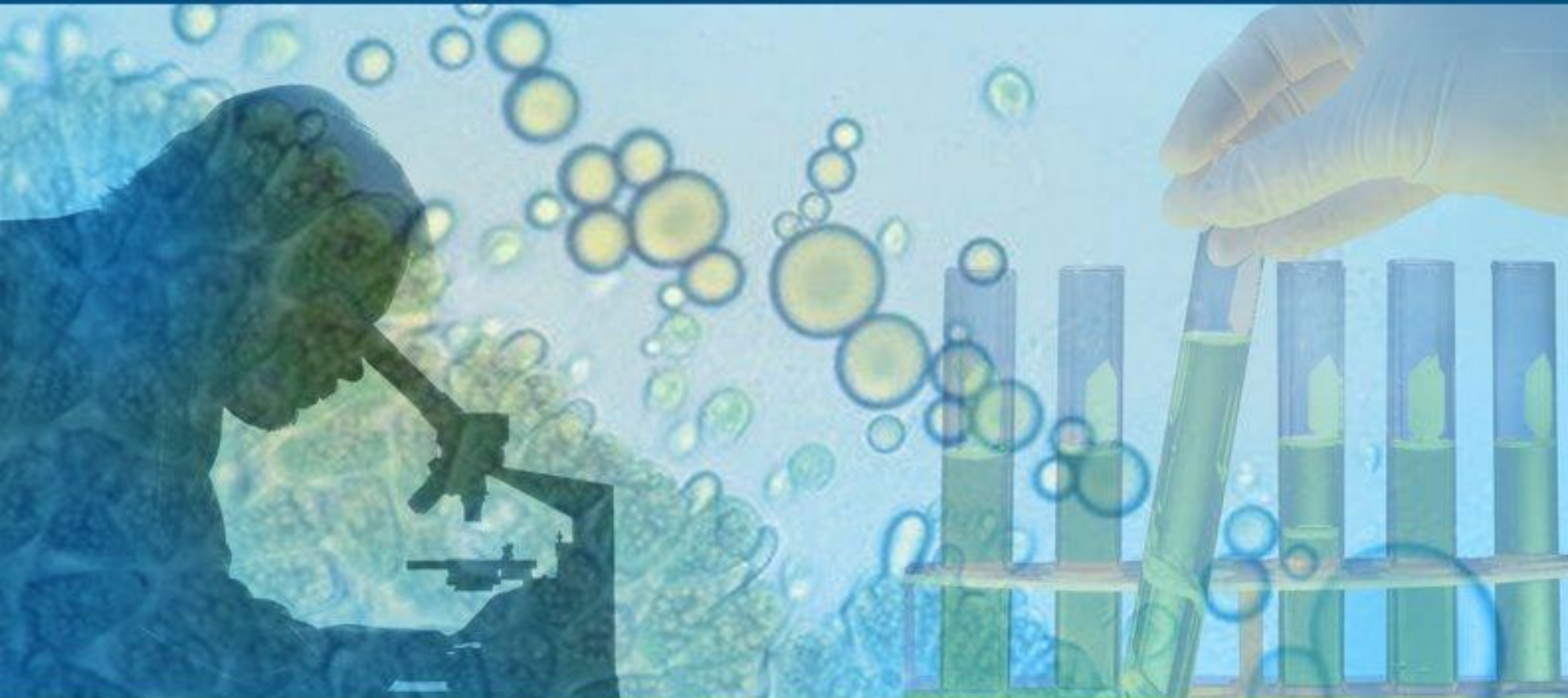
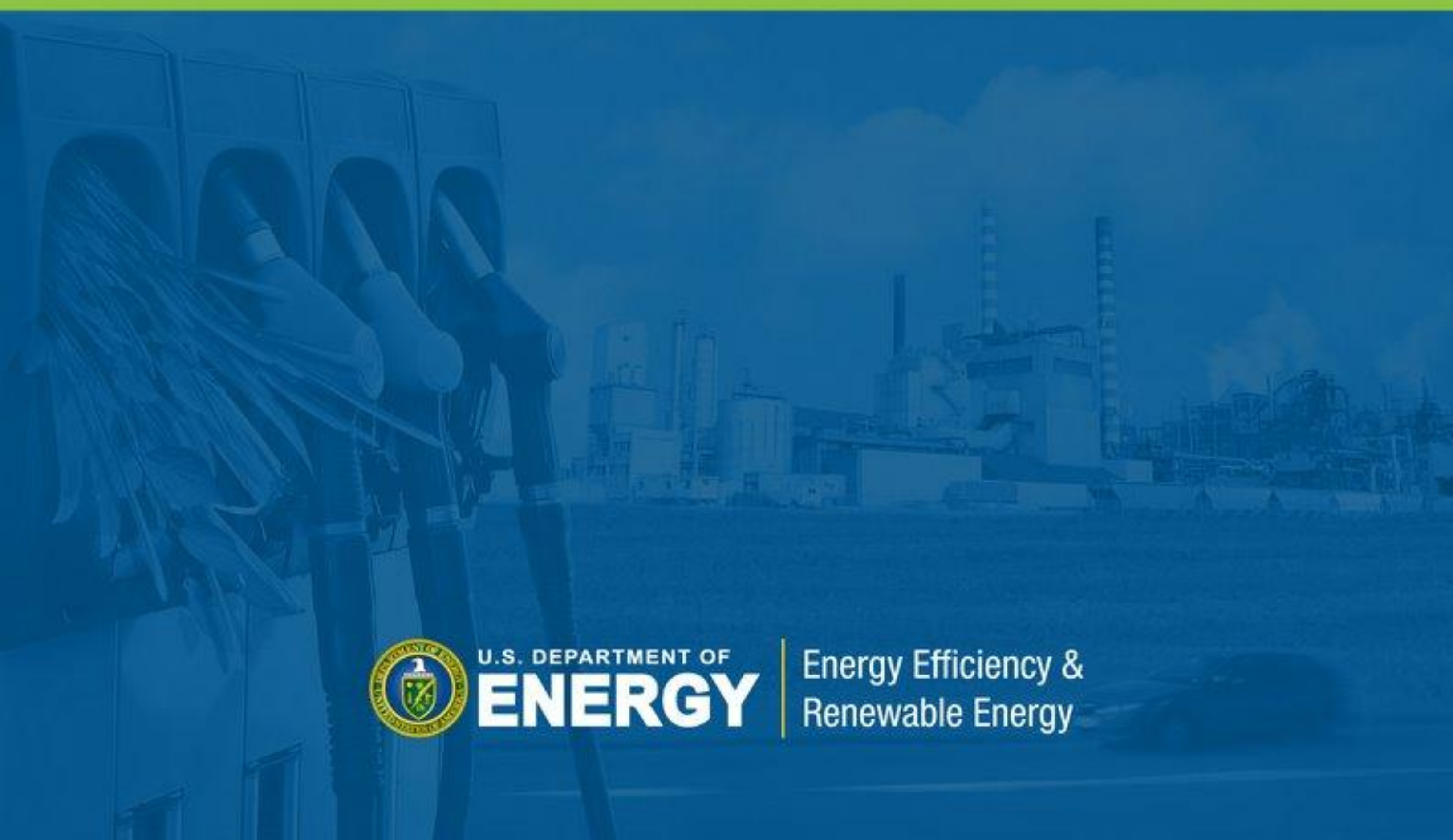
U.S. DEPARTMENT OF ENERGY BIOMASS PROGRAM



National Algal Biofuels Technology Roadmap



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

This page was left intentionally blank

Executive Summary

"We must invest in a clean energy economy that will lead to new jobs, new businesses and reduce our dependence on foreign oil," said President Obama. "The steps I am announcing today help bring us closer to that goal. If we are to be a leader in the 21st century global economy, then we must lead the world in clean energy technology. Through American ingenuity and determination, we can and will succeed."

—President Barack Obama

"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 said. "With American investment and ingenuity -- and resources grown right here at home -- we can lead the way toward a new green energy economy."

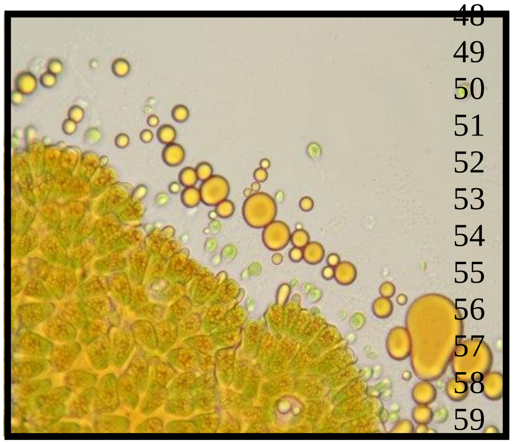
—Secretary of Energy Steven Chu

Speaking at the May 5th, 2009 White House ceremony announcing $800M in new biofuel research activities

The 2007 Energy Independence and Security Act (EISA) was enacted in response to concerns about global energy security and supply. The Act contains provisions designed to increase the availability of renewable energy that decreases greenhouse gas (GHG) emissions while at the same time also establishing an aggressive Renewable Fuels Standard (RFS). This new fuels standard mandates the production of 36 billion gallons of renewable fuels by 2022 of which at least 21 billion gallons must be advanced biofuels (i.e., non-corn ethanol). While cellulosic ethanol is expected to play a large role in meeting the EISA goals, a number of next generation biofuels, particularly those with higher-energy density than ethanol, show significant promise in helping to achieve the 21 billion gallon goal. Of these candidates, biofuels derived from algae, particularly microalgae, 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 biofuels created from algae, President Obama and Secretary of Energy Steven Chu announced on May 5th, 2009 the investment of $800M new research on biofuels in the American Recovery and Renewal Act (ARRA). This announcement included funds for the Department of Energy Biomass Program to invest in the research, development, and deployment of commercial algal biofuel processes.

Microalgae are unicellular, photosynthetic microorganisms that are abundant in fresh water, brackish water, and marine environments everywhere on earth. These microscopic plant-like organisms are capable of utilizing CO_2 and sunlight to generate the complex biomolecules necessary for their survival. A class of biomolecules synthesized by many species is the neutral lipids, or triacylglycerols (TAGs). Under certain conditions, some microalgae can accumulate significant amounts of lipids (more than 50% of their cell dry weight).



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 compared to typical terrestrial oil-seed crops, 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, and wastewater), and 5) Production of both biofuels and valuable co-products. More than 20 years ago, the Department of Energy-supported Aquatic Species Program (ASP), which represents the most comprehensive research effort to date on fuels from algae, illustrated the potential of this feedstock to be converted into liquid transportation energy. Much has changed since the end of the ASP. With rising petroleum prices and concerns about energy independence, security, and climate change, the quest to use of microalgal feedstocks for biofuels production has again been gaining momentum over the past few years. While the basic concept of using algae as an alternative and renewable source of biomass feedstock for biofuels has been explored over the past several decades, 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 the Department of Energy's Office of Biomass Program in the Office of Energy Efficiency and Renewable Energy (EERE). This two day event successfully brought together more than 200 scientists, engineers, research managers, industry representatives, lawyers, financiers and regulators. The workshop participants broadly represented stakeholders from different areas of industry, academia, the National laboratory system as well as governmental and non-governmental agencies and organizations. The primary purpose of the workshop was to discuss and identify the critical barriers currently preventing the economical production of algal biofuels at a commercial scale. The input to the roadmap document was structured around the Workshop's break-out sessions which were specifically created to address the various process operations that must be tackled in developing a viable algal biofuels industry. The workshop addressed the following topics/technical barriers:

- Algal Biology
- Feedstock Cultivation
- Harvest and Dewatering
- Extraction and Fractionation of Microalgae
- Algal Biofuel Conversion Technologies
- Co–Products
- Distribution and Utilization of Algal Based-Fuels
- Resources and Siting

- Corresponding Standards, Regulation and Policy
- Systems and Techno-Economic Analysis of Algal Biofuel Deployment
- Public-Private Partnerships

This document represents the output from the workshop and is intended to provide a comprehensive roadmap report that summarizes the state of algae-to-fuels technology and documents the techno-economic challenges that likely must be met before algal biofuel can be produced commercially. This document also seeks to explain the economic and environmental impacts of using algal biomass for the production of liquid transportation fuels Based on the outcome of the workshop, the technical barriers identified involve several scientific and engineering issues which together represent a significant challenge to the development of biofuels from microalgae. Taking these barriers into consideration, this roadmap also serves to make research and funding recommendations that will begin to lay the groundwork for overcoming the technical barriers that currently prevent the production of economically viable algal-based biofuels.

Viewpoints expressed during the DOE workshop and road mapping effort was that many years of both basic and applied R&D will likely be needed to overcome the current technical barriers before algal-based fuels can be produced sustainably and economically enough to be cost-competitive with petroleum-based fuels. Since both research and engineering improvements are absolutely critical components to implementing any commercial-scale, algal-based fuel production facility, it is also clear that a multidisciplinary research approach will be necessary to accelerate progress over the short term (0-5 years). For example, the ability to quickly test and implement new and innovative technologies in an integrated process setting will be a key component to the success of any such effort. Such an approach will ultimately serve as the engine that not only drives fundamental research and technology development but also demonstration and commercialization. Based on the work that needs to be accomplished, the proposed R&D activities will also require long-term coordinated support from relevant government agencies and national laboratories, private sector, academia, and the participation from virtually all interested stakeholders. Lastly, there is a need for a significant investment in our colleges and universities, as well as field experts, to train the professional work force that will be needed for developing the necessary infrastructure as well as the operation and maintenance of a robust and domestic algal biofuels industry.

Contents

1. Introduction

About the Roadmap

The framework for *National Algal Biofuels Technology Roadmap* was constructed at the Algal Biofuels Technology Roadmap Workshop, held on December 9 and 10, 2008 at the University of Maryland College Park. The Workshop was organized by the U.S. Department of Energy (DOE) Office of Energy Efficiency and Renewable Energy's Biomass Program to discuss and identify the critical barriers currently preventing the development of a domestic, commercial-scale algal biofuels industry.

Microalgae offer great promise to contribute a significant (=< 100%) portion of the renewable fuels that will be required to meet the U.S. biofuel production target of 36 billion gallons by 2022, as mandated in the Energy Independence and Security Act of 2007 under the Renewable Fuels Standard. In the longer term, biofuels derived from algae represent an opportunity to dramatically impact the U.S. energy supply for transportation fuels. The cultivation of algae at a commercial scale could provide sufficient fuel feedstock to meet the transportation fuels needs of the entire United States, while being completely compatible with the existing transportation fuel infrastructure (refining, distribution, and utilization). Further, algal biofuels could prove sustainable for generations – they consume CO_2 as a nutrient, have a much higher yield potential than other terrestrial biomass feedstocks, and can be grown with non-fresh water sources without needing to use high-value arable land. However, despite their huge potential, the state of technology for producing algal biofuels is regarded by many in the field to be in its infancy. There is a general consensus that a considerable amount of research, development, and demonstration (RD&D) needs to be carried out to provide the fundamental understanding and scale-up technologies required before algal-based fuels can be produced sustainably and economically enough to be cost-competitive with petroleum-based fuels. For this reason, a major objective of the Workshop was to help define the activities that will be needed to resolve the challenges associated with commercial-scale algal biofuel production and lay the framework for an algal biofuels technology roadmap.

The Algal Biofuels Technology Roadmap Workshop brought together the interdisciplinary expertise needed to fully discuss the promise and challenges of a commercial algal biofuels industry. The Workshop and the reporting process were designed to be as inclusive and transparent as possible. More than 200 participants were invited to attend the Workshop and broadly represented stakeholders from different areas of industry, academia, the United States national laboratory system, as well as governmental and non-governmental agencies and organizations. Over the course of the two days, the Workshop produced a very stimulating look at the growing algal biofuels industry and the opportunity to explore the science and engineering challenges that must be overcome to realize the sustainable production of algal biofuels at commercial scale. The Workshop participants drew on their experience and expertise during a series of technical discussions spanning all aspects of enabling a sustainable commercial algal biofuels industry. In these discussions throughout the Workshop, there was an underlying overwhelming consensus for the continued development of algal biofuels; participants agreed upon the need for DOE to coordinate with other federal agencies to support fundamental and applied research, infrastructure development, technology deployment, and information management at a national level, as well as to engage in the development of supportive policy, regulation, and standards for the emerging algal biofuels industry. These outcomes from the Workshop provided key inputs to the development of this Algal Biofuels Technology Roadmap.

The Workshop participants were provided with several valuable existing resource materials pertinent to algal biofuels in advance of the Workshop so as to ensure a uniform level of awareness of these materials. These materials included seminal literature references, general reviews and reports and are available at no cost to the general public for download and review by visiting the DOE Algae Biofuels Technology Roadmap Web site at http://www.orau.gov/algae2008/resources.htm. The available resources also contained materials sorted by topics of the Workshop's break-out sessions.

Developed from the discussions held at the Workshop, this roadmap presents information from a scientific, economic, and policy perspective that can support and guide R&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 R&D. In doing so, it lays the groundwork for identifying the technical barriers that likely need to be overcome for algal biomass to be used in the production of economically viable biofuels.

The roadmap is structured around the Workshop's break-out sessions—they were specifically created to address the various aspects that must to be tackled in developing a viable algal biofuels industry:

- Systems and Techno-Economic Analysis
- Algal Biology
- Algal Cultivation
- Processing (Harvesting and Dewatering)
- Extraction/Fractionation
- Conversion to Fuels
- Co-products
- Distribution & Utilization
- Resources and Siting
- Standards, Regulation, and Policy

America's Energy Challenges

As petroleum supplies diminish in the world, the United States becomes increasingly dependent upon foreign sources of crude oil. The United States currently imports approximately two-thirds of its petroleum and more than 60% of this petroleum is used for 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 over global warming effects due to greenhouse gas (GHG) emissions.

In response to these global energy concerns and in an effort to move the U.S. toward greater energy independence and security, President George Bush signed into law the Energy Independence and Security Act of 2007 (EISA), which contains new standards for vehicle fuel economy, as well as provisions that promote the use of renewable fuels, energy efficiency, and new energy technology research and development. The new energy legislation is designed to reduce the U.S. dependence on foreign oil by increasing the production of domestic alternative fuels and establishing a very aggressive Renewable Fuels Standard (RFS) (Table 1).

Table 1: EISA requirements under RFS

Renewable Fuels	Mandated Production by Volume
Corn Starch-Based Ethanol	15 billion gallons by 2015
Biodiesel	500 million gallons starting in 2009 and peaking at 1 billion gallons by 2012
Cellulosic Ethanol	100 million gallons in 2010, growing to 16 billion gallons by 2022
Other Advanced Biofuels (other than corn-based ethanol such as that produced from wood chips, agricultural waste or dedicated energy crops)	5 billion gallons by 2022

While cellulosic ethanol is expected to play a large role in meeting the EISA goals, it is unlikely that the supply of cellulosic ethanol will meet the EISA requirement of 100 million gallons by 2012 since most small-scale demonstration plants are not scheduled to begin production until the 2010-2011 timeframe.

Advanced biofuels also face significant challenges in meeting their targets set by EISA. As required by EISA, advanced biofuels must produce GHG emissions across their lifecycle that are at least 50% less than GHG emissions produced by petroleum-based transportation fuels. Moreover, the development of biofuels from oil crops and waste cooking oil/fats cannot realistically meet the demand for liquid transportation fuels because conventional oil yields per hectare from oil crops would require unrealistic acreages of land in excess of the total land area of the United States (Tyson et al., 2004). Further, more than 90% of the vegetable oil produced in the U.S. is used in the food products market, thereby severely limiting its use as a biofuel feedstock. Therein lies one of the main drivers in the development of microalgal diesel fuels—microalgae promises much higher productivities per unit area given its higher photosynthetic efficiency when compared to conventional crops. Table 2 contains data which demonstrates that potential oil yields from algae are also significantly higher than the yields of oilseed crops. Under the current yield scenarios, the potential oil yields from certain algae are projected to be at least 60 times higher than from soybeans per acre of land on an annual basis—approximately 15 times more productive than jatropha and approximately 5 times that of oil palm (Rodolfi et al., 2009). With these features of higher growth rates and increased oil yields, algae have the potential to replace a significant amount of the current U.S. diesel fuel usage while using only a fraction of the land equivalent what would be required from terrestrial crops.

Table 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	1,000-4,000[b]

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

Although a number of other proposed advanced biofuels show significant potential in helping to achieve the 21 billion gallon EISA mandate, biofuels derived from algal biomass feedstocks show considerable promise as a potential major contributor to the displacement of petroleum-based fuels. There are several aspects of algal biofuel production that have combined to capture the interest of researchers and entrepreneurs around the world:

- Unlike other oil crops, algae grow rapidly and many are exceedingly rich in lipid oil (oil levels of 20% to 50% are quite common).
- Using algae to produce feedstocks for biofuels production will not compromise the production of food and other products derived from terrestrial crops.
- The cultivation of algae does not entail land conflict for doing agriculture for food production.
- The water used to grow algae can include waste water and non-potable saline water that cannot be used by conventional agriculture or for domestic use.
- Algae have a tremendous technical potential for recycling CO_2-rich flue gases from coal burning power plants as well as from natural gas recovery operations.
- An algal biorefinery could potentially integrate several different conversion technologies to produce biofuels including biodiesel, green diesel, green gasoline, aviation fuel, ethanol, and methane as well as valuable co-products including oils, protein, and carbohydrates.

Advantages of Algal Biomass

- High per-acre productivity
- Non-food resource
- Use of otherwise non-productive, non-arable land
- Utilization of a wide variety of water sources
- Reduced GHG release into the atmosphere
- Production of biofuels and co-products

While the basic concept of using algae as an alternative and renewable source of biomass feedstock for biofuels has been explored in the past, a scalable, commercially viable system has not emerged. Past research investments have been intermittent and short-term thus insufficient to enable the development of an algae-based biofuels technology. Given recent and dramatic advances in relevant fields, in particular biology, and the fact that realizing the strategic potential of this feedstock will require critical engineering innovations and science breakthroughs, from understanding algal mass culture to downstream processing, a more substantial and sustained investment is paramount. This investment much include a significant R&D effort focused on answering fundamental biological questions related to algal physiology to support the engineering and scale-up effort..

The Algae-to-Biofuels Opportunity

Microalgae as a Feedstock for Fuel Production

In terms of chemical properties, the most important difference between fossil fuels and those derived from biomass feedstocks is that petroleum, natural gas, and coal are made of hydrocarbons—compounds composed entirely of carbon and hydrogen. In contrast, commercially available biomass-derived fuels (ethanol and biodiesel) contain oxygen (in addition to carbon and hydrogen), yielding different physical and chemical properties of the fuel and thus different combustion characteristics. As a result, the biomass-derived oxygenates have a reduced heating value compared to hydrocarbons. Oxygenates, which are in a partially oxidized state, release less energy upon combustion (complete oxidation) than do hydrocarbons, which are in a completely reduced state.

Table 3 compares the typical lower heating value (LHV) of several fuels in use today. Ethanol, for example, is more highly oxidized than a hydrocarbon since it contains oxygen (CH_3CH_2OH) and liberates significantly less energy on combustion than do petroleum-based components. Butanol ($CH_3(CH_2)_3OH$), on the other hand, has two additional carbon atoms, which makes it a higher energy density fuel. Alcohols are, nevertheless, beneficial fuel alternatives because the presence of oxygen allows these molecules to burn cleaner and more efficiently. Biodiesel, a renewable fuel currently produced commercially from vegetable oils (soy, canola, and sunflower), has significantly higher volumetric energy densities due to the presence of long chain fatty acids that contain carbon, hydrogen, and oxygen (e.g., $CH_3(CH_2)_{14}COOH$). The presence of oxygen in these fatty acid methyl esters has the added benefit of acting as an oxygenate and enhances engine performance in much the same fashion as the alcohols. Petroleum-derived diesel, which is comprised of approximately 75% saturated hydrocarbons (alkanes) and 25% aromatic hydrocarbons, has the highest energy density of all the fuels listed because the components in diesel contain only carbon and hydrogen substituents (no oxygen).

Table 3: Lower Heating Value (LHV)* of Various Liquid Transportation Fuels

Fuels	LHV (Btu/Gallon)
Ethanol	76,000
Butanol	99,840
Gasoline	115,000
Biodiesel (B100)	117,000
Petroleum Diesel	128,500

* The lower heating value or LHV of a fuel is the energy that can be recovered when the water of combustion is released as a vapor.

Source: *DOE, Hydrogen Analysis Resource Center*

Feinberg (1984) has discussed the issue comparison between the composition of various algal species with fuel chemical requirements. For this reason, only a brief characterization of the microalgae feedstock (as produced at the culture facility and fed to the fuel production facility) is presented here to establish the basis for determining appropriate process requirements for converting microalgal constituents into fuels.

Research conducted over the last 50 years has demonstrated that microalgae produce a diverse array of chemical intermediates and hydrocarbons and, therefore, offer promise as a potential substitute for products currently derived from petroleum or natural gas. Three major components can be extracted from microalgal biomass: lipids (including triglycerides and fatty acids), carbohydrates, and proteins. Bioconversion products include alcohols, methane, hydrogen, and organic acids, and catalytic conversion products include paraffins, olefins, and aromatics.

While each of the three main biochemical fractions of microalgae can be converted into fuels, lipids have the highest energy content and potential. The lipids of some species are composed of hydrocarbon molecules, similar to those found in petroleum feedstocks, while those of other species resemble vegetable oils (corn, soybean, canola, and others) that can be converted to a synthetic diesel fuel. Lipids are not the only potential biofuels feedstock from algae. Carbohydrates can be converted into ethanol by fermentation. Alternatively, all three components present in biomass can be converted into methane gas by an anaerobic digestion process or into syngas or pyrolysis oil by thermochemical conversion. Microalgae would thus be attractive feedstocks for fuel production if their productivity can be effectively exploited.

Although this report will briefly consider all the potential conversion processes to produce fuel from microalgal feedstocks, it will focus on the high-energy lipids. Many species have the ability to accumulate large quantities of these compounds, especially when cultivated under nutritive stress (Milner, 1976). Most lipids in algal cells are found in the membrane that surrounds the cell and cellular organelles. However, some strains produce a significant amount of storage lipids when grown under nutrient-limiting conditions. Oil levels of 20-50% are quite common (Chisti, 2007). The idea of generating biodiesel from the microalgal storage lipids was the main focus of DOE's Aquatic Species Program from 1978 to 1996 (Sheehan et al., 1998).

The Potential of Microalgal Oils

Numerous algal strains have been shown to produce more than 50% of their biomass (on a dry cell weight basis) as lipid with much of this present in the form of triacylglycerols (TAGs) (Hu et al., 2008). (It should be noted however, that like many aspects of algal biofuels research, the methodology generally used for algal lipid analysis - largely based on solvent extraction and gravimetric analysis - has yet to be standardized and thus the values published in the literature should be regarded, at best, as only an estimation of the lipid content.) Further, some algae accumulate high levels of lipids when cultivated under stress (e.g. limitations of certain nutrients) or in response to changes in culture conditions. For this reason, algal cellular lipid content can vary both in quantity and quality. Importantly, from a production point of view, accumulation of lipid produced under stress conditions is generally at the expense of significantly reduced biomass yields. Algae-derived oils contain fatty acid and triglyceride compounds, which like their

terrestrial seed oil counterparts, can be converted into biodiesel (via transesterification to yield fatty acid methyl esters) (Fukuda et al., 2001), and green diesel, green jet fuel, and green gasoline (produced by a combination of hydroprocessing and catalytic cracking to yield alkanes of various carbon chain lengths) (Kalnes et al., 2007).
Given that scalable algal biofuels are not yet attainable, applying a modest estimate of the potential productivity of oil from algae at 1,200 gallons/acre/year on the area of land equivalent to that used to produce the 2007 U.S. soybean crop (67 million acres) yields a figure greater than 100% of the petroleum diesel consumed annually in the U.S. Had the oil from the entire 2007 soybean crop been converted to biodiesel, on the other hand, it would have provided only 2.8 billion gallons of fuel. (Source: Soy Stats™, American Soybean Association). This amount of biodiesel would displace just 6% of the approximately 44 billion gallons of petroleum on-road diesel used annually in the U.S. Further, as a figure of merit (see Appendix), algae require approximately 2 kg of CO_2 for every kg biomass generated, therefore, this technology has the potential to recycle CO_2 emissions from power plants and other fixed sources of CO_2.

Improvements in either area productivity ($gm/m^2/day$) or lipid content (gm/dry cell weight) would significantly reduce the land area needed ultimately to produce this quantity of biofuel. The algal residue that remains after removal of the lipid component (i.e., largely carbohydrate and protein) could be used for the generation of energy (biopower), more liquid fuels through fermentation (ethanol, biobutanol, etc.), or gaseous (methane) fuels through anaerobic digestion, or serve as a feedstock for the generation of higher-value co-products. In the future, an algal-based biorefinery could potentially integrate several different conversion technologies to produce many biofuels as well as valuable co-products including oils, protein, and carbohydrates.

With concerns about petroleum supplies and costs as energy demands grow worldwide, energy independence, security, and global warming, the potential use of microalgal feedstocks for biofuels production, specifically lipids derived from them, has gained significant momentum over the past few years. It has been reported that the use of vegetable oil and fat-based feedstocks, which are widely used in world food markets, cannot realistically satisfy the ever-increasing demand for transportation fuels, nor are they likely to displace any significant portion of the U.S. petroleum fuel usage (Tyson et al., 2004). Algal oils do, however, have that potential because their oil yield/acre can be 5 to 60 times higher than that of terrestrial oil crops (see Table 2).

In addition to the production of energy-rich lipids, algae can also be regarded as an alternative source of carbohydrates. For example, some algae and cyanobacteria can accumulate large quantities of storage polysaccharides as a product of photosynthesis. These include starch, glycogen, and chrysolaminarin, three different polymers of glucose. Additionally, the main structural elements of algal cell walls have been shown to be composed of polysaccharides such as cellulose, mannans, xylans, and sulfated glycans. Algal-derived polysaccharides can be hydrolyzed (chemically or enzymatically) into sugars that can be fermented to ethanol.

Integrating With Biorefinery Concept

While the conversion of solar energy into renewable liquid fuels and other products from algal lipid feedstocks is technically feasible (Chisti, 2007), currently such biofuels cannot be produced economically enough to be cost-competitive with fossil fuels. A significant basic science and applied engineering R&D effort is required before the vision and potential of algae for biofuels can be fully realized. It is, however, conceivable that in the not too distant future, algae farms could become an integral part of a biorefinery concept that incorporates other advanced technologies to produce a variety of biofuels such as cellulosic ethanol, biodiesel, renewable "green" diesel, gasoline, jet fuel, and a wide range of co-products. This biorefinery could be integrated, at least initially, with a fossil fuel-based power plant. The CO_2 generated by this plant and from an integrated ethanol plant would serve as a rich source of nutrients for the growth of algae, as well as serve to mitigate the release of CO_2 by recycling it.

After extraction of the algal oils, the residue could be used as a starting feedstock to drive ethanol production (through the use of algal-derived sugars) or fed back into the power plant to be burned as a fuel source. To round out the biorefinery, a biodiesel plant or petroleum refinery (or both) would convert the algal lipids into the most cost-effective fuel depending on the economic situation. Ultimately, substantial R&D is needed to develop an algae-to-biofuels production system that can become an integrated component in a biorefinery that operates at high efficiency with minimal inputs at a low cost.

For these and other reasons, algae hold tremendous potential for the long-term biofuels strategy for transportation energy within the United States. Corn ethanol, though it poses longer-term sustainability challenges, can be used in the near term since the needed technologies and biomass production are readily available and it can help establish and exercise an ethanol-based biofuels economy. In the near to mid-term, cellulosic biofuels, starting with ethanol, present tremendous potential for replacing up to 30% of the U.S. gasoline usage, and cellulosic ethanol follows naturally from starch ethanol. Moving further out, other advanced biofuels from cellulosic biomass may provide reduced distribution costs and higher energy densities. Finally, in still longer term (perhaps 10 years), biofuels from algae present an opportunity at the greatest scale and with very attractive sustainability characteristics.

Investments So Far in Algal Biofuels Development

Early Work to 1996

Proposals to use algae as a means of producing energy date back to 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. Not until the energy price surges of the 1970s did the possibility of using algae as a fuel source receive renewed attention. 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 these lipid stores as a source of energy only gained serious attention during the oil embargo of the early 1970s, ultimately becoming the major push of DOE's Aquatic Species Program.

The Aquatic Species Program represents the most comprehensive research effort to date on fuels from algae. The program lasted from 1978 until 1996 and supported research primarily at DOE's 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 TAGs. *Cyclotella cryptica*, a diatom that is a attractive lipid producer, 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). 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. 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.

Metabolic engineering, which involves the modification of an organism at the genetic level to achieve changes in cellular metabolism, has proven successful for enhanced production of many compounds in industrial strains. Importantly, the genomics revolution has accelerated progress in metabolic engineering for many organisms. For this reason, metabolic engineering of microalgae has become increasingly accessible and could theoretically result in strains that produce more oil or produce it under different conditions (e.g., obviating the need for nutrient stress). Research during the latter years of the Aquatic Species Program focused on the metabolic engineering of green algae and diatoms that involved the development of basic genetic tools as well as actual pathway modifications.

The first successful transformation of microalgae with potential for biodiesel production was achieved in 1994 with the diatoms *C. cryptica* and *Navicula saprophila* (Dunahay et al., 1995). A second major accomplishment was the isolation and characterization of the gene from *C. cryptica* encoding the ACCase enzyme (Roessler and Ohlrogge, 1993), the first example of an ACCase gene from a photosynthetic organism. A key gene involved in carbohydrate biosynthesis was also isolated (US patent 5,928,932; Jarvis and Roessler, 1999).

Initial attempts at metabolic engineering using these tools were successful in altering the genes' expression levels, but no effect was seen on lipid production in these preliminary experiments (Sheehan et al., 1998). Termination of the Aquatic Species Program in 1996 prevented 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 not only for biological productivity, but also for 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 the process of collecting small microalgal cells from the dilute suspension of a growing culture—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 (OTF) in Roswell, N.M., 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 UC 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 United States. 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. 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/barrel (Sheehan et al., 1998). It was concluded that algal biofuels would never be cost competitive with petroleum, which was trading at less than $20/barrel in 1995. DOE estimated at that time that the cost of petroleum would remain relatively flat over the next 20 years. (Although, as we now know, the energy landscape has changed dramatically in the intervening 14 years.)

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 .

Research from 1996 to Present

Since the end of DOE's Aquatic Species Program in 1996, federal funding for algal research in general has been limited and intermittent. Federal funding is split between

DOE and the Department of Defense (DoD). Recent initiatives such as a major DARPA (Defense Advanced Research Projects Agency) solicitation 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. State funding programs and research support from private industry also make up a significant proportion of research funding. An ever-increasing level of research focus on algal biofuels has taken place at a number of U.S. national labs, including NREL, Sandia National Laboratories, National Energy Technology Laboratory, Los Alamos National Laboratory, and Pacific Northwest National Laboratory. Private investment in biofuels, in general, and algal biofuels, in particular, has been increasing at a dramatic pace over the last few years.

Not only in algae, investment in the clean fuels sector in general has been booming, with a major increase in cleantech capital investment during the past five years. Since 1999, investment in cleantech has increased almost five fold. The venture firms are looking at biomass, solar, and wind technologies, and in some instances, are investing in the construction of actual facilities for the production of fuels and electricity (Krauss, 2007). In the first three quarters of calendar year 2007, 168 deals were signed with a combined value of $2.6 billion (Gongloff, 2007). The total investment in cleantech in 2006 was between $1.8 billion, and $2.3 billion, depending on the study (Gongloff, 2007; Krauss, 2007). *The Wall Street Journal* (2007) reported that 180 deals with a total value of $1.8 billion were completed in 2006, an average value of $10 million per deal. In early 2007, the average deal value was $15 million, illustrating the increasing magnitude of investments that venture firms are completing.

With the increase in interest worldwide amongst the investment community in clean technologies, microalgae production has also received interest from the private sector. Energy companies, both large and small, are investing in demonstration plants, feedstock development, and process improvement. Many of these companies became interested in algae during the rapid rise in cleantech investment from 2004 to 2006 and as algae's advantages, such as its growth on traditionally underutilized land, production of high energy lipids, and high yield per land area, became more widely known. When tied with energy security and energy independence, the opportunity for algae-to-biofuels is significant, and the investment community is responding.

The investment community's focus is not always on utilization of the lipids. Some companies have identified niches based on the production of ethanol from algal biomass. Commercial entities are exploring all aspects of the algae-to-fuels process, including technologies based both on lipid conversion and the conversion of other algae components. Algae have been used to produce high value, small quantity products for decades, and new companies are looking to expand algae's impact.

In summary, the >150 algal biofuels companies in existence today worldwide are attempting to help make the algae-to-fuels concept a reality. Further, large existing companies with either market interest derived from their current business revenues (e.g. energy) or with know-how and technology potentially relevant to algal biofuels are beginning to show interest in algae as well. What's not known, of course, is which entities will undertake the major funding investments needed to realize sustainable, saleable algal biofuels.

Going Forward

Roadmapping a Strategy for Algal Biofuels Development & Deployment

The current state of knowledge regarding the economics of producing algal biofuels are woefully inadequate to motivate targeted investment on a focused set of specific challenges. Furthermore, because no algal biofuels production beyond the research scale has ever occurred, detailed life cycle analysis (LCA) of algal biofuels production has not been possible. For this reason, investment in algal biofuels research and development is needed to identify and reduce risk. This supports private investments aimed at producing algal biofuels at a commercial scale. In contrast, development of cellulosic biofuels benefits from direct agricultural and process engineering lineage to the long-standing agricultural enterprise of growing corn (a grass) for food (and recently, for conversion to starch ethanol). There is no parallel agricultural enterprise equivalent for cultivating algae at a similar scale. In short, the science of algae cultivation (algaculture), agronomy-for-algae, if you will, does not exist. It is thus clear that a significant basic science and applied engineering R&D effort including a rigorous techno-economic and LCA will be required to fully realize the vision and potential of algae. The techno-economic analysis can track the status of each contributing technology as per established benchmarks and help identify opportunities for cost reduction. Additionally, the pervasive interdependency of various processes and infrastructure in developing a cost-competitive algae-to-biofuels supply chain necessitates systems analysis to ensure these entities work together as an efficient system.

Thus a combination of systems, techno-economic, and life cycle analyses are critically needed to gain greater understanding for informed decision making so that investments can be targeted and optimized to greater positive effect. See section 11, Systems and Techno-Economic Analyses of Algal Biofuel Deployment (page 157) for detailed discussion and specifics.

Need for a Sizeable, Strategically Structured and Sustained Investment

In the years following the termination of the Aquatic Species Program, a small but growing body of work has been reported in peer-reviewed journals dealing with topics ranging from photobioreactor design to lipid metabolism, genetic manipulation, and genomic analysis. The total body of work in the past years is relatively small, reflecting a fairly low level of research funding. There is a large gap between the current reality of commercial microalgae production technology and the goal of producing a microalgae biomass with high oil content suitable for conversion to biofuels at a large scale.

One of the major unanimous conclusions of the Workshop was that a great deal of RD&D is still necessary to make the algae-to-fuels process a reality and to engage the private sector more aggressively, the associated level of risk must be reduced. The Workshop participants agreed that the obvious first step toward achieving sustainable, scalable biofuels from algae is long-term and sustained investment in research and development, whether at DOE national laboratories, universities, and/or in the private sector. Ultimately, a sizable and strategically structured investment to tackle the RD&D challenges of algal biofuels is needed to advance the knowledge and experience of the nation's research community, which can then support the commercialization activities led by venture-backed entrepreneurs, as well as existing business and technology leaders.

In addition, the Workshop participants identified the need for significant investment in our colleges and universities to train the professional work force for the new bioeconomy, including scalable algal biofuels. Over the past few years, U.S. academic laboratories interested in various aspects of algae-to-biofuels research have largely experienced inadequate levels of funding. Since the end of the DOE-sponsored Aquatic Species Program in 1996, there has been no significant or sustained mechanism for funding academic work in the development of algae-based biofuels (excluding biohydrogen from algae). More specifically, what's needed in algal biology is a new generation of applied biologists and engineers to design, build, and maintain large-scale systems to cultivate, harvest, and process algal biomass at scale. University graduate research in modern molecular biology needs funding to produce molecular biologists with skills in systems biology (e.g., genomics, proteomics, and metabolomics) as applied to algal biology to carry out the fundamental biology R&D to support this effort.

Further, the existing funding landscape is fractured, with most of the funding spread across a variety of federal agencies (DoD, DOE, Environmental Protection Agency), state governments, private industry, congressionally directed research, and internal institutional funds. The disconnect between the various small funding efforts and the absence of a centralized effort in this area has been a large source of frustration for the academic research community. The Workshop participants felt that funding agencies with varying missions need to work together to enable the development of partnerships that span not only basic and applied research arenas, but the various disciplines needed to tackle the diverse challenges algal biofuels present. A single federal agency coordinating studies in the field or making investments strategic enough can acquire a long-term leadership role and help tie in all the efforts across the nation toward the development of algal biofuels.

See section 12, Public-Private Partnerships for continued discussion and recommendations.

2. Algal Biology

Algae: Basic Biological Concepts

The term “algae” refers to a large group of simple plant-like photosynthetic organisms. Algae are typically subdivided into two major categories based on their relative size. Microalgae are defined as microscopic photosynthetic, free-living organisms that thrive in diverse ecological aquatic habitats such as freshwater, brackish (<3.5% salt), marine (3.5% salt), and hypersaline (>3.5% salt) environments within a wide range of temperature and pH (Falkowski and Raven 1997). Unicellular microalgae are easily distinguished from their larger counterparts, the macroalgae or “seaweeds,” which have cells organized into structures resembling leaves, stems, and roots of higher plants. Microalgae can be subdivided into two broad categories: the prokaryotic cyanobacteria and the true eukaryotic algae. Cyanobacteria, often referred to as the blue-green algae, have been included traditionally as “algae,” but these organisms are clearly photosynthetic “prokaryotes”—bacterial organisms that lack a defined nucleus. Because cyanobacteria do not typically produce much lipid (Hu et al. 2008), they are not a focus for this discussion. Nonetheless, as we will demonstrate below, there are reasons to consider cyanobacteria for certain aspects of research relevant for biofuel production.

Microscopic algae were among the first life forms to appear on our planet (Falkowski et al., 2004). They are responsible for fixing massive amounts of CO_2 while producing and sustaining the atmospheric oxygen that supports the majority of life on Earth (Falkowski and Raven, 1997). Microalgae play a significant role in global productivity capacity, with some strains capable of doubling their cell numbers several times per day. By some estimates, microalgae, though making up only 0.2% of global photosynthetic biomass, have been found to account for approximately 50% of the global organic carbon fixation (Field et al., 1998) and contribute approximately 40% to 50% of the oxygen in the atmosphere.

The biochemical mechanism of photosynthesis in microalgae is similar to that found in all plants. However, unlike their terrestrial counterparts, microalgae are particularly efficient converters of solar energy due to their simple structure. Free of the need to generate support and reproductive structures, and with a ready supply of water and nutrients, the microalgal cell can devote the majority of the energy it traps into biomass growth. Under the limitations of current technology, algae can convert up to 15% of the photosynthetically available solar irradiation (PAR), or roughly 6% of the total incident radiation, into new cell mass (Benemann et al., 1978). In contrast, terrestrial crops generally have lower photosynthetic conversion efficiencies. For example, the photosynthetic efficiencies for sugar cane, the most productive terrestrial crop, are no better than 3.5% to 4% (Odum 1971). But it is not only photosynthetic efficiency that makes algae attractive candidates for biofuel production, but also because, unlike terrestrial plants which produce specialized oil bearing seeds, every algal cell can be a lipid factory, greatly increasing the amount of oil that can be produced per acre. As a result, microalgae are a relevant target for scientific studies for biomass energy production, biofuels production, and utilizing the excessive amounts of CO_2 currently being released into the atmosphere through the heavy reliance on fossil fuels.

Algal Classification

The biodiversity of microalgae is enormous with tens of thousands of species being described and as many as 10 million extant (Metting, 1996). Microalgae have been isolated from diverse ecosystems such as freshwater, brackish, marine, hyper-saline, snow, and even hot springs, which require special adaptations in metabolism for survival. Furthermore, microalgae inhabit soil and biofilms, and are even found in symbiotic association with other organisms.

As a group, cyanobacteria hold important practical implications as transformers of solar energy. They range from simple, tiny unicellular organisms to multicellular colonies, from simple to branched filaments. The unicellular cyanobacterium *Synechocystis* sp. PCC6803 was the first photosynthetic organism whose genome was completely sequenced (Kaneko et al., 1996). It continues to be an extremely versatile and easy model with which to study the genetic systems of photosynthetic organisms. Cyanobacteria are not generally known to produce large quantities of lipids, though they have been shown to produce storage carbon in the form of starch or glycogen. Cyanobacteria are, nevertheless, important as potential production strains for a variety of chemical intermediates and fuels. For example, a recent report describes the production and secretion of sucrose by photosynthetic prokaryotes (US 20080124767). In addition, cyanobacteria have been engineered to produce ethanol through a photosynthetic process (Deng and Coleman, 1998).

Eukaryotic microalgae, on the other hand, are not a well-studied group from a biotechnological point of view. Among the species that are believed to exist, only a few thousand strains are kept in culture collections throughout the world, a few hundred are being investigated for their chemical content and just a handful are cultivated on an industrial scale (Chisti, 2007).

Algae can be further classified into at least 12 major divisions. Within those major divisions, some common classes of algae include the green algae (Chlorophyceae), diatoms (Bacillariophyceae), yellow-green algae (Xanthophyceae), golden algae (Chrysophyceae), red algae (Rhodophyceae), brown algae (Phaeophyceae) and picoplankton (Prasinophyceae and Eustigmatophyceae). Examples of each of these classes are known to produce high levels of lipids; these include *Chromonas danica*, *Phaeodactylum tricornutum*, *Nitzschia palea*, *Monallantus salina*, *Nannochloropsis sp*., and *Isochrysis sp* (Chisti, 2007). Several additional divisions and classes of unicellular algae have been described and details of their structure and biology are available (van den Hoek et al., 1995).

The commercial application of microalgal biotechnology began to develop in the middle of the last century. Today there are numerous commercial applications involving microalgae. Microalgal mass cultures have applications in the production of human nutritional supplements and specialty animal feeds (Becker 2004) and play a crucial role in aquaculture and wastewater treatment. They are cultivated as a source of highly valuable molecules such as polyunsaturated fatty acids (PUFAs) (Ward and Singh 2005) and pigments such as β-carotene and astaxanthin (Pulz and Gross, 2004).

Photosynthesis/CO_2 Fixation

Photosynthesis is a process whereby certain varieties of bacterial species, eukaryotic algae, and higher plants convert the potential of light energy into chemical energy. Carbon, in the form of CO_2 is recycled directly from the atmosphere generating biomass and oxygen in the process. In eukaryotic algae, photosynthesis takes place in specialized organelles called chloroplasts. Cyanobacteria are prokaryotes and do not possess chloroplasts or any other such organelles. In these organisms, photosynthesis takes place inside a membrane-bound sac known as a thylakoid. Cyanobacteria are widely believed to be the ancestor of the chloroplast, taken up by a protozoan billions of years ago and evolving into an endosymbiont. Photosynthesis is generally performed in two separate steps, known as the light and dark reactions. In the photosynthetic light reactions, photons of light are absorbed directly by chlorophyll and a variety of other accessory pigments to excite electrons to a higher energy state. In a series of reactions, the energy is converted into ATP and NADPH splitting water in the process and releasing oxygen as a by-product. In the light independent process (i.e., dark reaction), CO_2 from the atmosphere is converted (“fixed”) into sugar using ATP and NADPH generated during the light reaction.

There are generally two processes whereby algae fix CO_2: the C3 and C4 pathways Most algae and plants use the C3 pathway in which CO_2 is first incorporated into a 3-carbon compound known as 3-phosphoglycerate. The enzyme that catalyzes this reaction, ribulose-bisphosphate carboxylase (RuBisCo), is also the enzyme involved in the uptake of CO_2. The three carbon compound generated during the process enters the Calvin cycle leading to sugar formation.

Marine diatoms are responsible for up to 20% of the global CO_2 fixation. Marine diatoms use the alternative C4 pathway, and, as a result, generally have enhanced photosynthetic efficiencies over C3 pathway organisms (Kheshgi et al., 2000). These organisms concentrate CO_2 around Rubisco, thereby diminishing photorespiration, and the concomitant loss of energy. It is thought that this characteristic is responsible for the ecological significance of diatoms (Reinfelder et al. 2000), though it is not clear if this will provide a real advantage for diatoms cultivated in the presence of sufficient CO_2.

Strain Isolation, Selection, and/or Screening

Currently, a number of microalgal strains are 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 now for several decades, these strains may have lost part of their original properties such as mating capabilities or versatility regarding nutrient requirements (de la Jara et al, 2003). To obtain versatile and robust strains that can be used for mass culture in biofuels applications, it is, therefore, essential to consider the isolation of new, native strains directly from unique environments. For both direct breeding as well as for metabolic engineering approaches to improved biofuels production, it is vital to isolate a large variety of microalgae for assembly into a culture collection serving as a bioresource for further biofuels research.

The goals of isolation and screening efforts are to identify and maintain promising algal specimens for cultivation and strain development. However, 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. Further, it is recommended that the isolated strains be screened to develop baseline data on the effects of regional environmental variability on cultivars.

Isolation and Characterization of Naturally Occurring Algae Species/Strains

Algae occur in a variety of natural aqueous habitats ranging from freshwater, brackish waters, marine, and hyper-saline environments to soil as well as symbiotic associations with other organisms (Round, 1981). At this time most commercial microalgae production facilities use open raceway pond technologies (e.g., Earthrise and Cyanotech Corp) (Chisti, 2007) and rely on natural strain succession to maximize biomass production throughout the year. Therefore, sampling and isolation activities for new strains have to account for temporal succession of microalgae in natural habitats. Further, any large-scale sampling and isolation efforts should be coordinated to ensure broadest coverage of environments and to avoid duplication of efforts.

For isolation of new strains from natural habitats traditional cultivation techniques may be used including enrichment cultures (Andersen & Kawachi, 2005). However, traditional methods take weeks to months for isolation of unialgal strains. Also, as many colonies are obtained from single cells the strains are often already clonal cultures. For large-scale sampling and isolation efforts, high-throughput automated isolation techniques involving fluorescence activated cell sorting (FACS) have proven to be extremely useful (Sieraki et. al, 2005).

Natural Habitats: Marine, Freshwater, Brackish/Saline, Wastewater, And Extreme Environments

In addition to sampling from a variety of ecosystems, it is proposed that sampling strategies not only account for spatial distribution but also for the temporal succession brought about by seasonal variations of algae in their habitats. In addition, within an aqueous habitat some algae are typically found either in the planktonic (free floating) or benthic (attached) environments. Planktonic algae may be used in suspended mass cultures whereas benthic algae may find application in biofilm based production facilities. Thus, it is recommended to include sampling of both planktonic and benthic algae within the context of this roadmap.

Identification of Criteria for Screening

The 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 variability of *in situ* versus laboratory performance. Because all these parameters require significant experimental effort, it would be very helpful to develop automated systems that would provide information regarding all parameters simultaneously. Screening for metabolite production has to include not only the metabolite composition and content, but also the productivity of cells regarding metabolites useful for biofuels generation. Rapid oil analyses of strains could greatly facilitate this work. An ideal analytical method would allow for distinction between neutral and polar lipids, and would also provide fatty acid profiles.

At this time, bottleneck for screening large numbers of microalgae stems from a lack of high-throughput methodologies that would allow simultaneous screening for multiple phenotypes, such as growth rates and metabolite productivities. In terms of biofuel production, it would be beneficial to be able to screen in high throughput fashion for lipid content.

To improve the economics of algal biofuel production, other valuable co-products must be generated; this would require determining cellular composition regarding proteins, lipids, and carbohydrates. Further, many strains also excrete metabolites into the growth medium. These have been largely ignored, but they might prove to be valuable co-products, at least in systems that do not suffer from contamination. New approaches are necessary to develop screening methods for extracellular materials.

For mass culture of a given algal strain, it is also important to consider the strains robustness, which includes parameters such as culture consistency, resilience, community stability, and susceptibility to predators present in a given environment. Previous studies revealed that microalgae strains tested in the laboratory do not necessarily perform similarly in outdoor mass cultures (Sheehan et al., 1998). 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 will be essential to enable testing of hundreds to thousands of different algal isolates.

Development of Novel Concepts and Approaches for Strain Screening

Solvent extraction is the most common method for determination of lipid content in algal biomass, and it requires both a significant quantity of biomass and effort.   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. Further improvements in analytical methodology could be made through the development of solid-state screening methods.

Development of Strain Databases

Currently, no database(s) exists that would provide global information on the characteristics of currently available algal strains. Protection of intellectual property in private industry further exacerbates the flow of relevant strain data. Some minimal growth information is available from existing culture collections, but it is very difficult, if not impossible, to obtain more detailed information on growth, metabolites, and robustness of particular existing strains. To accelerate R&D of algae-based biofuels production system, it is recommended that a central strain, open access repository be created (major algae culture depositories may be potential sites).

Role of Algal Culture Collections

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 some other countries. Those responsible for culture maintenance already maintain thousands of different microalgal strains; they are experienced in strain cultivation 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.

As the major culture collections by their nature already collect and document data on strains, such existing collections could be nuclei for the development of a national algae resource center. It could prove to be very helpful to have culture collection organizations responsible for the gathering and dissemination of detailed information regarding potentially valuable strains such as:

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

Participants in the workshop recommended that funding be provided to support and expand at least one or both of the existing major collections as ***open source collections and national algae centers*** to fulfill the need of the algal biofuels community. Possibly, the UTEX and the CCMP algae collections can be developed in such a way. It is expected that the data generated from a publically funded research program will be made available either free of charge or for a minimal user fee. Development and maintenance of such comprehensive open source databases will require a commitment to long-term and stable baseline funding.

References

Andersen R.A. & Kawachi M. 2005. Traditional Microalgae Isolation Techniques, In: Algal Culturing Techniques (Ed. Andersen R.A.), Chapter 6, 83-102

Sieraki M., Poulton N., Chrosby N. 2005. Automated Isolation Techniques for Microalgae. In: Algal Culturing Techniques (Ed. Andersen R.A.), Chapter 7, 103-116

Cell Biology: Physiology and Biochemistry

Microalgae are photosynthetic microorganisms capable of harvesting solar energy while converting CO_2 and water to organic macromolecules (i.e. carbohydrates, proteins and lipids). Triacylglycerols (TAGs) are the main storage compound in many algae under stress conditions, such as high light or nutrient starvation. Certain algal species naturally accumulate large amounts of TAG (30-60% of dry weight) and exhibit photosynthetic efficiency and lipids/oil production potential at least an order of magnitude higher than terrestrial crop plants (Hu et al., 2008).

The major pathway for the formation of TAG in plants and algae involves *de novo* fatty acid synthesis in the stroma of plastids and subsequent incorporation of the fatty acid into the glycerol backbone, leading to TAG via three sequential acyl transfers from acyl CoA in the endoplasmic reticulum (ER) (Fig. 3). In algae, the *de novo* synthesis of fatty acids occurs primarily in the chloroplast. The committed step in fatty acid synthesis is the conversion of acetyl CoA to malonyl CoA, catalyzed by acetyl CoA carboxylase (ACCase). Overall, the pathway produces a 16- or 18-carbon fatty acid or both. These are then used as the precursors for the synthesis of cellular and organelle membranes as well as for the synthesis of neutral storage lipids, mainly TAGs. Triacylglycerol biosynthesis in algae has been proposed to occur via the direct glycerol pathway. Fatty acids produced in the chloroplast are sequentially transferred from CoA to positions 1 and 2 of glycerol-3-phosphate, resulting in formation of the central metabolite phosphatidic acid (PA) (Ohlrogge and Browse 1995). Dephosphorylation of PA catalyzed by a specific phosphatase releases diacylglycerol (DAG). In the final step of TAG synthesis, a third fatty acid is transferred to the vacant position 3 of DAG, and this reaction is catalyzed by diacylglycerol acyltransferase, an enzymatic reaction that is unique to TAG biosynthesis. PA and DAG can also be used directly as a substrate for synthesis of polar lipids, such as phosphatidylcholine (PC) and galactolipids. 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.

The aforementioned pathway (Kennedy Pathway) is believed to be the major pathway to accumulate TAG in plants and algae. However, the regulation of synthesis of fatty acids and TAG in algae is poorly understood at the physiological, biochemical and molecular biological levels. As a result, the lipid yields obtained from algal mass culture efforts performed to date fall short of the high values (50-60%) observed in the laboratory, adding to the problem of achieving economic algal oil production (Hu et al., 2008; Sheehan et al., 1998). Moreover, the alternate pathways to convert membrane lipids and/or carbohydrates to TAG have been recently demonstrated in plants and yeast in an acyl CoA-independent way (Arabolaza et al., 2008; Dahlqvist et al., 2000; Stahl et al., 2004) (see below). Such pathways have not yet been studied in algae.

Photosynthesis

There is little agreement on the theoretical maximum productivity of algae, though values in the 100-200 g^{-1} m^{-2} day^{-1} have been presented (references). Part of the difficulty here lies with the assumptions made for parameters such as light transmittance in culture, reflection, and absorption. Another problem shows up in calculations of photobioreactor productivity in which the area of the reactors themselves, not the area of the land that they occupy is used for the calculation. The theoretical productivity is an important parameter, however because can be used to set achievable goals for both cultivation process design as well as strain improvement projects. Similar work has been carried out with plants (Zhu et al., 2007; Zhu et al., 2008), and, these approaches could be useful for similar studies with algae. Detailed study of photosynthesis in algae would not only be useful for increased biomass productivity, but could also be useful in manipulation of lipid productivity. The redox state of the electron transport chain, the energy content ATP/ADP ratio, the availability of ATP/NAD(P)H, and cytosolic pH are known to regulate gene expression and cellular metabolism in yeasts, plants and algae (Felle 1989; Pfannschmidt et al., 2001; Rolland et al., 2001; Ryu et al., 2004). It has also been shown that some algae increase lipid production under limited light regimes (Klyachko-Gurvich et al. 1999). However, the photosynthetic regulation of lipid synthesis in algae needs to be studied with respect to the aforementioned mechanisms.

Metabolic Carbon Fluxes and Partitioning

Calculations based on the moderate assumptions of 25 g/m2/day and 50% lipid (See Appendix) suggest that annual oil production of over 5000 gal/acre/yr may be achievable in mass culture of microalgae. This oil yield, however, has never been demonstrated even at a laboratory level, in effect, reflecting the lack of a clear understanding of TAG synthesis, metabolic carbon fluxes and partitioning.

Metabolic flux analysis is a rapidly developing field concerned with the quantification and understanding of metabolism at the systems level. In microbial systems, powerful methods have been developed for the reconstruction of metabolic networks from genomic and transcriptonomic data, pathway analysis, and predictive modeling. Partitioning of carbon dominates intracellular fluxes in both photosynthetic and heterotrophic plants and algae, and has vast influence on both growth and development. Recently, much progress has occurred in elucidating the structures of the biosynthetic and degradative pathways that link the major and minor pools of intracellular intermediates to cellular polymers, in providing insight into particular fluxes such as those of the pentose phosphate pathway, and into general phenomena, such as substrate- or futile-cycles and compartmentation (Lytovchenko et al., 2007; Schwender et al., 2004). In most cases, the regulatory properties of these pathways have been elucidated, and the enzymes involved have been investigated. However, carbon fluxes and partitioning into lipid is less understood, and critical research on how algal cells control the flux of photosynthetically fixed carbon and its partitioning into various groups of major macromolecules (i.e., carbohydrates, proteins and lipids) are needed. A fundamental understanding of ‘global’ regulatory networks that control the partitioning of carbon between lipids and alternative storage products will be absolutely essential for metabolic engineering of algal cells for over-production of lipids.

