophic approach to microalgal biomass and biofuel production.

In addition to coastal and inland photoautotrophic microalgae production, off-shore marine environment concepts have been proposed. An off-shore scenario can be represented by extension of Figure 10.1 to conceptually include off-shore areas and structures equivalent to inland facilities. The integration of wind and solar energy on coastal and inland photoautotrophic microalgae cultivation sites has been proposed (Nair and Paulose 2014; Beal et al. 2015).

Heterotrophic Microalgae Approach

Heterotrophic microalgae biomass and metabolite production is based on the use of organic carbon feedstock in the form of sugars or other relatively simple organic compounds instead

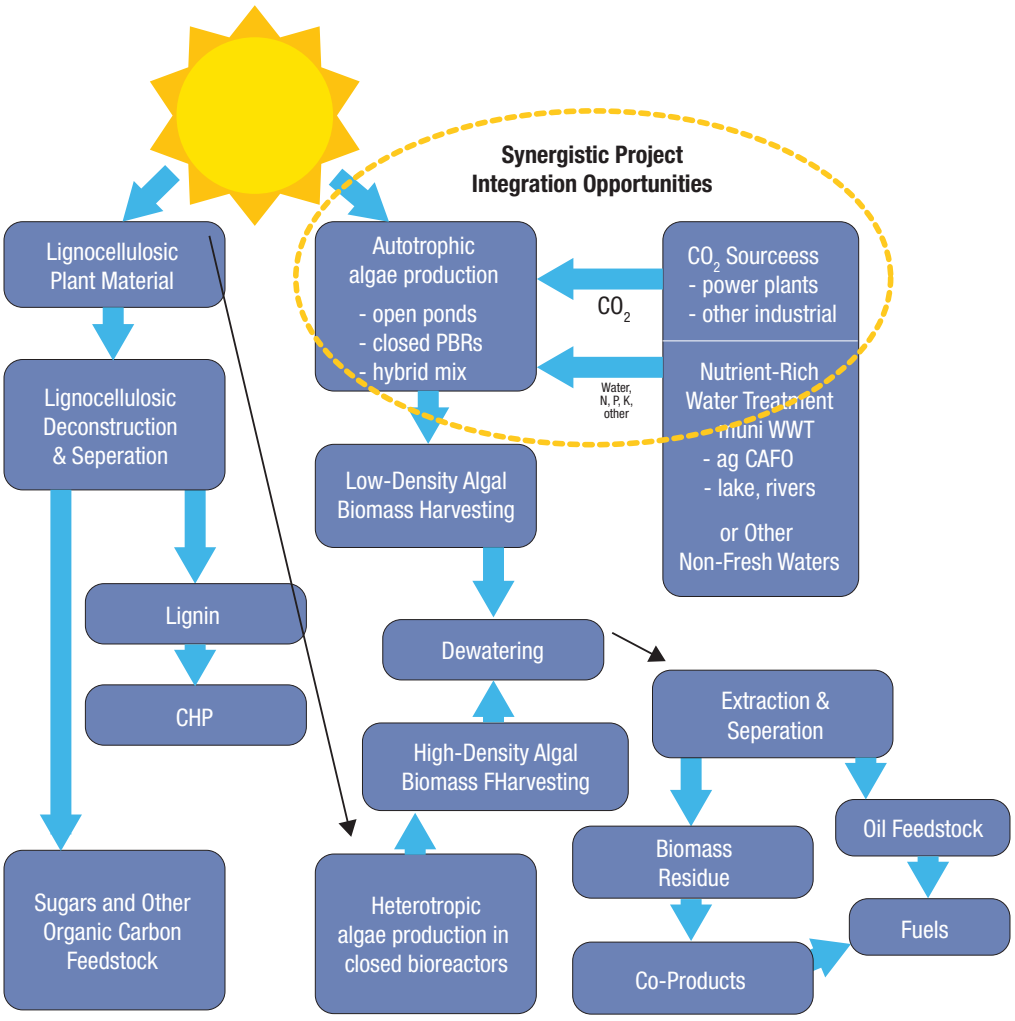

Figure 10.2. High-level illustration of heterotrophic and photoautotrophic approaches to microalgal biomass and biofuels production (Source: Adapted from the 2010 *National Algal Biofuels Technology Roadmap*.)

Table 10.1. Overview of Key Resource Constraints of Algae Production Approaches

Algae Production Approach	Key Resource Requirements
Photoautotrophic microalgae production	Climate, water, CO_2, other nutrients, required energy inputs, and land
Heterotrophic microalgae production	Sourcing of suitable organic carbon feedstock, water, energy, and other inputs required for siting and operating industrial bioreactor-based algae production and post-processing to fuels and other co-products
Mixotrophic microalgae production	A combination of resource requirements for photoautotrophic and heterotrophic microalgae production, depending on the process of production

of photosynthesis (see chapter 2). The algae are cultivated in the dark in closed industrial bioreactors that could potentially be established in many locations throughout the country. Achieving affordable scale-up and successful commercial expansion using the heterotrophic approach relies on the cost-effective availability of organic carbon feedstock—a resource that ultimately links back to a photosynthetic origin. Heterotrophic and photoautotrophic approaches to microalgae production have different siting and resource input implications, and thus present synergistic integration opportunities, but are not discussed in this review. Figure 10.2 provides an overview of the heterotrophic approach to microalgal biomass and biofuel production. Mixotrophic cultivation systems combine photoautotrophic and heterotrophic processes and have a range of resource requirements that are determined by the scale of production (Table 10.1; discussed further in chapter 4).

Heterotrophic production can be characterized as more of an industrial operation with a significant upstream logistics trail associated with the sourcing of the needed biomass-derived input feedstocks, whereas photoautotrophic production, in terms of cultivation and harvesting, is more akin to agriculture and serves as the point of origin for the biomass feedstock supply for the downstream value chain. Resource issues for the heterotrophic approach are more largely associated with the upstream supply of organic carbon feedstock derived from commodity crops, selected organic carbon-rich waste streams, and lignocellulosic biomass, thereby sharing many of the same feedstock supply issues with first- and second-generation biofuels (Table 10.1).

Use of sugars from cane, beets, other sugar crops, and from the hydrolysis of starch grain crops can lead to the problem of linking biofuel production and competition with food and feed markets. The preferred source of sugars and other appropriate biogenic carbon feedstocks for sustainable heterotrophic algae production are carbon-rich waste streams and the successful deconstruction of lignocellulosic materials. The latter has the greatest feedstock scale-up potential and is being pursued and reported elsewhere through bioenergy programs under DOE and USDA (e.g., Perlack et al. 2005; DOE 2006a). This work includes evaluation of siting and resource availability issues that are closely aligned with the production, availability, supply logistics, and pretreatment of lignocellulosic biomass feedstock that is expected to be capable of national scale-up to more than one billion tons annually (Perlack et al. 2005). Table 10.1 summarizes the key resource issues for different microalgae systems, including photoautotrophic, mixotrophic, and heterotrophic systems. Further discussion on the different approaches to microalgal biomass cultivation and production is in chapter 4.

Sustainability Indicators for Photoautotrophic Microalgae Biofuels

In addition to identifying the availability of resources for algal cultivation, assessments are also important to guide the responsible stewardship of resources toward environmental and socioeconomic sustainability. Sixteen largely quantitative, indicators of environmental sustainability have been proposed for algal biofuels (Table 10.2). These environmental sustainability indicators are categorized under soil quality, water quality and quantity, greenhouse gases, biodiversity, air quality, and productivity (Efroymson and Dale 2015). Other standards for environmental sustainability indicators have been proposed (GBEP 2011). Proposed socioeconomic sustainability indicator categories are social well-being, energy security, external trade, profitability, resource conservation, and social acceptability (Table 10.3, Dale et al. 2013). Throughout the chapter, the sustainability indicators will be described as they pertain to different resources for algae cultivation.

10.2 Resources Overview

Climate

Climate and temperature elements determine the overall viability of an algal biomass production site. As illustrated in Figure 10.1, key climatic factors include solar insolation, temperature,

Table 10.2. Environmental Indicators of Sustainability of Algal Biofuels and Proposed Units for Measurement

Category	Indicator	Units
Soil Quality	Bulk density	g/cm^3
Water Quantity	Peak storm flow	L/s
	Minimum base flow	L/s
	Consumptive water use (incorporates base flow)	Feedstock production: $m^3/ha/day$; Biorefinery: m^3/day
Water Quality	Nitrate concentration in streams (and export)	Concentration: mg/L; export: kg/ha/yr
	Total phosphorus concentration in streams and export	Concentration: mg/L; export: kg/ha/yr
	Salinity	Conductivity (no units)
Greenhouse Gases	CO_2 equivalent emissions (CO_2 and N_2O)	kgC_{eq}/GJ
Biodiversity	Presence of taxa of special concern	Presence
	Habitat of taxa of special concern	ha
	Abundance of released algae	Number/L
Air Quality	Tropospheric ozone	ppb
	Carbon monoxide	ppm
	Total particulate matter less than 2.5 µm diameter ($PM_{2.5}$)	$µg/m^3$
	Total particulate matter less than 10µm diameter (PM_{10})	$µg/m^3$
Productivity	Primary productivity or yield	gC/L/year or based on chlorophyll a

Source: Modified from Efroymson & Dale (2015).

precipitation, evaporation, and weather events. Each of these factors are described in the section below.

Sunlight and Temperature Needs

Growth of algae is technically feasible in many parts of the United States, but the availability of adequate sunlight and the suitability of climate and temperature are key factors that will determine economic feasibility. Availability of abundant sunlight is important for both photoautotrophic microalgae growth in open and closed cultivation systems. The average seasonal insolation is generally the dominant and rate-limiting factor for autotrophic algal productivity, and this factor varies widely across the country among inland, coastal, and offshore sites. Insolation has a strong influence on the spatial surface area of cultivation systems needed to achieve a set amount of product, downstream processing design capacity, the amount of CO_2 that can be captured, and the amount of culture that will need to be processed on a daily basis. The daily, seasonal, and annual variation in solar insolation, as well as other climate-related factors, such as temperature and weather (cloud cover, precipitation, wind, etc.) will also affect both the productivity and reliability of production.

Table 10.3. Socioeconomic Indicators of Sustainability of Algal Biofuels and Goals for Designing Sustainable Systems

Category	Indicator	Goal for design of sustainable system
Social Well-being	Employment	Provide a large number of high paying jobs
	Household income	Provide high-paying jobs and decrease fuel costs so that household income increases
	Work days lost due to injury	Select algal strains and conversion processes to minimize toxin production and toxicant exposure
	Food security	Develop algal biofuel systems on non-agricultural land and consider food coproduct options
Energy Security	Energy security premium	Maximize energy security dollar benefits of substituting algal biofuel for petroleum fuel
	Fuel price volatility	Reduce fuel price volatility below value without algal biofuel by contributing to reliable algal biomass and fuel supply with consistent prices
External Trade	Terms of trade	Create conditions so that less capital leaves a government entity to purchase petroleum
	Trade volume	Minimize net imports for fuel
Profitability	Return on investment (ROI)	Create a positive ROI
	Net present value (NPV)	Create a positive NPV
Resource Conservation	Depletion of non-renewable energy resources	Reduce amount of petroleum extracted per year, with a goal of zero
	Fossil energy return on investment (fossil EROI)	Increase fossil EROI above 1 and eventually above 3
Social Acceptability	Public opinion	Demonstrate high percent favorable opinion
	Transparency	Show a progressively increasing or high value
	Effective stakeholder participation	Show a progressively increasing or high value
	Risk of catastrophe	Frequency of catastrophic events based on current incidence or similar technology

Source: Modified from Dale et al. (2013) to emphasize algal feedstocks.

In general, the optimal temperature for algal biomass growth is between 20–35°C (68-95°F), though strains vary in temperature tolerance. Colder temperatures can lead to slower growth and productivity rates, while hotter temperatures can potentially reduce productivity rates or even cause the death of individual alga (Pate 2013). As a result, lower latitude areas are preferred for a more stable temperature range (Pate et al. 2011; Lundquist et al. 2010; Vasudevan et al. 2012; Pate 2013; Quinn et al. 2012). The operating temperature range used for algae biomass production can be altered through the use of

different open, closed, and hybrid cultivation systems and the use of co-located waste heat. Evaporative water loss, pond depth, pond mixing, solar gain during the day, radiative heat loss at night, and the thermal coupling and bidirectional heat flow through pond bottom and walls affect optimum tempature conditions in open ponds.

Closed photobioreactors (PBRs) are less sensitive to climate variability than open ponds due to their more controlled environment; however, temperature in a PBR needs monitoring due to limited evaporative cooling, as well as monitoring during severe weather. Temperature and availability of sunlight, both seasonally and annually, will most directly affect productivity, whereas precipitation, evaporation, and severe weather will affect water demand and water quality in open systems.

Additional factors could conceivably help producers overcome what might otherwise be unfavorable climate conditions for algae production. This could include situations where co-location of microalgae production might be possible with industrial operations capable of providing excess heat and power for cost-effective environmental control of algal cultivation (Khawam et al. 2014; Waller et al. 2012). This scenario, however, requires a more refined analysis for systems that are likely close and highly integrated with co-located industries providing synergistic opportunities for utilizing waste heat and energy.

Seasonal Considerations

A critical climate issue for both open and closed cultivation systems is the length of economically viable growing season(s) for the particular strains of algae available for productive cultivation. The primary geographical location drivers for determining length of growing seasons are latitude and elevation, which have major influence on the hours and intensity of available sunlight per day and the daily and seasonal temperature variations. Areas with relatively long growing seasons (for example, 240 days or more of adequate solar insolation and average daily temperatures above the lower threshold needed for economically viable growth) are the lower elevation regions of the lower latitude states of Hawaii, Florida, and parts of Louisiana, Georgia, Texas, New Mexico, Arizona, and California (NRC 2012). Other local climate and weather conditions will also have influence. While some analyses have been conducted and/or reported on seasonal variability (Davis 2012; Venteris et al. 2013; Venteris et al. 2014b; Coleman et al. 2014) further analysis (preferably on a site-by-site basis) with detailed data is needed to assess areas most suitable for algae production based on seasonal considerations. Discovery and development of algae species capable of increased productivity under wider ranges of light and temperature conditions and cultivation methods can also potentially lead to increased annual average productivities in more geographically diverse locations through rotating specialized summer and winter strains.

Availability and rotation of different algal species capable of good productivity in cold, temperate, and hot season conditions, would provide greater flexibility and could extend otherwise limited periods of commercially viable algae production (Pate 2013). Various scenarios involving the energy and cost considerations of algae during different seasons have been analyzed (Davis 2012; Davis et al. 2014a; Moody et al. 2014), and have found that seasonality had a profound impact on TEA and LCA, including optimal processing capacity and length of operations. From a TEA perspective, year-round operation was better, while a winter shutdown was more favorable for LCA (Davis et al. 2014a).

Preferred Geographic Regions for Algal Biomass Production

Early studies (Maxwell et al. 1985; Sheehan et al. 1998) focused on the Southwest and Southern U.S. regions as having the most optimal sunlight and temperature conditions. Studies performed by Sandia National Laboratories (Pate 2013), Wigmosta et al. (2011), and Vasudevan et al. (2012) utilized GIS-based tools that narrowed the scope to portions of Hawaii, California, Arizona, New Mexico, Texas, Louisiana, Georgia, and Florida for potential areas of adequate sunlight for optimal open cultivation of algal biomass. Other more recent studies using GIS-based tools (Davis 2012; Venteris et al. 2013, 2014 a-d; Davis et al. 2012; Davis et al. 2014b; Coleman et al. 2014; Bennett et al. 2014) have taken climatic factors into consideration within the tool, and generally correspond to suitable areas identified in previous studies, with greater emphasis on the South Atlantic and Gulf of Mexico areas (figs. 10.3, 10.4, and 10.5). Suitability of closed systems, such as photobioreactors, have been modeled by Quinn et al. (2012) and shown to have encouraging results for large-scale cultivation in southwestern United States. (More discussion on GIS-based resource assessment is available in chapter 11.)

Precipitation and Evaporation

Precipitation also affects water availability (both surface and groundwater) at a given location within a given watershed region. Areas with higher annual average precipitation (more than 40 inches), represented by specific regions of Hawaii, the Northwest, and the Southeast, are desirable for algae production from the standpoint of long-term availability and sustainability of water supply. Large-scale projects funded by DOE, such as Hawaii BioEnergy, the Cornell Consortium, and Algenol have been located in these high precipitation regions. Future research should consider seasonal variations of water availability (i.e., receiving annual precipitation amounts in a specific season, such as monsoons).

Evaporation is closely coupled with climate and affect the water requirements for algae growth systems. The western United Steates exhibits higher rates of evaporation, ranging from 4 to

21 ML.ha^{-1}.yr^{-1}, whereas the eastern United States generally is between <1 to 4 ML.ha^{-1}.yr^{-1} (Wigmosta et al. 2011). Suitable annual evaporation rates between 6–8 feet (1.8–2.4 meters) are generally agreed upon as a suitable level for algal biomass cultivation (Lundquist et al. 2010); Sapphire Energy, Inc., reports an evaporation rate of approximately 2 meters annually at Columbus Algal Biomass Farm in New Mexico (White and Ryan 2015).

Evaporation is utilized as a form of regulating the operating temperature of cultures. Evaporative water loss in open ponds is influenced by the degree of salinity of the water used for cultivation and the local latitude, elevations, daily ambient temperature variations, (Al-Shammiri 2002; Hutchison et al. 1978; Kokya et al., 2008; Oroud, 1995; Lundquist et al. 2010), and largely contributes to makeup water demand requirements (Pate et al. 2011; Wigmosta et al. 2011; Pate 2013). While closed cultivation systems have more control over the operating culture environment, evaporative cooling waters are still needed to prevent overheating of the system (Pate 2013).

SUSTAINABILITY INDICATORS FOR CLIMATE

Productivity and water quantity and quality are categories for environmental sustainability indicators (McBride et al. 2011; Efroymson and Dale 2015), severe weather also influences the risk of catastrophe socieoeconomic sustainability indicator for social acceptability (Efroymson, Dale, and Langholtz 2016). Therefore, in addition to influencing how a cultivation is operated, these abiotic factors contribute to the environmental and socioeconomic sustainability of the facility.

