IEA Bioenergy Task 39 Commercializing 1st- and 2nd- Generation Liquid Biofuels from Biomass

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Current Status and Potential for Algal Biofuels Production

A REPORT TO IEA BIOENEGY TASK 39

AUTHORS:

Al Darzins (NREL)

Philip Pienkos (NREL)

Les Edye (BioIndustry Partners)

BioIndustry Partners

NREL NATIONAL RENEWABLE ENERGY LABORATORY

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Background

One of the activities of IEA Bioenergy Task 39 is to commission state-of-the-art reports on some of the most important relevant clean energy, liquid biofuels technology topics. You can access many Task 39 past reports at www.Task39.org

One area that has received considerable recent attention is the potential of algae to produce low carbon energy dense liquid biofuels suitable for uses such as aviation, or as petrol/gasoline and diesel replacements.

IEA Bioenergy Task 39 is fortunate to have, within its extensive network, colleagues who have had long experience with algae technologies, both in terms of commercial growth of algae (as has occurred in Australia over many years of operating high-productivity open ponds) and in assessing the technical status and potential of algal biofuels (as carried out by the United States' National Renewable Energy Laboratory (NREL) during the Aquatic Species Program).

We want to thank the authors, Al Darzins and Philip Pienkos (NREL, US) and Les Edye (BioIndustry Partners, Australia) for their hard work in writing this report! Contributions of text and figures were provided by Wade Amos, John Benemann, Eric Jarvis, John Jechura, Anelia Milbrant, Matt Ringer, Kristi Theis, and Bob Wallace.

We also want to thank Don O'Connor of (S&T)^2 Consultants Inc. for the final editing and layout of this report as well all the Task 39 member Country Representatives and IEA Bioenergy Executive Committee members for providing excellent constructive feedback on initial drafts of the report.

With algal biofuels research and development evolving rapidly, we are confident that areas such as technical approach, process scale-up/commercial demonstration, life cycle/sustainability analysis, etc., of algal systems for liquid biofuels production will warrant further extended examination in the future. Such work will likely become one focus of future IEA Bioenergy Task 39 activities.

Jack Saddler/Jim McMillan

Co-Task Leaders

IEA Bioenergy Task 39; Liquid Biofuels

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Current Status and Potential for Algal Biofuels Production

Executive Summary

This IEA Bioenergy report, "Current Status and Potential for Algal Biofuels Production," seeks to examine the technical and economic feasibility of generating algal biomass for the production of liquid biofuels.

As worldwide petroleum reserves diminish due to consumption exceeding discoveries, many countries are becoming increasingly dependent upon imported sources of oil. The United States, for example, currently imports a full two-thirds of its petroleum from only a few countries around the world. The demand for energy is growing worldwide especially in many of the rapidly developing countries such as in China and India. Furthermore, the continued combustion of fossil fuels has created serious environmental concerns over global warming due to the increased release of greenhouse gases (GHG).

Biofuels are one of the potential options to reduce the world's dependence on fossil fuels but biofuels have their limitations. One of the recent concerns with respect to increased biofuels production is the availability of land. It is recognized that the GHG benefits of biofuels can be offset if land with existing high carbon intensity is cleared for the production of biofuel feedstocks. Biofuels that could be produced without large increases in arable land or reductions in tropical rainforests could be very attractive in the future. Algae may offer this opportunity.

The basic concept of using algae as a renewable feedstock for biofuels production has been known for many years. All of the elements for the production of lipid-based fuels from algae have been demonstrated.

- Algae can be grown in large outdoor cultures and harvested.
- The algal biomass will contain a certain percentage of lipids, though not necessarily all in the form of triacylglycerides (TAGs).
- Algal oil can be obtained from harvested biomass by known means, albeit with sub optimal yield, cost and thermodynamic efficiencies.
- Biodiesel (fatty acid methyl ester, FAME), hydrogenation-derived renewable diesel (HDRD) and synthetic jet fuel production from algal oil have been demonstrated at non-commercial scales.

However, past research and development funding in this field has been inadequate to facilitate the development of a robust algal biofuels industry.

Realizing the strategic potential of algal feedstocks will require breakthroughs, not only in algal mass culture and downstream processing technologies, but also in the fundamental biology related to algal physiology and the regulation of algal biochemical pathways.

Potential Benefits of Microalgal Oil Production

Microalgae include a wide variety of photosynthetic microorganisms capable of fixing CO_2 from the atmosphere and water to produce biomass more efficiently and rapidly than terrestrial plants. Numerous algal strains have been shown in the laboratory to produce more than 50 percent of their biomass as lipid with much of this as triacylglycerides (TAGs), also called triglycerides, the anticipated starting material for biodiesel fuels. Most of the observations of high lipid content come from algal cultures grown under nutrient (especially nitrogen, phosphorous, or silicon) limitation. Lipid content varies in both quantity and quality with varied growth conditions. While high lipid yields can be obtained under nutrient limitation, this is generally at the expense of reduced biomass yields. Nevertheless, the possibility that microalgae could generate considerably more oil than typical oilseed crops is an exciting opportunity.

An additional benefit of growing algae as a biofuels feedstock is that they can be cultivated on otherwise non-productive (i.e., non-arable) land that is unsuitable for agriculture or in brackish, saline, and waste water that has little competing demand, offering the prospect of a biofuel that does not further tax already limited resources. Using algae to produce feedstocks for biofuels production could have little impact on the production of food and other products derived from terrestrial crops, but will utilize water resources, which will need a life cycle assessment to identify areas for sustainable production.

Algae have the potential to reduce the generation of greenhouse gas (GHG) and to recycle CO_2 emissions from flue gases from power plant and natural gas operations as indicated by preliminary life cycle assessments. In the future, an algal-based biorefinery could potentially integrate several different conversion technologies to produce biofuels including biodiesel, green diesel and green gasoline[1] (generated by catalytic hydroprocessing and catalytic cracking of vegetable oils, respectively), aviation fuel (commercial and military), ethanol, and methane, as well as valuable co-products including oils, protein, and carbohydrates.

[1] Gasoline, jet fuel, and diesel are generally described as “renewable” or “green” if the feedstock material is derived from a biological source (such as biomass or plant oil) but has essentially the same chemical composition as that of crude oil.

Algal Cultivation

There are currently no meaningful amounts of microalgal biofuels produced commercially in the world. Approximately 9,000 tonnes of algal biomass is produced commercially today, mainly for the production of high-value, low-volume food supplements and nutraceuticals. In the U.S., three companies are responsible for the majority of commercial production. Two of these (Earthrise Nutritionals, LLC, in California, and Cyanotech Corp., in Hawaii) use raceway ponds for production. The third company (Martek Co., in Maryland) produces biomass by fermentation, in which the algae are grown in closed vessels on sugars in the dark, similar to yeast production. Cognis Australia Pty Ltd produce β-carotene from *D. salina* harvested from hypersaline extensive ponds in Hutt Lagoon and Whyalla. Hutt Lagoon has a total pond surface area of ca. 520 ha and Whyalla is ca. 440 ha. In terms of pond surface area, Hutt Lagoon and Whyalla are among the largest algal production systems in the world.

Proposed commercial algal biofuels production facilities employ both open (ponds) and closed (tubes, also known as photobioreactors) cultivation systems. Each of these has advantages and disadvantages, but photobioreactors are much more expensive to build than open ponds. Photobioreactors have not been engineered to the extent of other bioreactors in commercial practice, and so there is certainly room for cost reductions. Neither open ponds nor closed photobioreactors are mature technologies. Until large-scale systems have actually been built and can show demonstrated performance over many years of operation, many uncertainties will remain. Cultivation issues for both open and closed systems such as reactor construction materials, mixing, optimal cultivation scale, heating/cooling and CO_2 administration have been considered and explored to some degree, but more definitive answers await detailed and expensive scale-up evaluations. Nevertheless, as an important part of the development process, there are algal demonstration projects that are in progress.

Commercial algal growth will require the development of strains and conditions for culture that allow rapid production of algal biomass with high lipid content and minimal growth of competing strains. The economics of continuous algal propagation can be impacted by growth of contaminating algal species as well as the presence of grazers and pathogens. The rapid growth rate of algae relative to terrestrial crops and the expectation that algal culture for biofuels production will be a year-round process allows for opportunities to briefly and periodically shut down production to deal with competitors, grazers, and pathogens. Unlike terrestrial crops whose failure costs an entire growing cycle, an algal pond can be re-inoculated and resume production in a matter of days. Microalgae can thrive in a broad range of environmental conditions but specific strains are more limited by climatic conditions than terrestrial crops. In areas of high solar radiation, the theoretical maximum (based on the efficiency of incident light energy utilization) for algal productivity is 100 g/m^2/day. Most reports of high levels of sustained productivity in open ponds fall in the range of 15-30 g/m^2/day. The productivity rates are even lower when the area required for supporting activities (water supply, treatment, disposal, etc.) is included rather than just using the active pond area. Maximal volumetric productivities can be higher in a closed photobioreactor than in an open pond because the surface-to-volume ratio can be higher.

Algal Harvesting

The potential oil yields (litre/hectare) for algae are significantly higher than yields of oil seed crops (approximately 20 times higher than soybeans but again not including the area required for the supporting activities). Therefore, a smaller area is potentially required to produce triglyceride-rich oil from microalgae than from other types of biomass. Low cost algal harvesting options for biofuels applications do not currently exist. It is possible that new technologies are currently under development by the private sector. However, if these new techniques exist, they have not yet been assessed publicly and, therefore, are not documented in this report. Attempts in the past have taken advantage of spontaneous settling of the algal biomass, without benefit of chemical flocculants. Other mechanisms exist including the auto flocculation process, which depends on the co-precipitation of algal cells with calcium carbonate, and other precipitates that form in hard waters subject to high pH. Aside from settling, in some cases the algal biomass will float, either due to buoyancy (e.g., high oil content) or by using a dissolved air floatation (DAF) process. The use of small amounts of chemical flocculants (polymers) to aid in such a process could be cost effective, depending on the amount used. Nevertheless, a significant engineering research effort is needed to develop and prove out cost-effective algal harvesting techniques.

