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BIOMASS PROGRAM

National Algal Biofuels Technology Roadmap

MAY 2010

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

National Algal Biofuels Technology Roadmap

A technology roadmap resulting from the National Algal Biofuels Workshop
December 9-10, 2008
College Park, Maryland

Workshop and Roadmap sponsored by the U.S. Department of Energy
Office of Energy Efficiency and Renewable Energy
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Preface

Welcome to the U.S. Department of Energy (DOE) Biomass Program's National Algal Biofuels Technology Roadmap. Prepared with the input of more than 200 scientists, engineers, industry representatives, research managers, and other stakeholders, this document represents the synthesis of the Biomass Program's National Algal Biofuels Technology Roadmap Workshop, comments gathered during a public comment period, and supporting scientific literature. This Roadmap is intended to summarize the state of technology for algae-based fuels and document the research and development challenges associated with producing them at a commercial scale.

Renewable energy lies at the intersection of some of our nation's most pressing issues: our energy security, our economic wellbeing, and the stability of our global climate. These national challenges require near-term solutions as well as investments in nascent technologies that show promise for the future. Therefore, while DOE works to deploy renewable energy and energy-efficient projects across the country today, it remains committed to fostering technologies that could yield substantial benefits over time. Achieving cost-competitive, sustainable algal biofuels will entail years of research and careful planning, but their significant potential to serve as renewable transportation fuels warrants our thorough consideration of what efforts are necessary to make them a commercial-scale reality.

DOE has recently revived its investment in algal biofuels in response to the increased urgency of lowering greenhouse gas emissions and producing affordable, reliable energy, as well as the recognition that we will not likely achieve these goals via one technology pathway. Through appropriated dollars and the American Recovery and Reinvestment Act of 2009, DOE is investing in a variety of research, development, and demonstration (RD&D) projects that seek to tackle key technical hurdles associated with commercializing algal biofuels. Meanwhile, other federal agencies, private companies, and the academic community are also increasing their efforts to optimize and commercialize this renewable energy source.

This Roadmap lays the groundwork for identifying challenges that will likely need to be surmounted for algae and cyanobacteria to be used in the production of economically viable, environmentally sound biofuels. It is intended to serve as a resource for researchers, engineers, and decision-makers by providing a summary of progress to date and a direction for future algae RD&D activities, and we hope it fosters and informs participation from existing and new stakeholders as the next steps are taken to advance algal biofuels. DOE looks forward to continuing its work with diverse partners in evaluating renewable energy options and facilitating development of those that carry the greatest benefits today and in the years to come.

Valerie Sarisky-Reed

Acting Manager, Biomass Program

U.S. Department of Energy

Executive Summary

> Developing the next generation of biofuels is key to our effort to end our dependence on foreign oil and address the climate crisis – while creating millions of new jobs that can't be outsourced
>
> — *Secretary of Energy Steven Chu at the White House ceremony on May 5, 2009, announcing $800 million in new biofuel research activities*

In recent years, biomass-derived fuels have received increasing attention as one solution to our nation's continued and growing dependence on imported oil, which exposes the country to the risk of critical disruptions in fuel supply, creates economic and social uncertainties for businesses and individuals, and impacts our national security. The Energy Independence and Security Act of 2007 (EISA) established a mandatory Renewable Fuel Standard (RFS) requiring transportation fuel sold in the U.S. to contain a minimum of 36 billion gallons of renewable fuels, including advanced and cellulosic biofuels and biomass-based diesel, by 2022. While cellulosic ethanol is expected to play a large role in meeting the EISA goals, a number of next generation biofuels show significant promise in helping to achieve the goal. Of these candidates, biofuels derived from algae have the potential to help the U.S. meet the new RFS while at the same time moving the nation ever closer to energy independence. To accelerate the deployment of advanced biofuels, President Obama and Secretary of Energy Steven Chu announced the investment of $800M in new research on biofuels in the American Recovery and Renewal Act. This announcement included funds for the Department of Energy (DOE) Office of Energy Efficiency and Renewable Energy's (EERE) Biomass Program to invest in the research, development, and deployment of commercial algae-to-biofuel processes. Additional funding is being directed to algae-to-biofuel research both in EERE and other government agencies and programs.

The term algae can refer to microalgae, cyanobacteria (the so called "blue-green algae"), and macroalgae (or seaweed). Under certain conditions, some microalgae have the potential to accumulate significant amounts of lipids (more than 50% of their ash-free cell dry weight). These characteristics give great potential for an immediate pathway to high energy density, fungible fuels. These fuels can also be produced using other algae feedstocks and intermediates, including starches and

A culture of the microalgae Botryococcus. Photo courtesy of the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP).

sugars from cyanobacteria and macroalgae. In addition to fungible biofuels, a variety of different biofuels and products can be generated using algae precursors.

There are several aspects of algal biofuel production that have combined to capture the interest of researchers and entrepreneurs around the world. These include: 1) high per-acre productivity, 2) non-food based feedstock resources, 3) use of otherwise non-productive, non-arable land, 4) utilization of a wide variety of water sources (fresh, brackish, saline, marine, produced, and wastewater), 5) production of both biofuels and valuable co-products, and 6) potential recycling of CO_2 and other nutrient waste streams.

The DOE-supported Aquatic Species Program, an effort undertaken from 1978 to 1996, illustrated the potential of algae as a biofuel feedstock. Much has changed since the end of the program. Rising petroleum prices and a national mandate to reduce U.S. dependence on foreign oil, provide environmental benefits, and create economic opportunities across the nation have renewed interest in developing algal feedstocks for biofuels production.

While the basic concept of using algae as an alternative and renewable source of biomass feedstock for biofuels has been explored previously, a scalable, sustainable and commercially viable system has yet to emerge. The National Algal Biofuels Technology Roadmap Workshop, held December 9-10, 2008, was convened by DOE-EERE's Biomass Program. The two-day event brought together more than 200 scientists, engineers,

research managers, industry representatives, lawyers, financiers, and regulators from across the country to discuss and identify the critical challenges currently hindering the economical production of algal biofuels at commercial scale.

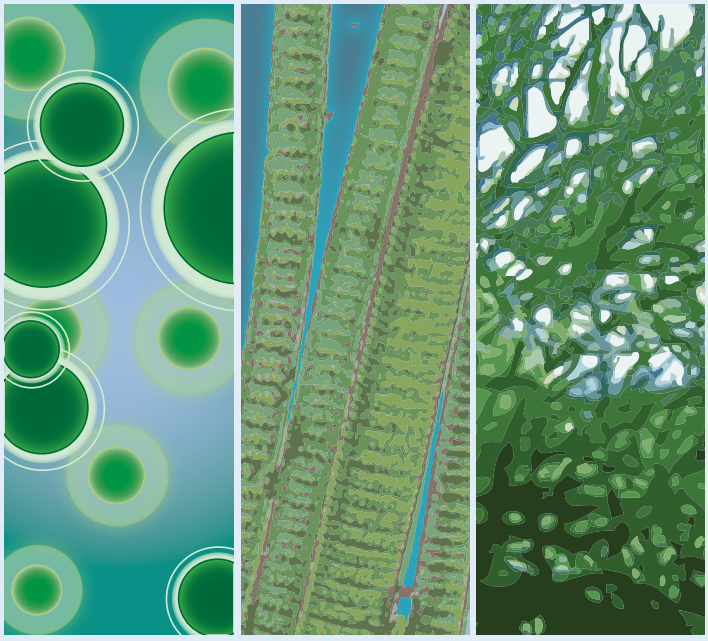
This document represents the output from the Workshop, supporting scientific literature, and comments received during a public comment period. The Roadmap document is intended to provide a comprehensive state of technology summary for fuels and co-products from algal feedstocks and to document the feasibility and techno-economic challenges associated with scaling up of processes. This document also seeks to explore the economic and environmental impacts of deploying algal biomass production systems at commercial scale. By documenting the challenges across the algal biomass supply chain and highlighting research and coordination needs and gaps, this document will serve to guide researchers and engineers, policymakers, federal agencies, and the private sector in implementing national research, development, and deployment efforts.

In summary, the Roadmap Workshop effort suggests that many years of both basic and applied science and engineering will likely be needed to achieve affordable, scalable, and sustainable algal-based fuels. The ability to quickly test and implement new and innovative technologies in an integrated process will be a key component to accelerating progress.

FROM ALGAE TO BIOFUELS

An Integrated Systems Approach to Renewable Energy that is

ALGAE FEEDSTOCKS



MICROALGAE CYANOBACTERIA MACROALGAE

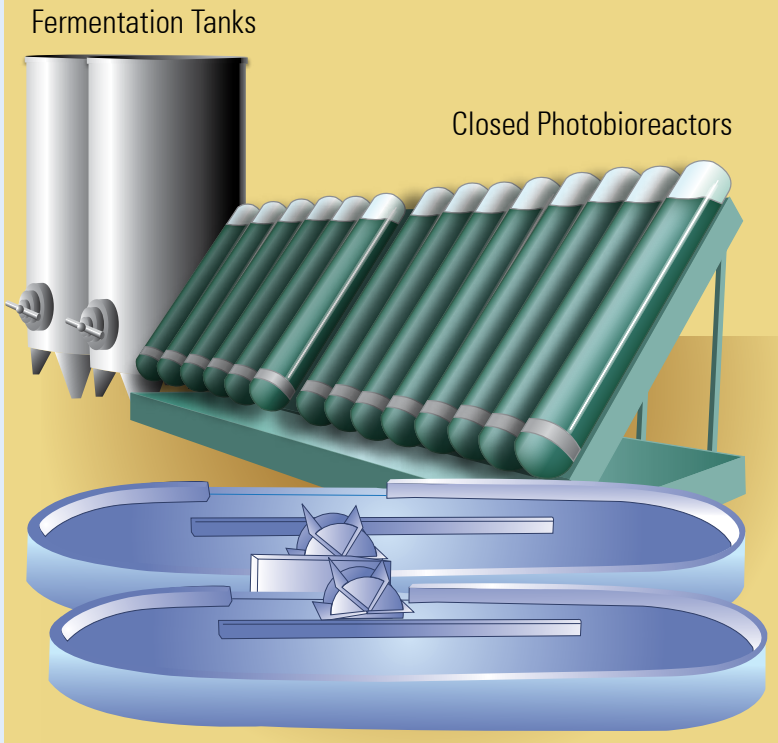
Algae as feedstocks for bioenergy refers to a diverse group of organisms that include microalgae, macroalgae (seaweed), and cyanobacteria (formerly called “blue-green algae”). Algae occur in a variety of natural aqueous and terrestial habitats ranging from freshwater, brackish waters, marine, and hyper-saline 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. Isolating new strains directly from unique environments will ensure versatile and robust strains for mass culture needed in biofuels applications.

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

POLICY SITING AND RESOURCES

Development Path Toward a

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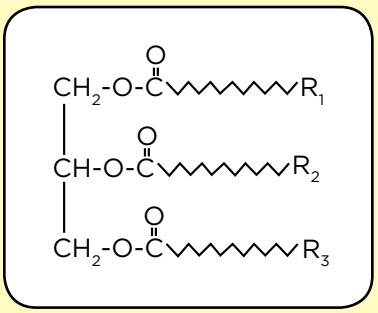
Abundant, Affordable, and Sustainable

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. While lipids and carbohydrates are fuel precursors (e.g., gasoline, biodiesel and jet fuel), proteins can be used for co-products (e.g., animal/fish feeds).

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

Commercially Viable Algal Biofuel Industry

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1. Overview

The Biomass Program (Program) 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 oil, provides environmental benefits, and creates economic opportunities across the nation. The Program's goals are driven by various federal policies and laws, including the Energy Independence and Security Act of 2007. To accomplish its goals, the Program has undertaken a diverse portfolio of research, development, and deployment (RD&D) activities, in partnership with national laboratories, academia, and industry.

Algal biofuels offer great promise in contributing to the Program'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 meet these l onger-term needs of the RFS and represent a significant opportunity to impact the U.S. energy supply for transportation fuels. Despite their potential, the state of technology for producing algal biofuels is regarded by many in the field to be in its infancy and there is a considerable amount of RD&D is needed to achieve affordable, scalable, and sustainable algal-based biofuels.

About the Roadmap

The framework for 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 the Biomass Program to discuss and identify the critical challenges currently 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 the Program's RD&D efforts.

More than 200 stakeholders were 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 algal biofuels industry. The Workshop was able to capture the participants' experience and expertise during a series of technical break-out sessions that spanned critical aspects of the algal biomass supply chain and cross-cutting issues. The outcomes from the Workshop provided key inputs to the development of this Algal Biofuels Technology Roadmap. The full proceedings of the Workshop can be found at http://www.orau.gov/algae2008pro/.

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. All comments are noted in the Appendix of this document. Every attempt was made to ensure that the Roadmap development process was transparent and inclusive.

This Roadmap presents information from a scientific, economic, and policy perspectives that can support and guide RD&D investment in algal biofuels. While addressing the potential economic and environmental benefits of using algal biomass for the production of liquid transportation fuels, the Roadmap describes the current status of algae RD&D. In doing so, it lays the groundwork for identifying challenges that likely need to be overcome for algal biomass to be used in the production of economically viable biofuels.

1.1 America's Energy Challenges

As global petroleum supplies diminish, the United States is becoming increasingly dependent upon foreign sources of crude oil. The United States currently imports approximately two-thirds of its petroleum, 60% of which is used for producing transportation fuels. The rising energy demand in many rapidly developing countries around the world is beginning to create intense competition for the world's dwindling petroleum reserves. Furthermore, the combustion of petroleum-based fuels has created serious concerns about climate change from the greenhouse gas (GHG) emissions.

In 2007, the *Energy Independence and Security Act* (EISA) was enacted, which set new standards for vehicle fuel economy, as well as made provisions that promote the use of renewable fuels, energy efficiency, and new energy technology research and development. The legislation establishes production requirements for domestic alternative fuels under the Renewable Fuels Standard (RFS) that increase over time (Exhibit 1.1).

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. Significant acreage and productivity will be required for biomass production to generate sufficient feedstock to meet the RFS mandates. Cellulosic feedstocks were identified by the Billion Ton Study as a significant source of biomass (Perlack et al., 2005). However, the study did not explore the potential of algae, while algae may offer comparable biomass productivity as lignocellulosic feedstocks – the key biomass resource factored in the study.

Many pathways are currently 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 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 (Exhibit 1.2). Potential oil yields from certain algae strains are projected to be at least 60 times higher than from soybeans, approximately 15 times more productive than jatropha, and approximately 5 times that of oil palm per acre of land on an annual basis (Rodolfi et al., 2009).

UNIQUE ADVANTAGES OF ALGAL FEEDSTOCK FOR ADVANCED BIOFUELS

- High area productivity
- Minimizes competition with conventional agriculture
- Utilizes a wide variety of water sources
- Recycles stationary emissions of carbon dioxide
- Compatible with integrated production of fuels and co-products within biorefineries

Exhibit 1.1 *Renewable Fuel Standard volume requirements (billion gallons)*
Cellulosic biofuels and biomass-based diesel are included in the advanced biofuel requirement.

	CELLULOSIC BIOFUEL REQUIREMENT	BIOMASS-BASED DIESEL REQUIREMENT	ADVANCED BIOFUEL REQUIREMENT	TOTAL RENEWABLE FUEL REQUIREMENT
2009	N/A	0.5	0.6	11.1
2010	0.1	0.65	0.95	12.95
2011	0.25	0.80	1.35	13.95
2012	0.5	1.0	2.0	15.2
2013	1.0	a	2.75	16.55
2014	1.75	a	3.75	18.15
2015	3.0	a	5.5	20.5
2016	4.25	a	7.25	22.25
2017	5.5	a	9.0	24.0
2018	7.0	a	11.0	26.0
2019	8.5	a	13.0	28.0
2020	10.5	a	15.0	30.0
2021	13.5	a	18.0	33.0
2022	16.0	a	21.0	36.0
2023	b	b	b	b

[a] *To be determined by EPA through a future rulemaking, but no less than 1.0 billion gallons.*
[b] *To be determined by EPA through a future rulemaking.*

Advantages of Algal Feedstocks

Algae can be preferred feedstock for high energy density, fungible liquid transportation fuels. There are several aspects of algal biofuel production that have combined to capture 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 waste water, produced water, and saline water, thereby reducing competition for limited freshwater supplies.
- Algae can recycle carbon from CO_2-rich flue 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.

Exhibit 1.2 *Comparison of oil yields from biomass feedstocks*[a]

CROP	OIL YIELD (GALLONS/ACRE/YR)
Soybean	48
Camelina	62
Sunflower	102
Jatropha	202
Oil palm	635
Algae	1,000-6,500[b]

[a] *Adapted from Chisti (2007)*
[b] *Estimated yields, this report*

1.2 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 emerged, 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 lipid 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 through the decade; this idea ultimately became a major push of 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, 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 project was completed (Sheehan et al., 1998). Some of the highlights are described briefly below.

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 U.S. 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 conditions, some species in the collection were shown to accumulate as much as 60% of their dry weight in the form of lipid, primarily triacylglycerides (TAGs) (Chisti, 2007).

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 et al., 1999; Sheehan et al., 1998).

Additional studies focused on storage carbohydrate production, as 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, chrysolaminarin in *C. cryptica* were characterized (Roessler, 1987 and 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 one of 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, demonstration-scale 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 inocula 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 the 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 unextracted 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/barrel in 1995.

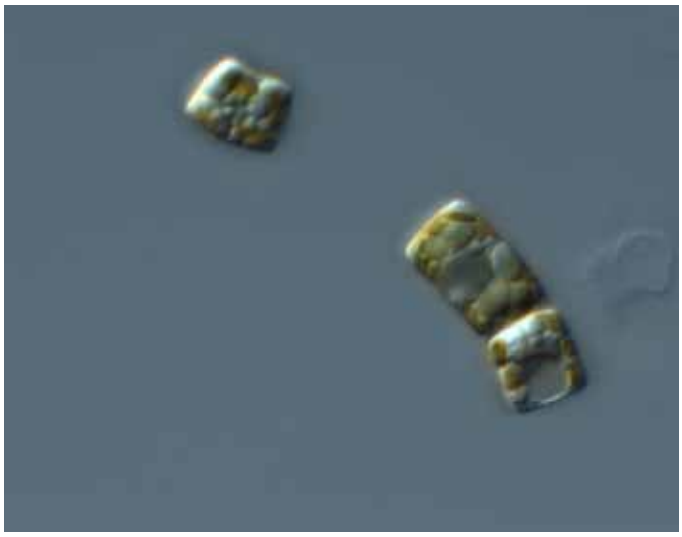
Overall, the Aquatic Species Program was successful in demonstrating the feasibility of algal culture as a source of oil and resulted in important advances in the technology. However, it also became clear that significant barriers would need to be overcome in order to achieve an economically feasible 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 Web site at www.nrel.gov/publications.

Also from 1968-1990, DOE 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 (SNG) 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 SNG would not be cost-competitive with fossil fuel gas.

Research from 1996 to Present

Since the end of DOE's Aquatic Species Program in 1996, federal funding for algal research has come from DOE, the Department of Defense, the National Science Foundation, and the Department of Agriculture. Recent initiatives, such as a major Defense Advanced Research Projects Agency solicitation, the Air Force Office of Scientific Research (AFOSR) algal bio-jet program, and several DOE Small Business Innovative Research (SBIR) request for proposals, suggest that funding levels are beginning to increase. Additionally, DOE's Advanced Research Projects Agency-Energy (ARPA-E), Office of Science, Office of Fossil Energy, and Biomass Program are all funding research activities that include investigating microalgae, cyanobacteria, and macroalgae for biofuels and beneficial re-use of CO_2.

Additionally, a number of U.S. national labs are increasingly focusing on algal biofuels research. State funding programs and research support from private industry also make up a significant proportion of research funding. Private investment in algal biofuels has been increasing at a dramatic rate over the last few years, significantly outpacing government funding.



Cyclotella cells, Courtesey of CCMP.

1.3 Algae-to-Biofuels: Opportunity and Challenges Today

Abundant, affordable, and sustainable feedstocks are the lifeblood of the burgeoning biofuels industry today. Algae must be considered as part and parcel of the feedstock mix for producing advanced biofuels. In contrast to the development of cellulosic biofuels which benefit from a direct agricultural and process engineering lineage, there is no parallel agricultural enterprise equivalent for cultivating algae at a similar scale. A sizable and strategically structured investment to tackle the challenges of algal biofuels is thus needed to support commercialization activities.

Based on the information provided at the Workshop, it was determined that a great deal of RD&D is still necessary to reduce the level of risk and uncertainty associated with the algae-to-biofuels process so it can be commercialized. Further, these activities must be accompanied with conducive developments on the non-technical fronts – regulations and standards, and public-private partnerships. By reviewing the technology gaps and cross-cutting needs, the Roadmap aims to guide researchers and engineers, policymakers, federal agencies, and the private sector in implementing a nationally coordinated effort toward developing a viable and sustainable algal biofuel industry.

Technology and Analysis Challenges

This Roadmap seeks to lay down the first comprehensive state of technology summary for fuels and co-products from algal feedstocks and to document the feasibility and techno-economic challenges associated with commercial scaling up of processes.