Metabolic Link between Starch and Lipid Metabolism

Starch is a common carbon and energy storage compound in plants and algae and shares the same precursors with the storage lipid TAG (Fig. 1). Therefore, TAG and starch may be inter-convertible. In young *Arabidopsis* seeds and *Brassica* embryos, starch was transiently accumulated and starch metabolism was most active before the oil accumulation phase (Kang and Rawsthorne 1994; Ruuska et al., 2002), indicating starch can be an important storage compound and its synthesis precedes oil accumulation. More recently, studies of higher plants showed that when starch synthesis was impaired or inhibited, the plant embryos or seeds accumulate 40% less oil (Periappuram et al., 2000; Vigeolas et al., 2004). While these results provide a clear indication that starch (carbohydrates) synthesis is linked to oil synthesis, the nature of the interaction is unknown. In algae, such interaction is also indicated by studies on the diatom *Cyclotella cryptica* (Roessler 1988) and some green algae. Therefore, it could be fruitful to initiate research on the metabolic link between starch and lipid metabolism. In this respect, *de novo* starch synthesis, degradation and interaction with lipid metabolism in algae need to be studied.

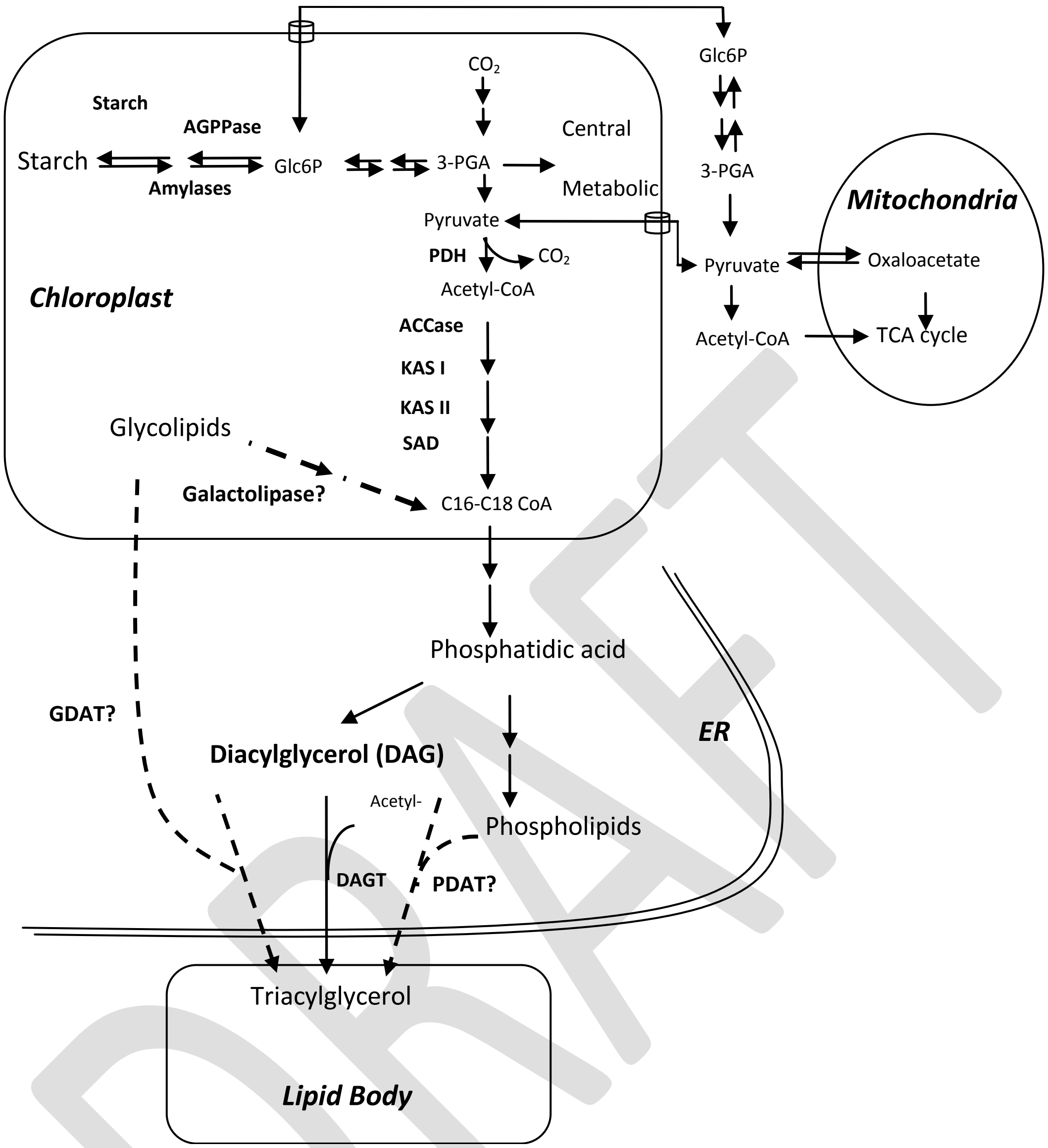


Figure 1: Major pathways for the fatty acid and TAG synthesis in plants and algae

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).

Lipid Synthesis and Regulation

Primary Pathway for Lipid Synthesis

The major pathway (Kennedy Pathway) for the formation of TAG involves *de novo* fatty acid synthesis in the stroma of plastids and subsequent incorporation of the fatty acid into the glycerol backbone, leading to TAG via three sequential acyl transfers from acyl CoA in the endoplasmic reticulum (ER) (Fig. 1). At the biochemical level, however, information about fatty acid and TAG synthetic pathways in algae is still fragmentary. We lack, for example, critical knowledge regarding both the regulatory and structural genes involved in these pathways and the potential interactions between pathways. 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 the two distinct destinations or the inter-conversion between the two types of lipids needs to be elucidated. Assuming that the ability to control the fate of fatty acids varies among algal taxonomic groups or even between isolates or strains of the same species, the basal lipid/TAG content may, in effect, represent an intrinsic property of individual species or strains. If this proves to be true, it will 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, d*e novo* fatty acid and lipid synthesis need to 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

Microalgae may possess multiple pathways for TAG synthesis and the relative contribution of individual pathways to overall TAG formation depends on environmental or culture conditions. As noted above, alternate pathways to convert membrane lipids and/or carbohydrates to TAG have been demonstrated in plants and yeast (Arabolaza et al., 2008; Dahlqvist et al., 2000; Stahl et al., 2004). For example, an acyl-CoA independent pathway for TAG synthesis is mediated by a phospholipid: DGAT acyltransferase (PDAT) that use phospholipids as acyl donors and DAG as an acceptor (Arabolaza et al., 2008; Dahlqvist et al., 2000; Stahl et al., 2004). In addition, the thylakoids of chloroplasts are the main intracellular membranes of algae, and their lipid composition dominates the extracts obtained from cells under favorable growth conditions. The algal chloroplasts have monogalactosyldiacylglycerol (MGDG) as their main lipid (~50%), with smaller amounts of digalactosyldiacylglycerol (DGDG, ~20%) and sulfoquinovosyldiacylglycerol (SQDG, ~15%) and phosphatidyglycerol (PG, ~15%) (Hardwood 1998). Under stress conditions, as the degradation of chloroplasts occurs, the fate of the abundant glycoglycerolipids remains unclear. . It has been proposed that a house-keeping pathway produces a basal/minimum level of TAG under favorable growing conditions, whereas alternative pathways that convert starch, excess membrane lipids, and other components into TAG play an important role for cell survival under stress. It has been further *hypothesized* that the chloroplast may be the major site for alterative pathways of TAG synthesis and is involved in biogenesis of cytosolic lipid bodies. To address the above hypothesis, studies that compare oleaginous algae (such as *Haematococcus pluvialis* and *Pseudochlorococcum* sp.) and the non-oleaginous algae (such as *Chlamydomonas reinhardtii*) are needed to elucidate four distinct pathways of TAG synthesis: 1) *de-novo* Kennedy Pathway, 2) TAG formation from starch reserves, 3) pathway to convert membrane phospholipid into TAG; and 4) pathway to convert membrane glycolipids into TAG.

Currently there are few algal species for which near-full genome information has become or will shortly become available, including *Chlamydomonas reinhardtti, Chlorella* NC64A, *Dunaliella salina, Cyanidioshyzon merolae, Ostreococcus tauri, Thalassiosira pseudonana and Phaeodactylum tricornutum* (http://www.jgi.doe.gov/genome-projects/pages/projects.jsf). A large-scale EST sequencing for oleaginous algae (such as *Pseudochlorococcum* sp. and *Haematococcus pluvialis*) under different cultural conditions will give us better knowledge on genes differentially expressed under different oil production conditions, and together with cDNA microarray and/or proteomic studies, will provide information about photosynthetic carbon partitioning and lipid synthesis in algae. Based on such information, metabolic engineering through genetic manipulation represents yet another promising strategy for the production of algal oils. The available approaches may include random and targeted mutagenesis and gene transformation. Cloning and transforming genes that influence the synthesis of lipids or improve robustness in growth performance in selected algal strains proven amenable to mass culture will enhance the overall performance and sustainable production of TAG or other lipids.

Organelle Interactions

The chloroplast boundary consists of two envelope membranes controlling the exchange of metabolites between the plastid and the extraplastidic compartments of the cell. The plastid internal matrix (stroma) is the primary location for fatty acid biosynthesis in plants and algae. Fatty acids can be assembled into glycerolipids at the envelope membranes of plastids or they can be exported and assembled into lipids at the ER to provide building blocks for extraplastidic membranes. Some of these glycerolipids, assembled at the ER, return to the plastid where they are remodeled into the plastid typical glycerolipids. As a result of this cooperation of different subcellular membrane systems, a rich complement of lipid trafficking phenomena contributes to the biogenesis of chloroplasts (Benning 2008). Considerable progress has been made in recent years towards a better mechanistic understanding of lipid transport across plastid envelopes in bacteria and plants. Such work is necessary in algae to better understand the interaction among organelles related to lipid formation and lipid trafficking phenomena.

Oxidative Stress and Storage Lipids

Under environmental stress (such as nutrient starvation), the algal cell quickly stops division and accumulates TAG as the main storage compound. Synthesis of TAG and deposition of TAG into cytosolic lipid bodies may be, with few exceptions, the default pathway in algae under environmental stress conditions. In addition to the obvious physiological role of TAG serving as carbon and energy storage, particularly in aged algal cells or under stress, the TAG synthesis pathway may play more active and diverse roles in the stress response. The *de novo* TAG synthesis pathway serves as an electron sink under photo-oxidative stress. Under stress, excess electrons that accumulate in the photosynthetic electron transport chain may induce over-production of reactive oxygen species, which may in turn cause inhibition of photosynthesis and damage to membrane lipids, proteins and other macromolecules. The formation of a C18 fatty acid consumes approximately 24 NADPH derived from the electron transport chain, which is twice that required for synthesis of a carbohydrate or protein molecule of the same mass, and thus relaxes the over-reduced electron transport chain under high light or other stress conditions. The TAG synthesis pathway is usually coordinated with secondary carotenoid synthesis in algae (Rabbani et al., 1998; Zhekisheva et al., 2002). The molecules (e.g. b-carotene, lutein or astaxanthin) produced in the carotenoid pathway are sequestered into cytosolic lipid bodies. The peripheral distribution of carotenoid-rich lipid bodies serves as a ‘sunscreen’ to prevent or reduce excess light striking the chloroplast under stress. TAG synthesis may also utilize phosphatidylcholine, phatidylethanolamine and galactolipids or toxic fatty acids excluded from the membrane system as acyl donors, thereby serving as a mechanism to detoxify membrane lipids and deposit them in the form of TAG. The exact relationship between oxidative stress, cell division and storage lipid formation in algae requires further study.

Lipid Body Formation and Relationship to Other Organelles

Despite the economic importance of microalgae as source of a wide range of lipophilic products, including vitamins, hydrocarbons and very long-chain ω-3 and ω -6 fatty acids, such as EPA and DHA, there have been relatively few studies on lipid bodies in algae compared with plants and fungi. In those cases where lipid-body accumulation in algae has been studied, cytosolic TAG-rich droplets ranging from 1–8 μm in size were observed. The proposal that lipid bodies in microalgae are not mere carbon stores but that they are more centrally involved in membrane lipid turnover is echoed by recent findings from higher plants—studies that also imply lipid-body TAG is metabolically active in seeds and other organs (Murphy 2001). The study of lipid-body biogenesis in plants has focused largely on the role of oleosins. 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 significant doubts about the role of oleosins in the biogenesis of plant lipid bodies. Rather, it is suggested, in the light of currently available evidence, oleosins may be primarily associated with the stabilization of storage lipid bodies during the severe hydrodynamic stresses involved in dehydration and rehydration in many types of seeds (Murphy 2001). Lipid bodies may dock with different regions of the ER and plasma membranes, or with other organelles such as mitochondria and glyoxysomes/peroxisomes, in order to load or discharge their lipid cargo. 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. This relationship may be both metabolic, with acyl and glycerol moieties exchanging between the different lipid classes, and spatial, with growing evidence of direct physical continuities between lipid bodies and bilayer membranes. In order to understand lipid metabolism in algae, the pathways/mechanisms for lipid biogenesis and composition, and the structure and function of lipid bodies and their interactions with other organelles related to storage lipid formation require further study.

References

Arabolaza A, Rodriguez E, Altabe S et al., (2008) Multiple pathways for triacylglycerol biosynthesis in *Streptomyces coelicolor*. Appl. Environ. Microbiol. 74:2573-2582

Benning C (2008) A role for lipid trafficking in chloroplast biogenesis. Prog. Lipid Res. 47:381-389

Chisti 2007 Biotechnology Advances 25:294-306 (2007).

de la Jara et al. J. App. Physiol. 15: 433-438 (2003)

Dahlqvist A, Stahl U, Lenman M et al., (2000) Phospholipid : diacylglycerol acyltransferase: An enzyme that catalyzes the acyl-CoA-independent formation of triacylglycerol in yeast and plants. Proc. Natl. Acad. Sci. U. S. A. 97:6487-6492

Falkowski, P.G.; Raven, J.A. (1997). Aquatic photosynthesis. Malden, MA: Blackwell Science

Felle H (1989) pH as a second messenger in plants . In: Second Messengers in Plant Growth and Development (Boss, W.F. & Morre, D.J. , eds) . New York:. Alan R. Liss, Inc., pp. 145–166.

Hardwood JL (1998) Membrane lipids in algae. In Siegenthaler PA and Murata N (eds): Lipids in Photosynthesis: Structure, Function and Genetics, pp. 53-64 (Kluwer, the Netherlands).

Hu Q, Sommerfeld M, Jarvis E et al., (2008) Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. Plant J. 54:621-639

Kang F, Rawsthorne S (1994) Starch and Fatty-Acid Synthesis in Plastids from Developing Embryos of Oilseed Rape (Brassica-Napus L). Plant J. 6:795-805

Klyachko-Gurvich, G. L., L. N. Tsoglin. J. Doucha, J. Kopetskii, I. B. Shebalina, and V. E. Semenko. 1999. Desaturation of fatty acids as an adaptive response to shirts in light intensity. Physiologia Plantarum 107: 240-249.

Lytovchenko A, Sonnewald U, Fernie AR (2007) The complex network of non-cellulosic carbohydrate metabolism. 10:227-235

Metting, F. B. 1996. Biodiversity and application of microalgae. J. Ind. Microbiol. 17:477-489.

Murphy DJ (2001) The biogenesis and functions of lipid bodies in animals, plants and microorganisms. Prog. Lipid Res. 40:325-438

Ohlrogge J, Browse J (1995) Lipid biosynthesis. Plant Cell 7:957-970

Periappuram C, Steinhauer L, Barton DL et al., (2000) The plastidic phosphoglucomutase from Arabidopsis. A reversible enzyme reaction with an important role in metabolic control. Plant Physiol. 122:1193-1199

Pfannschmidt T, Allen JF, Oelmuller R (2001) Principles of redox control in photosynthesis gene expression. Physiol. Plant. 112:1-9

Pulz, O.; Gross, W. (2004). "Valuable products from biotechnology of microalgae." Applied Microbiology and Biotechnology. (65); pp. 625-648

Rabbani S, Beyer P, Von Lintig J et al., (1998) Induced beta-carotene synthesis driven by triacylglycerol deposition in the unicellular alga *Dunaliella bardawil*. Plant Physiol. 116:1239-1248

Reinfelder et al. 2000 Nature 407:996-999.

Riekhof WR, Sears BB, Benning C (2005) Annotation of genes involved in glycerolipid biosynthesis in *Chlamydomonas reinhardtii*: Discovery of the betaine lipid synthase $BTA1_{Cr}$. Eukaryot. Cell 4:242-252

Roessler PG (1988) Effects of Silicon Deficiency on Lipid-Composition and Metabolism in the Diatom Cyclotella-Cryptica. J. Phycol. 24:394-400

Rolland F, Winderickx J, Thevelein JM (2001) Glucose-sensing mechanisms in eukaryotic cells. Trends Biochem.Sci. 26:310-317

The Ecology of Algae by Round, F.E (1981) London: Cambridge University Press

Ruuska SA, Girke T, Benning C et al., (2002) Contrapuntal networks of gene expression during Arabidopsis seed filling. Plant Cell 14:1191-1206

Ryu JY, Song JY, Lee JM et al., (2004) Glucose-induced expression of carotenoid biosynthesis genes in the dark is mediated by cytosolic pH in the cyanobacterium *Synechocystis* sp. PCC 6803. J. Biol. Chem. 279:25320-25325

Schwender J, Ohlrogge J, Shachar-Hill Y (2004) Understanding flux in plant metabolic networks. Curr. Opin. Plant Biol. 7:309-317

Sheehan J, Dunahay T, Benemann J et al., (1998) US Department of Energy's Office of Fuels Development, July 1998. A Look Back at the US Department of Energy's Aquatic Species Program – Biodiesel from Algae, Close Out Report TP-580-24190. Golden, CO: National Renewable Energy Laboratory.

Stahl U, Carlsson AS, Lenman M et al., (2004) Cloning and functional characterization of a Phospholipid: Diacylglycerol acyltransferase from Arabidopsis. Plant Physiol. 135:1324-1335

Vigeolas H, Mohlmann T, Martini N et al., (2004) Embryo-specific reduction of ADP-Glc pyrophosphorylase leads to an inhibition of starch synthesis and a delay in oil accumulation in developing seeds of oilseed rape. Plant Physiol. 136:2676-2686

Zhekisheva M, Boussiba S, Khozin-Goldberg I et al., (2002) Accumulation of oleic acid in *Haematococcus pluvialis* (Chlorophyceae) under nitrogen starvation or high light is correlated with that of astaxanthin esters. J. Phycol. 38:325-331

Zhu XG, de Sturler E, Long SP (2007) Optimizing the distribution of resources between enzymes of carbon metabolism can dramatically increase photosynthetic rate: A numerical simulation using an evolutionary algorithm. Plant Physiol. 145:513-526

Zhu XG, Long SP, Ort DR (2008) What is the maximum efficiency with which photosynthesis can convert solar energy into biomass? Curr. Opin. Biotechnol. 19:153-159

Biohydrogen: Direct Biophotolysis and Oxygen Sensitivity of the Hydrogen-Evolving Enzymes

Certain photosynthetic microbes, including algae and cyanobacteria, can produce H_2 from the world's most plentiful resources in the following reactions: $2H_2O$ + light energy → $O_2 + 4H^+ + 4e^- \rightarrow O_2 + 2H_2$. Two distinct light-driven H_2-photoproduction pathways have been described in green algae, and there is evidence for a third, light-independent, fermentative H_2 pathway coupled to starch degradation. All pathways have the reduction of ferredoxin (FD, Figure 2) in common as the primary electron-donor to a hydrogenase. Hydrogenases are enzymes that can reduce protons and release molecular H_2. The major types of enzymes contain either iron ([FeFe] hydrogenases, which generally are H_2-evolving) or both nickel and iron ([NiFe] hydrogenases, which are generally H_2-uptake enzymes) in their active sites. More information about these O_2-sensitive enzymes are available (Ghirardi et al., 2007). The light-driven pathways can either use water as the substrate (employing both photosystems II and I) or NADH from the glycolytic breakdown of stored carbohydrate (employing only photosystem I) to product H_2. Rather than utilizing light-driven reduction of FD, the dark, fermentative pathway may involve a pyruvate-ferredoxin-oxidoreductase (PFOR) enzyme, similar to those found in many anaerobic systems.

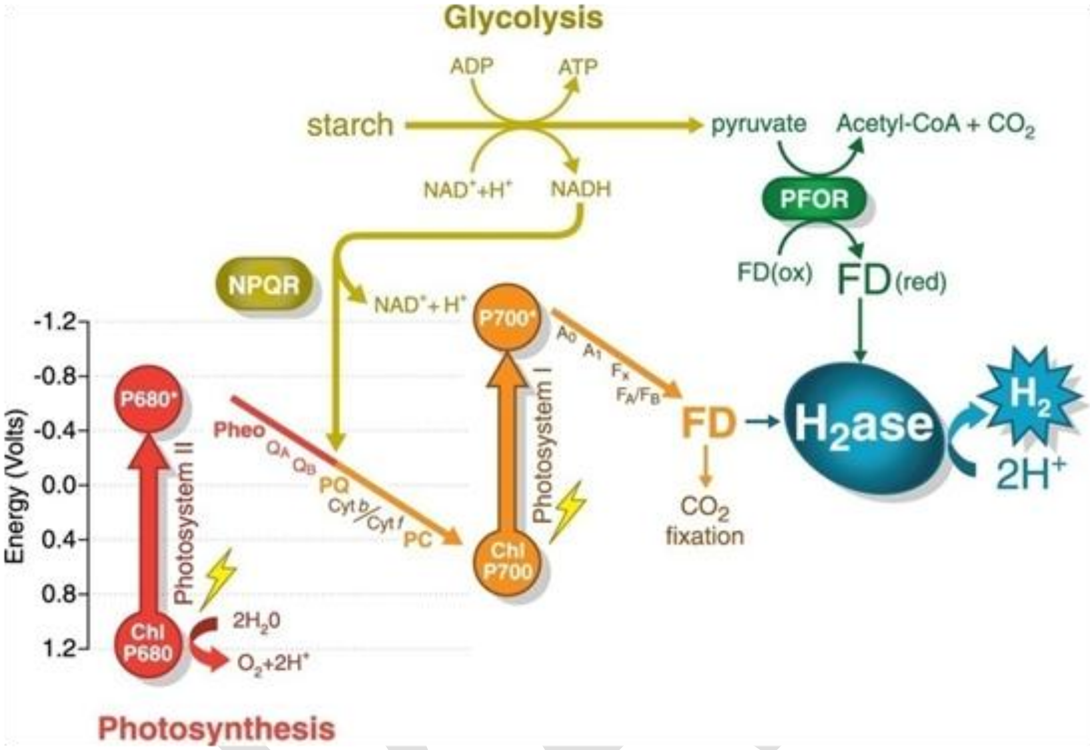


Figure 2: Three different pathways for H2 production

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 for the drawing).

Four biological challenges limiting biohydrogen production in algae have been identified as (Seibert et al., 2007) (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 at sunlight intensities. Many laboratories around the world are addressing these challenges by (a) engineering hydrogenases to improve the enzyme's tolerance to the presence of O_2 (Cohen et al., 2005), (b) identifying metabolic pathways that compete with hydrogenases for photosynthetic reductant by genomics approaches, and engineering their down-regulation during H_2 production, (c) engineering the photosynthetic membrane to significantly decrease the efficiency of photosynthetic-electron-transport-coupled ATP production (not depicted in Figure 2; ATP is required for carbon fixation but for not H_2 production), and (d) engineering the photosynthetic antenna pigment content to maximize the amount of solar light that can be used effectively in a photobioreactor (Polle et al., 2003). If all of the research challenges can be over come,

H_2-cost projections developed by the US Department of Energy suggest that biohydrogen could compete with gasoline at about $2.50 a kg (a gallon of gasoline contains the energy equivalent of about a kg of H_2)

Recently, researchers have begun to re-examining the prospects for using cyanobacteria to produce H_2. These studies are making use of bidirectional, [NiFe] hydrogenases that are found in some of these organisms rather than nitrogenases. While many of the same challenges identified in eukaryotic algae are also inherent in cyanobacteria, the advantages of working with these prokaryotic organisms are that they are more easily engineered than algae and have more O_2-tolerant hydrogenases (Ghirardi et al., 2009). On the other hand, the [FeFe] hydrogenases, found in algae, are better catalysts than the [NiFe] hydrogenases found in cyanobacteria (citation).

Other future areas of investigation that researchers are staring to examine, include the application of biological knowledge of photosynthesis and hydrogenase structure/function to developing biohybrid systems (those employing biological and synthetic components) and, ultimately, totally artificial photosynthetic systems that mimic the fuel-producing processes of photosynthetic organisms.

Fermentative Hydrogen Production (Indirect Biophotolysis)

Both algae and cyanobacteria carry out oxygenic photosynthesis. The former stores starch and the latter stores glycogen as the main carbon sink. 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 both microbes 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. 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 couple reductant to pyruvate-ferredoxin oxidoreductase, which reduces ferredoxin for subsequent H_2 production via either nitrogenases or hydrogenases (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* evolves H_2 in the resulting from the fermentation of stored glycogen (Serebryakova et al., 1998). Similarly under non-N_2 fixing condition, the hydrogenase from *Cyanothece* PCC 7822 produces H_2 in the dark and also excretes typical fermentation by-products including acetate, formate, and CO_2. (van der Oost et al. , 1989)

It is well established that dark fermentation suffers from low H_2 molar yield (less than 4 moles of H_2 per 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 pathway thus directing more of the cellular flux toward H_2 production; and (c) express multiple (both

[FeFe] and [NiFe])hydrogenases in green algae and cyanobacteria so that electrons from both ferredoxin (Fd) and NAD(P)H can serve as electron donor to support H_2 production.

References

Cohen, J., K. Kim, M. Posewitz, M.L. Ghirardi, K. Schulten, M. Seibert and P. King, “Finding Gas Diffusion Pathways in Proteins: Application to O_2 and H_2 Transport in CpI [FeFe]-Hydrogenase and the Role of Packing Defects”, *Structure* **13**:1321-1329, 2005.

Ghirardi, M.L., A. Dubini, J. Yu and P.C. Maness, “Photobiological Hydrogen-Producing Systems”, *Chemical Society Reviews* **38**: 52-61, 2009.

Ghirardi, M.L., M.C. Posewitz, P.C. Maness, A. Dubini, J. Yu and M. Seibert, “Hydrogenases and Hydrogen Photoproduction in Oxygenic Photosynthetic Organisms”, *Annual Review Plant Biology* **58**:71-91, 2007.

Kosourov, S.N. and M. Seibert, “Hydrogen Photoproduction by Nutrient-Deprived *Chlamydomonas reinhardtii* Cells Immobilized within Thin Alginate Films under Aerobic and Anaerobic Conditions”, *Biotechnology and Bioengineering* **102**:50-58, 2009.

Kruse, O., J. Rupprecht, K.P. Bader, S. Thomas-Hall, P.M. Schenk, G. Ginazzi and B. Hankamer, “Improved Photobiological H_2 Production in Engineered Green Algal Cells”, *Journal Biological Chemistry* **280**:34170-34177.

Laurinavichene, T.V., S.N. Kosourov, M.L. Ghirardi, M. Seibert and A.A. Tsygankov, “Prolongation of H_2 Photoproduction by Immobilized, Sulfur-Limited *Chlamydomonas reinhardtii* Cultures”, *Journal Biotechnology* **134**:275-277, 2008.

Melis, A., L. Zhang, M. Forestier, M.L. Ghirardi and M. Seibert, “Sustained Photobiological Hydrogen Gas Production upon Reversible Inactivation of Oxygen Evolution in the Green Alga *Chlamydomonas reinhardtii*”, *Plant Physiology* **122**:127-135, 2000.

Polle, J.E.W., S. Kanakagiri and A. Melis, “*tla1*, a DNA Insertional Transformant of the Green Alga *Chlamydomonas reinhardtii* with a Truncated Light-Harvesting Chlorophyll Antenna Size”, *Planta* **217**:49-59, 2003.

Seibert, M., P. King, M.C. Posewitz, A. Melis, and M.L. Ghirardi "Photosynthetic Water-Splitting for Hydrogen Production," *in* Bioenergy (J. Wall, C. Harwood, and A. Demain, Eds.) ASM Press, Washington DC, pp. 273-291, 2008.

Serebryakova, L.T., M. Sheremetieva, and A.A. Tsygankov, “Reversible hydrogenase activity of Gloeocapsa alpicola in continuous culture”, *FEMS Microbiol. Lett*. 166:89-94, 1998.

Stal, L.J., and W.E. Krumbein, “Temporal separation of nitrogen fixation and photosynthesis in the filamentous non-heterocystous cyanobacterium *Oscillatoria* sp.”, *Arch Microbiol*. 149:76-80, 1987.

Stal, L.J., and R. Moezelaar,. “Fermentation in cyanobacteria”, *FEMS Microbiol. Rev*. 21:179-211, 1997.

Toepel, J., E. Welsh, T.S. Summerfield, H.B. Pakrasi, and L.A. Sherman, “Differential transcriptional analysis of the cyanobacterium *Cyanothece* sp. strain ATCC 51142 during light-dark and continuous-light growth”, *J. Bacteriol*. 190:3904-3913, 2008.

Turner, J., G. Sverdrup, M.K. Mann, P.C. Maness, B. Kroposki, M. Ghirardi, R.J. Evans, and D.Blake, "Renewable hydrogen production", *Intl. J. Energy Res*. 32:379-407, 2008.

van der Oost et al. Archives of Microbiology 152:415-419 (1989).

Genomics and Systems Biology

Currently, there is a lack of understanding of the fundamental processes involved in the synthesis and regulation of lipid and other potential fuel products in microalgae. Proposing to develop large scale algal culturing technology for biofuels production without this understanding is analogous to establishing agriculture without knowing how plants grow. In the case of algal biofuels, gaining this information should require a much shorter time frame than that for agricultural development because high-throughput analysis tools including genomics, transcriptomics, proteomics, metabolomics, and lipidomics can be applied, enabling detailed analyses of multiple aspects of cellular metabolism simultaneously.

Development of Algal Model Systems

Criteria for Choosing Algal Model Systems

There are two general types of model system to consider: one would involve species or strains amenable to providing information on the basic cellular processes and regulation involved in synthesis of fuel precursors, and the other would involve 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 (ASP) that not all strains that grow well in the laboratory are suitable for large-scale culturing. Adapting the lessons learned on laboratory model species to species already known to be capable of growing in large scale might be easier, but as noted above, we cannot be certain that laboratory strains and productions strains will be sufficiently related to allow for lessons in the former to be applied to the latter.

Fuel/intermediate to be produced (H2, lipids, CHO, ethanol, co- products, etc.). One consideration in choosing model systems is the type of fuel or co-product to be produced. Possible fuel types 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, secondary metabolites), food supplements, or materials for nanotechnology in the case of the silica cell wall of diatoms (See Section 7). Discussions at the Workshop revealed that some commercialization strategies focused on the non-fuel co-product as the path to profitability. While this strategy may be successful, one can assume that the DOE will only be willing to support such an effort if the path to production of significant quantities of algal biofuel is clearly delineated. With decisions made about fuel product and additional co-products, a reasonable first approach to identify model species optimal for production of a desired fuel by surveying the literature or environment for species that naturally make abundant amounts of it. In such a strain, cellular metabolism is already geared towards production, which simplifies characterization and possible development for production.

Secretion of products/intermediates. The ability of an algal species to secrete fuel precursors may be attractive because it could reduce or avoid the cell harvesting step. However, there may be practical problems. If the desired product is volatile, then collection of the atmosphere above the culture may be required to isolate it, which will necessitate the use of closed photobioreactors (PBRs). An example of this is the Algenol process making use of engineered cyanobacteria to convert photosynthetically derived sugars to ethanol. Also to be considered is whether secretion actually makes the product more readily available. For example, although there are algae known to secrete lipids (e.g. *Botryococcus braunii*), they still are associated with the cells in a lipid biofilm matrix, and thus are not free to form an organic hydrocarbon phase in solution. (Bannerjee 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 available to contaminating microbes for catabolism. Pilot-scale experimentation and further metabolic engineering is required to evaluate possible advantages and disadvantages of secretion.

Characteristics pertaining to process demands. Culture stability over long periods will be a key to low cost production of biofuel, but very little is known about the characteristics of culture robustness. Certainly rapid growth is important both for overall productivity but also for 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 (See Section 9). Resistance to predators and to viruses could also be a useful phenotype. Finally 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.

Capability of 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, Miao et al., 2006), even under mixotrophic conditions where the substrate is not known to be transported into the cell (Ceron Garcia, Garcia Camacho et al., 2006). If the carbon source can be utilized by the cell, a potential advantage is growth in both light and dark periods. It is not clear what the relative amount of fuel precursor production under photosynthetic and heterotrophic conditions will be, but this can be determined. A potentially serious disadvantage of addition of external carbon sources is the possibility of increased contamination by undesired microbes living off the carbon source.

Survey the phylogenetic tree to expand number of potential candidates. Unicellular microalgae are the product of over 3 billion years of evolution, and are highly diverse (Falkowski, Katz et al., 2004). Multiple endosymbiotic events have occurred during the evolution of microalgae, and these are likely to have significant effects on 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, Buchel et al., 2006). Continued exploration of the evolutionary diversity of algae is important to identify species that are adept at making fuel precursors and those with high productivity under various environmental conditions.

Choice of the number of algal model systems to study. Given the phylogenetic diversity of microalgae, a large number of model systems could be studied. However, in a practical sense, the number to be studied in depth should be limited because a critical mass of researchers is required on a given species to make progress. In addition to the requirement for making fuel precursors, other factors related to what model species to study include ease of application of molecular and biochemical techniques, and transgenic capabilities. Having a sequenced genomic is critical, but lack of genome sequence at the outset should not be considered a barrier, considering that new sequencing technologies can generate a eukaryotic genome's worth of data in a week. It must be noted though, that the genomic data are only as useful as the annotation, so it will be important to provide sufficient resources to allow for detailed analysis of the data.

Cyanobacteria

Cyanobacteria generally do not accumulate storage lipids but they can be prolific carbohydrate and secondary metabolite producers, grow readily, and both fix atmospheric nitrogen and produce hydrogen. Moreover, they can be genetically manipulated, making them attractive organisms for biofuels production. A recent transgenic approach has enabled cyanobacterial cellulose and sucrose secretion (Nobles and Brown 2008), and previous work enabled ethanol production (Deng and Coleman 1999).

Cyanobacteria (blue-green algae) have many advantages over land plants, e.g., higher solar conversion efficiencies, much smaller land footprint, shorter growth cycle, and the ability to biosynthesize fuels and relevant biocatalysts. A significant advantage of cyanobacteria over green algae is that they are much easier to manipulate genetically, therefore allowing systematic genetic analysis and engineering of metabolic pathways. The model cyanobacterium *Synechocystis* sp. PCC 6803 has the potential to become a platform organism for the study of carbon metabolism toward production of hydrocarbon fuels and intermediates. The genome of this strain was sequenced over a decade ago, as the first among photosynthetic organisms. 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. However, a comprehensive understanding of carbon metabolism and regulation is not yet available, hindering the development of genetic engineering strategy for biofuel production.

In order to redirect carbon to a fuel production pathway, it will be necessary to remove the normal carbon sinks, and to understand the consequences at cellular and molecular levels. The important carbon storage compounds (sinks) in this cyanobacterium include glycogen, glucosylglycerol, sucrose, and polyhydroxybutyrate. Glycogen accumulates under normal growth conditions. Glucosylglycerol accumulates under salt stress. Sucrose accumulates when glycogen and GG sinks are not available, especially under salt stress. PHB accumulates under N depleting conditions. A systems biology approach on various carbon sink mutants will greatly advance our understanding of carbon metabolism and developing "designer organism" for biofuel production. The knowledge gained from cyanobacterial genetic analysis will also guide the development of biofuel production from green algae.

Several other cyanobacterial strains also have excellent genetic systems and are studied for the production of renewable fuels. For example, Synechococcus 7002 and Anabaena 7120 are studied for their hydrogen production potential. The latter is a filamentous strain that can form heterocysts, cells with specialized structure and metabolism for nitrogen fixation. Nitrogenase produces hydrogen as a by-product. Heterocysts are essentially anaerobic thus provide an environment for the nitrogenase and/or an oxygen-sensitive hydrogenase to operate. Synechococcus 7002 was also studied for ethanol production.

Eukaryotic Algae

Some eukaryotic algae are already fairly well-established model systems for biofuels production. They have a reasonable number of researchers working on them, have sequenced genomes, and have transgenic capabilities.

Green algae. *Chlamydomonas reinhardtii* is the most well-studied eukaryotic algae, and in addition to a sequenced genome and well developed transgenic capabilities, can be sexually crossed. It is not an abundant lipid producer, but can still serve as a model system for understanding the fundamentals of lipid synthesis and regulation. A possible serious drawback of *C. reinhardtii* is the fact that foreign genes introduced into the nucleus are silenced (Cerutti, Johnson et al., 1997) – hence no stable nuclear transgenic capability is yet possible. Chloroplast transformants are stable, and chloroplast protein expression systems are well developed, but since most genes are located in the nucleus, lack of stable nuclear expression is a barrier to analysis.

Chlorella is another well-studied class of green algae, and some species are abundant lipid producers. In *C. protothecoides*, 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, Miao et al., 2006). The genome sequence of *Chlorella* NC64A was recently completed (http://genome.jgi-psf.org/ChlNC64A_1/ChlNC64A_1.home.html), and several species of *Chlorella* have been transformed (Leon and Fernandez 2007).

Dunaliella salina has several useful characteristics for large-scale biofuels production. It produces abundant lipids (Weldy and Huesemann), and because it has outstanding salt tolerance (from 0.1 M to near saturation), it can be grown under extreme conditions that should reduce the growth of possible contaminating organisms. The genome sequence of

D. salina is currently being determined (est. size 130 Mbp), and transgenic strains have been reported (Li, Xue et al., 2007).

Diatoms. Diatoms were a major focus in the Aquatic Species Program, because as a class they tend to accumulate high amounts of lipid suitable for biofuels production (http://www.nrel.gov/docs/legosti/fy98/24190.pdf). Diatoms are highly successful and adaptable in an ecological sense and are responsible for 20% of the total global carbon fixation. A distinguishing feature of diatoms is their silica cell walls, 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 tightly coupled with the metabolism of other nutrients or involved in cellular macromolecule synthesis, therefore the silicon starvation induction response is simplified relative to other nutrient limitations. Two diatom genome sequences are complete (*Thalassiosira pseudonana* and *Phaeodactylum tricornutum*), and four more are underway (http://www.jgi.doe.gov/). None of the sequencing projects has focused on biofuels. Transgenic techniques are well established for several diatom species (Dunahay T.G., Jarvis E.E. et al., 1995; Apt K.E., Kroth-Pancic P.G. et al., 1996; Fischer, Robl et al., 1999; Zaslavskaia L.A., Lippmeier J.C. et al., 2000), and regulatable gene expression control elements have been identified (Poulsen N. and Kröger N. 2005). With the development of robust gene silencing approaches and possibly homologous recombination, the gene manipulation toolkit for diatoms will be fairly complete.

Sequencing and Annotation of Algal Genomes

The Value of Genome Sequences

Sequenced genomes are an essential information source for the interpretation of transcriptomic and proteomic data. Especially with the development of more powerful pyrosequencing methods, in which costs have been substantially reduced while more coverage is obtained in a shorter period of time, obtaining a genome sequence should be considered a necessity for any species to be developed for biofuels research or production.

Criteria for Selection/Prioritization of Organisms for Genome Sequencing & Annotation

Many of the same criteria cited above for the selection of model organisms pertain to strains chosen for genome sequencing. There are, however, additional criteria specific to sequencing projects.

Genome size and repeat structure. Genome size in microalgae can vary substantially, even in closely related species (Connolly, Oliver et al., 2008), and one reason for the variation is likely to be the accumulation of repeated sequences in the larger genomes (Hawkins, Kim 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; therefore, there will always be advantages to sequencing smaller genomes. Manipulation of smaller genomes should be simplified as well, since fewer copies of a given gene may be present.

Need to study diverse species (i.e., primary and secondary endosymbionts). Phytoplankton are distributed among at least eight major divisions or phyla, and represent a complex series of primary and secondary endosymbioses (Falkowski, Katz et al., 2004). It is likely that the different symbioses have affected communication between the plastid and nucleus (Wilhelm, Buchel 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, and a special focus on classes or individual species within classes that make abundant fuel precursors is essential.

Species to be considered for algal genomics include *Chlorella* sp., *Dunaliella* sp., *Nannochloropsis* sp., *Scenedesmus* sp., *Chlorococcum* sp., *Peudochlorococcum* sp., and a variety of diatom species.

Coordination with the Biological and Environmental Research (BER) Microbial Sequencing Program at the Joint Genome Institute

The advent of reasonable-cost high throughput sequencers and low cost commercially available sequencing services brings into question the need for coordination with established sequencing facilities such as JGI. Although JGI provides a tremendous service, because of high demand from diverse projects, access to sequencing is limited by one's queue position in the pipeline. Alternative approaches could be considered, for example, support of a stand-alone facility dedicated only to sequencing of algal biofuels candidate species.

Bioinformatics: Development of Streamlined Methods

Bioinformatics analysis of sequenced genomes, especially at the basic level of gene annotation, is essential to make sequence data usable, and if not properly done, can represent the largest stumbling block to achieving that goal. Quality standards and appropriate training should be established to ensure consistent and useful annotation. This could include the requirement of using a particular sequencing approach that provides sufficient coverage of ESTs to ensure accurate gene modeling. A stand-alone facility for sequencing and bioinformatics would facilitate high quality data production and analysis.

Comparative Analysis between Diverse and Closely Related Species

A benefit of comparing diverse species is that genes involved in the core production of a given type of biofuel precursor should be conserved between species and thus be more easily identifiable. However, genetic drift could complicate identification of certain types of genes that have low conservation. Comparison of organisms that specialize in production of different types of fuel precursors could enable identification of the genes involved by their presence or absence in the different species. Comparing closely related species that may make slightly different fuel precursors, or accumulate them to different levels, could be advantageous, since even though the overall gene content may be highly conserved, subtle differences in gene sequence could enable identification of the cause for the different phenotypes.

Identification of Species for Sequencing Efforts

Although sequencing of large numbers of candidate biofuels algal species is possible, excess data will result in incomplete interpretation and inefficient progress. It is recommended that a master plan for genome analysis of species be developed, with an initial focus on a small number of currently studied species. With these baseline data in place, the effort can branch out with a survey of major algal classes, and then species with specific desirable characteristics within the classes (the latter two can overlap). The information gained with each strain will provide the framework needed to facilitate the analysis of all subsequent strains.

Establishment of an Integrated Systems Biology and Bioinformatics Framework to Develop a Fundamental Understanding of Carbon Partitioning in Algae

Identification of important traits: Funnel-through systems analysis

Based on the criteria described above for strain selection, species will be analyzed using high throughput analysis approaches to determine the underlying cellular processes and regulation involved in producing the attributes of the strain. High throughput approaches enable in depth analyses to be performed in a whole cell context. Due to experimental variability, the highest potential can be realized by performing the various analyses on extracts from the same culture, and involving researchers from different laboratories in the process. To ensure the highest reproducibility in comparison between species, a standardized set of analysis approaches should be decided upon and implemented.

Transcriptomics

New, high-throughput sequencing technologies enable comprehensive coverage of transcripts, and quantification of their relative abundances. Most transcriptomic approaches evaluate mRNA levels, however small RNAs play major regulatory roles in eukaryotes (Bartel 2004; Cerutti and Casas-Mollano 2005), and have been identified in microalgae (Zhao, Li et al., 2007) and should be considered in investigations of gene expression regulation, especially with regard to translational regulation.

Proteomics

The cellular complement of protein reflects its metabolic potential. Mass-spectrometry-based proteomic analysis enables robust evaluation of soluble and membrane-associated proteins, and not only enables protein identification, but quantification and determination of whether post-translational modifications are present (Domon and Aebersold 2006; Tanner, Shen et al., 2007; Castellana, Payne et al., 2008). After annotation, protein databases on algal biofuel species should be established.

Metabolomics

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, including LC/MS, GC/MS, and NMR (Dunn, Bailey et al., 2005), have to be applied. There is a distinction between metabolomics, which involves identification and analysis of metabolites, and metabonomics which is ‘the quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification' (Nicholson, Lindon et al., 1999). In terms of algal biofuels research, the latter may be more important.

Lipidomics

Lipid analysis is done using mass spectrometry based approaches (Han and Gross 2005; Dettmer, Aronov et al., 2007). Quantitative comparison of lipid type and abundance are critical components of lipid-based biofuels approaches.

Integrated data analysis

To extract the most information from the "omic" approaches, an integrated analysis of data from each applied technique is desirable. For example, mRNA translatability is a significant regulatory step in gene expression, and determination of whether regulatory mechanisms are in place to control translation of mRNA into protein, requires a comparison of relative changes in transcript and protein. Enzymes have different rates of function that can be affected by feedback or posttranslational modification, therefore comparison of metabolite concentration in conjunction with protein level is required to determine the overall effect of protein induction on cellular metabolism.

Infrastructure and Investment

To maximize efficiency and reproducibility in analysis, it is recommended that a core "omics" facility dedicated to algal biofuels be established, where standardized equipment and procedures are used. Such a facility could serve as a central resource for algal biofuels researchers and be used for training programs to develop the next generation of trained experts.

Development & Adaptation of Genetic Tools and Deployment of Synthetic Biology Systems for Metabolic Engineering of Model Algal Organisms

Introduction

Development of algal biofuel technology will draw on past efforts in agronomy, plant breeding, genetics, molecular biology, and industrial biotechnology. Because it is clear that biological productivity is a key driver for economic viability (see Section 11), the ability to improve on native strains is a critical element in this research effort.

Develop a critical mass of expertise

Genetic manipulation approaches have been developed for microalgae, and the approaches are well defined conceptually. However, in a practical sense, the development of microalgal transformation systems requires a critical mass of researchers, takes a long time, and can be a risky endeavor for personnel at particular stages in career development (e.g. graduate students). Unless sufficient qualified researchers are interested in developing genetic manipulation tools for a particular species, the development of these tools will be slow. One solution would be to establish a center devoted to developing genetic manipulation tools for all candidate algal biofuel species. This would enable the coordinated development of tools for multiple species. As much as possible, tools should be developed that have application across multiple species, to reduce the development time for a particular species.

Genetic Toolbox

The ability of cells to grow on agar plates. One overarching requirement for genetic manipulation is the ability of a strain to grow on agar plates, because this is the usual way in which clonal populations of manipulated cells are isolated. Fortunately, most environmental strain isolation procedures involve plating, which automatically selects for that ability; however some modification of the procedure may be necessary, such as embedding cells in agar.

Identification of selectable markers, and development of universal transformation vectors. The fundamental basis of genetic manipulation is the ability to introduce DNA into the cell, and select for cells in which the DNA is present. Typically, this is accomplished by introducing an antibiotic resistance gene (Hasnain, Manavathu et al., 1985; Dunahay T.G., Jarvis E.E. et al., 1995), however complementation of mutants has also been achieved (Kindle, Schnell et al., 1989; Debuchy, Purton et al., 1989). Considerations of which antibiotic to use include whether the antibiotic is sensitive to light, and whether its potency is modulated by the salinity of the growth medium. Several markers have been developed for microalgae, including resistance to neomycin / kanamycin (Hasnain, Manavathu et al., 1985; Dunahay T.G., Jarvis E.E. et al., 1995), zeocin (Apt K.E., Kroth-Pancic P.G. et al., 1996; Hallmann and Rappel 1999), and nourseothricin (Poulsen, Chesley et al., 2006).

The mechanism of resistance can 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, Chesley et al., 2006), presumably because requirements for expression levels of the gene are lower.

Sophisticated metabolic engineering could require introduction of multiple selectable markers. Most current markers are derived from bacterially-derived genes, but in other unicellular eukaryotes, markers based on resistance generated by conserved ribosomal protein mutations have also been successful (Del Pozo, Abarca et al., 1993; Nelson, Saveriede et al., 1994). One caveat is that the mutated gene may need to be expressed at a higher level than the native gene (Nelson, Saveriede et al., 1994), or to completely replace the native gene in order to generate the phenotypic effect.

Once an appropriate antibiotic is identified, constructs need to be made to place the resistance gene under control of expression elements that function 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 T.G., Jarvis E.E. et al., 1995). This is highly desirable since isolation of control elements is time consuming. One goal of the development of transformation vectors for algal biofuels applications should be generate those that function in multiple species.

Transformation methods (Nuclear and chloroplast). A commonly successful method for introducing DNA into alga cells is the bolistic approach (Armaleo, Ye et al., 1990), which is useful for both nuclear and chloroplast transformation (Boynton, Gillham et al., 1988; Dunahay T.G., Jarvis E.E. et al., 1995). Other successful methods include electroporation (Shimogawara, Fujiwara et al., 1998), or vortexing with glass beads (Kindle, Richards et al., 1991) or silicon carbide whiskers (Dunahay 1993). The fundamental challenge to introducing DNA into a cell is the nature of the cell wall – hence, in certain species approaches may be limited. If methods exist to remove the cell wall, then chemically based methods of transformation could be attempted.

Sexual crossing (breeding). With the exception of *Chlamydomonas,* classical genetic approaches are not well developed in microalgae, but this methodology could be extremely important for following reasons:

- Some diatoms can be propagated vegetatively only for a limited number of generations and must be crossed periodically to maintain culture viability
- Breeding of desired characteristics from a number of phenotypic variants can allow for strain development without resorting to GM algae.
- Algal strains contain multiple copies of their genome and so recessive genotypes (like gene knockouts), may not be manifested by an altered phenotype unless that genotype is allowed to "breed true" though a series of sexual crosses.

Homologous recombination/ gene replacement vs. random insertion. DNA introduced into the nucleus of microalgal cells generally integrates randomly into the genome (Dunahay T.G., Jarvis E.E. et al., 1995). Gene replacement via homologous recombination can be more desirable because it is one method to overcome phenotypic dominance issues when a copy or copies of a wild type gene is/are present in addition to a modified gene. In addition, homologous recombination can be used to knockout genes. Obtaining successful homologous recombination has not always been straightforward; however, successful approaches include the addition of long flanking regions to the gene of interest (Deng and Capecchi 1992), use of single stranded DNA (Zorin, Hegemann et al., 2005), or co-introduction of recombinase genes (Reiss, Klemm et al., 1996).

Identification of useful gene expression control elements (constitutive and inducible). A useful aspect of a genetic manipulation toolkit is the use of gene expression control elements that drive expression to different mRNA accumulation levels. Frequently, transgenes are overexpressed by using very strong control elements, however, 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, and usually these control elements impart the same control over synthetic gene constructs using them. In addition, inducible and repressible promoters that can be actuated by simple manipulations are desirable, because they allow control over the timing of expression of a gene. 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 N. and Kröger N. 2005). Identification of other inducible or repressible control elements would be useful.

Downregulation approaches: RNA interference (RNAi). RNAi is a useful tool to downregulate gene expression. RNAi operates through double stranded RNAs that are cut down to small size and used to target suppression of expression of specific genes by base pairing. RNAi can inhibit transcription (Storz, Altuvia et al., 2005) and control translation by either cleaving specific mRNAs or sequestering them away from the ribosome (Valencia-Sanchez, Liu 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. In a practical sense, selecting for functional RNAi can be problematic. Even on vectors containing both a selectable marker and an RNAi construct, only a small percentage of selected transformants will have functional RNAi, which necessitates extensive screening (Rohr, Sarkar et al., 2004). One solution to this problem was developed in *C. reinhardtii* where the selection process was based on functional RNAi (Rohr, Sarkar et al., 2004). This approach requires that the transformed cell can transport tryptophan (Rohr, Sarkar et al., 2004).

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 tag, but others have demonstrated utility and some possible advantages (Gaietta, Deerinck et al., 2002; Regoes and Hehl 2005).

Isolation and Characterization of Mutant Species/Strains

The generation and characterization of mutants is a powerful approach to understand gene function and potentially generate strains with desirable characteristics.

Nondirected mutagenesis approaches. 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 identifying the locus of the mutations which requires a full resequencing of the entire genome.

Directed mutagenesis approaches. Targeted or tagged mutagenesis offer the advantage of simplified identification of the mutated gene since the gene is known at the outset, or the mutated gene incorporates an easily-identifiable foreign piece of DNA. Targeted approaches rely on homologous recombination (if the native gene is to be entirely replaced) or can involve changes in expression or modification of a modified copy of that gene that inserts elsewhere into the genome. Tagging can be accomplished by introducing a selectable marker randomly into the genome (Adams, Colombo et al., 2005), or through the use of transposons (Miller and Kirk 1999).

Screening approaches. 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, eg., reduced chlorophyll fluorescence (Polle, Kanakagiri et al., 2002) or increased neutral lipid accumulation via Nile red staining (Cooksey, Guckert et al., 1987) can be good screening criteria.

Given a well-developed screening approach, iterative selection could be used to generate algal strains with the desired properties but without the need to generate GM algae—something which may be desirable for large-scale algal production.

Directed evolution of enzymes/proteins. Especially with 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 favorably altered characteristics could be valuable for the development of algal biofuels technology, although the current state of transformation efficiency in algae would likely demand that the directed evolution take place in a more amenable host.

Gentically Modified Organisms (GMO)

There is a great deal of uncertainty regarding the need for or the wisdom of deploying genetically modified algae (GM algae, here defined as algal strains carrying coding sequences obtained from a foreign species). From the beginning of development of genetic engineering methodologies, it has been deemed worthwhile to build in safeguards to prevent release of genetically modified organisms (GMOs) to avoid disruption of ecosystems. The stringency of these safeguards varied with the size of perceived risk, and have been relaxed over the ensuing years in recognition that in most cases the risk was quite low. GM algae represent a novel situation, in consideration of plans for large scale deployment as well as an understanding of the basic biology that will inform such aspects as lateral gene transfer, potential for toxin production, or potential for large scale blooms and subsequent anoxic zone formation. Without a clear ability to judge these and other risks, it is likely that regulatory agencies will closely scrutinize the deployment of GM algae (See Section 10). Despite this uncertainty regarding the development of GM algae as production strains, development of genetic tools for this work is critical. In the first place, the desire for rapid commercialization of algal biofuels demands that all relevant approaches be tested in parallel. Secondarily, the data generated in the genetic manipulation of algal strains may provide the clues necessary to generate a comparable strain obtained through means that do not require use of foreign coding sequences.

References

Adams, J. E., S. L. Colombo, et al., (2005). "A mutant of *Chlamydomonas reinhardtii* that cannot acclimate to low CO_2 conditions has an insertion in the *Hdh1* gene." Function. Plant Biol. **32**: 55-66.

Apt K.E., Kroth-Pancic P.G., et al., (1996). "Stable nuclear transformation of the diatom *Phaeodactylum tricornutum*." Molecular and General Genetics **252**: 572-579.

Armaleo, D., G. N. Ye, et al., (1990). "Biolistic nuclear transformation of *Saccharomyces cerevisiae* and other fungi." Curr. Genet. **17**: 97-103.

Banerjee et al. 2002 Botryococcus braunii: a renewable source of hydrocarbons and other chemicals. Crit Rev Biotechnol 22:245-279.

Bartel, D. P. (2004). "MicroRNAs: Genomics, biogenesis, mechanism, and function." Cell **116**: 281-297.

Boynton, J. E., N. W. Gillham, et al., (1988). "Chloroplast transformation in Chlamydomonas reinhardtii with high-velocity microprojectiles." Science **240**: 1534-1538.

Castellana, N. E., S. H. Payne, et al., (2008). "Discovery and revision of Arabidopsis genes by proteogenomics." Proc Natl Acad Sci USA **105**: 21034-21038.

Ceron Garcia, M., F. Garcia Camacho, et al., (2006). "Mixotrophic production of marine microalga *Phaeodactylum tricornutum* on various carbon sources." J. Microbiol. Biotechnol. **16**: 689-694.

Cerutti, H. and J. A. Casas-Mollano (2005). "On the origin and functions of RNA-mediated silencing: from protists to man." Curr. Genet. **50**: 81-99.

Cerutti, H., A. M. Johnson, et al., (1997). "Epigenetic silencing of a foreign gene in nuclear transformants of *Chlamydomonas*." Plant Cell **9**: 925-945.

Connolly, J. A., M. J. Oliver, et al., (2008). "Correlated evolution of genome size and cell volume in diatoms (Bacillariophyceae)." J. Phycol. **44**: 124-131.

Cooksey, K. E., J. B. Guckert, et al., (1987). "Fluorometric determination of the neutral lipid content of microalgal cells using Nile red." J. Microb. Meth. **6**: 333-345.

Debuchy, R., S. Purton, et al., (1989). "The arginosuccinate lyase gene of *Chlamydomonas reinhardtii* - an important tool for nuclear transformation and for correlating the genetic and molecular maps of the Arg7 locus." EMBO J. **8**: 2803-2809.

Del Pozo, L., D. Abarca, et al., (1993). "Two different genes from *Schwanniomyces occidentalis* determine ribosomal resistance to cycloheximide." Eur. J. Biochem. **213**: 849-857.

Deng, C. and M. Capecchi (1992). "Reexamination of gene targeting frequency as a function of the extend of homology between the targeting vector and the target locus." Mol. Cell. Biol. **12**: 3365-3371.

Deng, M. D. and J. R. Coleman (1999). "Ethanol synthesis by genetic engineering in cyanobacteria." Appl. Environ. Microbiol. **65**: 523-528.

Dettmer, K., P. A. Aronov, et al., (2007). "Mass spectrometry-based metabolomics." Mass Spec. Rev. **26**: 51-78.

Domon, B. and R. Aebersold (2006). "Review - Mass spectrometry and protein analysis." Science **312**: 212-217.

Dunahay T.G., Jarvis E.E., et al., (1995). "Genetic transformation of the diatoms *Cyclotella cryptica* and *Navicula saprophila*." Journal of Phycology **31**: 1004-1012.

Dunahay, T. G. (1993). "Transformation of *Chlamydomonas reinhardtii* with silicon-carbide whiskers." Biotechniques **15**: 452.

Dunn, W. B., N. J. C. Bailey, et al., (2005). "Measuring the metabolome: current analytical technologies." Analyst **130**: 606-625.

Falkowski, P. G., M. E. Katz, et al., (2004). "The evolution of modern eukaryotic phytoplankton." Science **305**: 354-360.

Fischer, H., I. Robl, et al., (1999). "Targeting and covalent modification of cell wall and membrane proteins heterologously expressed in the diatom Cylindrotheca fusiformis (Bacillariophyceae)." Journal of Phycology **35**(1): 113-120.

Gaietta, G., T. J. Deerinck, et al., (2002). "Multicolor and electron microscopic imaging of connexin trafficking." Science **296**: 503-507.

Hallmann, A. and A. Rappel (1999). "Genetic engineering of the multicellular green alga Volvox: a modified and multiplied bacterial antibiotic resistance gene as a dominant selectable marker." Plant J. **17**: 99-109.

Han, X. L. and R. W. Gross (2005). "Shotgun lipidomics: multidimensional MS analysis of cellular lipidomes." Exp. Rev. Proteom. **2**: 253-264.

Hasnain, S. E., E. K. Manavathu, et al., (1985). "DNA-mediated transformation of Chlamydomonas reinhardtii cells - use of aminoglycoside 3' phosphotransferase as a selectable marker." Mol. Cell. Biol. **5**: 3647-3650.