Extraction of Algal Oils

The differences between microscopic microalgal cells and the seeds of oil-bearing plants demand that different processes be employed for recovery of the oil. The most likely technology for algal oil recovery involves some form of solvent extraction (though other methods such as mechanical extraction have been proposed), but any process option is likely to be complicated by the high water content of algal biomass. Once the algal oil is recovered, downstream processing to biodiesel or green diesel is well understood. Wet processing of microalgae may also emerge as a possibility to avoid significant dewatering costs if the appropriate processes can be developed.

Complications may still arise from differences in overall lipid content (i.e., relative levels of TAGs, phospholipids, and glycolipids) that will occur with changes in algal populations and climatic variations. The high inorganic content of algal biomass implies that there will be a cost to supply nutrients, but this may also mean that the extracted biomass will command a higher market price due to higher nutritional content in cases where it is not necessary to separate out unwanted inorganic materials. Substantial by-product credit can be obtained from algal wastewater treatment, conversion of biomass to energy, or higher value animal feed and veterinary nutraceuticals.

Fuel Production from Algal Feedstocks

Historically the emphasis of fuel products from microalgae has been on the high-energy lipid oils that, like their terrestrial seed oil counterparts, can be converted into alcohol esters (i.e., biodiesel) using conventional transesterification technology. The transesterification reaction is well-understood; however, there are still numerous approaches to optimizing the reaction for different feed compositions and different downstream processing requirements.

Alternatively, the oils can be used to produce a renewable or green diesel product by a process known as catalytic hydroprocessing. Vegetable oils and waste animal fats are being processed in a limited number of petroleum refineries to make renewable fuels. A major characteristic of petroleum-derived fuels is a near zero oxygen content. Biological sources have very high oxygen contents as compared to crude oil. The primary goal of making renewable gasoline, jet fuel, and diesel is to minimize the oxygen in the final fuel while maximizing the final energy content.

> ***The production of liquid transportation fuels from algal biomass is technically feasible. However there is a need for innovation in all elements of algal biofuels production to address technical inefficiencies, which represent significant challenges to the development of economically viable large-scale algal biofuels enterprises.***

The other issue addressed in this report is the economic feasibility of algal biofuel production at any scale and the feasibility of sustainable large scale production that contributes significantly to future liquid transportation fuel demand.

The economic analysis presented in this report clearly shows that algal biofuels production requires further research and development to become economically viable. Technical advances combined with emissions trading schemes, carbon tax or legislation to reduce CO2 emissions will obviously hasten the date when algal biofuel production can become a profitable enterprise. In markets with only a few small participants, algal biofuels production with the capture of the full worth of high value co-products may also be viable. The economic analysis also highlights the likely contribution that innovation will make to long-term profitability.

> ***With continued development, algal biofuels have the potential to become economically viable alternatives to fossil fuels.***

Notwithstanding the technical challenges, the availability of suitable land, in terms of soil type, elevation and slope, in suitable climates (incident radiation, temperature, precipitation/evaporation balances and severe weather), and the geographical nearness of this land to appropriate water and CO_2 inputs and possibly nearness to markets or transportation infrastructure may impose physical and economic limits to the contribution that algal biofuel can make to the world's future transportation fuel needs. For example, very few large CO_2 emissions sources are in close proximity to regions identified as being most suitable for year round, large scale open pond production systems. In fact, there is an absence of data that could be used in defining limits of production. Land use, land suitability and resource spatial mapping data compiled for the purpose of assessing the geographic potential of algal biofuels does not exist. Claims that algal biofuels could completely replace all petroleum derived transport fuels or even provide a significant contribution to liquid fuels on simple assessment seem improbable, but can be neither supported nor refuted. There is a need to develop this information.

The contribution of algal biofuels to future liquid transportation fuel supply is assessed against the US Energy Information Agency growth projections. By 2030, oil consumption is expected to increase to ca. 6.2 $TL \cdot yr^{-1}$ (106 million $bbl \cdot d^{-1}$) with 66% of this growth likely to occur in non-OECD countries in Asia. Transportation fuel use is expected to grow slightly to ca. 56% of total oil production. Over the same time period, biofuels will maintain a relatively steady share of unconventional liquid fuel production and grow to between 277 $GL \cdot yr^{-1}$ and 416 $GL \cdot yr^{-1}$ (4.8 to 7.2 million $Bbl \cdot d^{-1}$, or 8.0% to 12.0% of the liquid transportation fuel supply). The EIA uses a figure of ca. 340 $GL \cdot yr^{-1}$ as a reference case for total biofuel production in 2030.

A 5% contribution of algal biofuels to total biofuels supply by 2030 would require the construction of 170 100 ML facilities. When the technical uncertainty is considered it seems unlikely that the first large scale plant would be commissioned before the middle of the coming decade, and even this would be ambitious. Approaches that rely on molecular biology to achieve breakthroughs, e.g., the partnership between Synthetic Genomics Inc. and ExxonMobil Corp., are promising but will likely take more than a decade to reach commercial viability. Assuming success in the first commercial venture and accelerated rates of adoption beyond 2015-2020, 170 100 ML facilities could conceivably be operational by 2030 as this rate of construction is lower than the recent development rate of ethanol plants in the US and Brazil.

> ***Algal biofuels have the potential to replace a significant portion of the total diesel used today with a smaller environmental footprint. In addition, algal biofuel production can be carried out using marginal land and saline water, placing no additional pressure on land needed for food production and freshwater supplies.***

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1. Introduction

It seems probable that growth in human population, future climate change effects on freshwater resources, which are already stressed in some regions, and eventual shortages of unutilized arable land will encourage the exploitation of microalgae based production systems for both food and fuel. Claims that the ability to utilise non-arable land and waste water resources with few competing uses make algal biofuel production systems superior to biofuels based on terrestrial biomass has created great interest in governments, NGOs, the private sector and the research community. Current initiatives clearly indicate this interest at all levels of government and the in private sector in the development of algal biofuels technologies and enterprises.

This report examines the technology and economics of biofuel production from oil forming autotrophic microalgae. The context of this examination is an assessment of the likely contribution of algae derived biofuel to the world's future liquid transportation fuel needs. The current status of the technology is reviewed in section 2 of this report. The technology review covers algal biology, cultivation, harvest, extraction and conversion to liquid transport fuels. The sustainability of algal biofuel production systems is discussed in section 3 and the site requirements for large scale intensive pond algal production are considered in section 4. Section 5 presents economic analyses, which among other things explores the influence of proximity of resources to production sites. Section 6 addresses the likely contribution of algal biofuels to future liquid transportation fuel markets. The report also contains a number of appendices that contain a review of US algal biofuel research, development and demonstration, algal culture collections, the underlying assumptions and material balances used in the economic model (in section 5) and a photographic collection.

1.1 The World's Energy Challenges

As worldwide petroleum reserves diminish due to consumption exceeding discoveries, many countries are becoming increasingly dependent upon imported sources of oil. The United States, for example, currently imports a full two-thirds of its petroleum from only a few countries around the world. The demand for energy is growing worldwide especially in many of the rapidly developing countries such as in China and India. Furthermore, the continued combustion of fossil fuels has created serious environmental concerns over global warming due to the increased release of greenhouse gases (GHG).

Biofuels are one of the potential options to reduce the world's dependence on fossil fuels but biofuels have their limitations. One of the recent concerns with respect to increased biofuels production is the availability of land. It is recognized that the GHG benefits of biofuels can be offset if land with existing high carbon intensity is cleared for the production of biofuel feedstocks. Biofuels that could be produced without large increases in arable land, or reductions in tropical rainforests could be very attractive in the future. Algae may offer that opportunity.

The basic concept of using algae as a renewable feedstock for biofuels production has been known for many years. However, historical efforts in this field have been inadequate to facilitate the development of a robust algal biofuels industry. Realizing the strategic potential of algal feedstocks will require breakthroughs, not only in algal mass culture and downstream processing technologies, but also in the fundamental biology related to algal physiology and the regulation of algal biochemical pathways.

1.2 Algae: Basic Concepts

Algae belong to a large group of simple photosynthetic organisms. They are subdivided into two major categories based on their size. Microalgae, are small free-living microorganisms that can be found in a variety of aquatic habitats. They are able to thrive in freshwater, brackish, marine and hypersaline aquatic environments (Falkowski and Raven, 1997) and have been reported in desert crust communities thereby being able to endure temperature extremes and low water availability. Microalgae are easily distinguished from their larger photosynthetic cousins, the macroalgae, which have evolved defined anatomical structures resembling leaves, stems, and roots of higher plants.

The main advantages of using microalgal organisms in a variety of industrial applications are:

- they grow rapidly and have a higher solar conversion efficiency than most terrestrial plants;
- they can be harvested batch-wise or continuously almost all year round;
- algal production facilities can be collocated on otherwise non-productive, non-arable land;
- they can utilize salt and waste water sources that cannot be used by conventional agriculture;
- they can use waste CO_2 sources thereby potentially mitigating the release of GHG into the atmosphere; and,

- they can produce a variety of feedstocks that can be used to generate non-toxic, biodegradable biofuels and valuable co-products.