OVERCOMING BARRIERS TO ALGAL BIOFUELS: TECHNOLOGY GOALS

	PROCESS STEP	R&D CHALLENGES
FEEDSTOCK	Algal Biology	• Sample strains from a wide variety of environments for maximum diversity • Develop small-scale, high-throughput screening technologies • Develop open-access database and collections of existing strains with detailed characterization • Investigate genetics and biochemical pathways for production of fuel precursors • Improve on strains for desired criteria by gene manipulation techniques or breeding
	Algal Cultivation	• Investigate multiple approaches (i.e., open, closed, hybrid, and coastal/off-shore systems; phototrophic, heterotrophic, and mixotrophic growth) • Achieve robust and stable cultures at a commercial scale • Optimize system for algal productivity of fuel precursors (e.g., lipids) • Sustainably and cost-effectively manage the use of land, water, and nutrients • Identify and address environmental risks and impacts
	Harvesting and Dewatering	• Investigate multiple harvesting approaches (e.g., sedimentation, flocculation, dissolved air floatation, filtration, centrifugation, and mechanized seaweed harvesting) • Minimize process energy intensity • Lower capital and operating costs • Assess each technology option in terms of overall system compatibility and sustainability
CONVERSION	Extraction and Fractionation	• Investigate multiple approaches (e.g., sonication, microwave, solvent systems, supercritical fluid, subcritical water, selective extraction, and secretion) • Achieve high yield of desired intermediates; preserve co-products • Minimize process energy intensity • Investigate recycling mechanisms to minimize waste • Address scaling challenges, such as operational temperature, pressure, carrying capacity, side reactions, and separations
	Fuel Conversion	• Investigate multiple approaches to liquid transportation fuels (e.g., direct fuel production, thermochemical/catalytic conversion, biochemical conversion, and anaerobic digestion) • Improve catalyst specificity, activity, and durability • Reduce contaminants and reaction inhibitors • Minimize process energy intensity and emissions over the life cycle • Achieve high conversion rates under scale-up conditions
	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 enzymes, 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	• Assess and characterize land, climate, water, energy, and nutrient resource requirements for siting of microalgae (heterotrophic & photoautotrophic) and macroalgae production systems • Integrate with wastewater treatment and/or CO_2 emitter industries (in the case of heterotrophic approach) • Address salt balance, energy balance, water & nutrient reuse, and thermal management

PURSUING STRATEGIC R&D: TECHNO-ECONOMIC MODELING AND ANALYSIS

Given the multiple technology and system options and their interdependency, an integrated 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.

Regulations and Standards

While the Roadmap's primary objective is to highlight the technical challenges and opportunities associated with algal biofuels commercialization, it is recognized that RD&D activities need to be carried out under a framework of standards, regulation, and policy. Algal biofuel developers need to foresee and understand the potentially applicable legal requirements early on in the research and development process to help ensure algae are legally and safely developed and the end-products (i.e., biofuels and co-products) comply with applicable consumption standards. Being a nascent industry, there are no existing standards for various aspects of algal biofuels production. However, RD&D activities can inform further development of applicable laws and standards.

Public-Private Partnerships

A collaborative framework of public-private partnerships offers an opportunity to jointly address the technological, economic, and policy and regulatory challenges as resolution of these issues will likely require participation from multiple entities. However, structuring public-private partnerships for successful ventures is a challenge in itself given the myriad issues and interests, such as intellectual property rights. Also, supporting education will be critical to create intellectual talent and the workforce needed to allow this industry to grow.

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2. Algal Biology

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

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. Because it is not yet known how algae will be cultivated on a mass scale, new strains should be isolated from a wide variety of environments to provide the largest range in metabolic versatility possible.

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). Furthermore, large-scale sampling efforts should be coordinated to ensure broadest coverage of environments and to avoid duplication of efforts. The selection of specific locations can be determined by sophisticated site selection criteria through the combined use of dynamic maps, Geographic Information Systems (GIS) data and analysis tools. Ecosystems to be sampled could include aquatic (i.e., oceans, lakes, rivers, streams, ponds, and geothermal springs, which includes 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 ownership 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) and benthic (attached) environments. Planktonic algae may be used in suspended mass cultures, whereas 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 enrichment cultures (Andersen and Kawachi, 2005). However, some algal strains take weeks to months to be isolated by traditional methods (for a comprehensive review of algal culturing techniques, see Anderson, 2005). For large-scale sampling and isolation efforts, high-throughput automated isolation techniques involving fluorescence-activated cell sorting (FACS) have proven to be extremely useful (Sieracki et al., 2004). Because of morphological similarities when comparing many algal species, actual strain identification should be based on molecular methods such as rRNA sequence comparison, or in the case of closely related strains, other gene markers.

Screening Criteria and Methods

An ideal screen would cover three major areas: growth physiology, metabolite production, and strain robustness. The term growth physiology encompasses a number of parameters such as maximum specific growth rate, maximum cell density, tolerance to environmental variables (temperature, pH, salinity, oxygen levels, CO_2 levels), 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.

Screening for metabolite production may involve determining the cellular composition of proteins, lipids, and carbohydrates, 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 desired. For example, a helpful screen for oil production would allow for distinction between neutral and polar lipids, and would provide fatty acid profiles. Furthermore, many strains also secrete metabolites into the growth medium. Some of these could prove to be valuable co-products, and new 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, community stability, and susceptibility to 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 will need to be performed. The development of small-scale but high-throughput screening technologies is an important step in enabling the testing of hundreds to thousands of different algal isolates.

At this time, 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. Solvent extraction, for example, is the most common method for determination of lipid content in algae, but it requires a significant quantity of biomass (Ahlgren et al., 1991;) (Bligh and Dyer, 1959). Fluorescent methods using lipid-soluble dyes have also been described, and though these methods require much less biomass (as little as a single cell), it has not yet been established if these methods are valid across a wide range of algal strains (Elsey et al., 2007; de la Jara et al., 2003). Further improvements in analytical methodology could be made through the development of solid-state screening methods. Not only are rapid screening procedures necessary for the biofuels field, but could prove extremely useful for the identification of species (particularly in mixed field samples) necessary for the future of algal ecology. It could also reduce the number of redundant screens of algal species.

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. They may also support research on determining strain characteristics, 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 exacerbated 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 strain, open access repository could accelerate R&D of algae-based biofuels production systems.

A number of algal strains are currently available from culture collections such as UTEX (The Culture Collection of Algae at the University of Texas at Austin, Texas), with about 3,000 strains, and CCMP (The Provasoli-Guillard National Center for Culture of Marine Phytoplankton at the Bigelow Laboratory for Ocean Sciences in West Boothbay Harbor, Maine), with more than 2,500 strains. However, because many of the strains in these collections have been cultivated for several decades, some may have lost original properties, such as mating capability or versatility regarding nutrient requirements. To obtain versatile and robust strains that can be used for mass culture in biofuels applications, it would be prudent to consider the isolation of new, native strains directly from unique environments. For both direct breeding and metabolic engineering approaches to improve biofuels production, it will be important to isolate a wide variety of algae for assembly into a culture collection that will 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 algae resource centers. Culture collection organizations could be responsible for the gathering and dissemination of detailed information about potentially valuable strains. Information could include:

1. Strain name (species, subspecies name, taxonomy, reference)
2. Strain administration (number in collection, preservation)
3. Environment and strain history (specific habitat, collector)
4. Strain properties: Cytological, biochemical, molecular, & screening results
5. Mutants
6. Plasmids and Phages
7. Growth conditions (media, temperature, pH) & germination conditions
8. Biological interaction (symbiosis, pathogenicity, toxicity)
9. Practical applications (general and industrial)
10. Omics data (Genomics, Transcriptomics, Proteomics, or Metabolomics)

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 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. However, it was shown in the Aquatic Species Program that not all strains that grow well in the laboratory are suitable for large-scale culturing (Sheehan, 1998), so it is possible that other strains will be chosen for production. Adapting the lessons learned from laboratory model strains to strains known to be capable of large-scale growth may be feasible, but we cannot be certain that laboratory strains and production strains will be sufficiently related to allow for lessons from the former to be applied to the latter.

Useful Algal Characteristics

Culture stability over long periods will be a key to low cost production of biofuels. Rapid growth is important both for overall productivity and the ability to compete with contaminating strains. Other traits like 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. Resistance to predators and viruses could also be a useful phenotype. 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 H_2, 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 supplements, or materials for nanotechnology (in the case of the silica cell wall of diatoms). 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.

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 step. However, there may be practical problems to consider, such as, if the desired product is volatile, then collection of the atmosphere above the culture will be necessary to isolate it, which will necessitate the use of closed bioreactors. 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 sustainable secretion could be achieved, it is not clear what the effect of a lipid emulsion in an algal culture would be. For example, an abundance of exported lipids could unfavorably alter fluidics properties or provide a carbon source favoring growth of contaminants. Finally, secretion of either intermediates or products into the growth medium could make these compounds vulnerable to contaminating microbes for catabolism. Pilot-scale experimentation and further metabolic engineering is required to evaluate possible advantages and disadvantages of secretion.

Capability for Heterotrophic or Mixotrophic Growth

Heterotrophic or mixotrophic growth capabilities may be attractive attributes of algal strains. In some species, addition of supplemental carbon results in increased lipid accumulation (Xu et al. 2006), even under mixotrophic conditions where the substrate is not known to be transported into the cell (Ceron Garcia et al., 2006). If the carbon source is utilized by the cell, growth in both light and dark periods is possible, and high cell densities can be achieved. A potential disadvantage of the addition of external carbon sources is 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.

2.2 Algal Physiology and Biochemistry

Many algae are photosynthetic organisms capable of harvesting solar energy and converting CO_2 and water to O_2 and organic macromolecules such as carbohydrates and lipids. Under stress conditions such as high light or nutrient starvation, some microalgae accumulate lipids such as triacylglycerols (TAG) as their main carbon storage compounds. Certain microalgal species also naturally accumulate large amounts of TAG (30-60% of dry weight), and exhibit photosynthetic efficiency and lipid production at least an order of magnitude greater than terrestrial crop plants (Hu et al., 2008). Cyanobacteria and macroalgae, as a general rule, accumulate mostly carbohydrates, with 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). 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 biofuels production.

Photosynthesis and Light Utilization

When algae are cultivated photosynthetically, the efficiency of photosynthesis is a crucial determinate in their productivity, affecting growth rate, biomass production, and potentially, the percent of biomass that is the desired fuel precursor. Though theoretical biomass productivity values in the range of 100-200 g/m^2/day have been presented (Chisti, 2007), there is no current consensus on the true maximum productivity of algae. Theoretical productivity is an important concept, however, because it can be used to set achievable goals for both cultivation process design and strain improvement projects. It may be useful to carry out photosynthetic efficiency studies in algae similar to those that have been carried out for plants (Zhu et al., 2007 and 2008)

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). Regardless of the cultivation practices used to maximize light exposure (see Chapter 3), there remains limitations of algal photosystems regarding light utilization. The majority of light that falls on a photosynthetic algal culture at greater than laboratory scale 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 (Christi, 2007). Even when exposed to high light, algal photosystems have built-in strategies to prevent the over-absorption of light energy, which can lead to oxidative damage. A large majority of absorbed incident light is dissipated as heat and could be considered "wasted." Even with this photoprotection, under certain light regimes, photoinhibition or the reduction of photosynthesis due to light damage can still occur (Long et al., 1994; Foyer et al., 1994; and Niyogi, 1999). In an effort to overcome this barrier, it was shown that reducing the size of the chlorophyll antenna can increase the efficiency of light utilization (Polle et al., 2002).

There is still much to learn about the dynamics and regulation of the photosynthetic apparatus (Eberhard et al., 2008). 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.

Carbon Partitioning and Metabolism

Knowing how and when carbon is partitioned in a cell 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.

Further, a link between starch and lipid metabolism has been established. Starch is a common carbon and energy storage compound in plants and algae, and shares the same precursors with the storage lipid TAG (Exhibit 2.1). It is, therefore, possible that TAG and starch could be inter-convertible, a potentially important implication for biofuel production. In young Arabidopsis seeds and Brassica embryos, starch transiently accumulated and starch metabolism was most active before the lipid accumulation phase (Kang and Rawsthorne, 1994; Ruuska et al., 2002), indicating that starch is an important storage compound and its synthesis precedes oil accumulation. More recently, studies in higher plants showed that when

starch synthesis was impaired or inhibited, plant embryos or seeds accumulated 40% less oil (Periappuram et al., 2000; Vigeolas et al., 2004). While these results provide an indication that starch synthesis is linked to lipid synthesis, the nature of the interaction is unknown.

In microalgae, such an interaction has been also indicated by studies on the diatom Cyclotella cryptica (Roessler, 1988). It could, therefore, be fruitful to further research de novo starch synthesis, degradation, and interaction with lipid metabolism in algae.

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, it is important to determine the predominate types that are present.

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 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). These major storage polysaccharides represent potential biochemical feedstocks for conversion to liquid fuels. Microorganisms capable of fermenting laminarin and mannitol from *Laminaria hyperborea* to ethanol have been identified and partially characterized (Horn et al., 2000a and 2000b). Other abundant polysaccharides, for example 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. 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. 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 triacyglycerols (TAG), 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 synthesis of cellular and organelle membranes, as well as of neutral storage lipids such as TAG, use 16 or 18 carbon fatty acids as precursors. 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 (ER) (Exhibit 2.1).

TAG biosynthesis in algae has been proposed to occur via the above 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 (PA) (Ohlrogge and Browse, 1995). Dephosphorylation of PA 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, 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). Alternative pathways to convert membrane lipids and/or carbohydrates to TAG have recently been demonstrated in bacteria, plants and yeast in an acyl CoA-independent way (Arabolaza et al., 2008;

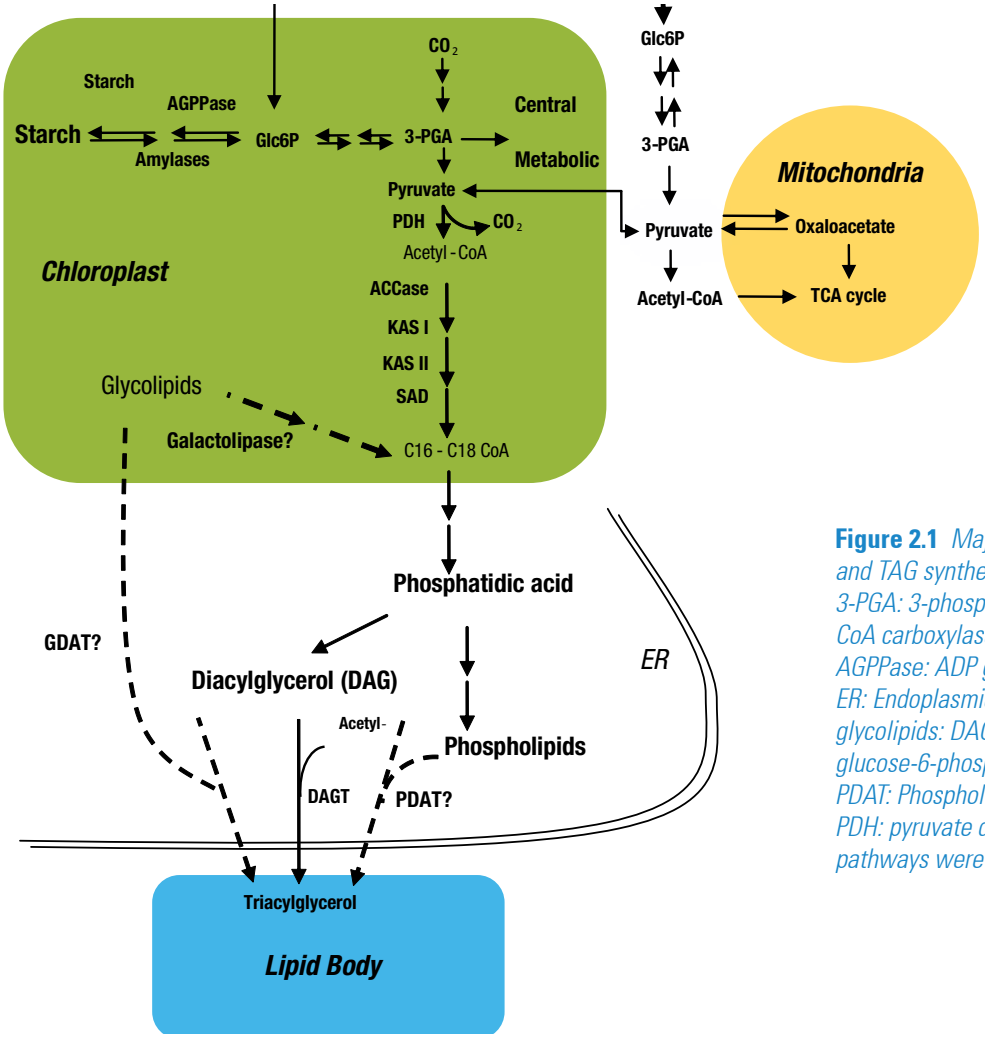

Figure 2.1 *Major pathways for fatty acid and TAG synthesis in plants and algae 3-PGA: 3-phosphoglycerate; Accase: acetyl CoA carboxylase; ACP: acyl carrier protein; AGPPase: ADP glucose pyrophosphorylase; ER: Endoplasmic reticulum; GDAT: putative glycolipids: DAG acyltransferase; Glc6P: glucose-6-phosphate; KAS: 3-ketoacyl-ACP; PDAT: Phospholipids: DAG acyltransferase; PDH: pyruvate dehydrogenase (putative pathways were proposed in dashed lines).*

Dahlqvist et al., 2000; Stahl et al., 2004). Such pathways have not yet been studied in algae. Moreover, PA and DAG can also be used directly as substrates for synthesis of polar lipids, such as phosphatidylcholine (PC) 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 relatively poorly understood. This lack of understanding 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). Understanding 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 starch, 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 ER and assembled into lipids for cellular membranes. Some glycerolipids assembled at the ER 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). 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. TAG synthesis may also utilize phosphatidylcholine, phatidylethanolamine or toxic fatty acids excluded from the membrane system as acyl donors, thereby serving as a mechanism to "detoxify" these compounds and deposit them in the form of TAG. 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

Despite the economic importance of algae as a source of a wide range of lipophilic products, including vitamins, hydrocarbons and very long-chain ω-3 and ω -6 fatty acids, there have been relatively few studies on lipid bodies in algae compared with plants and fungi. 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).

Lipid bodies may dock with different regions of the ER 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, 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 Expressed Sequence Tag (EST) analysis, cDNA microarray analysis, and proteomic studies, for example, would also 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.

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 Exhibit 2.2) (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 (FD) 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, those containing iron (which are generally H_2-evolving), or 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: (a) the O_2 sensitivity of hydrogenases, (b) competition for photosynthetic reductant at the level of ferredoxin, (c) regulatory issues associated with the over production of ATP, and (d) inefficiencies in the utilization of solar light energy (Seibert et al., 2008). These challenges could be addressed by (a) engineering hydrogenases with improved tolerance to O_2 (Cohen et al., 2005), (b) identifying metabolic pathways that compete with hydrogenases for photosynthetic reductant, and engineering their down-regulation during H_2 production (Mathews and Wang, 2009), (c) engineering the photosynthetic membrane for decreased efficiency of photosynthetic-electron-transport-coupled ATP production (ATP is not required for H_2 production), and (d) engineering the photosynthetic antenna pigment content for increased efficiency of solar light utilization (Polle et al., 2003).

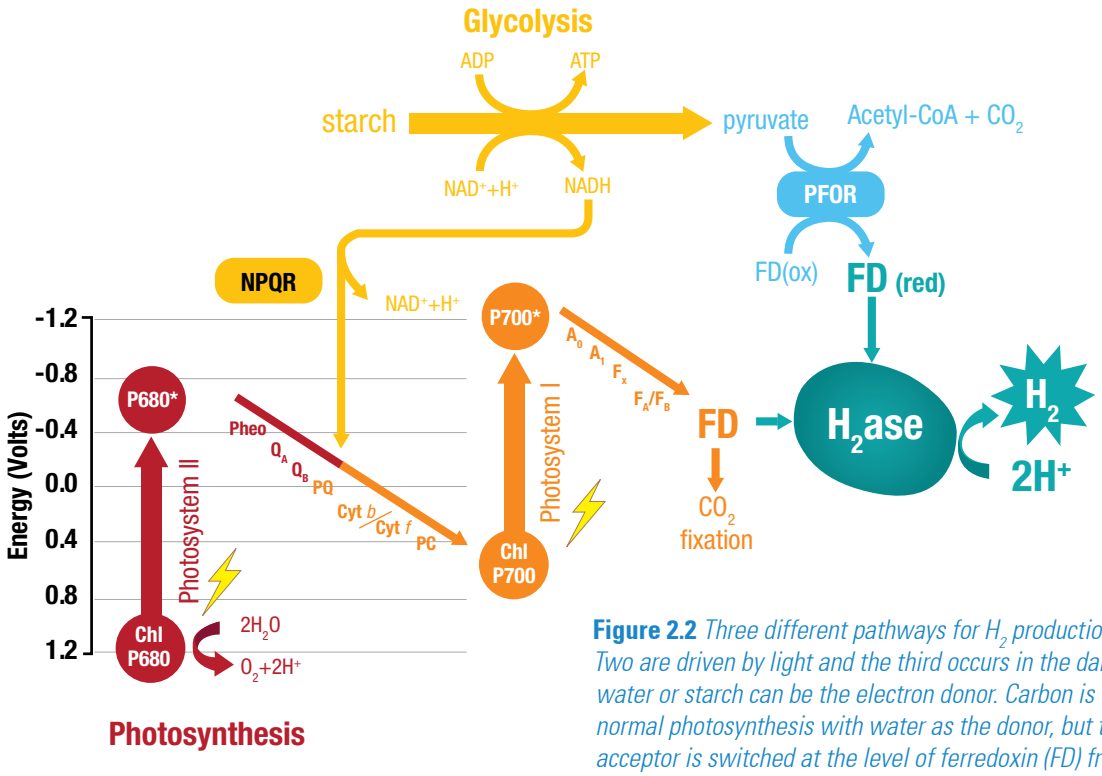

Figure 2.2 *Three different pathways for H_2 production Two are driven by light and the third occurs in the dark. Either water or starch can be the electron donor. Carbon is fixed under normal photosynthesis with water as the donor, but the electron acceptor is switched at the level of ferredoxin (FD) from CO_2 to protons under conditions that lead to H_2 production. (Drawing courtesy of Prof. M. Posewitz, Colorado School of Mines)*

Recently 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., 2009). A possibility to improve the efficiency of biological H2 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 required.