Severe Weather Events and Elements

Severe weather events, such as heavy rain and flooding, hail storms, dust storms, drought, and hurricanes pose serious concerns in the inland and coastal regions around the United States. However, photosynthesis does occur (although at a

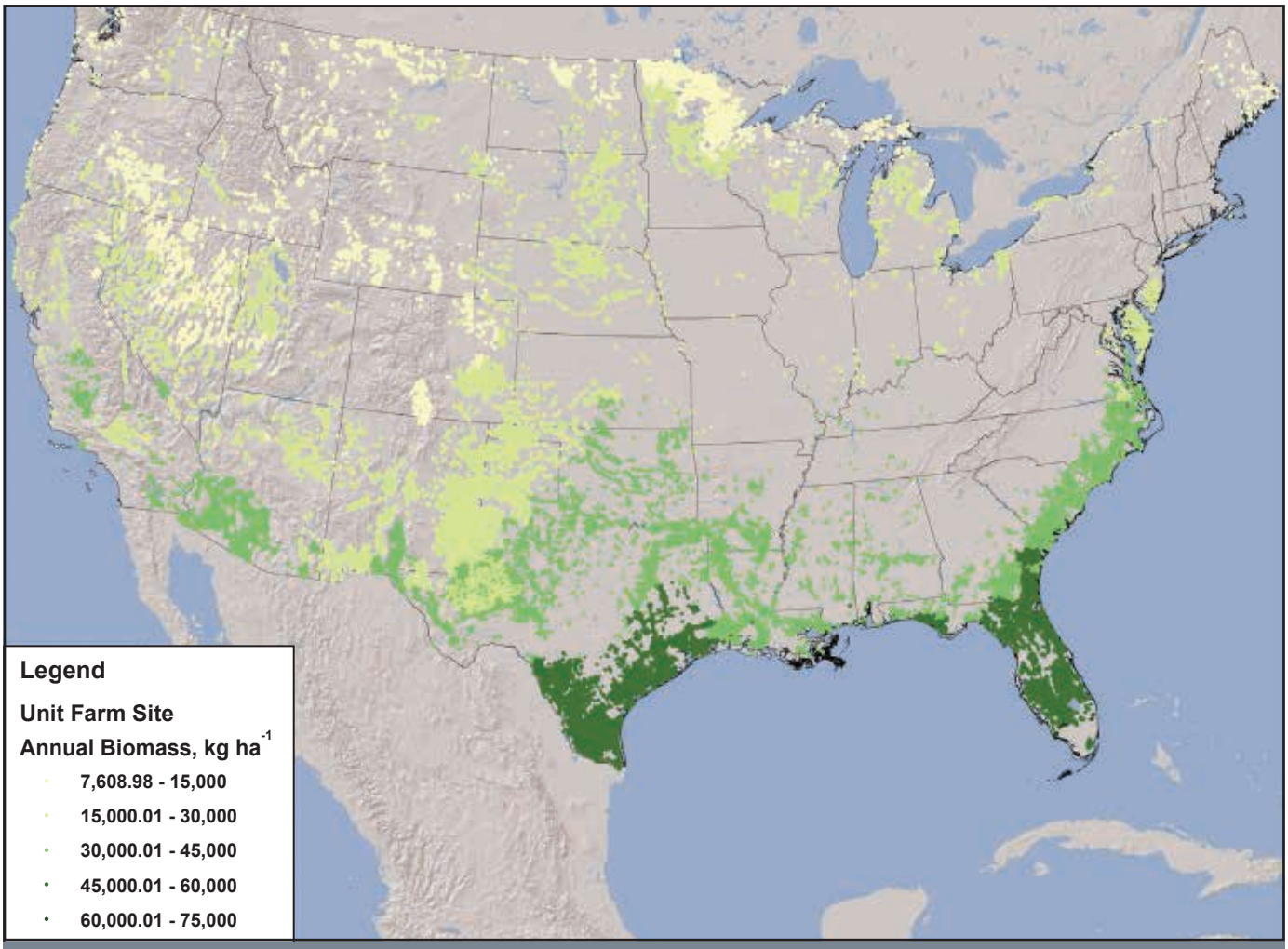

Figure 10.3. Examples of the preferred geographic regions for algal biomass production. Map of the potential unit farm sites and attendant productivity for open-pond growth of *Chlorella* assuming a pond salinity of 5g/kg. (Source: Venteris et al. 2014a)

reduced rate) during cloud cover and storms (Churkina and Running 1998). These weather events should be accounted when prospecting land for algae production as they can disrupt operating procedures through contaminating open systems of cultivation or causing physical damage to equipment. In the case a severe weather catastrophe results in release of algae or facility water into local ecosystems, social and environmental sustainability will be challenged by the risk of impact on biodiversity, water quality, and social acceptability (Efroymson, Dale, and Langholtz, 2016). As well, future considerations should include consideration of the impacts of sea-level rise for coastal facilities.

Water

Various efforts have been made by researchers to further understand water resources for algal cultivation, in the areas of water quantity, quality, and sustainability (Pate et al. 2011; Pate 2013; Wigmost et al. 2011; Batan et al. 2013; Venteris et al. 2012, 2013, 2014b,c). As illustrated in Figure 10.1, key water factors include source (surface/ground), siting, access, supply, salinity, quality, and sustainability. Each of these factors are described in the following sections:

Water Supply and Quantity Requirements

Suitable water supplies are a key input factor for cultivation, and are heavily dependent on geographical location and local conditions. Areas of the country with the highest solar resource best suited for algae growth also tend to be more arid and subject to more limited water supplies (Wigmosta et al. 2011; Venteris et al 2013; Wigmosta et al. 2014). In addition to geographic location, water use and consumption for algae-based biofuels will depend on the cultivation approach (photoautotrophic/heterotrophic/mixotrophic), growth system (open vs. closed. vs. hybrid combination). Mixotrophic and heterotrophic systems must also account for water used in the production of the upstream organic carbon feedstock. Different degrees of water usage are incurred if there is a need to replace water lost by evaporation in open pond systems, or to use water for evaporative cooling in closed systems.

Algal feedstock cultivation can be less, the same, or more water-intensive than the majority of terrestrial biofuel crops, depending on cultivation process, co-products, and location (Batan et al. 2013). Water utilization for algal biomass and downstream production of biofuels, both in terms of overall

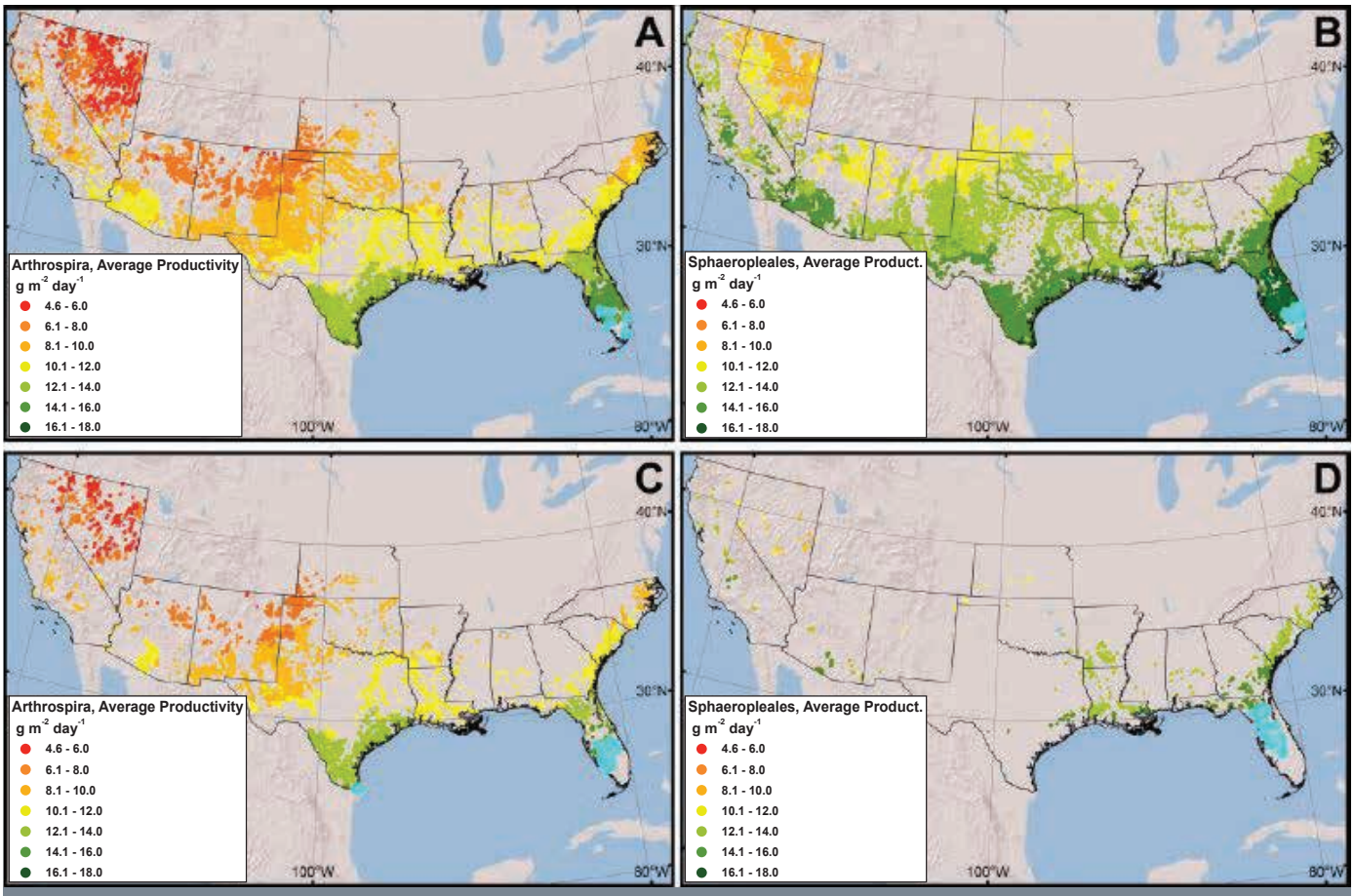

Figure 10.4. Examples of the preferred geographic regions for algal biomass production. Map of southern states showing productivities (annual average in g/m^2/day for (A) *Arthospira* and (B) *Sphaeropleales* for all sites considered in the study. "Base screen" scenario results for (C) *Arthospira* and (D) *Sphaeropleales*. The top 200 sites for all panels are highlighted in blue. (Source: Venteris et al. 2014c)

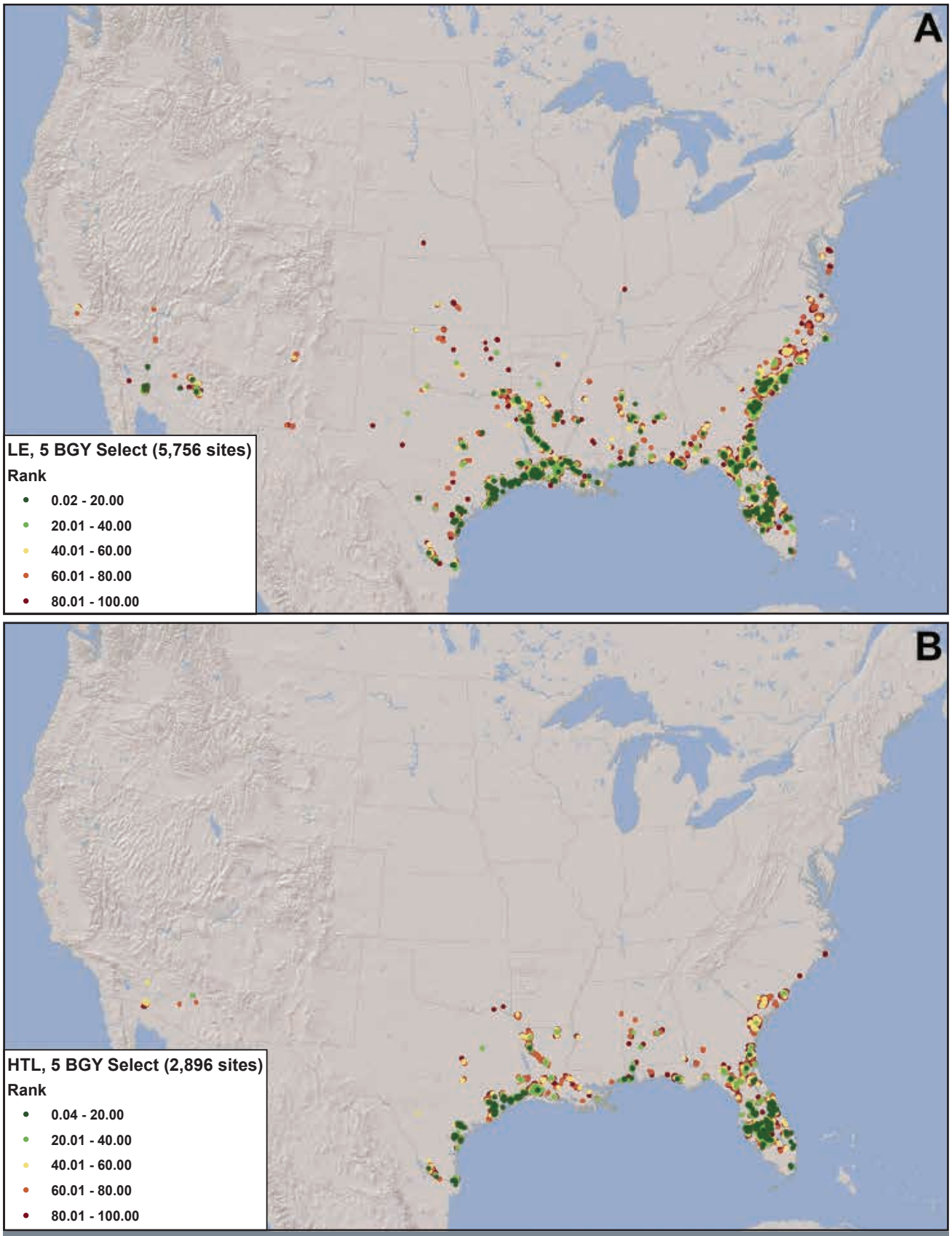

Figure 10.5. Examples of the preferred geographic regions for algal biomass production. Map showing 5 BGY site selections for LE (*A*) and HTL (*B*) scenarios. The sites are colored according to decimal rank, with the most cost-effective sites (selected first) having small numbers (and colored green). (Source: Venteris et al. 2014a)

input supply needs and consumption, warrants closer attention and assessment to better understand and refine water resource requirements. A key issue is the the uncertainty in quantity of freshwater available for algal cultivation.

Major questions that still need to be answered include

- How much surface water is actually available (especially in the eastern United States)?
- What are the economics of using saline groundwater, waste freshwater, and seawater?
- What are the economics and environmental sustainability of concentrate disposal from these sources?

Wigmosta et al. (2011) showed that under large commercial algae industry build-up, the amount of water required nationally could be 2.75 times the amount consumed by irrigated agriculture, due to open ponds being subject to evaporative loss. Therefore, it is important for efficient utilization practices to be put into place for large-scale cultivation. Existing water infrastructure, such as irrigation, would theoretically be able to supply the water quantity needed for large-scale growth, but at the cost of competing with existing agriculture uses (Pate et al. 2011). Even with increased use of non-freshwaters for cultivation, practices will continue to need improvements in efficiency (i.e., recycling), new technologies to reduce evaporative loss in open pond systems, and site-specific analyses on infrastructure needed and water sustainability (Venteris et al. 2014b,c).

From a resource use standpoint, integrating algae production with non-fresh wastewater resources for renewable fuels has the potential of putting less additional demand on limited freshwater supplies, reducing eutrophication of natural water bodies, and recycling nutrients. The unique ability of many species of algae to grow in non-freshwater over a range of salinities means that, in additional to coastal and possible offshore areas, other inland parts of the country can be targeted for algae production where brackish or saline groundwater supplies may be both ample and unused or underutilized. Furthermore, produced water has been shown as a promising alternative water source for cultivation in preliminary research (NAABB 2014). Produced water from petroleum, natural gas, and coal bed methane wells is a water resource that can range in quality from nearly fresh to hyper-saline. However, dependence on produced water alone will not support large cultivation efforts (Venteris et al. 2013). When considering the water resources needed for the future development and expansion of algal biofuel production, the use of non-freshwater sources should be considered in the face of the growing competition and demands on limited sustainable freshwater supplies, especially in the western United States (DOE 2006b; NAS 2007; Hightower et al. 2008; NRC 2012; Venteris et al. 2013). However, it should be noted that non-freshwater sources could potentially be more expensive than freshwater, depending on source (ground vs. surface waters) and transport distances (Venteris et al. 2013).

Capture and reuse of fresh and non-freshwater sources will be dependent on the geographical location, availability, affordability, and accessibility of such water sources. Modeling of water resources is important to understand species-specific requirements for siting and cultivation. One approach to efficient utilization would be to cultivate algae in areas that have the lowest freshwater used per liter of biofuel produced; Wigmosta et al. (2011) showed that the Gulf Coast, Eastern Seaboard, and the Great Lakes have less evaporative potential and more rainfall than locations in the southwest United States. Another approach is to utilize non-freshwater sources (e.g., saline waters), as arguably there is an "infinite" amount of saline water available from the ocean. Recent efforts by Venteris et al. (2014c) have modeled the near surface groundwater salinity of the southern continental United States (CONUS) for *Arthrospira* and *Sphaeropleales* (Figure 10.6).

Evaluation of water use and recycling for the overall algal biofuels production chain is also important for cost efficiency and sustainability (Yang et al. 2011) (see chapter 4 for discussion). Along the production pathway chain, additional water may be used and/or consumed (cultivation and harvesting), while at other times may also be saved, reclaimed, and recycled (harvesting, dewatering, and after some conversion processes). Additionally, transporting water through each component of the system is energy-intensive and has high costs. Quantifying the overall water requirements of the system is an important component for completing life-cycle assessments on algal biofuels.

Analysis of U.S. Water Supply, Consumption, and Management

Quantitative information remains limited on U.S. brackish and saline groundwater resources in terms of their extent, water quality and chemistry, and sustainable withdrawal capacity (Venteris et al. 2013). An improved knowledge base is needed to better define the spatial distribution, depth, quantity, physical and chemical characteristics, and sustainable withdrawal rates for these non-fresh groundwater resources, and to predict the effects of their extraction on the environment (Venteris et al. 2013; Alley 2003; Dennehy 2004). In response to this critical need to enhance and update information on national groundwater aquifers, USGS initiated a national study of brackish (including saline) groundwater in 2013 to improve the understanding of brackish aquifers at national and regional scales and locations (USGS 2013).