Microalgae were among the first life forms on earth (Falkowski et al., 2004). They are capable of fixing large amounts of carbon dioxide (CO_2) while contributing to approximately 40 percent to 50 percent of the oxygen in the atmosphere thereby helping to support the majority of life on our planet. Microalgae are highly productive on a global scale, with cell doublings of 1-4 per day. While microalgae make up only 0.2 percent of global biomass generated through photosynthesis, they account for approximately 50 percent of the total global fixed organic carbon (Field et al., 1998). Microalgae, like terrestrial plants, grow and multiply through photosynthesis, a process whereby light energy is converted into chemical energy by "fixing" atmospheric CO_2 by the following reaction:

$$6CO_2 + 6H_2O + \text{light energy} \rightarrow C_6H_{12}O_6 \text{ (sugars)} + 6O_2$$

The sugars formed by photosynthesis are converted to all the other cellular components (lipids, carbohydrates, and proteins) that make up the biomass.

The photosynthetic process in microalgae is similar to that found in terrestrial plants. However, microalgae, due to their simple structure, are particularly efficient converters of solar energy. Because microalgae do not need to generate elaborate support and reproductive structures, they can devote more of their energy into trapping and converting light energy and CO_2 into biomass. Microalgae can convert roughly 6 percent of the total incident radiation, into new biomass (Benemann et al., 1978). By comparison, terrestrial crops have generally lower photosynthetic conversion efficiencies. Sugar cane, one of the most productive of all terrestrial crops, for example has a photosynthetic efficiency of 3.5 to 4 percent (Odum, 1971). Based upon this distinguishing feature, microalgae have become a target for scientific studies on biomass energy production, biofuels production, as well as the potential utilization of CO_2 currently being released into the atmosphere through the use of fossil fuels.

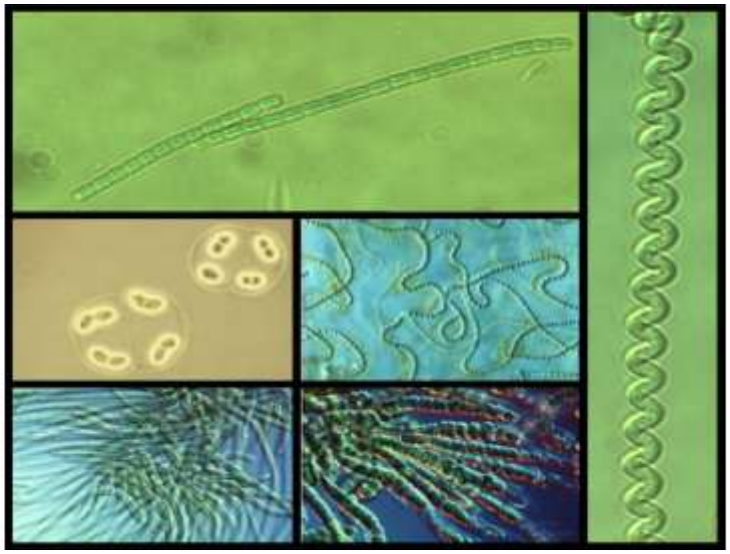

Figure 1-1 Examples of Cyanobacterial Diversity

Cyanobacteria range from simple unicellular organisms to colonies. Photo courtesy of Laura Beer, Colorado School of Mines and Review of the Universe (http://universe-review.ca).

The amount of microalgal biodiversity in nature is quite substantial with literally tens of thousands of different species described (Norton et al., 1996). Previously, microalgae were subdivided into two broad categories: cyanobacteria and the true algae. While cyanobacteria, often referred to as the blue-green algae, have been traditionally included as "algae," these organisms are clearly photosynthetic "prokaryotes"—bacterial organisms that lack a defined nucleus. Nevertheless, cyanobacteria along with the true algae are important transformers of solar energy. The structures of these photosynthetic microbes range from simple, tiny unicellular organisms to microorganisms with highly branched filamentous structures (Figure 1-1). The genome of the unicellular cyanobacterium Synechocystis sp. PCC680, which has served as a model system for prokaryotic photosynthesis, has been completely sequenced (Kaneko et al., 1996). While it continues to be an important model system, cyanobacteria, in general, are not generally known to produce large quantities of triglyceride lipids. They do, however, have the capability of producing large amounts of storage carbon in the form of starch or glycogen. Due to this and the advanced state of genetic techniques for cyanobacterial engineering, these important microbes will also be considered in this report.

The term "algae" is generally applied to "eukaryotes"—single or multicellular organisms whose cells contain a distinct membrane-bound nucleus. The eukaryotic algae also represent an extremely diverse assemblage of organisms with more than 40,000 species currently identified and many more yet to be identified. Despite this biodiversity, microalgae are still 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 a handful of culture collections throughout the world, a few hundred are being investigated for their chemical content and just a handful of important species are being cultivated on an industrial scale. Algae can be classified, based on a number of distinguishing characteristics, into at least 12 major divisions. These classes 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). Several additional divisions of unicellular algae have been described (van den Hoek et al., 1995). Figure 1-2 demonstrates only a very small sampling of the algal diversity observed in diverse aquatic environments.

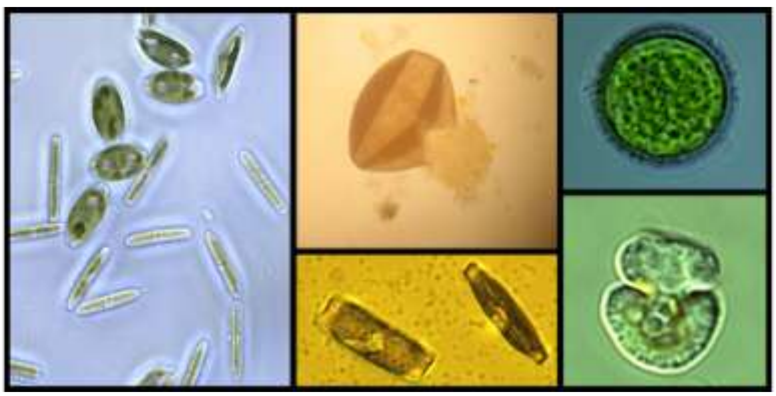

Figure 1-2 Microalgal Biodiversity

Examples of diatoms and green algae isolated from a variety of aquatic environments. The image located in the centre panel (top) is particularly striking because it shows a diatom in the process of rupturing and releasing distinct yellow-coloured lipid globules. Photo courtesy of Laura Beer, Colorado School of Mines.

1.3 Non-fuel Applications of Algal Cultivation

Microalgal biotechnology only began to develop in the middle of the last century. There are numerous commercial applications that involve the growth and processing of microalgae. Microalgae have current applications in the production of human nutritional supplements and specialty animal feeds (Becker, 2004). Microalgae are currently 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. They also play an important role in the aquaculture business and in wastewater treatment facilities. Most commercial microalgae production processes use “open pond” systems consisting of shallow recirculating raceways exposed to the atmosphere in which the water is mixed with a paddlewheel device (Spolaore et al., 2006). Currently, commercial production of microalgae biomass is limited to a few species, such as Spirulina, Chlorella, and Dunaliella, cultivated in open, CO_2 fertilized ponds for high value nutritional products. Worldwide the combined production rates for these algae are in excess of 5,500 tonnes/year (Pulz and Gross, 2004).

Unlike open pond systems, photobioreactors are closed systems that do not allow direct gas exchange with the atmosphere. These photobioreactors, in their simplest configuration, consist of an array of straight or curved tubular chambers that are usually constructed of plastic or glass. They can allow, at least in principle, better process control, higher biomass concentrations, and reduced contamination, while at the same time reducing evaporation and CO_2 losses (Janssen et al., 2003). At least two industrial international efforts are currently using photobioreactors in commercial algae production for nutraceutical applications. Open ponds and photobioreactors can be used as standalone production systems or in combination as a two-stage process in the production of algal biomass (Huntley and Redalje, 2007)

1.4 The Algal Biofuels Opportunity

More than 50 years of research have demonstrated the potential of various microalgal species to produce several chemical intermediates and hydrocarbons that can be converted into biofuels. Figure 1-3 is a schematic overview of microalgal chemical intermediates and the fuels that can be produced from these important components. The three major macromolecular components that can be extracted from microalgal biomass are lipids, carbohydrates, and proteins. These chemical components can be converted into a variety of fuel options such as alcohols, diesel, methane, and hydrogen.

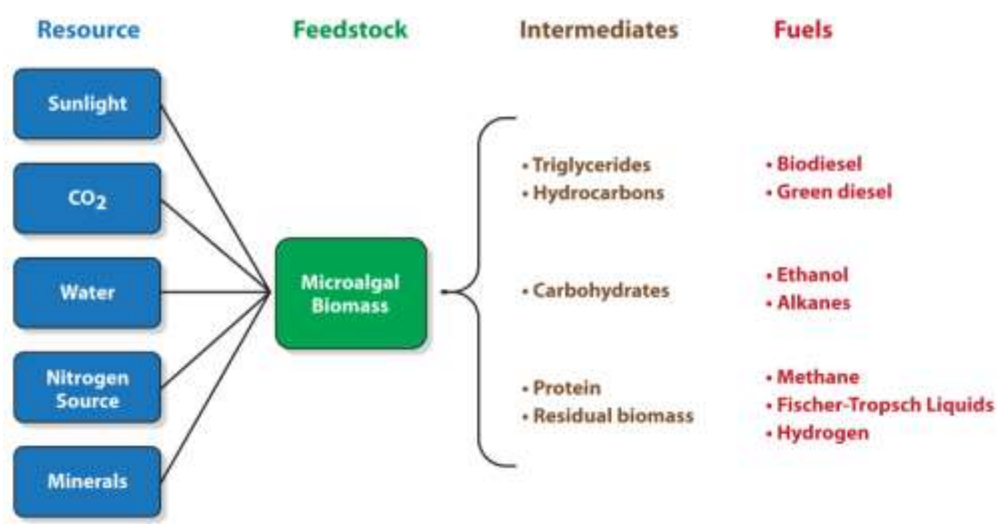

Figure 1-3 Fuel Production Options from Microalgal Cell Components

Of the three major microalgal fractions, lipids, by far, have the highest energy content. Some species, like Botryococcus, are capable of secreting hydrocarbon molecules like, those found in petroleum oil. Other microalgal species can accumulate significant amounts of triacylglycerides (TAGs). These lipids, which resemble the triacylglycerides from oilseed crops, can be converted into biodiesel and a synthetic "green" diesel. Microalgal-derived carbohydrates can also be converted into a variety of fuels such as ethanol or butanol by standard fermentation processes. Alternatively, the algal biomass residue remaining after oil extraction can be converted into methane gas using an anaerobic digestion process or into several different fuel intermediates through various thermochemical processes.