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 (EMP) pathway for degradation of glucose to pyruvate. From here, 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 (Toepel 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 *Gloeocapsa alpicola* can evolve H_2 from the fermentation of stored glycogen (Serebryakova et al., 1998). Similarly under non-N2 fixing condition, the hydrogenase from Cyanothece PCC 7822 produces H_2 in the dark and also excretes typical fermentation byproducts 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 mol 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: (a) improve photosynthetic efficiency to increase the yield of carbohydrate accumulation; (b) remove or down-regulate competing fermentative pathways thus directing more of the cellular flux toward H_2 production; and (c) learn to express multiple hydrogenases so that electrons from both ferredoxin (Fd) and NAD(P)H can serve as electron donors to support H_2 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 algae genetically remain far behind those developed for commonly used bacteria, fungi and higher plants. Efforts should 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 subsection seeks to 1) address the genetic tools available to modify algal strains, 2) describes enabling technologies and analyses that can be applied for biofuels and bioproducts, and 3) highlights 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 3 and 4.

The Genetic Toolbox

Because biological productivity is a 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 genes, to delete or disrupt genes, 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, the toolbox allows traits to 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 this approach include the introduction of multiple mutations in a genome and the difficulty in mapping the locus responsible for the phenotype.

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), or increased neutral lipid accumulation via Nile Red staining (Cooksey et al., 1987) 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 (GE) algae—something that could be desirable for large-scale algal production.

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 on an extra-chromosomal element called a plasmid. 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). An important consideration is that the use of antibiotics in large-scale production has 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 may need to be considered for production scale.

For research purposes, 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). 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.

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 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*), green algae (*Chlamydomonas, Dunaliella, Chlorella, Volvox*), diatoms (*Cyclotella, Navicula, Phaeodactylum, Thalassiosira*), dinoflagellates (*Amphidinium,*

Symbiodinium), red algae (*Cyanidioschyzon, Porphyridium, Gracilaria, Porphyra*), brown algae (*Laminaria, Undaria)* 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). Other successful methods include electroporation (Shimogawara et al., 1998), vortexing with glass beads (Kindle et al., 1991) or silicon carbide whiskers (Dunahay, 1993). 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 GE 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" though a series of sexual crosses. Many macroalgae species are capable of sexual reproduction, and traditional mutagenesis and breeding has been used to improve commercial varieties of seaweed since the 1950s (Bird and Benson, 1987). With the exception of *Chlamydomonas*, 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.

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).

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. 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 PCR or high throughput transcriptomics (i.e., microarrays). In addition, inducible and repressible promoters that can be actuated by simple manipulations are desirable, allowing for precise control over the timing of gene expression. 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). Identification of other inducible or repressible control elements would be useful for both research and commercial applications.

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 approaches have been investigated primarily in the model green alga, *Chlamydomonas reinhardtii*, although there is a recent report of the method being used in a red alga (Ohnuma et al., 2009). 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 *C. 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).

Directed Evolution of Enzymes/Proteins

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 so that they function more efficiently or have other favorable characteristics could be valuable for the development of algal biofuels technology.

Protein Tagging Technologies

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).

Enabling Technologies: "Omics" Approaches and Bioinformatics

High throughput approaches, such as genomics, transcriptomics, and proteomics, have enabled in-depth analyses to be performed in a whole cell context. Together, these methods have revolutionized the study of organisms both in culture and in natural habitats. These biological 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.

Sequencing and Annotating Algal Genomes

Sequenced genomes are an essential basis of information for the interpretation of transcriptomic and proteomic data. 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 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). Even though new sequencing technologies readily enable accumulation of data for large genomes, assembly of such data (especially with short read lengths) can be more challenging in repeat-laden genomes.

Eukaryotic algae constitute members from at least eight major phyla, all featuring a complex series of primary and secondary endosymbioses (Falkowski et al., 2004). 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. 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 over 20 completed genome sequences are available, the nuclear genomes of only a handful of microalgal species have been fully or partially sequenced to date.[1] These species include unicellular green algae (*C. reinhardtii*, *Volvox carteri*), a red alga (*Cyanidioschizon merolae*), several picoeukaryotes (*Osteococcus lucimarinus*, *Osteococcus tauris*, *Micromonas pussilla*, *Bathycoccus sp.*), a pelagophyte (*Aureococcus annophageferrens*), a coccolithophore (*Emiliania huxleyi*), and several diatoms (*Phaeodactylum tricornutum*, *Thalassiosira pseudonana*, *Fragilariopsis cylindrus*). Genome sequencing projects are also underway for the macroalga Porphyra purpurea at the Joint Genome Institute (U.S. Department of Energy) and for Ectocarpus siliculosus at Genoscope - Centre National de Séquençage (France). 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

[1] For a listing, visit http://genome.jgi-psf.org/euk_cur1.html

include the standardization of using a particular sequencing approach that provides sufficient coverage of ESTs 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.

Currently, at least10 other algal genomes, as well as another dozen or so EST projects, are underway. It is notable that while these projects represent a very useful survey, the rationale for sequencing these organisms was not related to lipid production or other biofuels efforts. Therefore, there are still a large number of useful algal species to sequence that are related to biofuels production, such as *Dunaliella sp., Nannochloropsis sp., Scenedesmus sp., Chlorococcum sp., and Pseudochlorococcum sp.*

Transcriptomics

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 (for example, 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.

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 (Bartel, 2004; Cerutti and Casas-Mollano, 2006). Small RNAs have been identified in microalgae (Zhao et al., 2007) and should be considered in investigations of gene expression regulation, especially with regard to translational regulation.

Proteomics

The cellular complement of proteins reflects its metabolic potential, and ultimately determines how a cell functions in response to the environment. Mass-spectrometry approaches allow for robust evaluation of soluble and membrane-associated proteins in the form of protein peptides. 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). In the absence of genomic information, proteomic approaches can also help assess the metabolic potential of organisms that may be difficult to isolate, or can determine functional diversity of a community of organisms.

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 liquid chromatography mass spectrometry, gas chromatography mass spectrometry, and nuclear magnetic resonance (Dunn et al., 2005). There is a distinction between metabolomics, which involves the identification and analysis of metabolites, and metabonomics, which is the quantitative measurement of the dynamic multi-parametric metabolic response of living systems to pathophysiological stimuli or genetic modification (Nicholson et al., 1999). Both metabolomics and metabonomics may be important in terms of algal biofuels research. Lipids are a subset of the molecular repertoire of the algae cell. Lipid analysis is done using mass spectrometry approaches (Han and Gross, 2005; Dettmer et al., 2007). 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. Monitoring lipid characteristics under a variety of different cultivation regimes can also help inform process engineering and improve yields (Yu et al., 2009).

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. 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).

Cyanobacteria generally do not accumulate storage lipids, but they are prolific carbohydrate and secondary metabolite producers. Some strains can double quickly (less than 10 hours), and some strains can fix atmospheric nitrogen and produce hydrogen. Moreover, many can be genetically manipulated, making them attractive organisms for biofuels production.

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 over 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 (Singh et al., 2008; Fulda et al., 2006; Koksharova et al., 2006; Eisenhut et al., 2008).

Transgenic approaches have enabled the production and secretion of cellulose, sucrose (Nobles and Brown, 2008), ethanol (Deng and Coleman, 1999), and isobutanol (Atsumi et al., 2009) in *Synechococcus*. Relatedly, *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).

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; Xiaoling Miao et al., 2003). It has also been shown that PHB accumulates under nitrogen depleting conditions (Miyake et al., 2000). It has not been shown how these pathways can be manipulated for the benefit of biofuels production. 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 over 3 billion years of evolution, and are highly diverse (Falkowski et al., 2004). Multiple endosymbiotic events occurred during the evolution of microalgae. These events likely had significant effects on the metabolic pathways and regulation of fuel precursor synthesis. 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 extents of endosymbiosis (Wilhelm et al., 2006). Continued exploration of the evolutionary diversity of algae is important to identify species that are adept at making fuel precursors and 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, it can be sexually crossed. It is not an abundant lipid producer, but nevertheless, *C. 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. A possible drawback of *C. reinhardtii* is the fact that nuclear expression of foreign genes is still problematic due to codon bias (Heitzer et al., 2007), RNA silencing (Cerutti et al., 1997), and positional effects (Ferrante et al., 2008). However, strategies to address these issues are being developed, and stable and successful expression of foreign genes in *C. reinhardtii* has been recently been reported (Neupert et al., 2009; Ferrante et al., 2008). Chloroplast transformants are also stable, and chloroplast protein expression systems are well developed.

Chlorella is another well-studied genus of green algae, and some species are abundant lipid producers. In *C. protothecoides*, the addition of an external carbon source induces heterotrophic growth, which increases both growth rate and lipid production, resulting in greater than 50% dry weight lipid (Xu et al., 2006). The genome sequence of *Chlorella* NC64A was recently completed.[2] Several examples of Chlorella transformation have been reported, although the stability of the expression of the foreign genes is still questionable (Leon and Fernandez, 2007).

Dunaliella salina has several useful characteristics for large-scale biofuels production. It produces abundant lipids (Weldy and Huesemann, 2007), and because it has outstanding salt tolerance (from 0.1 M to near saturation), it can be grown under extreme conditions that could reduce the growth of possible contaminating organisms. The genome sequence of *D. salina* is currently being determined (estimated size 130 Mb), and transgenic strains have been reported (Li et al., 2007).

Diatoms were a major focus in the Aquatic Species Program given their ability to accumulate high amounts of lipids (Sheehan et al., 1998). Diatoms are responsible for 20% of the total global carbon fixation (Armbrust et al., 2004), suggesting favorable growth rates for biomass production. A distinguishing feature of diatoms is their silica cell walls,

[2] http://genome.jgi-psf.org/ChlNC64A_1/ChlNC64A_1.home.html

and their requirement for silicon as a nutrient for growth. Silicon limitation is one trigger for lipid accumulation in diatoms. This is advantageous for studying the lipid induction response, because silicon metabolism is not believed to be tightly coupled with the metabolism of other nutrients. Two diatom genome sequences are complete (Armbrust et al., 2004; Bowler et al., 2008), and four more are underway.[3] None of the sequencing projects has thus far been focused explicitly on biofuels. Transgenic techniques are well established for several diatom species (Dunahay et al., 1995; Apt et al., 1996; Fischer et al., 1999; Zaslavskaia et al., 2000), and regulatory gene expression control elements have been identified (Poulsen and Kroger, 2005). With the development of robust gene silencing approaches and possibly with homologous recombination, the gene manipulation toolkit for diatoms will be fairly complete.

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. Unlike microalgae, they 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.

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 (Ali et al., 2001; Baldauf, 2003). In general, each of the major macroalgal groups has affinity with corresponding microalgal forms. For example, 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*.

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 (RFLPs) and random amplified polymorphic DNA (RAPD) 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., 2005), and strain selection and characterization (Jin et al., 1997; Meneses and Santelices, 1999; Niwa et al., 2005). 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.

Considerations of Genetic Modifications

Despite the great promise of GE 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 report, GE algae are defined as strains carrying coding sequences obtained from a foreign species. Since the beginning of the deployment of GE organisms, there have usually been built in safeguards to prevent the release of GE organisms to avoid potential disruption of ecosystems. However, even with these safeguards, there have been several unintended releases of GE organisms over the past 15+ 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 GE algae as production strains, development of genetic tools is still imperative from a research standpoint.

[3] http://www.jgi.doe.gov

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3. Algal Cultivation

3.1 Cultivation Pathways

Microalgae and Cyanobacteria

Given the multiple pathways to cultivating microalgae and cyanobacteria, it is premature to predict whether algal cultivation in closed (e.g., photobioreactors), open (e.g., open ponds) or hybrid systems will prevail in the industry. It is, therefore, important that cultivation R&D projects are closely associated with techno-economic (TE) analysis that can evaluate the cultivation system in the context of upstream and downstream processing to identify the best-suited cultivation system.

Broadly speaking, algae can be cultivated via photoautotrophic or heterotrophic methods, both varying in their challenges and advantages (Exhibit 3.1 compares the various features of both approaches).

Photoautotrophic Cultivation

For photoautotrophic cultivation strategies where algae require light to grow and create new biomass, capital costs for closed photobioreactor construction are currently higher than for open ponds raceways. However, it is important to acknowledge the advantages and disadvantages of both photoautotrophic cultivation approaches.

Traditionally, photobioreactors have suffered from problems of scalability, especially in terms of mixing and gas exchange (both CO_2 and O_2). Though photobioreactors lose much less water than open ponds due to evaporation, they do not receive the benefit of evaporative cooling and so temperature must be carefully maintained. Open ponds, however, are subject to daily and seasonal changes in temperature and humidity. Photobioreactors are unlikely to be sterilizable and may require periodic cleaning due to biofilm formation, 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 (Christi, 2007). Both types of cultivation systems must contend with maximizing light exposure. Many of these issues are being addressed through improved material usage and enhanced

Exhibit 3.1 *Comparative features of microalgal cultivation approaches*

		ADVANTAGES	CHALLENGES
Photoautotrophic Cultivation	Closed Photobioreactors	• Less loss of water than open ponds • Superior long-term culture maintenance • Higher surface to volume ratio can support higher volumetric cell densities	• Scalability problems • Require temperature maintenance as they do not have evaporative cooling • May require periodic cleaning due to biofilm formation • Need maximum light exposure
	Open Ponds	• Evaporative cooling maintains temperature • Lower capital costs	• Subject to daily and seasonal changes in temperature and humidity • Inherently difficult to maintain monocultures • Need maximum light exposure
Heterotrophic Cultivation		• Easier to maintain optimal conditions for production and contamination prevention • Opportunity to utilize inexpensive lignocellulosic sugars for growth • Achieves high biomass concentrations	• Cost and availability of suitable feedstocks such as lignocellulosic sugars • Competes for feedstocks with other biofuel technologies

engineering designs. Though TE analyses for both open pond and photobioreactor systems have been published or presented (see Chapter 10), much of the information used for these analyses is based on assumptions or proprietary data. As a result, it remains to be seen which system will be preferred at scale over long periods of operation. Additionally, in hybrid systems, photobioreactors could play a critical role as breeder/feeder systems linked to open raceways, providing high cell density algal inocula for production ponds (Ben-Amotz, 1995) or a series of linked turbidostats or chemostats (Benson et al., 2007).

Heterotrophic Cultivation

In heterotrophic cultivation, algae are grown without light and are fed a carbon source, such as sugars, to generate new biomass. This approach takes advantage of mature industrial fermentation technology, already widely used to produce a variety of products at large scale. Heterotrophic cultivation presents 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 inexpensive lignocellulosic sugars for algal growth. Heterotrophic cultivation also achieves high biomass concentrations that reduces the extent and cost of the infrastructure required to grow the algae (Xu, 2006). The primary challenges with this approach 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. A related approach is mixotrophic cultivation, which harnesses both the photoautotrophic and heterotrophic ability of algae.

Macroalgae

Macroalgae require unique cultivation strategies. 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 larger plant breeding community are now available to seaweed biologists and culturalists to advance the vegetative propagation of seaweeds through cell and tissue culture techniques. Although the field is still at an early stage of development, the micropropagation of plants is a concept that has been adopted by seaweed 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.et al., 2005, Reddy et al.et al., 2006) 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 off-shore, 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, 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. In the United States and Europe, environmental regulations and popular resistance against use of coastal regions for aquaculture represent challenges that will need to be overcome due to the conflicting uses of coastal zones.

Land-based pond systems have also been considered for macroalgal cultivation (Friedlander, 2008; Hanisak, 1987), 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). Advantages of the land-based systems over those in water have been listed as 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; and 5) possibility of farm operations located in close proximity to conversion operations (Chynoweth, 2002). 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 including the harvesting of naturally occurring marine algal blooms, and the use of algal mat or biofilm cultivation schemes. 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 a mixed or natural assemblage of organisms 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. The cost-benefit analysis of supplemental CO_2 in large-scale algal cultivation has yet to consider the intricacies of biological carbon concentration mechanisms (Wang and Spalding, 2006).

3.2 Scale-Up Challenges

The inherent difficulties of scaling up from laboratory to commercial operations present both technical and economic barriers to success. Because of the pervasiveness of issues related to scale, an investment in "open source" test bed facilities for public sector RD&D may foster more cultivation research.

Nutrient sources and water treatment/recycling are technically 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 unacceptable pathogens, chemical compounds, or heavy metals into the biomass stream (Hoffman et al., 2008; Wilson et al., 2009). Additionally, little is known about artificial pond ecology or pathology, and investigation into these areas will be important for the development of large-scale cultivation risk mitigation and remediation strategies.

Four broad cultivation challenges have emerged that are important to address for economically viable, commercial-scale algal cultivation:

- Culture stability;
- Standardized metrics for system-level productivity analysis;
- Nutrient source scaling, sustainability and management; and
- Water conservation, management, and recycling.

Stability of Large-Scale Cultures

Systems for large-scale production of biofuels from algae must be developed on scales that are orders of magnitude larger than all current worldwide algal culturing facilities combined. In certain cultivation systems, it will be challenging to maintain algal monocultures on this scale; it may become necessary to understand and manage the communities that will be present. Some members of the community will be of positive value, such as those that can scavenge and recycle nutrients or synthesize essential vitamins. Others will compete for shared resources, and still others will cause culture disruption. One of the more worrisome components of large-scale algae cultivation is the fact that algal predators and pathogens are both pervasive and little understood (Becker, 1994; Honda et al., 1999; Cheng et al., 2004; Brussaard, 2004). Fungal and viral pathogens are common, although current understanding of their diversity and host range is very limited. Wilson et al., (2009) point out that though there may be between 40,000 and several million phytoplankton species, there have only been 150 formal descriptions of phycoviruses. Chytrid fungi have also been known to cause the collapse of industrial algal cultivation ponds (Hoffman et al., 2008), but very little is known about host specificity and even less is known about host resistance mechanisms.

Important questions concerning this threat to large-scale algal cultures include:

- 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 bioreactors and open ponds?
- What prevention or treatment measures might limit such takeovers?

Methods for rapid, automated or semi-automated biological and chemical monitoring in production settings will be essential for assessing the health and compositional dynamics of algal cultures. The methods must be sensitive, selective, and inexpensive, as well as potentially provide for real-time monitoring. "Environmental" DNA sequence analysis can contribute to the development of PCR-based (Zhu et al., 2005; Boutte et al., 2006; Viprey et al., 2008) or flow-cytometry-based taxonomic assays, e.g., TSA-FISH (Marie et al., 2005). 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 for determining when decontamination and subsequent restart procedures represent the only alternative. This information will also inform efforts at developing 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 because of 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, a significant investment toward basic research in multi-trophic, molecular-level algal ecology will be an important component of the investment portfolio required for developing the potential of algae.

System Productivity

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 (Ketchum and Redfield, 1938; Shifrin and Chisholm 1981; Benemann and Oswald, 1996; Sheehan et al., 1998). More recent data suggest that high salt and high light stress in some marine phytoplankton may also result in increases in lipid content (Azachi et al., 2002). From a productivity standpoint, supplemental CO_2 has long been known to increase algal growth rate, and this area is receiving new attention from the search for renewable, sustainable fuels. New approaches are split between using algae to scrub CO_2 from emission gasses (Rosenberg et al., 2008; Douskova et al., 2009) and a focus on better understanding the mechanisms of biological CO_2 concentration from ambient air (Lapointe et al., 2008; Spalding 2008). There is justification to carry out R&D in both areas, as siting requirements for efficient algal cultivation may rarely coincide with high-volume point sources of CO_2 (see Chapter 9). The cost of CO_2 transportation and the volatile market for carbon credits will be a major challenge for TE feasibility studies; diverging business models are already apparent on these issues.

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 with mixed results (Cooksey et al., 1987; Reed et al., 1999; Eltgroth et al., 2005; Gao et al., 2008). These tools, as well as others such as Near Infra Red 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 Bodipy molecule with higher lipophilicity or lower quantum yields in aqueous solvent may prove to be more reliable indicators of algal lipid contents (Gocze and Freeman, 1994).