Hawkins, J. S., H. Kim, et al., (2006). "Differential lineage-specific amplification of transposable elements is responsible for genome size variation in *Gossypium*." Genome Res. **16**: 1252-1261.

Kindle, K. L., K. L. Richards, et al., (1991). "Engineering the chloroplast genome - techniques and capabilities for chloroplast transformation in *Chlamydomonas reinhardtii*." Proc Natl Acad Sci USA **88**: 1721-1725.

Kindle, K. L., R. A. Schnell, et al., (1989). "Stable nuclear transformation of *Chlamydomonas* using the *Chlamydomonas* gene for nitrate reductase." J. Cell Biol. **109**: 2589-2601.

Leon, R. and E. Fernandez (2007). "Nuclear transformation of eukaryotic microalgae - Historical overview, achievements and problems." Adv. Exp. Med. Biol. **616**: 1-11.

Li, H., L. Xue, et al., (2007). "The nitrate reductase gene-switch: A system for regulated expression in transformed cells of *Dunaliella salina*." Gene **403**: 132-142.

Miller, S. M. and D. L. Kirk (1999). "glsA, a Volvox gene required for asymmetric division and germ cell specification, encodes a chaperone-like protein." Development **126**: 649-658.

Nelson, J. A. E., P. B. Saveriede, et al., (1994). "The *CRY1* gene in *Chlamydomonas reinhardtii*: Structure and use as a dominant selectable marker for nuclear transformation." Mol. Cell. Biol. **14**: 4011-4019.

Nicholson, J. K., J. C. Lindon, et al., (1999). "'Metabonomics': understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological NMR spectroscopic data." Xenobiotica **29**: 1181-1189.

Nobles, D. R. and R. M. Brown (2008). "Transgenic expression of Gluconacetobacter xylinus strain ATCC 53582 cellulose synthase genes in the cyanobacterium *Synechococcus leopoliensis* strain UTCC 100." Cellulose **15**: 691-701.

Polle, J. E. W., S. Kanakagiri, et al., (2002). "Truncated chlorophyll antenna size of the photosystems - a practical method to improve microalgal productivity and hydrogen production in mass culture." Int. J. Hydrogen Energy **27**: 1257-1264.

Poulsen, N., P. M. Chesley, et al., (2006). "Molecular genetic manipulation of the diatom *Thalassiosira pseudonana*." J. Phycol. **42**: 1059-1065.

Poulsen N. and Kröger N. (2005). "A new molecular tool for transgenic diatoms - Control of mRNA and protein biosynthesis by an inducible promoter-terminator cassette." FEBS Journal **272**: 3413-3423.

Regoes, A. and A. B. Hehl (2005). "SNAP-tag mediated live cell labeling as an alternative to GFP in anaerobic organisms." Biotechniques **39**: 809.

Reiss, B., M. Klemm, et al., (1996). "RecA protein stimulates homologous recombination in plants." Proc. Natl. Acad. Sci. USA **93**: 3094-3098.

Rohr, J., N. Sarkar, et al., (2004). "Tandem inverted repeat system for selection of effective transgenic RNAi strains in *Chlamydomonas*." The Plant Journal **40**: 611-621.

Shimogawara, K., S. Fujiwara, et al., (1998). "High-efficiency transformation of *Chlamydomonas reinhardtii* by electroporation." Genetics **148**: 1821-1828.

Storz, G., S. Altuvia, et al., (2005). "An abundance of RNA regulators." Ann. Rev. Biochem. **74**: 199-217.

Tanner, S., Z. X. Shen, et al., (2007). "Improving gene annotation using peptide mass spectrometry." Genome Res. **17**: 231-239.

Valencia-Sanchez, M. A., J. D. Liu, et al., (2006). "Control of translation and mRNA degradation by miRNAs and siRNAs." Genes Dev. **20**: 515-524.

Weldy, C. S. and M. Huesemann "Lipid production by Dunaliella salina in batch culture: Effects of nitrogen limitation and light intensity." U.S. Dept. Energy J.Undergrad. Res. **http://www.scied.science.doe.gov/scied/JUR_v7/pdfs/Lipid%20Production%20by%20Dunaliella.pdf**: 115-122.

Wilhelm, C., C. Buchel, et al., (2006). "The regulation of carbon and nutrient assimilation in diatoms is significantly different from green algae." Protist **157**: 91-124.

Xu, H., X. Miao, et al., (2006). "High quality biodiesel production from a microalga *Chlorella protothecoides* by heterotrophic growth in fermenters." J. Biotechnol. **126**: 499-507.

Zaslavskaia L.A., Lippmeier J.C., et al., (2000). "Transformation of the diatom *Phaeodactylum tricornutum* with a variety of selectable marker and reporter genes." Journal of Phycology **36**: 379-386.

Zhao, T., G. L. Li, et al., (2007). "A complex system of small RNAs in the unicellular green alga *Chlamydomonas reinhardtii*." Genes Dev. **21**: 1190-1203.

Zorin, B., P. Hegemann, et al., (2005). "Nuclear-gene targeting by using single-stranded DNA avoids illegitimate recombination in *Chlamydomonas reinhardtii*." Euc. Cell **4**: 1264-1277.

3. Algal Cultivation

Introduction

Advantages of Algae as a Biofuel Crop

Microalgal cultivation affords the promise of renewable production of liquid transportation fuels with dramatically lower net carbon emissions than petroleum-based fuels. Although current production costs for algal biomass are not competitive with petroleum-derived fuel prices (Benemann and Oswald, 1996; Chisti, 2007), as noted above, microalgae have a number of compelling characteristics that argue for their development over other biofuel crops:

Figure 3: Microalgal production raceway in southeast New Mexico. Courtesy of the CEHMM, Carlsbad, NM, www.cehmm.org

Given the absence of economic impacts on food production and the large net CO_2 emissions predicted for scaling up other biofuel crops (Fargione et al., 2008), the use of microalgae for renewable transportation fuel appears very promising.

There are however, challenging technical, economic and regulatory barriers that must be addressed to support the development of a large-scale algal biofuel industry. Indeed, despite advances and over 50 years of pilot-scale algal research (Burlew, 1953), relevant information is so scarce that a recent review of nine alternative energy options fails to mention algal biomass (Jacobson, 2009). The most important fundamental limitation to overall algal productivity is the light-to-biomass conversion rate discussed in section 2, Algal Biology.

Algal Bioreactor Designs

It is too early to determine whether closed, open (see Figure 3) or hybrid designs will ultimately prevail, so it seems prudent to support cultivation R&D projects that are closely associated with ongoing TE analysis, as highlighted in the Introduction and discussed in detail in Section 11. While it is true that capital costs for photobioreactor construction are currently higher than for open ponds, it is important to acknowledge the advantages and disadvantages of both systems. Traditionally, photobioreactors have suffered from problems of scalability especially in terms of mixing and gas exchange (both CO_2 and O_2). They may require periodic cleaning because of biofilm formation and though they will lose much less water than open ponds due to evaporation, they will not receive the benefit of evaporative cooling and so temperature maintenance may be more of a problem. Though they are unlikely to be sterilizable, they can be cleaned and disinfected and so culture maintenance is likely to be superior to that of open ponds over long periods. Photobioreactors can also provide a higher surface to volume ratio and so it likely that they can support higher volumetric cell densities (though not areal productivities) reducing the amount of water that must be processed and thus the cost of harvest. Many of the disadvantages listed above are being addressed (mainly by algal biofuel companies) through improved material usage and improved engineering designs. Though TE analyses for both open pond and photobioreactor systems have been published or presented (See Section 11), much of the information used is either out of date, based on assumptions, or based on proprietary information. As a result, it remains to be seen which system will be superior at scale over long periods of operation. There seems to be a general agreement that photobioreactors could play a critical role as breeder/feeder systems linked to open raceways, providing high cell density unialgal inocula for production ponds (Ben-Amotz, 1995) or a series of linked turbidostats or chemostats (Benson et al., 2007).

Addressing Feedstock Productivity

Feedstock productivity can be defined as the quantity of desired product per unit area per time. It is important to note that feedstock productivity may NOT scale directly with total biomass productivity depending on cultivation methods (Bennemann and Oswald, 1996).

One approach to algal cultivation for biofuel production is to develop, grow and maintain highly productive strains to maximize the concentration of the desired chemical feedstock (e.g. TAGs) in harvested material. However, monocultures are inherently difficult to maintain and will require significant investment in methods for detection and management of competitors, predators and pathogens. At the other extreme, another approach is to cultivate a more stable, mixed or natural assemblage of organisms (i.e., an ecosystem) in an attempt to maximize total harvested biomass. This model would require a downstream biorefinery capacity to process simple and complex carbohydrates, protein, and lipids into a variety of useful products. The cultivation enterprise must accomplish these tasks while balancing daily and seasonal variations in light intensity and temperature. Nutrients, including CO_2, must also be managed in a way that balances productivity and pathogen sensitivity with the plasticity of algal physiological adaptation. For example, 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).

Other algal cultivation options are being discussed including off-shore cultivation, heterotrophic/mixotrophic cultures, and the use of algal mat cultivation schemes. Of these options, only the heterotrophic options have received much attention (e.g., the dark fermentation process under development by Solazyme).

Scale-Up Barriers

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, it was suggested at the Workshop that an investment in “open source” test bed facilities for public sector R&D may provide an opportunity for this sort of research to be carried out.

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 or other chemical compounds or heavy metals into the biomass stream (Hoffman et al., 2008; Wilson et al., 2009). Little is known about artificial pond ecology or pathology, and investigation in these areas will be critically important for the development of cultivation risk mitigation and remediation strategies. Large-scale culture stability requires a combination of fundamental research and laborious, empirical, optimization research. Finally, regulatory issues need to be coordinated with multiple regulatory agencies at both the federal and state level (see Section 10, Corresponding Standards, Regulation, and Policy). In particular, procedures for environmental risk assessment and review/approval for use of genetically modified algae need to be established and standardized. Also water management, agricultural and environmental concerns are not coordinated across multiple agencies.

Beyond these general concerns, four broad areas of R&D needs emerged from the Workshop that must be addressed for economically viable, commercial-scale algal cultivation:

i) Culture stability;
ii) Standardized metrics for system-level productivity analysis;
iii) Nutrient source scaling, sustainability and management; and
iv) Water conservation, management, and recycling requirements.

These barriers are discussed below with recommendations for each.

i) Stability of Large-Scale Cultures

Issues

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. Perhaps the most worrisome component of the large-scale algae cultivation enterprise 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 well-known although current understanding of their diversity or host ranges is embryonic. Wilson et al., (2009) point out that conservative estimates suggest there may be between 40,000 and several million phytoplankton species against only 150 formal descriptions of phycoviruses. Chytrid fungi have 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.

Questions raised at the Workshop concerning this threat to large-scale algal cultures included:

- Are agricultural or municipal waste streams—a potentially significant source of nutrients for algal cultivation—actually a major liability because of significant reservoirs of algal pathogens and predators?

- To what extent will local “weedy” phytoplankton invade and take over photobioreactors and raceways?
- What prevention or treatment measures might limit such take-overs?

Roadmap Recommendations

Methods for automated or semi-automated biological and chemical monitoring in production settings will be essential for assessing the health and compositional dynamics of algal ponds. 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). It bears repeating that monocultures are expensive to establish and maintain as predation, infection, and competition from “weedy” species is inevitable. Continuous monitoring will be critical since seasonal variation in competitors, predators and pathogens is expected (Hoffman et al., 2008; Wilson et al., 2009).

Also developing an understanding of pond speciation and ecology dynamics will be critical. Early detection schemes for invasive species, predators and pathogens will be the 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 equire decontamination/restarts will be an important parameter in the cost of production because of productivity lossed 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, physiological adaptations and/or genetic modifications to production strains that afford a growth advantage over competitors and pathogen resistance must also be a priority. 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 towards basic research in multi-trophic, molecular-level algal ecology will be an essential component of the investment portfolio required for developing the potential of algae.

Final stages of viral infection in the marine phytoplankton, *Pavlova virescens*. *(Wilson* et al.*, 2009)*

ii) Overall System Productivity

Issues

According to Oswald and Benemann (1996), there are four major areas of concern related to system productivity:

- species control;
- low-cost harvesting;
- production of biomass with high lipid content; and

- very high productivities near the efficiency limits of photosynthesis.

The issue of species control was addressed above, and low-cost harvesting is discussed in Section 4. This discussion will focus on the issue of CO_2 supplementation in context of large-scale cultivation productivity, followed by lipid content and production efficiency.

Addressing global warming and the need for international efforts to control GHG emissions provides both motivation and opportunity for microalgae science. From a productivity standpoint, supplemental CO_2 has long been known to increase the growth rate, yet this area is receiving new attention due to the search for renewable, sustainable fuels in the context of growing incentives for carbon sequestration technologies. These new approaches are split between using microalgae to scrub CO_2 from emission gasses (Rosenberg et al., 2008; Douskova et al., 2009) and approaches based on better understanding of biological CO_2 concentration mechanisms from ambient air (Lapointe et al., 2008; Spalding 2008). There would appear to be ample justification to support R&D on both approaches, as siting requirements for efficient microalgal cultivation may rarely coincide with high-volume point sources of CO_2 (Section 9). The cost of CO_2 transportation and the volatile market for carbon credits will be a major challenge for techno-economic feasibility studies, and diverging business models are already apparent on these issues.

Research at the interface between basic algal biology, and cultivation science and engineering must yield significant improvements in productivity while at the same time lower the cost of production. Utilization of existing and new knowledge related to physiological regulation of lipid accumulation with scalable cultivation schemes should lead to enhancements in productivity. Long ago, nitrogen nutrition was 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 of some marine phytoplankton may also result in increases of lipid content (Azachi et al., 2002). Finally, prospects for genetic engineering approaches to increasing the flux of carbon into lipid and pure hydrocarbon metabolites in microalgae are high.

Roadmap Recommendations

Fluorescent and Nuclear Magnetic Resonance-based methods for rapid lipid content screening in microalgae 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 pond monitoring. The synthesis of new non-toxic, permeable, fluorescent indicators other than Nile Red should be encouraged. 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 microalgal lipid contents (Gocze and Freeman, 1994).

Along these lines, 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. Some national and international efforts toward generating quality assurance policy standards early on in the development of an algal biofuel industry will likely pay large dividends.

Finally, there is a critical need to ensure that R&D teams are closely coordinating with TE assessment teams. The economic viability of the microalgal cultivation enterprise is a very interdependent equation involving multiple interfaces with technical research, integration and optimization research, and the changing world of regulatory and incentive policies (e.g. carbon credits).

iii) Nutrient Sources, Sustainability, and Management

Issues

The Workshop participants discussed several issues about nutrient supply for algal cultivation as they have a sizeable impact on cost, sustainability, and production siting. The primary focus was on the major nutrients nitrogen, phosphorous, iron, and silicon (for the case of diatoms) because they represent the biggest impacts on cost and sustainability. Phosphorous appears to be an especially contentious issue as there have been calculations that the world's supply of phosphate is in danger of running out within the century (reference). Requirements for additional nutrients, such as sulfur, trace metals, vitamins, etc. must be considered, but vary depending upon the specific strain and water source chosen. Strain selection (section 2, Algal Biology) should take nutrient requirements into account. The use of carbon-based nutrients (e.g., sugars) for heterotrophic growth systems was also outside the scope of this discussion but will ultimately affect the economics of such systems.

Microalgae have a high inorganic and protein content relative to terrestrial plants, and thus a high requirement for key inorganic nutrients. Nitrogen, phosphorous, and iron additions represent a somewhat 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. Note that commodity prices of this sort can fluctuate wildly. Nitrogen is typically supplied in one of three forms: ammonia, nitrate, or urea. The best 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 need to be accounted for in life cycle analyses. It is tempting to consider the use of nitrogen-fixing cyanobacteria as a way to provide nitrogen biologically, perhaps in co-culture with the eukaryotic algae that synthesize oil. However, such a scheme will certainly have some impact on overall productivity levels as photosynthetic energy will be diverted from carbon fixation to nitrogen fixation, which may or may not be compensated for by the "free" nitrogen (citation). Note also that flue gas fed to algal cultures may provide some of the nitrogen and sulfur needed from NO_x and SO_x (citation).

Additionally, careful control of nutrient levels is critical. Limitation of a key nutrient will have serious impacts on biomass productivity, but it may 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). 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 be a problem due to concerns over eutrophication of surface waters.

Finding inexpensive sources of nutrients will be important. Reagent grade sources of nutrients could easily take the price of a gallon of algal oil above $100 per gallon. 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. What makes this scenario particularly attractive is that the wastewater treatment component becomes the primary economic driver, with the oil-rich algae being simply a useful co-product. 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. Note also that this discussion ties into the Standards, Regulation, and Policy discussion in Section 10, as pathogen and heavy metal loads in wastewater could pose serious issues, particularly for disposal of blowdown water and utilization of biomass residues.

Another approach to reducing nutrient costs is to pursue a diligent recycle. The final fuel product of microalgae contains no nitrogen, phosphorous, or iron; these nutrients end up primarily in the spent algal biomass after oil extraction. If the protein content of the algae is used for animal feed, then the nitrogen will be lost to the system. If whole biomass is used as feed, all of the nutrients are lost. From an economic perspective, this is not a problem assuming that the value of animal feed exceeds the cost of nutrients, but from a sustainability perspective (especially considering the finite nature of the phosphate supply), nutrient recycle may prove to be more valuable than animal feed. Alernatively, it may be necessary to expand the limits of analysis to include recycling of nutrients from animal waste. But 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.

Roadmap Recommendations

Nutrient sourcing and the control of nutrient levels are vitally important factors for cultivation economics, productivity, and sustainability issues; therefore, this topic is recommended as a research priority for longer-term, government-sponsored research that is not being done in the private sector. The research priorities in this area include:

- TE and LCA to understand the cost, energy, and sustainability implications of various nutrient sources and recycle scenarios;
- Studies to explore the mechanisms of nutrient recycle, e.g., from anaerobic digestion sludges; and
- 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.

iv) Water Management, Conservation, and Recycling

Issues

One of the main advantages of using algae for biofuels production is their ability to thrive in water unsuitable for land crops, including 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 properly, 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. Of course, the water used to fill the pond can be saline, brackish, produced water from oil wells, municipal wastewater, or other low-quality water stream. However, the water being lost to evaporation is fresh water, and continually making up the volume with low-quality water will concentrate salts, toxics, 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 enclosed 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 through evaporative cooling under hot conditions. Closed systems that spray water on the surfaces or employ cooling towers to keep cultures cool will lose some if not all of the water savings of such systems under these conditions. A critical part of the analysis that goes into siting an algal facility will be to analyze the “pan evaporation” rates at specific sites to weigh in conjunction with water cost and availability (see Section 9).

Water recycle is essential, but the amount that can be recycled is strain-, water-, process- and location-dependent. An actively growing algal culture can easily double its 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.

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. Treatment (e.g., desalination, activated charcoal filtration, etc.) of the recycled stream would likely be cost prohibitive. The blowdown water exiting the process will also most likely require extensive 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 be a serious problem. Surface disposal and reinjection into wells may be an option as regulated by EPA and already practiced by oil industry, however, live cells could adversely affect biodiversity of neighboring ecosystems or result in the dissemination of genetically modified organisms. However, sterilization of blowdown water would be a very costly and energy-intensive proposition.

Roadmap Recommendations

Because of the importance of issues surrounding the use of water, Workshop participants agreed that government-sponsored research in this area is warranted. Recommendations included the following efforts:

- GIS analysis of water resources, including saline aquifers, and their proximity to utilizable cultivation sites that may have lower pan evaporation rates
- Studies aimed at 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, toxics (including heavy metals), and live cells
- Research at universities and/or private sector to develop cultivation systems with minimal water consumption. This could include reducing evaporative cooling loads through such means as selecting thermotolerant strains of algae.
- Research on water recycle and methods to maximize recycle (and minimize blowdown), while effectively managing the accumulation of salt and other inhibitors

References

Azachi, M., A. Sadka, M. Fisher, P. Goldshlag, I. Gokhman and A. Zamir (2002). "Salt induction of fatty acid elongase and membrane lipid modifications in the extreme halotolerant alga Dunaliella salina." Plant Physiology **129**(3): 1320-1329.

Becker, E. W. (1994). Microalgae: Biotechnology and Microbiology. Cambridge, Cambridge University Press.

Ben-Amotz, A. (1995). "New mode of *Dunaliella* biotechnology: two phase growth for beta-carotene production." J. Appl. Physiol. **7**: 65-68.

Benemann, J. R. and W. J. Oswald (1996). Final Report to US DOE NETL. Systems and economic analysis of microalgae ponds for conversion of CO_2 to biomass. . D. o. Energy.

Benson, B. C., M. T. Gutierrez-Wing and K. A. Rusch (2007). "The development of a mechanistic model to investigate the impacts of the light dynamics on algal productivity in a Hydraulically Integrated Serial Turbidostat Algal Reactor (HISTAR)." Aquaculture Engineering **36**(2): 198-211.

Boutte, C., S. Grubisic, P. Balthasart and A. Wilmotte (2006). "Testing of primers for the study of cyanobacterial molecular diversity by DGGE." Journal of Microbiological Methods **65**(3): 542-550.

Brussaard, C. P. D. (2004). "Viral control of phytoplankton populations: a review ." J. Eukaryot. Microbiol. **51**: 125-138.

Burlew, J. S. (1953). Algal culture: from laboratory to pilot plant. J. S. Burlew. Washington, D.C. , Carnegie Institution of Washington. **600:** 1-357.

Cheng, S. H., S. Aoki, M. Maeda and A. Hino (2004). "Competition between the rotifer Brachionus rotundiformis and the ciliate Euplotes vannus fed on two different algae." Aquaculture Engineering **241**(1-4): 331-343.

Chisti, Y. (2007). "Biodiesel from microalgae." Biotechnology Advances **25**: 294-306.

Cooksey, K. E., J. B. Guckert, S. A. Williams and P. R. Callis (1987). "FLUOROMETRIC-DETERMINATION OF THE NEUTRAL LIPID-CONTENT OF MICROALGAL CELLS USING NILE RED." Journal of Microbiological Methods **6**(6): 333-345.

Douskova, I., J. Doucha, K. Livansky, J. Machat, P. Novak, D. Umysova, V. Zachleder and M. Vitova (2009). "Simultaneous flue gas bioremediation and reduction of microalgal biomass production costs." Applied Microbiology and Biotechnology **82**(1): 179-185.

Eltgroth, M. L., R. L. Watwood and G. V. Wolfe (2005). "Production and cellular localization of neutral long-chain lipids in the haptophyte algae Isochrysis galbana and Emiliania huxleyi." Journal of Phycology **41**(5): 1000-1009.

Fargione, J., J. Hill, D. Tilman, S. Polasky and P. Hawthorne (2008). "Land Clearing and the Biofuel Carbon Debt." Science **319**(5867): 1235-1238.

Gao, C. F., W. Xiong, Y. L. Zhang, W. Q. Yuan and Q. Y. Wu (2008). "Rapid quantitation of lipid in microalgae by time-domain nuclear magnetic resonance." Journal of Microbiological Methods **75**(3): 437-440.

Gocze, P. M. and D. A. Freeman (1994). "Factors underlying the variability of lipid droplet fluorescence in MA-10 Lydig tumor cells." Cytometry **17**: 151-158.

Hoffman, Y., C. Aflalo, A. Zarka, J. Gutman, T. Y. James and S. Boussiba (2008). "Isolation and characterization of a novel chytrid species (phylum Blastocladiomycota), parasitic on the green alga Haermatococcus." Mycological Research **112**: 70-81.

Honda, D., T. Yokochi, T. Nakahara, S. Raghukumar, A. Nakagiri, K. Schaumann and T. Higashihara (1999). "Molecular phylogeny of labyrinthulids and thraustochytrids based on the sequencing of 18S ribosomal RNA gene." Journal of Eukaryotic Microbiology **46**(6): 637-647.

Jacobson, M. Z. (2009). "Review of solutions to global warming, air pollution, and energy security." Energy & Environmental Science **2**: 148-173.

Ketchum, B. H. and A. C. Redfield (1938). "A method for maintaining a continuous supply of marine diatoms by culture." Biological Bulletin **75**(1): 165-169.

Lapointe, M., T. D. B. MacKenzie and D. Morse (2008). "An external delta-carbonic anhydrase in a free-living marine dinoflagellate may circumvent diffusion-limited carbon acquisition." PLANT PHYSIOLOGY **147**(3): 1427-1436.

Marie, D., N. Simon and D. Vaulot (2005). Phytoplankton cell counting by flow cytometry. Algal Culturing Techniques. R. A. Anderson. San Diego, CA, Academic Press**:** 253-268.

Reed, D. C., M. A. Brzezinski, D. A. Coury, W. M. Graham and R. L. Petty (1999). "Neutral lipids in macroalgal spores and their role in swimming." Marine Biology **133**(4): 737-744.

Rosenberg, J. N., G. A. Oyler, L. Wilkinson and M. J. Betenbaugh (2008). "A green light for engineered algae: redirecting metabolism to fuel a biotechnology revolution." Current Opinion in Biotechnology **19**(5): 430-436.

Sheehan, J., T. Dunahay, J. Benemann and P. Roessler. (1998). "A look back at the U.S. Department of Energy's Aquatic Species Program-Biodiesel from Algae." Retrieved June 29, 2007, from www.nrel.gov/docs/fy04osti/34796.pdf.

Shifrin, N. S. and S. W. Chisholm (1981). "PHYTOPLANKTON LIPIDS - INTERSPECIFIC DIFFERENCES AND EFFECTS OF NITRATE, SILICATE AND LIGHT-DARK CYCLES." Journal of Phycology **17**(4): 374-384.

Spalding, M. H. (2008). Microalgal carbon-dioxide-concentrating mechanisms: Chlamydomonas inorganic carbon transporters.

Viprey, M., L. Guillou, M. Ferreol and D. Vaulot (2008). "Wide genetic diversity of picoplanktonic green algae (Chloroplastida) in the Mediterranean Sea uncovered by a phylum-biased PCR approach." Environmental Microbiology **10**(7): 1804-1822.

Wang, Y. and M. H. Spalding (2006). "An inorganic carbon transport system responsible for acclimation specific to air levels of CO_2 in Chlamydomonas reinhardtii." Proc. Natl. Acad. Sci. USA **103**: 10110-10115.

Weissman, J. C. and D. M. Tillet (1989). Design and operation of an outdoor microalgae test facility. . D. o. Energy.

Wilson, W. H., J. L. Van Etten and M. J. Allen (2009). The Phycodnaviridae: The Story of How Tiny Giants Rule the World. Lesser Known Large Dsdna Viruses. Berlin, Springer-Verlag Berlin. **328:** 1-42.

Zhu, F., R. Massana, F. Not, D. Marie and D. Vaulot (2005). "Mapping of picoeucaryotes in marine ecosystems with quantitative PCR of the 18S rRNA gene." Fems Microbiology Ecology **52**(1): 79-92.

4. Downstream Processing: Harvesting and Dewatering

Introduction

Conversion of algae in ponds or bioreactors to liquid transportation fuels requires processing steps such as harvesting, dewatering, and extraction of fuel precursors (e.g., lipids and carbohydrates). Cultures with as low as 0.02-0.07% algae (~ 1gm algae/5000 gm water) in ponds must be concentrated to slurries containing at least 1% algae or more given known processing strategies. The final slurry concentration will depend on the extraction methods employed and will impact energy input. Energy costs climb steeply above that achievable through mechanical dewatering as the desired percentage of dry mass increases. Final slurry concentration also impacts plant location because of transportation, and water quality and recycling issues. A feasible algae-to-fuel strategy, therefore, must consider the energy costs and siting issues associated with harvesting and dewatering. Addressing these issues require careful analysis of engineering designs, combined with appropriate R&D efforts of specific processing technologies to support those designs as well as a fundamental understanding of how algal biology can impact harvesting and dewatering strategies.

Processing Technologies

Flocculation and Sedimentation

Microalgae 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.

Chemical additives that bind algae or otherwise affect the physiochemical interaction between algae are known to promote flocculation. 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. Bioflocculation where algae are co-cultured with another organism that promotes sedimentation has also been considered. Sedimentation may produce slurries with up to 1% algae and over 80% algae recovery.

Optimizing flocculant type, mixtures, concentrations, and chemistry to maximize algae recovery will very likely depend on strain selection, understanding of the mechanisms 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:

- Flocculant recovery techniques are required to minimize cost and control water effluent purity.
- The effect of residual flocculent in recycled water on culture health and stability and lipid production must be understood and controlled. Likewise, the presence of flocculent in further downstream extraction and fuel conversion processes must be understood and controlled.
- The environmental impact of flocculent in released water effluent, and fuel conversion and use must be considered.
- Finally, optimized sedimentation tank designs with integration into further downstream dewatering techniques with 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. Flocculants are added 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. Suspensions with up to 1% algae with 98% algae recovery have been achieved.

All of the issues arising from the use of flocculants in 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

Filtration without prior flocculation can be used to harvest and dewater algae. Most strains considered for energy feedstocks have cell diameters less than 10 μm, which increases the challenge of filtering. Recovery rates are as high as 80% with slurry concentrations of 1.5-3% algae content.

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 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. Power costs will certainly influence design.
- Finally, and important step is recovering the algal biomass from the filter. Washing the filter is one practice, but doing so leads to a 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. Different configurations and collection designs have been tested with up to 20% algae content and recoveries in excess of 90% achieved. The efficiency is dependent on species selection (as related to size). Centrifugation technologies must consider large initial capital equipment investments and operating costs and high throughput processing of large quantities of water and algae. The current level of technology makes this approach cost prohibitive for most of the envisioned large-scale algae biorefineries; thus significant cost and energy savings must be realized before any widespread implementation of this approach can be carried out.

Other Techniques

A number of other techniques at various stages of R&D have been proposed to harvest and dewater algae. 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 easily removed; acoustic focusing to concentrate algae at nodes; the manipulation of electric fields; and bioharvesting where fuel precursors are harvested from higher organisms (e.g., shrimp and tilapia) grown with algae.

Drying

While flocculation, sedimentation, and DAF can achieve slurry concentrations up to 3% algae and centrifugation and belt filter presses up to 20%, drying is required to achieve higher dry mass 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 be require extra space and considerable time. Solutions involving either solar and wind energy are also possible.

Systems Engineering

While specific process technologies have been studied, given the importance as well as current cost and achievable scale of harvesting and dewatering, breakthroughs are needed in each. Further, new strategies should be developed to combine and integrate these processes into a pilot-scale or demonstration facility that takes an algae culture and converts it into a slurry of a specific concentration. This has yet to be accomplished and remains a significant challenge. Given the lack of obvious solutions, the energy requirements of these processes are not only largely unknown but unbounded. This has important implications for plant design in that 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?" cannot be answered. 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.

We do know that the cost of harvesting and dewatering will depend on the final algae concentration needed for the chosen extraction method. The cost 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 shown below provides food for thought regarding harvesting and dewatering technologies.

A 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. 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 the harvested biomass. The example illustrated in Figure 4 depicts energy needs for flocculation and sedimentation followed by a belt filter press and then a methane burning drum dryer. The likely operating curve would start with pond water having an algae concentration of 0.10 to 0.15 volume %. Flocculation and settling would increase this to approximately 0.7 volume %, and a gentle belt filter press would increase this further to 2 volume %, the maximum consistency which would be pumpable to and through the lysing and extraction operations.

The energy requirements for flocculation and sedimentation and the belt filter press are expected to be minimal (dewatering curve). However, the analysis does not include the cost of the flocculant (and energy required in its production) or the cost of flocculant recovery and water clean-up. Energy in the drum dryer is based on the latent heat of vaporization of water and is calculated at 0.54 watt-hours/gram. Further, the water lost to evaporation in the drum dryer is not insignificant in terms of both amount and importance, yet not included in this preliminary analysis. The drying energy curve does not include any inefficiency in the production or application of this energy, and therefore, represents the minimum theoretical energy required for drying. Nonetheless, this analysis shows that any harvesting/extraction scheme involving dry algae is energy prohibitive, requiring at least 60% of the energy content of algae. There is thus a need to develop strains of algae with much higher energy content than available today.

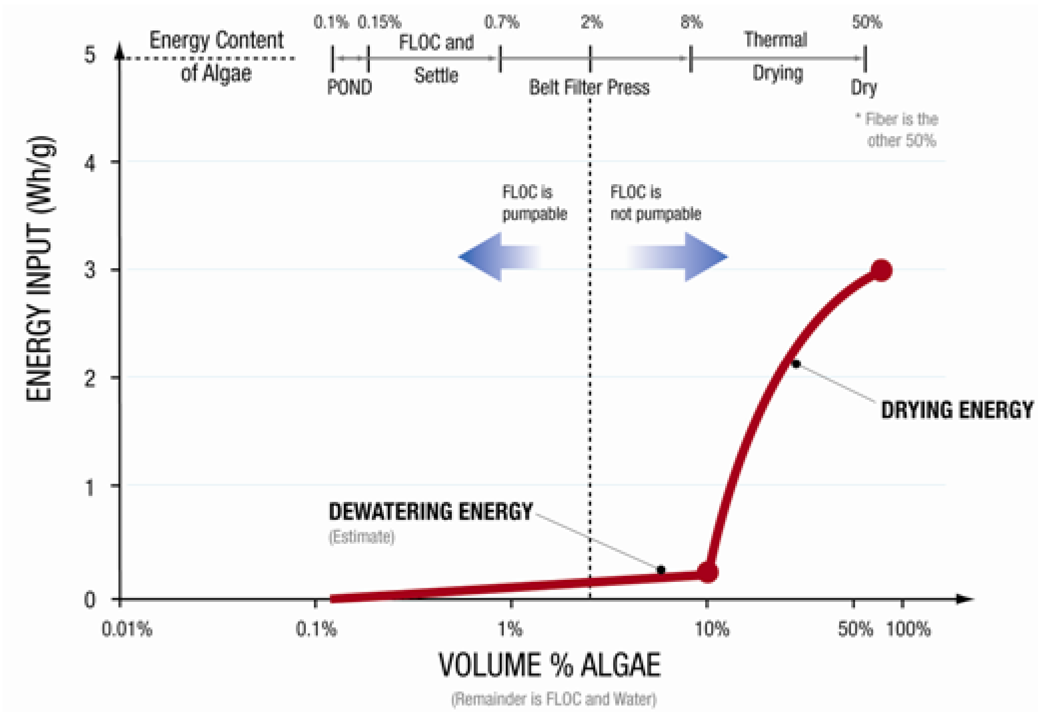


Figure 4: Approximate energy curve for harvesting, dewatering, and drying considering a process of flocculation, sedimentation, belt filter pressing, and drum oven heating.

The status line across the top shows the likely pond concentration, the consistency (volume percentage of algae) achievable by flocculation and settling, the consistency achievable by the belt filter press, and the region of dryness requiring thermal energy input. The point at which the floc is no longer pumpable is shown.

5. Extraction and Fractionation of Microalgae

Introduction

A wide variety of biomass feedstocks have been identified as suitable candidates for fractionation and conversion into biofuels. Feedstock sources range from agricultural and forestry residues, food crops such as soybeans and corn, municipal solid wastes (MSW), energy crops, transgenic species, biosolids, and manures (DOE&USDA 2005). Starch-based feedstocks have been converted into biofuels at the commodities level with terrestrial cellulosic feedstocks following on a rapid course for deployment. However, while many terrestrial feedstocks have defined routes for extraction and recovery of sugars and/or oils prior to their conversion into finished fuels, algal biomass suffers from a lack of well-defined and demonstrated industrial-scale methods of extraction and fractionation.

Microalgae's potential to produce high levels of lipids, carbohydrates and protein further complicates the extraction schemes for biofuels. Identifying the particular biological component for extraction depends heavily on the algal species and growth status, which is highly characterized for higher plants as compared to microalgae. Other challenges include difficulties in harvesting: while many feedstocks can be removed from their terrestrial environment at total solids >40%; by comparison, as discussed above algae require a high degree of concentration before extraction can begin. While extraction methods used for terrestrial oilseed plants have been proposed for microalgae, most are ineffective and have little utility.

The microalgae differ from traditional biomass feedstocks in several respects, such as in cell wall chemistry, the presence of large amounts of bulk water, smaller cell size, and the lack of standardized agronomic methods for harvesting or extraction; these differences identify some of the missing information required to extract and fractionate high-energy polymers from microalgae for biofuel production. To address the shortfall of relevant information a comprehensive research program needs to address barriers to algal-based biofuel development and begin to fund research groups to address these informational shortfalls. This section addresses the assumptions and potential scenarios for algal-based biofuels; review the existing technologies for extraction and fractionation of algal biopolymers, identifies gaps in the missing information, and lastly, discusses government's role and potential path forward toward algal-based biofuels.

Current Practices for Lipid Extraction/Fractionation

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. However, the dynamics of extraction in aqueous phase systems serves as a starting place for both continuous and industrial scale extraction operations.

Organic Co-Solvent Mixtures: The Origins of Two Solvent Systems

The concept of like dissolves like is the basis behind the earliest and well-known co-solvent extraction procedure of Bligh and Dyer, 1959. The method exposes the lipid (i.e. analyte) containing tissue to a miscible co-solvent mixture comprised of an alcohol (methanol) and an organic (chloroform). In this sense, methanol and chloroform combine to form a co-mixture solvent that favorably interacts (in terms of the four types of interactions mentioned previously) with the lipids, thus leading to their dissolution into the co-solvent. After the extraction reaction has been run to completion, 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. At this point the methanol and lipid molecules partition into the respective phases. As methanol is more "like" water (i.e., in terms of polarity) the great majority of methanol molecules partition into the water phase. As the lipid molecules are more "like" chloroform, the great majority of lipid molecules partition into the chloroform phase. More precisely, the molecular interactions between the water and methanol are stronger than they are between the methanol and chloroform while the interactions between the lipid and chloroform molecules are stronger than the interactions between the lipids and water/methanol solvent.

There is also the issue that chloroform will extract more than just the saphonifiable lipids (i.e. the unsaponifiable lipids such as pigments, proteins, amino acids, 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 et. al.1978); DMSO/petroleum ether for yeast (Park et. al. 2007); Hexane/ethanol for microalgae (Cartens et. al. 1996); and hexane/isopropanol for microalgae (Nagle et. al. 1990). The hexane system has been promoted because the hexane and alcohol will readily separate into two separate phases when water is added, thereby improving downstream separations.

Similarly, less volatile and toxic alcohols (ethanol, isopropanol) have been nominated in place of methanol because they are less toxic. One example is the hexane/ethanol extraction co-solvent system (Molina et. al. 1994). In other cases, single alcohol (1-butanol, ethanol) solvents have been tried (Nagle et. al. 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 partitions the extracted lipids into the nonpolar hexane (Fajardo et. al. 2007). In general, applications using pure alcohol (ethanol, 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 (i.e. butanol) have not compared well against the hexane/ethanol co-solvent system.

These results suggest that the two effects most important 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 is sufficiently porous and

(2) the ability of a second less polar co-solvent to better match the polarity of the lipids being extracted.

Also, if one wishes 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), a prior step to physically disrupt the cell membrane is useful.

Application of OrganicTwo-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. This suggests that when designing co-solvent systems to extract the entire range of lipids, one should be aware that while the use of more polar solvents will improve the range of lipids extracted, they may also decrease the carrying capacity of the solvent because, in general, solvents that extract polar lipids are not miscible with relatively high ratios of nonpolar lipids. The sequence of solvent addition can also affect extraction (Lewis et. al. 2000). Starting from freeze dried biomass, Lewis and coworkers 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). 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 addition agitation in the form of sonication, or the addition an additional methanol.

Direct Transesterification of Lipids into FAMES Using Organic Solvent Systems

The original work on lipid extraction, as defined above, was almost exclusively applied for the investigation of fatty acids in tissues. As such, the lipids were first extracted, purified, and then transesterified to fatty acid methyl esters before being characterized by gas chromatography (GC). As discussed above, these approaches were limited by incomplete recoveries owing to multiple factors such as low solvent carrying capacity and solvent-lipid polarity mismatch. To address this issue, 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 1 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. Rodriguez-Ruiz et al., (1998) applied this method to microalgal biomass and modified the approach to include hexane in the reaction phase in order to avoid a final purification step. Moreover, Rodriguez-Ruiz and coworkers found that the entire reaction could be shortened to 10 minutes if the mixture was incubated at 100°C under a sealed cap. Finally, Carvalho and Malcata (2005) found that when applying direct transesterification using an acid catalyst (i.e. acetyl chloride), the efficiency of the reaction is 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. 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.

The co-solvent system, however, remains largely a bench scale method that is difficult to scale up into an industrial process due to the actual toxicity of methanol and chloroform and the low carrying capacity of the solvent (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: (A) the elevated temperature and pressure increase the rate of mass transfer and degree of solvent access to all pores within the biomass matrix, and (B) 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. 1996). 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.

Temperature and pressure are two non-chemical parameters that increase solvation power of a particular solvent. The use of higher temperatures is assumed to increase the capacity of solvents to solubilize analytes because the thermal energy increase provided by the increase in temperature can overcome the cohesive (solute-solute) and adhesive (solute-matrix) interactions (e.g. by decreasing the activation energy required for the desorption process). Increased pressure facilitates increased transport of the solvent to the analytes that are trapped in pores. Pressure also helps to force the solvent into matrices that would normally not be contacted by solvents under atmospheric conditions. Despite these advantages, however, the application of pressure and temperature increase process energy and operating costs. These costs increases dramatically if water is present, and so the application of these process parameters favor the use of completely dried biomass.

Mechanical Disruption (i.e., Cell Rupture)

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 may present formidable barriers to solvent access. This generally requires that the native structure of the biomass must be mechanically disrupted prior to employment of a mixture of co-solvents, in order to favor the continuous penetration of persistent biomembrane-enclosed regions. Mechanical means are initially employed to disrupt the cell membrane prior to the application of the extraction solvents. The most common of these are (i) lyophilization followed by grinding in a pestle and mortar, (ii) grinding cells while frozen in liquid nitrogen, and (iii) other more intensive homogenization techniques such as bead beating, multi-pass homogenizers, and extreme ultrasonication. Efficient extraction requires that the solvent be able to fully penetrate the biomass matrix in order to contact the target analytes (i.e. lipids) wherever they are stored, and that the solvent's polarity must match that of the target analyte(s) (i.e. lipid). As such, this suggests mechanical disruption offsets the need to use elevated temperature and pressure processes that force the solvent into contact with the analyte.

Subcritical Water Extraction

Subcritical water extraction is based on the use of water, at temperatures just below the critical temperature, and pressure high enough to keep the liquid state (Ayala et. al. 2001). The technique, originally termed "pressurized hot water extraction", was initially applied to whole biomass hemicellulose and 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), the extraction of functional ingredients from microalgae (Herrero et. al 2006), and saponins from oil-seeds (Guclu-Ustundag 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 are 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 difficulty designing a system at large scale and the high-energy load required to heat the system up to subcritical temperatures. Large-scale design will require a significant cooling system to cool the product down to room temperature to avoid product degradation as well, generating significant additional energy use challenges.

Accelerated Solvent Extraction

Accelerated solvent extraction (ASE) was first proposed in the mid 1990's by Richter et al.,(1996). Accelerated solvent extraction uses organic solvents at high pressure and temperatures above their boiling point (Richter et. al. 1996). The solvents used are those normally used for standard liquid extraction techniques for Soxhlet or sonication. 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 (Schafer 1998). In addition to improving yields and dramatically 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. Various absorbents can also be added to the extraction cell in order to improve the purity of the final sample (Dionex 2007). For example, the addition of alumina (Al_2O_3 activated by placing in a drying oven at 350°C for 15 hour). In most cases, ASE can be an efficient technique assuming the extracting solvent, sample-solvent ratio, extraction temperature and time have been optimized. For example, Denery et al., examined these factors to optimize 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. What remains unclear is the effectiveness of such an approach at large scale in terms of how to handle large amounts of biomass as well as the energy cost. The latter is also noteworthy in the context that accelerated solvent extraction by definition uses non aqueous solvents and therefore must use dried biomass, a step that also requires the input of energy.

Supercritical Methanol or CO_2

Although supercritical fluid extraction is technically a solvent extraction technique, it has been separated from the discussion on solvent extraction above because supercritical fluids are a unique type of solvent. Supercritical fluid extraction is relatively recent extraction technique based upon the enhanced solvating power of fluids when above their critical point (Luque de Castro et. al. 1994). Its usefulness for extraction is due to the combination of gas-like mass transfer properties and liquid-like solvating properties with diffusion coefficients greater than those of a liquid (Luque de Castro et al. 1999). The majority of applications have used CO2 because of its preferred critical properties (i.e. moderate critical temperature of 31.1°C and pressure of 72.9 ATM), low toxicity, and chemical inertness, 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 process requires a dry sample that is placed into a cell that can be filled with the gas before being pressurized above its critical point. The temperature and pressure above the critical point can be adjusted as can the time of the extraction. Super critical extraction is often employed in batch mode, but the process can also be operated continuously. 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 lowed 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, as well as functional ingredients and lipids from microalgae (Herrero et. al. 2006). Lipids have been selectively extracted from macroalgae at temperatures between 40 to 50°C and pressures of 241 to 379 bar (Chueng 1999). Despite the range of products extracted from microalgae its application to the extraction of lipids for the production of biofuels is limited by both the high energy costs and difficulties with scale up.

"*Milking*"

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*. There 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 could be 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 technology that proposes to use solvents such as decane and dodecane in the presence of live microalgal cells that have been 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. The "Cell milking" technique, as described in this as context, has gained some attention in terms of patents and small-scale pilot applications by private companies. However, long-term testing of cell viability in the context of continual production remains to be done. If successful, this method does offer the possibility of selectively extracting lipids suitable for biofuels and excluding the extraction of lipids that cannot be transesterified and pigments (such as chlorophyll) that can be difficult to separate from the desired lipids and create a very viscous and tarry final product.

Nontraditional Extraction Approaches

In the existing marketplace, the number of companies producing algal-based products is quite modest. Even so, the business strategies of these companies often require extraction technologies to produce commercial products. Most of these companies focus on cultivating and producing green and blue-green algae for food supplements, beta-carotene, and related pigments for the nutraceuticals and food markets (Shahidi 2006). In many of these operations, the final product is the algal biomass itself. The algae are harvested, dried, and formulated into pellets, pills, or powders for consumption. Pigments and other nutraceuticals can be further extracted by grinding or ball milling the dried algae. In the future, using green solvents or supercritical extraction to increase the purity of the product may be the next step in product formulations. Commercially grown cyanobacteria are grown at large scale and are harvested using the cell itself as the finished product. 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, and the algae 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 (e.g. Solazyme). 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.

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 on both at many 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 principle 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. Consider for example, analytes in pores that have been sealed with water. While water might otherwise block access to an organic, at the elevated temperature, which reduces surface tension and reduces the polarity of water, the increased pressure will better force the solvent into the matrix where it can solubilize the analyte.

However, the cell wall needs to be understood in the context of the extraction process chosen. To use the emulsion technique, an algal strain that lacks a cell wall such that it is broken down during the centrifugation process must be used. If, however, a solvent extraction system that is based upon using dried biomass is designed, then microalgae without a cell wall would be problematic as most of the oil would be lost in the centrifugation step and the presence of emulsions would prove very problematic.

Energy Consumption and Water Recycle

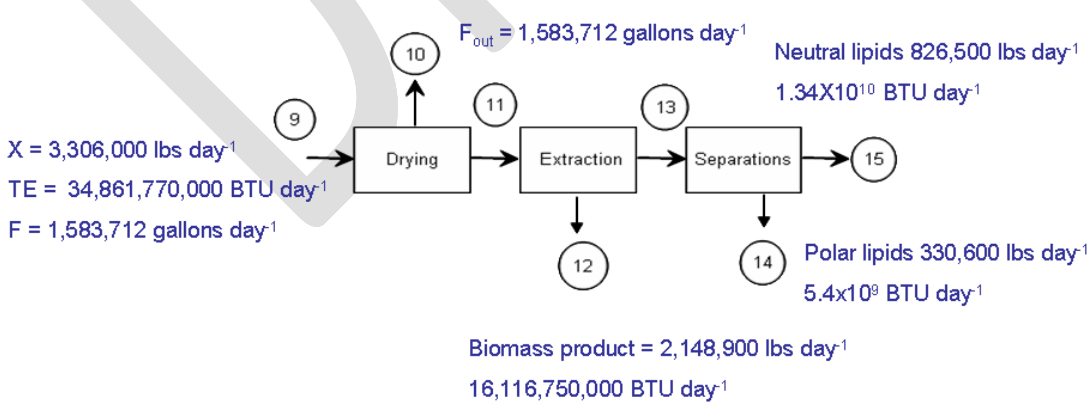


Figure 5 Typical Energy Calculation for Algal Biofuels Production System

Figure 5 shows a typical energy calculation for a production system that is scaled to produce over 3 million pounds of dried biomass per day. Assuming reasonable heats of combustion for lipid-free biomass, polar and neutral lipids, the total energy available is over 34 x 10^9 BTU per day. While this may seem significant, the value needs to be considered within the context of how much energy is used to produce, harvest, dewater, dry, and separate the final products. There is also the issue of the energy load of all the supporting operations. The breakout session at the DOE workshop set the following benchmarks: the extraction process, per day, should consume no more than 10% of the total energy load, as BTU, produced per day.

Goals

While much of the data and information needed to understand the relationship between water chemistry, cell lipid production, process economics, and algal cultivation are missing, specific characteristics of a "successful extraction process" can be outlined. Based on process economic models for cellulosic ethanol, the primary drivers for a cost-competitive process is the product yield and both capital and operating costs (Mosier et. al. 2005). The extraction yield depends not only on the efficiency of the extraction process, but on the primary productivity of the algal cultivation system as well. It should be noted that early pioneer processes used for both algal cultivation and lipid extraction/fractionation will not be efficient or cost-effective and must evolve to enable greater efficiency and less operating costs. The higher-level goals represent an advanced design that will incorporate high yields of extracted lipids, low energy consumption, efficient water recycle, minimal waste and impact on the environment. These goals should be used to guide future R&D. In moving from today's lipid-based extraction systems the more cost effective solutions of the future, other components such as carbohydrates and proteins may need multi-step processes to reduce cost and avoid waste discharges from the extraction facility. Specific goals for an extraction processes are:

1. Developing a 1st generation extraction process that recovers >75% of the algal bioproduct (includes lipids, protein, and carbohydrate)
    a. Efficient in a water rich environment (~85% moisture after harvesting)
    b. Consumes no more than 15% of the energy in the final product
    c. Recycles water from the process back to the cultivation process without impacting growth (avoiding chemical imbalances)
2. Developing nth generation extraction technology using "green technologies that recover >90% of the algal lipids, proteins and carbohydrates.
    a. Allows for 95% conversion of extracted materials into fuels or quality byproducts.
    b. Uses only 10% of the total energy in the harvested biomass
    c. Meets the water recycle requirements
    d. Integrated with other unit processes such as algal biology and cultivation
3. Minimal environmental impact
    a. Complete utilization of algal biomass (zero discharge)
    b. Limit/exclude discharges into air, water and soil from extraction process

Missing Science Needed to Support the Development of New Extraction and Fractionation Technologies

Algal Cell Wall Composition

Successful bioconversion of terrestrial cellulosic feedstocks requires advanced knowledge of how cultivation, plant growth, and harvesting affects the type, structure and amount of cellular carbohydrates. This knowledge is absolutely required for the development of efficient and effective conversion of cellulosic feedstocks into biofuels. To accomplish this goal, both new tools and capabilities have been adopted to address the lack of fundamental knowledge of terrestrial plant cell wall chemistry, carbohydrate deposition. The use of enhanced imaging and spectrophotometric tools to identify the structural components defining the cellular structure provides insights to barriers to both thermochemical pretreatment and enzymatic hydrolysis of the existing cell wall of terrestrial plants. Understanding the structural nature of cell wall chemistry is the goal for genomic control and production of feedstocks that have reduced recalcitrance to bioconversion and better yields in the field. This targeted approach to conversion has led to a better characterization of corn stover, the leading feedstock for DOE's 2012 cellulosic ethanol cost targets. Having demonstrated success with increasing the understanding of terrestrial plants, these tools and approaches could be applied to algal species to better understand the chemistry and compositional analysis for algal cell wall, ultra-structure and lipid chemistry, as a function of growth and cultivation practices.

Lipid Genesis, Chemistry, and Structure

As algal cells grow, the components for life are assembled and retooled as a function of growth. Knowledge of how lipids are produced, organized into cell membranes and other storage vessels, and how the controlling mechanisms affect the lipid composition will help in developing new extraction processes and understanding the effect of changes in lipid composition and cell wall structure through the cell cycle on the extraction processes. Can we modify the lipid composition to improve the efficiency of oil extraction? From the standpoint of algal biology, can we "customize" algal production strains for specific lipid characteristics that allow for low-energy extraction processes?

Development of Multitasking Extraction Processes

Algal lipids may be the first of several cellular components that will be fractionated from disrupted algal cells or removed through organic solvents. The ability to selectively remove desired components during the fractionation process is a hallmark for traditional petroleum refinery extraction processes. Using high temperatures and selective catalysts, a wide range of products and feedstocks are successfully removed from crude oil through selective processing. Algal-based biofuels would follow in the same vein, extraction and fractionation of multiple products in a minimal number of steps.

Early adopted extraction protocols may use organic solvent-based approaches. Ultimately, however, the development of "green" extraction systems would be needed to avoid issues with organic solvents, such as toxicity and costly solvent recycle.

Conclusion

Achieving significant petroleum displacement from biofuels using algal biomass requires an efficient and effective extraction/fractionation process that recovers lipids, proteins and carbohydrates from algal biomass, while preserving their potential for biofuels and other applications. There is wide gap between the existing technologies and an industrial-scale microalgal based biofuel process. There are large gaps in our knowledge needed to develop extraction/fractionation processes, such as cell wall composition, chemistry, and ultrastructure, the impact of high water content and chemistry on the extracted materials, and understanding the effect of cultivation and strain selection on the production of carbohydrates and lipids. Additionally, the need for demonstration facilities to provide standardized materials and to develop new tools and methods is critical to accelerate progress toward the goal for biofuel production from microalgae. Lastly, the development of algal-based biofuels can be accelerated by using many of the approaches, tools and governmental programs already established for cellulosic ethanol.

References

Biomass as Feedstock for a Bioenergy and Bioproducts Industry: The Technical Feasibility of a Billion-Ton Annual Supply: **April 2005 DOE and USDA publication**

Bligh, E.G. and Dyer, W.J., A rapid method for total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology, 1959. 37: p. 911 – 917

Fajardo, A.R., Cerdain, L.E., Medina, A.R., Fernandex, F.G.A., and Molina Grima, E., Lipid extraction from the microalgae Phaeodactylum tricornutum. European Journal of Lipid Science and Technology, 2007. 109: p. 120 – 126

Hara, A. and Radin, N.S., Lipid extraction of tissues with a low-toxicity solvent. Analytical Biochemisry, 1978. 90: p. 420 – 426

Park, P. K., Kima, E. Y. and Chub, K. H. (2007). "Chemical disruption of yeast cells for the isolation of carotenoid pigments." **53**(2): 148-152

[Cartens, M., Moina Grima, E., Robels Medina, A., Gimenez Gimenez, A. and Ibanez Gonzalez, J. (1996). "Eicosapentaenoic acid (20:5n-3) from the marine microalgae Phaeodactylum tricornutum." Journal of the American Oil Chemists' Society **73**: 1025-1031]

[Nagle, N. and Lemke, P. (1990). "Production of methyl ester fuel from microalgae." Applied Biochemistry and Biotechnology **24/25**: 355 – 361]

[Molina Grima, E., Robels Medina, A., Gimenez Gimenez, A., Sanchez, J.A., Garcia Camacho, F., and Garcia Sanchez, J.L., Comparison between extraction of lipids and fatty acids from microalgal biomass. Journal of the American Chemical Society, 1994. 71(9): p. 955 - 959]

[Iverson, S.J., Lang, S.L.C., and Cooper, M.H., Comparison of the Bligh and Dyer and Folch Methods for Total Lipid Determination in a Broad Range of Marine Tissue. Lipids, 2001. 36(11): p. 1283 - 1287.]**.**

[Lewis, T., Nichols, P.D., and McMeekin, T.A., Evaluation of extraction methods for recovery of fatty acids from lipid-producing microheterotrophys. Journal of Microbial Methods, 2000. 43: p. 107 - 116.]

[Lepage, R. and Roy, C.C., Improved recovery of fatty acid through direct transesterification without prior extraction or purification. Journal of Lipid Research, 1984. 25: p. 1391 - 1396.]

[Rodriguez-Ruiz, J., Belarbi, E.H., Sanchez, J.L.G., and Alonso, D.L., Rapid simultaneous lipid extraction and transesterification for fatty acid analysis. Biotechnology Techniques, 1998. 12(9): p. 689 - 691.]

Carvalho, A.P. and Malcata, F.X., Preparation of Fatty Acid Methyl Esters for Gas-Chromatographic Analysis of Marine Lipids: Insight Studies. Journal of Agricultural and Food Chemistry, 2005. 53: p. 5049 - 5059**.]**

[Herrero, M., Cifuentes, A., and Ibanez, E., Sub- and supercritical fluid extraction of functional ingredients from different natural sources: Plants, food-by-products, algae and microalgae. A review. Food Chemistry, 2006. 98: p. 136 – 148; Richter, B.E., Jones, B.A., Ezzell, J.L., Porter, N.L., Avdalovic, N., and Pohl, C., Accelerated solvent extraction: a technique for sample preparation. Analytical Chemistry, 1996. 68: p. 1033 - 1039**]**

[*Ayala, R. s. and Castro, L. (2001). "Continuous subcritical water extraction as a useful tool for isolation of edible essential oils." Food Chemistry **75**: 109 – 113*]

[*Mok, W. S.-L. and Antal Jr., M. J. (1992). "Uncatalyzed solvolysis of whole biomass hemicellulose by hot compressed liquid water." Industrial & Engineering Chemistry Research **31**(4): 1157 – 1161*].

[*Eikani, M. H., Golmohammad, F. and Rowshanzamir, S. (2007). "Subcritical water extraction of essential oils from coriander seeds (Coriandrum sativium L)." Journal of Food Engineering **80**: 735 – 740*]

[*Guclu-Ustundag, O., Balsevich, J. and Mazza, G. (2007). "Pressurized low polarity water extraction of sponins from cow cockle seed." Journal of Food Engineering **80**: 619 – 630*]

[*Richter, B. E., Jones, B. A., Ezzell, J. L., Porter, N. L., Avdalovic, N. and Pohl, C. (1996). "Accelerated solvent extraction: a technique for sample preparation."* Analytical Chemistry ***68**: 1033 -1039*]

[Denery, J. R., Dragull, K., Tang, C. S. and Li, Q. X. (2004). "Pressurized Fluid Extraction of carotenoids from Haematococcus pluvialis and Dunaliella salina and kavalactones from Piper methysticum." Analytica Chimica Acta ***501** 175 – 181*]

[*Schafer, K. (1998). "Accelerated solvent extraction of lipids for determining the fatty acid composition of biological material."* Analytica Chimica Acta ***358**: 69 – 77*]

[*Dionex, C. (2007). "Accelerated solvent extraction techniques for in-line selective removal of interferences."* Technical Note ***210**(LPN 1931): Sunnyvale, CA*].

[*Luque de Castro, M. D., Valcarcel, M. and Tena, M. T. (1994).* Supercritical Fluid Extraction*. Heidelberg, Springer Verlag*]

[*Luque de Castro, M. D., Jimenez-Carmona, M. M. and Fernandez-Perez, V. (1999). "Towards more rational techniques for the isolation of valuable essential oils from plants."* Trends in Analytical Chemistry ***18**(11): 708 – 715*].

[*Chueng, P. C. K. (1999). "Temperature and pressure effects on supercritical carbon dioxide extraction of n-3 fatty acids from red seaweed."* Food Chemistry ***65**: 399 – 403*].