While it is beyond the scope of this report to consider all the potential conversion processes to produce fuel from microalgal feedstocks, historically the emphasis has been on the high-energy lipids and oils. Many microalgal species have the ability to accumulate large amounts of triglycerides, especially under stress-induced growth conditions (Milner, 1976). The vast majority of lipids in most growing cells are typically found in the membrane that surrounds the cell. However, some strains produce significant amounts of storage lipids and can, when grown, for example, under nutrient limiting conditions, accumulate storage lipids up to 60 percent of their total weight. The notion of generating biofuels from these microalgal storage lipids was the main focus of the DOE Aquatic Species Program (1978 - 1996; Sheehan et al., 1998).

With the real potential for rising petroleum prices in the future and ever increasing concerns over energy independence, security, and global warming, the notion of using microalgal feedstocks for biofuels production has steadily gained momentum over the last few years. Lipids derived from microalgae have been the predominant focus of this interest because these oils contain fatty acid and triglyceride compounds, which, like their terrestrial seed oil counterparts, can be converted into alcohol esters (biodiesel) using conventional transesterification technology (Fukuda et al., 2001). Alternatively, the oils can be used to produce a renewable or "green" diesel product by a process known as catalytic hydroprocessing (Kalnes et al., 2007). The use of vegetable oil and waste fats for biofuel production cannot realistically begin to satisfy the increasing worldwide demand for transportation fuels nor are they likely in the near term to displace a significant portion of the U.S. petroleum fuel usage (Tyson et al., 2004). Algal-derived oils do, however, have the potential to displace petroleum-based fuels because their productivities (i.e., oil yield/hectare) can be 10 to100 times higher than that of terrestrial oilseed crops (see Table 1-1). These comparisons often do not include the land required to support the actual pond. Activities such as water supply, water treatment, waste disposal and other activities can significantly increase the area required for cultivation and reduce the effective production rates.

Besides having the capability of producing energy rich lipids, some microalgal species can also produce substantial amounts of carbohydrate related materials. For example, some microalgae and cyanobacteria have the capacity to accumulate large amounts of storage polysaccharides. These storage products include starch, glycogen, and chrysolaminarin. Furthermore, some microalgal cell walls are composed of cellulose, mannans, xylans, and sulfated glycans. These polysaccharides can be broken down chemically or enzymatically into monomeric (or oligomeric) sugars that can then be converted into ethanol and a variety of other fuels by select fermentation microorganisms.

The conversion of solar energy into renewable liquid transportation fuels from algal lipids has been shown to be technically feasible (Chisti, 2007). However, the economics of producing such biofuels is not currently cost competitive with fossil fuels and, therefore, precludes the widespread use of microalgae for biofuel production in the near term. It is clear that a significant R&D effort, involving basic science and applied engineering, will be required to fully realize the potential of microalgal feedstocks.

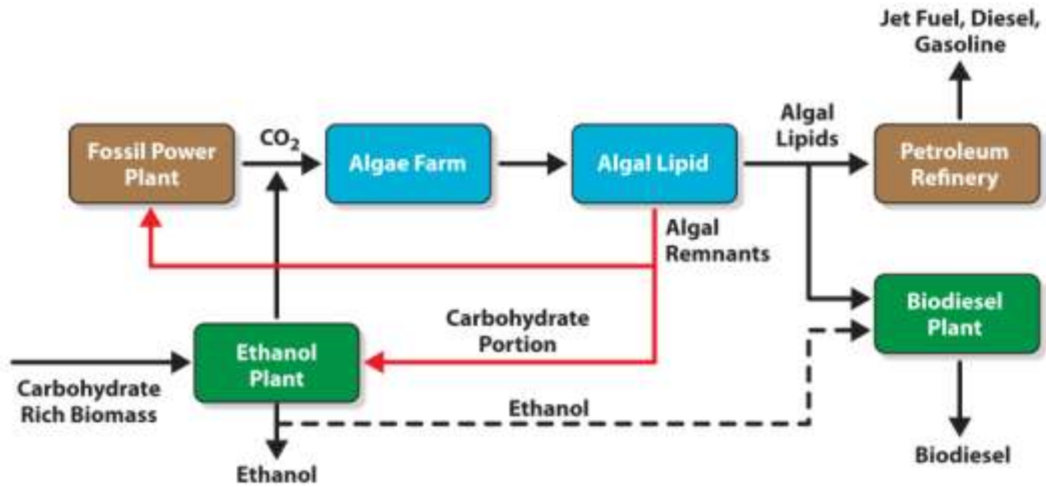

Figure 1-4 Example of an Algal Biorefinery

As depicted in the figure, the petroleum refinery and/or biodiesel plant would be an integral part of the algal cultivation facility. Alternatively, these two plants could be separate entities outside of the algal facility.

However, despite the challenges yet facing the establishment of a robust algal biofuels industry, it is still conceivable that algal cultivation farms could become integral part of a biorefinery that would also have the capability of producing other biofuels such as cellulosic ethanol, gasoline, biodiesel, renewable "green" diesel and jet fuel (Figure 1-4). This biorefinery could be co-located, for example, near a fossil fuelled electric power plant. The CO_2 generated by this power plant as well as from an integrated bioethanol plant could provide important nutrients for the growth of the microalgae. This would also serve to recycle the CO_2 at least one more time before it is released by combusting the algal-based biofuels.

Following extraction of the algal oils, the carbohydrate-rich residue could be used to produce ethanol. Alternatively, it can be fed into an anaerobic digester for methane production or recycled back into the electric power plant to be burned as a fuel. The algal oils themselves can be sent to, a biodiesel plant or petroleum refinery to convert the lipids into the most cost-effective fuel which would be dictated by the current economics. However, before this can occur at a large scale substantial R&D will be needed to develop a microalgal production system that operates at high efficiency and at a low cost.

1.5 Microalgae Oil Production: Comparison to Terrestrial Crops

There is a general consensus that the production of biofuels from oilseed crops and waste /fats cannot meet the worldwide demand for transportation fuels

(Tyson et al., 2004). In 2009, the U.S. used approximately 206 billion litres of petroleum diesel (http://eia.doe.gov). In 2009, U.S. biodiesel production decreased from to 2.56 billion litres to 1.85 billion litres (Biodiesel Magazine). Even though soy oil production for 2008-2009 totalled 9.3 billion litres, more than 90 percent of this oil is used in the food products market thereby severely limiting its use as a biofuel feedstock (Iowa State University). As of 2000, the U.S. produced in excess of 10.2 billion litres of waste vegetable oil that could be converted to transportation fuels. Combined with other U.S.-produced vegetable oils, along with fats and greases, these feedstocks, however, would only have the potential of replacing approximately five percent of the total petroleum diesel usage (Figure 1-5).

One of the main drivers in the development of microalgal diesel fuels is the higher photosynthetic efficiency of microalgae when compared to conventional crops, and hence the potentially higher productivities per unit area. Table 1-1 shows the potential oil yields from microalgae (based on calculations prepared for this report) under three different productivity scenarios. The first scenario with 10 $g/m^2/day$ matches long term productivity observed in Roswell, NM during the Aquatic Species Program despite the fact that the open ponds occasionally froze during the winter. The more productive scenarios would require warmer climates (or availability of waste heat) to maintain productivity during winter months and higher yield strains, but they are far below the theoretical maxima based on photosynthetic efficiency (Weyer et al. 2009).

Under all three scenarios, the productivity of algae could be significantly higher than that of soybeans (450 L/ha/yr) (Table 1-2). Depending on productivity, algae productivity could range from 65% of oil palm (6000 L/ha/yr) to surpassing that crop by nearly an order of magnitude. Using the higher oil content scenario (but demonstrated productivity), if the same amount of land currently devoted to the US soybean crop (75 million acres), microalgae could produce more than enough feedstock for biodiesel or green diesel to meet the current U.S. diesel fuel usage (Figure 1-5).