There is an immediate need to standardize productivity models and establish protocols for measurement of yields, rates, densities, metabolites, and normalization. Along with standards, coordinated research amongst 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, iron, and silicon (in the case of diatoms). Nitrogen, phosphorous, and iron additions represent a significant operating cost, accounting for 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 phosphate is in danger of running out (Abelson, 1999). 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, and the very large required energy inputs should be accounted for in life cycle analyses. 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. Note also that flue gas fed to algal cultures may provide some of the nitrogen and sulfur needed from NO_x and SO_x (Doucha, 2005; Douskova et al., 2009).

Careful control of nutrient levels is also critical. Limitation of a key nutrient will have serious impacts on biomass productivity, but 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 pose a problem for waste water 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 must 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 sources of nutrients will be important. Reagent grade sources of nutrients could make the price of a gallon of algal fuel cost-prohibitive. Agricultural- or commodity-grade nutrients are more applicable, but their costs are still significant. Therefore, utilizing the nutrient content of municipal, agricultural, or industrial waste streams is a very attractive alternative. 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 clean-up. Utilizing agricultural run-off also poses economic benefits by preventing eutrophication. A potential problem with this approach however is the impact on facility siting. 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 contains no nitrogen, phosphorous, or iron; these nutrients end up primarily in the spent algal biomass. From a sustainability perspective, nutrient recycle may prove to be more valuable than using the spent biomass for products such as animal feed. 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). 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 recycling of silicon, which is a component of the 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 vitally important factors for cultivation economics, productivity, and sustainability issues. Important research areas therefore include:

- TE and life cycle analysis to understand the cost, energy, and sustainability implications of various nutrient sources and recycling scenarios
- Studies to explore the mechanisms of nutrient recycling, e.g., from anaerobic digestion sludges
- Geographic Information System (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 Recycling

One of the main advantages of using algae for biofuels production is their 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 "show-stopper," either because of real economic or sustainability problems or because of loss of public support due to perceived problems.

With large cultivation systems, water demands will be enormous. For example, a hypothetical 1 hectare (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 (Weissman and Tillet, 1989), which is a loss of 13,000 gallons per day from the 1 ha pond. 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 fresh water, 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—a costly and often unsustainable option—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.

Conservation of water can be addressed to some extent through facility design and siting. An advantage of closed photobioreactors over open ponds is a reduced rate of evaporation. The added cost of such systems must be balanced against the cost savings and sustainability analysis for water usage for a given location. Note however that evaporation plays a critical role in temperature maintenance under hot conditions through evaporative cooling. Closed systems that spray water on the surfaces or employ cooling towers to keep cultures cool will lose some if not possibly all of the water savings of such systems under these conditions (Flickinger,1999). A critical part of the analysis that goes into siting an algal facility will be to analyze the "pan evaporation" rates at specific sites in conjunction with water cost and availability.

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 can 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. The blowdown water exiting the process will also most likely require treatment. 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. Surface disposal and reinjection into wells may be an option as regulated by the Environmental Protection Agency and 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.

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)

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4. Downstream Processing: Harvesting and Dewatering

Conversion of algae in ponds, bioreactors, and off-shore systems to liquid transportation fuels requires processing steps such as harvesting (Dodd and Anderson, 1977; Butterfi and Jones, 1969; McGarry and Tongkasa, 1971), dewatering, and extraction of fuel precursors (e.g., lipids and carbohydrates). These energy-intensive processes are only now being recognized as critically important. Cultures with as low as 0.02 - 0.07% algae (~ 1 gm algae/5000 gm water) must be concentrated to slurries containing at least 1% algae given the known processing strategies (Borowitzka, 1988). 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. 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. 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.

4.1 Approaches for Microalgae

Harvesting

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 Delanoue, 1987). Finally, electroflocculation and electrocoagulation offer the advantages of no added chemicals (Chen, 2004; Mollah et al., 2004; Poleman and Pauw, 1997).

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/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.
- 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) was established for sewage treatment and later studied in algae harvesting (Sim et al., 1988; Botes and Vanvuuren, 1991; Edzwald, 1993; Phochinda and White, 2003; Kwak et al., 2005; Bare et al., 1975; Koopman and Lincoln, 1983). 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 DAF. In addition to flocculant efficiency, recovery is largely dependent on bubble size and distribution through the suspension. DAF facilities require optimized integration with any engineered design for further downstream processing.

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 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 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 no washing requirements.

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 size). Centrifugation technologies must consider large initial capital equipment investments, operating costs, and high throughput processing of large quantities of water and algae. The current level of centrifugation technology makes this approach cost-prohibitive for most of the envisioned large-scale algae biorefineries. Significant cost and energy savings must be realized before any widespread implementation of this approach can be carried out.

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; acoustic focusing to concentrate algae at nodes; 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).

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 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.

4.2 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 direct 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 off-shore 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 compatible with their growth characteristics. In forms such as Laminaria grown on off-shore rings (Buck and Buchholz, 2004), 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 have stipulated the percentages of harvestable stock allowed to be harvested and the intervals between harvests to allow 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 hydrothermal liquefaction for bio-oils.

Macroalgae have less of a demand for dewatering as part of the pretreatment process. Anaerobic digestion, fermentation, and hydrothermal liquefaction 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 of seaweeds whose water content approaches 90% may be necessary, the exact ratio of water to solids for marine biomass remains to be determined.

4.3 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. Further, new strategies should be developed to combine and integrate these processes in order to take an algae culture and convert it into slurry of a specific concentration. This has yet to be demonstrated on a commercially relevant scale and remains a significant challenge.

A critical gap is the energy requirements of these processes are not only largely unknown but unbounded. This has important implications for plant design to answer simple questions like “What percentage of the total plant energy requirements or what percentage of that made available by algae must be directed toward harvesting and dewatering?”. Ultimately, a unit operations analysis of energy input for a range of dry weight content based on extraction needs is required with 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 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 watt-hours/gram if the energy content of lipids, carbohydrates, and proteins and the typical percentage of each in algae are considered (Illman et al., 2000). It is possible to estimate the energy requirements in watt-hours/gram of algae for harvesting, de-watering, and drying as a function of the volume percentage of algae in harvested biomass. The energy requirements for flocculation and sedimentation and the belt filter press are expected to be minimal. However, based on the latent heat of vaporization of water at 0.54 watt-hours/gram, energy balance can become an issue in systems that propose to take algal biomass and concentrate / dry it to enable downstream processing and extraction because of the high volumes of water that must be evaporated away. 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.

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5. Extraction of Products from Algae

While relatively limited volumes of bioproducts are currently produced from algal feedstocks, algal biomass suffers from a lack of well-defined and demonstrated industrial-scale methods for extracting and separating of oils and lipids required for enabling biofuel production. 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 competitive bulk commodity, extraction techniques employed must be efficient and effective.

Extraction depends on identifying the particular biological component for extraction, which is dependant on the algal species and growth status. Additionally, different harvest process operations (discussed in the Chapter 4) operations could affect extraction processes, as well as the fuel conversion process. While many terrestrial feedstocks can be removed from their environment at total solids >40%, microalgae and cyanobacteria may be cultivated as single cells suspended in water at concentrations below 1% solids. While macroalgae are more like traditional feedstocks, different logistical challenges arise from their offshore production and harvesting. Many effective extraction techniques require concentrated substrates, thus a high degree of concentration may be necessary before some types of extraction can begin. 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.

A shortfall of relevant information on efficient extraction of lipids and oils at larger-scale is limiting the algal-based biofuel development. Laboratory-scale comparisons of extraction of lipids from microalgae (Lee et al., 2010) and macroalgae (Aresta, Dibenedetto, and Barberio, 2005) 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 preparation for conversion to biodiesel (Zhang et al., 2003), little is known about the scale-up separation challenges for extracted algal lipids.

5.1 Current Practices for Lipid Extraction

The basis for lipid extraction from algal biomass is largely in the realm of laboratory-scale processes that serve analytical rather than biofuel production goals. Assuming a system that requires extraction of oils and lipids from harvested biomass—as noted, some systems are attempting to bypass this step—the dynamics of extraction in aqueous phase systems serves as a starting place for industrial-scale extraction operations.

Lipid extraction includes the following approaches: solvent-based extraction relying on microwaves and or sonication for cell disruption; using solvents to "milk" algal cells without disrupting cellular functions; and extraction bypass schemes that attempt to engineer algal systems that secrete products directly into the growth medium.

Mechanical Disruption (i.e., Cell Rupture)

Algal biofuel schemes that rely on the accumulation of intra-cellular lipids are the focus of this discussion. To be successful, any extracting solvent must be able 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 must be disrupted prior to extraction.

Effective mechanical disruption can help offset the need to use elevated temperature and pressure processes that force the solvent into contact with desired biopolymers. Different methods can be used to disrupt the cell membrane prior to the application of the extraction solvents. Mechanical disruption can include cell homogenizers, bead mills (or bead-beating), ultrasounds, and autoclaving (Mata et al., 2010). Non-mechanical methods include process such as freezing, application of organic solvents, osmotic shock, and acid, base, and enzyme reactions (Mata et al., 2010). The use of microwaves to disrupt cells and increase efficiencies of vegetable lipid and oil extraction is a promising development (Cravotto et al., 2008; Virot et al., 2008), though applications outside of analytical labs are unclear. For waste treatment, pretreatment of sewage sludge with "focused pulse" sonication has been shown to improve methane gas production and biosolids reduction in sludge digestion (Rittman et al., 2008). Recent work on extraction of lipids from three different types of oleaginous microalgae compared bead beating, sonication, autoclaving, osmotic shock, and microwaves and suggested that microwave disruption prior to solvent extraction is the most efficient method (Lee et al., 2010).

Organic Co-solvent Mixtures

The concept of like dissolves like 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.

Chloroform will extract more than just the saphonifiable 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 & Radin, 1978); dimethyl sulfoxide/petroleum ether for yeast (Park et al., 2007); hexane/ethanol for microalgae (Cartens et al., 1996); and hexane/isopropanol for microalgae (Nagle & 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.

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 (Grima et al., 1994). In other cases, single alcohol (e.g., 1-butanol and ethanol) solvents have been tried (Nagle & 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. More, 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 most 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.

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 be necessary.

Application of Organic Two-Solvent Systems for Lipid Extraction from Microalgae:

Iverson et al. (2001) found that the Bligh and Dyer method grossly underestimated the lipid content in samples of marine tissue that contained more than 2% lipids but worked well for samples that contained less 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.

Direct Transesterification of Lipids into Fatty Acid Methyl Esters (FAMES)

To increase analytical efficiency, Lepage and Roy (1984) proposed the direct transesterification of human milk and adipose tissue without prior extraction or purification for improved recovery of fatty acids. In general, this approach suggested that a one-step reaction that added the alcohol (e.g., methanol) and acid catalyst (e.g., acetyl chloride) directly to the biomass sample and followed with heating at 100˚C for an hour under sealed cap would 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 shortened to 10 minutes if the mixture was incubated at 100˚C under a sealed cap.

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 principle reasons:

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

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 - 200˚C) and pressure (500 - 3000 psi) conditions for short time periods (5 - 10 min). 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 (ref).

ASE is more efficient if 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 traditional Folch method for microalgae grown on dairy manure effluent (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.

Selective 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.

Subcritical Water Extraction

Subcritical water extraction (also known as hydrothermal liquefaction) is based on the use of water, at temperatures just below the critical temperature, and pressure high enough to keep the liquid state (Soto and Luque de Castro, 2001) . The technique, originally termed "pressurized hot water extraction," was initially applied to whole biomass hemicellulose as a pretreatment prior to its use as a fermentation substrate (Mok et. al., 1992). More recently, however, it has been applied for the selective extraction of essential oils from plant matter (Eikani et al., 2007), functional ingredients from microalgae (Herrero et al., 2006), and saponins from oil-seeds (Güçlü-Üstündağ et al., 2007). The basic premise to subcritical water extraction is that water, under these conditions, becomes less polar and organic compounds are more soluble than at room temperature. There is also the added benefit of solvent access into the biomass matrix that occurs at the higher temperatures as discussed above. In addition, as the water is cooled back down to room temperature, products miscible at the high temperature and pressure become immiscible at lower temperatures and can be easily separated. Some of the more important advantages described for subcritical water extraction include shorter extract times, higher quality of extracts, lower costs of the extracting agent, and environmental compatibility (Herrero et al., 2006).

With respect to microalgae, however, whether grown phototrophically or heterotrophically, one of the more attractive aspects is the use of water as the solvent, thereby eliminating the need for the dewatering step. A major constraint, however, as with accelerated solvent extraction, is the difficulty with designing a system at large scale and the high-energy load required to heat the system up to subcritical temperatures. Large-scale design will also require a significant cooling system to cool the product down to room temperature to avoid product degradation, creating additional energy use challenges.

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.

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). Lipids have been selectively extracted from macroalgae at temperatures of 40 - 50˚C and pressures of 241 - 379 bar (Chueng, 1999). 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 (see Chapter 6 for more detail).

Heterotrophic Production

Other methods for extraction and fractionation include the production of oils using heterotrophic algae. In this scenario, non-photosynthetic algae are grown using sugars as energy source and using standard industrial fermentation equipment (Barclay et al., 1994). Some private companies have engineered algae that secrete oil into the fermentation media that can be recovered and later refined into a biofuel; this approach significantly reduces the capital and operating cost for an extraction process. The potential benefits of this approach are the use of standard fermentation systems, higher productivity compared to photosynthetic systems, ease of scale-up, avoidance of expensive extraction scheme(s), the ability to

maintain the integrity of the fermentation catalyst and use of sugar-based feedstocks. However, significant downsides to this approach include many of the same feedstock logistics challenges faced by the nascent lignocellulosic industry. Chief amongst is the logistical challenge of securing a sustainable biomass feedstock to supply to feed large-scale heterotrophic "algal-refinery" operations.

5.2 Challenges

Presence of Water Associated with the Biomass

The extraction 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. 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.

Increasing the temperature helps to disrupt the solute-matrix interactions and to reduce the viscosity and surface tension of the water, thereby improving the contact between the solvent and the solute. Increased pressure facilitates enhancing the transport of the solvent to the analytes that have been trapped in pores. The pressure also helps to force the solvent into matrices that would normally not be contacted by solvents under atmospheric conditions. Mechanical disruption can reduce the pressure and temperature requirements.

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.

Energy Consumption and Water Recycle

For sustainable biofuels production, the following benchmark can be considered: the extraction process per day should consume no more than 10% of the total energy load, as Btu, produced per day. As discussed in Section 4.3, a preliminary look at the energy balance does not suggest that this is an insurmountable barrier, but it does serve to highlight the need for innovation to achieve sustainable commercial-scale systems.

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6. Algal Biofuel Conversion Technologies

Potentially viable fuels that can be produced from algae range from gaseous compounds like hydrogen and methane, to alcohols and conventional liquid hydrocarbons, to pyrolysis oil and coke. Attractive targets for this effort, however, are the liquid transportation fuels of gasoline, diesel, and jet fuel. These fuel classes were selected as the best-value targets because 1) they are the primary products that are currently created from imported crude oil for the bulk of the transportation sector, 2) they have the potential to be more compatible than other biomass-based fuels with the existing fuel-distribution infrastructure in the U.S., and 3) adequate specifications for these fuels already exist.

The primary objective of this chapter is to summarize a number of potentially viable strategies for converting algal biomass into 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, etc.), these desired fuel characteristics are driven primarily by customer requirements and are discussed later in Chapter 8. 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 emerged the Algal Roadmap Workshop in relation to conversion of algal feedstocks to fuels. These are noted here to help establish a reasonable framework for the most promising concepts for algal biofuels.

- First, 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 both composition and variability) is essential, since this is an upstream boundary condition for the entire downstream fuel-conversion process.
- Second, life cycle analysis of energy and carbon will be a key tool in selecting the preferred fuel conversion technologies from those discussed in this chapter.
- Third, the greatest challenge in algal fuel conversion is not likely to be how to convert lipids or carbohydrates to fuels most efficiently, but rather how best to use the algal remnants after the lipids or other desirable fuel precursors have been extracted. All of the petroleum feedstock that enters a conventional petroleum refinery must leave as marketable products, and this conservation law must also hold true for the algae biorefineries of the future if they are to achieve significant market penetration.

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 direct algal production of recoverable fuel molecules (e.g., ethanol, hydrogen, methane, and alkanes) from algae without the need for extraction;
2) Those that process whole algal biomass to yield fuel molecules; and
3) Those that process algal extracts (e.g., lipids, carbohydrates) to yield fuel molecules.

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 can be applied effectively. 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.

6.1 Direct Production of Biofuels from Algae

The direct production of biofuel through heterotrophic fermentation and growth from algal biomass has certain advantages in terms of process cost because it can eliminate several process steps (e.g., oil extraction) and their associated costs in the overall fuel production process. Heterotrophic growth also allows 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 (hundreds of grams per liter) and a high percentage of that biomass as lipid (well over 50%). The system is readily scaled up and there is an enormous potential to use various fixed carbon feedstocks (which would bring down the cost of production). These approaches are quite different from the usual algal biofuel processes that use algae to produce biological oils which is subsequently extracted and used as a feedstock for liquid fuel production, typically biodiesel. 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 anaerobic fermentation of these carbon sources to produce ethanol under dark conditions. If these alcohols can be extracted directly from the algal culture media, the process may be drastically less capital- and energy-intensive than competitive algal biofuel processes. The process would essentially eliminate the need to separate the biomass from water and extract and process the oils.

This process typically consists of closed photobioreactors utilizing sea-water 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). There have been reports of preliminary engineered systems, consisting of tubular photobioreactors (Hirano et al., 1997). One key aspect of the system is that a source of cheap carbon, such as a power plant, is typically used to supply CO_2 to the bioreactors to accelerate the algae growth. 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.

This technology is estimated to yield 4,000 - 6,000 gallons per acre per year, with potential increases up to 10,000 gallons per acre per year within the next 3 to 4 years with significant R&D. It is theoretically estimated that one ton of CO_2 is converted into approximately 60 - 70 gallons of ethanol with this technology. With such yields, the price of captured CO_2 becomes significant, and may require a price less than or equal to $10 per ton to remain cost-competitive. 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. 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 heterotrophic metabolic pathways using algae. 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 themselves 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, but more often are associated with the algae and thus must be recovered through dewatering and extraction. With further processing, a wide variety of fuels can be made. The process of growing the algae heterotrophically may present some advantages over typical photoautotrophic-based technologies. First, keeping the algae "in the dark" causes them to produce more alkanes than they do in the presence of sunlight. While their photosynthetic processes are suppressed, other metabolic processes that convert sugar into alkanes can become active. Second, some have shown the growth rate of the algae to be much higher than traditional methods (Xu et al., 2006). This is possible because instead of getting energy for growth from sunlight, the algae get concentrated energy from the sugars fed into the process. These higher cell concentrations reduce the amount of infrastructure needed to grow the algae, and enable more efficient dewatering if is actually required.

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.

Hydrogen

The production of hydrogen derived from algae has received significant attention over several decades. Biological production of hydrogen (i.e., biohydrogen) technologies provide a wide range of approaches to generate hydrogen, including direct biophotolysis, indirect biophotolysis, photo-fermentation, and dark-fermentation (see Chapter 2).

There are several challenges that remain before biological hydrogen production can be considered a viable technology. These include the restriction of photosynthetic hydrogen production by accumulation of a proton gradient, competitive inhibition of photosynthetic hydrogen production by CO_2, requirement for bicarbonate binding at photosystem II (PSII) for efficient photosynthetic activity, and competitive drainage of electrons by oxygen in algal hydrogen production.

The future of biological hydrogen production depends not only on research advances, i.e., improvement in efficiency through genetically engineered algae and/or the development of advanced photobioreactors, but also on economic considerations, social acceptance, and the development of a robust hydrogen infrastructure throughout the country.

6.2 Processing of Whole Algae

In addition to the direct production of biofuels from algae, the whole algae 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.

Macroalgae has specifically received some attention as a gasification feedstock and initial work shows that while some key differences exist as compared to terrestrial crops, certain species are suitable for gasification (Ross et al., 2008). Polysaccharides, such as mannitol, laminarin, and fucoidin, represent the main macroalgae biochemical feedstocks for conversion to liquid fuels (McHugh, 2003). Lipid content of a variety of macroalgal species is typically less than 5% of total dry weight (McDermid & Stuercke, 2003), too low for conversion to biodiesel, although concentrations approaching 20% are reported in some species (Chu et al., 2003; McDermid & Stuercke, 2003).

There are four major categories of conversion technologies that are capable of processing whole algae: pyrolysis, gasification, anaerobic digestion, and supercritical processing (Exhibit 6.1).

Pyrolysis

Pyrolysis is the chemical decomposition of a condensed substance by heating. It does not involve reactions with oxygen or any other reagents but can frequently take place in their presence. 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 has one major advantage over other conversion methods, in that it is extremely fast, with reaction times of the order of seconds to minutes.

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, a degradable alternative liquid called bio-oil can be produced. The bio-oil has an advantage that it can enter directly into the refinery stream and, with some hydrotreating and hydrocracking, produce a suitable feedstock for generating standard diesel fuel. Also, higher efficiency can be achieved by the so-called “flash pyrolysis” technology, where finely ground feedstock is quickly heated to 350 - 500˚C for less than 2 seconds. For flash pyrolysis, typical biomass feedstocks must be ground into fine particles. This is one area where algae have a major advantage over other biomass sources because it exists fundamentally in small units and has no fiber tissue to deal with. Several pilot plants for fast pyrolysis of biomass have been built in the past years in Germany, Brazil, and the United States, but bio-oil from pyrolysis is not a commercial product at the current time (Bridgwater, 2004). Even with the increased interest in converting biomass into liquid transportation fuels, it appears fast pyrolysis to create bio-oil, especially from algae, is a relatively new process (Bridgwater, 2007). There are several reports on the pyrolysis of algae in the scientific literature (Demirbas, 2006; Miao and Wu, 2004).