Total combined fresh and saline water withdrawals in the United States as of the year 2010 were estimated at 355,000 million gallons per day (Mgal/d), about 13% less than 2005 withdrawals (Maupin et al. 2014). Water withdrawal use is defined as the "water removed from the ground or is diverted

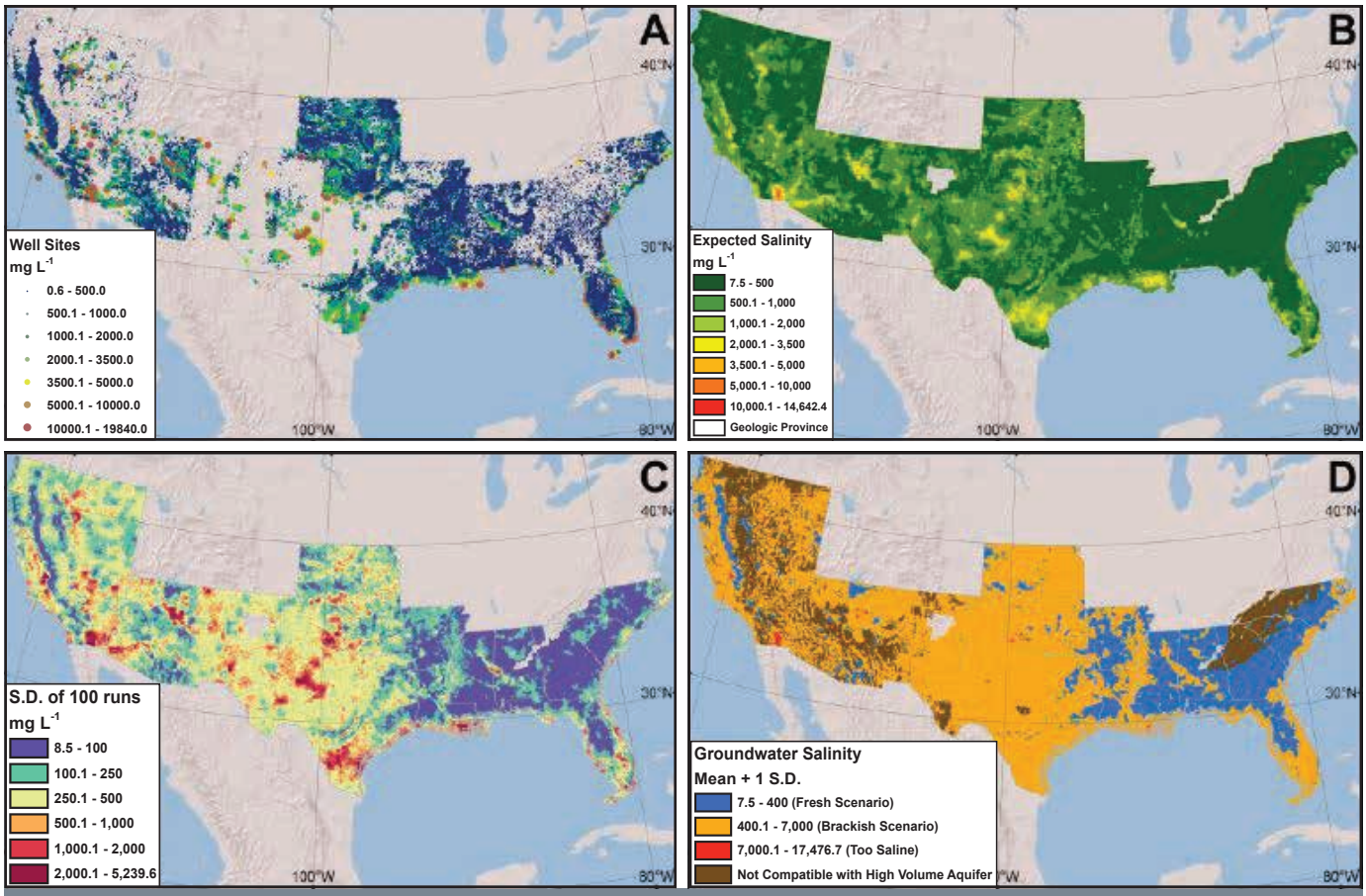

Figure 10.6. Maps illustrating the modeling of near surface groundwater salinity. (*A*) original well data, (*B*) expected value of salinities simulated using SGSIM with geologic province boundaries overlaid, (*C*) standard deviation of the expected value, and (*D*) map of regions with potential for high-volume aquifers and areas meeting the salinity criteria for *Sphaeropleales* and *Arthospira*. (Source: Venteris et al. 2014c)

from a surface-water source for use" (USGS 2016), whereas consumptive water use is defined as water withdrawn from a source and is unavailable for other uses (USGS 2011). Approximately 86% of withdrawals were freshwater, with about three quarters comprised of surface waters (Maupin et al. 2014). saline water (seawater and brackish coastal waters) were about 14% of the total water withdrawals, with about 93% comprised of surface-water (Maupin et al. 2014).

Electric power generation, irrigation (agriculture) and public supply make up 90% of total withdrawals in the United States (Maupin et al. 2014). Specifically, electric power generation freshwater consumptive use is about 18.4 Mm^3/d, and consumptive use is 18.4 Mm^3/d (3.9% of the total consumptive use of water in the United States) (Tidwell et al. 2014; Moore et al. 2015). Withdrawals for irrigation of crops and other lands totaled 115,000 Mgal/d (Barber, 2014), or 80.7% represented 33% of all water withdrawals, and 38% of all freshwater withdrawals (Barber 2014). At the national scale, total combined fresh and saline water withdrawals more than doubled from about 180 billion gallons per day in 1950 to more than 400 billion gallons per day in 1980. Total withdrawals since the mid-1980s remained relatively flat through 2005 at about 409,000 Mgal/d, and decreased in 2010 by 13% (355,000 Mgal/d) due to advances in technology and efficiency, as well as the migration of industrial manufacturing to outside of the United States (Barber 2014; Maupin et al. 2014).

The relatively flat national water withdrawal trend from 1980–2005, following a more than doubled water demand from 30 years prior, reflects the fact that freshwater in the United States has approached full allocation. Growing demand for limited freshwater supplies in support of development and population increase has thus far been offset by increased conservation, the increased re-use of wastewater, and advancements in water use efficiency, as explained by the decrease in the withdrawals in 2010. Many of the nation's fresh ground water aquifers are under increasing stress, and the future expansion of freshwater supplies for non-agricultural use must increasingly come from the desalination of saline or brackish water sources and from the treatment and reuse of wastewater, all of which have increasing energy demand implications (Moore et al. 2015; Barber 2014; DOE 2006b; Hightower et al. 2008; Kenny et al. 2009).

The stress on fresh water supplies in the United States is not restricted to the more arid western half of the country but is also becoming a local and regional concern at various locations throughout the East, where a growing number of counties are experiencing net freshwater withdrawals that exceed the sustainable supply from precipitation (DOE 2006b; Hightower et al. 2008). Overall, regional water availability is becoming an important topic in an overall energy-water nexus discussion. Climate change is also recognized as a factor that could have a major effect on all sectors of water resources supply and management in the future (USGS 2009).

Water Quality

Saline sources: One of the major benefits of growing algae is that, unlike most terrestrial agriculture, algal systems can potentially utilize non-freshwater sources that have few competing uses, such as saline and brackish groundwater, "co-produced water" from oil, natural gas, and coal-based methane wells, or municipal wastewater (Reynolds 2003; USGS 2002; Lundquist et al. 2010; NRC 2012; Venteris et al. 2013). However, open pond systems in more arid environments with high rates of evaporation, salinity, and water chemistry will change with evaporative water loss, thereby changing the culture conditions. These changes will require periodic blow-down of pond water after salinity build-up, periodic treatment of lower salinity make-up water to dilute the salinity build-up, the application of desalinization treatment to control salinity build-up, or high adaptive algae that can thrive under widely varying conditions (NRC 2012; Venteris et al. 2013). Open algal ponds may have to periodically be drained and re-filled, or staged as a cascading sequence of increasingly saline ponds, each with different dominant algae species and growth conditions.

Implementing desalination techniques to saline waters as an alternative to freshwater sources would likely impose additional capital, energy, and operational costs. Disposal of high salt content effluent or solid byproducts, from pond drainage and replacement, or from desalination operations, can also become an environmental problem for inland locations. Some salt byproducts may have commercial value, depending on the chemistry. As a water quality indicator, salinity of local water systems can be measured to provide assurance aginst unintentional leakage and salinization of surface or ground water. Measurements of nitrate and phosphorous concentrations are also indicators of water quality and are recommended to be monitored for algae cultivation (McBride et al. 2011, Table 10.2).

Municipal and organic rich sources: Municipal wastewater treatment facilities and agricultural dairy and feedlot operations located throughout the United States, particularly in the eastern half of the country, represent potential co-location sites for algae operations where nutrient-rich wastewater could be used for algae production, and the algae production can help provide nutrient removal service in the wastewater treatment. Two main types of algae production facilities are envisioned: (1) dedicated facilities, with the main purpose of biomass production, and (2) wastewater treatment facilities, which produce algal biomass as a consequence of the wastewater treatment. Dedicated biomass production facilities will also require wastewater treatment and nutrient recycling. A subset of wastewater treatment facilities consist of evaporation facilities, which are used to dispose of wastewater or brines. The major classes of wastewaters to be treated are municipal, organic industrial (e.g., food processing), organic agricultural (e.g., confined animal facilities), and eutrophic waters with low organic content but high nutrient content (e.g., agricultural drainage, lakes, and rivers). Whereas most wastewater treatment systems will use heterotrophic or mixotrophic cultures, for eutrophic water systems, photoautotrophic algae are uniquely positioned to provide this treatment service. Despite a seeming abundance of wastewater and waste nutrients, recycling of nutrients and carbon at algae production facilities will be needed if algae are to make a substantial contribution to national biofuel production. Even with internal recycling, importation of wastes and/or wastewater will still be needed in dedicated algae biomass production facilities to make up for nutrient losses (Brune et al. 2009).

Algae can be useful in the treatment of waters polluted with organic matter, excess nutrients (e.g., nitrogen, phosphorus, and potassium), metals, synthetic organic compounds, and potentially endocrine compounds (Oswald 1988; Woertz et al. 2009; Aksu 1998; Borde et al. 2002; Woertz et al. 2009; Lundquist et al. 2010; Lai et al. 2002; Shi et al. 2010; Zhang et al. 2014). The NAABB Consortium provided promising results in developing methods for cultivation in low-cost media using agricultural-grade nutrients, wastewater sources, and media recycling. Algae are similar to plants in that they both produce oxygen and assimilate nutrients. These reactions are also the best-known mechanisms of wastewater treatment by algae. Dissolved oxygen released by the algae can be utilized by bacteria to oxidize waste organic matter. The interaction of algae and bacteria in wastewater cultures leads to degradation of a wide variety of synthetic organic compounds such as phenol and acetonitrile (Borde et al. 2002; Muñoz et al. 2005). The ability of algae to assimilate dissolved nutrients down to trace concentrations is useful in wastewater treatment, if the nutrient-rich algae are then also removed from the water. Disinfection is promoted via the production of oxygen radicals in the presence of sunlight, dissolved oxygen, and naturally occurring organic catalysts (Sinton et al. 2002; Kohn et al. 2007). Heavy metals may be removed by adsorption to algal cells, which will be a benefit as long as the resulting metals concentrations in the algal biomass are not excessive or inhibitive for biomass production or later use in the processing of fuel and other co-products (NAABB 2014). Studies demonstrated that algae can grow in waters with high levels of heavy metals without much effect in growth rates, minimize microbial risks

from water from agricultural and municipal areas, as well as remediate produced water from oil and gas production sites (NAABB 2014). The bioaccumulation of trace contaminants in algae that would occur in the receiving waters, eventually harming higher organisms, might be prevented to a great extent by pond treatment followed by algae harvesting. The processing of the algal biomass for fuel and other co-products would presumably destroy and neutralize the contaminants, but further investigation is needed to confirm this. However, any heavy metals contaminating the algal biomass likely would remain in the waste from biofuel processing, potentially increasing the cost of waste disposal or recycling. For all biofuel feedstocks, routes of such contamination should be studied and preventative measures developed.

Wastewater Treatment

There have been many R&D projects investigating the integration of algal biomass production with wastewater treatment (see Lundquist et al. 2010; NAABB 2014; Orfield et al. 2014). Existing algae-based treatment facilities use relatively deep ponds (1–6 m). The great depths contribute to low algal productivity, but high productivity is not crucial to the treatment goals of these facilities (removal of organic matter and pathogens only). Ponds for more advanced treatment, including nutrient removal, need high algal productivities (as does biofuels feedstock production). These highly productive systems use shallow reactors, either high rate ponds (~30 cm) or algae turf scrubbers (~1 cm) (see chapter 4). High rates of algae production lead to high rates of nutrient removal and wastewater treatment. Thus, the objectives of biofuel feedstock production and wastewater treatment are aligned, at least in terms of maximizing biomass production. Maintenance of lipid-rich strains in wastewater, or manipulation of culture conditions to promote lipid production, is currently in development.

Algae-based wastewater treatment facilities are typically less expensive to build and to operate than conventional mechanical treatment facilities. For example, high-productivity algae ponds have a total cost that is about 70% less than activated sludge, which is the leading water treatment technology used in the United States (Downing et al. 2002). This cost savings, coupled with the tremendous need for expanded and improved wastewater treatment in the United States (EPA 2008) and throughout the world, provides a practical opportunity to install algae production facilities in conjunction with wastewater treatment.

In addition to the ability of algae systems to treat organic-rich wastewaters, their ability to treat high-nutrient, low-organic content wastewaters will expand the opportunities for algae production systems. Agricultural drainage and eutrophic water bodies (e.g., Salton Sea, California) is an example of such waters (Benemann et al. 2002). Treatment of nutrient-rich waters is likely to occur in more rural settings than treatment of municipal wastewaters, potentially leading to greater land availability and savings in land costs. Treatment of agricultural drainage with algae turf scrubbers (see chapter 4) without CO_2 addition and high rate ponds with CO_2 addition has been demonstrated in California's Central Valley and elsewhere (Craggs et al. 1996; Mulbry et al. 2008; Lundquist et al. 2004).

Water Sustainability Indicators

The environmental sustainability of water use for the production of algal biofuels can be evaluated by the water quanity used at the facility and the quality of effluents from the facility or receiving waters. Several strategies for evaluating water quantity indicators have been outlined (NRC 2012; GBEP 2011; McBride et al. 2011), and propose mesauring consumptive water use, or the water used in cultivation that cannot be recycled as it is lost to runnoff, evaporation, or incorporation into a product (Efroymson and Dale, 2015). The indicators presented by NRC (2012) recommend presenting consumptive water use with data that gives context to local water availability. For the environmental sustainability of algal biofuels, Efroymson and Dale (2015) recommends reporting minimum base flow, peak storm flow, and consumptive water use for other local activities as indicators of water quantity (Table 10.2). Environmental sustainability indicators have the potential to influence the socioeconomic sustainability indicators of food security and public opinion (Efroymson et al. 2016).

Land

Land availability will be important for algae production because either open or closed systems will require relatively large areas for implementation, as is expected with any photosynthesis-based biomass feedstock. Even at levels of photoautotrophic microalgae biomass and oil productivity that would stretch the limits of an aggressive R&D program (e.g., target annual average biomass production of 30 to 60 g/m^2 per day with 30% to 50% neutral lipid content on a dry weight basis), such systems would require in the range of roughly 800 to 2,600 acres of algae culture surface area to produce 10 million gallons of oil feedstock. In comparison, the average size of a commercial terrestrial agriculture farm (as of 2007) is 1,105 acres (MacDonald et al. 2013).

Land availability is influenced by various physical, social, economic, environmental, legal, and political factors. Wigmosta et al. (2011) demonstrated that appromizately 5.5% of the land area of the CONUS is required to to generate 220 x 10^9 L yr^{-1} of oil (or 48% of 2010 U.S. petroleum imports for transportation) through open pond production, compared to the results from Quinn et al. (2012) who presented that $1.853x10^8$ acres is required in the CONUS plus Hawaii to produce 315 BGY of lipid production using photobioreactors. When harmonized, the total land in the United States suitable for algal cultivation is approximately 75 million ha ($1.853x10^8$ acres), with the assumption of 18 $m^3ha^{-1}yr^{-1}$ yield (Quinn and Davis 2015).

A strength of algal biofuel production is the ability to cultivate biomass on non-arable lands, thus not competing against large-scale agricultural operations and other uses. Hundreds of millions of acres of relatively low-productivity, lower-value land exists in the United States (USDA 2006 and 2009), including pasture, grassland, and relatively barren desert land. Venteris et al. (2012) conducted an assessment of the amount and types of U.S. land available for conversion to algal biofuel production. Results indicate that even if currently productive croplands are excluded, on the order of $1.0e^{+6}$ km^2 (100 million hectares) are available. Pasture land, defined as a mix of grasslands and other non-forested pasture, range, and open grazing land, has been identified as very promising for cultivation, (Pate et al. 2011; Venteris et al. 2012; Langholtz et al. 2016). Areas with consistently warm temperatures and high solar activity, such as the Southwest, West Texas, Hawaii, Gulf Coast, and Florida, were identified as regions with high productivity potential, where the land is relatively flat and barren (Quinn et al. 2012). Marginal lands are also identified as a potential source of suitable land, with much of it located in farming regions in the Midwest and Plains for a low price. However, further study is needed on the availability of infrastructure in the surrounding region to support large-scale algal growth (Venteris et al. 2012). When considering trade-offs among a number of criteria including proximity to essential infrastructure, ideal locations for algae cultivation may be quite distant from regions of maximum growth potential (Venteris et al. 2014b).

Factors for Evaluating Land for Algal Production

Physical characteristics, such as topography and soil, limit the land available for open pond algae farming. Soils, and particularly their porosity and permeability characteristics, affect the construction costs and design of open systems by virtue of the need for pond lining or natural sealing. When approximately 66,000 candidate algae cultivation sites were analyzed for soil compaction suitability to avoid pond liners, more than half of the sites were eliminated due to high permeability coupled with insufficient clay content for adequate compaction (Venteris et al. 2014c). Topography would be a limiting factor for these systems because the installation of large shallow ponds requires relatively flat terrain. The bulk density of soil is proposed as a soil quality indicator for the environmental sustainability of algal biofuels, as changes in bulk density of subsoils below liners could influence the future productivity of the soil (Table 10.2).

Land prices and availability can also impact the cost of biofuel production at inland and coastal sites and should be considered in techno-economic analyses. Land that is highly desirable for development and other set-asides for publicly beneficial reasons may not be seen as suitable for algae production. There is very little conflict between algal biomass cultivation and terrestrial bioenergy feedstock production or other agricultural uses (e.g., livestock grazing) (Langholtz et al. 2016). For offshore sites, the right of access and use, and the associated logistics, risks, and costs of offshore marine operations will have a major impact on costs of production. Cost of site preparation and infrastructure facilities for offshore, coastal, and inland sites will all be location-dependent. It is reasonably straightforward to calculate the impact of the cost of land, and perhaps also for offshore sites, on the overall cost of total algal biomass and intermediate feedstock fraction (e.g., lipids, carbohydrates, proteins) production, but for each approach there will likely be an optimum range of sizes for a commercial production facility. If it is necessary to distribute the facility over a number of smaller parcels of land or offshore sites, it may not be possible to get the most benefit of economies of scale; to address this issue, the Pacific Northwest National Laboratory has begun conducting a sensitivity study for commercial production sites. The key tradeoffs will be between the cost of overall production (capital and operating costs) versus the matching of affordable production scale to the sustainable and affordable supply of the required input resources with the required output product processing and distribution infrastructure and markets.

Land ownership information provides valuable insights on which policies and stakeholders could affect project development. Publicly and privately owned lands are subject to different use, lease, and purchase requirements. For example, much of the land in the West is government owned, which means that environmental assessments and/or environmental impact statements could be required as part of the approval process. Indian reservations also comprise a significant portion of this land. There is a significant amount of marginal land ($6.9e^4$ km^2) owned by the federal government, but much of this land is located in areas of the CONUS that, if cultivated, would be subject to issues of sustainable water supply and infrastructure availability (Venteris et al. 2012).

Land Use and Environmental and Socioeconomic Sustainability

While the activity of a commercial algal biofuels industry offers socioeconomic benefits of energy security and non-renewable resource conservation, other environmental and socioeconomic factors can play a role in the commercialization of the industry (Efroymson and Dale 2015, Efroymson et al. 2016). In regards to land use, food security, a social wellbeing socioeconomic indicator, is pertinent. Even if marginal land is used, perceived conflict of food and feed production versus fuel could pose challenges for an algal biofuels operation. The influence of land conversion for an algal biofuel facility on the environmental sustainability indicator of biodiversity should also be considered (Efroymson and Dale 2015, Table 10.2). Finally, effective communication of the results of environmental and socioeconomic indicators, and discussion and incorporation of ideas from stakeholders (and demonstration of those incorporated ideas), is important to location selection and the overall socioeconomic sustainability of a biofuels operation (Efroymson et al. 2016, Table 10.3).