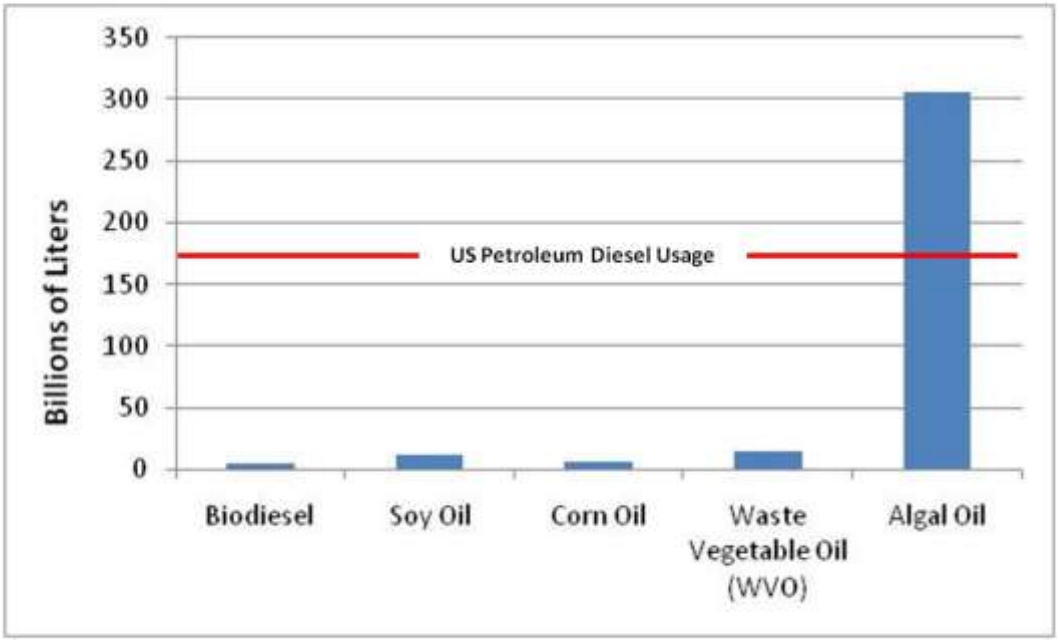

Figure 1-5 Potential of Various Oil-Based Feedstocks

Sources: Energy Information Administration (EIA), National Biodiesel Board, Corn Refiners Association.

	Demonstrated at Roswell	Higher oil content	Higher productivity
g/m^2/day	10	10	50
lipid content	15	40	40
days of operation per year	330	330	330
percent land devoted to ponds	70	70	70
L/ha/yr	3,800	10,200	50,800

Table 1-1 Microalgae Potential Yields

Crop	Oil Yield (Litres/ha/yr)
Soybean	450
Camelina	560
Sunflower	955
Jatropha	1,890
Oil palm	5,940
Algae	3,800-50,800[a]

[a] Estimated yields, this report

Table 1-2 Comparison of Oil Yields from Biomass Feedstocks

A number of other benefits also serve as driving forces for the development and deployment of algal-based technologies. The development of algal feedstocks offers the opportunity to utilize land and water resources that are, today, unsuitable for any other commercial use. Land use needs for the production of algal feedstocks complement, rather than compete with other biomass-based fuel technologies. Algae can be cultivated in areas that are far removed from farm and forest lands, thereby minimizing the damages caused to the eco- and food-chain systems, and obviating the food-versus-fuel dilemma. In addition, many species of algae that have the ability to accumulate oils can use brackish water or water from saline aquifers, making an algae feedstock production system complementary to existing agricultural practices.

Another benefit to using microalgae to produce biofuels is the mitigation of GHG due to the algae's natural ability to utilize concentrated forms of CO_2 (as provided by power plant flue gases) and to liberate oxygen. This technology could meet the potential need for carbon disposal in the electric utility industry while providing clean-burning alternatives in the transportation sector.

Despite the huge potential of algal feedstocks to replace significant quantities of petroleum based fuels, the technology is still regarded by many in the field to be in its infancy. There are many both basic and applied R&D milestones that need to be achieved before algal-based fuels can be produced economically enough to be cost-competitive with petroleum based fuels.

1.6 Summary of Benefits Using Algal Feedstocks for Biofuels

The potential benefits of algal feedstocks for biofuel production are summarized below.

- High per-acre productivity. Unlike other oil crops, algae grow rapidly and many are exceedingly rich in lipid oil (oil levels of 20 percent to 50 percent are quite common).

- Non-food resource. Using algae to produce feedstocks for biofuels production will not compromise the production of food and other products derived from terrestrial crops.
- Use of otherwise non-productive, non-arable land. Unlike terrestrial energy crops, the cultivation of algae will not need to compete with farmland for food production.
- Utilization of a wide variety of water sources. 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.
- Mitigation of GHG release into the atmosphere. Algae have a tremendous technical potential for GHG abatement through the use of CO_2-rich flue gases from coal burning power plants as well as from natural gas recovery operations. The abatement potential will vary depending on the end use of the products.
- Production of biofuels and co-products. 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.

There is a significant amount of work required in order to realize these benefits at a commercial scale. These issues and challenges are discussed in the following sections.

2. State of the Technology

This section of the report contains an assessment of technology for algal biofuels production. It provides an account of our understanding of algal biology, biomass production systems and downstream processing of algal biomass to fuels and other products. The need for innovation to overcome technical and economic barriers is addressed.

The cyanobacterium, Spirulina (recently reclassified as Arthrospira), has been used as a food source for century, harvested from natural blooms by meso-Americans before the conquest by Cortez (Diaz Del Castillo, B. The Discovery and Conquest of Mexico, 1517-1521. London: Routledge, 1928, p. 300). The idea of deliberately cultivating algae as a source of protein, using large scale open ponds, was pursued by German scientists during World War II. In the U.S., in the late 1940s and early 1950s, the Stanford Research Institute and the Carnegie Institute were involved in the first large-scale testing for algal food production. In the years that followed, Meier (1955) and Oswald and Golueke (1960) first proposed that algae could be cultivated as an energy source via anaerobic digestion. During the oil embargo of the 1970s, this concept was evaluated by Benemann et al. (1978) who concluded that methane produced from algal biomass could compete with projections of fossil fuel prices. Other researchers during this period observed that certain strains of algae were capable of accumulating high levels of lipid and that the level could be manipulated by changes in culture conditions especially by growth under nutrient (especially nitrogen and, in the case of diatoms, silicon) limitation.

2.1 Algae Biology

Algae are ubiquitous on earth, have adaptations to diverse environments and have greater photosynthetic CO_2 fixation efficiencies than terrestrial plants when comparisons are made based on land productivities. The prospects of commercial algal biofuels production are strengthened by this diversity, versatility and efficiency. Some physiological limits to algae growth may eventually be overcome or circumvented to some extent by advances in molecular biology. However, at present, there are significant gaps in our understanding of algae biology and this is salient to sustained commercial production of algal biofuels.

2.1.1 Algae Diversity and Versatility

Algae represent a diverse group of photosynthetic organisms spanning the simple unicellular cyanobacteria which lack an organized nucleus to the complex multicellular macroalgae known as seaweeds which possess organized nuclei and structurally distinctive organs and which grow to large size. Their diverse nature has made them ubiquitous on the earth (found in ecosystems ranging from hot springs to deserts and polar regions), though they are most common in aquatic environments. They can be found in fresh, brackish, saline and hypersaline waters, at acidic, neutral or basic pH. Algae grow rapidly and are key primary inputs to aquatic food chains. Consequently, there is rapid turnover of algal biomass and though algae make up less than 0.2% of the world's total photosynthetic biomass inventory at any moment, they are responsible for 50% of the world's oxygen evolution and CO_2 fixation (approximately 50 Gt carbon fixed per year).

Diatoms, a class of unicellular algae, play an important role in primary productivity, generating 20% of the global biomass. Many species of cyanobacteria are also capable of carrying out nitrogen fixation, by which they are able to grow without a need for nitrogenous fertilizers. Under the right conditions, algae are able to grow much more rapidly than terrestrial plants; algae can double in mass in as little as 8 hours. In an algal bloom cycle, this rapid growth is accompanied by nutrient depletion and followed by die off. The dead cells provide nutrients for other microorganisms, which in turn deplete O_2 in the water causing death of fish and other O_2 requiring organisms, and much degraded water quality (i.e. the process known as eutrophication). Algal blooms can also lead to release of toxins (e.g. red tide) which can affect fish populations and present public health hazards.

On the positive side, algae have long been used as a food source. The cyanobacteria spirulina, touted as a super food, is believed to have been a food source for the Aztecs and other Mesoamericans up to the 16th century, and seaweeds have been used in foods as diverse as sushi and ice cream. In addition, algal products such as thickening agents (agar, carageenan), colouring agents (astaxanthin) for farm-raised salmon, and nutraceuticals (β-carotene, omega-3 fatty acids), have developed growing markets. More than 1000 tonnes each of *Arthrospira* (spirulina), *Chlorella*, and *Dunaliella* are produced commercially each year. Algae have also been exploited in waste water treatment based on their ability to accumulate heavy metals and metabolize toxic compounds, while at the same time generating O_2. Algal ponds are often used for tertiary treatment of waste water. The fossil remnants of diatoms, a class of algae bearing cell walls made up predominantly of silicon, make up the filtering agent diatomaceous earth. The ability of diatoms to make their cell walls into intricate silica patterns is currently being explored for potential in nano-fabrication technology.

Over 40,000 separate species of algae have been identified, and that number almost certainly represents a small fraction of the true population (perhaps as high as 10,000,000 different species (Hu et. al., 2008). Because of the diverse nature of algae, it has been difficult to settle on a universally accepted classification system. For example, some experts will exclude cyanobacteria because of their simple cellular structure relative to other classes of algae. Others will focus on a separation of unicellular (microalgae) and multicellular (macroalgae). Much of the classification of algae depends upon photosynthetic pigments, whole organism morphology, cellular anatomy and ultrastructure, and metabolism and physiology. The biological divisions that encompass the various classes of algae are;

- Cyanophyta (cyanobacteria)
- Prochlorphyta
- Glaucophyta
- Rhodophyta (red algae)
- Cryptophyta (cryptomonads)
- Chlorophyta (green algae)
- Euglenophyta
- Chloroarachniophyta
- Pyrrophyta (dinoflaggellates), and
- Chromophyta (heterokonts)

Of these classes, those that produce significant amounts of lipids are considered to be of interest for the production of Biofuels. It is worth noting at this point that macroalgae, often referred to as seaweeds, are harvested both wild (990,000 tonnes in 2006, FAO) and cultivated (ca. 15 million tonnes in 2006, FAO). Macroalgae typically require deep bodies of water for growth, and generally are viewed to lack the potential to make a significant contribution to the world's future liquid transportation fuel needs. Notwithstanding this view macroalgae production is increasing and there is interest in the EU and Japan in its use as a feedstock for methane production by anaerobic digestion and ethanol production by saccharification and fermentation (e.g. the EU Regional Development Fund Biomara project).