A significant roadblock in using pyrolysis for algae conversion is moisture content, and significant dehydration must be performed upstream for the process to work efficiently. It is unclear exactly how much more difficult it would be to convert algae into a bio-oil compared to other biomass sources due to uncertainties in the ability to dehydrate the feedstock; no comprehensive and detailed side-by-side comparison was found in the scientific literature. It appears that pyrolysis will not be cost-competitive over the short-term unless an

inexpensive dewatering or extraction process is also developed. 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. While algal bio-oil may be similar to bio-oil from other biomass sources, it may have a different range of compounds and compositions depending on the type of algae and upstream processing conditions (Zhang et al., 1994). Another research paper demonstrated that the bio-oil produced by pyrolysis of algae can be tailored by carefully controlling the algal growth conditions (Miao and Wu, 2004).

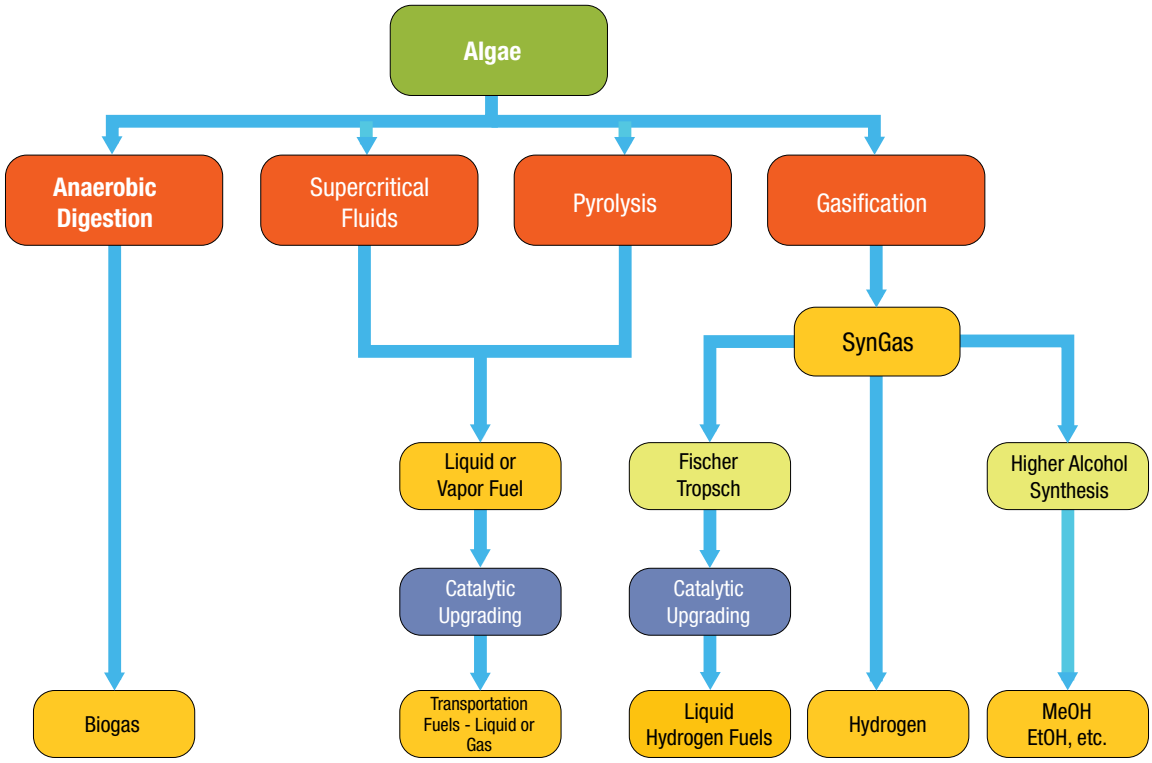
Unfortunately, there are also significant gaps in the information available about the specifications for converting algal bio-oil and the resulting products. The optimal residence time and temperature to produce different algal bio-oils from different feedstocks need to be carefully studied. Work also needs to be performed to understand the detailed molecular composition of the resulting bio-oils. Additionally, research is needed on the catalytic conversion of the resulting algal bio-oils. Another area of interest is the development of stabilizers for the viscosity of the bio-oil and acid neutralizing agents, so the bio-oil may be more easily transported throughout the upgrading process.

Gasification

Gasification of the algal biomass may provide an extremely flexible way to produce different liquid fuels, primarily through Fischer-Tropsch Synthesis (FTS) or mixed alcohol synthesis of the resulting syngas. 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 hydrogenation (Okabe et al., 2009; Srinivas et al., 2007; Balat, 2006).

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. It may be possible to feed algae into a coal gasification plant to reduce the capital investment required, address the issue of availability

Exhibit 6.1 *Schematic of the potential conversion routes for whole algae into biofuels*



for dedicated biomass plants, and improve the process efficiency through economy of scale. 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 regenerative 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 FT catalyst. FTS tends to require production at a very large scale to make the process efficient overall. However, the most significant problem with FTS is the cost of clean-up 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:

- Fluidized-bed gasification and catalytic reforming
- Fluidized-bed gasification and solvent tar removal
- Fluidized-bed gasification and subsequent thermal tar cracker
- Entrained-flow gasification at high temperature

A demonstration plant for gasification of wood chips with catalytic cracking of the tar is currently being built in Finland in a joint venture of the Technical Research Centre of Finland (VTT), Neste Oil, and Stora Enso. A solvent tar removal demonstration was installed in a plant in Moissannes, France in 2006.

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).

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. Also, it would be useful to leverage ongoing syngas-to-ethanol research using cellulosic.

Liquefaction

Direct hydrothermal liquefaction in subcritical water (defined as water held in a liquid state above 100°C by applying pressure) is a technology that can be employed to convert wet algal biomass to a range of liquid fuels (Patil et al., 2008). This technology is a representation of the natural geological processes known to be involved in the formation of petroleum-based fossil fuels realized over greatly shortened time scales. 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 this liquefaction process is a "bio-crude" that typically accounts for 45% wt. of the feedstock on a dry ash free basis and has energy content that is comparable to diesel and can be upgraded further. There are reports (Goudriaan et al., 2000) that claim the thermal efficiency, defined as the ratio of heating values of bio-crude products and feedstock plus external heat input, as high as 75%. Prior work in direct liquefaction of biomass was very active, and there are a few reports that used algal biomass as a feedstock. Liquefaction of *Dunaliella tertiolecta* with a moisture content of 78.4 wt% produced an oil yield of about 37% (organic basis) at 300˚C and 10 MPa (Minowa et al., 1995). The oil obtained at a reaction temperature of 340˚C and holding time of 60 min had a viscosity of 150 - 330 mPas and a calorific value of 36 kJ g−1, comparable to those of fuel oil. In the same report it was concluded that liquefaction technique was a net energy producer from the overall process energy balance. A maximum oil yield of 64% using *Botryococcus braunii* as a feedstock has also been reported. The algal biomass was processed by liquefaction at 300˚C, catalyzed by sodium carbonate (Sawayama et al., 1995). There have also been comparative studies that report the liquefaction technique was more effective for extraction of microalgal biodiesel than using the supercritical carbon dioxide (Aresta et al., 2005). Liquefaction of algae is considered a promising technological approach but due to limited information in hydrothermal liquefaction of algae to date, more research in this area is needed before it can become a commercially viable option.

Supercritical Processing

Supercritical processing is a recent addition to the portfolio of techniques 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 for 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, 2009). 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). Although it has been only demonstrated for the simultaneous extraction and transesterification of vegetable oils, it is envisioned as being applicable for the processing of algae. In this process variant, supercritical methanol or ethanol is employed as both the oil extraction medium and the catalyst 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 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.

Anaerobic Digestion of Whole Algae

The production of biogas from the anaerobic digestion of macroalgae, such as *Laminaria hyperbore* and *Laminaria saccharina*, is an interesting mode of gaseous biofuel production, and one that receives scant attention in the United States (Hanssen et al., 1987). The use of this conversion technology 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. Several studies have been carried out that demonstrate the potential of this approach. A recent 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). If this approach can be modified for the use of microalgae, it may be very effective for situations like integrated wastewater treatment, where algae are grown under uncontrolled conditions using strains not optimized for lipid production.

6.3 Conversion of Algal Extracts

The conversion of extracts derived from algal sources is the typical mode of biofuel production from 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. The most common type of algal extracts under consideration are lipid-based, e.g., triacylglycerides, which

can be converted into biodiesel. This section discusses chemical, biochemical, and catalytic processes that can be employed to convert algal extracts. (Exhibit 6.2).

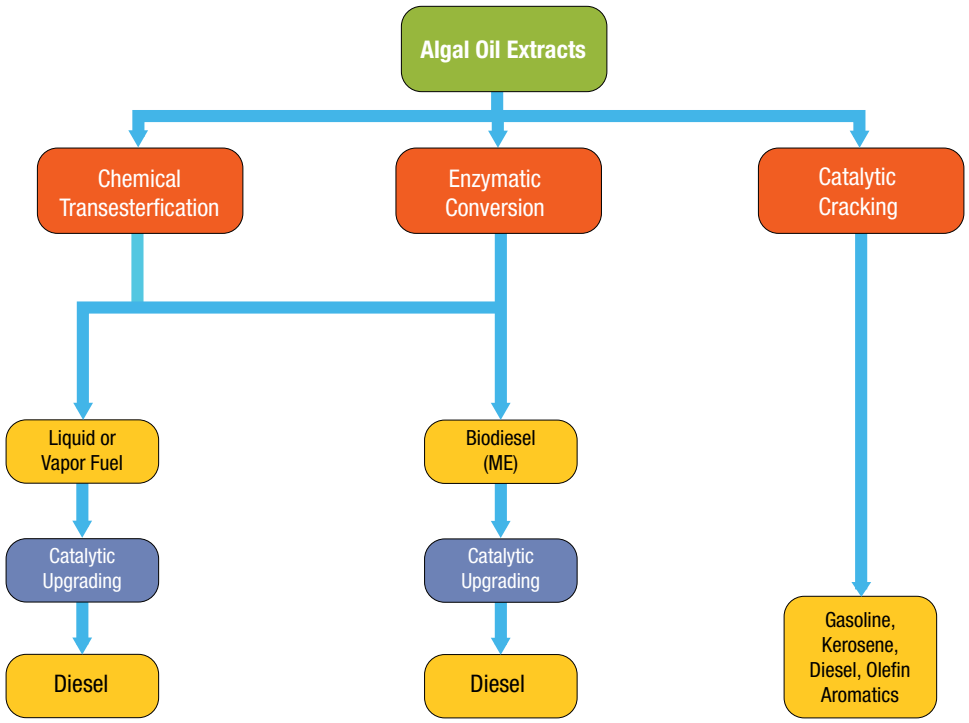
Chemical Transesterification

The transesterification reaction is employed to convert triacylglycerols extracted from algae to FAMEs (fatty acid methyl esters), which is simply a process of displacement of an alcohol group from an ester by another alcohol (Demirbas, 2009). 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 & 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.

Another route is found in acid-catalyzed transesterification reactions (Wahlen et al., 2008). The replacement of soluble bases by liquid acid catalysts such as H_2SO_4, HCl or H_3PO_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. Higher temperatures and longer reaction times are, therefore, generally required as a result. In order to compensate for this, heteropolyacids (HPA), such as $H_3PW_{12}O_{40}/Nb_2O_5$, 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 (L. Xu et al., 2008). The apparent 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 & 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

Exhibit 6.2 *Schematic of the various conversion strategies of algal extracts into biofuels*



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 provides simultaneously 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.

Biochemical (Enzymatic) Conversion

Chemical processes give high conversion of triacylglycerols to their corresponding esters but have drawbacks such as being energy-intensive, difficulty in removing the glycerol, and require removal of alkaline catalyst from the product and treatment of alkaline wastewater. Use of biocatalysts (lipases) in transesterification of triacylglycerols 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 triacylglycerols 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 some recent reports of using a solvent engineering method to enhance the lipase-catalyzed methanolysis of triacylglycerols 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).

Other important issues that need further exploration are developing enzymes that can lyse the 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.

Catalytic Cracking

The transesterification catalysts presented above are very strong and relatively mature in the field of biofuel production. Although very effective and relatively economical, these catalysts still require purification and removal from the product stream, which increases the overall costs. One potential solution to this is the development of immobilized heterogeneous and/or homogeneous catalysts that are very efficient and inexpensive (McNeff et al., 2008). Acid and basic 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 AlC_{l3} or ZnC_{l2}, have been proven as a viable means of converting triacylglycerols 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 HTiNbO3 as the catalyst. Vanadate metal compounds are stable, active catalysts during transesterification, with TiVO4 being the most active (Cozzolino et al., 2006). This catalyst is also more active than HTiNbO3, producing the same yields with lower residence times. Double-metal cyanide Fe-Zn 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 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 Al2O3.

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 - 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 8, 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. It is worth noting that the petroleum industry began by developing a replacement for whale oil, and now it is apparent that it is beginning to return to biological feedstocks to keep the pipelines full.

Gasoline, jet fuel, and diesel are generally described as "renewable" or "green" if it is 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 have already begun to explore the conversion of vegetable oils and waste animal fats into renewable fuels. 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 and algae) used in recent commercial jet test flights. 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 hydrothermal treatment (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 reduce double bonds to yield hydrocarbons. Glycerin can be converted to propane which can be used for liquefied petroleum gas.

The primary barrier to utilizing algae oils to make renewable fuels is catalyst development. 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 CO and CO_2 lost, 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 lipid as well as the tolerance of the catalyst for the contaminants to arrive at the most cost-effective process.

6.4 Processing of Algal Remnants after Extraction

One other critical aspect in developing a conversion technology that derives benefit from every potential input is the conversion of algal remnants after conversion of algal feedstock into fuel. This includes the anaerobic digestion of algal remnants to produce biogas, as well as the fermentation of any recoverable polysaccharides into biofuels.

Anaerobic digestion can be effectively used as a means of producing biogas from algae and algal remnants after extraction (Ashare and Wilson, 1979). In particular, the organic fractions of the algae remaining after oil extraction are amenable to anaerobic digestion. In addition, once the algae has been harvested, little if any pretreatment is required. 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. 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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7. Co-products

The "guiding truth" is that if biofuel production is considered to be the primary goal, the generation of other co-products must be correspondingly low since their generation will inevitably compete for carbon, reductant, and energy from photosynthesis. Indeed, 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. This chapter will address these options and discuss how some of them are better opportunities as they will not readily saturate corresponding markets in the long term.

This chapter will also address within the context of the biorefinery the possibility of coupling biomass cultivation with CO_2 mitigation (for carbon credits) 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 (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 6). The nature of the end products and of the technologies to be employed will be determined, primarily by the economics of the system, and they may vary from region to region according to the cost of the raw material (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 6); this chapter will focus on the non-fuel co-products.

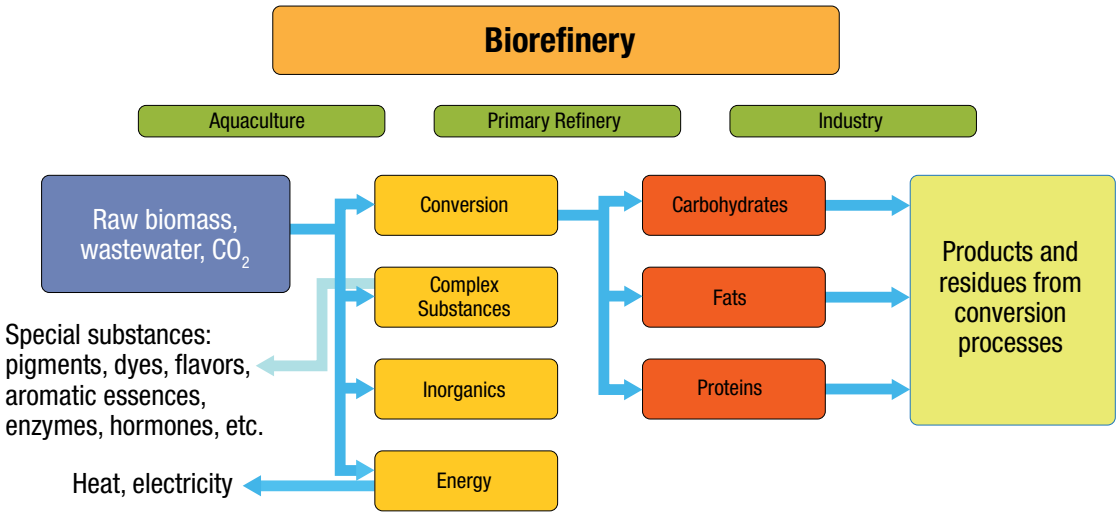
Under the biorefinery concept (Exhibit 7.1), the production of industrial, high-value and high-volume chemicals from amino acids, glycerol, and nitrogen-containing components of algal biomass becomes feasible (Mooibroek et al., 2007) and must be considered in determining the economics of the process.

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 considerably under a dollar for a commodity producer. For the purposes of the Roadmap, a reasonably valued chemical is defined as one that will cost roughly \$0.30 - \$1.00/lb, and can be produced at a volume of roughly 100 - 500 $x10^6$ lbs/yr.

7.1 Commercial Products from Microalgae and Cyanobacteria

A large number of different commercial products have been derived from microalgae and cyanobacteria. As summarized in Exhibit 7.2, 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.

Exhibit 7.1 *An overview of the biorefinery concept*



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. Yet the existing fossil fuels market is and the future algal-based biofuels market (designed in part to supplant the fossil fuels market) must be commodities based to meet required volumes at price points acceptable to the consumer. With the possible exception of the existing market for microalgal biomass for animal 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 less than those associated with the existing high-value algae-derived products.

Therein lies a major conundrum associated with the nascent algal-derived biofuels market: in the long term, massive lipid production dominates; yet in the short term, co-products of higher value in the marketplace must be pursued in order to offset the costs of production of algal-derived biofuels. 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 Roadmap for biofuel production have been overcome and associated life cycle costs are reduced to realize sustainable biofuel production at volumes and pricepoints that meet consumer demands or 2) new co-products that are low cost and have very large potential markets are developed.

Food and Feed

- Human Health Food Supplement: The consumption of microalgal biomass as a human health food supplement is currently restricted to only a few species, e.g., Spirulina (Arthospira), Chlorella, Dunalliella, and to a lesser extent, Nostoc and Aphanizomenon (Radmer, 1996; Pulz and Gross, 2004; Spolaore et al., 2006).

  The 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, currently at about 2.5 billion US$, is expected to grow in the future.

- Aquaculture: Microalgae are also used as feed in the aquaculture of mollusks, crustaceans (shrimp), and fish (Benemann, 1990; Malcolm et al., 1999). 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 market size, currently at ~700 million US$, is expected to expand significantly.

- Animal Feed Additive: 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 - 10% (wt.) can be safely used as a partial replacement for conventional proteins (Spoalore et al., 2006). The main species used in animal feed are Spirulina, Chlorella and Scenesdesmus. The market for microalgal animal feeds, estimated to be about 300 million US$, is quickly growing. However, it is important to note that since the flue gas from coal-fired power plants that will be used to supply carbon dioxide to the cultures will contain significant amounts of lead, arsenic, cadmium and other toxic elements, the resulting non-oil algal biomass is very likely to be unsuitable for use as an animal feed, particularly given the fact that algae are known to be effective at metal absorption.

Polyunsaturated Fatty Acids (PUFAs)

Microalgae can also be cultured for their high content in PUFAs, which may be added to human food and animal feed for their health promoting properties (Benemann, 1990; Radmer, 1994 and 1996). The most commonly considered PUFAs are arachidonic acid (AA), docohexaenoic acid (DHA), γ-linolenic acid (GLA), and eicosapentaenoic acid (EPA). AA has been shown to be synthesized by *Porphyridium*, DHA by *Crypthecodinium* and *Schizochytrium*, GLA by *Arthrospira*, and EPA by *Nannochloropsis*, *Phaeodactylum* and *Nitzschia* (Spolaore et al., 2006). 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 1.5 billion US$.

Anti-Oxidants

A number of anti-oxidants, 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 300 to 3,000 US$ per kg (Spolaore et al., 2006). The market size for β–carotene is estimated to be greater than 280 million US$.

Coloring Agents

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 *Hematococcus pluvialis*, has been successfully used as a 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 zeaxantin, have also been used in the feed of carp and even chicken (Puls and Gross, 2004; Spolaore et al., 2006). Phycobiliproteins, i.e., phycoerythrin and phycocyanin, produced by the cyanobacterium *Arthrospira* and the *rhodophyte Porphyridium*, are used as food dyes, pigments in cosmetics, and as fluorescent reagents in clinical or research laboratories (Spolaore et al., 2006).