Nutrients

The supply, availability, and cost of other nutrients (i.e., nitrogen, phosphorus, potassium) required as inputs for algae growth also play a role in commercial viability and extent of industrial build-up (Venteris et al. 2014a; Pate 2013; Williams and Laurens 2010; Liu et al. 2013; Ho et al. 2003) (see chapter 4). Under large commercial scale industrial build-up, the amount of nutrients required nationally could begin to approach the same order of magnitude as large-scale agriculture (Pate et al. 2011; Pate 2013). It is estimated that 7 million metric tons of nitrogen and 1.03 metric tons of phosphorus will be needed to support algal biofuel production ranging from 4.5 BGY to 12 BGY (Pate 2013). Increases in national nitrogen and phosphorus nutrient consumption are estimated to range from 1.4 to 4, and 1.3 to 2.9 times, respectively, for the production of 5 to 21 BGY of algal biofuel scenarios (Venteris et al. 2014a). Also, the nutrient requirement is determined by the specific pathway used; for example, hydrothermal liquefaction with combined catalytic hydrothermal gasification (CHG) resulted in a 34% improvement on nitrogen demand and a 52% decrease in phosphorus consumption compared to another method of lipid extraction (Venteris et al. 2014a).

Energy scale production without nutrient recycling was a "show stopper" due to the quantities of nitrogen and phosphorus required (Venteris et al. 2014a). The capture and reuse of nutrients from various agricultural and municipal waste streams (Woertz et al. 2009; Yang et al. 2011; Venteris et al. 2014a) can potentially help supply nutrients for algae production scale-up, but this will be dependent on the geographical location, availability, affordability, and accessibility of such nutrient sources (NRC 2012).

BETO has been addressing the issues of water quantity and nutrient supply concerns through the 2012 Advancements in Sustainable Algal Production FOA. Awards were made to the University of Toledo, Sandia National Laboratories, and California Polytechnic State University (Cal Poly). The University of Toledo partnership is investigating the utilization of dairy and municipal wastewaters for nutrient and water requirements, as well as nutrient recycling through anaerobic digestion of residual algae, to support algal production systems for biofuels. Sandia National Laboratories is developing a system to recapture phosphorus and nitrogen nutrients from residual algal biomass after oil extraction. Cal Poly is in the process of developing and demonstrating at least 75% water and nutrient recycle efficiency (with no adverse effects to the algal culture) while utilizing municipal wastewater.

Carbon Dioxide

The Carbon Capture Opportunity in Algae Production

Efficient algae production requires enriched sources of CO_2 because the rate of supply from the atmosphere is limited by diffusion rates. CO_2 availability and cost of delivery plays a major role in autotrophic microalgae cultivation scalability and operating expense. The use of CO_2 waste gas could be a potential source for free and low-cost CO_2 for algal cultivation, especially with select states implementing policy restrictions and/or a price on carbon. There is serious consideration that algae can convert emitted CO_2 into organic molecules that can be used as feed, building blocks for the biotechnology industry, and energy. Of the renewable energy sources being developed, bioenergy is uniquely positioned to produce energy and tangible products from waste sources

Flue gas, from coal-fired and natural gas power plants, and other CO_2 emitters, such as ethanol plants, could be attractive and widely-distributed sources of CO_2. Algae production could provide excellent opportunities for the utilization of fossil carbon emissions. Algae production does not directly sequester fossil carbon, but rather provides carbon capture and reuse in the form of fuels and other products derived from the algal biomass. Any greenhouse gas abatement credits would come from the substitution of renewable fuels and other co-products that displace or reduce fossil fuel consumption. Applications separating CO_2 in large industrial plants, including coal and natural gas combustion plants and ammonia production facilities, are already in operation today and under consideration for possible broader use for carbon capture and utilization as a climate change abatement strategy (Rubin 2005; Campbell et al. 2008). However, CO_2 co-location is only economically efficient at distances where the CO_2 costs less than purchased CO_2.

Likely Stationary CO_2 Emission Sources

Stationary CO_2 emission sources represent more than half of the more than 6.673 billion metric tons of CO_2 emitted annually in the United States (EPA 2015). Electric power generation alone (primarily from coal and natural gas-fired plants) represents more than 30% of the total, or more than 2.07 billion metric tons per year (EPA 2013).

Several major studies have been completed to study the availability of carbon dioxide from stationary sources for algal cultivation (Pate et al. 2011; Quinn et al 2012; Venteris 2014a). An estimated 10 billion gallons of fuel can be produced (given lipid yields at 19 $m^3\ ha^{-1}\ yr^{-1}$) with sufficient CO_2 from 19 lower-tier states (Arizona, Alaska, Alabama, California, Colorado, Florida, Georgia, Iowa, Kansas, Louisiana, Missouri, Mississippi, Nevada, Oklahoma, South Carolina, Texas, and Utah) from stationary sources (Pate et al. 2011). This amount of fuel production could account for approximately 20% of the U.S. waste carbon emitted per year (Venteris et al. 2014a). Waste carbon capture and utilization could be constrained by the infrastructure needed to transport the resource to large-scale algal facilities (Quinn et al. 2012).

The purity of CO_2 in emissions is a limiting factor for the distance of pumping flue gas to algae cultivation systems, because lower percentages of CO_2 in the emissions streams

means distribution pipelines are transporting larger volumes of gases the algae may not use (e.g. N_2) (Pate et al. 2011; Quinn et al. 2012; Lundquist et al. 2010; Venteris et al. 2014a). Ethanol production plants, compared to coal-fired power plants, have lower volumes of emissions, but higher purity of CO_2 in the emissions stream (97%) (Venteris et al. 2014a). However, a limitation of pure sources of waste carbon (such as from ethanol, ammonia, or hydrogen production plants) have comparable cost to purchasing medical-grade carbon dioxide (Middleton et al., 2014), or about $\$40T^{-1}$ (Venteris et al. 2014b). Natural gas-fired power plants are becoming a larger fraction of baseload generation and can also be considered as a source of CO_2 for algae production, although the concentration in the output emissions (4%–6%) is so dilute that the algae facility needs to be practically co-located to avoid excessive gas transport costs.

In general, baseload generators also emit CO_2 during periods of darkness when it cannot be utilized by the algae through photosynthesis. During those times, the CO_2 would be emitted to the atmosphere if not captured and stored by other means. Several companies have developed methods to store the CO_2 during nighttime hours so that it can be used by algae during daylight hours (see the following section).

A refinement of the inventory of stationary industrial CO_2 sources in the more promising regions of the country is needed for making refined assessments for algae production siting and CO_2 sourcing. The inventory would include characterization of the CO_2 emissions stream (e.g., rates and quantities of CO_2 produced, content, and description of substances toxic to algal growth and end-products) and the local availability and distance to suitable land for algae production.

Barriers to Viable Algae CO_2 Capture and Utilization Technology

The degree to which stationary CO_2 emissions can be captured and used affordably for algae production will be limited by the operational logistics and efficiencies (e.g., transport costs), composition of the flue gas, and the availability of land and water for algae cultivation scale-up within reasonable geographic proximity of stationary sources. The amount of land and water needed around a CO_2 point source depends on the productivity per unit area of the proposed algae system, and as well as other factors such as effective CO_2 uptake, closed vs. open bioreactors, pH tolerance of given organisms, incident light conditions, and the geochemistry of the water used in the bioreactor. Therefore, the requirements for CO_2 supply to enhance algae production, and the matching of CO_2 source availability with algal cultivation facilities, is not a simple issue.

The quality and location of the CO_2 source will also play a role for algal growth, and some sources are likely to require more cleanup than others (especially if there are plans for animal feed as a co-product and/or if the CO_2 source stream includes contaminants that inhibit algal growth). It will be necessary to provide a CO_2 source that is suitably free of materials potentially toxic to algae or toxic to co-products. Algae can be effective at capturing and concentrating heavy metal contaminants (Aksu 1998; Mehta and Gaur 2005; NAABB 2014), such as are present in some forms of flue gas. This could impact the suitability of residual biomass for co-products like animal feed, pharmaceuticals, and nutraceuticals, and is a consideration that requires further investigation (Huntley et al. 2015).

When algae facilities increase productivity or scale-up, one outcome scenario is the insufficient delivery of CO_2 to support cultivation from stationary industrial point sources due to prohibitive economics of CO_2 delivery (Venteris et al. 2014a). This co-location limitation could constrain the extent to which algal biofuels production can be affordably scaled up within any given region unless other factors drive the investment in carbon capture and delivery, or in expanding the nation's CO_2 pipeline infrastructure. Improvements in economically concentrating CO_2, developing technologies that decouple carbon capture and utilization (thereby obviating co-location restrictions), or advancements in direct air capture of CO_2 could all assist in enabling algae technology as a strategy to displace fossil oil products and capture fossil emissions.

A challenge for algae carbon capture and utilization is capturing a greater percentage of total CO_2 emissions from a given source. It is estimated that for algae production to fully utilize the CO_2 in the flue gas emitted from a 50-MWe semi-base load, a natural-gas-fired power plant would require about 2,200 acres of algae cultivation area, and that the CO_2 generated by the power plant can only be effectively used by the algae during the photosynthetically active sunlight hours (Brune et al. 2009). As a result, the greenhouse gas emissions offset will be limited to an estimated 20% to 30% of the total power plant emissions due to CO_2 off-gassing during non-sunlight hours and the unavoidable losses of CO_2 during algae production. While these results should be revisited based on more recent cultivation estimates (Davis et al. 2016), recent work conducted by Global Algae Innovations (GAI) suggests that greater utilization of total emissions is possible through conducting primary CO_2 capture with an absorber. GAI operates an 8-acre farm relying on CO_2 from flue gas from an adjacent power plant, and reports capturing of CO_2 for 24 hours a day, and storing excess CO_2 for use when power plant activity is down and for higher use on sunnier days. The system can capture 80% from low-concentration sources (flue gas with 4%–4.5% CO_2 concentration) and 90% from higher-concentration sources (coal); assuming the system has a utilization efficiency of 90%, the overall capture and use of the CO_2 is 70% to 80%. More recently, GAI has been able to operate their farm without the need for gas distribution and control. Despite the promising accomplishments shared by GAI, more work is needed on

storage options in media located at the site, so that CO_2 is not wasted during nighttime hours.

An additional challenge for algae based carbon capture and utilization is connecting a largely established industry (i.e., CO_2 point sources) with the developing industry of large-scale algae cultivation. From a utilities perspective, the value of collaboration will be to reduce emissions by renewable energy for the reduction of CO_2 as opposed to producing biofuels by utilization of CO_2 in an algal biofuels facility. Thus, mechanisms to encourage partnering between utilities and algae/fuel companies will be required, and new business models will be needed to commercialize this approach. Maintaining cultivation facilities during utility outages and through seasonal changes in algal growth rates, is a question that needs integration to answer. Gas-fired power plants that operate as peaking plants are not particularly suitable for use with algae facilities because of the intermittent and unplanned nature of the output emissions stream. Although often referred to as a "free" resource, the capture and delivery of concentrated CO_2 from stationary industrial sources as a supplement to enhance and optimize algae production will not be "free." Model estimates for capital costs for infrastructure and energy required to capture and deliver industrial CO_2 to ponds and grow/harvest algae should be refined with utility input.

While some applied R&D will benefit integration for many point sources of CO_2, some work will have greater impact on different sources. For example, improving technologies of CO_2 capture and concentration would likely expand co-location opportunities for natural gas greater than for coal-fired plants. While the opportunity exists to build new natural gas facilities with integrated elements for algae technology, in the United States, the mature, coal-fired power plant industry will largely need to be retrofitted to enable algae carbon capture and utilization. U.S. coal utilities could use retrofitting experience as a lower cost test run prior to designing new coal plants with algae carbon capture and utilization that are likely to be created in developing countries.

Economic incentivies or regulatory requirements could potentially support market investment in integration with algae cultivation facilities. Currently there is no nation-wide carbon credit or cap-and-trade framework that incentivizes this investment, although there are individual state incentives (e.g., California's Regional Greenhouse Gas Initiative). In August 2015, EPA published the final ruling for the Clean Power Plan, in an effort to reduce carbon pollution from power plants. The plan—stayed by the Supreme Court—in February 2016, put forward new guidelines that states must develop and implement plans for reducing carbon emissions from fossil fuel and natural gas-fired power plants. Carbon capture and utilization is broadly defined in the plan and includes algae utilization, providing the potential for an EPA-approved pathway using algae production as a method of carbon capture. However, it is still uncertain whether CO_2 emitters will take advantage of this potential pathway as a means to meet their emissions reduction targets.

DOE-Specific Activities

In 2014, BETO released the Targeted Algal Biofuels and Bioproducts (TABB) FOA, with a specific topic area on carbon dioxide utilization. Within this topic area, single investigator or small teams will pursue technology improvements that will result in increased biomass productivity leading to higher overall feedstock yields. Areas of interest include

- Enhancing transfer efficiency that could enhance productivity through ensuring adequate intracellular carbon stores, as well as lowering operating costs through more efficient utilization of carbon sources
- Target improvements may be measured through enhanced photosynthetic efficiency, increased carbon efficiency, and improved rates of transfer, either into carbon reservoirs, or uptake by algae from the reservoirs
- Improvements that result in improved productivity that could lead to higher feedstock yields.

Arizona State University has been selected under the TABB FOA to conduct research on atmospheric carbon dioxide capture, enrichment, and delivery to increase biomass production. GAI has also been selected to study increases in biomass yield by deploying an innovative system to absorb carbon dioxide from the flue gas of a power plant in El Cajon, California.

Macroalgae

One of the major benefits of macroalgae cultivation is the few resource requirements needed for cultivation; unlike microalgae, macroalgae generally only need open ocean space, nutrients, and infrastructure and energy for facility operations. Also, macroalgae cultivation can provide multiple ecosystem services, such as removal of CO_2 in the marine environment and utilizing excess nutrients in eutrophic areas. Most macroalgae in the United States is cultivated for food, food product additives, and pharmaceuticals. Based on the scale of macroalgae cultivation practices currently being used to meet non-fuel product demand (annual production of about 1.4 million dry metric tonnes for food products), the level of production would need to be greatly expanded for biofuels.

Research has been conducted on the feasibility of macroalgae for biofuels production, particularly focusing on methane (Ghosh et al. 1981; Debusk et al. 1986; Chynoweth et al. 2001; Langlois et al. 2012), and more recently on ethanol (Wargacki et al. 2012; Alvarado-Morales et al. 2013). However, limited data is available on resource and siting requirements for large-scale macroalgae cultivation for biofuels production. Previous studies have been conducted assessing the constraints (i.e., conflicting uses) from aquaculture in marine waters (Macleod 2007; Longdill et al. 2008; Broekhuizen et al. 2002; Perez et

al. 2003; Dolmer and Geitner 2004; Aguilar-Manjarrez and Ross 1995; Radiarta et al. 2008) as well as renewable energy (Cradden et al. 2016; Pérez-Collazo et al. 2015; DOE 2012; Janke 2010; Nobre et al. 2009; Dhanju et al. 2008).

Options for siting macroalgae (also known as seaweed or kelp) biomass production include offshore farms, near-shore coastal farms, and land-based ponds. However, the feasibility of land-based ponds for large-scale production is unlikely, unless the biomass is a part of a system that cultivates high-value organisms (e.g., abalone) or specialty markets (Roesijadi et al. 2010). While most macroalgae cultivation currently occurs in nearshore areas, limited available cultivation areas, competing uses, and user conflicts have spurred interest in offshore areas (Roseijadi et al. 2010, 2011).

Infrastructure is also a key requirement of large-scale macroalgal cultivation, with more research and development needed for technologies for harvesting, transporting, and processing the algae. Integration and collaboration with the offshore aquaculture industry will assist in establishing optimal infrastructure for larger economies of scale for macroalgal cultivation. Co-siting of macroalgal farms with other structures such as windfarms (Buck et al. 2004) and multitrophic aquaculture systems (Roseijadi et al. 2011) have also been proposed as a way of leveraging other technologies to facilitate the cultivation of macroalgae. The merits of each siting location should be carefully evaluated, taking into consideration factors such as the scale of farms required to meet production needs, cost and availability of space and nutrients, environmental impacts, and competition with other uses.

A major challenge lies in finding and developing new environments and cultivation approaches that will support production of macroalgae at the larger scales and lower costs needed to supply the biofuels market. Additional research, technology development, and favorable regulatory framework for coastal and offshore environment use could help enable cultivation at scales to meet production goals.

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11. Systems and Techno-Economic Analyses

Successful development of an algal biofuels industry requires the optimum combination of technical innovations in systems and processes, coupled with economic feasibility in the practical implementation and integrated scale-up for commercial production and marketing. Enabling successful advancement and commercialization in the algal biofuels field also requires the confidence and engagement of key public and private stakeholders so they can make necessary investments over time to reduce technical risks and overcome challenges to developing an algal biofuels industry. Toward this end, objective and quantitative modeling and analyses of systems and processes are needed that span different enterprise levels across the overall algae-to-biofuels supply chain. Such analyses can provide improved understanding and insight to help guide successful industry development within the real-world context of technical, environmental, political, infrastructural, market, and resource conditions and constraints.

This chapter provides a brief review of resource assessment (RA), TEA, and (LCA) work completed on algal biofuels and products. In addition, a systems analysis section will describe the work completed in design case pathways towards algal biomass production and downstream conversion. Finally, a brief discussion will provide a synopsis of the current and future challenges facing analyses.

11.1 Resource Assessment: Engineering Analysis, GIS-Based Resource Modeling, and Biomass Growth Modeling

RA can be utilized to model the potential constraints of scaling-up algal biofuels production, including sufficient solar resources, land, water, nutrient, CO_2 requirements, and transport infrastructure to access downstream processing systems. Current resource assessment activities utilized to understand the necessary resources for sustainable algal production systems include the identification of potential geographic locations for algae farms based on resource access and availability; cost estimates for current and future resources, and; the environmental sustainability of these resources (DOE 2016). The following section briefly reviews RA tools, such as engineering analyses, GIS-based resource modeling, and biomass growth modeling.

Engineering Analyses

Engineering analyses (technical and economic) at the unit operations level require the systematic calculation and tracking of mass and energy balances that include evaluation of the thermodynamic, hydrodynamic, biological, and chemical interactions of the systems and processes used. This may also require coupling with appropriate external data and driving functions such as time-dependent solar insolation, temperature, and other local environmental conditions. Mass, energy, and elemental balances must assess the conservation of mass, energy, and elements needed from end-to-end in the production pathway.

For a mass and energy balance of an algal biofuels production system, inputs to each system component are presented, and a numbered node represents a mass or energy balance calculation around a given unit operation. The inputs that are generally required for algae production and processing include carbon, nitrogen, phosphorus, processing chemicals, electricity, and heat. The value of this assessment is that it can help assess the overall viability of a given algal biomass production system and show what steps in the process are most energy and material intensive, thus highlighting areas for research and development. The development of mass- and energy-based systems models can help evaluate different proposed processes for overall viability and examine the sensitivity of different assumptions in individual processes to the overall system, as completed in a techno-economic analysis.

GIS-Based Modeling

GIS visualization and analysis tools are indispensable for algae production and supply chain systems analysis due to their ability to perform mapping and resource analysis spanning local, regional, and national scales. Critical climatic and natural resource data can be readily accessed, such as the following:

- Land and water resources (characteristics, availability, etc.)
- Climatic characteristics (temperature, precipitation, solar insolation, etc.)
- Water evaporation loss (function of climate, etc.)
- CO_2 resources (point source emitters, pipelines)
- Fuel processing, transport, storage infrastructure
- Other infrastructure and environmental features.