[*Hejazi, M. A., de Lamarlie, C., Rocha, J. M. S., Vermue, M., Tramper, J. and Wijffels, R. H. (2002)*

[*Hejazi, M. A., de Lamarlie, C., Rocha, J. M. S., Vermue, M., Tramper, J. and Wijffels, R. H. (2002). "Selective extraction of carotenoids from the microalgae Dunaliella salina with retention of viability."* Biotechnology and Bioengineering ***79(1): 29 - 36**: 29 – 36*].

(*Shahidi, F., (2006) Nutraceuticals and specialty lipids and their co-products CRS series Vol. 5. ,. Olaizola, M.* Biomol Eng. *2003 Jul; 20 (4-6):459-66*

Nathan Mosier a, Charles Wyman , Bruce Dale , Richard Elander ,Y.Y. Lee , Mark Holtzapple , Michael Ladisch *Features of promising technologies for pretreatment of lignocellulosic biomass* Biores. Tech. **96 (2005) 673–686**).

6. Algal Biofuel Conversion Technologies

Introduction (Producing "Fit for Purpose" Algal Biofuels)

Most of the preceding discussion in this roadmap has focused on making the technological advancements required to domestically produce large volumes of inexpensive, high-quality, algae-derived feedstocks that subsequently can be used to produce fuels. This step is of top priority since there is little hope for substantial displacement of imported petroleum without abundant, low-cost feedstocks. Nevertheless, the process step of converting an algal feedstock into a fuel that meets all customer requirements is not trivial and is equally essential for the successful deployment of algal biofuels.

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

The primary objective of this section is to summarize a number of potentially viable strategies for converting algal biomass into domestically produced, renewable replacements for petroleum gasoline, diesel, and jet fuel. These replacement fuels must be suitable for their applications in order to enable their widespread use. 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 section 8, Distribution and Utilization. This section 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 guiding truths became evident during the DOE's Algal Technology Roadmap Workshop in terms of addressing the conversion of algal feedstocks to fuels; these are noted here to help establish a reasonable framework for the most promising concepts identified in this roadmap.

- 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, lifecycle analysis of energy and carbon will be a key tool in selecting the preferred fuel conversion technologies from those discussed below.
- 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 also must hold true for the algae biorefineries of the future if they are to achieve significant market penetration and displace fossil fuels.

A large number of potential pathways exist for the conversion from algal biomass to fuels, and these are discussed below. The 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, 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 effectively 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 R&D opportunities are listed.

Direct Production of Biofuels from Algae

The direct production of biofuel from algal biomass has certain advantages in terms of process cost because it eliminates several process steps (e.g., extraction) and their associated costs in the overall fuel production process. These approaches are quite different from the usual algal biofuel processes that use algae to produce biological oils 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 through photosynthesis within the algae, or by feeding the algae sugar 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, which were 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. One example of this process technology links sugar production to photosynthesis with enzymes within individual algae cells. There are claims that this process may consume more than 90% of the system's CO_2 through photosynthesis, wherein the sugars are converted into ethanol (citation). The ethanol is secreted into the culture media and is collected in the headspace of the reactor 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 3-4 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 (citation). 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 also be required to produce a commercially viable organism. This appears to be the approach taken by Algenol in their efforts to commercialize ethanol production through cultivation of an engineered strain of cyanobacterium.

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. 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 (citation). 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 be easily recovered if freely secreted into the culture media and, if so desired, further processed to make a wide range of fuels.

This 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. Secondly, the growth rate of the algae can theoretically be orders of magnitude larger than traditional methods (citation). 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, indeed, dewatering is necessary.

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 to add process costs by requiring additional conditioning steps or by the concentration of biomass hydrolysate in the conversion step. Algae may prove to be more resistant to these compounds and allowing sugar conversion to occur more cheaply. Regardless of the source of sugars, however, there is limited availability and thus a zero sum game with other sugar-based biofuels. Only autotrophic algae provide an opportunity to increase the overall production of biofuels beyond that envisioned by the Renewable Fuel Standard.

Hydrogen

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

Green algae grown in photobioreactors for the production of hydrogen

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 CO2, 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 U.S.

Processing of Whole Algae

In addition to the direct production of biofuels from algae, it is also possible to process whole algae 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 consortium of algae, though at least some level of dewatering is still required. There are four major categories of conversion technologies that are capable of processing whole algae: pyrolysis, gasification, anaerobic digestion, and supercritical processing (Figure 6).

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, an alternative liquid (bio-oil) that is upgradable 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 divided feedstock is quickly heated to between 350 and 500^{o}C for less than 2 seconds. For flash pyrolysis, typical biomass must be ground into fine particles. This is one area algae have a major advantage over other biomass sources because it is already in fundamentally 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 U.S., 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).

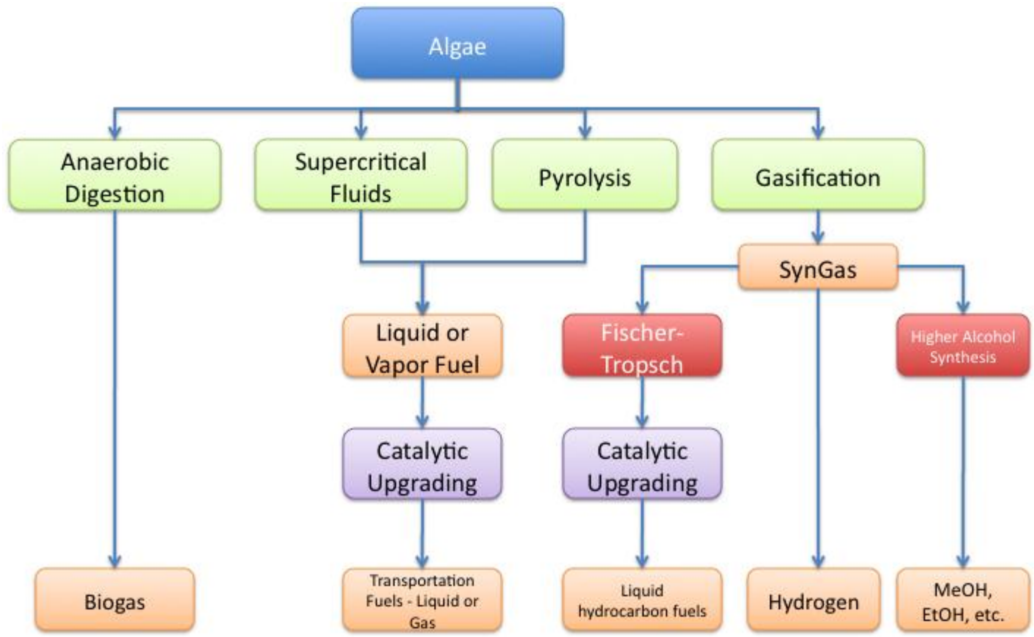


Figure 6: Schematic of the potential conversion routes for whole algae into biofuels

There are several reports on the pyrolysis of algae in the scientific literature (Demirbas, 2006; Wu and Miao, 2003).

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 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 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 on the catalytic conversion of the resulting algal bio-oil needs to be conducted. 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.,), 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 to 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 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 are high molecular weight molecules that can develop during the gasification process. The tars must be removed because they cause coking of the synthesis catalyst and any other catalysts used in the syngas cleanup process. The four basic mechanisms to deal with tar-related problems are:

- Fluidized-bed gasification + catalytic reforming
- Fluidized-bed gasification + solvent tar removal
- Fluidized-bed gasification + 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., 1994). 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 pre-treatment, but certain species of algae may not require pre-treatment 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 sources for realization of algal biofuels.

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-Fernandez 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 are not optimized for lipid production.

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. Biochemical, chemical, and supercritical transesterification processes, as well as the anaerobic digestion and fermentation process steps that can be employed are also discussed (Figure 10).

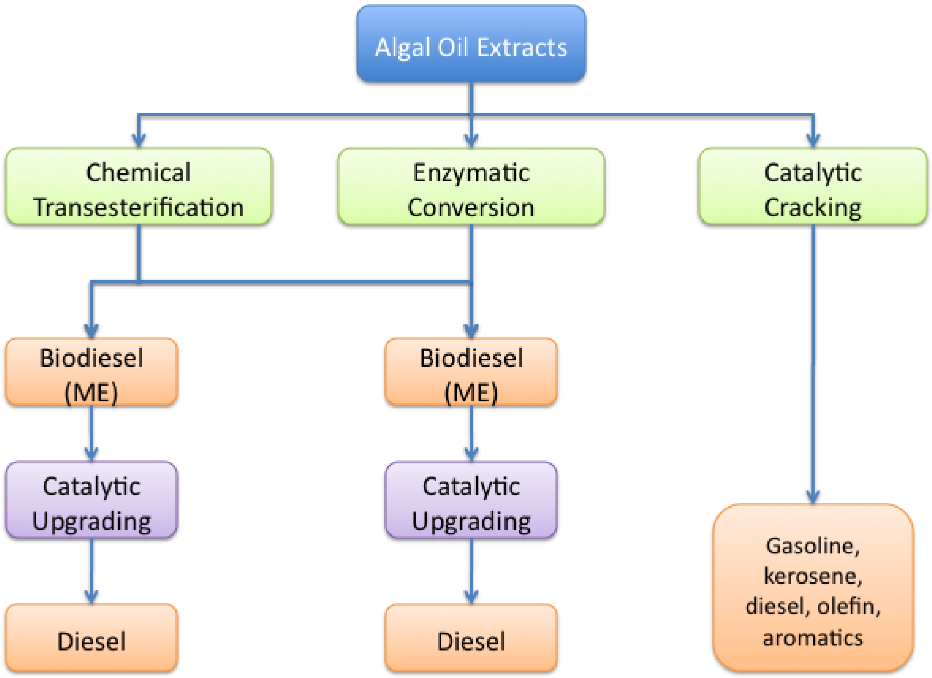


Figure 7: Schematic of the various conversion strategies of algal extracts into biofuels

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 and Boocock, 2006). The products of these reactions are typically separated by adding ether and salt water to the solution and mixing well. Finally, 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, thus enhancing product recovery (Ataya et al., 2008). 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 (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 and El Sheltawy, 2008). When the transesterification reaction is carried out in the presence of microwaves, the reaction is accelerated and requires shorter reaction times. As a result, a drastic reduction in the quantity of co-products and a short separation time are also obtained (Lertsathapornsuk et al., 2008). These preliminary results indicate that microwave processing may be cost-competitive with the more mature conversion processes currently available. In addition, catalysts may be used to enhance the impact of microwave irradiation (Yuan et al., 2009).

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., 2009). 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 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 Catalysis

Chemical processes give high conversion of triacylglycerols to their corresponding esters but have drawbacks such as being energy intensive, entail 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.

Chemical Catalysis

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, though in many cases, both types of sites could be present and it is not easy to evaluate the relative importance of the two types of sites in the overall reaction in terms of efficiency and cost. Lewis acid catalysts, such as $AlCl_3$ or $ZnCl_2$, have been proven as a viable means of converting 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 ATi_xMO, in which A represents a hydrogen atom or an alkaline metal atom, M a niobium atom or a tantalum atom, and x is an integer not greater than 7, were employed in vegetable oil transesterification. The catalysts obtained are stable and give high glycerol yield with high activities. A typical FAME yield of 91% and glycerol yield of 91% were obtained in a fixed-bed reactor at 200°C and 35 bar, using $HTiNbO_3$ as the catalyst. Vanadate metal compounds are stable, active catalysts during transesterification with $TiVO_4$ being the most active (Cozzolino et al., 2006). This catalyst is also more active than $HTiNbO_3$, producing the same yields with lower residence times. Double-metal cyanide 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 Al_2O_3.

One of the most difficult challenges is finding an ideal heterogeneous catalyst that has comparable activity in comparison to the homogenous catalyst at lower temperatures than the ones currently used (~220-240°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.

Supercritical Processing

Supercritical processing is a recent addition to the portfolio of techniques capable of simultaneously extracting and converting oils into biofuels (Demirbas, 2007). 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, 2008). 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, thus 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 (Ani 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., 2009; Vieitez et al., 2008). 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 this 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 transeterification conversion process, the overall algal biorefinery could become cost-competitive with fossil fuels. The clear immediate priority 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.

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 Section 8, gasoline, jet fuel, and diesel are 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 has 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, it 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. It is this process that provided the renewable jet fuel blends (derived from oils obtained from jatropha and algae) that have been 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 hydrotreating (basically, a chemical reductive process). Hydrotreating will convert the carboxylic acid moiety to a mixture water, carbon dioxide, or carbon monoxide, and reduce double bonds to yield hydrocarbons. Glycerin will be converted to propane which can be used for s 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 CO2 lost as well as the amount of H2 used. Refinery catalysts have also been developed to function within a certain range of chemical components 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.

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 polysachharides and other oligosaccharides present in the algal remnants that are well suited for traditional fermentation into ethanol and other biofuels.

References

Alsalme, Ali; Kozhevnikova, Elena F.; Kozhevnikov, Ivan V. Heteropoly acids as catalysts for liquid-phase esterification and transesterification. Applied Catalysis, A: General (2008), 349(1-2), 170-176.

Ani, Asnida Yanti; Abdullah, Mohd Fauzi; Ishak, Mohd Azlan Mohd; Ismail, Khudzir. Supercritical methanol extraction: a one-step process to produce biodiesel. Preprints of Symposia - American Chemical Society, Division of Fuel Chemistry (2008), 53(2), 865-866.

Anitescu, George; Deshpande, Amit; Tavlarides, Lawrence L. Integrated Technology for Supercritical Biodiesel Production and Power Cogeneration. Energy & Fuels (2008), 22(2), 1391-1399.

Armenta, Roberto E.; Vinatoru, Mircea; Burja, Adam M.; Kralovec, Jaroslav A.; Barrow, Colin J. Transesterification of fish oil to produce fatty acid ethyl esters using ultrasonic energy. Journal of the American Oil Chemists' Society (2007), 84(11), 1045-1052.

Ataya, Fadi; Dube, Marc A.; Ternan, Marten. Variables Affecting the Induction Period during Acid-Catalyzed Transesterification of Canola Oil to FAME. Energy & Fuels (2008), 22(1), 679-685.

Balat, Mustafa. Sustainable transportation fuels from biomass materials. Energy, Education, Science and Technology (2006), 17(1&2), 83-103.

Cao, Fenghua; Chen, Yang; Zhai, Fengying; Li, Jing; Wang, Jianghua; Wang, Xiaohong; Wang, Shengtian; Zhu, Weimin. Biodiesel production from high acid value waste frying oil catalyzed by superacid heteropolyacid. Biotechnology and Bioengineering (2008), 101(1), 93-100.

Cozzolino, M.; Tesser, R.; Di Serio, M.; Gaigneaux, E. M.; Eloy, P.; Santacesaria, E. Supported vanadium oxide nanoparticles: effect of preparation method, support and type of precursor on the catalytic performances in the ODH of methanol to formaldehyde. Studies in Surface Science and Catalysis (2006), 162(Scientific Bases for the Preparation of Heterogeneous Catalysts), 697-704.

Demirbas, A. Production of Biodiesel from Algae Oils. Energy Sources, Part A: Recovery, Utilization, and Environmental Effects (2009), 31(2), 163-168.

Demirbas, Ayhan. Biodiesel from waste cooking oil via base-catalytic and supercritical methanol transesterification. Energy Conversion and Management (2009), 50(4), 923-927.

Demirbas, Ayhan. Thermal degradation of fatty acids in biodiesel production by supercritical methanol. Energy Exploration & Exploitation (2007), 25(1), 63-70).

Demirbas, Ayhan. Oily products from mosses and algae via pyrolysis. Energy Sources, Part A: Recovery, Utilization, and Environmental Effects (2006), 28(10), 933-940.

Fang, Yaowei; Lu, Zhaoxin; Lv, Fengxia; Bie, Xiaomei; Liu, Shu; Ding, Zhongyang; Xu, Weifeng. A Newly Isolated Organic Solvent Tolerant Staphylococcus saprophyticus M36 Produced Organic Solvent-Stable Lipase. Current Microbiology (2006), 53(6), 510-515.

Guncheva, M.; Zhiryakova, D.; Radchenkova, N.; Kambourova, M. Acidolysis of tripalmitin with oleic acid catalyzed by a newly isolated thermostable lipase. Journal of the American Oil Chemists' Society (2008), 85(2), 129-132.

Hallgren, Anders; Andersson, Lars A.; Bjerle, Ingemar. High temperature gasification of biomass in an atmospheric entrained flow reactor. Adv. Thermochem. Biomass Convers., [Ed. Rev. Pap. Int. Conf.], 3rd (1994), Meeting Date 1993, 338-49.

Hanssen, Jon F.; Indergaard, Mentz; Oestgaard, Kjetill; Baevre, Olav Arne; Pedersen, Tor A.; Jensen, Arne. Anaerobic digestion of Laminaria spp. and Ascophyllum nodosum and application of end products. Biomass (1987), 14(1), 1-13.

Hawash, S.; Kamal, N.; Zaher, F.; Kenawi, O.; El Diwani, G. Biodiesel fuel from Jatropha oil via non-catalytic supercritical methanol transesterification. Fuel (2009), 88(3), 579-582.

Hirano, Atsushi; Samejima, Yasutaka; Hon-Nami, Koyu; Kunito, Shunji; Hirayama, Shin; Ueda, Ryohei; Ogushi, Yasuyuki. CO2 fixation and ethanol production by fermentative microalgae. Outdoor cultivation in a 50-L tubular bioreactor and co-fermentation with propionate in dark. Making a Business from Biomass in Energy, Environment, Chemicals, Fibers and Materials, Proceedings of the Biomass Conference of the Americas, 3rd, Montreal, Aug. 24-29, 1997 (1997), 2, 1069-1076.

Hirano, Atsushi; Ueda, Ryohei; Hirayama, Shin; Ogushi, Yasuyuki. CO_2 fixation and ethanol production with microalgal photosynthesis and intracellular anaerobic fermentation. Energy (Oxford) (1997), 22(2/3), 137-142.

Hirayama, S.; Ueda, R.; Ogushi, Y.; Hirano, A.; Samejima, Y.; Hon-Nami, K.; Kunito, S. Ethanol production from CO2 by fermentative microalgae. *Studies in Surface Science and Catalysis* (1998), 114 (Advances in Chemical Conversions for Mitigating CO2), 657-660.

Hon-Nami, Koyu. A unique feature of hydrogen recovery in endogenous starch-to-alcohol fermentation of the marine microalga, Chlamydomonas perigranulata. *Applied Biochemistry and Biotechnology* (2006), 129-132, 808-828.

Hossain, A. B. M. Sharif; Salleh, Aishah; Boyce, Amru Nasrulhaq; Chowdhury, Partha; Naqiuddin, Mohd. Biodiesel fuel production from algae as renewable energy. American Journal of Biochemistry and Biotechnology (2008), 4(3), 250-254.

Kalva, Abhishek; Sivasankar, Thirugnanasambandam; Moholkar, Vijayanand S. Physical Mechanism of Ultrasound-Assisted Synthesis of Biodiesel. Industrial & Engineering Chemistry Research (2009), 48(1), 534-544.

Lertsathapornsuk, V.; Pairintra, R.; Aryusuk, K.; Krisnangkura, K. Microwave assisted in continuous biodiesel production from waste frying palm oil and its performance in a 100 kW diesel generator. Fuel Processing Technology (2008), 89(12), 1330-1336.

Liao, Hui-Fen; Tsai, Wei-Chuan; Chang, Shu-Wei; Shieh, Chwen-Jen. Application of solvent engineering to optimize lipase-catalyzed 1,3-diglyacylcerols by mixture response surface methodology. Biotechnology Letters (2003), 25(21), 1857-1861.

Lopez-Hernandez, Arnoldo; Garcia, Hugo S.; Hill, Charles G., Jr. Lipase-catalyzed transesterification of medium-chain triacylglycerols and a fully hydrogenated soybean oil. Journal of Food Science (2005), 70(6), C365-C372.

McNeff, Clayton V.; McNeff, Larry C.; Yan, Bingwen; Nowlan, Daniel T.; Rasmussen, Mark; Gyberg, Arlin E.; Krohn, Brian J.; Fedie, Ronald L.; Hoye, Thomas R. A continuous catalytic system for biodiesel production. Applied Catalysis, A: General (2008), 343(1-2), 39-48.

Mendes, Rui L. Supercritical fluid extraction of active compounds from algae. Supercritical Fluid Extraction of Nutraceuticals and Bioactive Compounds (2008), 189-213.

Miao, Xiaoling; Wu, Qingyu. High yield bio-oil production from fast pyrolysis by metabolic controlling of Chlorella protothecoides. Journal of Biotechnology (2004), 110(1), 85-93.

Okabe, Kiyomi; Murata, Kazuhisa; Nakanishi, Masakazu; Ogi, Tomoko; Nurunnabi, Mohammad; Liu, Yangyong. Fischer-Tropsch Synthesis over Ru Catalysts by Using Syngas Derived from Woody Biomass. Catalysis Letters (2009), 128(1-2), 171-176.

Phillips, S. D. Technoeconomic Analysis of a Lignocellulosic Biomass Indirect Gasification Process To Make Ethanol via Mixed Alcohols Synthesis. Industrial & Engineering Chemistry Research (2007), 46(26), 8887-8897.

Raffelt, Klaus; Henrich, Edmund; Koegel, Andrea; Stahl, Ralph; Steinhardt, Joachim; Weirich, Friedhelm. The BTL2 process of biomass utilization entrained-flow gasification of pyrolyzed biomass slurries. Applied Biochemistry and Biotechnology (2006), 129-132, 153-164.

Refaat, A. A.; El Sheltawy, S. T. Time factor in microwave-enhanced biodiesel production. WSEAS Transactions on Environment and Development (2008), 4(4), 279-288.

Soriano, Nestor U., Jr.; Venditti, Richard; Argyropoulos, Dimitris S. Biodiesel synthesis via homogeneous Lewis acid-catalyzed transesterification. Fuel (2009), 88(3), 560-565.

Srinivas, S.; Malik, Ranjan K.; Mahajani, Sanjay M. Fischer-Tropsch synthesis using bio-syngas and CO2. Energy for Sustainable Development (2007), 11(4), 66-71.

Stavarache, Carmen; Vinatoru, M.; Maeda, Y.; Bandow, H. Ultrasonically driven continuous process for vegetable oil transesterification. Ultrasonics Sonochemistry (2007), 14(4), 413-417.

Svensson, Julia; Adlercreutz, Patrick. Identification of triacylglycerols in the enzymatic transesterification of rapeseed and butter oil. European Journal of Lipid Science and Technology (2008), 110(11), 1007-1013).

Su, Erzheng; Wei, Dongzhi. Improvement in lipase-catalyzed methanolysis of triacylglycerols for biodiesel production using a solvent engineering method. Journal of Molecular Catalysis B: Enzymatic (2008), 55(3-4), 118-125.

Vergara-Fernandez, Alberto; Vargas, Gisela; Alarcon, Nelson; Velasco, Antonio. Evaluation of marine algae as a source of biogas in a two-stage anaerobic reactor system. Biomass and Bioenergy (2008), 32(4), 338-344.

Vieitez, Ignacio; da Silva, Camila; Alckmin, Isabella; Borges, Gustavo R.; Corazza, Fernanda C.; Oliveira, J. Vladimir; Grompone, Maria A.; Jachmanian, Ivan. Effect of Temperature on the Continuous Synthesis of Soybean Esters under Supercritical Ethanol. Energy & Fuels (2009), 23(1), 558-563.

Vieitez, Ignacio; da Silva, Camila; Borges, Gustavo R.; Corazza, Fernanda C.; Oliveira, J. Vladimir; Grompone, Maria A.; Jachmanian, Ivan. Continuous Production of

Soybean Biodiesel in Supercritical Ethanol-Water Mixtures. Energy & Fuels (2008), 22(4), 2805-2809.

Wahlen, Bradley D.; Barney, Brett M.; Seefeldt, Lance C. Synthesis of Biodiesel from Mixed Feedstocks and Longer Chain Alcohols Using an Acid-Catalyzed Method. Energy & Fuels (2008), 22(6), 4223-4228.

Warabi, Yuichiro; Kusdiana, Dadan; Saka, Shiro. Biodiesel fuel from vegetable oil by various supercritical alcohols. Applied Biochemistry and Biotechnology (2004), 113-116 793-801.

Wu, Qingyu; Miao, Xiaoling. A renewable energy from pyrolysis of marine and freshwater algae. Recent Advances in Marine Biotechnology (2003), 9(Biomaterials and Bioprocessing), 111-125.

Xu, Leilei; Wang, Yuanhong; Yang, Xia; Yu, Xiaodan; Guo, Yihang; Clark, James H. Preparation of mesoporous polyoxometalate-tantalum pentoxide composite catalyst and its application for biodiesel production by esterification and transesterification. Green Chemistry (2008), 10(7), 746-755.

Yamane T; Iwasaki Y; Roxana R; Shimidzu N; Doisaki N Multiple intensified performance of an enzyme-catalyzed reaction in organic medium. Annals of the New York Academy of Sciences (1998), 864 171-9.

Yang, Yong; Xiang, Hongwei; Zhang, Rongle; Zhong, Bing; Li, Yongwang. A highly active and stable Fe-Mn catalyst for slurry Fischer-Tropsch synthesis. Catalysis Today (2005), 106(1-4), 170-175.

Yuan, H.; Yang, B. L.; Zhu, G. L. Synthesis of Biodiesel Using Microwave Absorption Catalysts. Energy & Fuels (2009), 23(1), 548-552.

Yung, Matthew M.; Jablonski, Whitney S.; Magrini-Bair, Kimberly A. Review of Catalytic Conditioning of Biomass-Derived Syngas. Energy & Fuels ACS ASAP.

Zhang, Bing; Wu, Qing-Yu; Sheng, Guo-Ying; Fu, Jia-Mo. Influences of bacterial degradation on gases generated by pyrolysis of heterotrophically yellowing algae. Chinese Science Bulletin (1994), 39(23), 2018-22).

Zhou, Weiyang; Boocock, D. G. B. Phase distributions of alcohol, glycerol, and catalyst in the transesterification of soybean oil. Journal of the American Oil Chemists' Society (2006), 83(12), 1047-1052.

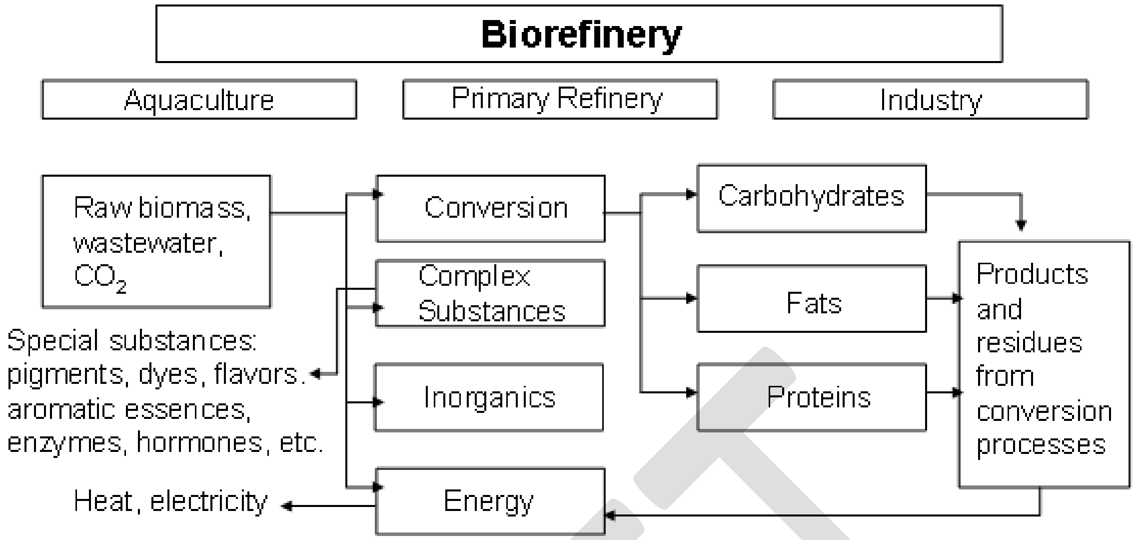
7. Co-products

Introduction

The amount of fossil-derived diesel used for transportation in the U.S. today is about 44 billion gallons/year (Source: 2007 data from U.S. Energy Information Administration; www.eia.doe.gov). Based upon calculations assuming the moderate productivity of 25 g/m2/day and 50% lipid (See Appendix) and assuming an 80% yield on biofuel from lipid, it would take 10 million acres (4 million hectares) of cultivation systems to displace this amount of fuel with algal biofuel, and it would result in the co-generation of about 190 million tons of lipid-extracted biomass per year. The "guiding truth" is that if biodiesel 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 section will address these options and discuss how relatively few of these options will not readily saturate corresponding markets in the long term.

This section will also address within the context of the biorefinery the possibility of coupling biodiesel generation 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 means. 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 Section 6); this section will focus on non-fuel co-products.



Modified from Kamm and Kamm, 2007

Figure 8: An Overview of the Biorefinery Concept

Under the biorefinery concept (Figure 8), 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 this report, 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 - 500×10^6 lbs/yr.

Commercial Products from Microalgae

A large number of different commercial products have been derived from microalgae. As summarized in Table 4, 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.

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. This situation, is anticipated to continue until 1) a sufficient number of the challenges outlined earlier in this roadmap for biofuel production will have been overcome and associated lifecycle costs 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.

i) 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 *Nosto*c; 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.

ii) 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, 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$.

iii) 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$.

iv) 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).

v) 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).

vi) 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 rather large cost.

Table 4: 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		300	Pulz&Gross (2004)
POLY-UNSATURATED FATTY ACIDS (PUFAs)			
ARA		20	Pulz&Gross (2004)
DHA	<300	1,500	Pulz&Gross (2004) Spolaore et al., (2006)
PUFA Extracts		10	Pulz&Gross (2004)
GLA			Spolaore et al., (2006)
EPA			Spolaore et al., (2006)
ANTI-OXIDANTS			
Beta-Carotene	1,200	>280	Pulz&Gross (2004) Spolaore et al., (2006)
Tocopherol CO_2 Extract		100-150	Pulz&Gross (2004)
COLORING SUBSTANCES			
Astaxanthin	< 300 (biomass)	< 150	Pulz&Gross (2004) Spolaore et al., (2006)
Phycocyanin		>10	Pulz&Gross (2004)
Phycoerythrin		>2	Pulz&Gross (2004)
FERTILIZERS/SOIL CONDITIONERS			
Fertilizers, growth promoters, soil conditioners		5,000	Pulz&Gross (2004) Metting&Pyne (1986)

Potential Options for the Recovery of Co-products

Co-products from microalgae, to be commercially viable and acceptable, must address one of these three criteria:

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 is 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 such replacements as algal proteins that can replace distiller's dried grains from corn dry grind ethanol processing. Price becomes less of an issue if the product can be labeled "organic", and thus sold for a premium.

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.

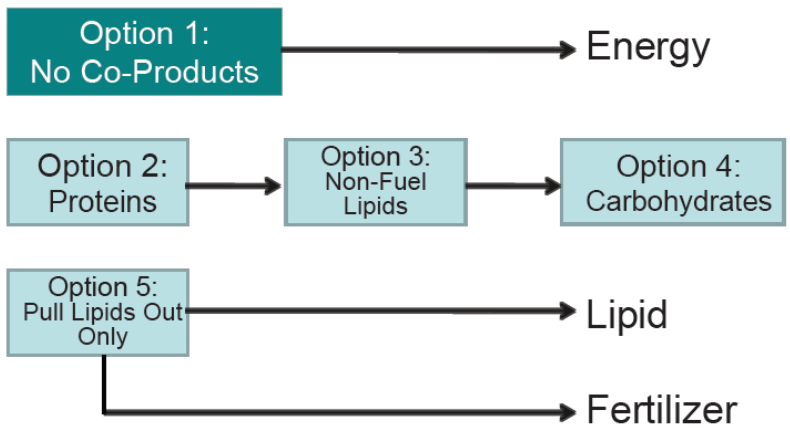


Figure 9: Overview of the five potential options for the recovery and use of co-products

As shown in Figure 9, there are at least five different options for recovering economic value from the lipid-extracted microalgal biomass. 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 the 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. As reviewed in Huesemann and Benemann (2009), anaerobic digestion of whole (i.e., non-extracted) micro and macro-algal biomass has been successfully demonstrated, with reported methane yields of about 0.3 L per gram volatile solids. 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 Section 6). 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, the thermochemical conversion of lipid-extracted biomass has the potential advantage that the resulting nitrogen-containing gases (e.g., ammonia, 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.

The R&D needs for Option 1 are as follows:

- Maximize the efficiency of the conversion of lipid-extracted biomass to energy by both anaerobic digestion and thermochemical processes. Identify appropriate catalysts and determine the optimal process conditions and the net energy balances.
- Better understand the characteristics of lipid-extracted microalgal biomass as a feedstock for thermochemical conversion and anaerobic digestion. Find out if certain species are better suited for use in these processes.
- Gain an increased understanding of nutrient recycling and recovery. Can the gaseous nutrients be directly recycled into culture ponds or is some pre-treatment needed?
- Better understand the fertilization potential of residual product. Can the residues from anaerobic digestion and the ash generated by thermochemical processes be safely used as soil fertilizers or conditioners?

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, 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 a 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.

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. It must be said that entry into the cellulase market is fraught with uncertainty based on market share for industrial enzymes controlled by a handful of companies. Desire to reduce ethanol production cost by production of enzymes on site and move towards consolidated biorprocess in which the enzymes are produced by the ethanologen eliminating the need to purchase enzymes from an external source.

The R&D needs for Option 2 are as follows:

- Better knowledge of the market for food, feed, and industrial enzymes. What is the potential market size? Who are the competitors? What are the price constraints?
- Improved understanding of the protein/enzyme extraction and recovery process. What extraction process is most effective and compatible with end-product use?
- Quality requirements for food/feed protein. What is the effect of CO_2 source (flue gas) on the quality of the protein (i.e., avoid problems of heavy metal toxicity)? Are there any other impurities in the protein fraction that could be cause of concern? What is the shelf-life of the protein? How does the type of microalgal species affect the quality of the food/feed protein, i.e., are certain species more suitable than others? To what extent are microalgal proteins assimilated by humans and animals and do they have beneficial effects? What are the regulatory requirements in terms of assuring the safety of microalgal proteins for human/animal consumption?
- Build molecular genetic tools for optimizing protein synthesis in microalgae. Can we increase the yield of the desired protein or enzyme fraction without jeopardizing lipid productivities?
- Better understanding of amino acid recycling. What tests can be done to ensure the continued quality of the protein, i.e. the amino acids?
- Assessment of additional opportunities for protein-based chemicals. Are there protein-based chemicals, e.g., glutamic acid, with large market potential other than industrial enzymes that could be recovered from the lipid-extracted microalgal biomass?

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, jetfuel 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 s for the manufacture of surfactants, bioplastics, and specialty products such as urethanes, epoxies, lubricants, etc.

The R&D needs specific to Option 3 are stated below.

- Better knowledge of the market for surfactants, biodegradable plastics, and specialty chemicals. What is the potential market size? Who are the competitors? What are the price constraints?
- Improved understanding of the fatty acid composition of microalgae used for biofuels production. What is the fatty acid composition of the non-fuel fraction? Are there any impurities that could interfere with the manufacture of the desired co-products?

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, butanol), and organic acids (formic, acetic, propionic, butyric, succinic, 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 section 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.

The R&D needs for Option 4 are as follows:

- Better knowledge of the market for fermentation-derived solvents, acids, and other specialty chemicals. What is the potential market size? Who are the competitors? What are the price constraints?
- Improved understanding of the market value of using algal carbohydrates as industrial starches vs. refined products including fuels and chemicals
- Overcome knowledge gaps related to the fermentation of microalgal sugars. What are the conversion yields and economics? What type of carbohydrate is specifically amenable to fermentation? How competitive is the process with sugars derived from agriculture (e.g., corn) or agricultural wastes? How will impurities in the biomass feedstock (from flue gases?) impact the bioconversion of algal sugars to fuels and chemicals?
- Analysis of the impact of bioconversion of sugars on the complexity of biorefinery operations? How clean does the sugar stream have to be to be suitable as a fermentation feedstock?
- Availability of bioresource support services, such as easily accessible strain collections and data resources. This is really an overarching R&D need for Option 4 to be applicable.

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.

The R&D needs for Option 5 are as follows:

- Better knowledge of the market for soil fertilizers and conditioners derived from lipid-extracted biomass residues. What is the potential market size? Who are the competitors? What are the price constraints?
- Improved understanding of the fertilization and soil conditioning potential of the residual biomass. What are the effects on soil quality? Is pretreatment needed? Is regulatory approval required?

Crosscutting Areas / Interfaces

There are a number of different interfaces with the other areas addressed in this roadmap that should be addressed. In order to determine which co-products will be valuable to a particular algal plant process, it is first necessary to have an understanding of the chosen process up until the point of lipid and co-product extraction.

One other option, that should be noted here, is that it could be feasible or more cost effective to extract the co-product from the algae first and then remove the lipids later in the process. This would need to be addressed before the process for lipid extraction is defined by the algal plant process model. For the purposes of the scope of this document, it is assumed that the lipids are the primary product and as such they would be extracted first. However, further studies founded on this perspective might be particularly useful and can appropriately be carried out by industrial entities—either alone or perhaps with government investment or partnership.

When addressing the issue of co-products, it is most important to have knowledge of algal composition and lipid extraction. A number of different products can already be produced using algae, but it is necessary to determine whether these same products can be produced from the residual biomass after lipid extraction. This issue interfaces with the issues concerning conversion technologies (i.e., transesterification or thermochemical conversion) because depending on the conversion method used, a particular co-product may not be feasible. After the composition of the algae is broken down and the lipids are extracted, the residual biomass composition (i.e., proteins, carbohydrates, and fats) may have structurally changed to the point where it is unusable for its intended purpose, for things such as animal feed or fertilizer.

This issue has been observed with the dry feedstocks and is known as “brown intractable material.” After the carbohydrates are extracted from the feedstock (corn, corn stover, etc.) for the production of ethanol, if the correct conditions are not met, then the residual biomass composition is virtually unusable for value-added co-products. This material can be burned to produce little heat, but this is highly variable depending on the material that is left. This same problem could be faced after the lipid extraction from algae and therefore, needs to be addressed.

In order to determine whether co-products are a viable option for algal plants wishing to produce them in conjunction with the production of fuel from lipids, studies must first be conducted to determine whether co-products can actually be extracted from algae after the lipids are removed, which could potentially change a great deal depending on the conversion method. It must then be determined whether the extracted co-products can be produced cost competitively. Expectations for co-product revenues should be consistent with current market size for similar co-products. Two such product analysis studies, which were conducted by the DOE on sugars and lignin, produced a list of "Top Ten Products" from sugars and from lignins (Bozell, J. J., Holladay, J. E., Johnson, D., White, J. F, 2007; Werpy, T. and G. Petersen, 2004). It is recommended that the same methodology from these two studies be used to determine a list of the top cost-competitive co-products derived from algae.

If it is possible to produce a cost-competitive co-product after removing the lipids, then issues related to siting and resources factor significantly into the discussion. The logistics related to the production and distribution of the chosen co-product(s) must be addressed in order to determine what the parameters are for scale-up production of the co-product. The stability/sustainability of the residual biomass could potentially be a barrier to the production of the co-product on a large scale, so a determination of whether the co-product can be produced, maintained, and then shipped is also a factor that needs to be addressed. After the process for the scale-up production of a co-product is determined, an assessment must then be made as to whether the co-product still remains cost competitive.

Some of the risks associated with the logistics of co-product production may be alleviated by taking advantage of the size and siting of the algae plant itself. A study should be conducted that relates to the potential benefits and advantages of the size and siting of the algal plant with regard to the residual biomass streams. If the sustainability of the produced co-product is limited, then a site close to the intended distribution might be necessary. For example, if a suitable source of animal feed with a short life span is produced, then the site of the algae plant should be located near the animals that will consume it.

There are also policy and regulatory implications and issues associated with the successful production and distribution of a cost-competitive co-product from algae. The issues of quality and safety should coincide with both the production of the co-product and the scale-up production, because if the co-product does not meet safety standards, then it will not be worthwhile to invest in its production. Health and safety codes must be maintained for any of the identified potential co-products from algae. If the residual biomass is being used for animal feed, food supplements, fertilizer, etc., then it must first be determined to be safe, whether this be by current standards of health and safety regulations for the applicable industry, or under new regulations specific to algal co-products. It is recommended that DOE, and other third parties like national labs, participate in the development of the quality standards and regulations relating to the development of algal biofuels (section 10, page 144). If biomass is produced that cannot be used as a valuable co-product, then the process for waste handling is also an area of concern that must be addressed.

The topic of co-products from algae interfaces both with the process steps before and after their extraction. It may not be possible to extract valuable, cost-competitive co-products from algae once the process for removing the lipids has occurred. If it is possible, then the issue becomes whether it is cost competitive to scale up the production of the co-product(s) after they have been removed from the residual biomass. Also, the issues relating to the quality of the co-products must be addressed in order to determine whether it is worthwhile to invest in the creating the co-product for market. These are key barriers that interface with other sections in this roadmap.

References

Benemann, J.R., "Microalgae Products and Production: An Overview", *Developments in Industrial Microbiology*, **31:**247-256, 1990 (*Journal of Industrial Microbiology*, Suppl. No. 5).

Berger, L.L. and D. L. Good. 2007. "Distillers Dried Grains Plus Solubles Utilization by Livestock and Poultry," in Corn-Based Ethanol in Illinois and the U.S. A Report from the Department of Agricultural and Consumer Economics, University of Illinois, Champaign-Urbana.

Borowitzka, M.A., "Microalgae as Sources of Fine Chemicals", *Microbiological Sciences*, **3(12):**372-375, 1986.

Bozell, J. J., Holladay, J. E., Johnson, D., White, J. F., Top Value Added Chemicals from Biomass. Volume II – Results of Screening for Potential Candidates from Biorefinery Lignin, U.S. Department of Energy, October 2007, Report PNNL-16983, available at: www.pnl.gov/main/publications/external/technical_reports/PNNL-16983.pdf.

Burja, A.M., B. Banaigs, E. Abou-Mansour, J.G. Burgess, and P.C. Wright, "Marine Cyanobacteria – A Prolific Source of Natural Products", *Tetrahedon*, **57:**9347-9377, 2001.

Chew, T.L. and S. Bhatia, "Catalytic processes towards the production of biofuels in a palm oil and oil palm biomass-based refinery", *Bioresource Technology* **99**: 7911-7922, 2008.

Choi, W.J., "Glycerol-based Biorefinery for Fuels and Chemicals", *Recent Pat. Biotechnol*. **2**: 173-180, 2008.

Datta, R., S.P. Tsai, P. Bonsignore, S.H. Moon and J.R. Frank, "Technological and Economic Potential of Poly(lactic acid) and Lactic Acid Derivatives*", FEMS Microbiological Review* **16**:221-231.

Hu, Q., M. Sommerfeld, E. Jarvis, M. Ghirardi, M. Posewitz, M. Seibert, and A. Darzins, "Microalgal Triacylglycerols as Feedstocks for Biofuel Production: Perspectives and Advances", *The Plant Journal*, **54:** 621-639, 2008.

Huesemann, M.H., and J.R. Benemann, "Biofuels from microalgae: Review of products, processes, and potential, with special focus on *Dunaliella sp*.", In: *The Alga Dunaliella: Biodiversity, Physiology, Genomics, and Biotechnology*, A. Ben-Amotz, J.E.W. Polle, and D.V. Subba Rao, editors, Science Publishers, New Hampshire, 2009.

Kamm, B. and M. Kamm, “Biorefineries – Multi Product Processes”, *Adv. Biochem. Engin./Biotechnol.* **105:**175-204, 2007.

Kawaguchi H, K. Hashimoto, K. Hirata, and K. Miyamoto, “H_2 production from algal biomass by a mixed culture of *Rhodobium marinum* A-501 and *Lactobacillus amylovorus*”, *J. Biosciences Bioengineering* **91**:277-282, 2001.

McCausland, M.A., M.R. Brown, S.M. Barrett, J.A. Diemar, and M.P. Heasman, “Evaluation of Live Microalgae and Microalgal Pastes as Supplementary Food for Juvenile Pacific Oysters (*Crassostrea gigas*)”, *Aquaculture*, **174:**323-342, 1999.

Metting, B., and J.W. Pyne, “Biologically Active Compounds from Microalgae”, *Enzyme Microbial Technology*, **8:** 386-394, 1986.

Metting, B., W.J. Zimmerman, I. Crouch, and J. van Staden, “Agronomic Uses of Seaweed and Microalgae”, In: *Introduction to Applied Phycology*, I. Akatsuka, editor, SPB Academic Publishing, The Hague, Netherlands, 1990, pp. 589-627.

Mooibroek, H., N. Oosterhuis, M. Giuseppin, M. Toonen, H. Franssen, E. Scott, J. Sanders and A. Steinbüchel, “Assessment of Technological Options and Economical Feasibility for Cyanophycin Biopolymer and High-Value Amino Acid Production”, *Applied Microbiology Biotechnology* **77**:257-267, 2007.

Olaizola, M., “Commercial Development of Microalgal Biotechnology: From the Test Tube to the Marketplace”, *Biomolecular Engineering*, **20:** 459-466, 2003.

Philip, S., T. Keshavarz, and I. Roy, “Polyhydroxyalkanoates: Biodegradable Polymers with a Range of Applications (Review)”, *Journal of Chemical Technology and Biotechnology*, **82:**233-247, 2007.

Pulz, O., and W. Gross, “Valuable Products from Biotechnology of Microalgae”, *Applied Microbiology and Biotechnology*, **65:**635-648, 2004.

Radmer, R.J., and B.C. Parker, “Commercial Applications of Algae: Opportunities and Constraints”, *Journal of Applied Phycology*, **6:**93-98, 1994.

Radmer, R.J., “Algal Diversity and Commercial Algal Products”, *BioScience*, **46(4):**263-270, 1996.

Richmond A. and Z. Cheng-Wu, “Optimization of a Plate Glass Reactor for Mass Production of *Nannochloropsis sp*. Outdoors”, *J. Biotechnol*. **85:**259-269, 2001.

Singh, S., B.N. Kate, and U.C. Banerjee, “Bioactive Compounds from Cyanobacteria and Microalgae: An Overview”, *Critical Reviews in Biotechnology*, **25:**73-95, 2005.

Spolaore, P., C. Joannis-Cassan, E. Duran, and A. Isambert, “Commercial Applications of Microalgae”, *Journal of Bioscience and Bioengineering*, **101(2):**87-96, 2006.

Werpy, T. and G. Petersen, 2004, “Top Value Added Chemicals from Biomass: Volume 1 Results of Screening for Potential Candidates from Sugars and Synthesis Gas,” National Renewable Energy Laboratory, Department of Energy (August, 2004).

Willke, T.H. and K.D. Vorlop, 2004. “Industrial Bioconversion of Renewable Resources as an Alternative to Conventional Chemistry”, *Applied Microbiology and Biotechnology* **66**, 131-142.

Wu, G.F., Q.Y. Wu, and Z.Y. Shen, “Accumulation of Poly-b-hydroxybutyrate in Cyanobacterium Synechocystis sp. PCC6803”, *Bioresource Technology*, **76:**85-90, 2001.

Yazdani, S.S. and R. Gonzalez, “Anaerobic Fermentation of Glycerol: a Path to Economic Viability for the Biofuels Industry”, *Current Opinion Biotechnology* **18**:213-219, 2007.

Yildiz, G. and F. Kadirgan, “Electrocatalytic Oxidation of Glycerol”, *J. Electrochem.Soc.* **141**:723-730, 1994.

Zerlov, V.V., O. Berezina, G.A. Velikodvorskaya, and W.H. Schwarz, “Bacterial Acetone and Butanol Production by Industrial Fermentation in the Soviet Union: Use of Hydrolyzed Agricultural Waste for Biorefinery”, *Applied Microbiology and Biotechnology*, **71:**587-597, 2006.

8. Distribution and Utilization

The final two steps for successful large-scale production of algae-derived blendstocks and their penetration into existing petroleum-fuel markets are:
1) cost-effective distribution from the point of production to refueling locations; and
2) end-use that is clearly beneficial to the customer.

In considering distribution and utilization, several different issues arise depending on the biofuels' molecules (i.e., are they in the range of C_4 to C_{10} molecules or diesel/jet fuel range hydrocarbons with C_{11} to C_{20} molecules); the fractional contribution of particular hydrocarbon species in the final fuel; and their degree of oxygenation of the fuel.

Distribution

In general, the transportation from refinery to refueling stations of non-oxygenated hydrocarbon biofuels produced from algae do not pose any unique challenges as compared to fossil-derived fuels. Fuels that are blends, which include biodiesel (FAMEs) or hydrotreated algal oils, are readily compatible with current pipeline and tanker distribution systems. It is also anticipated that gasoline range fuels that include higher alcohols, such as butanol or other advanced (synthetic) pure hydrocarbons (e.g., those derived from isoprenoids), will not require significant distribution system modifications.

In contrast, the same cannot be said for all transportation biofuels. In particular, ethanol presents several challenges with respect to distribution and blending into finished gasoline blends. Ethanol is not directly compatible with existing pipeline equipment and practices. Early trials of shipping ethanol containing gasoline blends in pipelines revealed a potential for accelerated corrosion of the pipelines. Additional tests are being conducted to learn how to avoid the potential accelerated corrosion. Further, petroleum product pipelines do not originate near the ethanol production facilities. Finally, it is well known that the ethanol in ethanol-gasoline blends can be extracted out of the gasoline phase if the blend comes in contact with an aqueous phase. This phenomenon is referred to as a low "water tolerance" and it is problematic since tanks and vessels used to store and blend petroleum products typically have an aqueous phase at the bottom of the tank/vessel. For this and other reasons discussed in this report, algae hold tremendous potential for the long-term biofuels strategy for transportation energy within the United States. And while, in the longer term (10 years), biofuels from algae present an opportunity at the greatest scale with very attractive sustainability characteristics and concurrent opportunities for both co-product development and utilization of existing petrochemical infrastructure (from refining to distribution), the longer investment timeframe required for algae fits nicely with a strategic biofuels portfolio which also includes starch ethanol now, cellulosic ethanol soon followed by other cellulosic biofuels shortly thereafter, and finally algal biofuels in the longer term but at the greatest scale.

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 section 6, Algal Biofuel Conversion Technologies, 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 to oil shales to tar sands to gasified coal to chicken fat and are thus among the stakeholders in reducing the uncertainty about the suitability of algal lipids 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, 2008a; ASTM, 2008b; and ASTM, 2008c). Failure of a fuel to comply with even one of the many allowable property ranges within the prevailing specification 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 (USEPA, 2007). In addition to meeting fuel standard specifications, algal biofuels, as with all transportation fuels, must meet Environmental Protection Agency regulations on combustion engine emissions.

The Workshop discussions on utilization issues surfaced another guiding truth that it is unreasonable to expect new specifications to 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, researchers should be continually re-evaluating the conversion process to seek 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, 2008d; and ASTM, 2008e]).

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, 2008d). 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, 1998). The primary end-use issues for plant-derived biodiesel are: lower oxidative stability than petroleum diesel, higher emissions of nitrogen oxides (NO_x), and cold-weather performance problems (Knothe, 2008). 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, 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 (e.g., [Aatola, et al., 2008]). 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 NO_x emissions. Nevertheless, unless they are heavily isomerized (i.e., transformed from straight- to branched-chain paraffins), renewable diesel and SPK will have comparable cold-weather performance problems as 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 butanols 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 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.

Research Needs

The primary research efforts required to enable optimal algae-derived blendstock utilization are relatively independent of whether oxygenates or hydrocarbons are produced. These efforts are: 1) characterization studies to quantify contaminants and end-product variability depending on the production process; 2) engine performance and emissions testing for early identification of undesired characteristics; and 3) tailoring the algal fatty-acid profile to mitigate fit-for-purpose issues and to ultimately enhance value relative to corresponding petroleum products.

References

Aatola, H., Larmi, M., Sarjovaara, T., and Mikkonen, S., "Hydrotreated Vegetable Oil (HVO) as a Renewable Diesel Fuel: Trade-off between NO_x, Particulate Emission, and Fuel Consumption of a Heavy Duty Engine," SAE Paper 2008-01-2500, *SAE Trans.* 117, Sect. 3, 2008.

ASTM International, "Standard Specification for Diesel Fuel Oils," ASTM International Report: ASTM D 975, 2008a.

ASTM International, "Standard Specification for Automotive Spark-Ignition Engine Fuel," ASTM International Report: ASTM D 4814, 2008b.

ASTM International, "Standard Specification for Aviation Turbine Fuels," ASTM International Report: ASTM D 1655, 2008c. http://www.epa.gov/otaq/consumer/fuels/mtbe/mtbe.htm.

ASTM International, "Standard Specification for Biodiesel Fuel Blend Stock (B100) for Middle Distillate Fuels," ASTM International Report: ASTM D 6751, 2008d.

ASTM International, "Standard Specification for Diesel Fuel Oil, Biodiesel Blend (B6 to B20)," ASTM International Report: ASTM D 7467, 2008e.

Graboski, M.S. and McCormick, R.L., "Combustion of Fat and Vegetable Oil Derived Fuels in Diesel Engines," *Progress in Energy and Combustion Science* **24**, pp. 125-164, 1998.

Knothe, G., "'Designer' Biodiesel: Optimizing Fatty Ester Composition to Improve Fuel Properties," *Energy & Fuels* **22**, pp. 1358-1364, 2008.

USEPA, "State Actions Banning MTBE (Statewide)," U.S. Environmental Protection Agency Report: EPA420-B-07-013, 2007. Online source,

9. Resources and Siting

Introduction

Successfully developing and scaling algal biofuels production, as with any biomass-based technology and industry, is highly dependent on siting and resources. Critical requirements, such as suitable land and climate, sustainable water resources, CO_2 and other nutrients must be appropriately aligned in terms of their geo-location, characteristics, availability, and affordability. Technical and economic success concurrent with minimal adverse environmental impact necessitates the matching of both, the siting and resource factors to the required growth conditions of the particular algae species being cultivated and the engineered growth system designs being developed and deployed.

Assessments of the resource requirements and availability for large-scale autotrophic algal cultivation were conducted during the Aquatic Species Program [e.g., Maxwell, et.al., (1985)], primarily in the Southwest region of the United States. Many of the findings of this and other earlier assessments still hold true today. Sufficient resources were identified by Maxewell, et.al. (1985) for the production of many billions of gallons of fuel, suggesting that algae have the potential to significantly impact 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 others. Figure 9-1 provides a simple high-level illustration of the major resource and environmental parameters that pertain to the inputs of climate, water, and land. These parameters are of greatest importance to siting, design, production efficiency, and costs. For each parameter, a variety of conditions may be more or less cost-effective to siting and operation of algal biomass production.

In this section an overview of the critical resources for algal growth systems, specifically climate, water, carbon dioxide, and land, is presented. This is followed by in-depth discussion of algae biomass production relative to wastewater treatment and to CO2 sequestration, both of which determine relevant siting opportunities for algal biofuel production. Analysis of current algal-based wastewater treatment techniques showing potential technical considerations for co-producing algal biofuel, such as recycling of wastewater is included. Similarly, the challenges associated with algae production from CO2 emitters are outlined.

Finally, the focus of this section is on siting and resource issues associated with algae biomass production based on autotrophic growth using energy from sunlight and the need for inorganic carbon and other key nutrients. It should be noted that heterotrophic algae that do not require light energy can be cultivated in waste treatment facilities or in closed industrial bioreactors in many locations throughout the country, and thus for the use of algae in this approach, an entirely different set of siting and resource criteria come into play. However, the affordable scale-up and successful commercial expansion via heterotrophic algae still requires an organic carbon feedstock – sugars - that ultimately links back to a photosynthetic origin (see Appendix Figure A-1). Given that the use of sugars from cane, beets, and other sugar crops or from the hydrolysis of starch grain crops retains the problematic linkage of biofuel production with competing food markets, the preferred source of sugars or other appropriate organic carbon feedstocks for use with heterotrophic algae would be based on the successful deconstruction of lignocellulosic materials given their scale-up potential. Obtaining these sugars for conversion to fuels is being undertaken and reported elsewhere (e.g., DOE 2006). For that reason, the siting and resource issues for heterotrophic algal production will not be further addressed in this section.

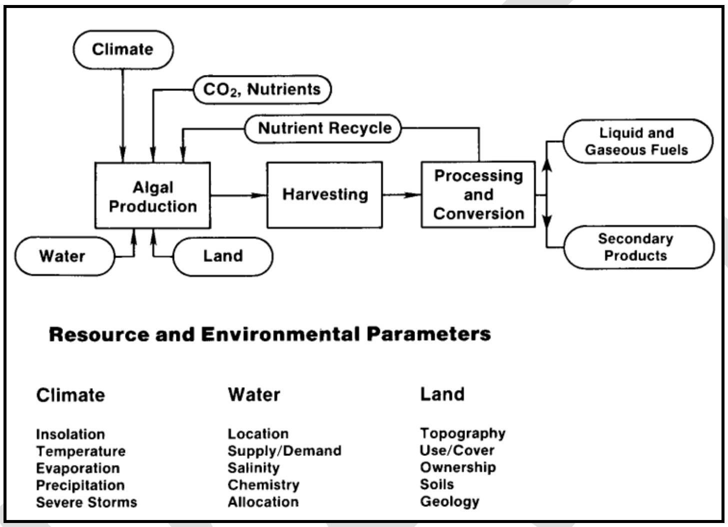


Figure 9-1. Land, water, climate, CO_2, and other nutrients represent key siting and resource elements for algae biofuel production. Additional resources include power, energy, materials, capital, labor, and other inputs associated with establishing facilities infrastructure and conducting operations and maintenance. *Source: Maxwell, et.al. (1985)*

Resources Overview

Climate

Various climate elements affect algae production. As illustrated in Figure 9-1, these include solar radiation, temperature, precipitation, evaporation, and severe weather. Closed photo-bioreactors are less sensitive to climate variability than open ponds due to the more controlled environment that closed systems can provide. Of all the key climate elements, temperature, availability of sunlight and the length of growing season will most directly affect productivity, whereas precipitation, evaporation, and severe weather will affect water supply and quality. These factors are discussed in more detail in the Appendix.

Equally important for algae growth with both open and closed cultivation systems is the availability of abundant sunlight. The majority of the country is suitable for algae production from the standpoint of having sufficiently high solar radiation (with parts of Hawaii, California, Arizona, New Mexico, Texas, and Florida being most promising).

Some northern areas, such as Minnesota, Wisconsin, Michigan, and New England would have very low productivity in the winter months. Growth of algae is technically feasible in all parts of the U.S., but the availability of adequate sunlight and the suitability of climate and temperature are key siting and resource factors that will determine economic feasibility. Additional factors could conceivably mitigate what might otherwise appear to be uneconomical resource conditions, however, this would require systems that would likely be closed and highly integrated with co-located industries providing synergistic opportunities for utilizing waste heat and energy and are thus not analyzed at length here. Such analyses would need to include assessment of the monthly or seasonal solar radiation, ambient temperature ranges, and establish minimum economically-feasible operational requirement values for the winter months.

Various species of microalgae of interest for biofuel feedstock production grow under a wide range of temperatures. High annual production for a given species, 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 open pond systems is the length of economically viable growing season for the particular strains of algae being cultivated. The analog for this with more conventional terrestrial crops is the length of time between the last killing frost in the spring and the first killing frost in the fall, although this terrestrial crop definition does not precisely apply to algae. Like terrestrial crops, however, the primary factors for determining a growing season length does correlate with latitude and altitude. Areas with relatively long growing seasons (240 days or more) are the lower elevation regions of the lower latitude states of Hawaii, Florida, and parts of Louisiana, Georgia, Texas, Arizona, and California. Thorough analysis (preferably on a state-by-state basis), supplied with detailed data is needed to assess the areas most suitable for open pond systems based on this climate factor. It is encouraging that researchers today are not only concerned with finding algae with high oil yield, but also with algae that grow well under severe climate conditions, particularly extreme temperature.