Exhibit 7.2 *Summary of microalgae commercial products market*

COMMERCIAL PRODUCT	MARKET SIZE (TONS/YR)	SALES VOLUME (MILLION $US/YR)	REFERENCE
BIOMASS			
Health Food	7,000	2,500	Pulz&Gross (2004)
Aquaculture	1,000	700	Pulz&Gross (2004) Spolaore et al., (2006)
Animal Feed Additive	No available information	300	Pulz&Gross (2004)
POLY-UNSATURATED FATTY ACIDS (PUFAs)			
ARA	No available information	20	Pulz&Gross (2004)
DHA	<300	1,500	Pulz&Gross (2004) Spolaore et al., (2006)
PUFA Extracts	No available information	10	Pulz&Gross (2004)
GLA	Potential product, no current commercial market		Spolaore et al., (2006)
EPA	Potential product, no current commercial market		Spolaore et al., (2006)
ANTI-OXIDANTS			
Beta-Carotene	1,200	>280	Pulz&Gross (2004) Spolaore et al., (2006)
Tocopherol CO_2 Extract	No available information	100-150	Pulz&Gross (2004)
COLORING SUBSTANCES			
Astaxanthin	< 300 (biomass)	< 150	Pulz&Gross (2004) Spolaore et al., (2006)
Phycocyanin	No available information	>10	Pulz&Gross (2004)
Phycoerythrin	No available information	>2	Pulz&Gross (2004)
FERTILIZERS/SOIL CONDITIONERS			
Fertilizers, growth promoters, soil conditioners	No available information	5,000	Pulz&Gross (2004) Metting&Pyne (1986)

Fertilizers

Currently, macroalgae (i.e., seaweeds) are used as a plant fertilizer 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. 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), cosmetics (Spolaore et al., 2006), pharmaceuticals and bioactive compounds (Burja et al., 2001; Metting and Pyne, 1986; Olaizola, 2003; Singh et al., 2005; Pulz and Gross, 2004), polysaccharides (Benemann, 1990; Borowitzka, 1986; Pulz and Gross, 2004), and stable isotopes for research (Benemann, 1990, Radmer, 1994; Pulz and Gross, 2004). The market for these specialty products is likely to be very small due to their specialized applications.

7.2 Commercial Products from Macroalgae

Macroalgae possess high levels of structural polysaccharides that are extracted for their commercial value (Exhibit 7.3). They include alginate from brown algae and agar and carrageenen 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).

7.3 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 (shutdown economics). This sets a high bar but 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 dried grains from corn dry grind ethanol processing. Price becomes less of an issue if the product can be labeled "organic" and thus saleable at a premium.

Exhibit 7.3 *Global value of seaweed products per annum (McHugh, 2003)*

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
Carrageenen (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**

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 recovering economic value from the lipid-extracted microalgal biomass (Exhibit 7.4). These are:

- Option 1 – Maximum energy recovery from the lipid extracted biomass, with potential use of residuals as soil amendments
- 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/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 as Soil Amendments

Given the large amounts of lipid-extracted biomass residues that will likely 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.

The most promising energy recovery technology, both from a practical and economic perspective, is the anaerobic digestion of lipid-extracted biomass. 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 per 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, as is currently already done for certain waste water treatment sludges (see http://www.unh.edu/p2/biodiesel/pdf/algae_salton_sea.pdf).

In addition to anaerobic digestion, thermochemical conversion technologies, such as pyrolysis, gasification, and combustion, could also be potentially considered for the recovery of energy from the lipid-extracted biomass (see Chapter 6). These technologies are able to convert a much larger fraction of biomass into fuels. However, these technologies are still in the testing and development stage, and because of their large energy inputs (temperature and pressure), could have poor or even negative energy balances (Huesemann and Benemann, 2009). Nevertheless,

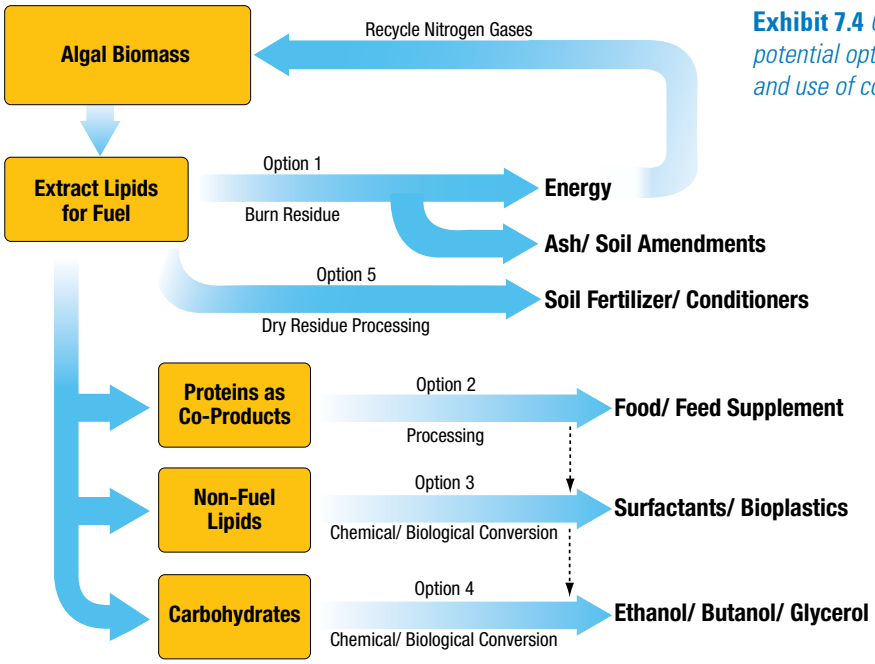


Exhibit 7.4 *Overview of the five potential options for the recovery and use of co-products*

the thermochemical conversion of lipid-extracted biomass has the potential advantage that the resulting nitrogen-containing gases (e.g., ammonia and nitrous oxides) could be recycled 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.

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. 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 distillers dry grains plus soluble (DDGS)) (Berger and Good, 2007). The current price for DDGS ranges from $110 - 150 per ton (http://www.ams.usda.gov/mnreports/sj_gr225.txt). 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.

The byproduct material, which contains proteins, might make a useful animal feed. However, feeding studies indicate that algae cannot be used as a high percentage of feed, due to issues such as taste of the meat or eggs, and interactions with animal digestion. Furthermore, the overall size of the animal feed market is small, relative to the amount of byproduct that would be produced, and the individual local markets for animal feed are often not located adjacent to areas where algae may be produced. As a result, byproduct markets are likely to be overwhelmed, and byproduct prices will be greatly depressed versus current levels.

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 C_{10} to C_{24}, 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 C_4 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., 1995).

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 (http://www.biodieselmagazine.com/article.jsp?article_id=377). As the current production levels for biodiesel (700 million gallons in 2008) 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 under 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 desired products, including carbohydrates, if desired.

Option 5. Recovery (Extraction) 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. As was mentioned above, the market for organic fertilizer is large and potentially growing.

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8. 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, biofuels, and bioproducts. Being intermediate 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.

8.1 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 the substrate energy-density and stability;
- Maximizing compatibility with existing infrastructure (e.g. storage tanks, high capacity; delivery vehicles, pipelines, dispensing equipment, and end-use vehicles); and
- Optimizing the scale of operations to the parameters stated above.

Distribution is complicated by the fact that several different fuels from algae are being considered, as discussed in detail in Chapter 6 (Algal Biofuel Conversion Technologies). Ethanol, biodiesel, biogas, renewable gasoline, diesel, and jet fuels are all possible products from algal biomass. Each of these different fuels has different implications for distributions. Some of these fuels appear to be more compatible with the existing petroleum infrastructure. 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 and Backs, 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.

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

- First, 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, and the quality of the final fuel product.
- Second, an issue that impacts 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).
- Third, 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. 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.

Ethanol is another likely fuel from algae. With over 10 billion gallons per year produced and consumed domestically, distribution-related issues for ethanol has 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% to 15%); as the ethanol content in the blend increases, the transport costs will also increase due to the lower energy density of the fuel.

8.2 Utilization

The last remaining hurdle to creating 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 6, such a fuel is said to 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 lipids and carbohydrates as a feedstock for fuel production.

Typically, compliance with specifications promulgated by organizations such as ASTM International ensures that a fuel is fit for purpose (ASTM International, 2009a, 2009b, 2009c, 2009d, and 2009e). 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. Some notable examples included: elastomer-compatibility issues that led to fuel-system leaks when 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 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 Environmental Protection Agency regulations on combustion engine emissions.

As is true of any new fuel, it is unlikely that new specifications will be developed for algal fuels in the near term (i.e., at least not until significant market penetration has occurred); hence, producers of algal fuels should strive to meet prevailing petroleum-fuel specifications. Nevertheless, 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).

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, 2009b). 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). 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 synthetic paraffinic kerosene (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. 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. 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 technology/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 are the most common biofuels currently being considered for use in gasoline, and these alcohols can be produced from fermentation of starches and other carbohydrates contained in algae.

Additionally, the hydro-treating 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.

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9. Resources and Siting

The development and scale-up of algal biofuels production, as with any biomass-based technology and industry, needs to be analyzed from a site location, as well as from a resource availability and use perspective. Critical requirements—such as suitable land and climate, sustainable water resources, supplemental CO_2 supply, and other nutrients—must be appropriately aligned in terms of their geo-location, characteristics, availability, and affordability. To achieve success regarding both technical and economic performance without adverse environmental impacts, the siting and resource factors must also be appropriately matched to the required growth conditions of the particular algae species being cultivated and the engineered growth systems being developed and deployed. The sustainability and environmental impacts of national algae production capacity build-up and operation over time will be important complementary aspects of the siting and resources issues that will also need careful consideration and analysis using tools and methodologies discussed later in chapter 10.

This chapter provides an overview of the issues associated with site location and key resources for various microalgae and macroalgae production approaches (Exhibit 9.1). Further, an in-depth discussion is included on the potential to couple land-based microalgae biomass production with wastewater treatment and industrial sources of concentrated CO_2, both of which influence siting decisions for algal biofuel production. Integration with wastewater treatment can play an additional important role in the sourcing of nutrients from both the input wastewater and from possible nutrient recycling from residual algal biomass. The status of algae-based wastewater treatment and necessary technical improvements for co-producing algal biofuels are described. Similarly, the challenges associated with coupling industrial CO_2 sources with algae production are outlined.

9.1 Resource Requirements for Different Cultivation Approaches

Photoautotrophic Microalgae Approach

Assessments of resource requirements and availability for large-scale, land-based photoautotrophic microalgal cultivation were 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; Lansford et al., 1990; 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.

Exhibit 9.2 provides a simple high-level overview of the major resource and environmental parameters that pertain to the algae biofuels production inputs of climate, water, CO_2, energy, nutrients, and land. These 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.

In addition to coastal and inland photoautotrophic microalgae production, off-shore marine environment concepts are also being proposed. This scenario

Exhibit 9.1 *Key resource issues for different algae systems*

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
Photoautotrophic macroalgae production	Availability of suitable coastal and off-shore marine site locations

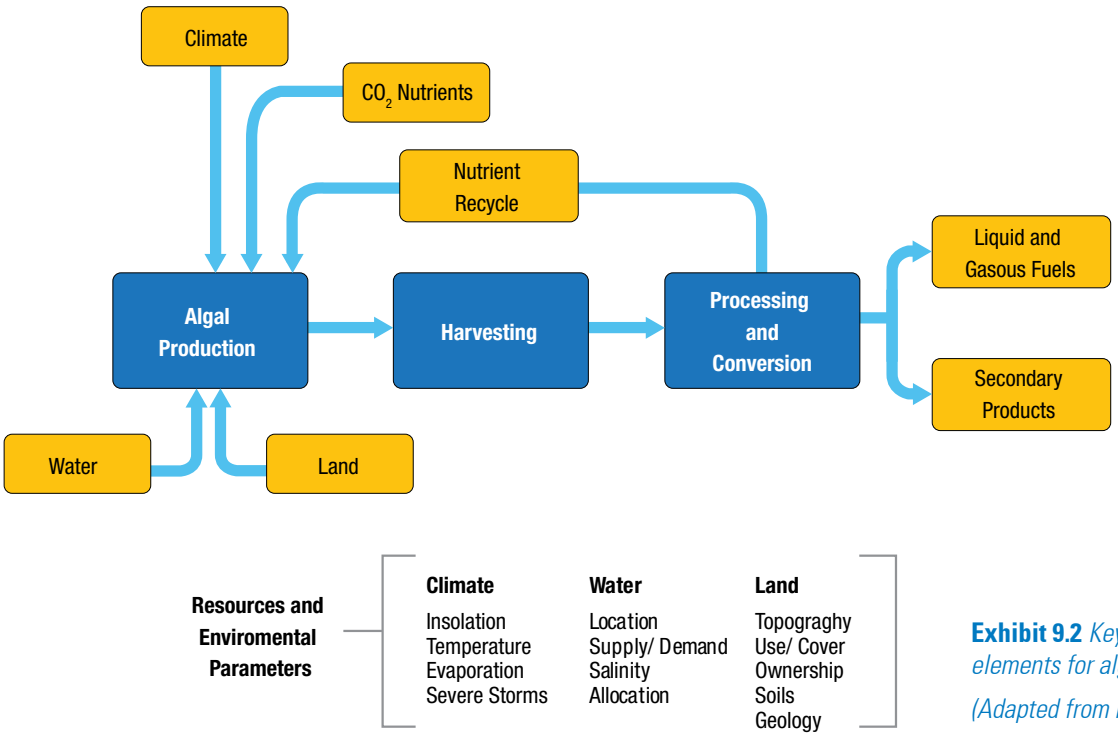


Exhibit 9.2 *Key siting and resource elements for algae biofuel production (Adapted from Maxwell et al., 1985)*

can be represented by extension of Exhibit 9.2 to conceptually include the equivalence of land and culture facilities with off-shore areas and structures. A similar conceptual extension holds for the application of Exhibit 9.2 to off-shore macroalgae production.

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 of photosynthesis. 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 (Exhibit 9.3). Heterotrophic and photoautotrophic approaches to microalgae production have different siting and resource input implications and thus present synergistic integration opportunities. 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.

Use of sugars from cane, beets, other sugar crops, and from the hydrolysis of starch grain crops can, after sufficient scale-up of production and demand, lead to the problem of linking biofuel production with competing food and feed markets. The preferred source of sugars and other appropriate organic carbon feedstocks for greatest sustainable scale-up potential and avoidance of adverse food/feed market impacts would be based on the use of 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 Department of Agriculture (USDA; e.g., Perlack et al., 2005; DOE, 2006a). This includes siting and resource issues that are closely coupled with the production, availability, supply logistics, and pretreatment of lignocellulosic biomass feedstock that is expected to be capable of national scale-up to over one billion tons annually (Perlack et al., 2005).

Photoautotrophic Macroalgae Approach

Options for siting macroalgae (also known as seaweed or kelp) biomass production include offshore farms, near-shore coastal farms, and land-based ponds. The merits of each should be carefully evaluated, taking into consideration factors such as the scale of farms required to meet production needs, cost and availability of space

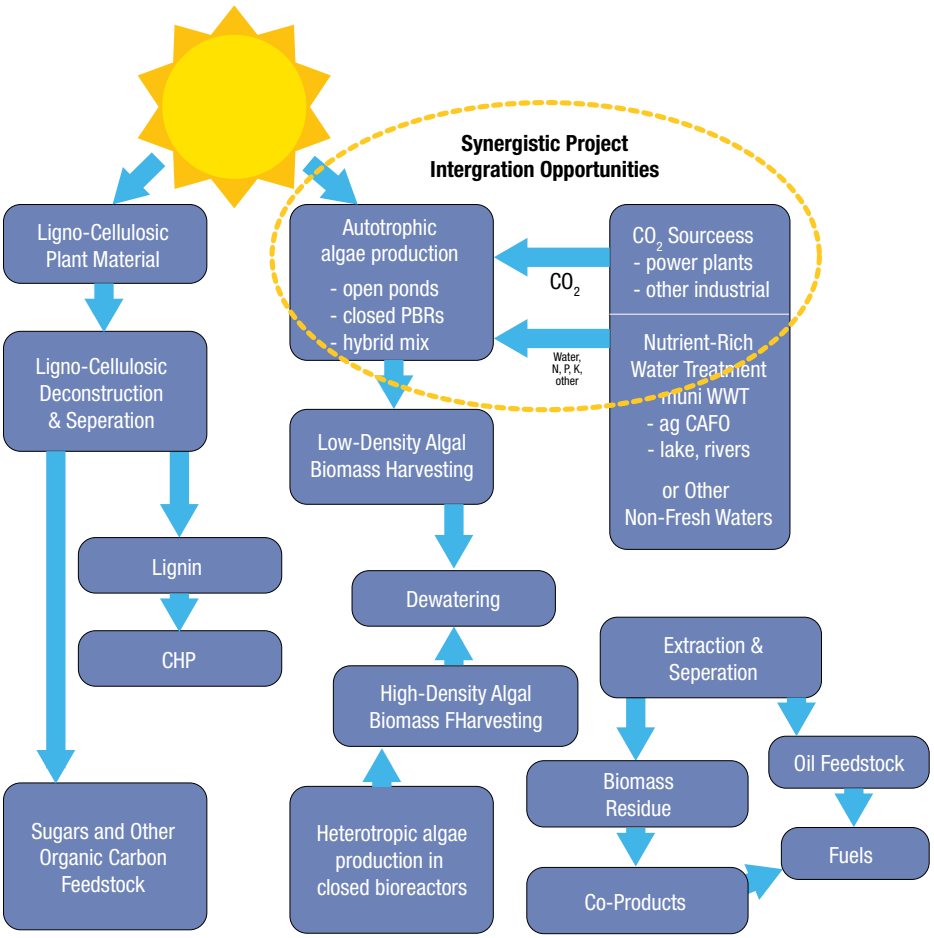


Exhibit 9.3 *High-level illustration of heterotrophic and photoautotrophic approaches to microalgal biomass and biofuels production*

and nutrients, environmental impacts, and competition with other uses. The co-siting of macroalgal farms with other structures such as windfarms (Buck et al., 2004) has also been proposed as a way of leveraging other technologies to facilitate the cultivation of macroalgae. Based on the scale of macroalgae cultivation practices currently being used to meet non-fuel product demand (annual global production of about 1.4 million dry metric tonnes for food products), the level of production would need to be greatly expanded for biofuels.

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.

9.2 Resources Overview

Climate

Sunlight and Temperature Needs

Various climate elements affect photoautotrophic algae production, particularly in the case of land-based microalgae production. As illustrated in Exhibit 9.4, key factors include solar radiation, temperature, precipitation, evaporation, and severe weather. Closed photobioreactors are less sensitive to climate variability than open ponds due to the more controlled environment that closed systems can provide. Temperature and availability of sunlight, both seasonally and annually, will most directly affect productivity, whereas precipitation, evaporation, and severe weather will affect water supply and water quality in open systems. Heterotrophic algae production in closed industrial bioreactor systems utilize mature bioreactor technology that can provide relatively clean and protected environmental control needed to optimize growth conditions for higher biomass density cultures. Heterotrophic algal cultures can be one to two orders of magnitude higher in biomass density than possible with open photoautotrophic systems that typically achieve no more than about 1 gram per liter culture density. From a resource use perspective, the heterotrophic approach can reduce both water and energy-use intensity in the algae production operation. Closed heterotrophic bioreactor systems are decoupled from the variable ambient outdoor climate, weather conditions, and day/night light and temperature cycles. This is done at the expense of

high-cost systems, controls, and enclosures requiring energy input for environmental control. For the case of offshore marine macroalgae production, the ocean or sea environment provides a buffer that moderates temperature variation, with the major climate element affecting algal growth being the availability of sunlight. Storm and ocean dynamics affecting waves, currents, and marine nutrient transport cause additional impacts on productivity.

Equally important for photoautotrophic microalgae growth with both open and closed cultivation systems is the availability of abundant sunlight. A significant portion of the United States is suitable for algae production from the standpoint of having adequate solar radiation (with parts of Hawaii, California, Arizona, New Mexico, Texas, and Florida being most promising). The more northern latitude states, including Minnesota, Wisconsin, Michigan, and the New England states, would have very low productivity in the winter months. 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 siting and resource factors that will determine economic feasibility.

Preferred Geographic Regions for Algae Production

Exhibit 9.4 presents some simple GIS-based scoping conducted by Sandia National Laboratories. The goal was to provide a preliminary high-level assessment identifying preferred regions of the United States for photoautotrophic microalgae production based on the application of selected filter criteria on annual average climate conditions, the availability of non-fresh water, and the availability of concentrated sources of CO_2. The climate criteria used to narrow down the geographical regions were: annual average cumulative sun hours ≥ 2800, annual average daily temperature ≥ 55°F, and annual average freeze-free days ≥ 200.

It was recognized from the outset that the correlation of these annual average climate criteria with what might actually be achieved with annual average algae productivity in large-scale deployed systems could only be taken as a gross indicator. Within that context, however, the results are suggestive and consistent with the more recent assessment by Pacific Northwest National Laboratory (PNNL) using higher GIS resolution and more information-rich climate and meteorological data. Projections of annual average algae biomass production from the PNNL study show clear patterns relating climate to total biomass growth, with the higher growth regions having gross qualitative similarity to Exhibit 9.4 and the southern tier states showing greatest productivity potential based on the modeling assumptions used. In Exhibit 9.4 (a), the lack of attractiveness of the Gulf Coast region from southeast Texas to northwest Florida is attributed to the lower annual average solar insolation available, whereas PNNL study suggests projected productivities in this region could be relatively high. This is likely due to more moderate annual average temperatures despite lower solar insolation. These results should be considered simply indicative at this point rather than predictive, but suggest preference for lower latitude, i.e., lower elevation sites.