Most of the algae known to produce more than 20% of their biomass as lipids fall into the divisions Cryptophyta, Chlorophyta, and Chromophtya.

Cryptomonads are biflagellate unicellular algae carrying the photosynthetic pigments chlorophyll a and c, α-carotene and β-carotene giving them the colours green, olive, brown, yellow, red, or blue. They are found in waters ranging from fresh to hypersaline, sometimes in great abundance. *Rhodomonas salina* (also known as *Chroomonas salina*) is a cryptomonad known to produce lipids at high levels.

Chlorophyta or green algae range from unicellular forms to large seaweeds. Their photosynthetic pigments are similar to those in higher plants and include chlorophyll a and b, α-, β-, and γ-carotene, and various xanthophylls. Their cell walls contain cellulose and they often use starch as an energy reserve (attributes of potential feedstocks for ethanol production). *Chlamydomonas reinhardtii*, a chlorophyte, was selected as a model system for the study of plants, and is one of the few algae whose entire gene sequence is known. *C. reinhardtii* can grow autotrophically on a simple medium of inorganic salts and in the presence light and CO_2, but can also grow heterotrophically in total darkness using acetate as a carbon source and O_2. Several Chlorophytes are known to produce high levels of lipids including *Botryococcus braunii, Chlorella vulgaris, Neochloris oleoabundans*, and *Nannochloris sp*.

The chromophyta contain chlorophyll a and b, α-, β-, and γ-carotenes, zeaxanthin and several other xanthophylls. They comprise many different classes of algae including the Chrysophyceae (golden-brown algae), Bacillariophycease (diatoms), Xanthophyceae (yellow-green algae), Eustigmatophyceae, and Prymnesiophyceae. Examples of each of these classes are known to produce high levels of lipids including *Ochromonas danica, Phaeodactylum tricornutum, Nitzschia palea, Monallantus salina, Nannochloropsis sp.*, and *Isochrysis sp.*

Special consideration will be given to the division cyanophyta also known as cyanobacteria or blue green algae. Unlike the other divisions of algae described so far, this division is prokaryotic, that is, they lack nuclei and are members of the bacterial kingdom. They contain many different photosynthetic pigments including chlorophyll a and d, phycobilins, β-carotene, zeaxanthin, and other xanthophylls, and phycobilins. Although a *Nostoc* commune has been shown to produce triacylglycerides, cyanobacteria rarely produce more than 20% of their cell weight as lipids, but they will be included in this discussion because they have been shown to accumulate high levels of glycogen (as much as 60% of dry weight) as a storage material, and it is possible to divert the carbon flux from carbohydrate production to lipid production. In addition, cyanobacteria have long-established commercial production methods (mainly for food supplements and nutraceuticals) and genetic techniques have been developed for many different strains.

2.1.2 Photosynthesis and CO_2 Fixation

Photosynthesis is a process carried out by some bacterial species, algae, and higher plants. It refers to the methods by which these organisms convert the energy of light into chemical energy, harvesting light to drive CO_2 fixation. In this way, carbon is recycled from the atmosphere into biomass. An added feature of photosynthesis as carried out by algae and higher plants is the water-splitting reaction, resulting in the production of oxygen. Without this reaction, animal life on Earth would not be possible.

Photosynthesis is carried out in two separate series of steps, the light and dark reactions. The light reaction refers to the absorption of photons of light by chlorophyll and accessory pigments such as carotenes and xanthophylls. In eukaryotic algae, chlorophyll and the photosynthetic reactions take place in specialized organelles known as chloroplasts. Cyanobacteria are prokaryotes and do not possess chloroplasts or any other organelle. In these organisms, photosynthesis takes place in a membrane-bound intracellular system known as a thylakoid.

Absorption of a photon by chlorophyll leads to a series of reactions that result in the conversion of light energy to chemical energy used for the conversion of CO_2 to intermediate sugars and ultimately biomass (CO_2 fixation) and to the generation of O_2.

There are two methods by which algae fix CO_2. Most algae use the C3 pathway (otherwise known as the Calvin Cycle). In this pathway, CO_2 is combined with a 5-carbon compound to yield two 3-carbon compounds. The enzyme that catalyzes this reaction, ribulose-bisphosphate carboxylase (RuBisCo), will also act as an oxygenase when the oxygen concentration is high. Under these conditions, no carbon is fixed and a toxic compound is produced resulting in net energy loss. This phenomenon is known as photorespiration and is an example of the disadvantage faced by photosynthetic, oxygen producing organisms. Work is being done to improve efficiency of RuBisCo in plants (Spreitzer and Salvucci, 2002). This work will likely provide information relevant for improvement of algal strains as well.

Diatoms, on the other hand, along with many important terrestrial crop plants (including maize, sorghum, sugarcane, and millet), are known as C4 plants because they use a different, more efficient pathway for CO_2 fixation. Rather than use RuBisCo to form two three-carbon compounds, C4 plants combine CO_2 with a three-carbon compound to yield a four-carbon compound, eliminating energy losses due to photorespiration and improving the efficiency of CO_2 fixation. It has been calculated that C4 plants have twice the photosynthetic efficiency as C3 plants (Kheshgi et al., 2000), though this difference becomes less notable under conditions of high CO_2.

Most algae are photoautotrophs meaning that they can derive all of their energy from photosynthesis and all of their carbon requirements from the fixation of CO_2. Consequently, they require only sunlight, CO_2, and simple inorganic nutrients to thrive. Some (e.g., Chlamydomonas) are capable of growing heterotrophically in the dark which means that they can utilize exogenous carbon sources and can be cultivated in standard fermentors, much like yeast, rather than in ponds or photobioreactors. This capability has been exploited to develop tools for genetic manipulation of the photosynthetic pathway and more recently by Solazyme for the production of algal lipids by heterotrophically grown algae. The Solazyme process offers certain advantages in terms of elimination of contamination problems and higher volumetric productivity, but eliminates the primary thermodynamic advantage of algal biofuels that derives from the algal cell's ability to harness light energy to drive CO_2 fixation.

2.1.3 Physiological Limits to Growth

There are inherent limitations to photosynthetic growth. Principal among these is the level of solar flux or solar radiation. Based on the understanding of the energetics of photosynthesis and CO_2 fixation, it is possible to determine the maximum theoretical growth rate for algae. In areas of high solar radiation (receiving >6 kWh/m^2/day), the theoretical maximum growth rate for algae is approximately 100 g/m^2/day. This theoretical maximum will be lower in areas receiving less solar radiation input. There are other limitations that further reduce the growth rate (discussed below), but it is thermodynamically impossible to exceed this rate in sunlight regardless of whether the algae are grown in pond or in photobioreactors. Observations of algal growth at a rate of 50 g/m^2/day have been made both in natural blooms (Field et al., 1998) and in open pond systems (Sheehan et al., 1998), but these were not sustainable over an extended period. High values of productivity observed over extended periods in both open and closed systems tend to fall within the range of 20-30 g/m^2/day based on illuminated culture surface area (Lee, 2001).

Other limitations to growth of algae include:

Biosynthetic rates. It is necessary for an algal cell to synthesize enough of the essential cellular components for two separate cells before it can divide. All of these components derive from CO_2 and simple inorganic nutrients. It is thought that growth rates are limited not by photosynthesis or CO_2 fixation, but rather by subsequent steps of conversion of precursor sugars to biomass (Keem and Low, 1992; Ma et al., 1997).

Temperature. Algae, like all living organisms, have an optimum narrow temperature range for growth. As water temperature decreases or increases beyond the optimum range, growth is inhibited, then halted and then cell death occurs. In algal production systems temperature can be controlled to maintain optimal growth, but in some cases, this will be prohibitively expensive. In cooler climates where heating of algal systems is required, the waste heat in power plant flue gas may provide an economic means for temperature control.

CO_2 limitation. The rate at which atmospheric CO_2 can diffuse into an algal culture would significantly limit growth. This can be overcome by sparging algal cultures with CO_2. Though this adds expense to the process, it represents a means to capture CO_2 from point sources such as power plants.

Other nutrient limitations. Algae require nitrogen, phosphorous, sulphur, and other trace nutrients to grow; diatoms also require silicon for construction of the cell wall. The nitrogen and sulphur could be provided by the power plant as NOx and SOx, along with the CO_2, and many of the other nutrients would be present in the water used for culture (especially water from a waste water treatment facility). Silicon for diatom growth will likely require supplementation. It must be noted, that nutrient requirements, like growth temperature, must be maintained within an optimal range to promote maximal growth. Too little of a nutrient will reduce the growth rate and too much can prove toxic. Nutrient limitation, as discussed previously, can result in increased overall lipid content in algal cells, but it comes at the expense of overall productivity.

Self-shading. In high cell density cultures, cells nearer to the light source absorb all incoming light preventing it from reaching the more distal cells. This limitation is reduced somewhat by providing good mixing to prevent cells from spending too much time in the shade and high surface-to-volume ratios for ponds and photobioreactors.