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 United States, are very desirable for algae production from the standpoint of long-term availability and sustainability of water supply. Evaporation increases water requirements for an open algae growth system, making it a critical factor to consider when choosing locations for open pond farming. Evaporation is a less important criterion for selecting locations of closed photobioreactors, although evaporative cooling is often considered as means to address increased culture temperatures associated with photobioreactors. Southwestern states and Hawaii have the highest evaporation rates in the country, with more than 60 inches annually. A thorough evaluation of this climate factor will contribute to the assessment of water requirements, implications for sustainable production scale-up, and overall economics. Severe weather events, such as heavy rain and flooding, hail storms, dust storms, tornadoes, and hurricanes pose serious concerns in regions of the Central states, Southwest, Southeast, and coastal areas. These weather events can contaminate an open pond environment or cause physical damage to both open and closed systems, and needs to be taken into account when looking at prospects for algae production in inland and coastal regions of the United States.

Water

One of the major benefits of growing algae is that, unlike terrestrial agriculture, algal culture can utilize impaired water (water with few competing uses), such as saline and brackish water, or “co-produced water” from oil, natural gas, and coal-bed methane wells. For open pond systems with high rates of evaporation, however, salinity will tend to increase over time meaning that it is likely that some non-saline make-up water will be required, or some form of desalination treatment applied, to maintain water chemistry within range limits that are suitable for algal growth. Alternatively, open algal ponds may have to periodically be drained and re-filled, or staged as a cascading sequence of increasingly saline ponds with different algae species and growth conditions, to maintain water chemistry required for successful algal cultivation. Implementing water desalination would impose additional capital, energy, and operational costs. Disposal of high salt content effluent or solid by-products, from pond drainage and replacement, or from desalination operations, also becomes an environmental problem for inland locations. Some salt by-products 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.

In 2000, total U.S. freshwater and saline-water withdrawals were estimated at 408,000 million gallons per day (Mgal/d), as shown in Figures 9-2 and 9-3. Saline water (seawater) withdrawals were about 15% of the total, as illustrated in Figure 9-3. Almost all saline water, more than 96%, is used by the thermoelectric-power industry to cool electricity-generating equipment. Naturally, the coastal states make the most use of saline water with California, Florida, and Maryland accounting for 50% of all saline water withdrawals. Saline groundwater is used by geothermal power plants in Nevada (78.7 Mgal/d), California (32.9 Mgal/d), and Utah (0.87 Mgal/d), as well as by the thermoelectric power plants in Hawaii (1,200 Mgal/d). Saline groundwater withdrawals are not included in the groundwater withdrawals shown in the graph on the right side in Figure 9-3 (USGS 2000).

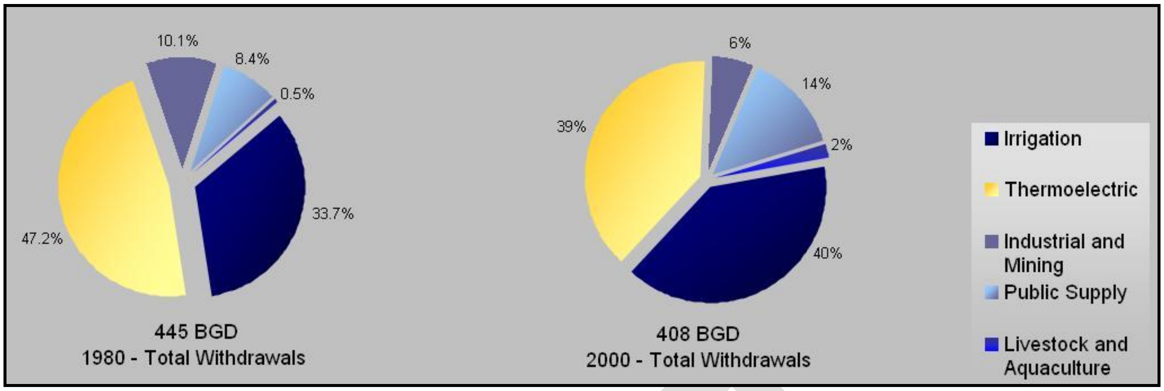


Figure 9-2. Estimated fresh water use in the United States by sector in the years 1980 and 2000. Source: Hutson, et.al. (2004).

Figure 9-3 indicates a growing withdrawal of water between 1950 and 1980. Between 1980 and 1990, the withdrawal dropped and remained fairly constant. The recent trend may indicate that fresh water sources in the U.S. is approaching full allocation as well as emphasis towards conservation. Future expansion of fresh water supplies for non-agricultural use is expected to come from the desalination of saline or brackish water sources and from the treatment and reuse of wastewater (DOE 2006b).

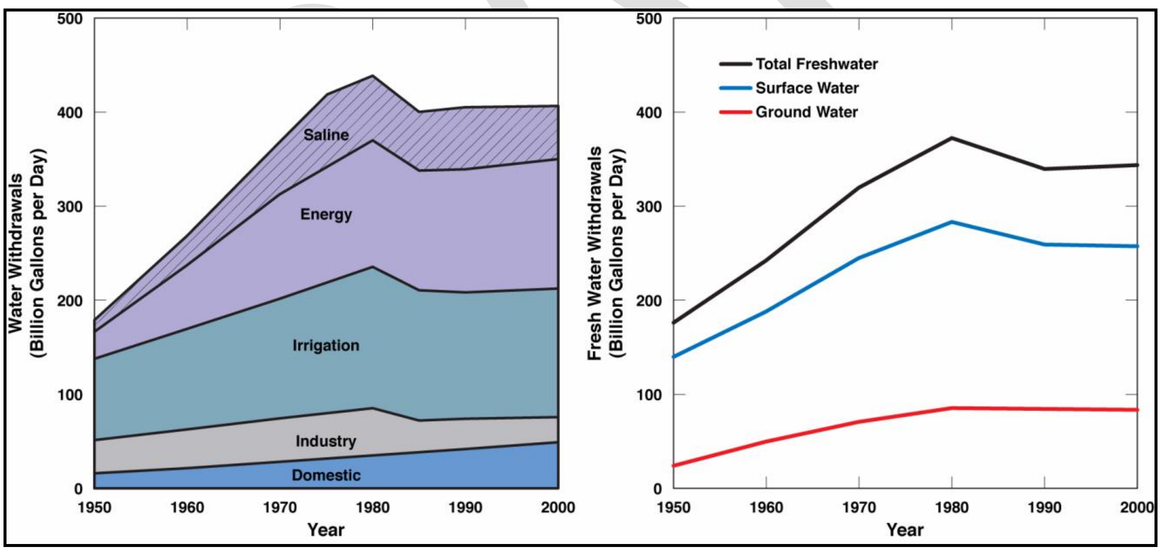


Figure 9-3. Estimated surface and ground water use in the United States during the years 1950-2000. Source: Hutson, et.al. (2004).

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; Pate, et.al., 2007; NAS 2007; Hightower, et.al, 2008). Climate change is also recognized as a factor that could affect all sectors of water resources supply and management in the future (USGS 2009). Integrating algae production with wastewater treatment, discussed later in this section, leverages water that is potentially available.

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. Data on brackish and saline groundwater resources is very outdated. 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 its extraction on the environment (Alley 2003). Saline groundwater resources, particularly deeper aquifers that are largely unregulated by state engineers and water authorities, are also increasingly being looked at as a source of water for treatment and use to meet growing needs for other industrial, commercial, and residential development in water sparse regions of the country, such as high population growth areas of 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)]. Locations closer to the surface would provide a cost effective way for algae production. The locations and depths of saline aquifers in the United States is based on data from 1965, the last time this sort of survey was taken, therefore newer and more up-to-date information needs to be collected to improve our understanding of this resource in support of more detailed algae siting analyses. Produced water from petroleum, natural gas, and coal bed methane wells is an additional underutilized water resource that can range in quality from nearly fresh to hyper-saline.

Location, depth, potential yield, recharge rate, sustainability of supply, and quality (chemical components and characteristics) are critical in assessing non-fresh groundwater aquifer resource availability and suitability for algae production. Some of this information is available for major aquifers. However, if these aquifers are spread over large geographic areas, detailed analysis is difficult. Data on small, local aquifers may be available through state agencies and private engineering companies, but a significant effort would be required to identify, collect, and analyze this information.

Water use and consumption for algae-based biofuels will clearly be dependent on the type of growth systems used (open vs. closed vs. combination) and site-specific details of climate, solar insolation, weather conditions (cloud cover, wind, humidity, etc.). Another complicating factor will be the degree of salinity of the water used for cultivation.

Beyond evaporative water loss associated with algae cultivation (See Appendix), which can be expected to be significantly reduced if closed or hybrid systems are used, it will also be important to consider water use for the overall value chain from algal cultivation through harvesting and post-processing into fuels and other products. Along the way, additional water will be used and consumed, and may well also be saved, reclaimed, and recycled, depending on systems and processes details.

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 use requirements. There is considerable untapped potential for utilizing brackish, saline, and co-produced water, and analysis and experiments are both needed to leverage those resources.

Carbon Dioxide

Optimal algae growth occurs in a CO_2 enriched environment. Dedicated algae production could provide excellent opportunity for the utilization of fossil carbon emissions and serve as a complement to subsurface sequestration.

The largest anthropogenic source of CO_2 emissions in the United States is the combustion of fossil fuels used in power generation, transportation, industrial processes, and residential and commercial buildings. About 6 billion metric tons of CO_2 are emitted annually from these sources in the United States with power generation (mainly coal) alone representing 40% of the total, or more than 2 billion metric tons per year (EIA 2008). If half of current U.S. power plant emissions, or 1-billion metric tons of CO_2 per year, could be effectively captured and used for algae biomass growth, the result could be the annual production of an estimated 200 to 600 million gallons of algal-based biofuels, as further discussed in the Systems and Techno Economic Analysis section of this report. This volume of diesel-equivalent fuel represents on the order of 50% to 150% of current U.S. use of diesel fuel for transportation.

Not all CO_2 emissions are suitable for capture and use with algae production although CO_2 could be captured from large stationary emission sources, such as power plants and industrial facilities. Table 9-1 provides more information on the major CO_2 sources in the United States. The concept of co-locating these facilities with an algae farm (discussed later in length) provides an effective approach to recycle the CO_2 into a useable liquid fuel. Applications separating CO_2 in large industrial plants, including natural gas treatment plants and ammonia production facilities, are already in operation today (Rubin 2005). Algae will only utilize CO_2 during daylight hours when photosynthesis is active with the rate of effective CO_2 uptake varying 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 that would be toxic to algae. For example, excessive amount of sulfur compounds typically found in coal-fired flue gas will be toxic to algae cultivation. Detailed analysis of industrial CO_2 emissions from point sources would provide a more refined estimate of this resource availability for algae production. Utilization of CO2 by algae is further illustrated in the Systems and Techno-Economic Analysis section of this report.

Table 9-1. Major stationary CO_2 sources in the United States [NATCARB (2008)]

CATEGORY	CO2 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**

Land

Land availability is important for algae production because either open ponds or closed systems would require relatively large areas for implementation. Land availability is influenced by many physical, social, economic, legal, and political factors. Large surface area is required for algal production systems because of the limits on available sunlight energy and the photosynthesis-based conversion efficiency for algae biomass production. Despite having higher photosynthetic efficiencies than terrestrial plants, algae will be constrained by a practical upper limit on the amount of biomass growth that can be achieved per unit of illuminated surface. Also contributing to overall limitations of productivity per unit of surface area is the fact that algal cells nearest the illuminated surface absorb the light and shade their neighbors farther from the light source. Algal productivity is measured in terms of biomass produced per day per unit of available surface area (typically in units of grams/meter2/day or tons/acre/year of dry-weight-equivalent biomass). Even at levels of productivity that would stretch the limits of an aggressive R&D program (e.g., annual average of 60 g/m2/day with 50 % oil content on a dry weight basis), such systems will require 500 acres of land to produce 10 million gal/yr of oil feedstock, as discussed further in the Systems and Techno-Economics section of this report.

To put land requirements for biofuel production in perspective, the amount of cropland that would be required to replace half of the 64 billion gallons/year of petroleum currently used in the U.S. (which includes 44 billion gallons of petroleum diesel for transportation) would require unrealistically and unsustainably large cultivation areas using conventional oilseed crops. Soybeans, with an average oil yield of about 50-gal per acre, would require a land area equivalent to approximately 1 million $miles^2$ or roughly 1.5 times the current amount of U.S. cropland (as illustrated by the larger rectangle in Figure 9-5). Based on the higher yields possible with algae, the equivalent volume of oil feedstock could potentially be produced with only 10,000 $miles^2$ of land area, as illustrated by the contrasting land footprint areas shown in the rectangles in Figure 9-4. This is illustrated further in the Systems and Techno-Economics section of this report.

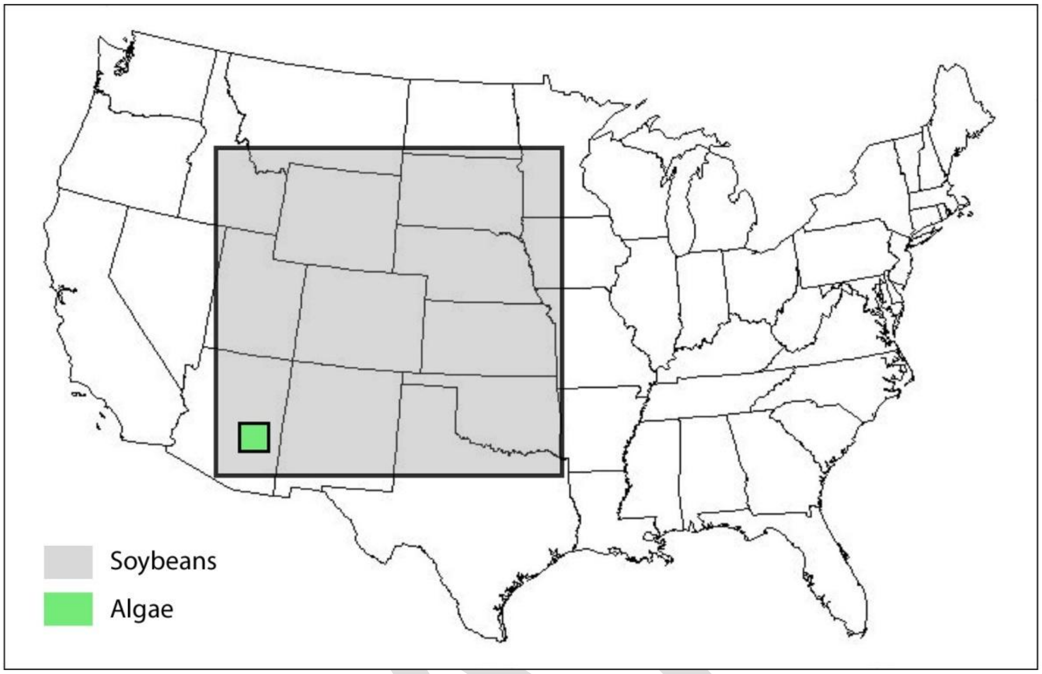


Figure 9-4. Land requirement. The amount of land required to replace 50% of the current petroleum distillate consumption using soybean (gray) and algae (green). *Adapted from Bryan, et.al. (2008)*

Millions of acres of relatively low productivity/low value land exists in the United States (USDA, 2006; USDA, 2009), including pasture, grassland, and relatively barren desert land). For a realistic appraisal of land for algae production (i.e., land that could actually be suitable and 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. 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 can be effectively eliminated from consideration for site development not only due to the intrinsic needs of the technology, but also due to the increased costs of site development. These considerations can significantly reduce the land area available for algae development. Soils, and particularly their porosity / permeability characteristics, affect the construction costs and design of open systems by virtue of the need for pond lining or sealing.

Land ownership information provides valuable insights on which policies and parties could affect project development. Publicly and privately owned lands are subject to variable use, lease, and purchase requirements. Much of the land in the West is government owned, which means that environmental assessments and/or environmental impact statements would be required as part of the approval process. Indian reservations also comprise a significant portion of this land. In effect, land ownership represents political constraints on land availability (Maxwell 1985).

Land use and land value affect the land affordability. By reviewing historical economic analyses for lipid production to date, the cost of land is either not considered or relatively small compared to other capital cost, as discussed in the Systems and Techno-Economics section of this report. Land in high demand is therefore not desirable and not targeted for algae growth. Sensitive environmental or cultural land constraints will also reduce the overall land availability [Maxwell (1985)]. Examples of this type of constraint include parks, monuments, wildlife areas, archaeological sites, and historical monuments. 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.

Integration with Water Treatment Facilities, Power Utilities, Other Industries

This subsection addresses the technical and economic challenges water and power utilities should consider with co-production of algae biomass. Both wastewater sources and industrial sources of CO_2 that could be utilized for algae production are numerous and widely distributed in the U.S. Nevertheless, most barriers to algae production by utilities are common to all potential algae producers.

Water Treatment Applications

Figure 9-5 shows national-level point sources for wastewater treatment facilities and feedlot operations. These represent the potential sites for algae operations. Two main types 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. A subset of wastewater treatment facilities is 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 below.

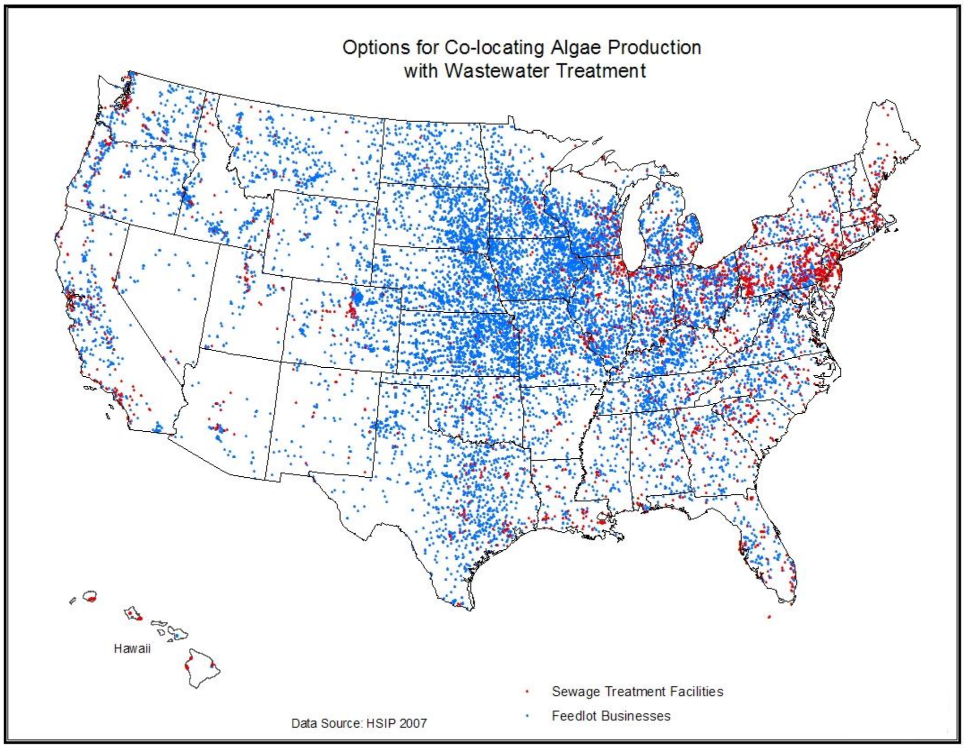


Figure 9-5. Map of major wastewater treatment facilities and confined animal feedlot operations in the United States that could provide wastewater and nutrients for co-located algae production.

Algae can be useful in the treatment of waters polluted with organic matter, excess nutrients (e.g., nitrogen, phosphorus, potassium), metals, synthetic organic compounds, and potentially endocrine disrupting compounds (Oswald 1988, Woertz et al. 2009, Aksu 1998, Borde et al. 2002). 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 U.S. (Downing et al., 2002). This cost savings, coupled with the tremendous need for expanded and improved wastewater treatment in the U.S. (USEPA 2008) and throughout the world, provides a practical opportunity to instal 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 an abundance of wastewater and waste nutrients, recylcing will be needed to have substantial impact on GHG abatement or to operate affordably and sustainably. Importation of wastes and/or wastewater will still be needed in dedicated algae treatment facilities (Brune et al., 2009) and on-site wastewater treatment would still occur as a consequence of this waste importation.

Algae Production Techniques for Water Treatment Plants

Integration of algae production with wastewater treatment is illustrated schematically in Figure 9-6. 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 feedstock production). These productive systems use shallow reactors, either high rate ponds (~30 cm) or algal turf scrubbers[1] (~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.

Biofixation of CO_2 by waste-grown algae has been demonstrated. In fact, supplementation of wastewater with CO_2 eliminates the carbon limitation that is typical in wastewater treatment ponds, resulting in accelerated treatment and nearly complete nutrient removal (Woertz et al., 2009; Fulton and Lundquist, in preparation). The use of flue gas as a CO_2 source for algae production has been successful (as discussed elsewhere in this document), but it has not been demonstrated for wastewater treatment.

As with other algae production systems, harvesting is a crucial step in wastewater treatment systems. The standard method is chemical addition for coagulation/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) is high for biofuel production (Maglion 2008). Non-chemical flocculation processes (bioflocculation and autoflocculation) are far less costly, but research is needed to improve the reliability of these processes (as discussed elsewhere in this report).

Mechanical treatment technologies have short hydraulic residence times and consequently activated sludge (the leading process) is not able to effectively treat high storm-related flows. Pond facilities with residence times of days are able to accumulate high flows, buffering their adverse effect on effluent quality and preventing the discharge of partially treated wastewater.

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 treatment mechanisms. 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

[1] The productivity of algal turf scrubbers, in particular, must be reported in terms of organic matter since the turf scrubbers entrap silt and precipitates leading to over-estimates of productivity based on total solids production.

waste organic matter, as noted in the diagram in Figure 9-6. The ability of algae to assimilate dissolved nutrients down to trace concentrations is most useful in water 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 newly discovered trace contaminants (e.g., endocrine disrupting compounds such as human hormones and antibiotics from animal facilities) is an area in need of study.

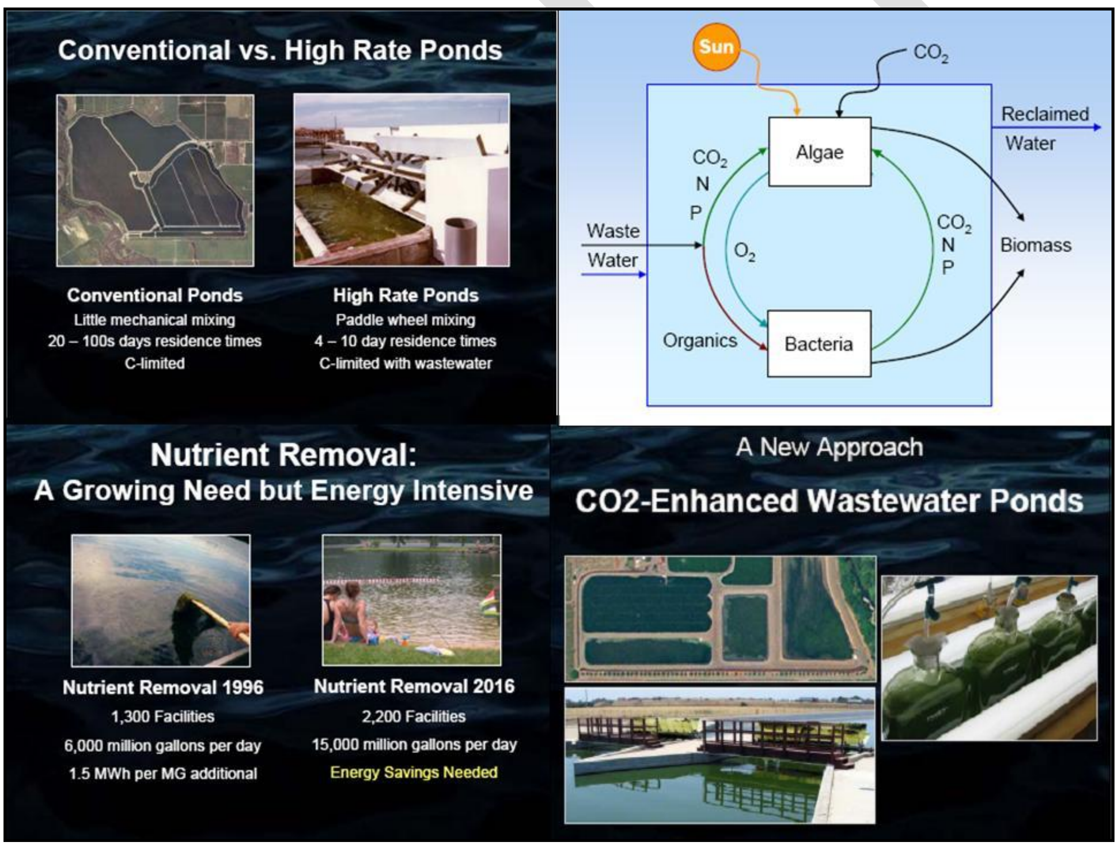


Figure 9-6. Integration of algae production with wastewater treatment for nutrient removal and biomass production (Lundquist, 2008).

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 that would occur in the receiving waters, eventually harming higher organisms, might be prevented to a 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. Metal contaminants can cause problems with themochemical processing steps for fuel production, and would almost certainly need to be removed prior to some forms of fuel processing. These potentials should be investigated, as they would be a significant advantage for the algae-producing technologies.

Treatment of Inorganic Wastewaters for Algae Production

In addition to the ability of algae systems to treat organic-rich wastewaters, their ability to treat organic-depleted but otherwise nutrient-rich wastewaters such as agricultural drainage or eutrophic water bodies (e.g., Salton Sea, Calif.) will expand the opportunities for algae production systems. 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 organic-depleted wastewaters, CO_2 addition or atmospheric absorption is essential since inorganic carbon generation from decomposition of organic matter is not 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 part of the evaporation process thereby creating 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.

Finally, algae cultivation in evaporation ponds would create a product in conjunction with the water disposal service. Ponds are used for evaporative disposal in closed hydrologic basins or where saline waters cannot be discharged to receiving waters due to regulatory salinity limits. Algae production could be quite high in the early, less-saline stages of an evaporation pond system.

Summary of 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
- 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
- Potential for complete nutrient recycling
- Potential to be integrated with power plant or other CO_2 emitting industry operations

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

This subsection includes findings from discussions held at the DOE Algae Biofuels Roadmap Workshop break-out sessions, and additional input sought from 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 the recommended role of the federal government. Findings from these interviews and conference calls were integrated with the workshop inputs in developing this subsection.

A particularly promising aspect of algal cultivation for production of biofuels is the ability of algae to metabolize CO_2 and store carbon released from fossil-fuel burning power plants and other CO_2-emitting industrial sources. This provides both a source of carbon for enhance algal growth, and a means for capturing CO_2 before it is released to the atmosphere. This combination of potential net greenhouse gas (GHG) emissions reduction through enhanced algal growth for production of biofuels makes co-location of algal cultivation with industrial CO_2 sources a promising area for further research.

While the information in this subsection 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 may ultimately be reused and re-released to the atmosphere when algal-derived fuels are used for transportation. While re-use of the carbon 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.

Figure 9-7 illustrates the distribution of various types of industrial CO_2 sources in the United States. A quantitative breakdown is also listed in Table 9-1. Stationary industrial sources of CO_2 are widely distributed throughout the United States. Table 9-1 notes that fossil-fired power plants represent the majority of CO2 emissions from stationary sources. A number of large coal-burning power plants in the southern tier of states provide ample sources for algal growth on a large scale. Figure 9-8 illustrates the concept of utilizing power plant flue gas for algae production. To put the nationwide CO_2 resource from stationary emitter sources into perspective, capturing around 20% of the 6 Gt of CO_2 released into the atmosphere from stationary sources by algae for conversion to fuels would be enough to replace nearly all of the distillate fuels used annually in the United States (further discussion in the Systems and Techno-Economic Analysis section of this report). This is based upon an estimated 300 pounds of algal oil per ton of CO_2 consumed during algal biomass production (at 30% lipid algal content by weight), which at about 7.7 lbs/gallon yields about 40 gallons per ton; or 40 billion gallons per Gt of CO_2. Thus, while it will not be practical to use algal cultivation to absorb all CO_2 emissions from US stationary sources, the CO_2 resources available can yield very large quantities of algal oils and ultimately transportation fuels.

Results of discussions at the workshop break-out sessions and subsequent discussions with EPRI and several electric utility companies identified a number of advantages and barriers to co-location of algal cultivation facilities with industrial CO_2 sources, as well as recommendations on areas for research and regulatory/policy evaluations. An overriding theme of the discussions 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 will 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.

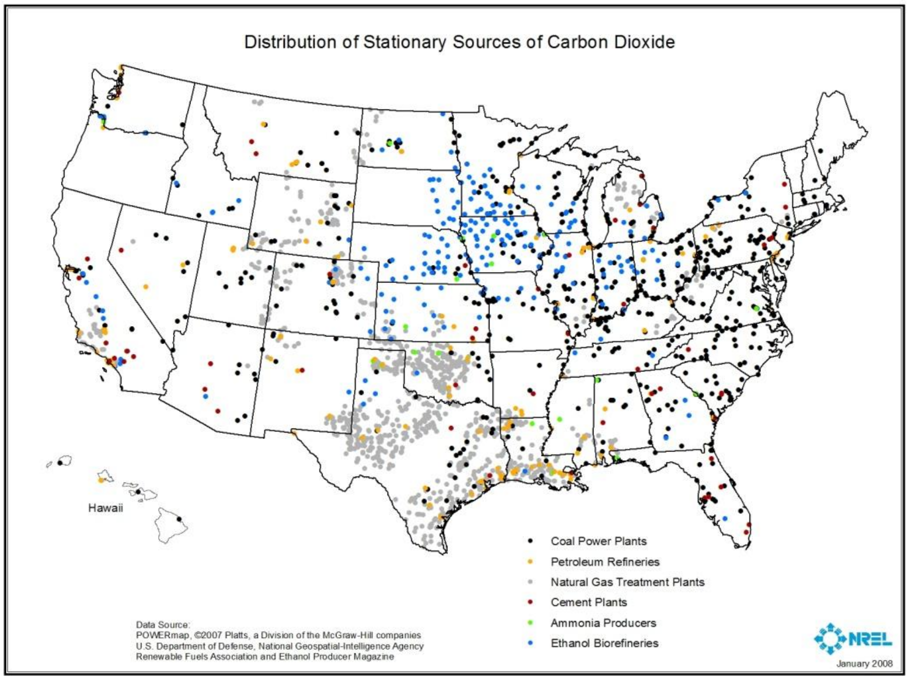


Figure 9-7. Select Large Stationary Sources of CO_2

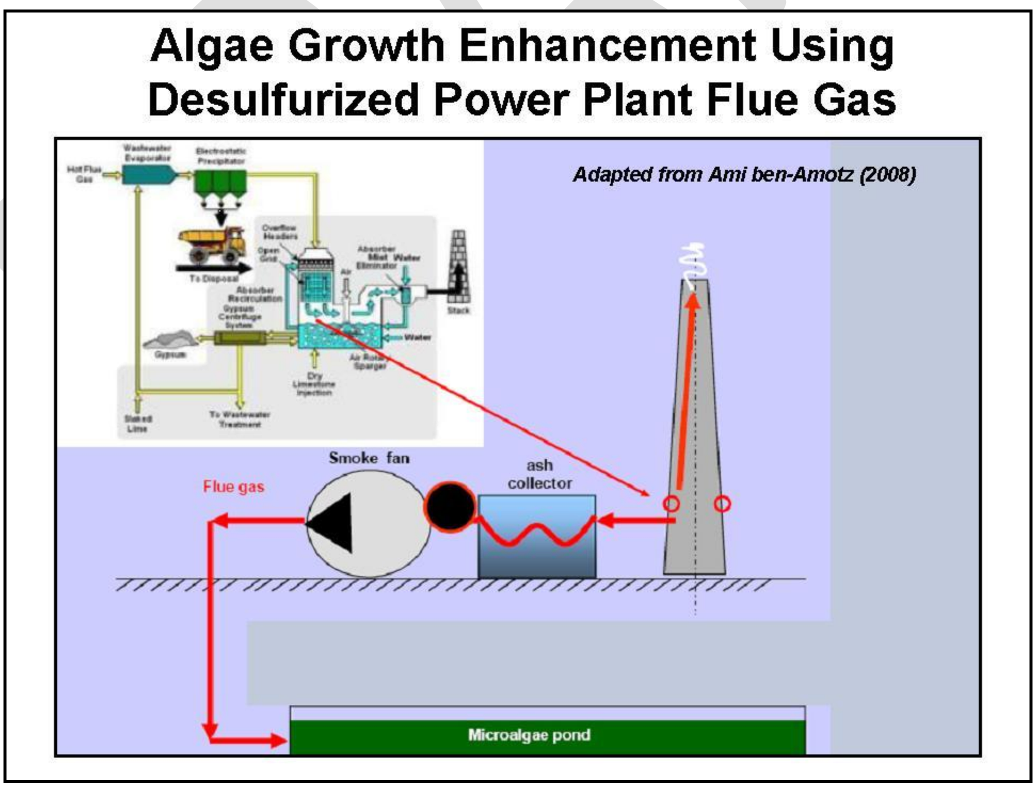


Figure 9-8. Illustration of integration of algae cultivation with electric power generation for enhanced algal biomass growth using desulfurized fossil-fired power plant flue gas (adapted from ben-Amotz 2008).

Furthermore, research efforts and policy evaluations will need to focus on both carbon capture and biofuels/co-product production to overcome technical and economic barriers (technical, regulatory and economic) for algae facilities that are co-located with electric utilities and other industrial CO_2 sources. Identified advantages, barriers, and recommended areas for further research and policy evaluation are summarized below.

Advantages of Co-location of Algae Production with Stationary Industrial CO_2 Sources

The following is a summary of the potential advantages of co-locating algal cultivation facilities with stationary industrial CO_2 sources:

- Availability of abundant CO_2 to stimulate algal growth at low cost –a fraction of the CO_2 released by US industrial sources could be converted to enough fuel to displace our current diesel use.
- Excess heat available to heat algae ponds as required at minimal cost – This will allow development of algal cultivation facilities in virtually any region of the US on a year-round basis.
- Power plants are often located near abundant non-potable water supplies, and excess wastewater or cooling water may be available – This may help overcome one of the primary resource challenges for algae cultivation at scale and provide beneficial re-use of cooling water and wastewater.
- Potential carbon credit for utilities – This will require establishing a US policy on carbon absorption and re-use as transportation fuel in lieu of permanent sequestration.

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

- Need for nutrient sources – While stationary CO_2 sources provide ample carbon for algal growth, in most cases there will not be a complementary nutrient 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.
- Regulatory framework for carbon-capture credits is not clear – 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.
- 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, which will require new regulatory policies.

- Parasitic losses from power required to deliver CO_2 to ponds and grow/harvest algae – We need to evaluate these losses, minimize them, and compare them to other approaches to carbon sequestration
- 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. Also, CO_2 is only absorbed during periods when sunlight is available and photosynthesis is active in the algae.
- Maintaining algal cultivation facilities during utility outages and through seasonal variability 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.
- Electric utilities are not in the fuels business – These regulated PUCs will be constrained in entering nines areas, and 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.

Recommended Areas for Research and Policy Evaluations

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 are some specific recommendations:

- 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 (5-10 acres). The testbeds would be located at power plants, wastewater treatment facilities, ethanol plants or other CO2 emitting industry facilities, and agricultural drainage/water body restoration sites. This effort could involve a consortium of R&D organizations, universities, algal cultivation companies, algal technology companies, refiners, distributors, and other participants coordinated by DOE at the national level. Specific testbed R&D topics include:
    - Technology evaluation
    - Determination of algae production facility model parameters

  - Flue gas CO_2 absorption/biofixation efficiency given seasonal and diel variations in photosynthesis and various water chemistries
  - Control of algal biomass quality (ratios of lipids:proteins:carbohydrates & 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
- Establish Government policies and regulations regarding biofixation of CO_2 for biofuels as opposed to geologic sequestration
- 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.

Conclusions and Recommendations

Siting and resource issues for algal biofuels scale-up are dominated by land use, water supplies, nutrient supplies, required energy inputs, and related regulatory policies. The recommendations made in this overall section place emphasis on areas that overlap strongly with the mission space of DOE. Discussion and findings pertaining to siting and resource issues include the recognition that adequate land, CO_2, water, and sunlight appear to exist at numerous locations throughout the United States where algal biomass cultivation could be undertaken and could potentially generate significant volumes of biofuel. Emphasis has been placed here primarily on the photoautotrophic approach. The heterotrophic approach using organic carbon sources without the need for light energy is acknowledged, but not addressed in detail.

Siting and resource requirements for land, water, CO_2 and other nutrients, sunlight, and other resource inputs will depend on the algal biology and cultivation systems approaches used and their productivity. Improved siting and resource assessments for algal biofuel scale-up will require more detailed biological and system performance metrics and data. However, the technologies and processes associated with algal biomass production for biofuels remains immature, include numerous potential pathways for implementation, and currently lack the needed establishment of detailed requirements for siting & input resource utilization. The ability to successfully and affordably scale-up algal biofuel production, and the associated siting and resource needs and consequences, will thus clearly depend on future progress made in addressing numerous other technical and economic performance issues tied to the biology, technologies, systems, and processes discussed elsewhere in this report.

Specific recommendations related to Siting and Resource issues where DOE mission interests and technologies are most relevant were discussed earlier in detail, and are summarized below:

- Provide or enable development of objective information, data, and technical and economic assessments critical to the establishment of siting and resource requirements for algal biomass and biofuel production
- Enable or facilitate assessment and characterization of non-fresh water resources and their suitability for growing algae and impact on operations
- R&D investment in assessments and technology development tied to improved CO_2 and nutrient sourcing, utilization, and reuse integrated with algal biomass production;
- R&D investment in technologies, systems, and processes requirements and designs matched to various siting and resources availability options;
- R&D investment in assessing specific technologies, systems, and processes appropriate to:
    - Integration with wastewater treatment and/or CO_2 emitter industries
    - Smaller scale, distributed vs. larger scale centralized options
    - Inland vs. coastal vs. off-shore marine options
    - Synergistic co-location and integration of algal biofuels & co-products with other product and service industries and their market infrastructures
    - Addressing salt management, energy balance, water & nutrient reuse, and thermal management (or lack thereof) associated with the algae growth and processing systems that impact on siting and resource requirements through
        - reduced water loss algae production systems and processes
        - lower energy-intensity water desalination technology & systems
        - innovative systems integration for improved use of waste heat and overall thermal management
    - Leverage and application of eco-system management techniques, resources and skills to the siting & resource utilization aspects of algal biomass and biofuel production
- Strategic partnering with other agencies, industry, and environmental stakeholder communities to establish constituency for algae R&D and applications development
    - Joint Studies / Assessments
    - Pilot projects
    - Educational outreach and human resource development
- Develop and disseminate objective authoritative information for other agencies, stakeholders and general public

Section 9 Appendix – Additional Figures

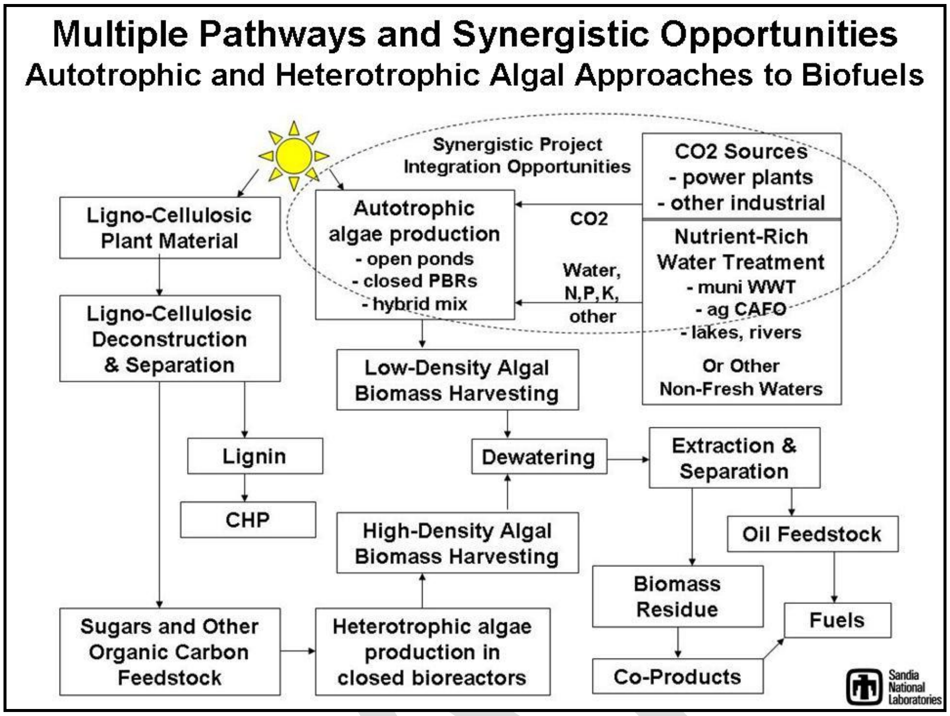


Figure A-1. Autotrophic and heterotrophic paths to algal biofuels have different siting and resource input implications and synergistic integration opportunities.Emphasis in Siting & Resources Section is on the autotrophic algae approach.

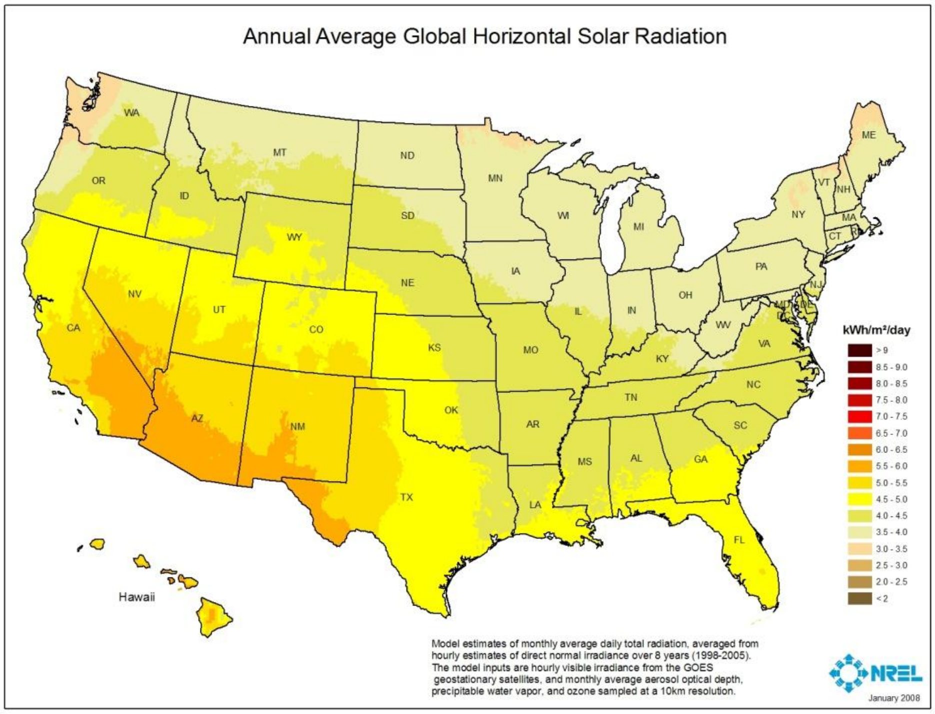


Figure A-2. Annual average solar radiation

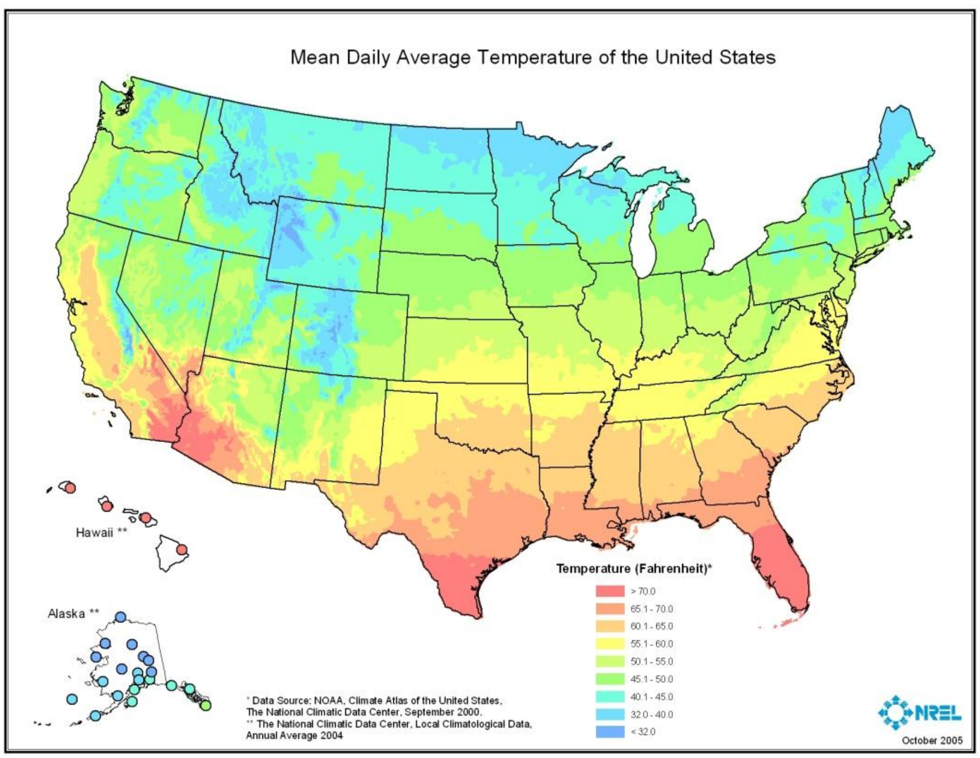


Figure A-3. Mean daily average surface temperature

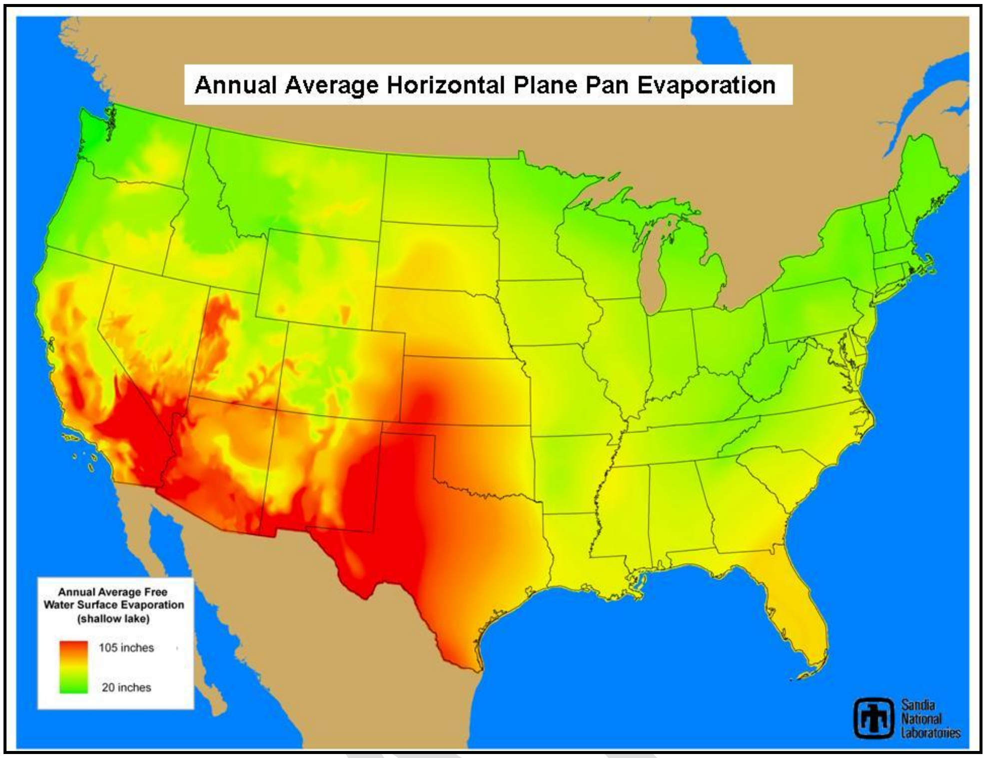


Figure A-4. Map of horizontal plane pan water evaporation (an approximate measure of the water loss that can be expected from open pond algae production)

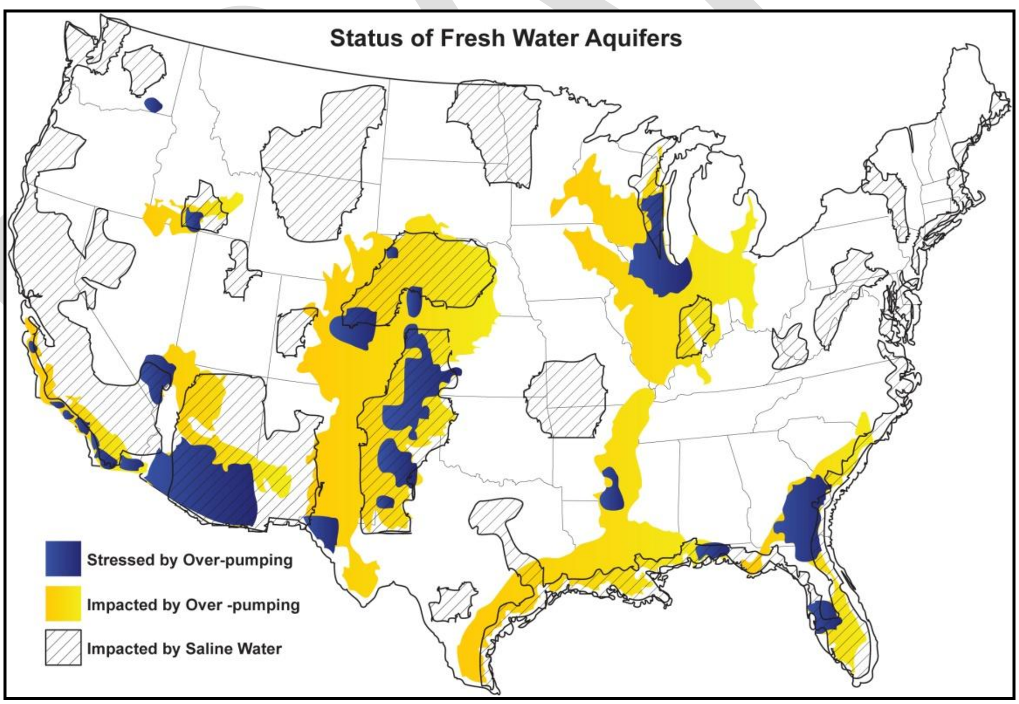


Figure A-5. Fresh water aquifers impacted by over pumping and water quality concerns (Shannon, 2006)

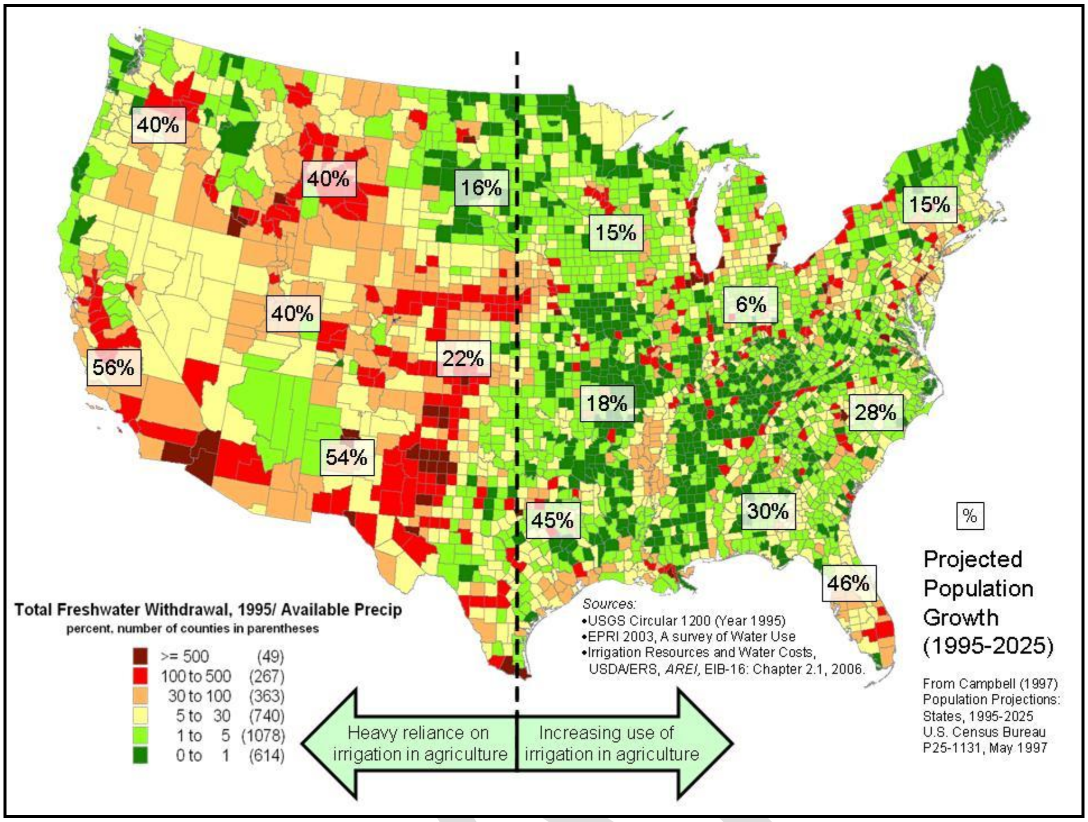


Figure A-6. Emerging fresh water resources stress and projected population growth in the United States (DOE 2006b; Pate, et.al., 2007; Hightower, et.al, 2008).