The impact that availability and cost of these resources can have on algal biofuel production scale-up was discussed in chapter 10. The cost and benefit tradeoffs of CO_2 capture through biofixation using photoautotrophic microalgae cultivation enhanced by CO_2 from industrial sources will affect the economics of algal biofuel production.

In 2011, a national-scale resource and production assessment tool screened the United States for suitable areas for the production of algal biomass in freshwater open pond systems (Wigmosta et al. 2011). BAT takes into consideration upstream resource requirements, as well as the geographical suitability of areas around the CONUS for optimal algal growth. Original data parameters of the model included elevation, hydrography, land cover and classifications, existing transport infrastructure, protected areas, and average climatic conditions. Results of the study identified 430,803 km^2 of suitable land (approximately

5.5% land area of the CONUS) that could produce approximately 58 billion gallons of biofuel, when assuming there are no constraints on the availability of resources. The analysis concluded that there is a sufficient amount of suitable land and water available for large-scale production, calling out the Gulf Coast region as the most optimal location in regards to the availability of resources (Wigmosta et al. 2011). However, the authors emphasized that the availability of sustainable sources of water would be a future limiting factor to the growth of algal biomass production facilities in the United States (discussed in chapter 10).

Since the development of the BAT model, a number of GIS-based studies have been conducted to examine the resource availability and requirements of large-scale algal biofuel production in the United States. Specific analyses have been conducted on land use (Pate et al. 2011; ANL, NREL, PNNL 2012; Quinn et al. 2012a; Venteris et al. 2012; Moody et al. 2014; Venteris et al. 2014b; Coleman et al. 2014; Langholtz et al. 2016), water source and requirements (Pate et al. 2011; ANL, NREL, PNNL 2012; Fortier and Sturm 2012; Batan et al. 2013; Venteris et al. 2013; Venteris et al. 2014b,c), CO_2 requirements (Pate et al. 2011; Quinn et al. 2012b; Venteris et al. 2014b), nutrient requirements (Pate et al. 2011; Quinn et al. 2014a), infrastructure (Venteris et al. 2014c), and downstream conversion processing (Venteris et al. 2014a,b).

The U.S. Billion-Ton Update indicates there is sufficient land available to meet the DOE goal of producing 5 BGY of algal biofuels by 2030, and this is validated by the harmonization survey published by Quinn and Davis (2015). Non-arable land in the southwest and southeast regions are the most suitable areas for algal biomass production, with a specific interest in the coastal areas of the Gulf of Mexico (ANL, NREL, PNNL 2012; Venteris et al. 2014b; Coleman et al. 2014; Langholtz et al. 2016), southern Atlantic coast, and south central Arizona regions (Quinn et al. 2012a; Venteris et al. 2014b), due to abundant and inexpensive freshwater supplies in the Gulf states (Venteris et al. 2013) and incorporation of less-efficient sites and more realistic salinities (Venteris et al. 2014a). The viability of seawater utilization (by facilities within 20 km of the coast) and saline groundwater, as potential alternatives to freshwater, have been modeled for cultivation (NAABB 2014; Huntley et al. 2015).

Growing algal biomass on land that is classified as pasture has little potential for conflict with terrestrial dedicated bioenergy feedstocks, and it also has alternative uses for agricultural production (Langholtz et al. 2016); however, the issue of land use conversion in the Southeast continues to be problematic, potentially making the inexpensive, non-arable lands in the Southwest more attractive (Venteris et al. 2014b). Nutrient requirements will depend on the type of production pathway used and the ability to reuse or recycle nutrients (Venteris et al. 2014a; Quinn and Davis 2015). CO_2 emission sources are generally shown to be denser in the eastern United States, which reflects the current infrastructure network (Venteris et al., 2014b,c). Finally, downstream conversion processes are shown to have different resource footprints, with the hydrothermal liquefaction pathway generally having smaller land, water, CO_2, and nutrient requirements than traditional lipid extraction (Venteris et al. 2014a). A more detailed discussion on the implications of the resource availability and requirements is available in chapter 10.

Overall, uncertainty still exists in resource modeling due to varying assumptions used for productivity levels and the scope of the assessment (Quinn and Davis 2015). While research has begun to compare results of the productivities of different algal strains, more data is needed from test-bed and commercial-scale facilities to reduce this uncertainty (Venteris et al. 2014b,c).

Growth Modeling

Productivity continues to be one of the major barriers to economical, large-scale algal biomass production. As such, focusing on increasing the productivity, in conjunction with combined lower energy and developing inexpensive technologies, will drive costs and emissions down and advance the industry towards meeting the goal of 5 BGY of algal biofuels by 2030 (ANL, NREL, PNNL 2012). As with terrestrial feedstocks, algal biomass productivity will be constrained by the available energy density in sunlight and the relatively low efficiencies of photosynthetic processes coupled with other systems losses. It is still a major challenge to replicate productivity numbers for lab-grown strains in outdoor ponds (Huesemann et al. 2016). Focusing on closing the lab-to-field yield gap is crucial to the development of large-scale production (White and Ryan 2015).

Biomass growth models project productivity by estimating light attenuation within a culture and by predicting the rate of growth as a function of incident or absorbed light (for a more detailed overview of algal biomass productivity, see chapter 4). As previously discussed in chapter 4, autotrophic algal biomass cultivation generally occurs in closed photobioreactors or open ponds/raceways. For outdoor pond cultivation, more than 40 models have been developed to predict algal productivity (for a review of many of these models, see Béchet et al. 2013). Many models that were used previously to measure productivity were not validated or were simply too complex for use (e.g., too many variables) (Huesemann et al. 2013). A biomass growth model was developed to predict outdoor performance of a given strain by measuring a limited number of strain-specific model input parameters within the lab, while using sunlight intensity and pond temperature data from the BAT (Huesemann et al. 2013). The biomass growth model was further developed to predict productivity in outdoor ponds under conditions of diurnally fluctuating light intensities and water temperatures and replete nutrient levels for three different strains (Huesemann et al. 2016).

Despite the validation work completed on open pond cultivation, there are a number of uncertainties that should be addressed in future work, including the effect of vertical mixing induced light/dark cycles on recovery from photo-inhibition and biomass loss in the dark zone during daylight hours, maximum specific growth rate measurements with diurnal light/dark cycles, the loss of biomass during overnight dark periods in addition to the shorter periods of darkness (i.e., circulation within the aphotic zone), the effect of light acclimation on predictions, and inhibition by photosynthetic oxygen in other algal strains (Huesemann et al. 2016).

The possibilities of highly productive algal strains have been major drivers of research into using algae as a biofuels feedstock. The upper theoretical production limit for microalgae oil has been estimated to be between the order of 240 $m^3ha^{-1}yr^{-1}$ (approximately 26,000 gal/acre/year) to 350 $m^3ha^{-1}yr^{-1}$ (approximately 38,000 gal/acre/year) of raw algal oil (Zemke et al. 2010; Weyer et al. 2010). This range is based on expected losses, photosynthetic efficiency, and other assumptions made in the analysis (including the availability of high solar insolation consistent with lower latitudes and/or high percentage of clear weather conditions, 50% oil content). A best-case limit of 40 $m^3ha^{-1}yr^{-1}$ (4,000 gal/acre/year) has been presented (Weyer et al. 2010). Taking into account significant variation of assumed solar radiation, harmonization of these studies resulted in a maximum theoretical yield of 350 $m^3ha^{-1}yr^{-1}$ (37,000 gal/acre/year) for an ideally situated location at the equator (Quinn and Davis 2015). However, an important limitation of these results is their sole focus on algal lipid content; with the introduction of the HTL and the combined algal processing conversion pathways, discussed later in this chapter and in chapter 7, productivity modeling is beginning to consider the contribution of carbohydrates, proteins, and other metabolites to raise fuel yields or produce co-products.

The potential for high algal biomass productivity for biofuels at commercial scale remains hypothetical. Current large-scale commercial production of high-value algae products in open ponds could serve as a baseline reference for fuels, but they reflect lower biomass productivities currently in the range of 10–20 $g/m^2/day$ (Ramachandra et al. 2013; Moheimani 2013; Handler et al. 2014; Guieysse et al. 2013; Passell et al. 2013; Collet et al. 2014; Adesanya et al. 2014). This is significantly lower than future target projections for biomass feedstock of 30–60 $g/m^2/day$ (Amer et al. 2011; Brentner et al. 2011; Quinn et al. 2012b; Nagarajan et al. 2013; Mata et al. 2010).

Davis et al. (2016) presents a literature review of future estimates of production yield ranging between 15–60 $g/m^2/day$ annual average, as shown in Figure 11.1, based on current baseline estimates ranging from 7-35 $g/m^2/day$ annual average. Variations are based on wide ranging assumptions in cultivation productivity and system design nuances.

In outdoor photobioreactor cultivation, data collected over 2-1/2 years on the productivity of *Nannochloropsis oculata* and *Nannocloropsis salina* cultivated in multiple photobioreactors demonstrated annualized volumetric growth rates of 0.16 $g\ L^{-1}\ d^{-1}$ (peak at 0.37 $g\ L^{-1}\ d^{-1}$) for *N. oculata* and 0.15 $g\ L^{-1}\ d^{-1}$ (peak at 0.37 $g\ L^{-1}\ d^{-1}$) for *N. salina*, and an overall average lipid production of 10.7 $m^3\ ha^{-1}\ yr^{-1}$ (peak at 36.3 $m^3\ ha^{-1}\ yr^{-1}$) (Quinn et al. 2012b). Previous work on photobioreactor cultivation on *Monodus subterraneus* demonstrated a productivity of 0.03–0.20 $g\ L^{-1}d^{-1}$ but was only cultivated from a single photobioreactor for a study duration of 3 months (from Bosma et al. 2007).

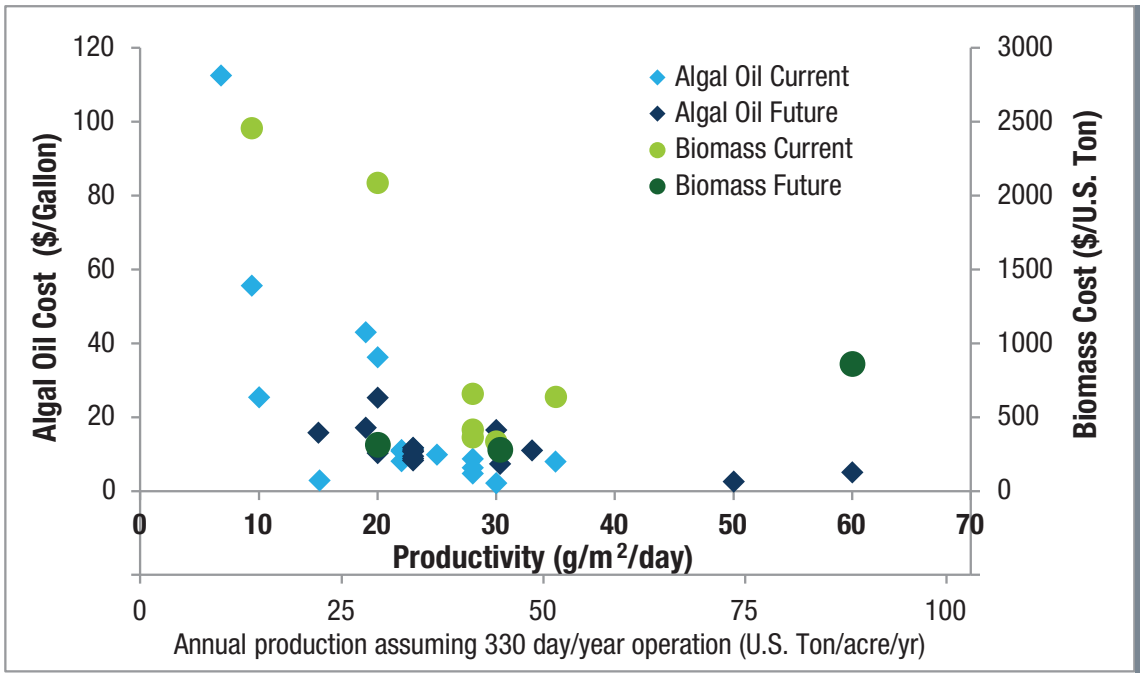

Figure 11.1. Literature scan demonstrating variability in cultivation yields and estimated cost of algal biomass and bio-oil, differentiating between "current performance" versus "future projections." Figure is limited to open pond studies alone, excluding PBRs. (Source: Davis et al. 2016).

Next Steps in Research

The following are recommendations for next steps in resource assessment development:

- Increase focus on CO_2 availability, delivery costs, and co-location opportunities
- Conduct further analysis of alternative water sources beyond freshwater (saline, brackish, etc.) and the possible ramifications on brine wastewater management and the local ecosystem
- Focus on site screening for suitable locations that do not require full plastic pond liners (high-clay soils, etc.)
- Consider different farm sizes or collections of smaller nearby farms
- Consider the environmental and economic impacts of land-use conversion
- Further analyze the potential of nutrient recycling
- Expand in focus to minimize seasonal variability (e.g., through strain rotation, varying water depths, and other temperature control options, etc.)
- Further analyze on the effect of short light/dark cycles on biomass growth
- Examine inhibition by photosynthetic oxygen in algal strains during growth.
- Further validate large-scale growth modeling.

11.2 Life-Cycle Analysis

LCA is a "cradle-to-grave" analysis approach for assessing the resource use and environmental impacts and tradeoffs of industrial systems and processes. LCA is important for assessing relative GHG emissions and other resource utilization (e.g., water, energy) impacts among different approaches to algal biofuels production, and in comparison with fuels based on other renewable and non-renewable feedstocks.

The term "cradle-to-grave" refers to accounting for all activities related to the production and use of a product, including activities like recovering natural resources (ores, petroleum, natural gas, coal), converting them to required chemicals and energy utilities, direct energy use during manufacturing the products under study, transportation of materials to the manufacturing plants, transportation of goods to consumers, and use of the goods. This analysis can also consider the effects associated with construction of the manufacturing infrastructure. The scope of activities included is called the *system boundary*. To compare the LCA results of one product with another, the system boundaries must be compatible (i.e., must be encompassing enough to include all differences that result when one product is chosen over another).

The term "life cycle" refers to the major activities in the course of the product's lifespan, from manufacture, use, and maintenance, to final disposal, including the raw material acquisition required to manufacture the product (EPA 2009). The process employs a phased approach that consists of four major components: goal definition and scoping, life-cycle inventory analysis, life-cycle impact assessment, and interpretation (ISO 1997). LCA methodologies, modeling, database resources, and tools have been developed that include Argonne National Laboratory's GREET "well-to-wheels" model (ANL 2009, 2015), the University of California, Davis' Lifecycle Emission Model (Delucci 2004), and numerous others (EPA 2009). In addition to net GHG emissions, LCA for biofuels can also assess impacts and tradeoffs associated with utilization intensity for water, energy, nutrients, and other resources.

LCAs are a particularly important aspect of presenting the environmental sustainability of algal biofuels (NRC 2012). Algal biofuels potentially have lower GHG emissions rates versus conventional petroleum fuel production. However, the level of sustainability of algal biofuels production is still uncertain due to relatively little data available on outdoor, sustained algae cultivation at a meaningful scale (a challenge common to TEA modeling as well, discussed below).

LCAs that present a net energy return (energy in/out ratio) less than one are desirable, as more fuel energy is produced than energy consumed. Net energy ratios presented in the literature generally range from 0.7 (Luo et al. 2011 to 7.8 (Brentner et al. 2011). Thermochemical pathways, such as HTL, have shown to have a high net energy ratio due to yield sensitivities, with the drying of biomass for pyrolysis also affecting the net energy ratio (Bennion et al. 2015). Lipid extraction pathways are seen as having a more favorable net energy ratio when anaerobic digestion is incorporated (Quinn et al. 2014a,b).

LCA results for GHG emissions from algal biofuels production are presented in three stages: (1) well to pump, encompassing all activities from nutrient manufacturing and recovering of algal feedstocks, to transport of fuel to a station; (2) pump to wheels, which is considering the combustion of the fuel within a vehicle; and (3) well to wheels, which is the sum of well to pump and pump to wheels (ANL, PNNL, NREL 2012). The overall range in GHG emissions in each pathway is determined by the varying cultivation methods and end products (Bennion et al. 2015; Quinn and Davis 2015). (Unless otherwise noted in the literature, the combustion emission for petroleum used in this section is 73 g-$CO_{2\text{-eq}}MJ^{-1}$.)

There have been several conversion pathways examined through LCA to quantify GHG emissions, although lipid extraction and HTL have been the main focus for algae conversion. The lipid extraction-modeled pathways include wet solvent extraction (Frank et al. 2011; Quinn et al. 2014a; Collet et al. 2014; Woertz et al. 2014; Sills et al. 2013; Soh et al. 2014; Passel et al. 2013; ANL, PNNL, NREL 2012), dry solvent extraction (Azadi et al. 2014; Adesanya et al. 2014; Sills et al. 2013; Vasudevan et al. 2012), and secretion

(Vasudevan et al. 2012). The results from the literature show that the best-case scenario for well to wheels (assuming a combustion emission of 72 g-$CO_{2\text{-eq}}MJ^{-1}$) for lipid extraction is 31.3 g-$CO_{2\text{-eq}}MJ^{-1}$ (Quinn et al. 2014b). Anaerobic digestion of lipid-extracted algae shows a potential positive affect on GHG emissions due to the processing of resulting methane for heat and power, as well as enabling the recycle of nitrogen and phosphorus nutrients, resulting in reduced demand for fossil-derived energy at the production facility (Frank et al. 2012; Quinn et al. 2014b). However, it has also been shown that there is an overestimation of methane yields in the sub-process model validation, relative to anaerobic digestion yields typically discussed in the literature (Quinn and Davis 2015). It is important for pathways employing anaerobic digestion to fully consider the fate of nitrogen that is not recovered from the process residuals and to evaluate fugitive methane emissions from the anaerobic digestion process and from the methane combustion technology (common methane-fired internal combustion engines have higher fugitive emissions than do turbines) (Frank et al. 2012).

The current best-case GHG emissions scenario for well to wheels for HTL is 29 g-$CO_{2\text{-eq}}MJ^{-1}$, when biogas produced from waste streams is utilized for process energy (Frank et al. 2013). A study updating this theoretical scenario with process data, including upgrading data, increases emissions to 38 g-$CO_{2\text{-eq}}MJ^{-1}$ (Davis et al. 2014b). In comparison, pyrolysis pathway scenarios range between 283 g-$CO_{2\text{-eq}}MJ^{-1}$ (Bennion et al. 2015) and 363 g-$CO_{2\text{-eq}}MJ^{-1}$ (Grierson et al. 2013). Comparing the data with other reports of GHG emissions from HTL processing (106 g-$CO_{2\text{-eq}}MJ^{-1}$ by Liu et al. [2013] and 108.2 g-$CO_{2\text{-eq}}MJ^{-1}$ by Fortier et al. [2014]), as well as to the pyrolysis and subcritical water pathways (Ponnusamy et al. 2014), shows how sensitive the overall analysis is to different processing technology details and assumed performances (Bennion et al. 2015; Quinn and Davis, 2015).