Light saturation and photoinhibition. Algae can absorb more light than they can utilize for energy via photosynthesis and light saturation occurs at lower levels of light than found at high solar radiation areas, thus the measured growth rate will be lower than the maximum predicted by thermodynamic calculations. Above a certain light intensity, the growth rate does not just level off, but actually drops because of photoinhibition or photooxidative death (viz. irreversible damage to the photosynthetic machinery) (Smith et al., 1990). Furthermore, photooxidative death is exacerbated by high concentrations of O_2.

From a techno-economic perspective, growth rate and maximum cell density are important parameters. Growth rate determines how quickly the biomass can be produced and maximum cell density determines the amount of water that must be processed to recover the oil. To a certain extent the maximum cell density is controlled by light input and mixing (avoidance of self-shading), but it is also controlled by algal physiology. In addition, cell die off at the maximum cell density can have an impact on harvestability or on the quantity and quality of the oil product. Due to the higher surface-to-volume ratios of closed photobioreactors, the volumetric cell densities can reach levels greater than 4 g/L, more than 10 times higher than that achieved in open ponds (Chisti, 2007). Compare this value to 100 g/L routinely achieved in commercial Escherichia coli or yeast fermentations.

2.1.4 Lipid Biosynthesis

Triacylglycerides (or triglycerides, TAG), the most common lipids in algal cells, are formed from fatty acids and glycerol (Figure 2-1). These lipids are used by algal cells as a storage compound and are similar to the lipids found in vegetable oil. In addition TAGs or neutral lipids algae produce a number of other lipid types including phospholipids (polar lipids, two fatty acids and a phosphate group on glycerol, an essential component of the cell membrane), glycolipids (glycerol molecule combined with two fatty acids and a sugar molecule, e.g. galactosyldiacylglycerides, found in chloroplast membranes) and sulfolipids (sulphate esters of glycolipids also found in chloroplast membranes).

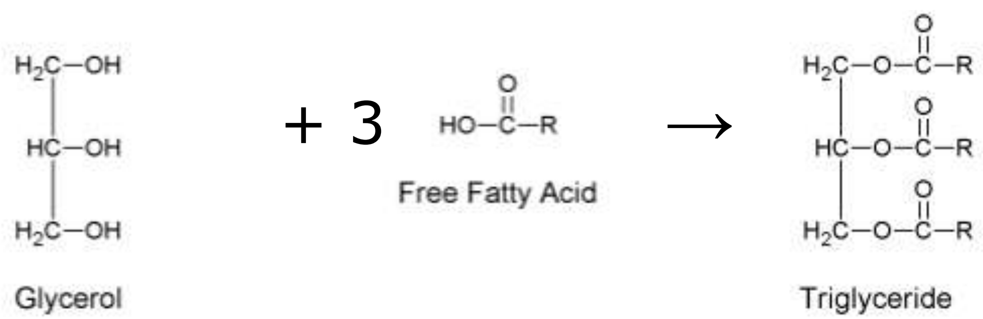

Figure 2-1 Structure of Triacylglycerides

The lipid content in algae can range from 1 % to >50 % and can vary greatly with the growth conditions. High levels of TAGs are seen mostly in eukaryotic algae and are not common in cyanobacteria or other prokaryotes in general, though some bacteria have been shown to produce up to 87 % of their dry weight as TAG (Alvarez and Steinbuchel, 2002).

TAG storage is an important adaptation for photosynthetic organisms that feast and famine with the diurnal cycle; TAG produced during the day provides a carbon and energy source for the night. The fatty acid composition of membrane lipids will vary with temperature to maintain the ideal membrane fluidity and triglyceride carbon chains can vary in length depending upon the algae species and environmental conditions during growth, as can the hydrogen-to-carbon ratio (or number of C=C alkene bonds). The lipids range in chain length from relatively short (C8-10) to very long (>C35) though the TAG fatty acids in most algae typically range from C14-C18. The lipids are mainly saturated and monounsaturated, though C12 saturated and C20 polyunsaturated fatty acids are also observed. This compares well with the fatty acid composition of palm oil and soybean oil commonly used for biodiesel.

Botryococcus braunii produces up to 50 % of its cell mass as hydrocarbon. Oils produced by this strain have generated significant interest because they can be fed into standard hydrocrackers to produce gasoline and other transportation fuels. Though this algal strain offers promise of direct production hydrocarbons for fuel, its slow growth rate is a drawback. Whereas other algal strains can double in biomass as often as every four hours, *B. braunii* has a doubling time on order of three to four days (Banerjee et al., 2002).

In eukaryotic algae, lipid content is typically inversely proportional to growth rate, increasing when growth is inhibited by lack of nutrient (especially nitrogen or silicon) or when the cell reach stationary phase (Borowitzka, 1988). These changes in growth rate can result in an increase in lipid content by as much as two fold. This is not the case in cyanobacteria, where the lipid content remains more or less constant throughout the growth cycle as well as during growth under nitrogen limitation (Piorreck and Pohl, 1984). The increase in lipid content observed with growth limitation is not thought to be due to increased biosynthetic rates, but rather to reduction in other cellular components, leaving a higher proportion of lipid overall.

In the red alga *Porphyridium cruentum*, the concentration of total lipid increased with decreasing light intensity (Klyachko-Gurvich et al., 1999). This was also seen in red alga *Tichocarpus crinitus*, though at the same time, the proportion of TAG as ratio of total lipid fell (Khotimchenko and Yakovleva, 2005). This could be a useful characteristic for large scale lipid production because cells are likely to be light limited in high cell density cultures. Increasing light intensity can also lead to increases in TAG concentration (Roessler 1990). This is also seen in freshwater algal biofilm (Napolitano, 1994) where fatty acid composition of lipids is also a function of light intensity. In the diatom *Chaetoceros muelleri*, total lipid increased from approximately 7 % to ca. 40 % to 50 % percent when cells were starved for nitrogen (McGinnis et al., 1997). Similar results were seen with other diatoms and other classes of algae as well (Roessler, 1990). Diatoms require silicon for construction of their cell wall, and growth under silicon limitation can also increase the lipid content of cells (Roessler, 1990). Temperature changes can influence the fatty acid composition of cells as changes in membrane fluidity are required, but variable results in total lipid production in response to temperature changes have been seen with different algal species (Roessler, 1990). The halotolerant alga, *Dunaliella salina*, has been shown to produce lower levels of lipids at high salinity but the proportion of neutral lipids did not change (Roessler, 1990). Phosphate limitation also leads to a slight increase in total fatty acid content in the fresh water eustigmatophyte *Monodus subterraneus* along with a large increase in the proportion of TAG (Khozin-Goldberg and Cohen, 2006).

It is interesting to note that limitation of nitrogen, phosphate, and silicate leads to increased density and sinking of diatoms, with silicon limitation providing the most pronounced change. This phenomenon is thought to allow the diatoms to move in the water column to zone of higher nutrients (Bienfang et al., 1982). As efforts are made to regulate lipid production in diatoms, it may also be possible to manipulate the density for enhanced harvest efficiency.

2.1.5 Culture Collections

Culture collections are necessary to preserve the diversity of natural habitats, protect genetic material, and provide basic research resources. These collections are supported by national governments, universities, industry and private organizations. The host facilities of culture collections carry out a wide variety of services including patent deposit, storage, distribution, identification, training, and consultation services. According to the World Federation for Culture Collections (WFCC), there are 574 registered collections of microorganisms in 68 different countries http://www.wfcc.info/index.html. As many as 90 of these registered collections contain some algae strains. The WFCC website contains a searchable database of collections which provides information on main focus of the collection, contact details, status of the collection, sponsors and budgets, availability of cultures and catalogues, and links to strain lists. Culture collections identified by the WFCC as containing significant algae species are listed in Appendix B. Slightly more than 1700 algal strains are held in these collections.

2.1.6 Oil Producing Algal Strain Selection

Successful commercial algal growth will require the development of strains and conditions for culture that allow rapid production of biomass with high lipid content and minimal growth of competing strains. Microalgae can thrive in a broad range of environmental conditions but specific strains are more limited by climatic conditions than most terrestrial crops. Various approaches are being implemented to identify potential production strains.

Testing strains in established culture collections offers the advantage of simplicity in that axenic strains are readily available along with prior knowledge (e.g. of habit) on the strain of choice. The disadvantages of this approach lie in the fact that very few strains have been deposited because of their lipid production characteristics. In addition, many culture collections have maintained their strains for years by serial transfer in liquid culture or on agar and this practice inadvertently selects for superior growth under these conditions (not in outdoor culture). In fact, it was shown in the US DOE Aquatic Species Program that common laboratory strains are rarely capable of outcompeting wild strains.

Isolation and screening of novel native algae allows for the selection for strains adapted to particular climate, water chemistry and other selective conditions such as extremes of pH, temperature, salinity, or ability to grow with unscrubbed flue gas as CO_2 source. Until recently, the effort needed to generate axenic cultures was significant and time consuming. The recent introduction of fluorescence activated cell sorting instruments (FACS) has greatly accelerated this process by allowing single particles with the fluorescent signal of chlorophyll (i.e. single organisms) to be identify and sampled from microdroplets of environmental samples or enrichment cultures. Thousands of axenic cultures can be generated daily basis by the use of FACS and liquid handling robots. These cultures can be screened for lipid production with the same equipment and lipid soluble fluorescent dyes (i.e. Nile red or BODIPY). Ultimately, fluorescent screening for lipid production must be confirmed by gravimetric analysis which requires extraction of on the order of 1 g of dried biomass and significant effort that is not amenable to high throughput.