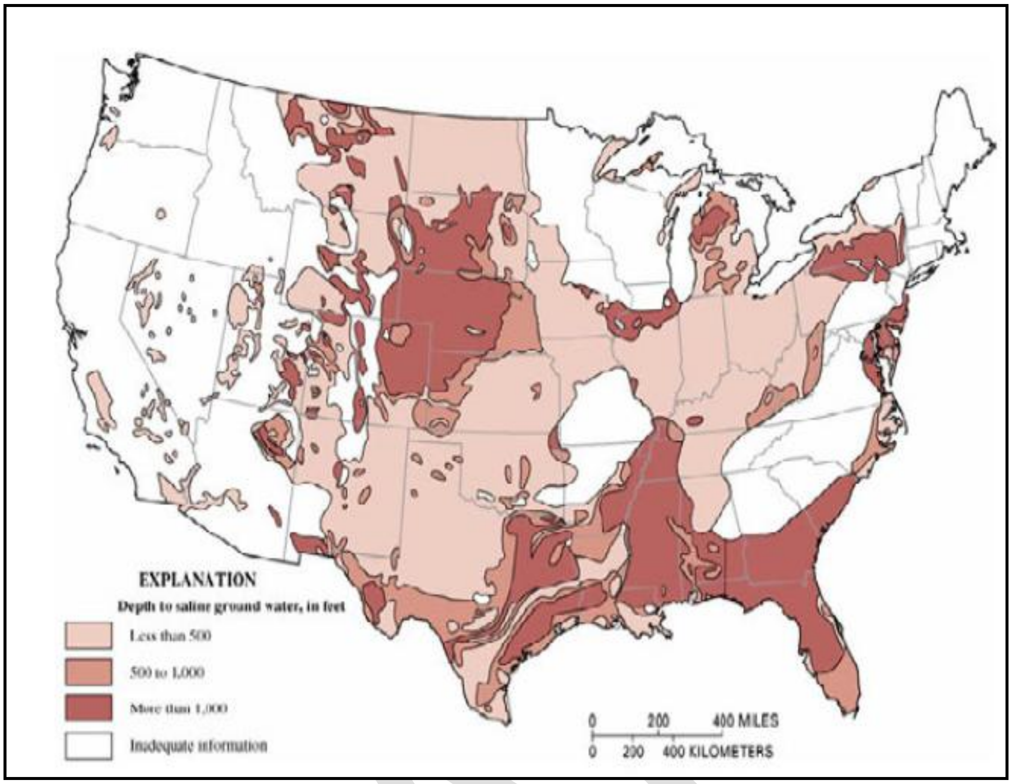


Figure A-7. Depth to saline groundwater resources *(Feth et al., 1965)*

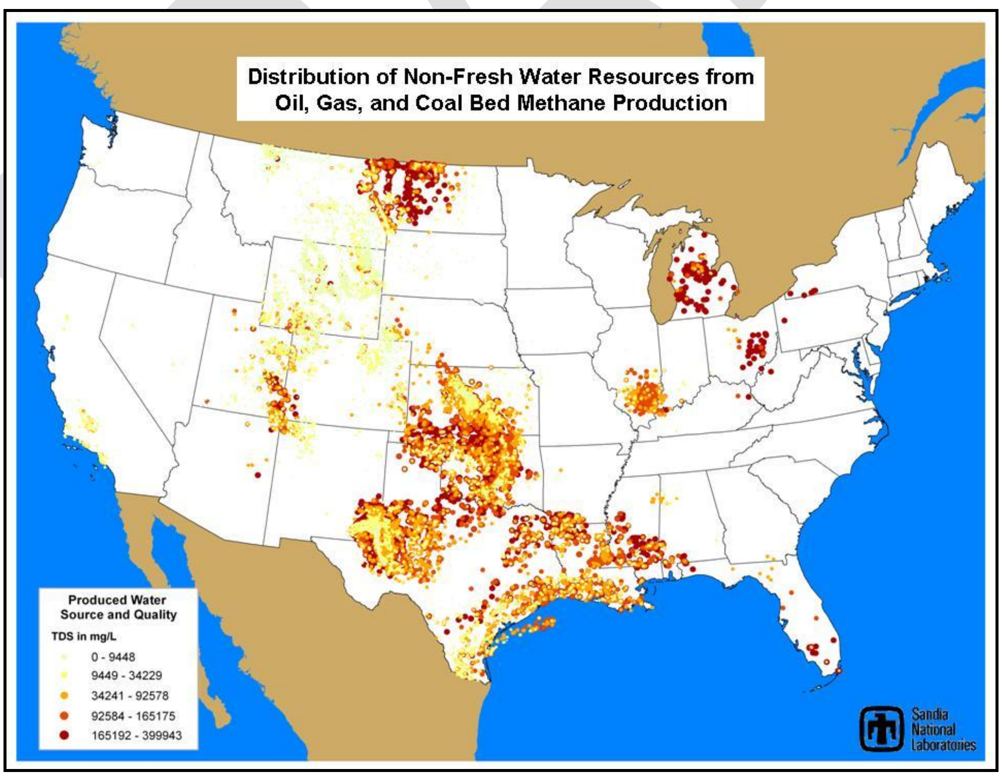


Figure A-8. Map of produced water resources from energy mineral extraction

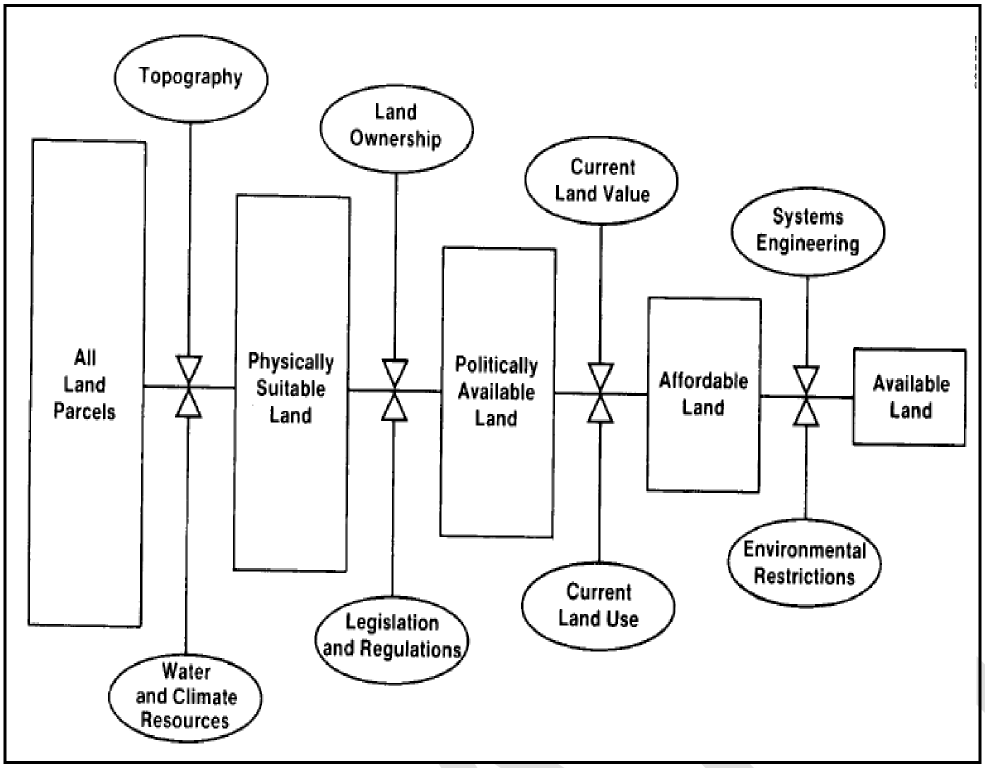


Figure A-9. Process of evaluating and constraining land available for algae production
Source: Maxwell, et.al., (1985)

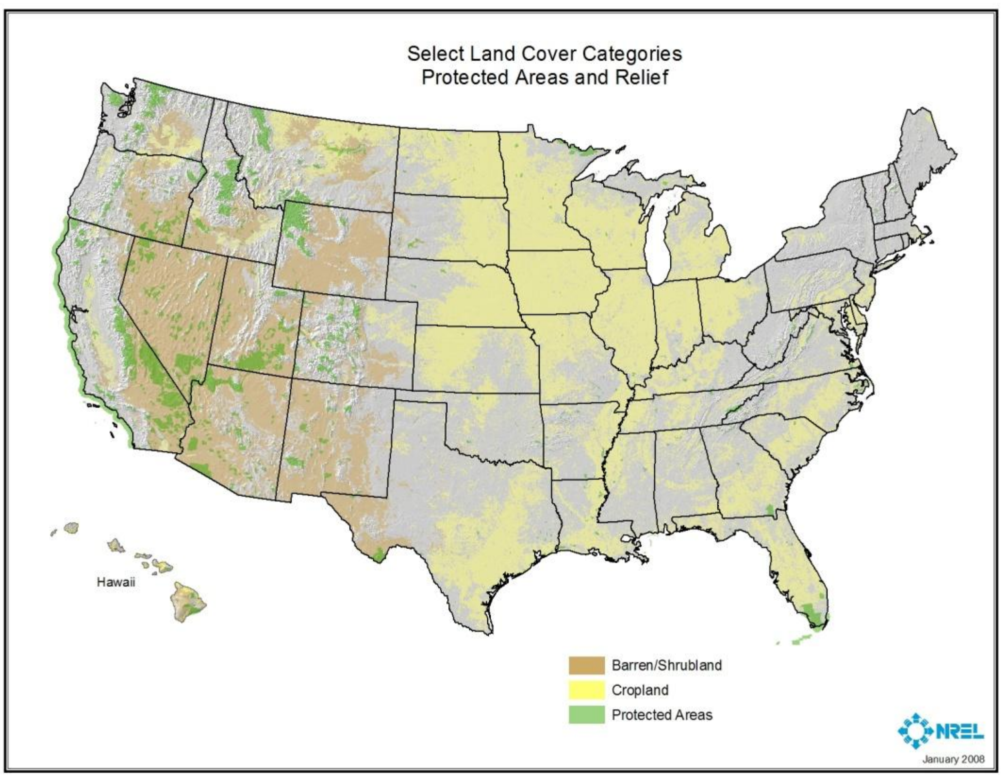


Figure A-10. Select land cover categories, protected areas, and relief

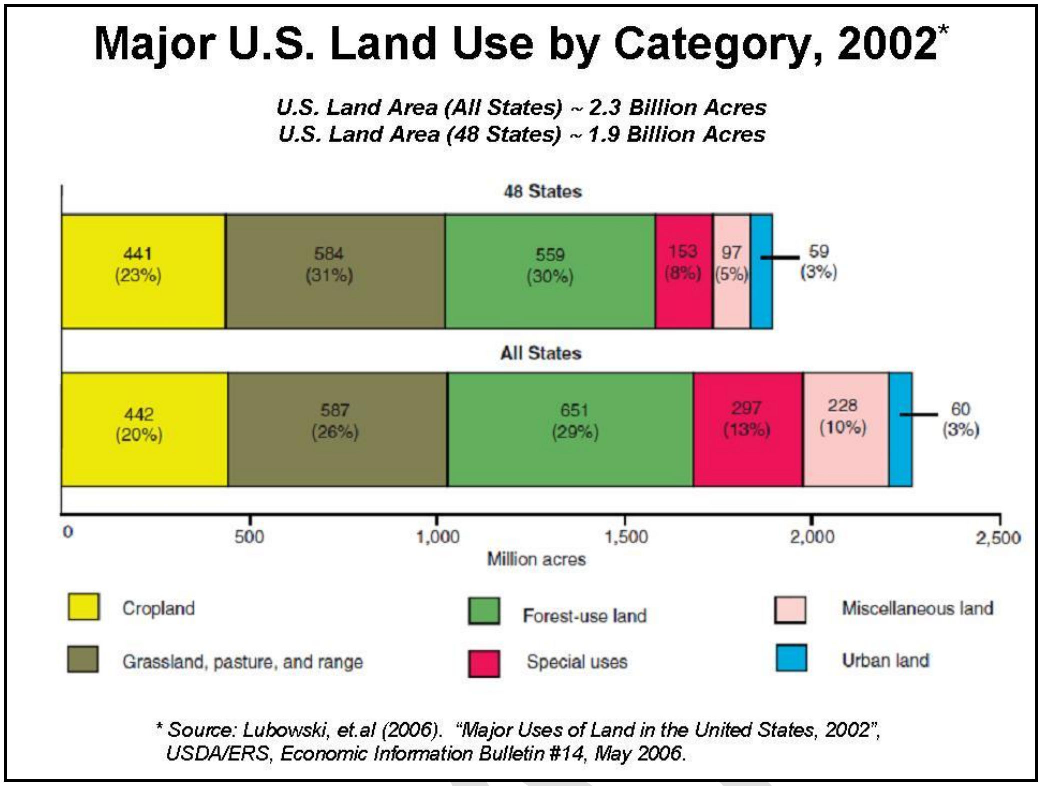


Figure A-11. Land use by category in the U.S. (USDA, 2006).

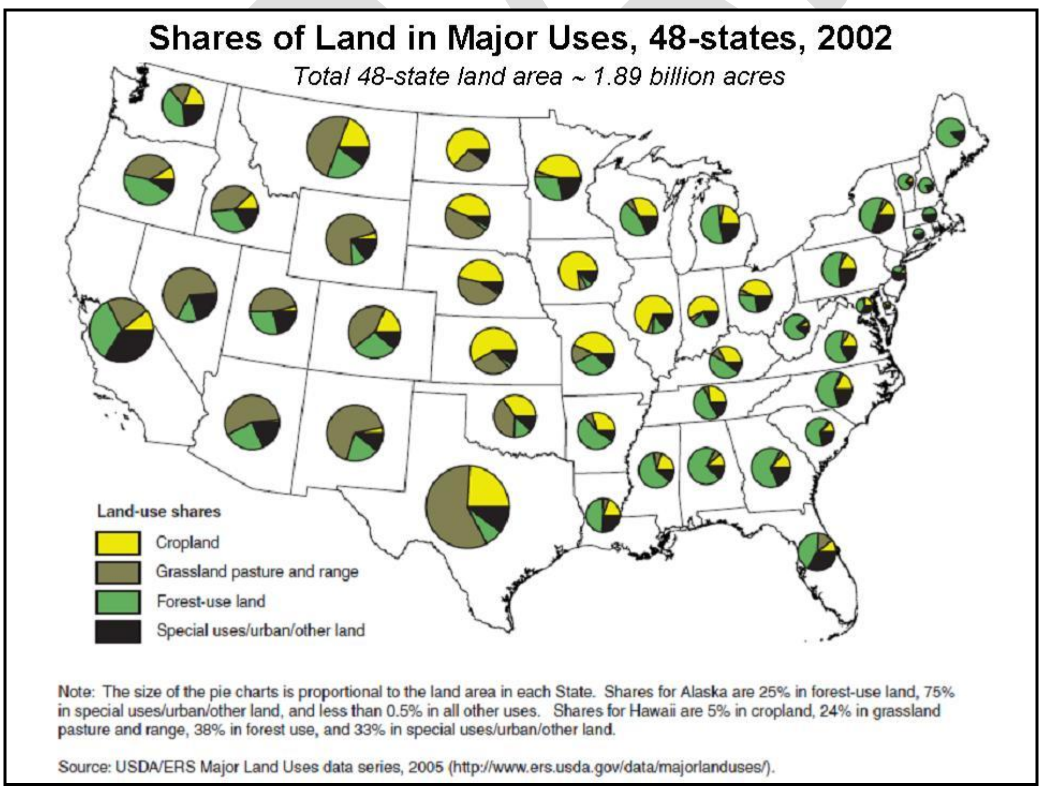


Figure A-12. State distribution of land use by category (USDA, 2006).

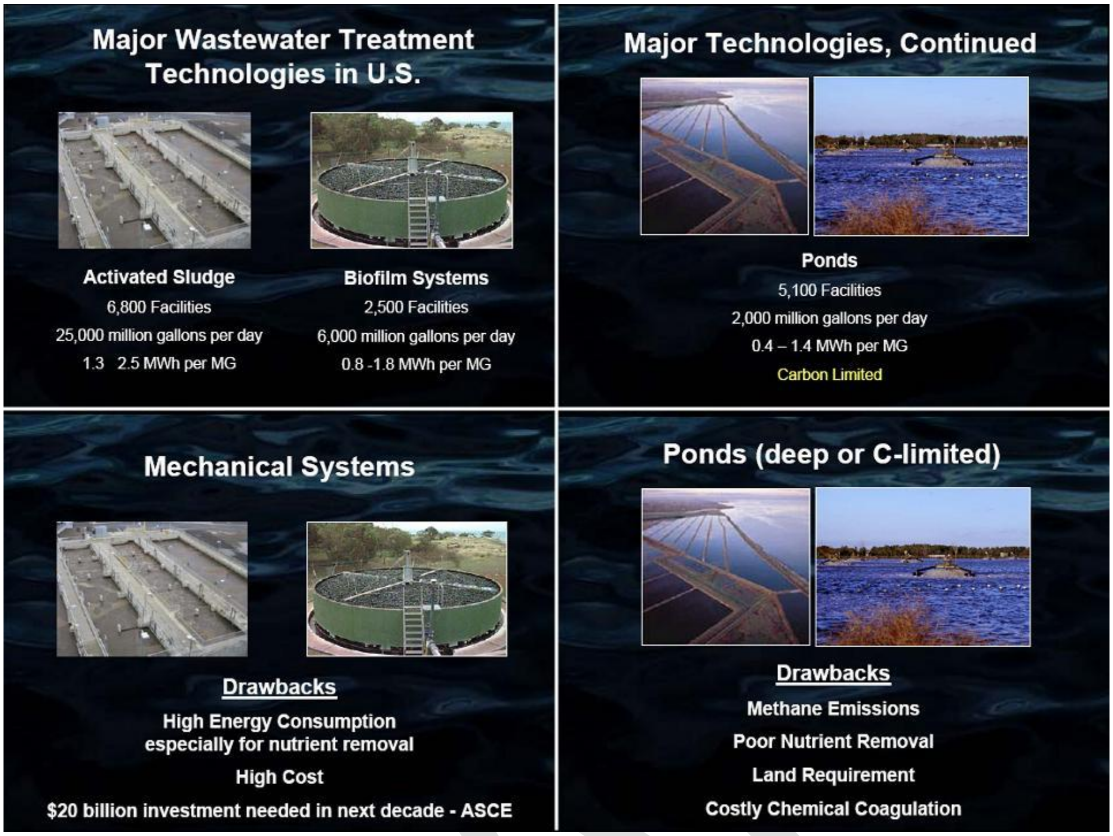


Figure A-13. Major wastewater treatment technologies currently used in the U.S., along with their drawbacks (Lundquist, 2008).

Figure A-14. Typical algae harvesting options with wastewater treatment (Lundquist, 2008).

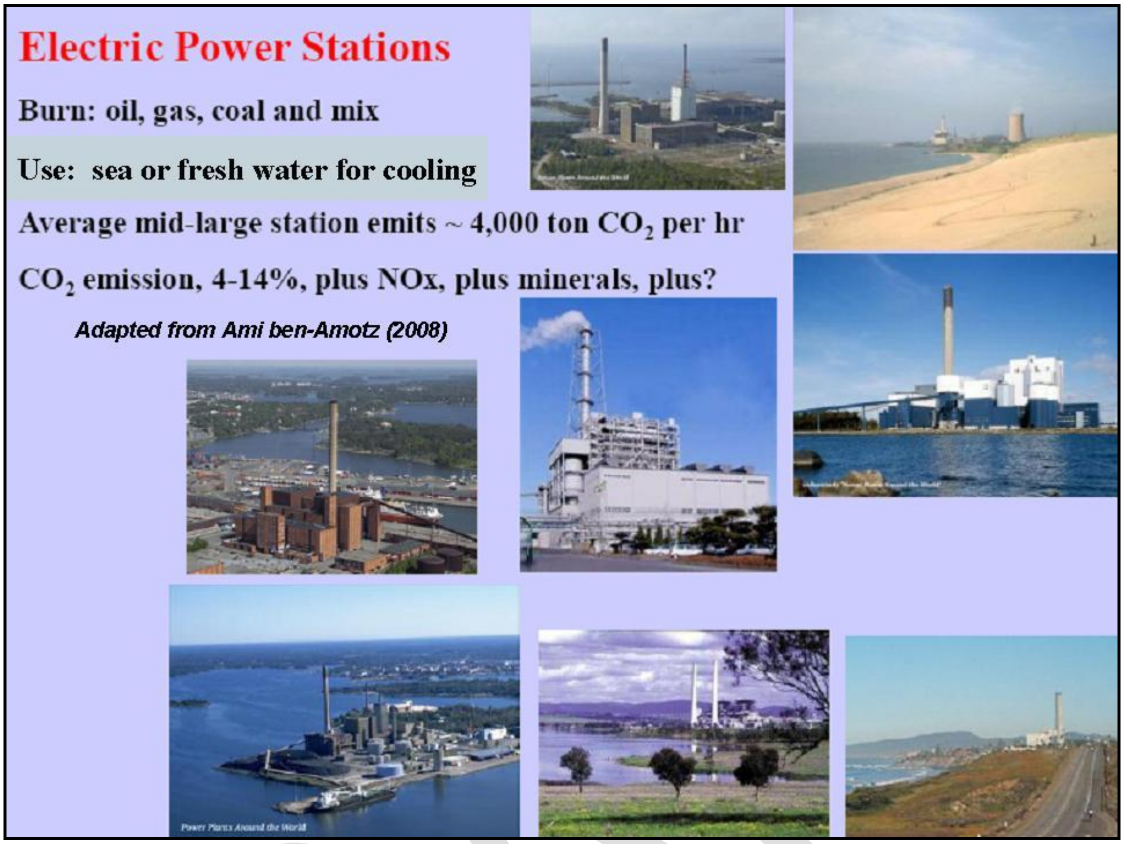


Figure A-15. Examples of fossil-fired power plants that represent stationary point sources of CO2 that could be utilized to enhance algae growth while capturing and re-using a portion of the fossil carbon emissions (adapted from ben-Amotz, 2008).

10. Corresponding Standards, Regulation, and Policy

Introduction

Two separate breakout sessions on standards, regulation, and policy were held at the Workshop, indicating the importance of these topics for the successful commercialization of algal biofuels. These sessions were attended by algal biofuel companies, academia, service providers, biofuel end users, national labs, state and federal regulatory agencies, environmental groups, and DOE.

It was widely understood that these topics were essential for the successful “birth” of a new 21st century form of agriculture – cultivation of algae at scale, built on the foundation of biotechnology and industrial microbiology rather than agronomy. Perhaps because of this foundation, the issue of genetically modified (GM) algae did not emerge as a major topic of discussion. Rather, the case was made that the challenges ahead for large-scale cultivation and processing of algae for biofuels exist at a much more fundamental level. This is evidenced by the repeated call for LCA and environmental impact studies to be used to guide regulatory and policy decisions. These sorts of studies are inextricably linked to TE analyses, which for now must be based on an assortment of assumptions and data extrapolated from small-scale laboratory work or from the cultivation of algae for higher-value products, as an algal biofuel industry does not presently exist anywhere in the world. This analysis will also be complicated by the requirement to cover many potential process options, as it is not yet clear which ones have the most commercial potential. These efforts, however complicated, must be carried out immediately as they are essential to inform both R&D and business plans and will help to point out barriers, greatly facilitating the development and commercialization of an algal biofuel industry.

Rationale for Standards and Regulations Development

Regulatory ambiguities represent uncertainty that increases risk and adds costs and time for companies trying to build their business. However, it is also important to point out that regulation is based on laws, and laws are written for existing industries, not for potential industries; thus, although there are laws presently on the books that will cover some aspects of the algal biofuel industry, they were not crafted with this industry in mind.

The algal biofuel industry has the potential scale necessary to play a significant role in our national energy needs and will impact our society in far reaching ways. It is beyond the scope of this exercise to consider the societal benefits or challenges (both national and international) that will result from a project of this magnitude that could provide true energy security and availability of renewable transportation fuels. However, it is important to anticipate all aspects of an algal biofuels industry that will draw regulatory scrutiny, especially environmental issues.

Many of the changes inherent in a novel, large-scale, 21st century agricultural development are anticipated to be beneficial to the environment overall (e.g. CO_2 mitigation and wastewater remediation), but some aspects will require significant changes to the way we presently use land, water and other resources. Thus any regulatory framework must consider the overall impact on society and the environment, and provide the opportunity for the industry to flourish (assuming, of course, that the benefits will heavily outweigh the disadvantages), while maintaining our environment in the most reasonable and responsible manner possible. With such potentially significant changes for both our society and our environment from an industry that has yet to fully define itself, we need to maintain maximum flexibility while establishing standards and a regulatory framework that can function at the earliest possible time. To accomplish this, a sensible science-based policy needs to be established. Initial standards and a regulatory framework must be developed that can reduce the uncertainty associated with this new industry, while maintaining the ability to respond to the many challenges that seem likely to be associated with any industry that has the scale and significance of a biofuels industry.

Status of Standards and Regulations Relating to the Algal Biofuels Industry

As standards and regulations are written for existing industries and not for potential industries, the current status provides for a position to build a significant industry in a very short period of time using a regulatory framework that has been cobbled together for related but distinct industries. To accelerate the development of standards and regulations that are relevant for a nascent algal biofuel industry, it may be prudent to make educated assumptions on what an "algal biofuel industry" might entail, and then determine what aspect of this new industry may already fall under existing standards and regulation, and what aspects should be considered for standards and regulations in the near term. It might also be prudent to decide the boundaries of this new industry, and what should be regulated: air, water, soil, organism, Environmental Health and Safety, Food and Feed, just to name a few.

Standards and Regulations Issues

Any regulation should be based on a set of standards, and these standards need to be defined in a scientific, transparent and credible manner. Standards will need to be established for all aspects of this new industry, from how to catalog species of algae to establishing native versus non-native strains, to GMO classification (description, handling, levels of hazard etc), and especially, to the products of the process, including both biofuel products and non-fuel products. Because this industry is essentially being built from the ground up, the existing regulatory processes that potentially impact this industry must first be identified, including the role of federal, state and local agencies that presently regulate one or more aspects of growing or processing algae. Anticipating future potential roles for agencies that will become essential as the industry develops will also be an important step.

These regulatory and standards issues can be addressed with the following questions:

- What aspects of the algal biofuel industry are likely to be regulated – land use, water use, air emissions, water emissions, public health, algal strains, production plant safety, etc.?
- What federal, state and local agencies have an interest in this industry?
- What federal, state and local agencies presently have regulatory responsibilities that could potentially affect the industry?
- What are the areas in which standards will be needed, and when?
- How can standards be established in a way that will accelerate the development of this industry?
- As there is presently a lack of scientific data required for meaningful standards and regulations, how can the required scientific data be generated in the shortest possible timeframe?
- What are the long-term implications of the proposed standards and regulatory framework as regards accelerating the development of this industry?
- What are the potential conflicting intersections between the proposed regulatory framework and existing regulations? How can these be resolved appropriately?

Developing Standards

Areas in Which Standards Are Needed

Although the algal biofuel industry has not yet grown to be a commercial entity, the products of algal biomass are expected to add to or displace existing feedstocks for established industries (e.g. lipid for production of biodiesel or green transportation fuels, and delipidated biomass for production of animal feed or biogas) which currently do have standards. The finished product standards will inform the development of algal feedstock standards which may affect the entire value chain. As an illustration, we will compare standards that would be involved for algal biomass to be used as a feedstock for transportation biofuels (e.g. biodiesel) as well as a higher value co-product (e.g. animal feed). This illustration is in no way meant to recommend the development of algal biomass for animal feed, but rather draw attention to ways that decisions based on economic or market analyses can affect fundamental aspects of the production process. In this example we might find the following set of standards applied to the entire process from cultivation to lipid extraction and purification:

- Biodiesel feedstock
  - Chemical characteristics
    - Fatty acid chain length
    - Free fatty acid level
    - Percentage of TAG
    - Degree of instauration
    - Amount of color
    - Amount and identity of additional extractable materials
- Animal feed feedstock
  - Chemical characteristics
    - Percentage of protein, carbohydrate, and nucleic acids
    - Amount and identity of organic and inorganic micronutrients

    - Ash content
        - Silicon from diatom cell wall
        - Heavy metals or sulfur from flue gas or water source
    - Digestibility
    - Solvent contamination from extraction
- Source biomass characteristics
    - Algal strain composition
        - Percentage of contaminating algal strains
        - Percentage of other microorganisms
        - Natural species of GMO

Because the standards for the animal food industry are more stringent than for the biodiesel industry, the ensuing standards for the algal biomass production and processing will take precedence and will work their way through the entire process to the very front end – algal strain development. In this example, the standards for the animal feed producers will likely be as important in strategic planning as the regulations established by the EPA and USDA, and more difficult to influence with scientific data (that is to say, product sales may depend more upon public opinion than on data).

Alternatively, conversion of delipidated biomass to methane by anaerobic digestion would have a completely different (and less stringent) set of standards, but some level of standards may still be necessary because it is not clear that all algal strains can be readily converted in an anaerobic digester.

Status of Algal Biofuels Industry Standards

There is a decades-old history of commercial production of algal biomass for dietary supplements and nutraceuticals. There does not appear to be a universal set of standards for this industry, but standards exist for several aspects of the production process, informed both by the regulations of the Food and Drug Administration (FDA), as well as by the needs of the commercial organizations responsible for marketing the final product (e.g. requirements for "organic" labeling.) These standards are likely to be more stringent and significantly different from those established for algal biofuels, but will likely provide guidance for companies intending to pursue these markets for byproduct disposition.

The Algal Biomass Organization (ABO), a 501C-6 trade association formed in 2007, has begun an effort to establish a comprehensive list of standards to cover the entire algal biomass value chain, from raw materials to finished product (Figure 10). These include industries that impact algal biomass production, such as biotechnology, input groups (e.g., wastewater treatment organizations and CO_2 sources) and support industries (e.g., equipment manufacturers and algal cultivation facility engineering firms).

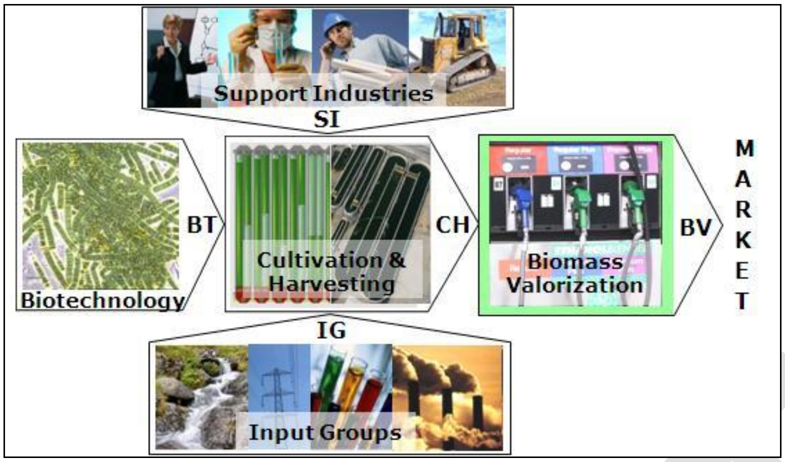


Figure 10: Algal biomass value chain

The ABO's effort is meant to facilitate the growth of this nascent industry by reducing uncertainty, promoting communications among potential partners, reducing the costs of technical progress and helping establish a basis for regulatory oversight. It is modeled after the IEEE Standards Association, a unit of the Institute of Electrical and Electronics Engineers, an international non-profit, professional organization for the advancement of technologies related to electricity. The standards cover a wide-range of industries that fall within the scope of the IEEE, including power and energy, biomedical and healthcare, information technology, telecommunications, and others.

As noted above, the development of a comprehensive list of standards could do much to eliminate the uncertainties in commercialization of algae-based technologies, thus encouraging investment and promoting partnering opportunities. Given that only a small subset of standards will relate directly to biofuel production, DOE is not suitably aligned to take a lead on this effort. Nonetheless, DOE could be instrumental in supporting this effort by providing funding for the accumulation of data needed to craft the standards. It could also help by promoting cooperation of federal regulatory agencies (e.g. USDA, EPA, and FDA) that will have jurisdiction over various aspects of the algal biomass industry. Representatives of these agencies at the Workshop made it clear that regulations already exist that were written without taking algae into consideration but that will nonetheless govern the algae industry. But they also indicated that the regulatory agencies do not wish to deliberately or even inadvertently hinder the growth of the industry. *It would be a great aid to the industry if DOE were to facilitate the sharing of information among the regulatory agencies and individuals charged with the task of drafting the standards.* This sort of effort could be modeled after the work led by the DOE's Office of Energy Efficiency and Renewable Energy to draft new model codes and standards for domestic and international production, distribution, storage, manufacturing and utilization of hydrogen.

Timeline for Completing Actions

As noted above, the ABO has taken the first steps in establishing a comprehensive list of standards for the algal biomass industry by completing a first draft of a list of 20 standards to serve as a guide. Individuals, both within and outside the ABO, will have an opportunity to participate in the subsequent tasks of data accumulation and draft standards writing; other organizations may also choose to contribute to this effort either by cooperating with ABO or by acting independently. It is expected that the first of these standards will be published by early 2010. The body of standards, regardless of the source, will likely be a living document with regular evaluation and updating as the industry matures.

Building a Regulatory Structure

The Case for Regulation

The current state of uncertainty caused by regulatory ambiguity serves to increase the risk and could significantly delay the development of an algal-based biofuel industry. Rapid progress toward commercialization requires a best effort at establishing a productive regulatory framework as soon as possible that is clear but flexible. In order to develop a reasonable regulatory process in the shortest period of time, we first need to understand what regulations are presently in place at local, state and federal levels, and identify the agencies responsible, including USDA, EPA, and additional state and local authorities. The impact of existing regulations on the immediate deployment of first generation algal growth efforts must also be identified. It may be necessary to obtain a federal waiver of local regulation of algal biofuels, as a way to mitigate risk for early stage investment. As has been pointed out, the scientific data do not yet exist for any informed regulatory guidelines to be developed. It is thus important for algal biofuel proponents to proactively work in partnership with regulatory agencies like USDA's Animal and Plant Health Inspection Service, rather than presume that these agencies will automatically assume the worst when examining the potential for algae growth to impact existing agriculture.

Existing regulations may not apply and many may conflict and overlap. The regulatory process is fragmented. Defining algal cultivation as an agriculture process rather than an industrial process may result in less stringent regulations, but downstream processing aspects may not allow that to happen. Fully integrated cultivation-lipid recovery facilities may be necessary for economic viability, but the current regulatory situation discourages integrated facilities, and it may be necessary to remedy this situation.

As we move toward algae that produce feedstocks that are closer to hydrocarbon-based fuels, it may become more complicated to regulate this new industry using existing agricultural or industrial guidelines. For example, lessons learned from soybean oil extraction may not be relevant when viewed in the light of the potential scale of algal biofuels, which far exceeds that of any existing agricultural oils.

Considerations for developing a regulatory framework for the algal biofuel industry should include:

- Which regulatory agencies should be viewed as stakeholders for developing this new biofuel industry?

- How can we develop and standardize a process to improve the understanding of what it takes to utilize algal production strains, including strains imported from other locations, strains bred for specific qualities, and GM algae?
- EPA, USDA are presently sharing regulation
    - EPA: microorganisms used for industrial purposes are in its purview (industrial biotech organism)
    - USDA: all aspects of animal and plant health fall under its purview
- Regulation is based on existing laws, and few existing laws directly address algal cultivation or harvesting.
- Changing laws or getting new laws passed may be difficult and time consuming and may slow the progress of developing an algal biofuel industry. Is it, therefore, better to work within the current framework?
- The ideal regulatory process, although not presently achievable, would be a *single lead federal agency with responsibility to direct agencies at the state and local levels to add consistency and uniformity to developing regulations.*

Status of Algal Biofuels Industry Regulation

At present, the EPA regulates microorganisms used for industrial purposes, including industrial biotech organisms; algae used for biofuel production could certainly fall under this category. The FDA has largely been responsible for safe use of recombinant microorganisms, as well as for large-scale culture of cells, microorganism and viruses; standards of safety for all aspects of microbiological RD&D are largely informed by, if not completely encompassed by, FDA Biosafety regulations. The USDA regulates crops and any potential for pests brought in from other countries. Although algal growth is not obviously regulated under this authority, one can imagine that the large-scale production of algae will more closely resemble agriculture than industrial biotechnology, and for these reasons, the USDA is likely to be the agency to examine regulatory issues as they develop for this industry.

In addition to these federal agencies, there are state and local regulations for several aspects of the algal biofuel industry, including limitations on the import of non-native or GM algal strains, growing non-native or GM algal strains in open ponds, and the discharge of any water in which a species of algae was grown. There are also local land and water use issues that will apply to any algal biofuel industry that seeks to establish a production facility at any significant scale.

Although it is outside the scope of this roadmap to identify all of the state and local regulations that can impact an algal biofuel industry, it is clear that state and regional differences regarding regulations for large-scale algal cultivation already exist. These issues will need to be addressed early on, preferably at a federal level, so that a consistent set of standards and regulations can be adopted for the industry as a whole. The central questions and actions required to develop such a framework are listed below.

Actions required

- Identify impact of existing regulations on industry.
- Identify issues unique to algal biofuels.

- Identify areas of regulatory interest (e.g., air, water, soil, land use).
- Develop a LCA of probable algal biofuel production scenarios.
- Develop an Environmental Impact Assessment of probable algal biofuel production scenarios.
- Develop an efficient, integrated working group on regulatory issues, including identification and coordination of the regulatory agency responsibilities. (e.g., regulated as agricultural, industrial or a hybrid?)
- Support the acquisition of scientific data required for regulation and standards.
- Develop a database of existing regulatory policy.
- Build information resources, perhaps by providing a link to a central database on the NEPANet
- Help develop a regulatory roadmap (creating the framework) that identifies the agencies and laws that pertain to algal-based biofuels.
- Help coordinate the many groups that are already working on one aspect or another of the algal biofuel industry to increase efficiency and reduce redundancy in all of these tasks.

Timeline for Completing Actions

Year 1:

1. DOE should put in place a process that can help define a clear picture of the regulatory and standards policy for algal biofuels.
2. An LCA should be developed to help determine the challenges and opportunities of the biofuels industry, and as a way to define the areas where standards and regulation may be needed. As an LCA may not be sufficient, we also need to:
    a. develop a comprehensive sustainability analysis; and
    b. start an environmental impact assessment/statement/report, cross-cutting intra-agency process
3. Promote the definition of an algal biofuel industry as agriculture.
4. Include perception and social impacts into all aspect of standards, regulation and policy.

Year 2:

1. Complete Phase II Environmental Impact Statement by the end of Year 2.
    a. Include the environmental groups at the outset. They are forward thinking as it relates to biofuels.
    b. Ensure that municipal wastewater and CO_2 abatement as well as more regional environmental issues (i.e. nutrient load reduction in the Mississippi River) are included in the EIS.
    c. Roll local, regional, and national landscapes/issues together to generate information to provide to regulators.
    d. Keep municipal and industrial wastewater as a separate issue.

Policy Framework for Algal Biofuels

Policy Objectives

It is clear that a thriving industry based on the large-scale production of algal biofuels can significantly impact a number of national energy policy goals, including energy security, greenhouse gas abatement, and reduction of competition for strategic resources such as water and agricultural land. Equally important to the nation are the opportunities for creating new jobs, novel approaches for water remediation, alternate source for chemical feedstocks not based on petroleum, and new sources for food and feed. Therefore, it is in the best interest of the federal government to develop a set of policies that will promote the development of this industry beyond the R&D phase to large-scale commercialization. At the highest levels, these policies will reduce uncertainty and risk, thus encouraging scientists, entrepreneurs and investors to enter the arena in large numbers and remain for the time needed to bring this industry to fruition.

Probably the most serious area of *uncertainty* at this moment involves the regulatory landscape as described above. The size and unprecedented nature of projected algal biofuel production facilities will call for scrutiny not just on issues such as release of non-indigenous algal strains or toxic chemical handling, but also ecological impacts due to engineering of multi-acre cultivation systems and local climate changes due to large-scale evaporation. Small, algal biofuel companies with limited resources are faced with a complex overlapping set of regulations established by a number of agencies at federal, state, and local levels that were designed for significantly different industries. State agencies are beginning to address algal biofuel issues in response to plans for pilot- or demo-scale cultivation and processing facilities. This movement may be informed by the contradictory drivers of fear of unknown biohazards and desire for growth in commerce, but not necessarily by scientific fact. Based on input at the Workshop, it appears that states are beginning to line up into two camps, ones that will promote the growth and others that will restrict the growth of a local algal biofuel industry.

A second area of uncertainty lies in the sustainability aspect of algal biofuels. It has been assumed that algal biofuels will have a significantly smaller carbon footprint than corn ethanol, and perhaps even cellulosic ethanol, but no LCA has yet been published. Biofuel companies may not have the resources to unravel the regulatory tangle, or to carry out LCAs, or to proactively deal with agencies setting up new policies. In addition, because intellectual property may be the primary asset of biofuel companies, they may be disinclined to reveal the information necessary for these studies to be carried out. Finally, because private companies have a financial stake in the successful development of the industry, the credibility of studies carried out by the industry that will benefit from the outcome of the studies may not be high; such studies should therefore be undertaken by a neutral party, such as perhaps one or a combination of the DOE national labs.

In terms of the intersection between policy and launching an industry to produce algal biofuels at scale, many productive actions can be taken. DOE and USDA have been supporting R&D efforts for cellulosic ethanol for nearly 30 years, and it will take a few more before this industry becomes economically viable. Risk reduction, through sustained support for a continuum of basic to applied research efforts, combined with changes in policies relating to gasoline formulation, provided the foundation for the establishment of a number of startup companies poised to begin commercial production in the next few years. The development of the cellulosic ethanol industry represents a useful model for support of the algal biofuels industry, though there are key differences:

1. Cellulosic ethanol R&D grew out of an effort conducted mainly at academic and national labs. The push for commercialization began only after a significant level of risk had already been eliminated. In contrast, support for algal biofuel R&D has never been great and has languished for the past decade. Commercial algal biofuel enterprises are springing up rapidly and have taken the lead in R&D despite the risk of failure at this early stage. As a result, the bulk of progress in the future will be protected as valuable IP.
2. Cellulosic ethanol R&D is based on well-understood biotech fundamentals including enzymatic hydrolysis, microbial genetics, and industrial fermentation. Research at bench scale could reasonably be expected to scale to industrial levels, and economic models could be based on a broad base of technological precedents. In addition, the scale up of corn ethanol processes provided much valuable data, not to mention hardware, to facilitate the scale up of cellulosic ethanol production. Algal biofuel technology is based on a rather limited understanding of algal biology that comes from academic labs, combined with understanding of large-scale algal growth obtained from the production of food supplements and nutraceuticals, but these studies have little precedent for production of low-cost commodities on a scale that can impact the fuel industry. Bench-scale work has uncertain predictive value for large-scale production, and it is unlikely that the equipment exists that can be downscaled from the pilot or demo scale to something that can be carried out in a lab. Therefore, the only way to guarantee that lab-scale experimental work is relevant to commercialization is to work within a fully integrated facility that allows for evaluation of algal growth characteristics, productivity, harvest, and conversion. This is beyond the capabilities of most academic and national labs, again limiting R&D to the commercial enterprises.
3. Cellulosic ethanol benefits from its direct agricultural and process engineering lineage to starch ethanol. Starch ethanol in turn benefits from generations of traditional crop breeding and, more recently, genetic engineering efforts to improve yields. The manifold precedents that inform the cultivation of cellulosic energy crops at scale (e.g. switchgrass) and the fermentation processes for converting the sugars to ethanol are derived from our existing agricultural economy for growing corn for food and converting starch to ethanol. Despite this heritage, decades have been spent tackling the challenge of breaking down the lignocellulose biopolymer and streamlining the conversion to fuel process. For algae, on the other hand, there is no existing agricultural economy for producing algal biomass at any appreciable scale. The knowledge about the biology of algae as a potential energy crop is currently limited. Further, the science and engineering of algal biomass processing/oil extraction draws little from any single existing industrial process and currently depends upon the application and adaptation of methodologies from a wide spectrum of industries. Even these may be insufficient, and the successful commercialization of algal

biofuels may require the invention of novel process technologies, designed specifically for algae.

Policy Options

In terms of attenuation of uncertainty, it would be advantageous to establish a lead agency to help reduce the complexity of the regulatory framework. Recently, Secretary of the Interior Ken Salazar stated that he is considering allowing “one-stop” permitting for electrical transmission lines rather than demanding that developers apply for permits from each federal agency involved (i.e., EPA, DOE, and the U.S. Fish and Wildlife Service). A similar approach could be applied for algal cultivation facilities. Alternatively, a R&D Board or a senior-level council co-chaired by DOE and USDA (also includes DOI, DOT, EPA, DOC, DOD, NSF, Treasury, OFEE, OSTP, OMB) could provide the platform. DOE and USDA could take a lead role by supporting transparent efforts to carry out LCAs and environmental impact analyses. Environmental groups should be invited to participate. The Natural Resources Defense Council has already demonstrated an interest in algae and has begun to explore sustainability issues. These efforts would require specific sets of assumptions (e.g. cultivation technology, location, etc.) and so would not be sufficiently inclusive to cover all process permutations, but ultimately could serve as the basis for the development of a more flexible model that could be used to carry out sustainability studies as well as TE analyses for pre-commercial processes under development.

In terms of reducing business risk, federal policy is also critical to ensure that the level of effort needed to achieve commercialization can be sustained over the long run. Although there have been announcements of very large private investments in algal biofuel companies, these are exceptions, and the majority of companies labor with a small financial base. The oil industry has begun to show interest in algal lipids as a feedstock for renewable fuels, and this is appropriate since their valuation is tied to proven reserves, and algae have the potential to provide an above-the-ground renewable oil reserve. To date, though, their support for the R&D effort has been minor, making it hard to foresee how the current industry players can stay in the game long enough to bring about the technological developments necessary to achieve commercialization without federal support. It might be said that the state of algal biofuels technologies is comparable to that of the oil industry in the early 20^{th} century. That industry is currently worth more than $1 trillion. How much will it cost (and how much is it worth) to bring the state of algae technology to the point where it is operating on a comparable level?

Risk reduction, therefore, comes in the form of vastly increased support across the range of R&D activities from basic research to scale up to pilot and demo facilities. In terms of basic research, increased support would facilitate work at the grass roots level, providing much needed information regarding algal ecology, physiology and molecular biology and at the same time increasing the number of trained researchers, engineers, and biofuel plant operators who will be needed to carry commercial development forward. This support would not just be an investment in the commercialization of algal biofuels but also a tactical tool in support of continued U.S. competitiveness; it could also provide an opportunity for training and high-tech job creation in areas where these sorts of prospects are currently lacking in the U.S. Risk reduction for scale-up efforts can use cellulosic ethanol approaches as a tool, offering loan guarantees and cost shares to make it possible for under-funded organizations to test their systems sooner rather than later.

Additionally, market incentives can do much to give confidence to investors so they can begin to see a returns on their investments sooner rather than later. Policies should motivate innovation rather than prescribing pathways and be drawn on lessons learnt from past mistakes, especially from past experience with corn ethanol. It was a common complaint at the Workshop that algal biofuels do not compete on a level playing field, especially due to the loud voices of lobbyists for established biofuel interests. This is another area where LCA analysis could prove very helpful in encouraging growth and development. Most proponents of algal biofuels believe that they will prove to be more sustainable than any alternative, and so the sooner the analyses are completed, the quicker the algal biofuel industry would receive that recognition. Subsidies and tax incentives could provide motivation for increased investments, but it must be pointed out that these incentives must be of a magnitude that makes sense in light of the size of the potential industry. Market incentives in the form of offtake guarantees at specified prices or novel mechanisms to stimulate demand such as a strategic fuel reserve for algal biofuels could alter the risk/reward calculation.

Much of the above assumes fully integrated algal biofuel companies responsible for all aspects from basic biology of algae to supply of biofuels at filling stations. It is quite possible, however, that the industry will be modular in form, with many separate companies contributing the necessary expertise and infrastructure to bring a portfolio of products to market. These may include the following technologies:

1. Algae biomass producers
2. Biofuels processors
3. Fuel blenders
4. Co-product manufacturers
5. Co-service providers (e.g., wastewater treatment, carbon capture and recycling for GHG emissions abatement)

Each of these already exists in one form or another, but each addresses either a different market, a different technology set, or a different source of feedstock. Each of these individual components must come together in an integrated fashion to allow for the successful establishment of an algal biofuel industry. Each segment will face different economic challenges to develop the necessary technology, and different sorts of incentives will need to be identified and implemented to ensure that companies enter the field and remain long enough for achieving commercialization.

Actions Required:

1. Identify the impact of existing policies (e.g. the definition of "advanced biofuel") on the algal biofuels industry.
2. Determine and document policy areas of importance for the algal biofuels industry. This means not just fiscal policy but regulatory and IP ownership policy as well. Policies at many levels may impact development of the industry:
    a. Support for technology R&D
    b. Support for technology demonstration

    c. Financial incentives, e.g., tax credits, loan guarantees
    d. Support for education of scientists and engineers
    e. Resource use policy
    f. Intellectual property treatment
3. Determine organizations with policy-related responsibilities.
4. Develop coordination mechanism for organizations with policy responsibilities.

11. Systems and Techno-Economic Analysis of Algal Biofuel Deployment

Introduction

Successful development of an algal biofuels industry requires the proper combination of technical innovations in systems and process coupled with economic feasibility in the practical implementation, integration and scale-up for commercial production. Prior to such development, confidence that the entire system can operate economically and sustainably in order to merit investment and engagement from necessary stakeholders is necessary. Toward this end, the modeling, simulation, and analyses of systems and processes at multiple levels are critical for developing improved understanding and insight for how an algae-to-biofuels and co-products system can best be implemented and operated within its natural, political, infrastructural, and market constraints. As significant R&D will be required to overcome the technical challenges discussed throughout this Roadmap, modeling and analyses can offer guidance on the wise investment of resources toward those actions, processes, and or systems that show promise of the greatest return on investment.

Recognizing the interdisciplinary nature of systems and techno-economic modeling and analysis, this section addresses only a fraction of possible methodologies associated with large-scale deployment analyses. A discussion of a systems modeling framework within which these analyses can be constructed and conducted is presented as well. This section concludes by describing the additional actions needed to further refine this systems modeling framework and facilitate achievement of desired goals.

First, techno-economic modeling and analysis are presented in some detail, as well as the process of organizing and creating an analysis framework, including the development of a conceptual process flow diagram. Next, a brief overview of complementary approaches and types of analysis techniques that can be utilized in the process are presented. An estimation of cost uncertainty per gallon of algae crude is described. Bounding calculations on estimated CO_2 sequestration using algae is presented. A brief description of ways that resource availability can impact biofuel production economics will be presented with more detailed discussion available in the Appendix. Currently, because the needed information for such modeling and analysis spans science, engineering, and business and is highly uncertain, such analyses are best accomplished through computer modeling, which can capture and accumulate these uncertainties and communicate them in a manner that allows information-driven decision-making with the benefit of the most comprehensive information available.

As a part of the development of this roadmap, the DOE's OBP has sponsored the development an algae techno-economic model framework based on a system dynamics methodology. While uncertainties in the parameters, lack of data, and analysis questions remain to be resolved, such a model framework will ultimately provide a first-order, dynamic outlook for helping to guide R&D investments and (thereby it is hoped) provide useful information for successful commercial scale-up of algal biofuel production. At this early stage, this model framework is intended to demonstrate what such a model could include and how it can be used to identify and guide research and technology development for algal biofuels. In this context then, this report and this section in particular does define a systems modeling framework but does not create a complete systems model for algal biofuel production. Rather this section defines what is necessary to create such a model and suggests a path forward to achieve that goal. Ultimately, more thorough analysis and model refinement will reveal the critical challenges and guide our progress towards economical, scalable, and sustainable algal biofuels.

Workshop Results and Discussion

The following discussion focuses on the workshop outcomes and recommendations in light of systems modeling. Discussions in preparation for the DOE workshop, and discussions among participants during and after the workshop, have acknowledged the need to define scope and determine the role that systems Techno-Economic modeling and analysis can or should play, and the range of approaches, scales, and level of detail that could or should be addressed. Figure 11-1 illustrates the essential factors of the techno-economic modeling and analysis to be taken into consideration for a comprehensive analysis of the developing industry. These factors provide a broad systems perspective that integrates the interdependent science and engineering aspects of algae biofuels with environmental, economic, and policy aspects to provide critical insight and information needed for decision-support. Within an overall systems context, Figure 11-2 is a chart showing major topic areas that align with the roadmap workshop breakout session topics. These categories deliberately follow a supply-chain process whose findings and recommendations can directly impact concurrent modeling and analysis efforts.

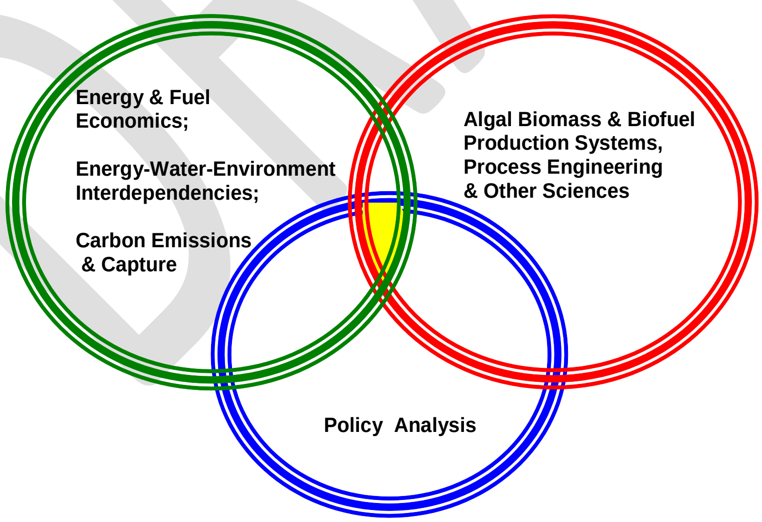


Figure 11-1. Techno-economic modeling and analysis addresses interdependent issues spanning science and engineering, environmental and economic issues, and policy.

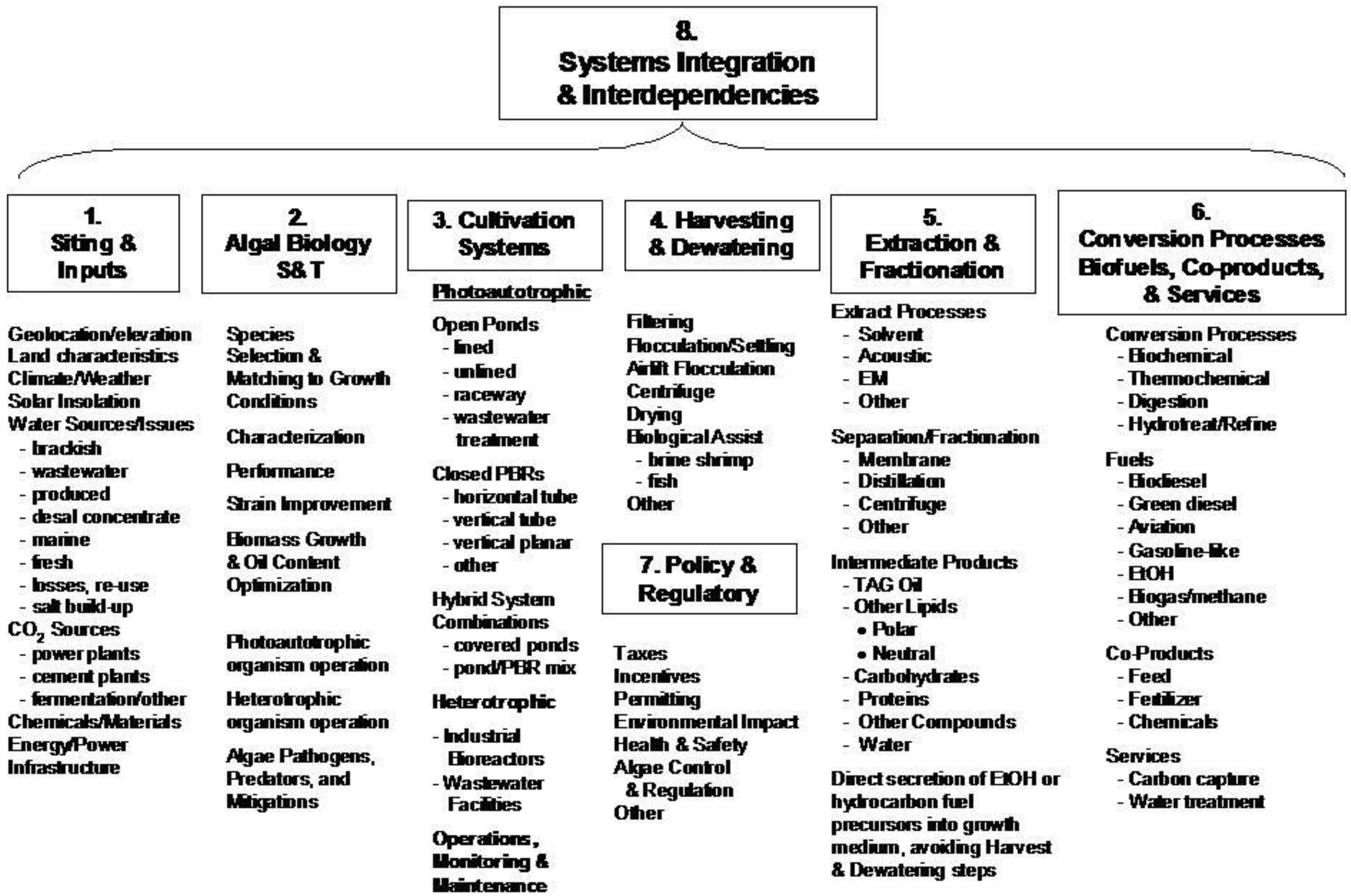


Figure 11-2. Organizational chart identifying and summarizing key topic areas and issues for the overall algal biofuels value chain, which provides guidance for the scope of content that should be integrated into systems techno-economic modeling and analysis

Figure 11-3 illustrates various complementary approaches and techniques that may come into play within the systems-level scope of modeling and analysis for algal biofuels. Due to more limited and immediate analysis objectives, individualized modeling effort tend to focus on modeling at a plant level rather than broader integrated systems modeling and analysis at a regional or national scale. Others have noted there is a need and role for both integrated systems modeling as well as detailed process modeling, and that the two can be coupled. It was generally agreed that modeling and analysis needs to be a critical part of a national algae biofuels research program and industry development effort, similar to the modeling effort in support of the lignocellulosic ethanol biofuel program. It is also expected that the needs for coupled models of differing fidelity and scales will be defined in the early stages of the systems modeling R&D effort.

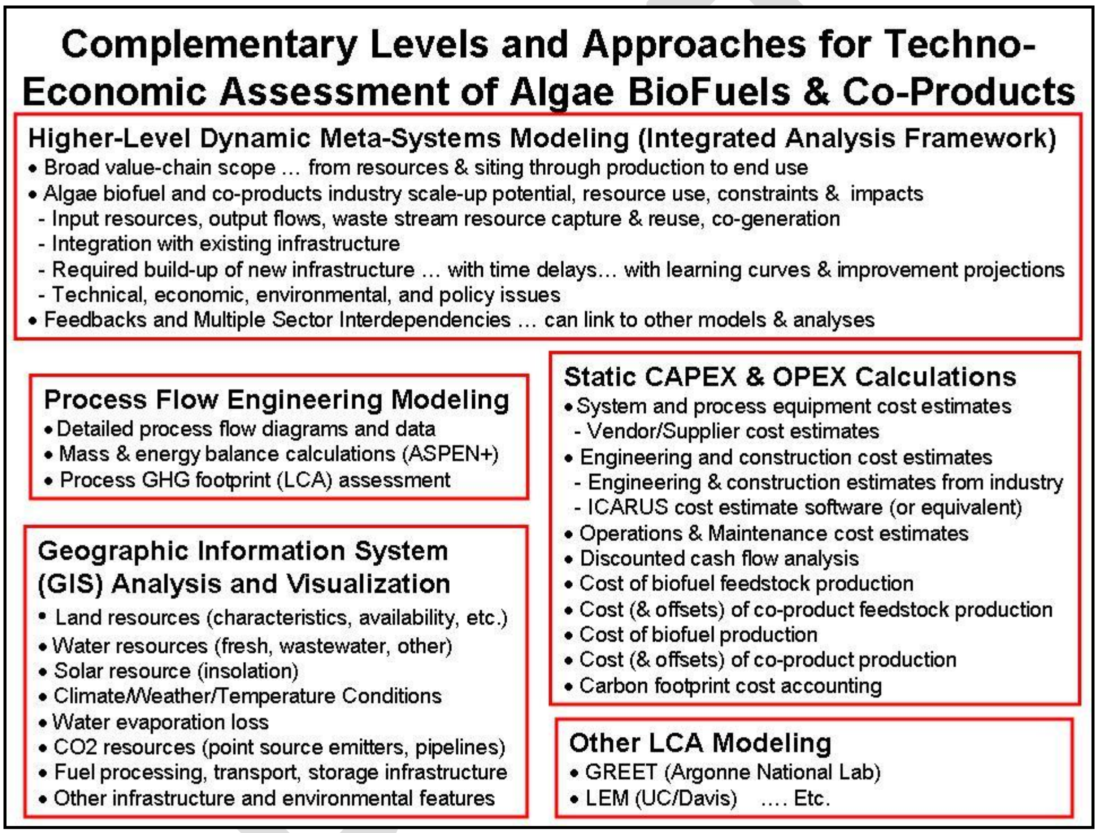


Figure 11-3. Multiple levels and complementary approaches available for Systems, Processes, and Techno-Economic Modeling and Analysis of Algal Biofuels.

Included in the workshop discussions was the question of how best to approach addressing the multiple paths and configurations of rapidly evolving systems and processes that should be considered in the modeling and analysis effort. For example, one approach is modeling major cultivation system categories of open pond, closed PBR, or a hybrid combination and activating only those portions of the hybrid configuration model desired. Beyond this, how to best conceptualize a model that inclusively cover the overall "beginning to end value chain" for algal biofuels production is still emerging. The size of the "matrix" of possibilities in this design could quickly become unmanageable. Having many multiple models that are each uniquely customized to a specific combination of systems and processes and performance parameters is another approach, and essentially represents what exists today with various groups doing modeling and assessment specifically focused on their chosen approach. The disadvantage of such a distributed approach is obviously that it does little to inform the regional, national, or optional technology needs/options.

The desired goal is to develop a "generalized" and flexible modeling and assessment framework and platform that incorporate the key technical information distilled by methods outlined in Figure 11-3. Some sort of standardized interface requirements or definitions should be established for system and process functional blocks that would enable the development of an open-source modeling and assessment platform with "plug & play" flexibility. Much more detailed or custom models of an individual subsystem or process blocks could then be developed by various others in industry, universities, and national labs using different techniques such as high performance physics-based modeling (e.g., Sandia's CFD open raceway pond model) or process engineering models using widely accepted and used commercial process modeling tools like AspenPlus^{TM}, or customized spreadsheets. Flexibility in being able to link custom subsystem or process models into an overall meta-system modeling and analysis platform would provide a capability that could be of significant value and benefit to different stakeholder communities that could include:

- DOE & national labs doing R&D, assessment, & tracking of program investments
- Other Federal and State Agencies (DOD, USDA, EPA, etc.)
- Universities doing a wide range of technical/economic/ policy R&D and assessment
- Industry developing & commercializing technologies, systems, processes
- Private investment / funding sources

Systems Analysis

This section provides an overview of key system modeling components that are believed to be required for a fully functional multi-scale model framework for realization of algal biofuel production goals.

System analysis is foundational to designing a strategy for algal biofuel deployment. A system is an aggregation of subsystems interacting such that the system is able to deliver an over-arching functionality. Figure 11-4 illustrates the interdependent character of the overall algae biofuels value chain that involves a broad range of systems, processes, and other technical and non-technical issues. To facilitate system-level thinking during the workshop, a process flow diagram was developed to reveal the intricate interdependency of algal biofuel production and present at every track discussion. Figure 11-5 shows a number of process options available for every step in the algal biofuel production chain, from algae growth to fuel processing. Sub-level processes that made up different thematic sessions in the workshop, are all inter-related. Collecting and understanding key information from each node in the process becomes the primary task of the system analysis.

Both Figure 11-4 and Figure 11-5 indicate that there are large permutations of potential pathways to algal biofuel production, most of which are still immature and emerging. In fact, there are more than 2000 unique pathways from strain selection to final product and co-product. Even that is an underestimate since many of the process steps will differ depending on the product or co-product chosen. Though it may seem daunting to attempt to develop a comparative analysis based on so may process permutations, there is precedence for this sort of undertaking in DOE's H2A program. Established in 2003 in response to President Bush's Hydrogen Fuel Initiative, H2A was designed to consider various pathways toward a hydrogen economy, evaluate costs, energy and environmental tradeoffs and set research priorities and inform policy by sound analysis. The options for hydrogen production include goal gasification, nuclear energy, wind electrolysis, and organic molecule reforming. This program could serve as a guide for moving forward with analysis of algal biofuel production.