Next Steps in Research

The following are recommendations for next steps in LCA of algal biofuels production:

- Consider LCA/GHG tradeoffs for on-site oil upgrading (hydrotreating) that allow for nitrogen recycle back to cultivation (primarily from HTL), instead of off-site upgrading, which may allow for lower-cost processing to combine products from several conversion facilities (or blending to a refinery) but which loses the ability to recycle nutrients and incurs additional transportation penalties
- Given pond mixing (power) typically being one of the largest drivers on LCA, consider alternative possibilities such as shutting pond circulation down at night, going to airlift circulation, adding pond-side photovoltaic power, etc. Paddlewheel efficiency varies considerably in the literature (e.g., 10% in Beal et al. [2015], and 40% in Weissman [1988]). Data are required to remove this uncertainty.
- Consider that productivity values are typically reported without associated mixing power, which leaves the concern that productivities have been overestimated through cultivation experiments with untenable mixing rates or overly dilute growth conditions.
- Conduct further analysis of emissions from production facilities construction, which have generally been excluded in analyses (e.g., further analysis of GHG emissions attributed to pond liners and pond concrete [Adesanya et al. 2014; Canter et al. 2014]).
- Consider large soil disturbance, including soil removal and grading, during pond construction (Davis 2016). Changes in soil organic carbon have not been evaluated adequately. Liners and soil compaction form a barrier to water penetration, including rainfall. The consequences of possible changes in water flows within the local ecosystem requires further consideration.
- Examine the role of co-products and/or examine different CO_2 sources as inputs and implications for carbon neutrality.
- Further examine regionalized LCAs (effects of specific grid mixes at play and different water consumption considerations).
- Increase focus on CO_2 sourcing logistics, in harmonization with RA and TEA.

11.3 Techno-Economic Analysis

TEA is designed to determine the economic feasibility of technology processes (see www.nrel.gov/analysis/tech_bio_analysis.html). TEAs have become the primary assessment tool in understanding cost benchmarks for algal biofuels state of technology, as well as the potential feasibility and required research and development areas for advancing the processes (Quinn and Davis 2015).

The scope of TEA includes the capability to apply and integrate detailed process engineering and thermodynamic modeling at the unit operations level with resultant economic estimates for system costs. Capital and operating cost estimates are established to analyze annual cash flows for a production facility and estimate economic viability metrics, such as minimum fuel/product selling price, rate of return, or net present value. TEAs employed by BETO include unit operation design flow and information models, processing design and modeling, capital costs and operating cost determination, discounted cash-flow analysis, and sensitivity analysis and risk assessment (DOE 2016). The overall goal is to capitalize on the flexibility and insight available through application of well-developed computer modeling and engineering analysis tools combined with disparate database information that exists

or can be developed. The characteristics of the biological system at the algal cellular level affect the performance of the engineered cultivation system and processes at a higher integrated level. The integrated systems must then in turn function within climate and weather conditions that vary with geographical location, so these approaches need to be both multi-level and multi-scale.

System and process simulation and optimization under a systems engineering framework can prove very beneficial for system design and operation. An algal biofuels and co-products supply chain is a complex interdependent system with numerous alternative pathways and functional elements and feedbacks at various spatial and temporal scales and resolutions. Sensitivity analyses on these alternatives and comparative tradeoff assessments across a range of approaches and conditions are among the critical modeling and analysis needs. GIS-based data integration into TEAs is also key to siting and resource assessment and the design, analysis, and optimization of facilities location and supply chain logistics (from algae cultivation through end-use fuels and products biorefining, transport, and distribution).

There have been numerous TEAs completed focusing on the feasibility of various pathways to algal biofuels. The completed TEAs present a variable opinion of biofuel cost from the literature, as shown in Figure 11.2 (Quinn and Davis 2015); the range of biofuel cost is between \$1.65 gal^{-1} (Benneman and Oswald 1996) and \$33.16 gal^{-1} (Richardson et al. 2012) (converted to 2014 dollars with an inflation rate of 2.4%). Disparities among the analyses conducted include cultivation (open ponds vs. photobioreactors) and processing pathways (pyrolysis, solvent extraction, and HTL), current vs. future technologies being modeled (including achievable productivities/yields), and boundaries of the analysis.

Efforts have been made to harmonize the different variables used in the analyses (see Sun et al. 2011; ANL, PNNL, NREL 2012), including growth method, conversion pathway, productivity projections, seasonal variability, and type of focus, which have shown that the large disparities in analysis outputs may be reduced when first agreeing on a common set of inputs. An analysis of twelve studies that examined cost estimates for the production of 1 gallon of algal oil (specifically, triglyceride), originally ranging from \$10.61 gal^{-1} to \$25.22 gal^{-1} (s.d. \$7.18/gal^{-1}, resulted in a harmonized mean value of \$11.57 gal^{-1} associated with a much narrower range of \$10.87–\$13.32 gal^{-1} (s.d. \$1.17 gal^{-1}) after harmonizing four independent TEA models to a common set of key assumptions attributed to a "near-term" biofuels cost scenario (Sun et al. 2011). This work was supplemented in 2012 by ANL, PNNL, and NREL under guidance from DOE, to jointly develop an integrated baseline incorporating harmonized data and inputs for RA, TEA, and LCA models, resulting in an annual average algal oil selling price of \$16.64 gal^{-1} and seasonal selling price

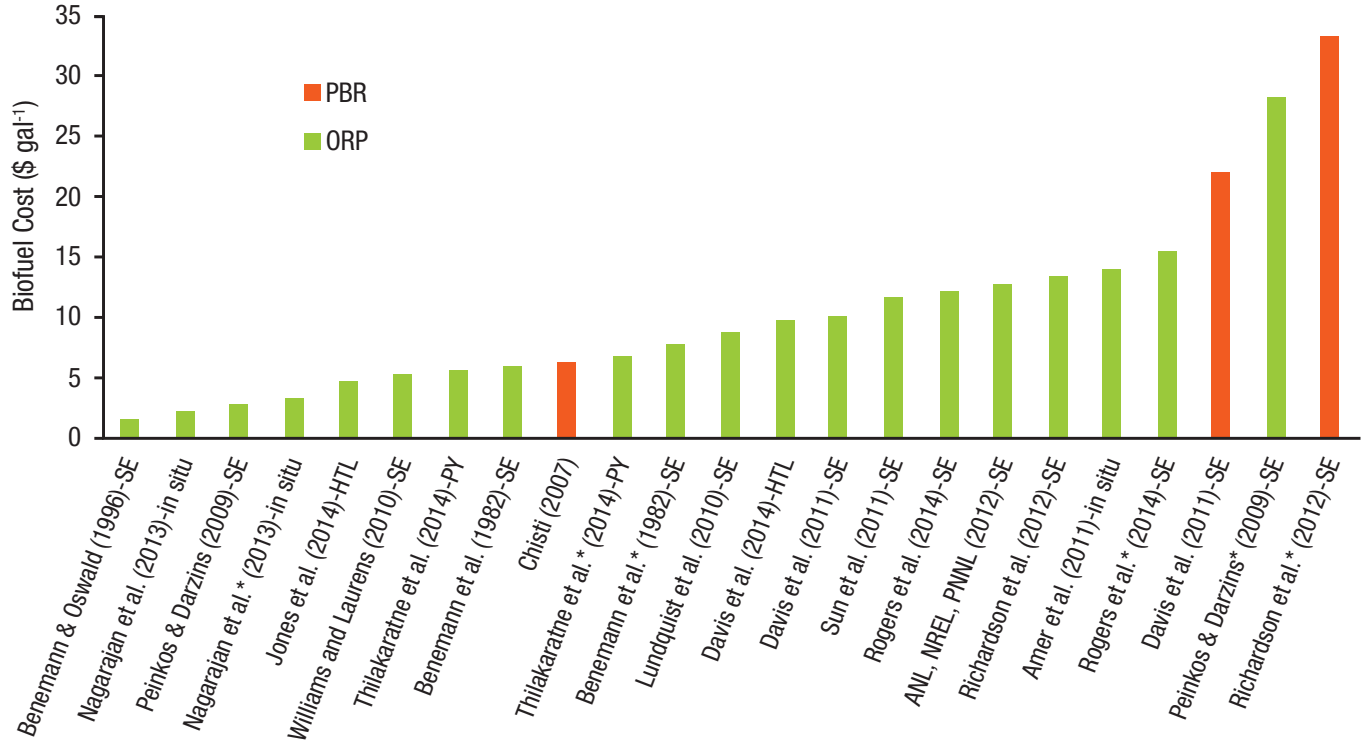

Figure 11.2. A comparison of TEA results from PBRs and open raceway ponds (Source: Quinn and Davis 2015)

of $17.49 gal^{-1}. Further details on this harmonization effort will be discussed later in this chapter.

The majority of TEAs from the literature focus on open raceway pond cultivation processes (Benemann and Oswald 1996; Nagarajan et al. 2013; Pienkos and Darzins 2009; Jones et al. 2014; Williams and Laurens 2010; Thilakaratne et al. 2014; Benemann et al. 1982; Lunquist et al. 2010; Davis et al. 2011, 2014a; Sun et al. 2011; Rogers et al. 2014; ANL, NREL, PNNL 2012; Richardson et al. 2012; Amer et al. 2011; Beal et al. 2015; Huntley et al. 2015). There have been fewer studies that looked at PBR cultivation (Chisti 2007; Davis et al. 2011; Richardson et al. 2012; Richardson et al. 2014). When the two cultivation pathways are compared, previous results show that the cost of production from PBR cultivation is roughly 2 to 2.5 times greater than open raceway pond cultivation ($8.52 gal^{-1}-$12.73 gal^{-1} open raceway ponds vs. $18.10 gal^{-1}-$32.57 gal^{-1} PBRs) (Davis et al. 2011; ANL, PNNL, NREL 2012; Richardson et al. 2012). Sensitivity analyses for both cultivation systems show that the greatest contributors to production cost are growth rate and composition (i.e., lipid content in the case of lipid extraction approaches), but specifically for PBRs, there is room for improvement of capital costs (Davis et al. 2011). Overall, there is a need to improve productivity while simultaneously decreasing capital and operating expenses for both PBR and open raceway pond systems.

A significant capital cost associated with open pond cultivation is pond liners. While liners provide advantages to algal biomass cultivation (namely reducing leakage, contamination, silt suspension during circulation, and the costs of potential regulatory permitting for these issues) they are a tremendous capital cost that may prove to be prohibitively expensive in the context of low-cost commodity fuels. Based on the harmonization model cited earlier (Davis et al. 2012), with an installed cost of $0.47/ft^2, the addition of full liners more than doubled the pond cost (and add $5.50/gal to the base case fuel selling price), translating to the single largest cost impact in the harmonized model. A similar penalty was presented in NREL's 2016 algal biomass design report, which showed the addition of full pond liners add nearly $130/ton to a base-case biomass selling price of $491/ton for minimally lined pond systems (Davis et al. 2016). However, it may be possible to avoid this high capital cost if ponds are located in areas with high soil clay content or if local regulation does not require liners (Davis et al. 2012). In the future, alternative solutions may be identified to mitigate pond leakage/percolation.

It is important to factor in seasonality when calculating the overall costs of the production system. The variability in seasonal productivity can be as much as 5- or 10:1 variation between summer peak production and winter minimum production (Quinn and Davis 2015). For example, since algae have a very high moisture content, summer production cost estimates will have to take into consideration either (a) significant conversion facility equipment over-design for use in a small fraction of the year, or (b) the energy and costs required to dry excess biomass during peak summer productivity, as well as other inputs, in order to make use of in winter months to reduce lower overall seasonal throughputs (Davis et al. 2012; Davis et al. 2014a,b; Jones et al. 2014). Future research will need to take into consideration construction and storage possibilities that address the complexities around seasonal algal biomass production, and also must work to better quantify compositional quality/degradation changes that may occur during storage.

Financial feasibility analyses build on TEAs and are a necessary tool for investors to evaluate the potential commercialization of algal biofuels. Like the TEAs, financial feasibility analyses use a systems modeling approach to analyze the multitude of algal production pathways. The goal is to provide financial information in addition to the cost of production. This financial information includes the firm's annual income statement, cash flow, and balance sheets, as well as annual debt servicing costs on capital expenses along with replacement and financing of machinery over time. Additionally, annual dividend payments to investors and annual federal income taxes, along with costs from cash flow deficit financing are calculated. Other outputs include rate of return on investment, probability of positive ending year cash reserves, annual net worth, and net present value, along with other financial variables important for determining the financial viability and sustainability of a business. Lastly, the probability of economic success for the business is determined and is used in defining the economic viability of the business. Examples of existing algae financial feasibility analyses include Richardson et al. (2012) and Richardson et al. (2014). To attract investors and make algal biofuels a commercial realization, these metrics will be required, in addition to the cost of production.

Next Steps in Research

The following are recommendations for next steps in developing TEAs for algal biofuel production:

- Improve understanding of algal oil upgrading costs from HTL, lipid extraction, and other pathways, including on-site versus central upgrading, potential for blending into refinery infrastructure, and better understanding fuel/blendstock properties from different oil feedstocks and upgrading steps.
- Investigate the technical, economic, and market potential for value-added co-products from algal biomass to reduce fuel costs at national commodity scales of production.
- Reduce uncertainties and improve understanding of PBR production economics.
- Develop metrics for financial feasibility, such as probability of positive cash flows, debt repayment capacity, positive net cash income, and increases net worth to attract investors.

- Understand and harmonize data on risk with modeled analyses.
- Continue harmonization of RA, LCA, and TEA.
- Further investigate regulatory issues around pond liner requirements specific to algae ponds for biofuel production.
- Increase focus on CO_2 sourcing logistics, in harmonization with RA and LCA.

11.4 Harmonization of Modeling Efforts

In 2012, ANL, NREL, and PNNL (Davis 2012) completed work to harmonize RAs, LCAs, and TEAs for the algal biofuels production supply chain. Upstream processing—including siting, cultivation, harvest, dewatering, and conversion to fuels via lipid extraction (as the best-understood conversion pathway at the time)—were applied to data from LCA and TEA analyses using a concurrent set of assumptions to establish an integrated baseline for algal biofuels production. The purpose of the harmonization initiative was to establish a framework to assess the impacts of new and improved technologies within the field (ANL, NREL, PNNL 2012), and to ensure that RA, LCA, and TEA model outputs were based on an internally consistent framework so that their results carried the same meaning.

The overall results of the harmonization modeling presented higher costs and emissions associated with the production of algal biofuels than what had been independently generated previously from RA, TEA, and LCA, respectively. Specifically, alignment of TEA, LCA, and RA models resulted in a lower annual average productivity baseline than previously assumed (13 g/m^2/day vs. 25 g/m^2/day) (ANL, NREL, PNNL 2012). The authors suggested that this new lower-productivity model that results in a fuel yield of 1,000 gal/acre/year represents a more realistic baseline, compared to prior studies on the order of 1,500–2,000 gal/acre/year for near-term potential (Davis 2012), which is validated with more recently published cultivation productivity data (White and Ryan 2015).

A similar harmonization modeling exercise was conducted of costs, emissions, and resource availability for the production of 5 BGY of renewable diesel in the United States by HTL (Davis et al. 2014b. Even though screening down to 5 BGY significantly reduced spatial and temporal variability relative to higher national-scale production thresholds, variations in site-to-site, season-to-season, and interannual productivity estimates still affected economic and environmental performance. Performance metrics based on annual average or peak productivity were again shown to be inadequate; temporally and spatially explicit computations allowed more rigorous analysis of these dynamic systems. For example, three-season operation with a winter shutdown was favored from an LCA standpoint to avoid high greenhouse gas emissions, but economic performance was harmed in the TEA model by underutilized equipment during the winter. Thus, analysis of algal biofuel pathways must combine spatiotemporal resource assessment, economic analysis, and environmental analysis integrated over many sites when assessing national scale performance.

These harmonized baselines are different than "state of technology" analyses due to the theoretical nature of the data utilized in the harmonization model. That is, the data uses assumptions that were derived from literature for small-scale experiments that came from other industries (e.g., wastewater processing), or that were modeled rather than experimentally validated. The third type—modeled data—was dictated, in general, by insufficient, publicly available data on algal biomass production and conversion to fuels up through the 2012 and 2014 studies referenced previously. This longstanding issue around data availability is beginning to improve given recent information made available over the past two years on both cultivation and conversion (data available from ATP^3; White and Ryan, 2015; Beal et al. 2015; Huntley et al. 2015; Jones et al. 2016; Dong et al. 2016; Laurens et al. 2015).

A review of TEA, LCA, and RA modeling up to 2015 reveals a large variation in the productivity, GHG emissions, biofuel costs, and yield (Quinn and Davis 2015). With this variation, there is on-going need to further collaborate to harmonize data from modelling efforts. These efforts should primarily involve making use of new published experimental data from industry, testbeds, and other organizations for sustained, real-world cultivation and subsequent conversion of biomass.

11.5 Systems Analysis

Systems analysis is foundational to designing a strategy for algal biofuels and co-products deployment. A system is an aggregation of subsystems interacting, such that the system is able to deliver an over-arching functionality. Figure 11.3 is a revised adaptation of a process flow diagram that was developed and presented at the 2008 Algae Roadmap Workshop to illustrate the representative number of multiple-path process options available for each step in the algal biofuel production chain, from algae growth to fuel and co-product processing and end-use. There are a large number of permutations of potential pathways to algal biofuel production, most of which are still being developed. Process steps vary depending on the product or co-product chosen.

Other chapters of this document point out the limited availability of detailed information about the characteristics of algae themselves and the characteristics (energy requirements and costs) of the systems and processes involved in the algal biofuel supply chain. Systems analysis can help manage the complexity of pathways to algal biofuels and co-products by quantifying uncertainties, identifying and appropriately modeling interdependencies and feedbacks, and comparing trade-offs from various scenarios with regard to cost, risk, technical performance, and environmental impacts.