Additional factors could conceivably overcome what might otherwise appear to be uneconomical site and resource conditions for algae production. This could include situations where co-location of inland microalgae production at higher latitudes might be possible with

Exhibit 9.4 *Rough scoping assessment of preferred site locations for outdoor algae production*

a) Regions with annual average climate conditions meeting selected criteria: ≥ 2800 hour annual sunshine, annual average temperature ≥ 55° F, and ≥ 200 freeze-free days

b) Fossil-fired power plant sources of CO_2 within 20 miles of municipal wastewater facilities in the preferred climate region

industrial operations capable of providing excess heat and power for cost-effective environmental control of the algae cultivation. This would, however, require a more refined analysis for systems that would likely be closed and highly integrated with co-located industries providing synergistic opportunities for utilizing waste heat and energy. Such analyses should include assessment of the monthly or seasonal solar radiation and ambient temperature ranges; it should also establish minimum economically feasible operational requirement values for heating in the winter and, for closed reactors, cooling in the summer.

Seasonal Considerations

Various species of microalgae have potential for biofuel feedstock production and are capable of growing under a wide range of temperatures. High annual production for a given species grown photoautotrophically outdoors, however, will require that suitable climatic conditions exist for a major part of the year (Maxwell et al., 1985). Therefore, a critical climate issue for both open and closed photobioreactor systems is the length of economically viable growing season(s) for the particular strains of algae available for productive cultivation. For outdoor ponds, the conventional crop analogy for this is the length of time between the last killing frost in the spring and the first killing frost in the fall. For closed photobioreactors, the conventional crop analog is the greenhouse and the limiting energy and cost needed to maintain internal temperature throughout the seasons. Availability and rotation of different algal species capable of good productivity in cold season and hot season conditions, respectively, would provide greater flexibility and could extend otherwise limited periods of commercially viable algae production.

The primary geographical location factors for determining length of growing season 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. Other local climate and weather conditions will also have influence. Thorough analysis (preferably on a state-by-state basis) with detailed data is needed to assess areas most suitable for algae production based on this climate factor. Discovery and development of algae species capable of increased productivity under wider ranges of light and temperature conditions can also potentially lead to increased annual average productivities in more geographically diverse locations. It is encouraging that researchers today are not only concerned with finding algae with high biomass productivity and oil content yield, but also with algae that grow well under severe climate conditions, particularly extremes in temperature, both high and low.

Water Requirements

Precipitation 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. Evaporation, discussed later in this section, is closely coupled with climate and will increase water requirements for an open algae growth system. Evaporative loss can be a critical factor to consider when choosing locations for open pond production. Evaporation is a less important concern for selecting locations of closed photobioreactors, although evaporative cooling is often considered as a means to reduce culture temperature. The southwestern states (California, Arizona, and New Mexico) and Hawaii have the highest evaporation rates in the United States, with more than 60 inches annually. A thorough evaluation of evaporative water loss is needed to assess water requirements, implications for sustainable production scale-up, and overall economics. Evaporative water loss is discussed in a later subsection.

Severe Weather Events and Elements

Severe weather events, such as heavy rain and flooding, hail storms, dust storms, tornadoes, and hurricanes pose serious concerns in the inland regions of the central states, Southwest, Southeast, and coastal areas. These weather events can contaminate an open system environment or cause physical damage to both open and closed systems, and need to be taken into account when looking at prospects for algae production in both inland and coastal regions of the United States. Offshore marine algae production will be subject to severe weather impacts in the form of wind, waves, and currents that can cause disruption or damage to physical structures and operations. The marine environment can also be highly corrosive to materials and usually demands both the use of higher quality and more costly materials and greater maintenance.

Water

General Water Balance and Management Needs

One of the major benefits of growing algae is that, unlike most terrestrial agriculture, algal culture can potentially utilize non-fresh water sources having few competing

uses, such as saline and brackish ground water, or "co-produced water" from oil, natural gas, and coal-bed methane wells (Reynolds, 2003; USGS, 2002). However, for 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. This will require periodic blow-down of ponds after salinity build-up, periodic addition of non-saline make-up water to dilute the salinity build-up, the application of desalination treatment to control salinity build-up, or highly adaptive algae that can thrive under widely varying conditions. 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 water desalination would 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. Water balance and management, along with associated salt build-up and management issues, from both a resource perspective and an algal cultivation perspective, are important areas for future research, modeling, and field assessment.

Analysis of U.S. Water Supply and Management

Total combined fresh and saline water withdrawals in the United States as of the year 2005 were estimated at 410,000 million gallons per day (Mgal/d), or 460,000 acre-feet per year (Kenny et al., 2009). Saline water (seawater and brackish coastal water) withdrawals were about 15% of the total. Almost all saline water, about 95%, is used by the thermoelectric-power industry in the coastal states to cool electricity-generating equipment. In 2005, nearly one-half of the total U.S. withdrawals (201,000 Mgal/d) were for thermoelectric-power generation, representing 41% of all freshwater, 61% of all surface water, and 95% of all saline-water withdrawals in 2005. Withdrawals for irrigation of crops and other lands totaled 128,000 Mgal/d and were the second-largest category of water use. Irrigation withdrawals represented 31% of all water withdrawals, and 37% of all freshwater withdrawals (Kenny et al., 2009). At the national scale, total combined fresh and saline water withdrawals more than doubled from about 180 billion gallons per day in 1950 to over 400 billion gallons per day in 1980. Total withdrawals since the mid-1980s have remained relatively flat at slightly over 400 billion gallons per day, with the majority (85%) being fresh (Hutson et al., 2004; Kenny et al., 2009).

The relatively flat national water withdrawal trend over the past 25 years, following the more than doubling of water demand over the 30 years prior to that, reflects the fact that fresh water sources in the Untied States are approaching full allocation. Growing demand for limited fresh water supplies in support of development and population increase has thus far been offset by increased conservation and by the increased re-use of wastewater. Many of the nations' fresh ground water aquifers are under increasing stress, and the future expansion of fresh water 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 (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 eastern half of the country, where a growing number of counties are experiencing net fresh water withdrawals that exceed the sustainable supply from precipitation (DOE, 2006b; Hightower et al., 2008;). Climate change is also recognized as a factor that could have major effect on all sectors of water resources supply and management in the future (USGS, 2009).

Scoping Out Water Requirements for Algae Production

Water use and consumption for algae-based biofuels will depend on the cultivation approach (photoautotrophic/heterotrophic), with water use in upstream organic carbon feedstock production needing to be part of the heterotrophic assessment. Water use will also depend on the type of growth systems used for photoautotrophic microalgae (open vs. closed vs. hybrid combination), whether evaporative cooling is used for closed systems, and the site-specific details of climate, solar insolation, and weather conditions (cloud cover, wind, humidity, etc.). Also a complicating factor for evaporative water loss in open systems will be the degree of salinity of the water used for cultivation and the local latitude, elevation, ambient temperature variations, solar insolation, humidity, and wind conditions (Al-Shammiri, 2002; Hutchison et al., 1978; Kokya et al., 2008; Mao, 1999; Oroud, 1995). A significant source of water demand with inland algae production operations could be for the replacement of water continuously lost to evaporation from open cultivation systems. Whether or not this is a problem for sustainable algal industry scale-up will depend on the geographic location, climate conditions, and locally available water resources. This will be of greatest impact and concern in water-sparse locations, which also tend to be in the more arid and higher solar resource regions like the Southwest. A rough upper bound estimate of evaporative water loss

with open systems was done as part of a hypothetical scale-up scenario study in preparation for the National Algae Roadmap Workshop, held in December 2008. The notional scale-up scenarios and assumptions made are discussed later in this chapter (see Exhibit 9.7).

The evaporative loss estimates from the notional scenarios provide an indication of the potential magnitude of the issue, and were based on simply applying fresh water open horizontal pan evaporation data (Farnsworth et al., 1982a and 1982b; Shuttleworth, 1993; Woolhiser et al., 1984) to large area scale-up of algae cultivation with open systems. Extrapolations based on fresh water pan evaporation data will be worst-case and will likely be an over-estimate of what can be expected under actual operating conditions in the field. Open bodies of brackish and saline water will usually experience less evaporative loss than fresh water, as noted earlier.

The evaporation estimates suggest that water loss on the order of several tens of gallons of water per kilogram of dry weight biomass produced, or several hundreds of gallons of water per gallon of algal biofuel produced, could be a consequence of open system operation in the more arid and sunny regions of the country. The most optimistic production scenario was for the southwestern United States, which assumed annual average algal biomass and oil productivities of nearly 31 g/m^2 per day with 50% dry weight oil content. If less optimistic productivities are assumed, the estimated evaporative water loss intensities will be greater.

Evaporative water loss associated with algae cultivation can be significantly reduced if closed systems are used. Evaluation of water use for the overall value chain from algal cultivation through harvesting and post-processing into fuels and other products will also be important. Along the way, additional water will be used and consumed, and may well also be saved, reclaimed, and recycled, depending on systems and process specifics. Water must be considered a key element of life cycle analysis for algal biofuels, as with other forms of bioenergy (NAS, 2007; Gerbens-Leenes et al., 2009).

In summary, water utilization for algal biomass and downstream production of biofuels, both in terms of overall input supply needs and consumption, warrants closer attention and assessment to better understand and refine water resource requirements. Water requirements information will not be well characterized until larger scale systems are implemented, monitored, and evaluated under a range of site locations and conditions. There is considerable untapped potential for utilizing brackish, saline, and co-produced water. Additional analysis, experimental investigation, and field trials at larger scales of operation are needed to understand how best to leverage these resources.

Developing and Mapping Water Resources for Algae Production

When considering the water resources needed for the future development and expansion of algal biofuel production, the use of non-fresh water sources will need to be emphasized in the face of the growing competition and demands on limited sustainable fresh water supplies (DOE, 2006b; NAS, 2007; Hightower et al., 2008). From a resource use standpoint, integrating algae production with wastewater treatment, discussed later in this chapter, has the potential benefits of productively using non-fresh wastewater resources for renewable fuels, putting less additional demand on limited fresh water supplies, reducing eutrophication of natural water bodies, and recycling nutrients.

The unique ability of many species of algae to grow in non-fresh water over a range of salinities means that, in addition to coastal and possible off-shore 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. Unfortunately, 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. 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 ground water resources, and to predict the effects of their extraction on the environment (Alley, 2003; Dennehy, 2004). Saline groundwater resources, particularly deeper aquifers that are largely unregulated by state engineers and water authorities, are also of increasing interest and potential competition for access as a source of water for treatment and use to meet commercial and residential development needs in high growth rate water-sparse regions like the Southwest (Clark, 2009). Depth to groundwater is pertinent to the economics of resource development. Along with geological data, depth information determines the cost of drilling and operating (including energy input requirements for pumping) a well in a given location (Maxwell et al., 1985). Suitable aquifers located closer to the surface and nearer to the cultivation site would provide a more cost-effective source of water for algae production than deeper sources located longer distances from the cultivation site. The location, depth, and chemical characterization of saline aquifers in the United States are areas of investigation in need of greater investment. The maps of saline groundwater

resources are based on incomplete data that was largely developed by the USGS prior to the mid-1960s (Feth, 1965; Dennehy, 2004). More detailed and up-to-date information is needed to improve our understanding of this resource in support of algae production siting analyses (Dennehy, 2004; NATCARB, 2008b). Produced water from petroleum, natural gas, and coal bed methane wells is another closely related and underutilized water resource that can range in quality from nearly fresh to hyper-saline (Reynolds 2003; USGS, 2002).

The geographical location, spatial extent, depth, potential yield, recharge rate, sustainability of supply, and quality (chemical components and characteristics) are important information for assessing non-fresh groundwater aquifer resource availability and suitability for economic uses (Shannon, 2006), including algae production. A limited amount of this information is available for major aquifers. However, if these aquifers are spread over large geographic areas, detailed analysis is difficult and often lacking. Data on small, local aquifers may be available through state agencies and private engineering companies, but a significant effort will still be needed to locate, identify, collect, and analyze this information.

Carbon Dioxide

The Carbon Capture Opportunity in Algae Production

Efficient algae production requires enriched sources of CO_2 since the rate of supply from the atmosphere is limited by diffusion rates through the surface resistance of the water in the cultivation system. Flue gas, such as from fossil-fuel-fired power plants, would be a good source of CO_2. Algae production could provide excellent opportunities for the utilization of fossil carbon emissions and complement subsurface sequestration. However, algae production does not actually sequester fossil carbon, but rather provides carbon capture and reuse in the form of fuels and other products derived from the algae 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. In addition, at some large scale of algae production, parasitic losses from flue gas treatment, transport, and distribution could require more energy input than the output energy displacement value represented by the algae biofuels and other co-products.

Likely Stationary CO_2 Emission Sources

Major stationary CO_2 emission sources that could potentially be used for algae production are shown in Exhibit 9.5. The sources shown (NATCARB, 2008) represent over half of the more than 6 billion metric tons of CO_2 emitted annually in the United States (EPA, 2009; EIA, 2008 and 2009). Power generation alone (mainly using coal) represents over 40% of the total, or more than 2 billion metric tons per year (EIA, 2008 and 2009).

Barriers to Viable CO_2 Capture and Utilization

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, and the availability of land and water for algae cultivation scale-up within reasonable geographic proximity of stationary sources.
As an example, a recent analysis suggests that for algae production to fully utilize the CO_2 in the flue gas emitted from a 50-MWe semi-base load natural-gas-fired power plant would require about 2,200 acres of algae cultivation area (Brune et al., 2009). The CO_2 generated by the power plant can only be effectively used by the algae during the photosynthetically active sunlight hours. 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 parasitic losses of algae production (Brune et al., 2009). Larger coal-fired base-load generators that typically output a steady 1,000 to 2500 MWe of power would each require many tens of thousands of acres of algae production and large volumes of water to provide a similar effective offset of 20% to 30% of the CO_2 emitted.

The distance for pumping flue gas to algae cultivation systems will become a limiting factor that requires capture and concentration of CO_2 from the flue gas for longer distance transport and distribution. Applications separating CO_2 in large industrial plants, including natural gas treatment plants and ammonia production facilities, are already in operation today and under consideration for possible broader use for carbon capture and storage (CCS) in response to climate change (Rubin, 2005; Campbell et al., 2008). Photoautotrophic algae will only utilize CO_2 during daylight hours when photosynthesis is active. The rate of effective CO_2 uptake will also vary with the algae species, biomass growth rate, and details of growth system and incident light conditions. 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. In addition, it will be necessary to provide a CO_2 source that is suitably free of materials potentially toxic to algae.

An inventory of stationary industrial CO_2 sources in the more promising regions of the country, including 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 the local availability and distance to suitable land for algae

production, is needed for making refined assessments for algae production siting and CO_2 sourcing. One outcome of a hypothetical algae production scale-up scenario is the limited quantity of CO_2 that would likely be available from stationary industrial point sources (e.g., Exhibit 9.5) within practical transport distances of suitable algae production sites in a given geographical region. This can be expected to constrain the extent to which algal biofuels production can be affordably scaled up within any given region unless other factors drive the investment in expanding the nation's CO_2 pipeline infrastructure.

Land

Factors for Evaluating Land for Algal Production

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 2600 acres of algae culture surface area to produce 10 million gallons of oil feedstock, as will be discussed further in chapter 10.

Land availability is influenced by various physical, social, economic, legal, and political factors, as illustrated in Exhibit 9.6. 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. For a realistic appraisal of land for algae production (i.e., land that would be both suitable and potentially available for siting algae production facilities), several characteristics need to be considered.

Physical characteristics, such as topography and soil, could 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 sealing. Topography would be a limiting factor for these systems because the installation of large shallow ponds requires relatively flat terrain. Areas with more than 5% slope could well be eliminated from consideration due to the high cost that would be required for site preparation and leveling.

Land ownership information provides valuable insights on which policies and stakeholders could affect project development. Publicly and privately owned lands are subject to variable use, lease, and purchase requirements. For example, much of the land in the West is government owned, which means that environmental assessments and/

Exhibit 9.5 *Major stationary CO_2 sources in the United States (NATCARB, 2008a)*

CATEGORY	CO_2 EMISSIONS (Million Metric Ton/Year)	NUMBER OF SOURCES
Ag Processing	6.3	140
Cement Plants	86.3	112
Electricity Generation	2,702.5	3,002
Ethanol Plants	41.3	163
Fertilizer	7.0	13
Industrial	141.9	665
Other	3.6	53
Petroleum and Natural Gas Processing	90.2	475
Refineries/Chemical	196.9	173
Total	**3,276.1**	**4,796**

or environmental impact statements could be required as part of the approval process. Indian reservations also comprise a significant portion of this land. Land ownership can represent and impose political and regulatory constraints on land availability (Maxwell et al., 1985).

Further, as with any form of biomass, algae productivity will be constrained by the available energy density in sunlight and the relatively low efficiencies of photosynthetic processes coupled with other systems losses. The result will be theoretical and practical upper limits on the amount of biomass growth that can be achieved per unit of illuminated surface (Weyer et al., 2009). Contributing to productivity limits per unit of illuminated surface area is the fact that algal cells nearest the illuminated surface absorb the light and shade their neighbors farther from the light source. Optimizing light utilization in algae production systems includes the challenge of managing dissipative energy losses that occur when incident photons that cannot otherwise be effectively captured and used by the algae in photosynthesis are instead converted to thermal (heat) energy in the culture media and surrounding cultivation system structures. Depending on the algae strain and culture system approach used, the dissipative heat loading can be a benefit in moderating culture temperatures and improving productivity under colder ambient conditions, or can lead to overheating and loss of productivity during hotter ambient conditions. Loss mechanisms that restrict the fraction of incident photon flux that can be effectively used to drive photosynthesis ultimately places practical upper limits on the biomass productivity. Despite the practical upper limits that will naturally exist for algae productivity, the potential remains for high algae biomass production relative to more conventional crops, (chapter 10). It must be stressed that "potential" algal biomass and bio-oil production projections at commercial scale currently remain hypothetical rather than real, given that large-scale algae biomass production intended for bioenergy feedstock does not yet exist. Relatively large-scale commercial algae production with open ponds for high-value products can serve as a baseline reference, but currently reflect lower biomass productivities in the range of 10 - 20 g/m^2 per day. This is significantly lower than the more optimistic target projections for biofuel feedstock of 30 - 60 g/m^2 per day. However, such systems have not been optimized for higher-volume, lower-value production with algal strains developed and improved over time to be more suitable and productive for biofuel feedstock rather than those used for today's high-value algae biomass product markets.

Land Availability Constraints

Land use and land value affect land affordability. By reviewing the more recent economic analyses for algae biomass and projected oil production, the cost of land is often not considered or is relatively small compared to other capital cost. Land that is highly desirable for development and other set-asides for publically beneficial reasons may

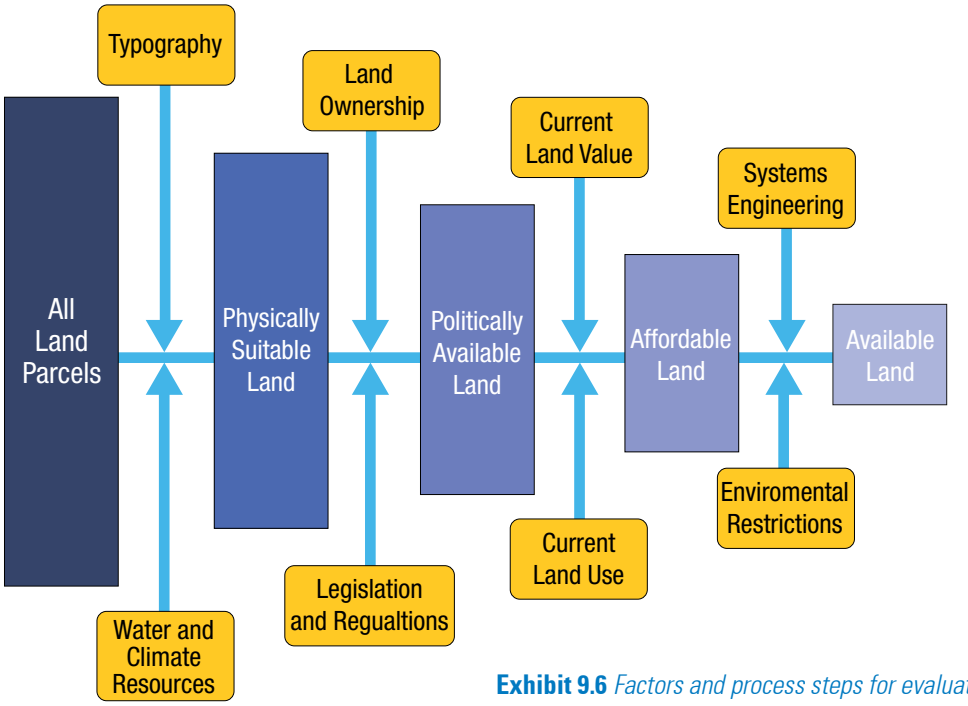


Exhibit 9.6 *Factors and process steps for evaluating and constraining the available and appropriately suitable land for algae production (Adapted from Maxwell et al., 1985)*

not be seen as suitable for algae production. The same applies to land highly suited for higher-value agricultural use. Beyond economics, this also avoids the perception and potential conflict of food and feed production versus fuel. Sensitive environmental or cultural land constraints will also reduce the overall land availability (Maxwell et al., 1985). Examples of this type of constraint include parks, monuments, wildlife areas, archaeological sites, and other historical sites. On the other hand, some land cover characteristics could present excellent opportunities for algae farming. Land cover categories such as barren and scrubland cover a large portion of the West and may provide an area free from other food-based agriculture where algae growth systems could be sited (Maxwell et al., 1985). The availability and sustainability of water supplies in the West will also be a key consideration, as noted earlier.