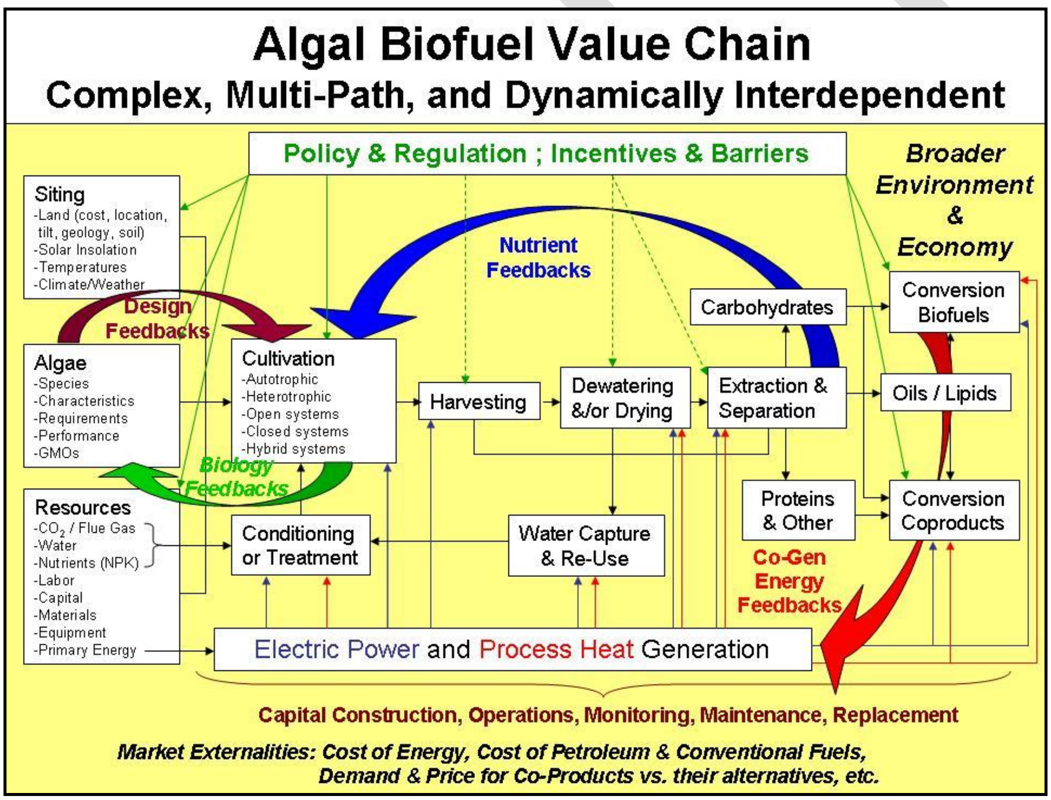


Figure 11-4: Illustration of the broad systems analysis perspective needed to address the dynamic coupling and interdependencies across the overall algal biofuels and co-products value chain.

Process Flow Diagram for Algae Biofuel Production

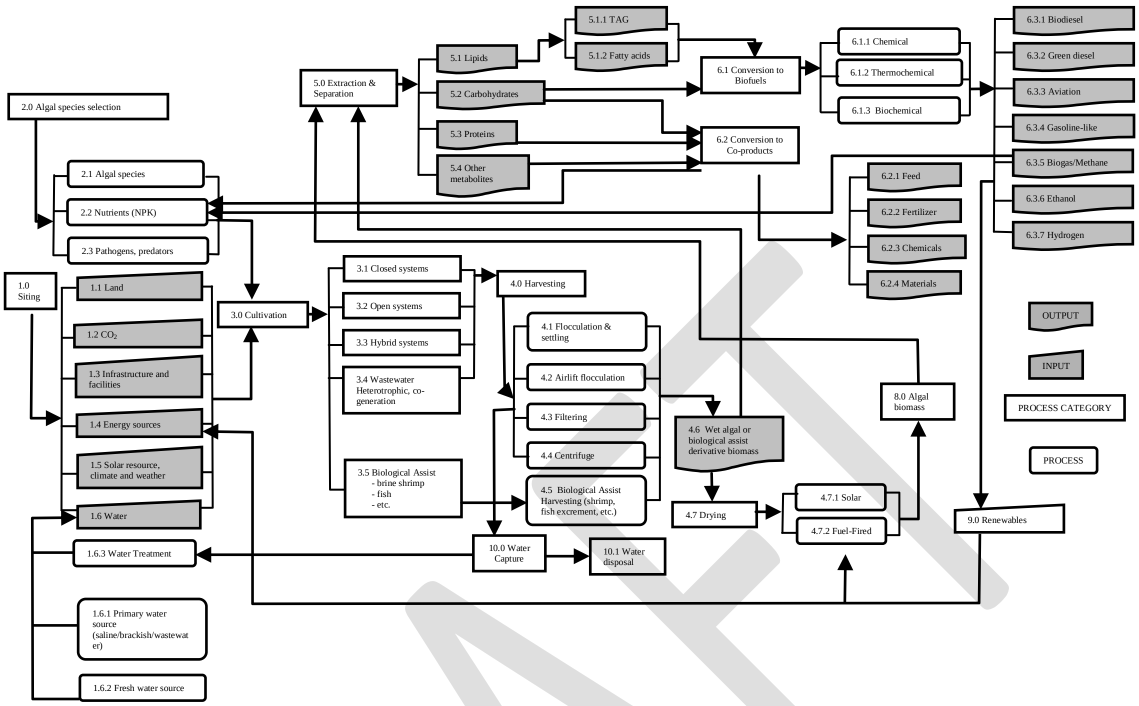


Figure 11-5 – Multi-pathway algae biofuel process flow diagram for tracking inputs, outputs, and feedbacks across the entire system.

Other sections of this report point out the lack of information about the characteristics of algae themselves and the characteristics (energy requirements and costs) of the processes that are described in the process flow diagram. A substantial number of barriers are enumerated and designated as goals to be achieved. Systems analysis can help quantifying the complexity of producing algal biofuel by quantifying uncertainties, identifying and correctly modeling interdependencies and feedbacks, and comparing trade-offs from various scenarios with regard to cost, risk, etc.

At a subsystem level, analysis methodologies and tools exist for resolving process development, each providing a unique method for addressing technical and economic concerns. Broadly, engineering analyses require automated mass, momentum, and energy balances that evaluate the thermodynamic or hydrodynamic limits of processing units. Example tools include AspenPlus™, FLUENT™., among others.

Geographic Information System (GIS) Analysis and Visualization tools(described in detail in Section 10) are indispensible for algal systems analysis due to their ability to perform regional mapping and resource analysis. Critical climatic and natural resource data can be readily accessed, such as

- Land and water resources (characteristics, availability, etc.)

- Climatic change: temperature, precipitation, solar
- Water evaporation loss
- CO2 resources (point source emitters, pipelines)
- Fuel processing, transport, storage infrastructure
- Other infrastructure and environmental features.

Economic analysis tools for static CAPEX & OPEX calculations are also integral to system analysis as they reveal financial investment or market incentives needed for algae biofuel deployment. Some examples are

- POLYSYS
- ICARUS cost estimate software (or equivalent)
- Equipment, Operation & Maintenance cost estimates
- Discounted cash flow analysis
- Cost (& offsets) of co-product feedstock production
- Cost of biofuel production
- Carbon footprint cost accounting

Specific life-cycle analysis modeling tools include GREET (Argonne National Lab, 2009) and Lifecycle Emission Model (Delucci, 2002).may also be employed. Multiple models and model results will be required at multiple scales and incorporated into the systems model framework to adequately address the scope of the algal biofuel technical challenge.

Algae Production Cost Uncertainties – Illustrative Example

Data gathering for an industry that has yet to be realized can be one of the biggest challenges in techno-economic analysis. To facilitate the objectives of participating experts during the roadmapping workshop, cost analysis based on published data was carried out using twelve references and summarized graphically in Figure 11-7. Using existing sources of information available in the open literature and through initial collaboration amongst NREL, Sandia, and several university and industry participants, including:

- Benemann & Oswald T-E Assessment of Open Ponds (1996)
- Presentations from 2007 and 2008 Algae Biomass Summit meetings
- Other available T-E assessments
  - SNL Analysis
  - CSU/Solix Analysis
  - NMSU Analysis
- Other open literature papers & reports

While the sampling size is small relative to the available information, the range of estimates already reveals discrepancies in cost by three orders of magnitude. These estimates include both actual and hypothesized values that span 10+ years and 3 continents. They also span several technologies (open pond, PBRs, etc.). The only real data available for algal biomass production comes from the food supplement/nutraceutical industry. Extrapolation of cost data for β-carotene and eicosapentaenoic acid production, using relatively conservative assumptions for lipid content (35%), leads to figures on the order of $1000 per gallon lipid. These numbers are absurdly high for biofuel production, but serve as an entry point into this analysis. A summary of consolidated TE modeling efforts is shown in Figure 11-6 and the basis for these calculations is shown in Table 11-1. In presentations at Algae Biomass Summits in 2007 and 2008, Ben-Amotz of Seambiotics explored process options (some actually implemented and some hypothetical)in a transition from β-carotene production to algal biofuel production, yielding a more reasonable value of $25 per gallon (Figure 11-6). Other lessons to be learned from this exercise is that when raceway ponds are compared head to head with photobioreactors (as in the case of the the two values generated by Sandia, below) increased capital costs led to an almost two-fold increase in estimated production costs. The impact of increased productivity is demonstrated by the various cost estimates provided by Benemann's original model and NREL's updated version. NMSU's model also demonstrates the value of improved productivity as well as the impact of economies of scale. The Solix model, alone of those evaluated, demonstrated the value of improved productivity, reduced energy costs and co-product credit. It is remarkable (though possibly coincidental) that the various base case estimates employing tested process steps all fall in the range of $20-40 dollars per gallon, despite large differences in process details and economic parameters used. details and , there are indications that a combination of improved biological productivity and fully integrated production systems can bring the cost down to a point where algal biofuels can be competitive with petroleum at approximately $100 per barrel.

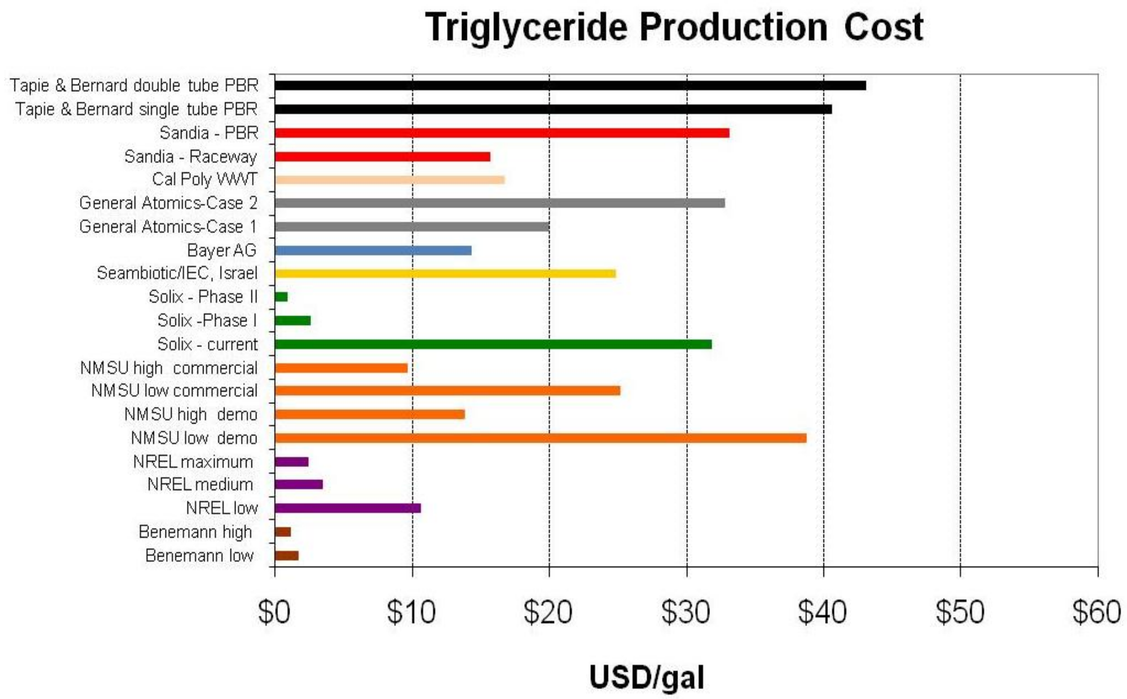


Figure 11-6 Per gallon triglyceride cost from different publically available estimates. Benemann (1996); NREL & NMSU (private communications, 2008); Solix; Bayer; General Atomics; Cal Poly (2008); Seambiotic, Israel, (2008); Tapie & Bernard (1987); Sandia (2007).

Table 11-1 – Summary of assumptions from the various sources shown in Figure 11-6.

	SCENARIO	Reactor Type	Lipid yield (wt% of dry mass)	Areal Dry Algae Mass Yield (g/m2/day)	Loan Period (yrs)
Benemann	per ha basis	open pond	50%	30	5
Benemann	per ha basis	open pond, max	50%	60	5
NREL	Current Case	open pond	25%	20	15
NREL	Aggressive Case	open pond	50%	40	15
NREL	Maximum Case	open pond	60%	60	15
NMSU	current yield	open pond	35%	35	20
NMSU	highest yield	open pond	60%	58	20
Solix	Current	hybrid	16% - 47%	0 - 24.5	unk
Solix	Q2, 2009	hybrid	16% - 47%	30-40	unk
Seambiotic/IEC, Israel	Best Yield	open	35%*	20	unk
Sandia	Raceway&PBR	both	35%	30	20
Bayer Tech Services	Germany	PBR	33%	52	10
Bayer Tech Services	El Paso, TX	PBR	33%	110	10
General Atomics	100 acres	open/hybrid	unk	unk	unk
Cal Poly, Case1	100 ha	wastewater treatment + digester	25%	20	8
Tapie & Bernard	10 ha	T-PBR	35%*	20	5

* Assumed quantity required to convert from weight-basis to oil-basis

Impact of Geographic Variability of Inputs on Algal Biofuel Production Costs.
The various inputs necessary for algal biofuel production have been described in previous sections. Certain elements, like cost of power and water vary over the U.S. but these variations, though important for overall TE analysis, are not unique to the development of algal biofuel technology. There are, on the other hand, aspects of large scale autotrophic algal cultivation, for which geographical variation of resource availability will have major impacts on cost of production, even commercial viability. These aspects are discussed at length in the Appendix, but it is appropriate that they are briefly mentioned here.

- The average annual insolation is inarguably the rate limiting factor for algal productivity, and this factor varies widely across the country. This variation will determine the area of cultivation systems needed to achieve a set amount of product; it will affect the amount of CO2 that can be captured; and it will affect the amount of culture that will need to be processed on a daily basis.
- CO2 availability and cost will play a role in cultivation scalability and operating expense. As noted in Section 9 and in this section, it will be advantageous to co-locate cultivation facilities with fixed CO2 sources, but this will not be feasible in all instances and thus, it may be necessary to transport CO2 over some distance. Even in the case of co-location, the size of an algae facility will require extensive

pipeline systems, adding to the cost. Quality of CO2 will also play a role for algal growth, and some sources are likely to require more cleanup than others (especially if there are plans for animal feed as a co-product). Finally, carbon credits must also enter into this analysis, though it is not yet clear how to factor this into the calculation. Land prices and availability can also impact the cost of biofuel production. It is reasonably straightforward to calculate the impact of the cost of land on the overall cost of lipid production, but it is likely that there is an optimum minimum size for a production facility. If it is necessary to distribute the facility over a number of smaller parcels of land, it may not be possible to get the most benefit of scale economy.

- As in traditional agriculture, the temperature during the growing season will restrict the ability to cultivate specific strains for extended durations. In the summer, evaporation rates may provide some level of temperature control but evaporation will also add to operating cost (for water replacement). Waste heat from the CO2 source may allow for growth during periods of suboptimal temperature, but moving this heat to the extensive algal cultivation systems will provide the same engineering problems as moving the CO2.

In summary, then, it is clear that calculations for the cost of of algal biofuel production will require detailed inputs that take into consideration the variations in cost and availability of the essential elements for cultivation. While these variations may be minor relative to the technical uncertainties, it must be stressed that a technology that will require immense volumes to play a role in the energy economy cannot afford to miss the economic target by a fraction of a penny.

Algae Techno-Economic analyses: System Dynamics modeling

Systems dynamics modeling is a powerful, rigorous, and flexible modeling approach that can foster collaborative analysis. A dynamics simulation model will also provide an integrated analysis framework and will include:

- Broad value-chain scope: from resources and siting through production to end use
- Algae biofuel and co-products industry scale-up potential, resource use, constraints and impacts
- Input resources, output flows, waste stream resource capture and reuse, co-generation
- Integration with existing infrastructure
- Required build-up of new infrastructure with time delays, learning curves and improvement projections
- Technical, economic, environmental, and policy issues
- Feedbacks and Multiple Sector Interdependencies with links to other models and analyses

The framework for a systems dynamics model of commercial scale algal biofuels operations is described. The preliminary model uses the yield and land availability assumptions from the same data sources used in Figure 11-10 and Tables 11-4 & 11-5.

By using an interactive graphical user interface, the model can be used to conduct rapid 'what if' analyses. For example, by selecting a specific yield constrained by the land availability constraint (all land by sun hour class, land constrained by CO_2 availability, land constrained by access near CO_2 sources) results in estimates of total algae production in g/day/meter squared. Figure 11-11 shows the results from a demonstration run in which the "NREL current open pond" yield is chosen and land is limited by sun hour (least constrained). Note the slider settings that also influence model output. The yield is 20 g/day/meter^2. The results show a cumulative production of approximately 5.6 billion kilograms (dry) of algae by the year 2030. With 50% oil content, this would result in 2.8-billion kg of oil, which is about 0.81-billion gallons.

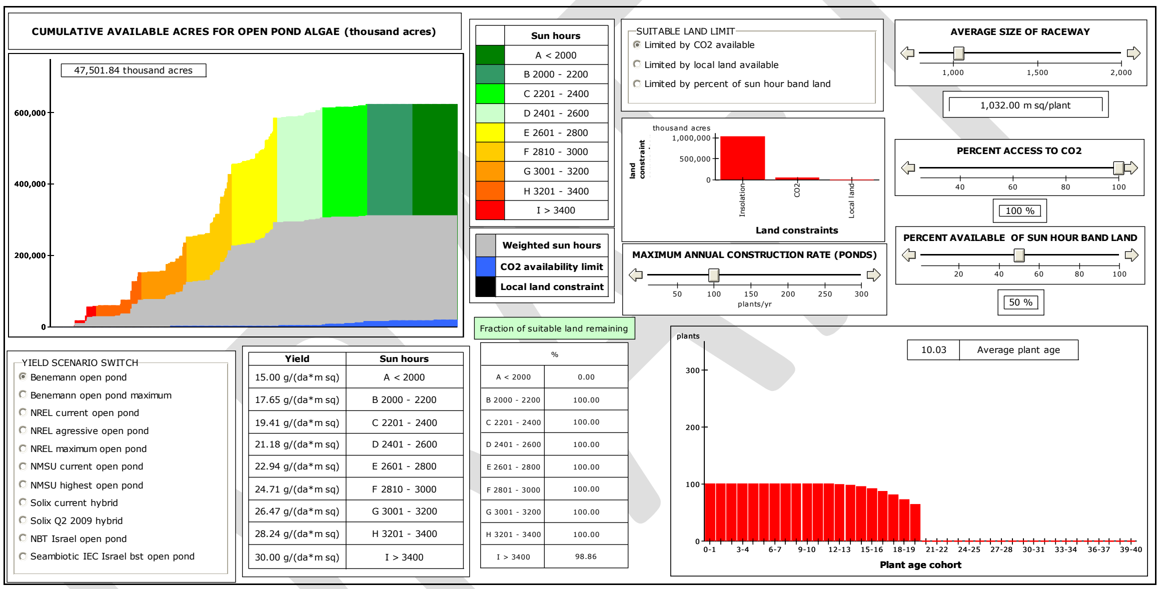


Figure 11-11: Sample preliminary model interface

This result requires building ponds on approximately 33,000 acres. This amount of land is constrained by the 100 plants/yr of 5 raceways of 1032 acres each, which is a constraint that was activated during this run. The model will eventually include the ability to do Monte Carlo simulation, varying parameters values within pre-set ranges in order to describe the uncertainty or robustness of model output.

Recommended Priorities and R&D Effort

The DOE model described above was initially prepared in outline form for the algae roadmap workshop, and has been developed further since the workshop. The model currently includes only a limited amount of available data. To adequately inform research and investment devisions for algal biofuel deployment, continued progress in techno-economic analysis can provided needed additional information. Workshop participants specifically suggested that the following areas be addressed in the modeling and analysis.

- Determine the current state of technology
- Identify critical path elements that offer opportunities for cost reduction
- Identify research areas most in need of support
- Identify external factors that will impact cost
- Provide plan for entry of algal biofuel into a renewable fuel portfolio
- Inform and perhaps guide formation and/or modifications to public policy
- Incorporate appropriate insights and benefit from alliances with industry associations

The Techno-Economic Analysis can accomplish this by:

- Stressing dynamics over detail
- Employing modular modeling, e.g. ISBL and OSBL approaches*
- Establishing interface requirements between sub-systems
- Leveraging university resources
- Maintaining industry standard notation, units, etc.

To process the above suggestions with sufficient fidelity to inform R&D investment and guide technology risk management, an concentrated effort to construct a useful system analysis model is recommended. While we initially provided an illustrative system dynamics framework, a more comprehensive, phased approach is outlined in Table 11-6.

* ISBL – Inside Boundary (or Battery) limits, OSBL – Outside Boundary Limits

Table 11-6: Phased approach with capability targets and deliverables as guidelines and suggestions set by Roadmap participants.

Phase	Tasks	Deliverable	Capability
1	Develop the framework to include the entire algal biofuel life cycle. This would include constraints on algae production, processing technology, and production cost estimates.	Model 0.1 Beta – dynamics accounted for	Model runs with notional data and or data ranges. Rudimentary user interface
2	Populate the model with data obtained from commercial firms including an estimate of the technology's Technology Readiness Level.	Model 1.0 Beta	Model runs with commercial data. Ability to see bounds on parameters and the resulting life cycle uncertainty.
3	Confidence building and model sensitivity runs. Probable re-work to include any changes to the algal biofuel system.	Model 1.0 – detail accounted for	Completed user interface, populated with vetted and protected data sets. Ability to run policy scenarios and determine investment priorities.

Throughout the Workshop, significant algae to biofuel process uncertainties were identified along all steps of the process. These have been noted in earlier sections. Addressing these uncertainties in a systematic and integrated modeling assessment could help speed the deployment of an algal biofuels industry.

References

Chisti, Y., "Biodiesel from Microalgae," Biotechnology Advances 25 (2007) 294-306.

Molina Grima, E., E.-H. Belarbi, F.G. Acien Fernandez, A.R. Medina, Y. Chisti, "Recovery of microalgal biomass and metabolites: process options and economics," Biotechnology Advances, 20 (2003) 491-515.

Lundquist, T.J., "Engineering & economic Assessment of algae biofuel production," Algae Biomass Summit 2008.

Tapie, P and A. Bernard, "Microalgae production: technical and economic evaluations," Biotechnology and Bioengineering," 32, (1988) 873-885.

Huntley, M.E. and D.G. Redalje, "CO_2 mitigation and renewable oil from photosynthetic microbes: a new appraisal," Mitigation and Adaptation Strategies for Global Change, 2006.

Ringer, M., R. Wallace, P. Pienkos, NREL Techno-economic analysis, private communications, 2008.

Starbuck, M. and P. Lammers, Techno-economic analysis, private communication, 2008.

Willson, B., "Low cost photobioreactors for algal biofuel production & carbon capture," 2nd Bundes-Algen-Stammtisch, Hamburg, 2008.

Weyer, K., D. Bush, A. Darzin, B. Willson, "Theoretical maximum algal oil production," Algae Biomaass Summit, 2008.

Steiner, U. "Biofuels' cost explosion necessitates adaptation of process concepts," European White Biotechnology Summit, Frankfurt, Germany, 2008.

Benemann J.R. and W.J.S. Oswald, "Systems and economic analysis of microalgae ponds for conversion of CO2 to biomass," Final Report to the DOE-PETC under Grant #DE-FG22-93PC93204, 1996.

Hazlebeck, D. "General Atomics Algae Biofuel Program," October, 2008.

Wu, B. and R. Pate, "Algal oil production notional "Baseline" scale-up assessment," Sandia presentation, 2007.

The Greenhouse Gas, Regulated Emissions, and Energy Use in Transportation (GREET) model. http://www.transportation.anl.gov/modeling_simulation/GREET/

Overview of the Lifecycle Emissions Model (LEM) http://pubs.its.ucdavis.edu/publication_detail.php?id=305

Forrester, Jay W. 1961. Industrial Dynamics. The M.I.T. Press.

Attra (2006). "Biodiesel: The Sustainability Dimensions", National Sustainable Agriculture Information Service, National Center for Appropriate Technology, 2006 http://www.attra.ncat.org

Ben-Amotz, Ami (2007). "Production of Marine Unicellular Algae on Power Plant Flue Gas: An approach toward bioenergy and global warming", Algal Biomass Summit, San Francisco, CA, November 15-16, 2007.

Ben-Amotz, Ami (2008). "Biofuel and CO2 Capture by Marine Microalgae", Algal Biomass Summit, Seattle, WA, October 24-24, 2008.

Benneman, J.; Oswald, W. (1996). "Systems and Economic Analysis of Microalgae Ponds For Conversion of CO_2 to Biomass". Report prepared for the Pittsburg Energy Technology Center under Grant No. DE-FG22-93PC93204.

Benemann, J.R. (2002). "A Technology Roadmap for Greenhouse Gas Abatement with Microalgae." Report to the U.S. Department of Energy, National Energy Technology Laboratory, and the International Energy Agency Greenhouse Gas Abatement Programme. Prepared for the International Network on Biofixation of CO_2 and Greenhouse Gas Abatement with Microalgae.

Bill A., Griffin T, Marion, J. and Nsakala ya Nsakala (2001). "Controlling Power Plant CO2 Emissions: A Long range View", Conference Proceedings, Power Gen. Europe 2001, Brussels, Belgium. www.netl.doe.gov/publications/proceedings/01/carbon_seq/1b2.pdf

Bryan, P. and Miller, S. (2008). "Algal and Terrestrial Second-Generation Biofuels – Chevron and the *New Energy Equation,"* Chevron Biofuels, Chevron ETC, Presentation at Scripps Institution of Oceanography, March 26, 2008. http://spg.ucsd.edu/algae/pdf/2008-03-26-Scripps.pdf

Campbell, Peter, Tom Beer, David Batten (2009). "Greenhouse Gas Sequestration by Algae – Energy and Greenhouse Gas Life Cycle Studies", Transport Biofuels Stream, CSIRO Energy Transformed Flagship PB1, Aspendale, Vic. 3195, Australia 2009. http://www.csiro.au/org/EnergyTransformedFlagship.html

Chisti, Y. (2007). "Biodiesel from microalgae." *Biotechnology Advances*. (25); pp. 294-306.

Kadam, K.L. (1997). "Power Plant Flue Gas as a Source of CO_2 for Microalgae Cultivation: Economic Impact of Different Process Options", Energy Convers. Mgmt, v.38, Suppl., pp. S505-S510, 1997.

Kadam, K.L. (2002). "Environmental implications of power generation via coal-microalgae cofiring", Energy v.27, pp. 905-922, 2002.

Massingill, Michael, James Carlberg, Gregory Schwartz, Jon Van Olst, James Levin, and David Brune (2008). "Sustainable Large-Scale Microalgae Cultivation for the Economical Production of Biofuels and Other Valuable By-Products", Algal Biomass Summit, Seattle, WA, October 24-24, 2008.

McKinsey&Company (2008). Warren Campbell, et.al., "Carbon Capture & Storage: Assessing the Economics", Report prepared under the McKinsey Climate Change Initiative, September 22, 2008. http://www.mckinsey.com/clientservice/ccsi

ORNL (2003). "Bioenergy Conversion Factors", http://bioenergy.ornl.gov/papers/misc/energy_conv.html http://www.localenergy.org/pdfs/Document%20Library/Bioenergy%20conversion%20factors.pdf

Pate, R. (2008). "Algal Biofuels Techno-Economic Modeling and Assessment: Taking a Broad Systems Perspective", Plenary presentation at DOE Algae Biofuels Technology Roadmap Workshop, University of MD Inn and Conference Center, 9-10 December 2008.

Rubin, E. (2005). "Carbon Dioxide Capture and Storage." IPCC Technical Summary.

Schenk, Peer., Skye R. Thomas-Hall, Evan Stephens, Ute C. Marx, Jan H. Mussgnug, Clemens Posten, Olaf Kruse, and Ben Hankamer (2008). "Second Generation Biofuels: High-Efficiency Microalgae for Biodiesel Production", Bioenerg. Res. v1:20–43 Published online: 4 March 2008.

Sheehan, J., T. Dunahay, J. Benemann, and P. Roessler (1998). "A Look Back at the U.S. DOE's Aquatic Species Program – Biodiesel from Algae", NREL/TP-580-24190, July 1998.

Sun, Sally, and Raymond Hobbs (2008). "Power Plant Emissions to Biofuels", Presentation by Arizona Public Service Company at NREL-AFOSR Workshop on Algal Oil for Jet Fuel Production, Washington, DC, 19-21 February 2008.

Tyson, K.S.; Bozell, J.; Wallace, R.; Peterson, E.; Moens, L. (2004). *Biomass Oil Analysis: Research Needs and Recommendations.* NREL/TP-510-34796. Golden, CO: National Renewable Energy Laboratory.

USDA (2006). "Major Uses of Land in the United States, 2002", USDA Economic Research Service, Economic Information Bulletine Number 14, May 2006.

USDA (2009a). "Land Use, Value, and Management: Major Uses of Land", USDA Economic Research Service On-Line Briefing Room http://www.ers.usda.gov/Briefing/LandUse/majorlandusechapter.htm

USDA (2009b). "United States Census of Agriculture 2007: Summary and State Data", v.1, Geographic Area Series, Part 51, Issued February 2009 by the USDA National Agricultural Statitics Service. http://www.agcensus.usda.gov/Publications/2007/Full_Report/usv1.pdf

Van Harmelen, T.; Oonk, H. (2006). "Microalgae Biofixation Processes: Applications and Potential Contributions to Greenhouse Gas Mitigation Options." Prepared by TNO Built Environment and Geosciences for the International Network on Biofixation of CO_2 and Greenhouse Gas Abatement with Microalgae. Available from jbenemann@aol.com.

Weyer, Kristina, Daniel Bush, Al Darzins, Bryan Willson (2008). "Theoretical Maximum Algal Oil Production", Algal Biomass Summit, Seattle, WA, October 24-24, 2008.

Surface Evaporation Map of US - Evaporation for the United States," NOAA Technical Report NWS 34, Washington, D.C., 82 p.

Farnsworth, R.K., E.S. Thompson, and E.L. Peck (1982). "Evaporation Atlas for the Contiguous 48 United States," NOAA Technical Report NWS 33, Washington, D.C.

Produced Water Quality Map of US - The produced water map shows locations of brackish water generated during oil and gas recovery. Water quality, date sample was collected, sample depth, geologic formation samples was collected from, and wellname are examples of data included in this dataset. It is available at http://energy.cr.usgs.gov/prov/prodwat/dictionary.htm

CO2 Source Map of US - CO2 sources were compiled by NATCARB as part of the national Carbon Sequestration Program. An example of some of the sources included in the database are power generating facilities, refineries, manufacturing, natural gas transmission and cement plants. http://www.natcarb.org/Atlas/data_files.html

Saline aquifer data was compiled by NATCARB as part of the national Carbon Sequestration Program. Information includes geologic basin and formation, as well as formation surface area. http://www.natcarb.org/Atlas/data_files.html

12. Public-Private Partnerships

Introduction

As noted several times throughout this document, the participants of the Roadmap Workshop voiced a strong consensus regarding the need and opportunity for the continued development of algal biofuels. Of equal importance was the participants' agreement regarding the need for leadership from DOE in several areas including:

1) coordinating with other federal agencies to support fundamental and applied research, infrastructure development, technology deployment, and information management at a national level, and
2) promoting the development of enabling policy, regulations, and standards for the emerging algal biofuels industry.

The Workshop participants emphasized the critical need for DOE and other federal agencies to partner with national laboratories, academia, and most importantly, with industry. The participants, however, also noted the uniqueness of the partnerships environment in algal biofuels development, given the fact that the algal biofuels industry is still in its infancy. More specifically, given the current state of this industry, the business strategies of many existing companies are focused on some aspect of algae, but not necessarility producing transporation biofuels from cultivated algal biomass at scale. Some would advance the view that no recognized "industry" at all given that there are no profitable concerns current producing algal biofuels. Given this situation, the framework and leadership needed to carry out the fundamental R&D needed to help launch a US algal biofuels industry is considerable yet still in the formative stages.

With concerns over intellectual property (IP) rights and future earnings, most companies in this emerging industry have not adopted the openness found in other industries in terms of sharing data and science learnings with the larger international research community (including US national laboratories and universities). At the same time, value proposition presented by algal biofuels is widely recognized by the nation's top scientists and engineerings, environmentalists, and entrepreneurs; many believe that algae holds significant—if longer term—promise to address nation's energy challenges, especially in an anticipated carbon-constrained world . Given this landscape, overcoming the technical challenges to realizing the potential of algal biofuels will require inspired and empowered leadership and strategic partnerships.

This section discusses the rationale for public-private partnerships in general and specifically, as related to algal biofuels. Further, various models for such partnerships employed in past efforts are discussed in the context of applicability to the algal biofuels challenge, including criteria for formation of public-private partnerships (i.e., characteristics for membership), and in particular, intellectual property models. In addition, several options for action and anticipated timelines are presented and discussed.

Building Successful Public-Private Partnerships

People and organizations partner when they believe it is in their best interest to do so rather than “going it alone.” They recognize that some characteristic of the challenges they face (financial investment, risk, technical capability, etc.) present a significant barrier to their success and that the odds of success can be attractively enhanced if tackled with partners.

However, deciding to partner is one thing; building a successful partnership is another entirely. At the highest level, successful partnerships have several key attributes (J. Micheau, 2008):

- The partners collaborate on the basis of common interest.
- The benefits of partnership outweigh the cost of collaboration.
- The partners can achieve more through collaboration than they can individually.
- The benefits received from the partnership should be proportional to the value of the contribution.
- The partnership should not openly conflict with the interest of other groups.

As ideas to encourage and enable partnerships to advance algal biofuels across private and public organizations are contemplated, it is important to keep these attributes in mind.

Partnerships may bring together parties that have not worked together before, which could both be a benefit (new complimentary capability) and a challenge (the understanding of how to work together). Finding a basis of common interest for an algal biofuels partnership is possible, as this industry has many needs where multiple players can benefit, especially when teams are formed around the unique, differentiating, and yet complimentary strengths of their members. Implicit in the concept of collaborating on a common basis is the sharing of pre-competitive research results, which allows the advancement of technology and know-how to levels far beyond the capability of any individual entity. Generally, this need is met via public funding for research institutions (e.g. universities and national laboratories) for whom this kind of work is particularly aligned with both their missions and strengths. Networking events such as workshops, conferences, and seminars are important tools for creating such a collaborative environment.

From the taxpayer’s perspective, the government is the steward of taxpayer funds and its role in a national public-private partnership should be to ensure that access to the partnership is open to all who can contribute so as not to benefit only a few industrial players while at the same time catalyzing commercialization of technology derived from the fundamental and applied science and engineering advances achieved via public funding. For this reason, efforts to matriculate and enable national public-private partnerships be open and inclusive, and encourage generating and sharing ideas from every corner of the algal biofuels industry, formulating new approaches that will benefit all and aid industry growth towards addressing the US agenda for sustainable transportation energy security. At the same time, these efforts must respect and enable investment aimed at building commercial-scale for-profit industry ultimately required to meet U.S. needs in sustainable transportation energy.

The Benefits of Algal Biofuels Public-Private Partnerships

The algal biofuels industry is evolving with numerous players, many focusing on their specialty along one to a few elements in algal biofuels value chain. Partnerships, based on sharing of knowledge and capabilities for mutual benefit, are needed to pull together the expertise and facilities, accelerating growth and enabling the development of a sustainable, algal biofuels system for this industry. Figure 11 shows the benefits of collaboration between private entities (e.g. industry) and public entities (e.g. national laboratories and universities) for development of the algal biofuels.* While benefiting both private and public entities from shared investment toward mutual objectives, public-private partnerships have the potential to accelerate commercialization of algal biofuel technology, leading to rapid industry growth and a stable market.

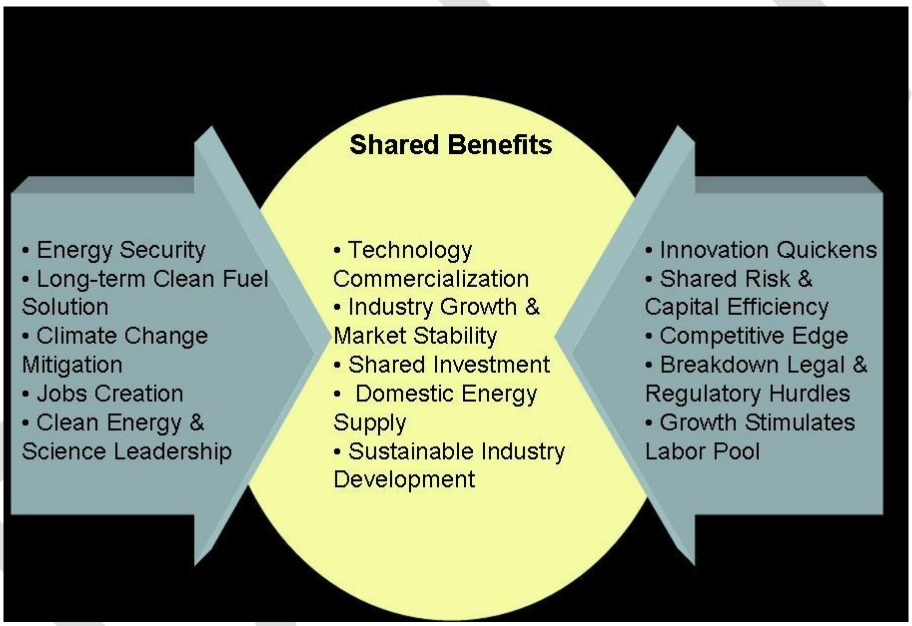


Figure 11: Benefits of algal biofuels public-private partnerships

Industry benefits from public-private partnerships from the exposure to fundamental science and engineering R&D through collaboration, thereby quickening the pace of innovation. This, in turn, increases the capital efficiency of commercial firms, many of which may be investor-backed and pre-revenue as well as reduces the risk of to private investment.

* In this situation, academia can be either public or private, realizing benefits in both categories.

Partnership Environment in the Algal Biofuels Industry

The algal biofuels industry ecosystem includes a broad cross-section of parties from multiple segments of industry and venture-backed investment, academia, government agencies, and national laboratories. As this industry moves forward, each of these entities will play a vital role in fundamental science and engineering R&D, technology development, addressing technical and regulatory hurdles, and creating jobs and a labor pool of talent.

The algal biofuels industry is currently comprised primarily of small technology-rich firms. These players are focused on various aspects of the algal biofuels value chain from algal growth to harvesting, extraction, and ultimately to refining and conversion to fuels. Some larger players, with experience from other industries, also contribute in the refining element, although many relevant larger companies appear to be following the algal biofuels industry without yet aggressively engaging. The product users include larger companies across the petrochemical (and it's feedstock customers) industry, agriculture, and the aviation industry.

Additionally, this industry is characterized by limited sharing of information, as industry players strive to protect their intellectual property. Given that no single player is yet ready to work the entire the algal biofuels value chain, an approach of limited commercial collaboration, combined with relative little federal investment as yet for pre-competitive R&D results in slower progress with higher risk for all. Rather, in all likelihood, the algal biofuels industry will be built by those who figure out how to work together, sharing information and allowing multiple links in the value chain to work in concert. Collaborations between publically and privately funded researchers are key to enabling the formation of these partnerships. Carefully conceived partnerships that promote sharing of information and technology while at the same time ensuring for-profit companies to provide a return to their investors through the commercialization and application of the resulting technology represent the best hope for accelerating the establishment of this industry.

Challenges for Algal Biofuels Public-Private Partnerships to Address

The key challenges that partnerships must address for the algal biofuels to become viable include the following:

- Technology development in algal biology, growth and harvesting, oil extraction, and fuel conversion;
- Pre-commercial-scale user facilities that are accessible to researchers and developers to evaluate their technologies;
- Clear regulations allowing for siting of algal facilities and production of acceptable algal products;
- Labor force and intellectual talent to draw upon; and
- An open environment that stimulates the sharing of ideas and technology across the entire algal biofuels value chain.

Challenges that are particularly pertinent to be addressed by a public-private partnership are highlighted below, loosely sorted according to the earlier sections in this document.

Algal Biology

The Workshop participants identified several areas that could benefit from some form of public-private partnership(s):

i) Share Understanding of Existing Current Strains & Coordinate Efforts to Identify New Strains

IP issues currently exacerbate the slow flow of relevant strain data. To optimize the investment of resources from both the public and private sector and accelerate progress in the industry, large-scale sampling and isolation activities for new strains of algae need to be conducted with careful coordination of the publically funded activities. Such efforts must account for the temporal success of microalgae in natural habitats and allow results to be assembled into a culture collection serving as a bioresource for further biofuels research. Given the phylogenetic diversity of microalgae, a large number of model systems could be studied; however, in a practical sense, the number to be studied in depth should be limited because a critical mass of researchers is required on a given species to make progress. A public-private partnership would be useful to fund, develop, and maintain a central strain, open access repository; perhaps, such a capability could be located at the culture collection centers at University of Texas at Austin (UTEX) and/or in West Boothbay Harbor, Maine (CCMP).

ii) Assist in Development of Basic Methods & Standards

It is often difficult to compare data generated by different labs. This is especially true in a new field where basic methods and standards have not yet been established. There is a need for a common database for global information on the characteristics of currently available algal strains. Of particular importance is the need to establish voluntary or otherwise common units of biomass productivity (e.g., gm dry weight/L/day). The central strain, open access repository noted above would assist by using common units of measurement.

iii) Coordinate Genome Sequencing Efforts between Public Sector & Private Industry

To accelerate progress and minimize duplication, efforts between the public-sector genome sequencing capabilities (e.g. DOE's JGI) and the efforts of the private sector might be coordinated so that taxpayer funds would be leveraged to those strains that the scientific community (spanning both the public and private sectors) concur as showing the most promise. A public-private partnership would be a useful vehicle to identify the criteria for selection and then prioritizing the organisms for genome sequencing and annotation. Clearly, while private concerns interested in particular strains will fund the sequencing of whatever strains present a value proposition for their interests, the issue here is one of overall leveraging of taxpayer dollars. With this in mind, the need for validated data and the consensus of the scientific community should be used to determine a prioritized list of target strains for public sequencing. In some sense, this would serve as a “master plan for genome analysis,” described earlier in the Algal Biology section (page 15).

iv) Develop a Robust Bioinformatics Infrastructure

A bioinformatics infrastructure that facilitates shared understanding and communication across the scientific community with respect to algal biofuels is non-existent. The absence of such a resource thwarts progress, but so too would the creation of such an infrastructure if ill-conceived. Quality standards and appropriate training should be developed and established to ensure consistent and useful annotation thus ensuring that the resulting annotated sequence data is usable by the larger scientific community. A standardized set of analysis approaches should be decided upon and implemented, particularly in the areas of transcriptomics, proteomics, metabolomics, lipidomics, and integrated data analysis. By its very nature, the development of such an infrastructure demands that stakeholders from all key customer, user, sponsoring groups, etc. come together to address the corresponding issues and chart a path forward to development.

v) Develop Key Facilities that are too capital intensive, risky, or both for either party

As described earlier, sustained RD&D efforts at the necessary scale will promote significant capital investment. Such investment is frequently too risky or simply out of the question (in terms of acquiring such capital) for the average start-up firm. Further, much of the RD&D needed is in areas that are sufficiently pre-competitive, and as such, no single entity in private industry will dare to bear the effort and risk to gain insight or overcome a challenge that will benefit the entire industry overall. Moreover, technologies developed in the laboratory have traditionally not translated well to the field, since the environment has a significant impact on algae performance. Open, collaborative facilities that allow precompetitive R&D and new technologies to be tested would accelerate technology development. Feedback from the Roadmap Workshop suggest that a core “omics” facility dedicated to algal biofuels and a facility devoted to the development of genetic manipulation tools that have application across multiple species would significantly reduce the development time for individual strains. The investment required to develop and maintain such a facility for some period of time is most appropriately within the purview of the government.

To leverage government investment (taxpayer funds), however, both the development of facilities for RD&D efforts and the efforts conducted therein should be coordinated. The suggestion of such coordination, however, may strike a disconcerting chord with many who perceive that in general, quasi-government and/or government performed research takes longer to accomplish particular milestones than if the same R&D were performed outside of the government environment. Consequently, a public-private partnership with mechanisms (paths) for both precompetitive R&D as well as private R&D could be envisioned to generate the IP necessary to establish and capitalize for-profit commercial entities. Such a partnership would be particularly useful in establishing the functional requirements so that ultimately the design, implementation, use and maintenance of such a facility meet the requirements of a broad user base.

Algal Cultivation and Processing

For the same reasons as above, public-private partnership(s) are also the most viable means to fund, design, develop, start up, operate and maintain joint-use, open-access facility(ies) for large-scale R&D that address cultivation and downstream processes. Dynamic pond monitoring will be important for both wild-type and genetically modified algae whose competitiveness in the field cannot be accurately predicted. This effort will require a significant investment toward basic research in multi-trophic, molecular-level algal ecology, the costs and risks of which are perhaps best borne by a public-private partnership.

Further, algal biomass suffers from a lack of well-defined and demonstrated industrial-scale methods of extraction and fractionation. Inextricably linked with the processing subsystems are the significant issues of energy requirements and the associated costs (with cultivation, harvesting, etc.). Sharing of the costs and insights gained would be particularly useful in focusing further investments in preferred methods, and process and tool development, and in providing critical data to techno-economic modeling efforts.

Conversion to Fuels "Fit for Use", Distribution & Utilization

Today, all of the petroleum feedstock that enters a conventional petroleum refinery must leave as marketable products, and this conservation law also must hold true for the algae biorefineries of the future if they are to achieve significant market penetration and displace fossil fuels. The feedstock, conversion process, and final fuel specifications are highly interdependent and must be considered together if an optimal process is to be identified. However, the greatest challenge in algal fuel conversion is likely to be how to best use the algal remnants after the lipids or other desirable fuel precursors have been extracted. 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. Lifecycle analysis of energy and carbon will be a key tool in selecting the winning fuel conversion technologies.

Resources & Siting, Regulations & Policy, and Systems Analysis & Techno-Economic Modeling

Resources and siting, regulations and policy, and systems analysis and techno-economic modeling are highly interdependent topics. Singly and together, they may perhaps represent the loudest cry-out for public-private partnerships as all other efforts associated with research, technology development, processing systems, proof of pathways, etc., must be undertaken within the context and framework associated with these systems issues.

Resources and siting issues for algal biofuels scale-up are dominated by land use, water supplies, nutrient supplies, required energy inputs, and related regulatory policies, some of which are outside the purview of DOE. Given U.S. needs in sustainable transportation energy, the potential presented by algal biofuels, and the current state of knowledge and commercial activity, the birth of a new industry "from the ground up," is anticipated. Hence, the existing regulatory processes that potentially impact this industry, including the role of federal, state and local agencies that presently regulate one or more aspects of growing or processing algae, need to be identified. Future potential roles for agencies that will become essential as the industry develops need to be anticipated and addressed.

The challenges ahead for large-scale cultivation and processing of algae for biofuels are significant and R&D teams should include techno-economic assessment efforts. The economic viability of sustainable microalgal cultivation enterprise is a very interdependent equation involving multiple interfaces between technical research, integration and optimization research, and the changing world of regulatory and incentive policies (e.g. carbon credits). At the Workshop, there were repeated calls from various stakeholders for life cycle analyses and environmental impact studies to be used to guide regulatory and policy decisions. Such studies are inextricably linked to technoeconomic analyses, which for now must be based on an assortment of assumptions and data extrapolated from small-scale laboratory work or from the cultivation of algae for higher-value products, as an algal biofuel industry does not presently exist anywhere in the world. For example, when trying to model a subsystem level (e.g., large-scale cultivation process), the modelers will require input in terms of assumed values or ranges (for production unit costs, etc.). Without a fully developed industry, standards therein and any model likely to be useful to many must be non-proprietary and include data based on average or assumed values.

A feasible algae-to-fuel strategy must consider the energy costs and siting issues associated with each subsystem (e.g., cultivation, harvesting, dewatering, etc.). Cost estimates for lifecycle modeling of a particular process will be needed, but lacking any public-private partnership, it will be difficult to validate enough cost data points for a particular process to know that the model has much validity at all.

Lastly, systems analysis and techno-economic modeling will also be complicated by the requirement to cover many potential process options, as it is not yet clear which ones have the most commercial potential. One or more public-private partnerships could serve valuable roles as the interface/broker to provide data, feedback, and to ensure accountability and coordination along these fronts.

Various Roles Anticipated by Stakeholders

Government

Government, including DOE, its national laboratories, and other agencies (e.g, USDA, DOD, NASA, and FAA), can bring significant value to public-private partnerships for algal biofuels. They can conduct unique research requiring multidisciplinary approaches and differentiating R&D infrastructure. Further, government laboratories house world-class user facilities. Government can also bridge knowledge gaps across the algal biofuels value chain and through technology development, from foundational research to commercialization. Working with algal trade organizations, government can also help disseminate critical information to facilitate sharing of research, helping to advance the algal biofuels industry.

There is a role for government at each stage of the process from fundamental research to pilot-scale testing and cost-sharing of first-generation algal-based biorefineries. As the algal biofuels industry does not yet have a product in any meaningful quantity in the biofuels market, requiring matching funding from this nascent industry would likely possible primarily in a research context. Government should seek to disseminate pre-competitive research towards accelerating industry growth and decreasing the time required for the industry to bring product to market and becoming economically sustainable.

Federal leadership and investment towards developing a successful algal-based biofuels industry has several advantages. Through this process, the federal government can leverage both funding and cross-cutting collaborative efforts to fulfill the gaps in scientific knowledge, provide support for novel approaches and pilot demonstrations that will reduce risk for investors and speed deployment of algal biofuels.
Government can play the following roles in advancing the algal biofuels industry, serving the interests of society, nation, as well as business:

- encourage the formation of partnerships and successful technology transfers;
- provide funding, taking on early high risk in the development of critical, sustainable technologies;
- establish clear regulations (discussed in greater detail in the Policy section);
- implement unbiased assessments of technology advancements and the associated societal benefits (e.g. sustainability) in the form of publicly available reports;
- commission national resources to advance algal biofuels, including unique areas of research and environmental impact studies; and
- coordinate policymaking and funding for algal biofuels research, development, demonstration and deployment (RDD&D) initiatives among U.S. government agencies.

One way to implement interagency coordination is to adapt existing policy instruments that foster collaborations across agencies for producing lignocellulosic biofuels to also include algal biomass. Among these, the Biomass R&D Board, whose appointees include both federal agency leadership as well as external experts, is a good example of interagency coordination. Setting up clear and transparent funding guidelines will be important to ensure government-funded research is unique and relevant

Algal-based biofuel development can leverage the lessons learned from DOE's cellulosic ethanol biofuels program and apply many of the same tools and insights that have led toward funding cross-cutting research leading to new insights and achieving technical targets needed to bringing cellulosic-based ethanol closer to fruition.

Individual Companies within the Private Sector

Given the present state of the industry, the role that individual companies might play is unclear and best left to market-driven evolution. Rather, the government's interest must lie in ensuring that public-private partnerships receiving taxpayer funds serve the national interest as well as individual commercial concerns.

Emerging Trade Organizations

As discussed in the Regulatory & Policy section, the Algal Biomass Organization (ABO), a 501C-6 trade association formed in 2007, has begun an effort to establish a comprehensive list of standards to cover the entire algal biomass value chain, from raw materials to finished product modeled after the IEEE Standards Association.[*] Other collaborative efforts related to one or more subsystems of the overall algae-to-fuel lifecycle already exist (e.g., California Biomass Collaborative, Southwestern Biofuels Association, San Diego Center for Algae Biotechnology, etc.). Participation of trade organizations in public-private partnership model would be highly valuable.

Academia

Universities and community colleges have an important role to play in the development of this industry. Academic training will be critical to prepare scientists, engineers, operators, economists, and technology managers who will make up the intellectual workforce for algal biofuels. Universities also function as a place to stimulate the exchange of ideas by enabling an open environment for scientific exchanges, conducting high quality research, especially at the individual investigator scale, and serving as environments to develop and implement new tools, analyses and processes (such as genomic information, highly sensitive imaging and chemical detection technologies, high-throughput devices, catalysts, supercomputers, modeling software, and separation technologies). The means of transitioning IP from academia to industry should be enhanced to quicken the pace of commercialization for the benefit of both academia and industry. As such, members of academic institutions should be encouraged to join appropriate public-private partnerships.

Partnership Models

There are many models for public-private partnerships but none specific to the unique space occupied by the algal biofuels industry. The problem with four illustrative models presented below (Table 5) and many others that exist is that they were all formed relative to an existing industry, not one where the goal is to develop the industry from its emergence stage. Therefore, it should not be expected that any one specific model will meet all of the needs of the algal biofuels industry.

Nevertheless, one approach that might prove useful to conceptualizing the various models

- for public-private partnerships is to think in terms of the five attributes of successful partnerships discussed earlier within the context of particular scenarios (e.g. particular algal strain, dewatering pathway, conversion process, etc.) or end goals (specific intended use, performance aspects of the fuel, etc.). Doing so may help define the boundary problem(s) for focus by the public-private partnership and bring clarity to the composition, requirements, and expected contributions of the membership

[*] IEEE Standards Association: A unit of the Institute of Electrical and Electronics Engineers, an international non-profit, professional organization for the advancement of technologies related to electricity.

A model may be evaluated for applicability against the following criteria:

- Openness – How inclusive is the membership to its industry (or segment thereof)?
- Technology Commercialization – Is it structured to develop and commercialize new technology?
- Industry Growth – Does it seek to grow the industry?
- Shared Investment – Does it share investment equitably?

Table 5 compares several existing public-private partnership models against these four external characteristics and other characteristics (e.g., number of members, type of legal entity, etc.) The models presented are intended to serve only as examples of these four external characteristics for the algal biofuels industry and to prompt members of a would-be algal biofuels public-private partnership to consider these attributes and models, discuss and debate the merits of each, and select the best or optimal combination of attributes to meet the specific mission of their partnership.

Models for Openness

AGATE, CITRIS, NINE, and SEMATECH were all examples of partnership models with a high degree of openness in terms of membership and sharing of knowledge through various kinds of activities. AGATE, CITRIS, and NINE each had over 60 participants in their organizations, while SEMATECH had over 50% of the global semiconductor production through its 16 members. With the high-level of participation from their sectors, these organizations can effectively represent and address critical needs for their industry. These organizations facilitate new approaches to address critical needs through periodic technical meetings and forums that foster cross-collaboration amongst participants. The open-membership aspect of these models allows for new ideas to be injected into the collaborative environment, accelerating technology development. These four organizations varied in the type of entity they created (501c6, consortium, and university institute), the lead organization (DOE, NASA, DARPA, and the University of California), and the funding they received; but all of them had a common objective to maximize industry involvement within their organization.

Models for Technology Commercialization

SEMATECH and AGATE offer the best models for technology commercialization. Commercialization is more likely to occur when industry collaborates in research and development; this is absent in the models for NINE and CITRIS. DOE's Bioenergy Research Centers offered reasonable approaches for technology commercialization, but industry involvement is small as compared to SEMATECH and AGATE. SEMATECH has the most sophisticated process for technology commercialization; collaborative pre-competitive research is selected and conducted by the membership, ensuring that the membership perform work of common interest and benefit and avoid competing interests; the results are transferred by publication and/or the member-only website. Further development to a commercially viable solution occurs with external partners maintained by SEMATECH; SEMATECH provides non-exclusive, royalty-free licenses to its members and preserves the ability to license technology to outside parties for commercialization. Moreover, with over 40 industrial members from the general aviation industry, the AGATE consortium was able to successfully focus on critical needs in lightweight, affordable jet engine design, multifunction display for navigation and power control, streamlined flight training curriculum, real-time weather data link technology, and lightning protection. Recognizing the importance of IP issues, AGATE members agreed to cross-license background IP, as well as newly developed IP, at reasonable rates, avoiding roadblocks in commercialization. Facilitated through the AGATE consortium, these technologies moved from research concepts to adoption into the marketplace. Both of these examples indicate how many partners were able to come together and determine in what areas they can collaborate to their mutual benefit, while reducing IP concerns so that technologies can be effectively commercialized.

Models for Industry Growth

The Bioenergy Research Centers, AGATE, and SEMATECH offer the best models for industry growth. Each of these organizations was designed to attack specific technical, operational, or regulatory hurdles limiting industry growth. SEMATECH was organized to increase competitiveness of U.S. semiconductor industry as a result of the market threat from Japanese semiconductor firms. AGATE, the largest industrial consortium of its time with both large established firms and small businesses in the general aviation market, was designed to develop technology and standards that would create operational efficiencies for all market firms, which had seen a dramatic decrease in small aviation market demand. The Bioenergy Research Centers were conceived to address high risk, game-changing technical challenges that need to be resolved to make the cellulosic biofuels industry economically and environmentally sustainable. While each has been successful in aiding industry growth, the Bioenergy Research Centers are more closely aligned with the needs of the algal biofuels industry in terms of the maturity of the market and the overwhelming number of start-ups in the industry.

Models for Shared Investment

The partnership models shown in Table 6 indicate varying degrees of shared investment between the government and its partners. The Bioenergy Energy Centers, NINE, and CITRIS were funded predominately by government with some participation by industry. SEMATECH and AGATE are models based on significant government funding and matching funding from industry; SEMATECH and AGATE were designed to support industries with an existing market. The algal biofuels industry does not yet enjoy such investment.

Recommendations and Timeline

The challenges that seem most amenable to being addressed through public-private partnership are aligned with DOE's mission but are not solely within its mission space. As such, the Workshop participants agreeably felt that a lead agency such as DOE would need to serve as the "sponsor" or "lead" public organization to ensure clarity in terms of relative authority within a public-private partnership. Several key efforts that might be sponsored by the government within the context of some public-private partnership(s) are highlighted below. The government should support each to varying degrees:

1. Commercialization of algal cultivation facilities co-located with industrial CO_2 sources and/or wastewater treatment facilities.
2. Establishment of national algae biomass production test-beds to conduct research at the pilot scale (5-10 acres). These testbeds could be located at power plants, wastewater treatment facilities, and agricultural drainage/water body restoration sites to allow for adequate investigation of the role of these input groups in the overall economic viability of production processes. This effort could involve a consortium of R&D organizations, universities, algal cultivation companies, algal technology companies, refiners, distributors, and other participants coordinated by DOE at the national level.
3. Independent evaluation of any given technology's TRL (Technology Readiness Level) so that government agencies can fund in the earlier stages with the knowledge and "interest and pull" of other industrial entities who would assume the handoff to further the TRL and commercialize.
4. Education and development programs to grow the specific labor pool needed to run algal-biofuels related operations, develop new algae-based fuels and co-products, and innovate new cost-cutting measures.
5. Clarification of pertinent regulations and development of a comprehensive list of standards to eliminate the uncertainties in commercialization of algae-based technologies, thus encouraging investment and promoting partnering opportunities. DOE cannot be expected to take a lead on this effort because only a small subset of standards will relate directly to biofuel production. Nonetheless DOE could be instrumental in supporting this effort by providing funding for the accumulation of data needed to craft the standards. It could also help by promoting cooperation of federal regulatory agencies (e.g. USDA, EPA, and FDA) that will have jurisdiction over various aspects of the algal biomass industry.
6. Development of computer models of algae production facilities that will aid in: rapid and consistent engineering design; techno-economic analyses; LCA/GHG abatement analysis; the evaluation of economies of scale vs. advantages of decentralized production considering parasitic losses of CO_2 transport, etc.; the evaluation of temperature control (power plant cooling and algae pond heating); and the development of efficiently designed and operated test-bed facilities.