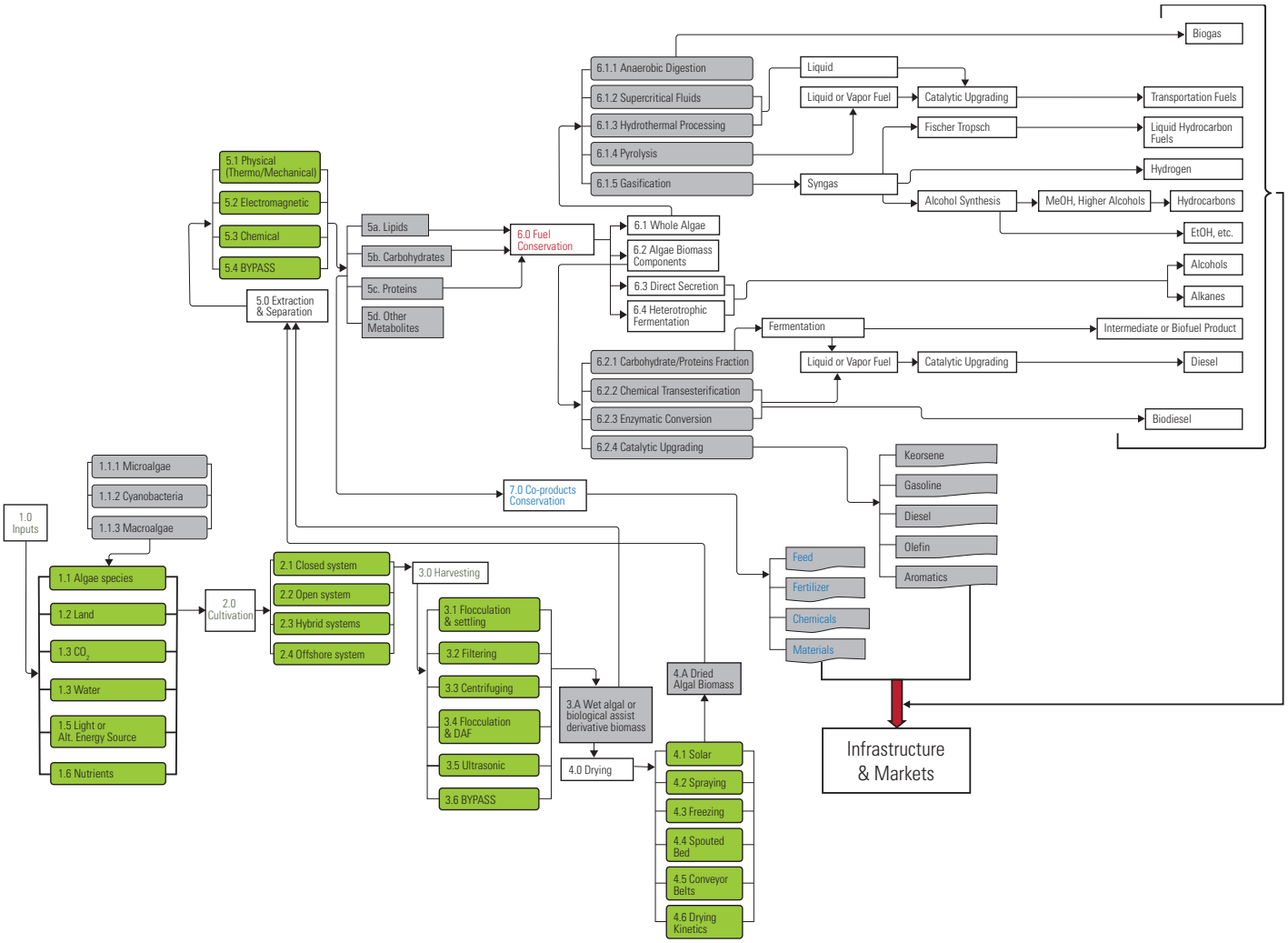

Figure 11.3. High-level multi-pathway algae biofuel process flow diagram for the algal biofuels and co-products supply chain. (Source: Adapted from the 2010 *National Algal Biofuels Technology Roadmap*.)

The key barriers to the development of algal feedstocks are the cost, quality, and volume of available, sustainably-grown biomass to supply the growing biobased industry for biofuels, bioproducts, and biopower. Analysts use modeled scenarios, developed in close collaboration with researchers, to perform conceptual evaluations termed "design cases," which typically are aspirational models projecting potential future process performance and resulting production costs that may be achievable by a given target year for a mature "n^{th}-plant" commercial facility. Design cases and accompanying "state-of-technology" reports (the latter focused on current experimental benchmarks as supported by available data) are used to describe discreet barrier areas to achieving large volumes of low-cost, high-quality algal biofuel intermediates. These design cases provide a detailed basis for understanding the potential of production and conversion technologies and help identify technical barriers where research and development could lead to significant cost improvements.

Critical emphasis areas that have been identified as a result of these analyses include

- Developing biology and culture management approaches to unlock algal biomass productivity potential and stable cultivation
- Developing low-cost, scalable cultivation systems that maximize reliable annual yield and quality, and minimize energy use, water consumption, land use, and nutrient additions
- Developing low-cost, high-throughput harvest technologies that can be integrated with cultivation systems
- Performing integrative analysis to identify critical barriers and evaluate impacts on overall yield to developments in biology, cultivation, and processing.

The first step in drafting a design case is to design the algae production facility and downstream processing pathway. Sufficient detail is needed in the facility design, cultivation methods, and processing pathway to reduce uncertainty. Detailed production system designs for an envisioned algal production farm have been developed (Lundquist et al. 2010; Beal et al. 2015; Davis et al. 2016). A variety of pathway technologies have been evaluated with systems design (Richardson et al. 2014; Quinn et al. 2014b; Davis et al. 2014a; Beal et al. 2015). Generally speaking, the field is currently divided into two pathways: (1) whole biomass thermochemical conversion (such as HTL), and (2) fractionation of biomass into lipids and one or more other component fractions (Valicor, OpenAlgae, "combined algal processing", hexane extraction, etc.). These two routes create notable tradeoffs with respect to nutrient recycling, quantity of fuel produced, and whether or not co-products, such as animal feed ingredients, chemicals, or other fuels, are generated. Quinn et al. (2014b) and Beal et al. (2015) provide a side-by-side comparison of several processing routes.

BETO has completed analysis on two initial downstream pathways representing these two general conversion strategies, both identified as promising approaches to achieving R&D targets in 2022:

- Combined algae processing (pretreatment of algal biomass to hydrolyze carbohydrates followed by fermentation of carbohydrates and extraction and upgrading of lipids) (Davis et al. 2014a).
- Whole algae HTL and upgrading (Jones et al. 2014).

The two conversion design cases assume an "n^{th} plant"[1] scenario for conversion of algal biomass to fuels through a hypothetical commercial-scale algal biorefinery. The pathways differ in types of algae cultivated upstream, as well as harvesting, preprocessing, conversion, and recycle/wastewater treatment operations, although both include significant nitrogen and phosphorus recycle.

In 2016, an additional design case was published to more explicitly define a set of process, design, and cost goals for the cultivation, harvesting, and dewatering of algal biomass (relative to prior projections, e.g., as documented in the 2012 harmonization report), envisioned to be achieved by 2022, for incorporation into the downstream conversion pathways:

- Biomass production in open systems and processing through dewatering for downstream conversion (Algae Farm Design Case) (Davis et al. 2016).

Alternative designs will need to be compared and validated as additional R&D data become available. Other critical areas must be evaluated, including methods of cultivation (batch, semi-continuous, fully continuous, etc.), harvest operations fully integrated with upstream cultivation and downstream conversion (including resultant, harvest efficiencies), separations and recycle cost and efficiency, oil upgrading, and opportunities for conversion to co-products including the types of co-products which can be produced and sold.

Combined Algae Processing Pathway

The combined algae processing conversion pathway represents many processing options for conversion of algae-derived carbohydrates and lipids to fuel and blendstock end products.

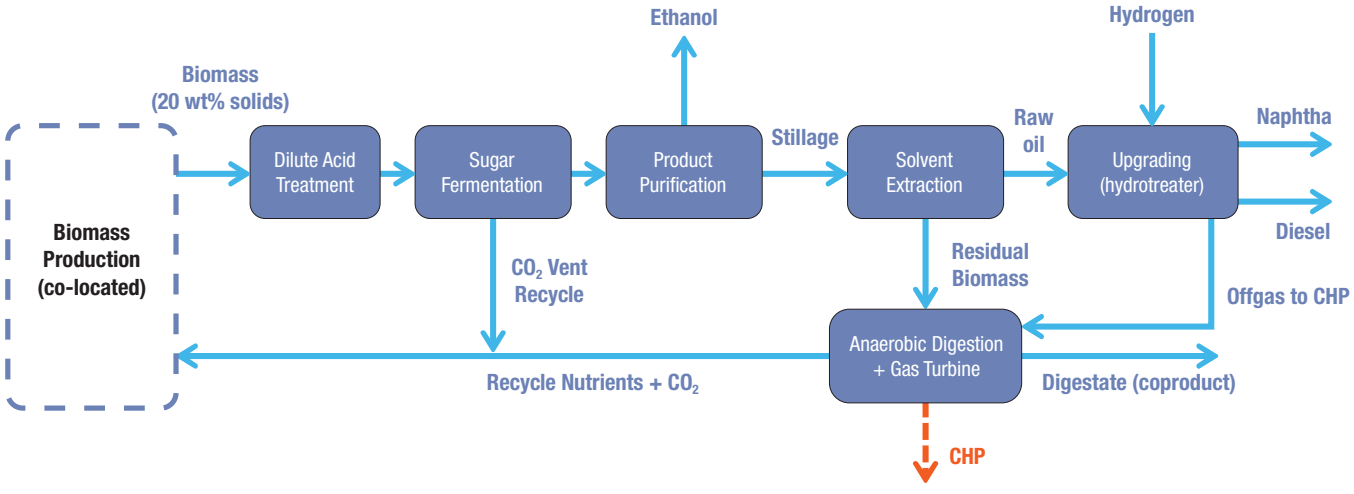

Figure 11.4. CAP process flow diagram (Source: DOE 2016)

[1] n^{th} plant analyses assume advances in technology and commercial activity, and this analysis includes future-looking assumptions for strain performance including robustness (resistance to crashes), productivity, and composition (lipid content) above what has currently been demonstrated at small scale outdoors.

Conversion processes focused on in this pathway include acid pretreatment of algal biomass followed by fermentation of sugars and lipid extraction/upgrading with a high fractional energy yield to produce hydrocarbon products (such as renewable diesel), as well as additional yield to ethanol from fermented sugars. Priority areas, technical targets, and accompanying cost projects for conversion of algal biomass to fuels and co-products are documented in the 2014 Algal Lipid Upgrading Design Case (Davis et al. 2014a), and were further refined in a subsequent state-of-technology report that includes the process steps for combined algae processing.. This design case serves to describe a single, feasible conversion pathway to transparently document the assumptions and details that went into its design, and is a relevant pathway for the production of high-value products (DOE 2016). In addition to this base case focused on sugars-to-ethanol and lipids-to-renewable diesel, alternative options exist within a CAP processing approach to convert sugars to other fuel products (e.g., yeast lipids) or co-products (e.g., organic acids), as well as protein to high-value co-products (e.g., animal/fish feed, mixed alcohols, and bioplastics).

The process described in the design case uses mild dilute-acid pretreatment of algal biomass delivered after upstream dewatering to 20 wt% solids, which hydrolyzes carbohydrates to monomeric sugars and makes the biomass more amenable for downstream extraction; this is followed by whole-slurry fermentation of the resulting monomeric sugars to ethanol, followed by distillation and solvent extraction of the still-age to recover lipids (primarily neutral lipids with inclusion of polar lipid impurities). The process design also includes lipid product purification, product upgrading (hydrotreating) to diesel and naphtha blend stocks, anaerobic digestion and combined heat and power (CHP) generation, product storage, and required utilities.

The resulting cost projections (DOE 2016, Table 11.1) emphasize that the greatest opportunity to reduce costs in the combined algae processing pathway is in the production systems through improved biomass yield and the subsequent costs of the feedstock. Additional improvements can be made through increasing the yields of the fermentable sugars, decreasing the costs of acid pretreatment, and increasing the yields of lipids. Based on such improvements, fuel costs from the baseline combined algae processing pathway may plausibly be reduced from a current estimated benchmark $13.89/GGE to $5.90/GGE by 2022. Opportunities for improvement also exist moving forward to leverage the non-destructive fractionation nature of the combined algae processing pathway to pursue isolation and/or upgrading to value-added products.

The GHG emissions of this pathway have also been estimated, including biomass production, conversion to biofuel, transport to consumer facilities, and combustion of fuel in vehicles. The resulting analysis estimated that the pathway requires 0.5 MJ of fossil fuel and 0.07 MJ of petroleum per MJ of renewable diesel produced, and that the total GHG emissions from the pathway is 37 gCO_2e per MJ of total fuel produced (renewable diesel and ethanol combined) (Davis et al. 2014a).[2]

Algal Hydrothermal Liquefaction Pathway

The Algal HTL Design Case (Jones et al. 2014) documents a pathway model for conversion of whole algae to fuel and other products. The process described in the design case uses dewatered algae (20 wt% on an ash-free basis) that is pumped to the HTL reactor, where condensed phase liquefaction occurs. The

Table 11.1. Summary of Cost Contributions for Combined Algae Processing Design Case and State-of-Technology Report

Production Cost Breakdown, $/GGE (2014$)	2015 State of Technology (SOT)	2015 SOT + Pond Liners	2022 Projection
Feedstock	$11.25	$15.05	$4.23
Conversion	$1.95	$1.95	$1.35
Hydrotreating	$0.81	$0.81	$0.46
Anaerobic Digestion[a]	($0.27)	($0.27)	($0.25)
Balance of Plant	$0.15	$0.15	$0.11
Total	**$13.89**	**$17.69**	**$5.90**

[a] AD contribution includes coproduct credits attributed to nutrient + CO_2 that recycles back to production ponds.

[2] This total is for the fuel plus infrastructure cycles (i.e., the total emissions in a scenario where excess biomass is dried and stored).

products of the liquefaction process (oil, solid, aqueous phase, and gas) are then separated, with the oil hydrotreated to generate diesel and naphtha-range fuels. The HTL aqueous phase is sent to catalytic hydrogasification to convert all organics to CO_2 and methane before recycling the treated water back to the ponds. As a result, there is a reduced level of fresh nutrient demands during cultivation. Process off-gas from HTL may be used to generate hydrogen, heat and/or power for the facility. A hydrogen source is included in the hydrotreating step and is assumed to be co-located with the biomass production and conversion facility. Nutrient recovery occurs through recycling-treated waste (containing dissolved carbon dioxide and ammonia), CO_2 containing flue gas, and phosphorus recovered from treated HTL solids back to the algae ponds for further biomass cultivation. This process is shown in Figure 11.5.

Similar to the combined algae processing pathway, the cost to produce dewatered algae feedstock is the most significant factor affecting the final cost of the fuel (see DOE 2016, table 11-2). Additional algal strain development is needed to optimize the desirable characteristics for HTL conversion. Key opportunities for improvements identified in the pathway include improved HTL oil separation for the HTL aqueous phase, and optimization of value obtained from that aqueous phase. Based on such improvements, fuel costs from the baseline HTL pathway may plausibly be reduced from a current estimated benchmark $14.78/GGE to $4.72/GGE by 2022.

The sustainability of this pathway has been analyzed, with a scope spanning from biomass production to burning of the fuel in a vehicle. The specific pathway utilizes a validated model of biomass production, as well as mass and energy balances from Jones et al. (2014) for the cultivation and conversion of algal biomass, which also includes the drying and storage of surplus biomass in the summer for use in winter months when levels of biomass growth are low. The resulting analysis presents that the pathway utilizes 0.45 MJ of fossil fuel and 0.02 MJ of petroleum per MJ of renewable diesel product generated; the total GHG emissions from the pathway is 37 gCO_2e per MJ of produced fuel products (renewable diesel and naphtha-range products) (Frank et al. 2013).

Algae Farm Design

The Algae Farm Design Case was completed (Davis et al. 2016) to more explicitly define a set of process, design, and cost goals projected to be achieved by 2022 for the cultivation and harvesting/dewatering of algal biomass in open ponds and to refine the delivered biomass cost in the two conversion-focused design cases and annual state-of-technology updates. The design case analyzed the utilization of a 5,000-acre facility (based on cultivation area) consisting of large open freshwater ponds for continuous production of algal biomass with the freshwater strain *Scenedesmus actus*, grown to a mid-level 27% FAME lipid content and harvested at a steady-state density of 0.5g/L AFDW. The biomass is concentrated to a 20 wt% solid through a three-step dewatering series, including gravity settlers, membranes, and centrifugation. Consideration is also taken for on-site inoculum propagation, water circulation, and CO_2 delivery sourced from an off-site power plant. The pathway is shown in Figure 11.6.

Based on "n^{th}-plant" design assumptions, project costs, financing, and strain/process targets projected to be demonstrated by the year 2022, the minimum biomass selling price was estimated to range between $392–$649/ton (ash free dry weight) associated with eight distinct pond size/design scenarios. The MBSP is inversely proportional with individual pond size, varying from an average $612/ton of dewatered biomass (ash free dry weight) for "small" 2-acre pond designs, $491/ton for "medium" 10-acre pond designs, and $406/ton for "large"

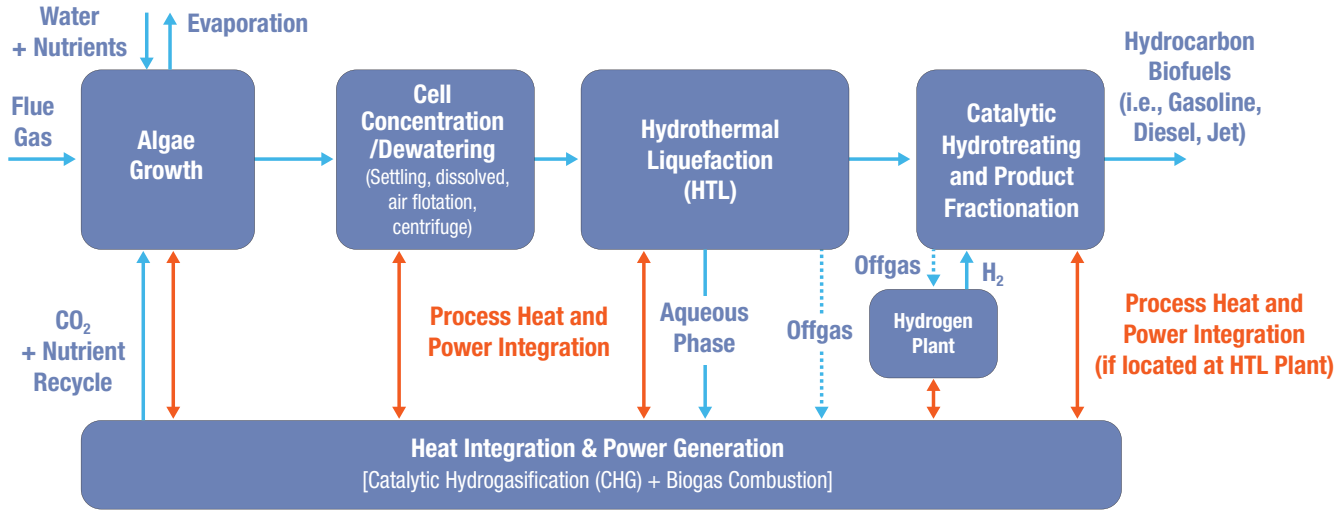

Figure 11.5. AHTL process flow diagram (Source: DOE 2016)

Table 11.2. Summary of Cost Contributions for HTL Design Case and SOT

Production cost breakdown, $/GGE (2014$)	2015 SOT [a]	2015 SOT [b] + pond liners	2022 projection[c]
Feedstock	$11.33	$15.15	$3.18
Hydrothermal liquefaction	$1.18	$1.18	$0.49
Hydrotreating upgrade to finished fuels	$0.44	$0.44	$0.31
Catalytic hydrothermal gasification	$1.54	$1.54	$0.57
Balance of plant	$0.29	$0.29	$0.17
Total	**$14.78**	**$18.60**	**$4.72**

[a] 1340 ton per day (tpd) ash-free dry weight (AFDW) algae @ $1091.59; naphtha valued at $3.25/gal
[b] 188 tpd AFDW algae @ $1222/ton; naphtha valued at production cost
[c] 568 tpd AFDW algae @ $491/ton; naphtha valued at production cost

hypothetical 50-acre designs (all in 2011 dollars). A 2015 state of technology, with a liner scenario and without, and a 2022 projection for a medium pond design, were published in the 2016 *BETO Multi-Year Program Plan* (see summary in Table 11.3). Results from this analysis reiterate that recycling nutrients fixed in the biomass back to the production ponds is critical for controlling costs as well as minimizing greenhouse gas emissions. The work also reiterates that it is critical to avoid the use of fully lined ponds if possible, and instead situate ponds in locations with high native clay content and use liners only as needed for erosion control in small areas. If ponds were fully lined across the full 5,000 acres of cultivation area, the minimum biomass selling price would increase more than $125/ton, on average, for a 10-acre pond design scenario. The report also includes a high-level discussion on the tradeoffs between sourcing CO_2 via carbon capture from power plant flue gas versus direct utilization of bulk flue gas, with concentrated CO_2 costs adding significantly to the minimum biomass selling price, while flue gas is challenged by substantial logistical and practicality constraints for a facility of this size. Finally, the report highlights that it would be challenging to reduce biomass costs below $400/ton without aggressive further improvements to both productivity and "farm" system costs, a biomass price point that further indicates a need to pursue co-products alongside fuels in order to achieve viable algal biofuel production costs (Davis et al. 2016).