9.4 Integration with Water Treatment Facilities

Inevitably, wastewater treatment and recycling must be incorporated with algae biofuel production. The main connections of algae production and wastewater treatment are the following:

- Treatment technology is needed to recycle nutrients and water from algae biofuel processing residuals for use in algae production.
- Imported wastewater provides nutrients and water to make-up inevitable losses. The imported wastewater would be treated as part of the algae production.
- Algae-based wastewater treatment provides a needed service.
- Algae-based wastewater treatment can be deployed in the near-term and provides workforce training and experience in large-scale algae cultivation that would translate to future dedicated algae feedstock production facilities.

For large-scale algae biofuel production, nutrients from wastewater (municipal and agricultural) would be captured by algae and then recycled from the oil extraction residuals for additional rounds of algae production. Nutrient recycling would be needed since wastewater flows in the United States are insufficient to support large-scale algae production on the basis of a single use of nutrients. Inevitable nutrients losses during algae production and processing could be made up with wastewater nutrients, which can also help supplement and off-set the cost of commercial fertilizers for algae production. Supply and cost of nutrients (nitrogen, phosphorus, and potassium) be a key issue for achieving affordable and sustainable scale-up of algae biofuels production.

POTENTIAL BENEFITS OF ALGAE PRODUCTION WITH WASTEWATER TREATMENT

Although algae-based wastewater treatment requires many times more land area than mechanical treatment technologies, in suitable climates, algae-based treatment has the following advantages:

- Early opportunity to develop large-scale algae production infrastructure
- Development of skilled algae production workforce
- Potential for nutrient recycling at algae biomass production facilities
- Wastewater treatment revenue that offsets algae production costs
- Lower capital and O&M costs than conventional wastewater treatment
- Lower energy intensity than conventional wastewater treatment (a greenhouse gas benefit)
- Potential to be integrated with power plant or other CO_2-emitting industry operations
- Potential to treat agricultural drainage and eutrophic water bodies

Wastewater Treatment and Recycling Applications

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 facility are envisioned: dedicated facilities, with the main purpose of biomass production, and 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 roles of these facility types in the development of an algae biofuels industry are discussed in this subsection.

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 disrupting compounds (Oswald, 1988; Woertz et al., 2009; Aksu, 1998; Borde et al., 2002). 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, or manipulation of culture conditions to promote lipid production, have yet to be demonstrated consistently for ponds, including wastewater treatment ponds.

Algae-based 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. 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). 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 Production Techniques for Wastewater Treatment Plants

Integration of algae production with wastewater treatment is illustrated schematically in Exhibit 9.7. Existing algae-based treatment facilities use relatively deep ponds (1-6 m). The great depths contribute to low algae 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 algae productivities (as does biofuels feedstock production). These highly productive systems use shallow reactors, either high rate ponds (~30 cm) or algal turf scrubbers (~1 cm). Closed photobioreactors are not emphasized in this wastewater treatment discussion since they are likely to be economical only when also producing high-value products (>$100/kg biomass), which is unlikely when wastewater contaminants are present.

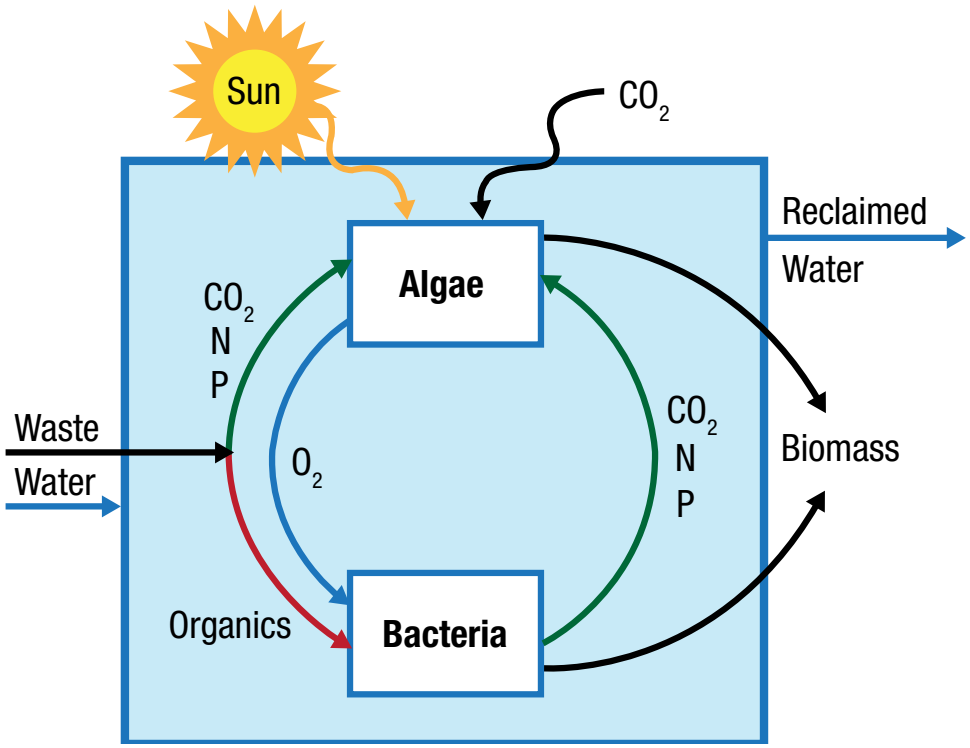
As with other algae production systems, harvesting is a crucial step in wastewater treatment systems. The standard method is chemical addition to achieve coagulation and flocculation, followed by algae separation in dissolved air flotation units or sedimentation clarifiers. The cost of chemical addition ($0.10 - $0.17 per m3 treated) (Maglion, 2008) is high for biofuel production. Non-chemical flocculation processes (bioflocculation and autoflocculation) are far less costly, but research is needed to improve the reliability of these processes (as discussed in chapter 4). As noted above, the major types of wastewaters available for combined algae production and water treatment are those contaminated with organic matter and nutrients (e.g., municipal and industrial sources) and wastewaters mainly contaminated with inorganic nutrients (e.g., agricultural drainage, rivers, and lakes).

Treatment of Organic Wastewaters for Algae Production

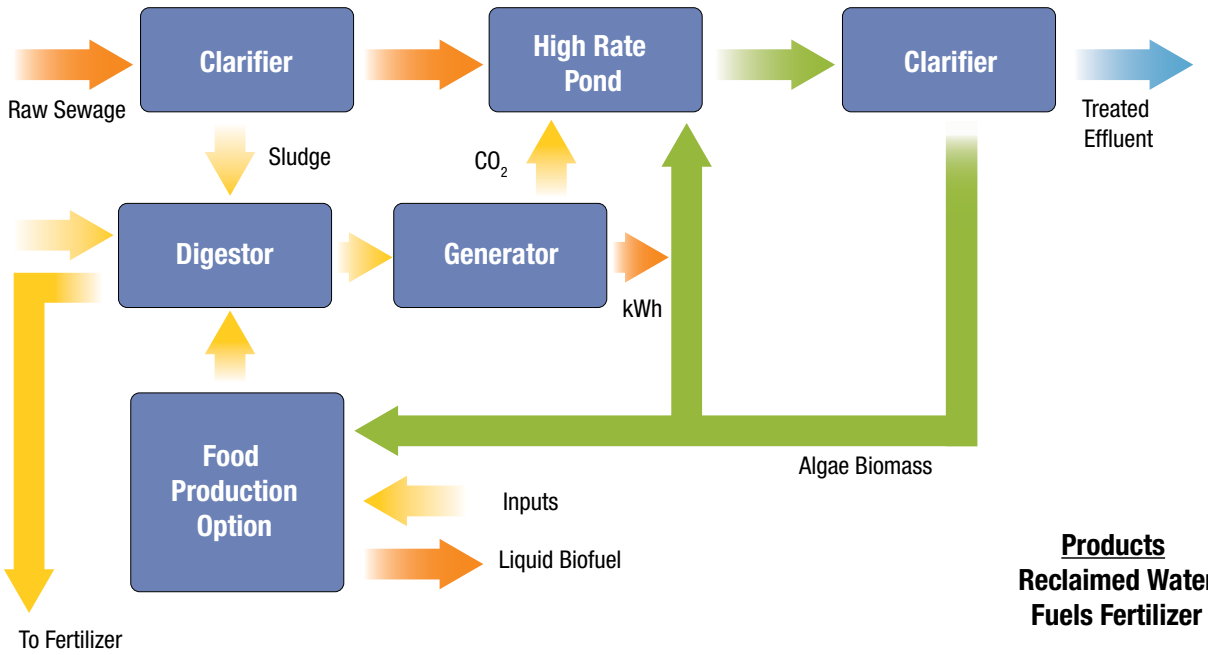
Organic-rich wastewaters usually also contain nutrients, requiring two types of treatment. Algae are similar to plants in that they both produce oxygen and assimilate nutrients. These reactions are also the best-known mechanisms of wastewater treatment by algae. The dissolved oxygen algae release is used by treatment bacteria to oxidize waste organic matter (Exhibit 9.7). 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. Less well-known are the ability of algal systems to provide natural disinfection and remove trace contaminants. 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 algae biomass are not excessive or inhibitive for later use in the processing of fuel and other co-products. Finally, 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., 2003, Muñoz et al., 2005). The removal of trace contaminants (e.g., endocrine disrupting compounds such as human hormones and antibiotics from animal facilities) is an area in need of study.

Mechanical treatment technologies typically hold the wastewater for less than 12 hours, whereas pond technologies hold the wastewater for at least several days and in an environment similar to many natural receiving waters. The bioaccumulation of trace contaminants in algae that would occur in the receiving waters, eventually harming higher organisms, might be prevented to a

Exhibit 9.7 *Integration of algae production with wastewater treatment for nutrient removal and biomass production:*



a) *Basic principles of operation;*



b) *Simplified conceptual system block diagram*

great extend 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.

Treatment of Inorganic Wastewaters for Algae Production

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, Calif.) are examples 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.

For algae-based treatment of low-organic content wastewaters, CO_2 addition or slow atmospheric absorption is essential since inorganic carbon generation from decomposition of organic matter would not be significant. Treatment of agricultural drainage with algal turf scrubbers 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).

High rate ponds might be used as components of evaporation systems needed to dispose of blow-down or other wastewater. The high rate ponds could create an algal product while performing the service of water evaporation. Evaporation ponds are currently used to dispose of agricultural drainage, oil field produced water, mine drainage, etc. As with any evaporation pond system, hazards to wildlife from toxic compounds (e.g., selenium, chromium) must be carefully evaluated.

Main Research Needs for Algae Production with Wastewater

Successful use of high rate ponds specifically for nutrient removal/recycling requires resolution of several issues, as follows:

- Large-scale (3-5 acre) demonstration of CO_2-enhanced high rate ponds for nutrient removal
- Determine CO_2 biofixation efficiency
- Determine growth model parameters
- Develop algae grazer control strategies
- Develop reliable low-cost algae harvesting techniques, such as bioflocculation, autoflocculation, micro-screening, etc.
- Demonstrate recycling of biofuel processing wastes for algae production
- Determine the cost savings and greenhouse gas (GHG) emissions avoidance benefits compared to conventional wastewater treatment technologies.

9.5 Co-location of Algal Cultivation Facilities with CO_2-Emitting Industries

This section includes findings from discussions held at the National Algal Roadmap Workshop break-out sessions, and additional input sought from managers at major electric utilities through later meetings and conference calls. These follow-on efforts were coordinated with the Electric Power Research Institute (EPRI), and included several large municipal electric utilities. The topics of discussion included the value proposition, desired outcomes, integration opportunities and challenges, market drivers, technical and market challenges, constraints on large-scale development, co-products, and potential opportunities for the federal government. Findings from these interviews and conference calls were integrated with the Workshop inputs in developing this section. It is important to point out that amongst the numerous barriers to co-location of algal cultivation facilities with industrial CO_2 sources identified at the Workshop and subsequent discussions with electric utilities, an overriding theme was that electric utilities primarily view algae cultivation as a means of CO_2 capture as opposed to a method for producing biofuels and co-products. Thus, electric utilities may need to partner with algae cultivation/technology companies and fuel refiners/distributers with very different business models and goals for algae production in order for this type of co-location to be widely commercialized. Furthermore, research efforts and policy evaluations would likely need to focus on both carbon capture and the production of biofuels and co-products to overcome barriers (technical, regulatory and economic) for algae facilities that are co-located with electric utilities and other industrial CO_2 sources.

The Opportunity in Co-Locating with CO_2-Emitting Sources

Since photoautotropic algae growth requires CO_2, and productivity can be enhanced by supplementing the limited CO_2 available from the atmosphere, concentrated sources flue gas from fossil-fuel burning power plants and other CO_2-emitting industrial sources can be beneficially used in algae production. Resulting costs for CO_2 will be site-specific and dependent on methods of capture, conditioning, and distance of transport to algae cultivation sites. Costs are expected to be lower than for pure commercially supplied CO_2, but economic viability must be determined case by case. Offset of fossil fuel consumption by algae biofuel and other co-products must be done with approaches that provide a net GHG emissions reduction. Co-location of algal cultivation with industrial CO_2 sources is a promising area for further research.

While the information in this section focuses on fossil-fired power plants, it is also relevant to other CO_2-intensive industries (e.g., cement manufacturing, fossil fuel extraction/refining, fermentation-based industries, some geothermal power production, etc.). The emissions from many of these facilities have higher CO_2 concentrations compared to power plant flue gas, which typically ranges from about 5% to about 15%, depending on the type of plant and fuel used. This higher concentration would affect the sizing and operations of algae production facilities—an aspect that could be incorporated into engineering models described in more detail in the Systems and Techno-Economic Assessment section of this report.

An important policy question to consider is the value of CO_2 absorption by algae in any carbon-credit or cap and trade framework, in that the carbon will be re-released to the atmosphere when algal-derived fuels are combusted. While algae biofuels can be expected to result in a net reduction of overall GHG emissions, the process of capturing flue-gas CO_2 to make transportation fuels may not rigorously be considered carbon sequestration. The regulatory implications of this will need to be addressed before utilities and fuel companies are likely to widely adopt algal cultivation co-located with industrial CO_2 sources. A variety of stationary industrial sources of CO_2 are distributed throughout the United States. The quantitative breakdown, introduced earlier in Exhibit 9.5, shows that fossil-fired power plants represent the majority of CO_2 emissions from stationary sources. A number of large coal-burning power plants distributed across the southern tier states provide ample sources for algal growth on a large scale.

ADVANTAGES OF CO-LOCATION OF ALGAE PRODUCTION WITH STATIONARY INDUSTRIAL CO_2 SOURCES

- Abundant quantities of concentrated CO_2 available from stationary industrial sources can supplement low concentration CO_2 from the atmosphere.
- Excess heat or power may be available to provide heating or cooling for improved thermal management of algae cultivation systems – this will allow developing algal cultivation facilities under a broader range of geographic and climate conditions on or near a year-round basis.
- Excess wastewater or cooling water may be available, found often in proximity of power plants – overcoming a primary resource challenge for algae cultivation at scale, while providing beneficial re-use of cooling water and wastewater.
- Potential carbon credit for utilities. This will require establishing a U.S. policy on carbon absorption and re-use as transportation fuel in lieu of permanent sequestration.

Coal-fired power plants may be a convenient source of CO_2 for algae production, but from an emissions control perspective, construction of algae systems at natural gas-fired power plants may be a better investment. The reason is that coal-fired power plants have higher CO_2 emissions per unit energy produced than natural gas-fired power plants. Thus, using algae to capture the maximum amount of CO_2 emissions from coal-fired plants would require proportionally larger algae production systems per unit energy produced and higher costs per unit energy produced. However, coal-fired plant flue gas typically has about a factor of two greater CO_2 concentration (10 - 15%) than natural gas plants (5 - 6%), which can bring some advantage in terms of efficiency of capture, transport, and delivery of CO_2 from the power plant to the algae cultivation site. Also, gas-fired power plants that operate as peaking plants rather than base-load generators will have intermittent operation that would introduce intermittency in the supply of CO_2 for algae growth. The impact on algae production would depend on the phasing of the intermittency with respect to the daylight hours when photosynthesis is active. Gas-fired baseload generators would not be intermittent, but, as with baseload coal-fired plants, would 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 sequestered by other means (Rubin, 2005).

Barriers to Co-Location of Algae Production with Stationary Industrial CO_2 Sources

- Need for nutrient sources: While stationary industrial sources of concentrated CO_2 can potentially provide ample carbon for photosynthesis-driven algal growth, in most cases there will not be a complementary nutrient (N, P, K) supply. Therefore nutrients must be brought in from other sources, or in some cases algal cultivation could be co-located with both stationary CO_2 sources and nutrient sources such as wastewater treatment facilities and agricultural waste streams.
- Unclear regulatory framework for carbon-capture credits: Until there are regulations in place that quantify carbon credits from algal growth facilities, the uncertainty may pose a barrier for wide commercial adoption of the technology.
- Land availability: Suitable and affordable vacant land may not be available adjacent to or near major power plants
- Emissions from ponds are at ground level: Regulatory requirements from power plants and other stationary sources are governed by the Clean Air Act, and are based upon point-source emissions from high elevations. The use of flue gas to cultivate algae will involve non-point source emissions at ground level.
- Capital costs and operational costs: There exists a need to evaluate capital costs and parasitic operational losses (and costs) for infrastructure and power required to capture and deliver industrial CO_2 to ponds and grow/harvest algae. These costs and losses must be minimized and compared to other approaches for the capture and sequestration or reuse of carbon. Current estimates are that approximately 20% - 30% of a power plant's greenhouse gas emissions can be offset by algae biofuel and protein production (Brune et al., 2009). 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".
- Too much CO_2 near plants for realistic absorption: Large power plants release too much CO_2 to be absorbed by algal ponds at a realistic scale likely to be possible near the power plant facility. The same generally holds true for other stationary industrial sources of CO_2 (cement plants, ethanol plants, etc.). Also, CO_2 is only absorbed during periods when sunlight is available and photosynthesis is active in the algae.
- Maintaining cultivation facilities during utility outages and through seasonal changes in algal growth rates: Detailed models will be needed to develop and evaluate approaches for managing the variable nature of both CO_2 emissions and algal growth rates/CO_2 uptake.
- Resistance from electric utilities: Electric utilities are not in the fuels business and regulated public utility commissions will be constrained in entering the fuel production arena. Their fundamental objective will be to capture CO_2 as opposed to producing biofuels and co-products. 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.

Directions for Research and Development

Several areas for research, as well as policy-development efforts, will be required for commercialization of algal cultivation facilities co-located with industrial CO_2 sources and/or wastewater treatment facilities. The following directions have been identified:

- Develop computer models of algae production facilities that will aid the following:
  - Rapid and consistent engineering design
  - Techno-economic analyses
  - Life Cycle Analysis and GHG abatement analysis
  - National inventory of potential production sites
  - Evaluation of economies of scale vs. advantages of decentralized production considering parasitic losses of CO_2 transport, etc.
  - Evaluation of temperature control (power plant cooling and algae pond heating)
  - Development of efficient test-bed facilities
- Establish national algae biomass production test-beds to conduct research at the pilot scale (3 - 10 acres). The test-beds would ideally be located at power plants, wastewater treatment facilities, ethanol plants or other CO_2 emitting industry facilities, and agricultural drainage/water body restoration sites that also represent a range geographical locations, solar resource, and climate conditions. This effort could involve a consortium of R&D organizations, universities, algal cultivation companies, algal technology companies, refiners, distributors, and other participants coordinated at the national level. Specific test-bed R&D topics relevant to power and wastewater utilities include:
  - Technology evaluation at larger scales
  - Determination of algae production facility model parameters

- Flue gas or other industrial source CO_2 supply logistics, costs, and absorption/biofixation efficiency and algal biomass productivity given seasonal and diel variations in photosynthesis and various water chemistries
- Control of algal biomass quality (ratios of lipids: proteins: carbohydrates and C:N:P)
- Methods of nutrient and water recycling within production facilities; salinity and blowdown management.
- Algal biomass handling, storage, and processing prior to fuel extraction; flocculation harvesting; pathogen safety
- Beneficial management of residuals for soil carbon development, crop fertilization, etc.
- Development of algal strains and their cultivation techniques
- Investigate the safety of ground-level flue gas emissions from ponds including plume modeling and regulatory analysis
- Effects of various flue gases on algae production and co-product quality
- Scrubbing of flue gas for NOx, SOx, etc.
- Power plant cooling with treated wastewater in conjunction with algae production

- Evaluate policies that would encourage partnering between public utilities/other industrial CO_2 sources and algal cultivation/technology companies and refiners/distributors.
- Develop and train the future algae production/algae biomass processing workforce at the national test-bed and other sites. Develop university training programs.

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Appendices

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