Table 5: Comparison of Public-Private Partnership Models

PPP Model	Entity Type	Openness of PPP	Technology Commercialization	Industry Growth	Shared Investment
SEMATECH[1, 2]	Non-Profit Corporation (501c6) with two facilities and three subsidiaries	• Open membership • 16 members, 50% of global semiconductor production • Forums inspiring cross collaboration amongst members • Public conferences through Knowledge Series	• Collaborate on pre-competitive R&D selected by membership • Transfer of technology by publication or member-website data transfer • Technology further developed to manufacturing solutions with external partners; then adopted • SEMATECH owns created IP and provides non-exclusive, royalty-free license to members • SEMATECH can also license IP to third parties	• Designed to increase competitiveness of existing US firms in the semiconductor marketplace • Provides commercialization network that drives economic development • Develops coordinated industry roadmap to focus R&D and spur on economic growth	• Government funding: $500M over 5 years • Industry: Match government funding • Now funded solely by industry
DOE BIOENERGY RESEARCH CENTERS (BRCs): JBEI[3], GLBRC[4], BESC[5]	DOE Center - Not a separate legal entity with employees	• Initial partners defined; • JBEI has partner slots open • BRCs have 1 or 2 industrial partners, with several university partners • Public conferences and workshops at GLBRC create opportunity for collaboration	• Research conducted at BRC, other DOE facilities, or partner facilities by DOE and/or partners • Few industry partners to commercialize developed IP • IP licensed to interested parties with an evaluation of commercialization potential • Transfer of technology by publication	• Address significant technical game-changing, high-risk barriers for cellulosic biofuels • Industry growth supported through education • Limited industry involvement to address issues on industry growth	• Government funding: $125M over 5 years • Significant funding from State of Wisconsin and private sources for GLBRC • Significant funding from State of Tennessee for BESC • Potential cash and in-kind contributions for JBEI
NATIONAL INSTITUTE FOR NANOTECHNOLOGY EDUCATION - NINE[6]	Non-Profit Corporation (501c6) with one host facility	• Open membership • Consortium of 60 industry, academic and national lab partners;	• Pre-competitive research selected by all NINE members • Research conducted by Sandia and member universities • NINE provides non-exclusive,	• Industry growth through education • Technology only licensed to members	• Government funding approved for $10M over 5 years; not yet appropriated • Founding member commitment of $300K over 3 years

		• Technical workshops technical, business, and social issues inspire cross collaboration amongst members	paid-up license to industry members		
ADVANCED GENERAL AVIATION TRANSPORT EXPERIMENTS - AGATE[7, 8]	Industry consortium with NASA & FAA - Not a separate legal entity with employees	• Open membership, • Consortium of 76 industry, academic and government partners, including more than 40 representatives from industry;	• Collaborative research amongst consortium members designed to reduce technical, operational, and regulatory bottlenecks • IP licensed exclusively or non-exclusively • Commercialization afforded as members agree to cross license background and newly developed IP non-exclusively to each other royalty-free • Transfer of technology by publication, and transfer of knowledge through member-only database	• Technology development and standardization designed to reduce operational costs and increase general aviation market, including large established firms and small businesses • Significant industry involvement provides market focus and commercialization network	• Government funding of $100M over 8 years • Industry match of $100M over 8 years
CENTER FOR INFORMATION TECHNOLOGY RESEARCH IN THE INTEREST OF SOCIETY - CITRIS[9]	University of California Institute - Not a separate legal entity with employees	• Donor-driven model • Institute involving over 60 IT industry partners and 4 UC campuses • Numerous Forums inspiring cross collaboration amongst researchers and inaction with industry	• Research conducted by four University of California campuses • Software is open source licensed • Other IP is either licensed non-exclusively, royalty-free basis or exclusively, royalty basis as needed to achieve the widest possible dissemination.	• Industry growth supported through education • Limited industry involvement through Advisory committee to address issues on industry growth	• Funding of $200M over 4 years provided by State of California, UC Campus funds, and industry gifts • No Federal funding

Appendix:

Scenarios Illustrating Preliminary Consequence Assessment:

Land, Water, and CO_2 Demand for Algal Biofuels Scale-up

Establishing the Basis for Initial Algal Production Scale-up Assessments

Autotrophic algal productivity is typically measured in terms of dry weight biomass produced per day per unit of illuminated cultivation system (open pond, closed photobioreactor, or hybrid combination of open and closed systems) surface area. Typical units of measure include annual average grams/meter^2-day, metric-tonnes/hectare-year, or tons/acre-year of dry-weight-equivalent biomass. Neutral lipid (oil) content in algae is typically measured in terms of percentage of dry weight biomass, resulting in oil productivity typically being measured in terms of metric-tonnes/hectare-year or gallons/acre-year.

Unit conversion factors useful for translation among the various units of measure can be found at the end of this Appendix.

The high energy density neutral lipid oils of immediate interest as biofuel feedstock from algae, as well as from other more conventional oil crops and waste oil sources (Tyson, et.al. 2004), consists largely of triacylglycerol (TAG). The volumetric density of TAG vegetable oils is ~ 0.92-grams/ml, which is equivalent to about 7.6-lbs/gal.

Assuming an annual daily average algal biomass productivity of P_{BD} [grams/m^2-day] and an annual average oil content of L [%] produced over the period of a full 365-day year, the resulting annual average biomass production P_{BA} [mt/ha-yr] and annual average oil production P_{OA} [gal/ac-yr] is be given by:

$$P_{BA}\text{ [mt/ha-yr]} = 3.65\text{ [mt-m}^2\text{-d/g-ha-yr]} \times P_{BD}\text{ [gram/m}^2\text{-day]} \qquad \text{(Eq B-1)}$$

$$P_{OA}\text{ [gal/ac-yr]} = 1.17\text{ [gal-ha/mt-ac]} \times L\text{ [\%]} \times P_{BA}\text{ [mt/ha-yr]} \qquad \text{(Eq B-2)}$$

$$P_{OA}\text{ [gal/ac-yr]} = 1.17 \times 3.65 \times L\text{ [\%]} \times P_{BD}\text{ [g/m}^2\text{-d]}$$

$$= 4.27\text{ [gal-m}^2\text{-d/g-ac-yr]} \times L\text{ [\%]} \times P_{BD}\text{ [g/m}^2\text{-d]} \qquad \text{(Eq B-3)}$$

As an example, if we assume P_{BD} = 30 g/m^2-day and L= 25 % oil content, using the above equations gives (without specifying the units on the leading coefficient 4.27):

$$P_{OA}\text{ [gal/ac-yr]} = 4.27 \times 25\text{ [\%]} \times 30\text{ [g/m}^2\text{-day]} \sim 3200\text{ gal/ac-yr.}$$

Figure B-1 provides a parametric mapping of annual average algal oil production, P_{OA}, in gal/ac-yr as a function of annual average daily biomass productivity, P_{BD}, in g/m^2-day and annual average neutral lipid content L in percent of dry weight algal biomass, as described in above equations. The example calculation above is also plotted in Figure B-1 for illustration. The simple conversion equations given above, and the parametric plot in Figure B-1, provide a quick means of translating annual average daily algal biomass productivities and oil content into annual production projections on a gallons per acre basis.

A key attraction of algae for biofuel feedstock production is the potential for high annual oil productivity per unit of area (i.e., P_{OA}). Projections for achievable annual average productivities for large commercial scale operations have ranged widely in the public domain and continue to be the subject of uncertainty and debate. Table B-0 includes the results of productivity calculations assuming Weyer's (Weyer, et.al. 2008) theoretical maximum (red row) as well as more moderate assumptions of productivity (green row).

g/m2/day	percent lipids	gal/acre/year	liter/ha/year
15	10	633	5929
25	25	2639	24705
25	50	5278	49410
50	50	10556	98821
100	50	21113	197642
180	70	53204	498057

Table B-0: Algae Productivity Calculations

Figure B-2 presents the results of Weyer's recent analysis (Weyer, et.al. 2008) suggesting an upper theoretical limit on the order of 50,000-gal/ac-yr and perhaps a practical limit on the order of 5000-6500 gal/ac-yr, based on the assumptions made in the analysis (high solar insolation consistent with lower latitudes and/or high percentage of clear weather conditions, 50 % oil content). An interesting feature of the assessment is the comparison with other projections from the open literature noted in Figure B-2.

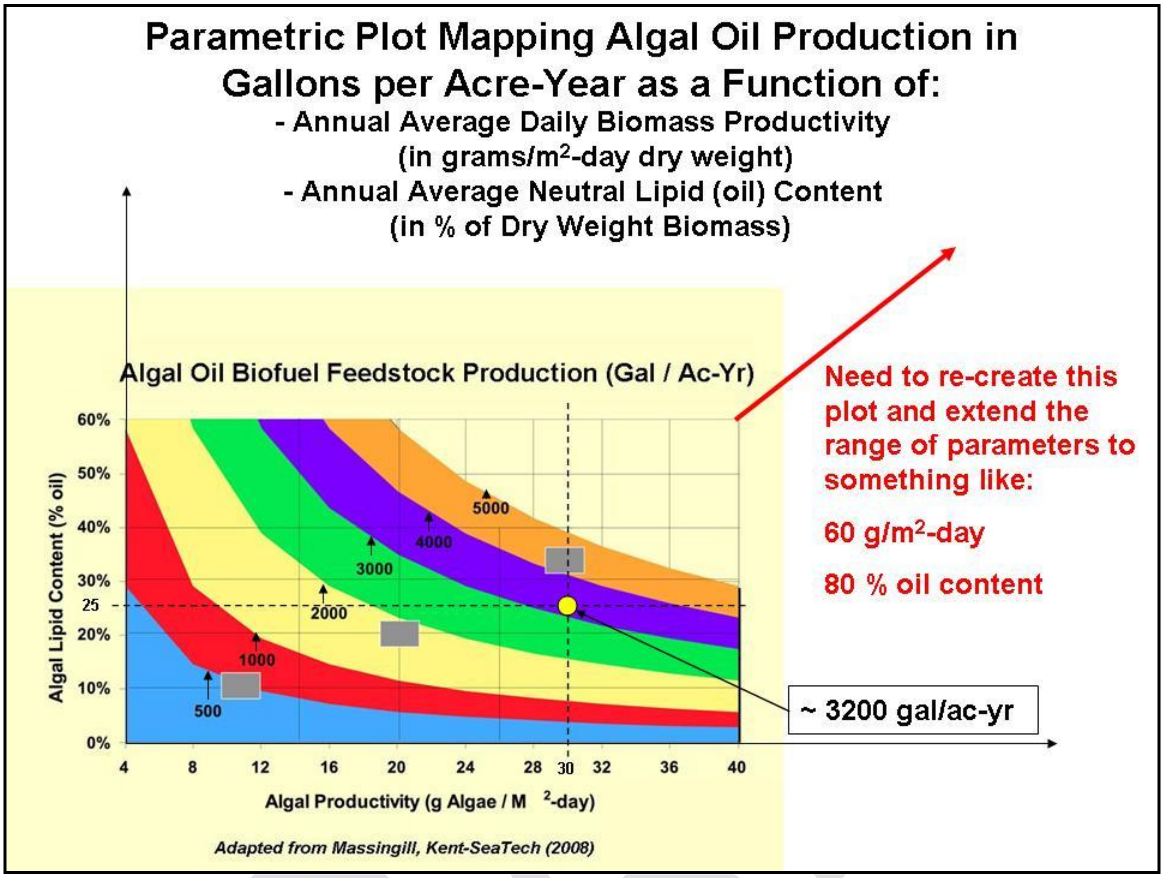


Figure B-1. Mapping of estimated annual algal oil production in gallons per acre as a function of annual average algal biomass productivity, in grams per square-meter per day, and algal neutral lipid (oil) content as a percentage of dry weight biomass. (Adapted from Massingill 2008). … Place-holder figure… need to re-do.

For the sake of the preliminary consequence assessments presented here, we assume that algal oil productivities at scale under high solar resource and suitable temperature conditions will have a practical upper limit on the order of 6500-gal/ac-yr. We also assume that this may be achieved without specifying cultivation system details or configuration, other than to allow that it may be possible with open systems subject to maximum evaporative water loss, as will be discussed later. As noted above, the critical drivers for overall algal oil productivity will be the tradeoff between the achievable annual average daily biomass productivity and the average oil content, which can be expected to depend on the complex combination of algal strain, cultivation system, and local growing conditions, as discussed at length in other sections of this report. For the simple scaling assessments presented here, we simply ignore the complexities and details that will ultimately need to be addressed, and generally acknowledge that affordable and reliable optimization of the combination of these two critical production metrics will be key to favorable techno-economics for algal biofuels.

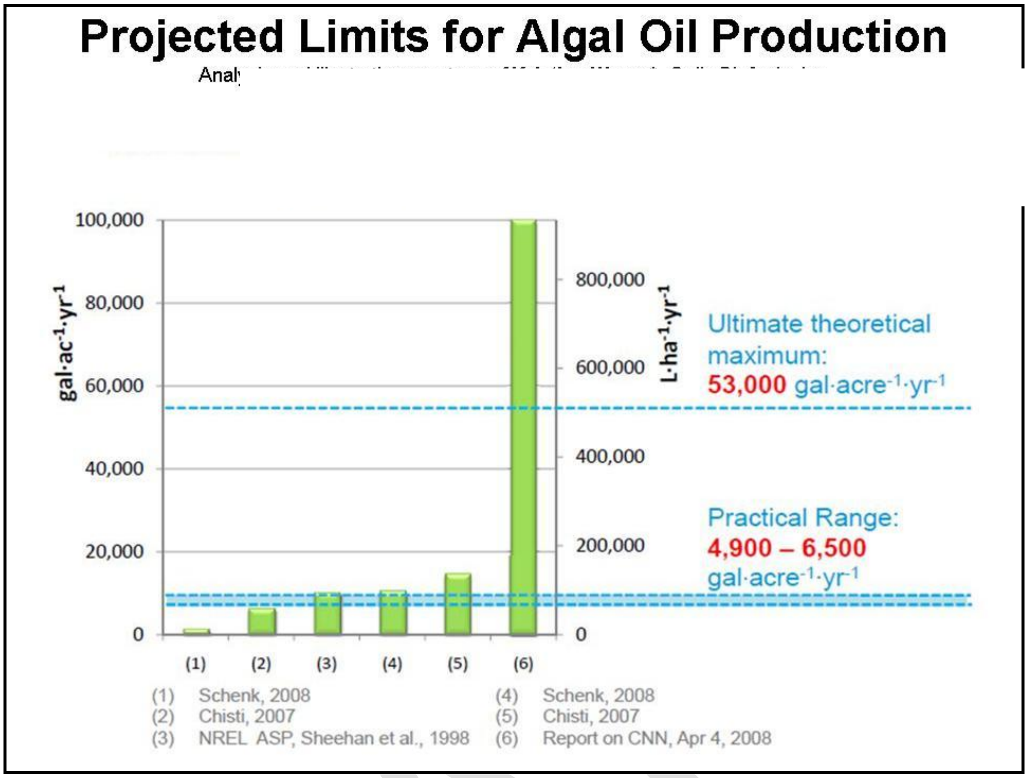


Figure B-2. Projected maximum theoretical and practical limits for algal oil production at nominal 20-degree latitude under high solar insolation conditions; compared with estimates reported from other open sources (Weyer, et.al. 2008).

Scenario-1: Projected Land Requirements for Algae as Compared to Corn and Soy Oils

As a first scenario example, it is instructive to compare the projected land footprint requirement among corn, soy, and algae for producing the volume of bio-oil feedstock needed to displace half of the roughly 44 billion gallons of petroleum diesel fuel currently used annually in the U.S. for transporation. Table B-1 provides a list of numerous conventional oil crops and representative yields (Attra 2006). Corn and soy do not have particularly high oil productivities on average, but they are interesting from the standpoint of being major U.S. commodity crops, with large acreages in production and yields that vary depending on geographic location and whether irrigation is used (USDA 2009b). Corn has relatively low average oil productivity on the order of about 18-gal/ac-yr, while soy has somewhat higher average oil productivity on the order of about 48-gal/ac-yr, as noted in Table B-1. For algae, we will assume a productivity on the order of 5000-gal/ac-yr, which is consistent with the practical limits discussed earlier.

The production target for this scenario is to displace 50% of the petroleum-based diesel fuel currently used for transportation, or 22-billion gallons, with biofuel in the form of biodiesel or green diesel derived from the corn, soy, or algae derived vegetable oil. We will assume that the volumetric conversion efficiency (gallons of biofuel produced per

Table B-1. Conventional Oil Crops and Yield Estimates (Attra, 2006).

Conventional Oil Producing Crops and Typical Oil Yield

Plant	Latin Name	Gal Oil/ Acre	Plant	Latin Name	Gal Oil/ Acre
Oil Palm	*Elaeis guineensis*	610	Rice	Oriza sativa L.	85
Macauba Palm	*Acrocomia aculeata*	461	Buffalo Gourd	*Cucurbita foetidissima*	81
Pequi	*Caryocar brasiliense*	383	Safflower	*Carthamus tinctorius*	80
Buriti Palm	*Mauritia flexuosa*	335	Crambe	*Crambe abyssinica*	72
Oiticia	*Licania rigida*	307	Sesame	*Sesamum indicum*	71
Coconut	*Cocos nucifera*	276	Camelina	*Camelina sativa*	60
Avocado	*Persea americana*	270	Mustard	*Brassica alba*	59
Brazil Nut	*Bertholletia excelsa*	245	Coriander	*Coriandrum sativum*	55
Macadamia Nut	*Macadamia terniflora*	230	Pumpkin Seed	*Cucurbita pepo*	55
Jatropa	*Jatropha curcas*	194	Euphorbia	*Euphorbia lagascae*	54
Babassu Palm	*Orbignya martiana*	188	Hazelnut	*Corylus avellana*	49
Jojoba	*Simmondsia chinensis*	186	Linseed	*Linum usitatissimum*	49
Pecan	*Carya illinoensis*	183	Coffee	*Coffea arabica*	47
Bacuri	*Platonia insignis*	146	Soybean	*Glycine max*	46 *
Castor Bean	*Ricinus communis*	145	Hemp	*Cannabis sativa*	37
Gopher Plant	*Euphorbia lathyris*	137	Cotton	*Gossypium hirsutum*	33
Piassava	*Attalea funifera*	136	Calendula	*Calendula officinalis*	31
Olive Tree	*Olea europaea*	124	Kenaf	*Hibiscus cannabinus L.*	28
Rapeseed	*Brassica napus*	122	Rubber Seed	*Hevea brasiliensis*	26
Opium Poppy	*Papaver somniferum*	119	Lupine	*Lupinus albus*	24
Peanut	*Ariachis hypogaea*	109	Palm	*Erythea salvadorensis*	23
Cocoa	*Theobroma cacao*	105	Oat	*Avena sativa*	22
Sunflower	*Helianthus annuus*	98	Cashew Nut	*Anacardium occidentale*	18
Tung Oil Tree	*Aleurites fordii*	96	Corn	*Zea mays*	18 *

gallon of input vegetable oil feedstock) will be about 80%, regardless of the final type of fuel, with the understanding that there will also be other by-product fractions. This requires that 22/0.8 (=27.5) billion gallons of vegetable oil feedstock must be produced annually.

Corn, at 18-gal/ac, would require just over 1.5-billion acres of land (~ 2.3-million square miles), which is about 80 % of the total land area of the lower-48 states (~ 1.9-billion acres), is about factor of three and a half times greater than the entire cropland of the U.S. (~ 440-million acres) and is about a factor of eighteen higher than the current U.S. corn acreage of about 86-million acres (USDA 2006; USDA 2009b). Soy, at 48-gal/ac, would require about 570-million acres (slightly below 0.9-million square miles), which is 130 % of all U.S. cropland and is over a factor of seven greater than the current U.S. soy acreage

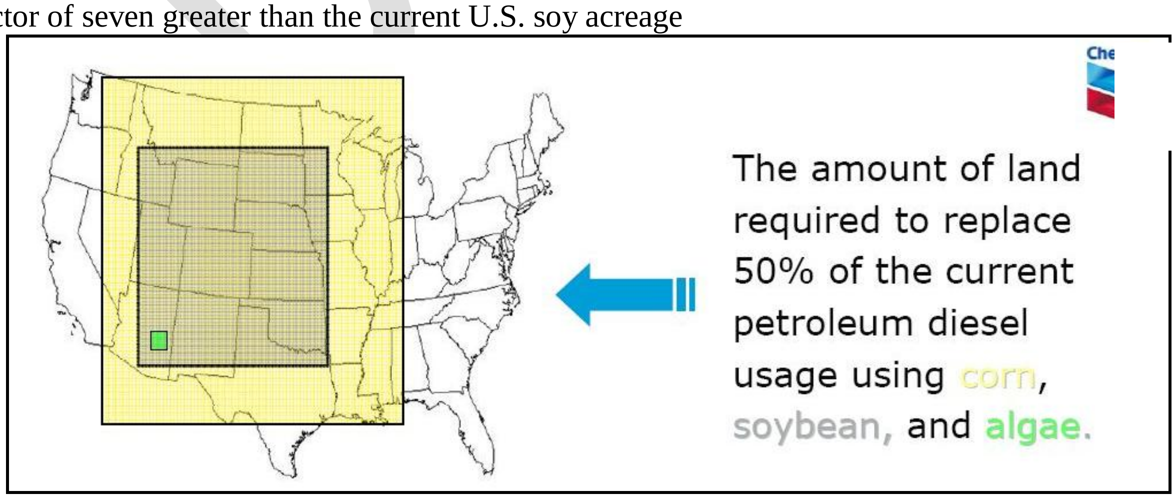


Figure B-3. Land footprint and oil production tradeoffs of corn, soy, and algae(adapted from Bryan, et.al. 2008).

(about 80-million acres). Algae, at 5000-gal/ac, would require 5.5-million acres (about 8500 square miles), which is about 7.6 % and 7.0 %, respectively, of the land area of the State of AZ and the State of NM. Figure B-3, adapted from Chevron (Bryan, et.al. 2008), provides illustration of this scenario.

Scenario-2. Land Area for Commercial Scale Algae Biofuel Feedstock Production

If we assume that a commercial scale algal biofuel feedstock production operation would be on the order of 10-million gal/yr to 50-million gal/yr of oil feedstock output, then with an area productivity target of 5000-gal/ac-yr, this would require from 2000-ac to 10,000-ac of algal cultivation area. A glance at the parameter map in Figure B-1 suggests that this productivity target could be achieved, for example, with an annual average 20-gram/m^2-d at 60% oil content, or with 40-g/m^2-day at 30% oil content.

Basis for Order-of-Magnitude Projections of CO_2 Utilization with Algae Production

Autotrophic algae growth and biomass production can be enhanced with CO_2 from stationary sources, such as flue gas from fossil-fired power plants (Kadam, 1997; Kadam, 2002; Sun, et.al, 2008; ben-Amotz 2007; ben-Amotz 2008). Rough estimates of CO_2 utilization are discussed here as a useful exercise to gain insight and appreciation for the opportunities and challenges for carbon capture in algae biomass and reuse in the form of algal based transportation fuels.

The carbon mass balance for algal biomass growth using the metabolic breakdown and conversion of CO_2 during photosynthesis results in approximately 1.6 to 2 mass units of CO_2 being consumed for every mass unit (dry weight equivalent) of biomass produced (Van Harmelen, et.al, 2006; Chisti, 2007; Schenk, et.al. 2008; Sun, et.al. 2008). This “CO2 utilization factor” depends on algae type, growth conditions, and relative percentage of carbon partition within the biomass. The process of bio-fixation of CO2 takes place only during sunlight hours when photosynthesis is active. In the absence of storage, only the CO2 emitted during the sunlight hours can be captured and incorporated into the algal biomass.

The efficiency with which CO2 will actually be taken up by the algae will be a function of the algae, the growth system size and configuration, and the dynamic operational conditions. The resulting efficiency will be less than 100%. Efficiencies in excess of 90% have been reported (Sheehan, et.al. 1998; Van Harmelen, et.al. 2006), but for this discussion we will assume that an efficiency of ~ 80% can be achieved on an annual average basis during sunlight hours. The remaining fraction of CO2 not taken up by the algae will escape into the environment, unless other measures are taken.

A simple way to view this “capture efficiency” is to think of the cultivated algae as a “sponge” or “sink” that has the capability to absorb and consume the CO2. The size or capacity of the “sponge/sink” must be appropriately matched to the volume of CO2 being made available to maximize the capture and consumption of the CO2. For algae under cultivation, this means that the productive area of the algae farm and the algae culture density and growth rates must be such that maximum use can be made of the available CO2. If the "sponge/sink" is too small, less of the CO2 can be effectively utilized and the capture/consumption efficiency will be lower. Here, we assume that the algae "sponge/sink" can be made large enough to capture/consume 80% of the CO2 delivered. For simplicity, we ignore the details that involve the design of the cultivation system, the way the CO2 is distributed and injected into the system, and the measures that must be taken to assure the appropriate maintenance of other key nutrient levels and growing conditions, all of which will can contribute to achieving higher CO2 use efficiency.

The effective sunlight hours per day at any given site will vary as a function of latitude and season, and will also be modulated by weather conditions such as cloud cover. We assume for simplicity that the effective annual average daily sunlight period when photosynthesis is active will be 8-hours, or one third of the 24-hour day. The rate of CO2 emissions from fossil-fired power plants will vary with the type of plant technology and type of fuel used. Figure B-4 provides representative CO2 emission rates for typical coal, oil, and gas fired plants in units of kg-CO2 per MWh of power generation (Bill, et.al. 2001).

Natural gas fired power plants emit about 450-kg of CO2 per MWh of operation, while coal-fired power plants emit about 920-kg of CO2 per MWh of operation, roughly a factor of two greater. A simplified illustration of the CO2 mass flows and use by algae that is assumed in this discussion is shown in Figure B-5.

Using these assumptions, we consider several scenarios that provide simplified projections for CO2 utilization by algae.

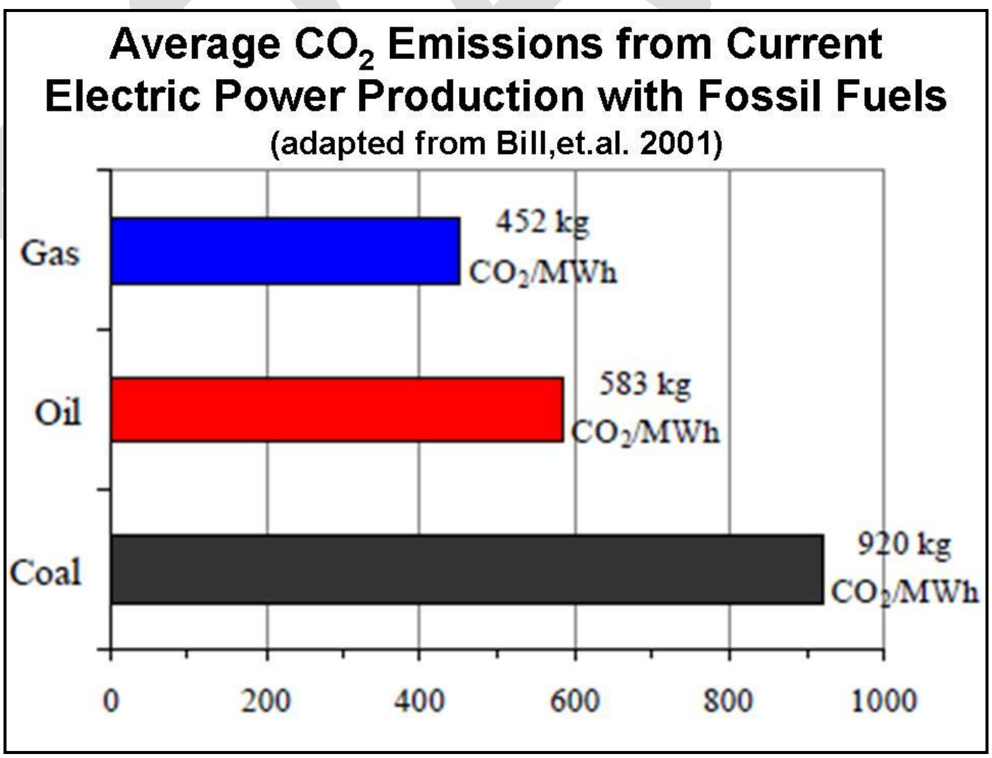


Figure B-4. Average estimates of CO2 Emission Rates in kg per MWh from Fossil-Fired Electric Power Generation Plants (Bill, et.al. 2001).

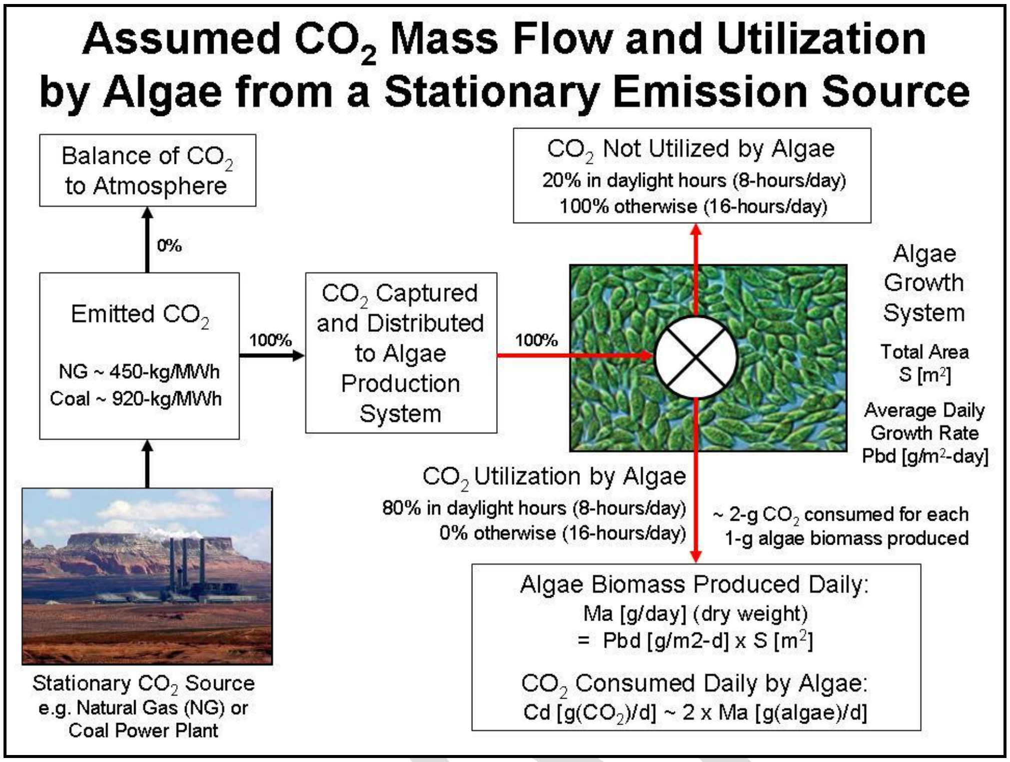


Figure B-5. Process diagram and assumptions used for utilizing CO_2 from stationary emission sources, such as fossil-fired power plants, to enhance algae growth while capturing carbon emissions for re-use in algae-based biofuels and other co-products.

Scenario-3. Capture of CO2 emissions from a 200-MW natural gas power plant

This scenario focuses on a 200-MW natural gas fired power plant operating 24-hours per day (a plant this size would more realistically be a “peaker” plant that only operates at peak periods). We’ll use the rule of thumb (see above) that 2 mass units of CO2 will be consumed for every mass unit of dry weight equivalent biomass grown. As noted earlier, the actual number will vary, depending on algae type and growth conditions, which is where a lot of complications come in that we will conveniently ignore is this discussion.

From Figure B-4, we have natural gas power plant emissions of 450-kg per MWh = 990 lbs CO2 per MWh = 0.495 U.S. tons CO2 per MWh. For a 200-MW power plant operating at rated capacity, that gives 99-tons of CO2 per hour of operation. Assuming an average of 8-hours per day of sunlight-enabled algae biomass production, this gives 792 tons CO2 per day that could potentially be utilized for algal biomass production. The other 2/3 of a day worth of CO2 emission from the power plant (1585 tons CO2) would be emitted to the atmosphere unless something else were done to capture and store the CO2. Assuming that of the 792 tons of CO2 emitted during sunlight hours, 80% can be utilized by the algae (factoring in less than perfect capture and uptake by the algae, as discussed earlier), this gives about 633 tons of CO2 per day actually metabolized by the algae. With two mass units of CO2 consumed for every mass unit of algae biomass grown, this would support production of about 316 tons of algae biomass per day.

Assuming average algae cultivation yields of 20-grams of biomass per square meter per day, that would mean a yield per hectare (10,000-square meters) of 200-kg = 440-lbs = 0.22-tons/ha-day. Converting to acres (2.47-acres/ha) gives 0.089-tons of algal biomass per acre per day. At this level of biomass productivity, 316 tons of algae per day would consume about 633-tons of CO2 emissions per day. This would require ~ 3550-acres of algae farm. Achieving higher algae productivities or higher CO2 uptake efficiencies would clearly reduced the required algae farm size accordingly. Coal plants emit about twice the amount of CO2 as natural gas plants on a per energy unit generated basis, as noted earlier. Thus, using an algae farm to capture an equivalent fraction of CO2 emissions from a coal power plant would require approximately twice the farm size as required for a natural gas plant.

Scenario-4. Capture & use of 1-billion metric tonnes of CO2 for algal oil production

Using the rough rule-of-thumb (see references and discussion above) that two mass units of CO2 will be used and consumed in the production of one mass unit of algae biomass, it follows that one billion metric tonnes of CO2 could be captured through the production of 0.5-billion metric tones of algae biomass. At 2200 pounds per metric tonne, this gives 1100-billion pounds of algal biomass (dry weight equivalent). For algae biomass with 30% oil content, this would yield 330-billion pounds of oil. Assuming oil density of 7.6-lb/gal, the result would be 43.5-billion gallons of algal oil. This volume of algal oil feedstock converted to biodiesel or green diesel (assuming a volumetric conversion factor of about 80% for either fuel, as discussed earlier) would yield about 35-billion gallons of diesel-type biofuel, which could displace approximately 80% of the total petroleum-based diesel fuel currently used annually in the U.S. for transportation.

Scenario-5. Notional scale-up scenarios to assess land, CO2, & water consequences

As a final example we consider a preliminary analysis of algal biofuel scale-up that investigates the projected requirements and consequences for land, water, and CO2 supply. The scenario consists of assuming the scale-up of algal oil production, in each of three different regions of the country, to the levels of 20-billon, 50-billion, and 100- billion gallons per year. We assume production scale-up within each of three multi-state groups located in three different regions of the United States: Southwest (California, Arizona, New Mexico), Midwest (Nebraska, Kansas, Iowa, and Missouri), and Southeast

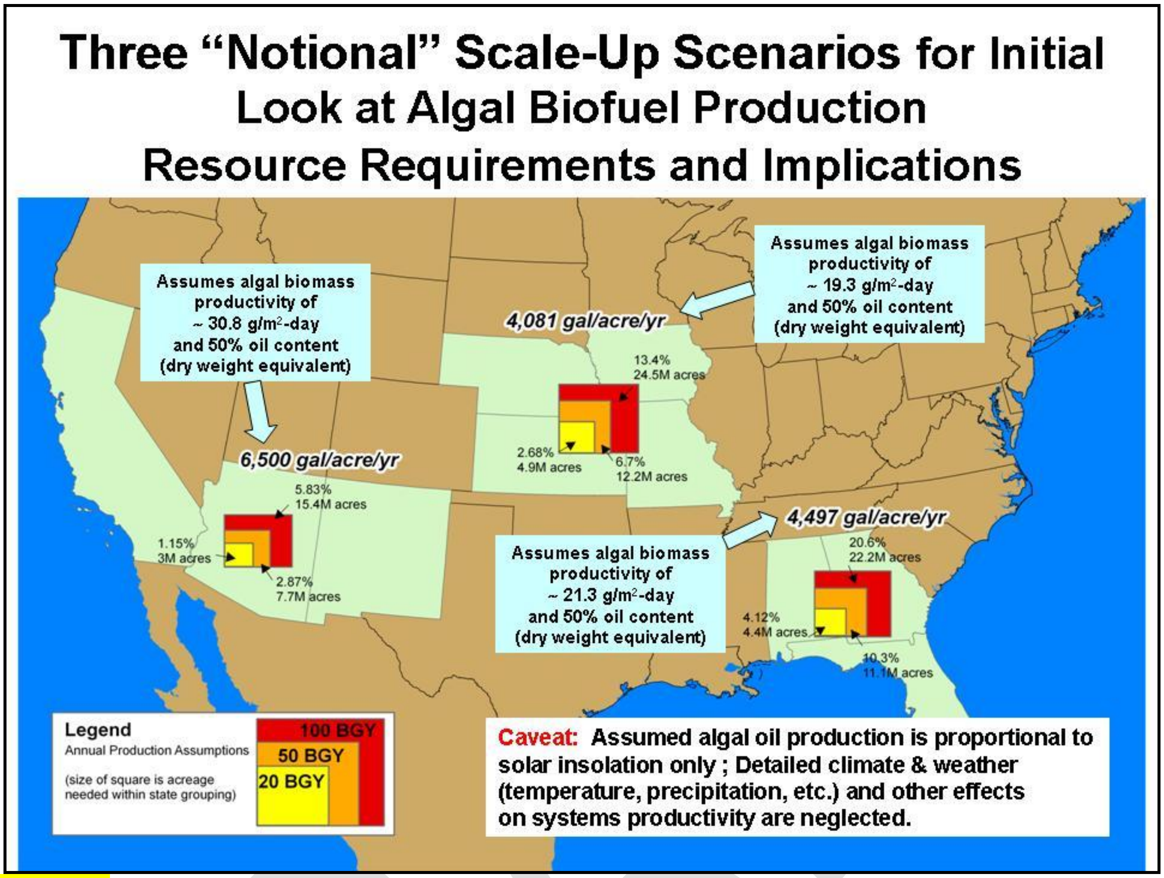


Figure B-6. Acreage needed in three different regions of the U.S., as a percentage of multi-state areas shown, for algae production with the assumed productivities shown based on available solar resource.

(Alabama, Geogia, and Florida). Algal oil productivity of 6500 gallons/acre-yr is assumed for the highest solar resource conditions, as discussed earlier. This maximum level of productivity is assumed for the SW region. Productivities for the other two regions are reduced in proportion to the average annual solar resource available in those regions as an average across the states in each group. Figure B-6 illustrates the scenario and shows the results and productivity assumptions used. The number of acres required to achieve the three algal oil production target levels in each of the three geographic regions is represented by the areas of the rectangles associated with each production level.

The projections of land required for the SW region scenario are shown in Figure B-7, along with the actual land use profile by category for those states based on USDA estimates of land use by class. This information is also presented in Table B-2. Using the algae CO2 utilization assumptions discussed earlier, the projections for CO2 required for the SW region scenario are shown in Figure B-8, along with the profile of CO2 emissions from stationary sources in those states reported in the NATCARB data base.

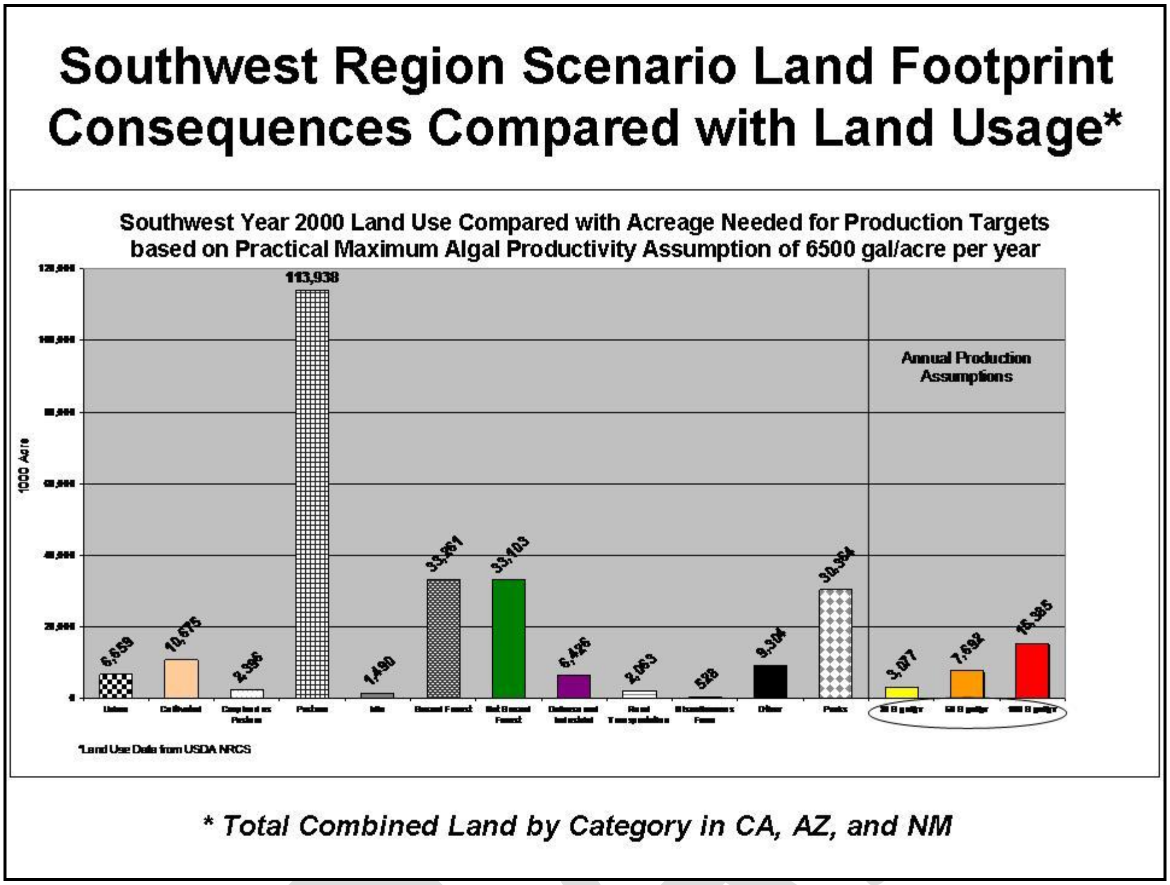


Figure B-7. Profile of land usage in the SW region states compared with the projected land required for algal oil production scale-up to 20, 50, and 100-billion gallons per year.

Figure B-9 shows the projected evaporative water loss from open systems in the SW region scenario, along with a profile of actual water use in those states. This water loss is expected to be a significant *over*-estimate, due to factors that are discussed at greater length in Section 10 of this report and further assumes no mitigating strategies for reducing evaporative water loss from open ponds. The projected CO2 and water usage impacts for the three scale-up scenarios in all three geographic regions is summarized in Table B-4.

Table B-3: Land availability by class and land requirements for algae production Southwest (CA, AZ, NM)

			Percent of land class required		
Land class	**Estimated acreage * ('000 acre)**	**Percent**	**20 BGY (3,077,000 acre)**	**50 BGY (7,692,000 acre)**	**100 BGY (16,385,000 acre)**
Urban	6659	2.66%	NA	NA	NA
Cultivated	10675	4.27%	28.82%	72.06%	153.49%
Cropland as pasture	2396	0.96%	128.42%	321.04%	683.85%
Pasture	113938	45.54%	2.70%	6.75%	14.38%
Idle	1490	0.60%	206.51%	516.24%	1099.66%

Grazed forest	33261	13.29%	9.25%	23.13%	49.26%
Non-grazed forest	33103	13.23%	NA	NA	NA
Defense and industrial	6426	2.57%	NA	NA	NA
Rural transportation	2063	0.82%	NA	NA	NA
Miscellaneous farm	528	0.21%	NA	NA	NA
Other	9304	3.72%	33.07%	82.67%	176.11%
Parks	30364	12.14%	NA	NA	NA

* thousands of acres

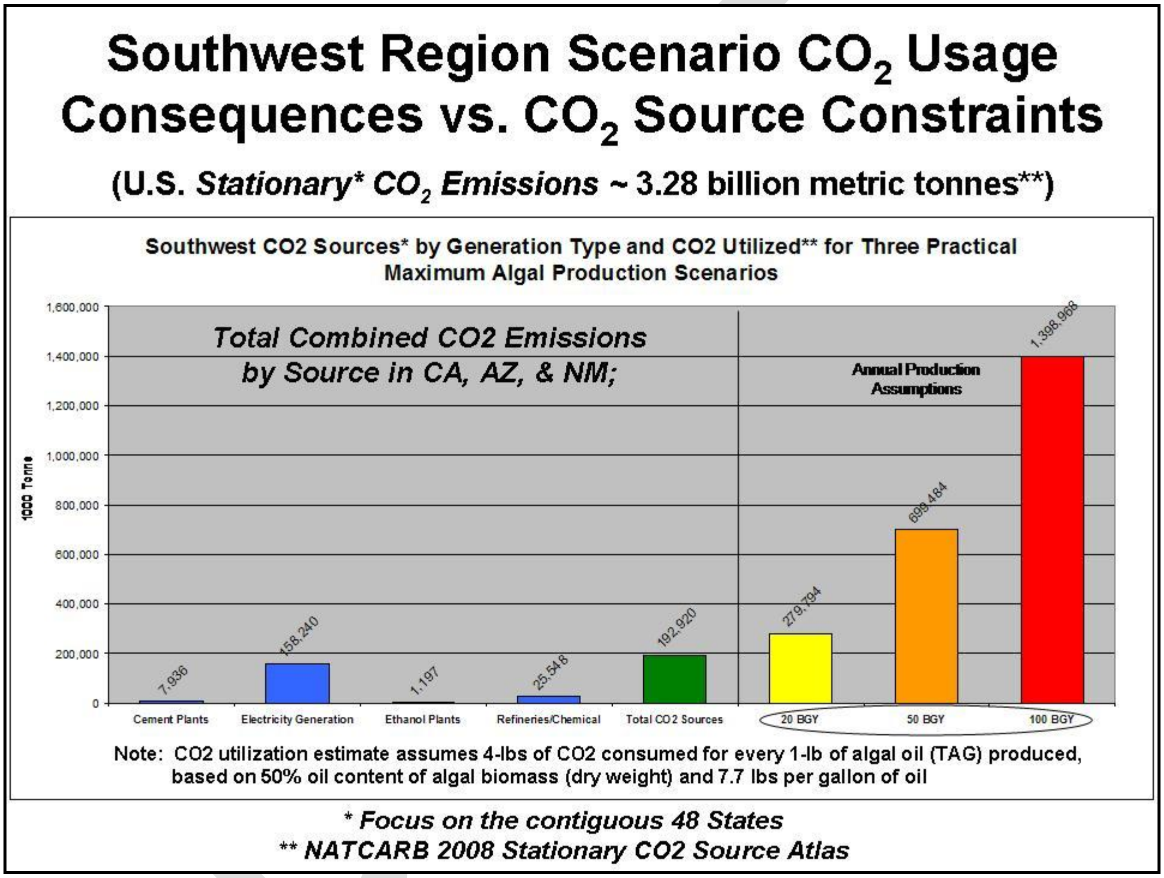


Figure B-8. Profile of stationary CO2 emissions in the SW region states compared with the projected CO2 required for algal oil production scale-up to 20, 50, and 100-billion gallons per year (Pate, et.al. 2008).

Table B-4. Preliminary assessment of potential CO2 demand and evaporative water loss for three hypothetical algal oil production volume scale-up scenarios implemented separately in three multi-state regions of the U.S.: Southwest (CA, AZ, NM), Midwest (NE, KS, IA, MO), and Southeast (Al, GA, FL) (Pate, et.al., 2008)

CO2 Usage	20 BGY	50 BGY	100BGY	20 BGY	50 BGY	100BGY
	% of 2008 CO2 emission from electricity generation			% of 2008 total CO2 emission		
Southwest	176%	440%	880%	144%	361%	722%
Midwest	161%	404%	807%	128%	320%	640%
Southeast	94%	235%	470%	45%	112%	223%
Water Evaporation	20 BGY	50 BGY	100BGY	20 BGY	50 BGY	100BGY
	as % of 1995 total irrigation			as % of 1995 total water use		
Southwest	26%	585%	108%	12%	266%	49%
Midwest	70%	162%	304%	25%	56%	106%
Southeast	48%	119%	239%	12%	31%	62%

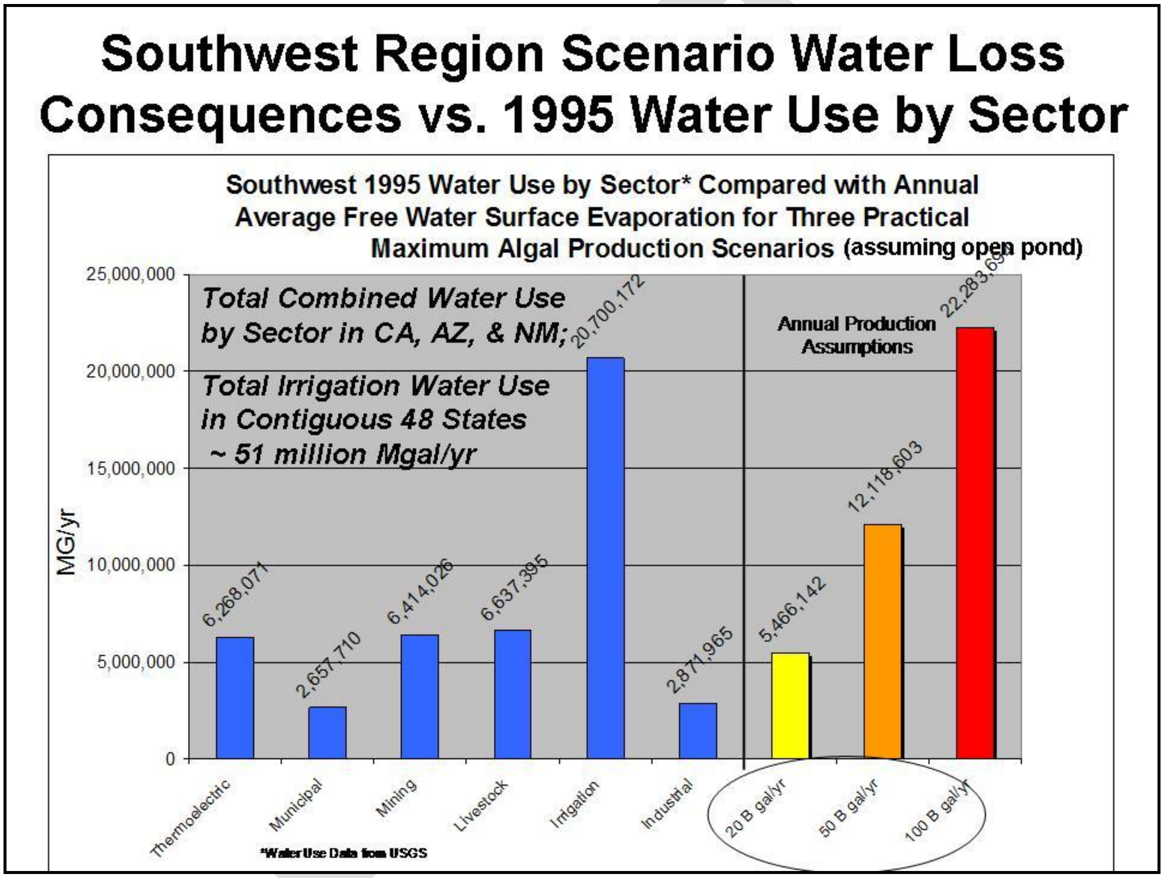


Figure B-9. Profile of water use in the SW region states compared with projected water loss from open ponds for oil production scale-up to 20, 50, and 100-billion gallons per year (Pate, et.al. 2008). Pan evaporation data for fresh water was used, which is worst-case and will likely be a significant over-estimate.

The data in Table 3-4 reinforces the necessity of developing a model with the required data to address the uncertainty in production constrained by input resource availability. A preliminary system dynamics model of algal biofuel production was built to examine the land availability issue by looking at land class, sunlight hours, location of CO2 point sources, and land limitations around point sources of CO2 (see section 11 of this report).

References

Attra (2006). "Biodiesel: The Sustainability Dimensions", National Sustainable Agriculture Information Service, National Center for Appropriate Technology, 2006 http://www.attra.ncat.org

Ben-Amotz, Ami (2007). "Production of Marine Unicellular Algae on Power Plant Flue Gas: An approach toward bioenergy and global warming", Algal Biomass Summit, San Francisco, CA, November 15-16, 2007.

Ben-Amotz, Ami (2008). "Biofuel and CO2 Capture by Marine Microalgae", Algal Biomass Summit, Seattle, WA, October 24-24, 2008.

Benneman, J.; Oswald, W. (1996). "Systems and Economic Analysis of Microalgae Ponds For Conversion of CO_2 to Biomass". Report prepared for the Pittsburg Energy Technology Center under Grant No. DE-FG22-93PC93204.

Benemann, J.R. (2002). "A Technology Roadmap for Greenhouse Gas Abatement with Microalgae." Report to the U.S. Department of Energy, National Energy Technology Laboratory, and the International Energy Agency Greenhouse Gas Abatement Programme. Prepared for the International Network on Biofixation of CO_2 and Greenhouse Gas Abatement with Microalgae.

Bill A., Griffin T, Marion, J. and Nsakala ya Nsakala (2001). "Controlling Power Plant CO2 Emissions: A Long range View", Conference Proceedings, Power Gen. Europe 2001, Brussels, Belgium. www.netl.doe.gov/publications/proceedings/01/carbon_seq/1b2.pdf

Bryan, P. and Miller, S. (2008). "Algal and Terrestrial Second-Generation Biofuels – Chevron and the *New Energy Equation,"* Chevron Biofuels, Chevron ETC, Presentation at Scripps Institution of Oceanography, March 26, 2008. http://spg.ucsd.edu/algae/pdf/2008-03-26-Scripps.pdf

Campbell, Peter, Tom Beer, David Batten (2009). "Greenhouse Gas Sequestration by Algae – Energy and Greenhouse Gas Life Cycle Studies", Transport Biofuels Stream, CSIRO Energy Transformed Flagship PB1, Aspendale, Vic. 3195, Australia 2009. http://www.csiro.au/org/EnergyTransformedFlagship.html

Chisti, Y. (2007). "Biodiesel from microalgae." *Biotechnology Advances*. (25); pp. 294-306.

Kadam, K.L. (1997). "Power Plant Flue Gas as a Source of CO_2 for Microalgae Cultivation: Economic Impact of Different Process Options", Energy Convers. Mgmt, v.38, Suppl., pp. S505-S510, 1997.

Kadam, K.L. (2002). "Environmental implications of power generation via coal-microalgae cofiring", Energy v.27, pp. 905-922, 2002.

Massingill, Michael, James Carlberg, Gregory Schwartz, Jon Van Olst, James Levin, and David Brune (2008). "Sustainable Large-Scale Microalgae Cultivation for the Economical Production of Biofuels and Other Valuable By-Products", Algal Biomass Summit, Seattle, WA, October 24-24, 2008.

McKinsey&Company (2008). Warren Campbell, et.al., "Carbon Capture & Storage: Assessing the Economics", Report prepared under the McKinsey Climate Change Initiative, September 22, 2008. http://www.mckinsey.com/clientservice/ccsi

ORNL (2003). "Bioenergy Conversion Factors", http://bioenergy.ornl.gov/papers/misc/energy_conv.html http://www.localenergy.org/pdfs/Document%20Library/Bioenergy%20conversion%20factors.pdf

Pate, R. (2008). “Algal Biofuels Techno-Economic Modeling and Assessment: Taking a Broad Systems Perspective”, Plenary presentation at DOE Algae Biofuels Technology Roadmap Workshop, University of MD Inn and Conference Center, 9-10 December 2008.

Rubin, E. (2005). “Carbon Dioxide Capture and Storage.” IPCC Technical Summary.

Schenk, Peer., Skye R. Thomas-Hall, Evan Stephens, Ute C. Marx, Jan H. Mussgnug, Clemens Posten, Olaf Kruse, and Ben Hankamer (2008). ”Second Generation Biofuels: High-Efficiency Microalgae for Biodiesel Production”, Bioenerg. Res. v1:20–43 Published online: 4 March 2008.

Sheehan, J., T. Dunahay, J. Benemann, and P. Roessler (1998). “A Look Back at the U.S. DOE’s Aquatic Species Program – Biodiesel from Algae”, NREL/TP-580-24190, July 1998.

Sun, Sally, and Raymond Hobbs (2008). “Power Plant Emissions to Biofuels”, Presentation by Arizona Public Service Company at NREL-AFOSR Workshop on Algal Oil for Jet Fuel Production, Washington, DC, 19-21 February 2008.

Tyson, K.S.; Bozell, J.; Wallace, R.; Peterson, E.; Moens, L. (2004). *Biomass Oil Analysis: Research Needs and Recommendations.* NREL/TP-510-34796. Golden, CO: National Renewable Energy Laboratory.

USDA (2006). “Major Uses of Land in the United States, 2002”, USDA Economic Research Service, Economic Information Bulletine Number 14, May 2006.

USDA (2009a). “Land Use, Value, and Management: Major Uses of Land”, USDA Economic Research Service On-Line Briefing Room http://www.ers.usda.gov/Briefing/LandUse/majorlandusechapter.htm

USDA (2009b). “United States Census of Agriculture 2007: Summary and State Data”, v.1, Geographic Area Series, Part 51, Issued February 2009 by the USDA National Agricultural Statitics Service. http://www.agcensus.usda.gov/Publications/2007/Full_Report/usv1.pdf

Van Harmelen, T.; Oonk, H. (2006). “Microalgae Biofixation Processes: Applications and Potential Contributions to Greenhouse Gas Mitigation Options.” Prepared by TNO Built Environment and Geosciences for the International Network on Biofixation of CO_2 and Greenhouse Gas Abatement with Microalgae. Available from jbenemann@aol.com.

Weyer, Kristina, Daniel Bush, Al Darzins, Bryan Willson (2008). “Theoretical Maximum Algal Oil Production”, Algal Biomass Summit, Seattle, WA, October 24-24, 2008.

NOAA (????). “Surface Evaporation Map of US” - Evaporation for the United States," NOAA Technical Report NWS 34, Washington, D.C., 82 p.

Farnsworth, R.K., E.S. Thompson, and E.L. Peck (1982). "Evaporation Atlas for the Contiguous 48 United States," NOAA Technical Report NWS 33, Washington, D.C.

Produced Water Quality Map of US - The produced water map shows locations of brackish water generated during oil and gas recovery. Water quality, date sample was collected, sample depth, geologic formation samples was collected from, and wellname are examples of data included in this dataset. It is available at http://energy.cr.usgs.gov/prov/prodwat/dictionary.htm

NATCARB (2008a). CO2 Source Map of US - CO2 sources were compiled by NATCARB as part of the national Carbon Sequestration Program. An example of some of the sources included in the database are power generating facilities, refineries, manufacturing, natural gas transmission and cement plants. http://www.natcarb.org/Atlas/data_files.html

NATCARB (2008b). Saline aquifer data was compiled by NATCARB as part of the national Carbon Sequestration Program. Information includes geologic basin and formation, as well as formation surface area. http://www.natcarb.org/Atlas/data_files.html