Next Steps in Research

One of the biggest challenges for systems analysis and connecting TEA, LCA, and RA models is data gathering and

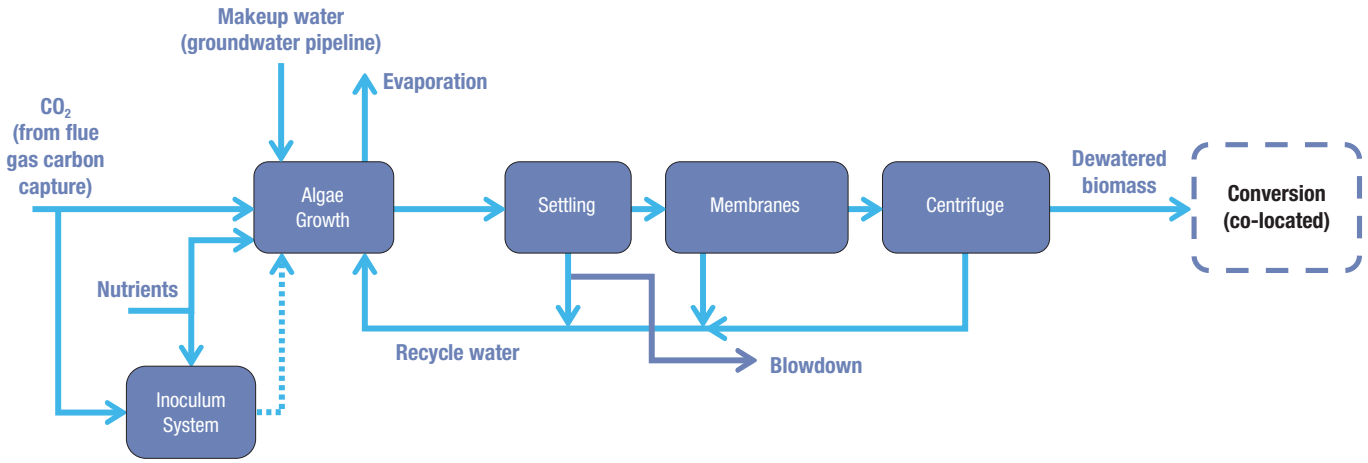

Figure 11.6. Algae CO_2 pathway (Source: MYPP 2016)

Table 11.3. Summary of Cost Contributions for Algae Farm Design Case and SOT

Production cost breakdown, $/ton AFDW (2014$)	2015 SOT	2015 SOT + pond liners	2022 projection
Production cost	$1,069	$1,483	$409
Harvest/dewater	$116	$116	$64
Other (facility circulation, storage)	$42	$42	$21
Biomass selling price	**$1,227**	**$1,641**	**$494**

validation of the technical and economic system performance of algae technologies that have very few pilot- or demonstration-scale projects. Until very recently, there has continued to be a wide variability in basic assumptions on many parameters, from algal productivity to capital and operating costs. These shortcomings of the existing literature and modeling knowledge base have presented an on-going challenge in designing commercial-scale systems and reducing uncertainties in realizable economic and sustainability metrics for algal biofuels. This challenge is beginning to be recognized an addressed, thanks to the recent advents of the design reports, coupled with new literature from industry and consortia organizations that document outdoor cultivation performance and modeling analysis in increasing levels of detail (Beal et al., 2015; Huntley et al., 2015; White and Ryan, 2015). Still, more work remains.

Based on completed modeling analyses for algal biomass to fuels and products, as well as input from the algae sector,[3] the following are recommendations for systems analysis next steps (DOE 2013, 2014):

- Encourage data sharing, especially of algae growth rates and biomass compositional analyses
- Examine additional, novel, cultivation pathways
- Incorporate risk analyses
- Incorporate and define multiple sustainability metrics beyond GHG emissions (e.g., resource use and ecological impact)
- Standardize methods and analysis used to evaluate algal biomass and biofuel production.

BETO works with project partners, particularly in the national laboratories, to address these recommendations for improvements in systems analysis.

[3] Bioenergy Technologies Office Algae Strategy Workshops, 2013–2014; http://energy.gov/eere/bioenergy/algal-biofuels-strategy-workshop

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12. Conclusion

The 2010 *National Algal Biofuels Technology Roadmap* sought to comprehensively summarize the state of technology for fuels and bioproducts from algal feedstocks and to document the feasibility and techno-economic challenges associated with commercial scaling. Since that initial review, there have been significant advancements in the field, as well as the articulation of new challenges, lessons learned, and critical next steps, which have been detailed in this update, and are summarized in this chapter.

12.1 Advancements in the Field

One of the most critical areas of focus in algal biofuels R&D—algal biology—has benefited from dedicated researchers advancing understanding of the true requirements of outdoor algae cultivation. The field has recognized that strain robustness, not just lipid content, is critical for large-scale cultivation. Molecular technologies have been developed to make the necessary improvements in robustness and productivity, including molecular toolboxes for strain improvement and advanced genomics, transcriptomics, proteomics, metabolomics, and phenomics platforms. Rapid advances in molecular biology tools have allowed scientists to manipulate algal genomes to express new or altered proteins, including those involved in metabolism and photosynthesis. Work in directed evolution and high-throughput selection systems have led to the development of advanced algal strains. In addition, multiple libraries of catalogued species from marine, freshwater, brackish, or otherwise low-quality water environments have been collected. Some researchers have also discovered that "superior strain" development may not hold the whole answer and that beneficial symbioses and ecosystem responses exist within certain bacteria, microbes, and algal strain communities. Development of standardized protocols for the quantification and characterization of biomass and cellular composition has allowed for the establishment of a common language and consistent metrics for success among researchers. This has also enabled the valorization of algal biomass potential across multiple products and end uses, from biofuels to animal feed to specialty platform chemicals.

Moving many of these biological advances to outdoor cultivation environments has been a major success and is still an area of continued research effort. Development of laboratory tools and methods that mimic outdoor conditions has allowed for the ability to predict pond performance. Pond crashes are being addressed by species-specific pathogen and predator prevention methods, as well as approaches to create a stable diversified culture less sensitive to predation. Several specific molecular tools have been developed to monitor pond health and species composition. Novel cultivation designs have demonstrated productivity improvements at increasing scales, including systems capable of using waste industrial carbon dioxide, nutrient-rich impaired water, or wastewater streams. Nutrient and water recycle strategies have proven necessary for both economic and environmental sustainability, and advances in these strategies are consistently improving system viability. Much of this cultivation data has been made publicly available via the ATP^3 experimental testbeds program.

Major advances have also been made in feedstock processing and conversion to biofuel intermediates and finished fuels. Innovations in hydrothermal liquefaction have demonstrated the conversion of wet biomass into crude oil at high yield, with low energy costs, in a continuous process. Wet solvent extraction processes have also improved total fuel yields. Researchers have demonstrated the effectiveness of an integrated technology based on moderate temperatures and low pH to convert the carbohydrates in wet algal biomass to soluble sugars for fermentation, while making lipids more accessible for downstream extraction and leaving a protein-enriched fraction behind. Algal oil has been successfully converted to jet and biodiesel meeting the ASTM standards. Algae companies are beginning to see off-take agreements with fuel producers such as Tesoro, Phillips 66, and others. Test runs in aviation and cross-country road trips have demonstrated high fuel performance.

12.2 New Challenges

In recent years, the algal biofuels RD&D has achieved technological advancements that can bring about transformational changes, including the ability to predict, breed, and select the best-performing strains; the ability to monitor and control system inputs in a dynamic and integrated fashion; the ability to harvest algae at high throughputs; and the ability to extract and convert more algal biomass components into fuels. However, there is still much work left to do to achieve cost-competitive algal biofuels. Table 12.1 outlines the current challenges in the field.

12.3 Lessons Learned

Through its efforts to address these challenges, the algal research field has learned lessons that can be applied to support future efforts. Although there has been progress, translating lab-scale results to production systems continues to be a significant hurdle. Investigators have learned that success at the bench does not always mean success outdoors. Outdoor strain growth and development is better able to incorporate actual regional and environmental conditions. In addition, algal biology efforts must be compatible with downstream processes, such as harvesting and conversion. Strains that perform well in terms of productivity must have a tailored harvesting and conversion regime to perform well in terms of biofuel intermediate yield, and it is important to consider the variability in the complex natural systems involved as well. The enormous diversity of strains means that specific techniques and specific molecular tools are required. These complex and diverse

systems have led to diverse laboratory methodologies and data collection procedures, which researchers have learned makes comparative analysis problematic, and which has instigated an industry standardization effort.

It is now understood that water and nutrient recycle and energy conservation are necessary considerations to ensure that production of algal biofuels and bioproducts is environmentally and economically sustainable. The current nutrient requirements for carbon dioxide, nitrogen, and phosphorous are significant. In terms of energy return on investment, the industry has learned that wet extraction processes are essential. Drying algal feedstock has significant impacts on greenhouse gas emissions, as well as the total economics of the system. Dewatering technologies are a major design consideration when scaling, and they impact not only energy use, but also capital and operating costs. Delivery of CO_2 to the facility is also a considerable constraint, and the co-location of facilities with carbon emitters—while a rational design for individual pilot companies—may be constrained in full nation-wide deployment due to limited location availability. Calculations for scaling and facility deployment must be inclusive of mass and energy balances, resource constraints, capital expenditures, and incorporate whole-system data collection from pilot projects. Researchers have learned that real-world data is essential and that the field needs to find ways to disseminate it publicly without jeopardizing intellectual property.

In general, the algal biofuels RD&D field has learned that industry dogma must be reassessed. Old truisms, such as the need for nitrogen starvation for cell growth, and that genetic modification is the only answer to productivity, have been increasingly challenged. As the knowledge base continues to evolve and build on prior learnings, disruptive breakthroughs

Table 12.1. Algal Feedstocks R&D Technical Challenges and Barriers

Process step	Technical barrier	Challenges
Feedstock	Algal biology	• Advance understanding of basic algal biology across species (including photosynthesis and carbon management) • Establish an algal database for identification, proteomics, genomics, and transcriptomics for all known species; improve open access data sharing of existing and emerging research (i.e., testbeds, online omics databases) • Develop and advance molecular toolboxes for heterologous gene expression in potential production strains • Advance understanding of open pond production health (i.e., ecology, predators, crashing) • Advance understanding of safety, policy oversight of genetically-engineered organisms.
	Algal cultivation	• Advance understanding of culture dynamics and stability (i.e., crop protection, nutrient addition and limitation) • Improve on ability to translate performance from bench-scale experiments to large process-development scale • Identify standardized metrics for system-level productivity analysis • Sustainably and cost-effectively manage resources for biomass production (i.e., water and nutrient conservation and recycling) • Advance understanding of CO_2 utilization at industrially relevant scale.
	Harvesting and dewatering	• Develop and demonstrate harvesting, dewatering, and drying technologies at industrially relevant scales • Assess the economic viability, energy requirements, and environmental sustainability of harvesting and dewatering technologies at industrially relevant scales • Examine performance of existing and new harvesting and dewatering technologies over long durations of operation • Advance understanding of species-specific effects on harvesting and dewatering.

Table 12.1. *(continued)*

Process step	Technical barrier	Challenges
Conversion	Extraction and fractionation	• Investigate the techno-economic and systems impacts of scale up of extraction technologies • Advance understanding of the impact of feedstock composition on end products • Examine performance of existing and new extraction technologies at industrially relevant scales • Address scaling challenges, such as the presence of water, side reactions, separations, operational temperature, and pressure.
	Fuel conversion	• Assess and seek to achieve high conversion rates at industrially relevant scales. • Optimize fuel recovery at industrially relevant scales • Examine and understand coproduct recovery in relation to fuel recovery at all scales • Advance understanding of nutrient recycling with new and existing conversion technologies • Examine and minimize conversion technology energy use, emissions, and contaminants over the life cycle at industrially relevant scales. • Advance understanding of algal species-specific conversion technology requirements and limitations.
	Co-products	• Identify and evaluate the co-production of value-added chemicals, energy, and materials from algal remnants (e.g., biogas, animal/fish feeds, fertilizers, industrial enzyme, bioplastics, and surfactants) • Optimize co-product extraction and recovery • Conduct market analyses, including quality and safety trials to meet applicable standards.
Infrastructure	Distribution and utilization	• Characterize algal biomass, intermediates, biofuel, and bioproducts under different storage and transport scenarios for contamination, weather impacts, stability, and end-product variability • Optimize distribution for energy and costs in the context of facility siting • Comply with all regulatory and customer requirements for utilization (e.g., engine performance and material compatibility)
	Resources and siting	• Integrate modeling efforts to capture multiple dimensions of effects from production of algal biomass, including sustainable resource use • Standardize methods and analysis for modeling resource characteristics and requirements • Investigate the impacts of carbon capture and utilization of algal biomass production • Address salt balance, energy balance, water and nutrient recycling, and thermal management • Advance understanding of integration of CO_2 waste emitting industries and wastewater treatment plant co-location with algal cultivation facilities

are going to be necessary to achieve cost-competitive and commodity-scale quantities of algal biomass for biofuel and bioproduct production.

12.4 Critical Next Steps

Near-term actions critical to progress in the field include collection and dissemination of quality and standardized data. Technology solutions are dispersed among many companies and research laboratories. The protection of intellectual property is a concern, but open-access data, information, and tools, such as those provided through the DOE-funded testbed programs and the Los Alamos National Laboratory 'Omics Database, are critical to prevent duplication of mistakes and to advance the field. Defining standards, metrics, and best practices for analysis and quality controls for data will facilitate data management and dissemination programs. Collaborative sharing of raw biomass and feedstock for downstream processing and conversion researchers to test would also benefit the entire field. In addition, communication of successes and accomplishments can help to not only provide lessons to fellow researchers, but can also help garner investor and public interest.

Given the multiple technology and system options and their interdependency, a continued focus on integrating and harmonizing techno-economic modeling and analysis spanning the entire algae to biofuels supply chain is crucial in guiding research efforts along select pathways that offer the most opportunity to practically enable a viable and sustainable algae-based biofuels and co-products industry. Additional data is also critically needed to develop systematic performance models. Models can be used to address high-impact sustainability drivers, such as greenhouse gas emissions and water consumption, from the feedstock generation facility to the downstream conversion processes. Modeling toolsets can support reverse engineering and sensitivity analyses, probability charts, and process unit validations, and can also facilitate sharing of information among user groups. Close collaboration among modelers and experimentalists can help to identify critical focus areas to improve economic and sustainability metrics and can identify operational requirements for large-scale algae production facilities.

Project data from integrated and semi-integrated designs can support optimization of cultivation, harvesting, and processing unit operations. Sharing of data from reactor design and balance of plant studies can support optimization of scaled pathway details, such as heat integration and strategies to leverage existing sources of energy. Data from cost-effective culture monitoring systems are needed to identify and remedy pond crashes. Data and information is needed on point source CO_2 including uptake efficiency and potential bioaccumulation of pollutants.

In order to facilitate implementation of CO_2 point source solutions, inter- and intra-agency coordination is needed at multiple levels. DOE's Fossil Energy Office is currently investigating algal carbon capture and utilization strategies. EPA has included algal carbon capture and utilization in its Clean Power Plan as a means for states to meet regulatory requirements for point source pollutants. Concerns around the stability and environmental impact of genetically modified algae also dictates engagement by EPA in this field. DOE can support scientific data sharing for the regulatory community's consideration in this matter.

In general, a dedicated research and development focus on cost-effective solutions for simple, low-energy inoculum and culture production, product extraction, and conversion systems is required. BETO's Advanced Algal Systems Program is focused on demonstrating progress toward achieving high-yield, low-cost, environmentally sustainable algal biofuel production systems, and is actively working with the R&D community to make algal biofuel a part of a diversified energy future.

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Appendix D: List of Acronyms

ABY – Algal Biomass Yield

ACCase – acetyl-CoA carboxylase

AFDW – ash free dry weight

ASE – accelerated solvent extraction

ASP – Aquatic Species Program

ATP – adenosine triphosphate

ATP^3 – Algae Testbed Public-Private Partnership

BAT – Biomass Assessment Tool

BD20/BD40 – 20% and 40% biodiesel

BETO – Bioenergy Technologies Office

CAB-Comm – Consortium for Algal Biofuels Commercialization

CAP – Combined Algal Processing

CCM – carbon concentrating mechanism

CHG – catalytic hydrothermal gasification

DAG – diacylglycerol

DDG – distillers dry grain

DDGS – distillers dry grain plus soluble

DGAT – diacylglycerol acyltransferase

DHA – docosohexaenoic acid

DOE – U.S. Department of Energy

EISA – Energy Independence and Security Act

EPA – U.S. Environmental Protection Agency

ePBR – environmental photobioreactor

FACS – fluorescence-activated cell sorting

FAME – fatty acid methyl ester

FOA – funding opportunity announcement

FTS – Fischer-Tropsch Synthesis

GAI – Global Algae Innovations

GGE – gallons of gasoline equivalent

GHG – greenhouse gas

GIS – geographic information system

GLA – y-linolenic acid

HPA – heteropolyacid

HTL – hydrothermal liquefaction

IBR – integrated biorefinery

LCA – life-cycle analysis

LEAPS – Laboratory Environmental Algae Pond

LED – light-emitting diode

MAE – microwave-assisted extraction

NAABB – National Alliance for Advanced Biofuels and Bioproducts

NO_x – nitrogen oxides

NREL – National Renewable Energy Laboratory

NSF – National Science Foundation

PAP – Parallel Algal Processing

PBR – photobioreactor

PEF – pulsed electric field

PNNL – Pacific Northwest National Laboratory

pR&D – research and development

PUFA – polyunsaturated fatty acids

RA – resource assessment

RAFT – Regional Algal Feedstocks Testbed

RD&D – research, development, and demonstration

RFS – Renewable Fuel Standard

RNAi – ribonucleic acid interference

RON – research octane number

SABC – Sustainable Algal Biofuels Consortium

SAG – Culture Collection of Algae at Goettingen

SEGHTL – two-step sequential hydrothermal liquefaction

SHS – single-component system

SPK – synthetic paraffinic kerosene

SSS – two-component system

TABB – Target Algal Bioproducts and Biofuels

TAGs – triacyglyerols

TALE – transcription activator-like effectors

TALEN – transcription activator-like effector nuclease

TEA – techno-economic analysis

USDA – U.S. Department of Agriculture

UTEX – University of Texas

ZFN – zinc-finger nucleases

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