Strains that have already shown some promise for lipid productivity may be further improved through the classical genetic techniques of mutagenesis and breeding. While mutagenesis is random and may or may not lead to superior organisms that grow well in large scale open cultivation systems, it has been used successfully in terrestrial plants. Likewise, breeding has been used for millennia in the service of agriculture especially for traits that have a number of genetic loci, but the techniques for genetic crossing of algae are limited to a few strains and have not yet been demonstrated for lipid production.

The application of genetic engineering techniques and modern methods of systems biology (viz. genomics, proteomics, transcriptomics and metabolomics) for improvement of algal strains *prima facie* has considerable promise. Genomics and the genetic engineering of algae are discussed in the next section of this report.

2.1.7 Genomics

Genomics is a relatively new branch of biological science that involves the study of an organism based on the complete genetic sequence of the entire genome. By late 2007, the complete sequences nearly 2,000 viruses, 600 prokaryotes, and about 25 eukaryotes have been recorded. This number continues to grow as sequencing technologies become faster and cheaper. Sequences have been completed for over 20 cyanobacteria and are publicly available (http://genome.jgi-psf.org/mic_cur1.html). Of the lipid-producing microalgae (viz. belong to the class of Chlorophyceae, Bacillariophyceae, or Chrysophyceae) only 11 species have been sequenced. *C. reinhardtii*, *Volvox carteri* (uni-cellular green algae), the free living strain *Chlorella vulgaris* C-169 and the symbiotic *Chlorella sp*. NC64A - *Cyanidioschizon merolae* (red alga), *Osteococcus lucimarinus* and *Osteococcus tauris* (marine picoeukaryotes), *Aureococcus annophageferrens* (a harmful algal bloom component), *Emiliania huxleyi, Micromonas pusilla* (two strains), *P. tricornutum*, and *T. pseudonana* (diatoms) and *Bathycocc sp.*, have been fully or partially sequenced to date (http://genome.jgi-psf.org/euk_cur1.html).

Currently at least another 10 algal genomes, as well as another dozen or so Expressed Sequence Tag (EST) projects, are underway. It is notable that while these projects represent a very useful survey, in no case was the rationale for sequencing of these organisms related to lipid production or other biofuels efforts. From the perspective of the molecular biology of algal oil production there are still a large number of useful algal species to sequence.

Some of the relevant information derived from algal genomics (Grossman, 2007) include the observations that the red alga *C. merolae*, has no genes coding for fatty acid desaturases, explaining the observations that it does not synthesize unsaturated fatty acids with two or more double bonds. Genomic analysis of *C. reinhardtii* provided information regarding photosynthetic apparatus (Grossman, 2007). Analysis of the genomes of the diatoms *T. pseudonana* and *P. tricornutum* provides insights into the way diatoms position themselves in the water column; the function, structure and evolution of light harvesting components; mechanisms associated with the dissipation of excess absorbed light energy; the role of the C4 pathway in CO_2 fixation; and the biosynthesis of long chain polyunsaturated fatty acids (Grossman, 2007).

2.1.8 Genetic Engineering of Algae

Gene transfer systems have been established in many algal strains, including cyanobacteria of the genera *Synechococcus, Synechocystis, Anabena, Nostoc,* and *Arthrospira* (spirulina) (Koksharova and Wolk, 2002), green algae (*Chlamydomonas, Dunaliella*, and *Chlorella*), diatoms (*Cyclotella, Navicula, Phaeodactylum*), dinoflagellates (*Amphidinium* and *Symbiodinium*), red algae (*Cyanidoschyzon* and *Porphyidium*) and euglenoids (*Euglena*) (Walker et al., 2005).

Genetic manipulation of cyanobacteria is more advanced than that of eukaryotic algae because many of the tools for molecular biology developed for bacterial manipulation also work in cyanobacteria. Since self-replicating plasmids have not been effective for eukaryotic algae, gene insertion into the nuclear genome or into chloroplast DNA is the most common tool. Though there have been a number of successful examples of gene transfer in algae, none of the strains amenable to genetic engineering make much lipid and development of the methodology in any new strain is not assured.

Promoter gene sequences are a necessary for the expression of foreign genes in all transgenic organisms. Promoters are inserted in front of foreign genes and effect (and often amplify) transcription of the foreign DNA sequence into messenger RNA. Promoters typically used in manipulation of higher plants do not work well in algae. Gene expression in cyanobacteria has been driven both by promoters derived from bacterial genomes and by native promoters (Ditty et al., 2005). Foreign gene expression in eukaryotic algae has relied on native algal promoters, notably, the promoters of the *C. reinhardtii* RuBisCo small subunit, the *C. reinhardtii* heat shock protein 70A, and the *P. tricornutum* fucoxanthin-chlorophyll binding protein. Inclusion of introns along with the cloned gene can enhance gene expression (Lumbreras et al., 1998). Stability of gene expression can be a problem without constant selection through a poorly understood mechanism known as transcriptional gene silencing (Wu-Scharf et al., 2000).

It is not always a simple matter to identify recombinant strains following gene transfer. The task is greatly simplified by the inclusion of an additional gene (know as a selectable marker) that provides either a growth selective advantage or an easily identified trait. Examples of the former are antibiotic or herbicide resistant genes and genes that restore an essential metabolic activity (Koksharova and Wolk, 2002; Leon-Banares, 2004). Reporter genes often provide visual evidence that gene transfer has succeeded. Luciferase, the enzyme responsible for light emission in fireflies, has been expressed in both cyanobacteria and eukaryotic algae.

Success in genetically modifying algae has led to attempts to develop them as production vectors for recombinant proteins, including human therapeutic proteins and vaccines (Siripornadulsil et al., 2007). In some cases, the recombinant algae are meant to be fed whole to a selected target organism (e.g., production of a toxic gene product for use as an insecticide or production of a growth hormone to enhance growth of fish in aquaculture). Further developments in genetic manipulation of algae for these sorts of applications can be expected to accelerate progress on other fronts as well, including the development of algal biomass as a source of biofuels.

Examples of engineering efforts in eukaryotic algae relevant to biofuel production include an effort to manipulate lipid production of diatoms *C. cryptica* and *N. saprophila* by transformation (Dunahay et al., 1996). The acetyl-ACP carboxylase gene (thought to be the rate limiting step in fatty acid biosynthesis) was cloned from *C. cryptica* and used to transform *C. cryptica* and *N. saprophila*. Transformants were identified with higher carboxylase activity than the wild-type, but they did not produce higher lipid levels. More recently, RNAi technology, a method used to silence specific gene expression, was used in *C. reinhardtii* to down-regulate the entire light-harvesting antenna complex (Mussgnug et al., 2007). As in the work described previously (Lee et al., 2002), the engineered strain exhibited increased photosynthetic efficiency and provided higher yields. The cyanobacteria *Synechocystis* 6803 has been engineered to alter expression of fatty acid desaturase genes. The saturation level of membrane lipids is important in controlling the fluidity of membranes in response to temperature fluctuations. Engineered strains have been developed that are more cold-tolerant (by expressing an additional desaturase gene) or cold-sensitive (by removing a desaturase gene).

Although molecular biology tools have been developed for a number of cyanobacteria and eukaryotic algae, methodology remains far behind that developed for commonly used bacterial strains and higher plants. Many strains that might be of interest for oil production have proved to be unyielding to attempts at genetic manipulation. Even in algal strains that are amenable to genetic manipulation, technical deficiencies reduce the rate of progress. These include:

- A limited number of antibiotic and herbicide selectable markers that are effective in eukaryotic algae, though many more are available for cyanobacteria.
- Recessive markers (e.g. mutated genes that disrupt essential pathways, causing recipients to depend upon additional nutrients for growth) do not work in diploid cells.
- The sexual reproduction processes are not understood for most algae and so it is difficult to carry out sexual crosses reliably.
- It is not yet possible to generate monoploid cell lines in diatoms.
- It is difficult to maintain a selective trait over many generations due to the poorly understood phenomenon of transcriptional gene silencing.

Metabolic engineering in *E. coli* has been developed to the point where new biosynthetic pathways can be created and carbon can be channelled into those pathways to achieve commercially significant yields. While this is not yet achievable for algae, current significant research efforts may deliver engineered algae with superior growth rate, lipid content, culture density and climate tolerance.

2.2 Algae Cultivation

There are four algae cultivation technologies currently in use for commercial microalgae production and proposed for algal biofuel production (viz. extensive or open ponds, intensive or raceway ponds, closed photobioreactors in many designs and closed fermenter systems).

2.2.1 Extensive Ponds

Large extensive or open pond systems are currently in use for wastewater treatment and *Dunaliella salina* production. Oxidation ponds in wastewater treatment systems (see Figure 2-2) are not in the true sense for algae production as no algae are harvested. Cognis Australia Pty Ltd produce β-carotene from *D. salina* harvested from hypersaline extensive ponds in Hutt Lagoon and Whyalla (see Figures 2-3 and 2-4). The halotolerant *D. salina* dominates naturally in brine at salt concentrations >100 $g \cdot L^{-1}$ but grows relatively slowly (producing perhaps not much more than 2.2 $t \cdot ha^{-1} \cdot yr^{-1}$). Hutt Lagoon has a total pond surface area of ca. 520 ha and Whyalla is ca. 440 ha. In terms of pond surface area, Hutt Lagoon and Whyalla are among the largest algal production systems in the world. These extensive pond algae production systems have limited mixing, and rely on natural selection and the bounty of nature with minimal intervention.

Figure 2-2 Extensive Oxidation Ponds for Sewage Treatment

Melbourne Water Corporation's Western Treatment Plant, Werribee, Australia

Figure 2-3 Extensive Pond Production of *Dunaliella salina*

Hutt Lagoon, Australia (Google Earth)

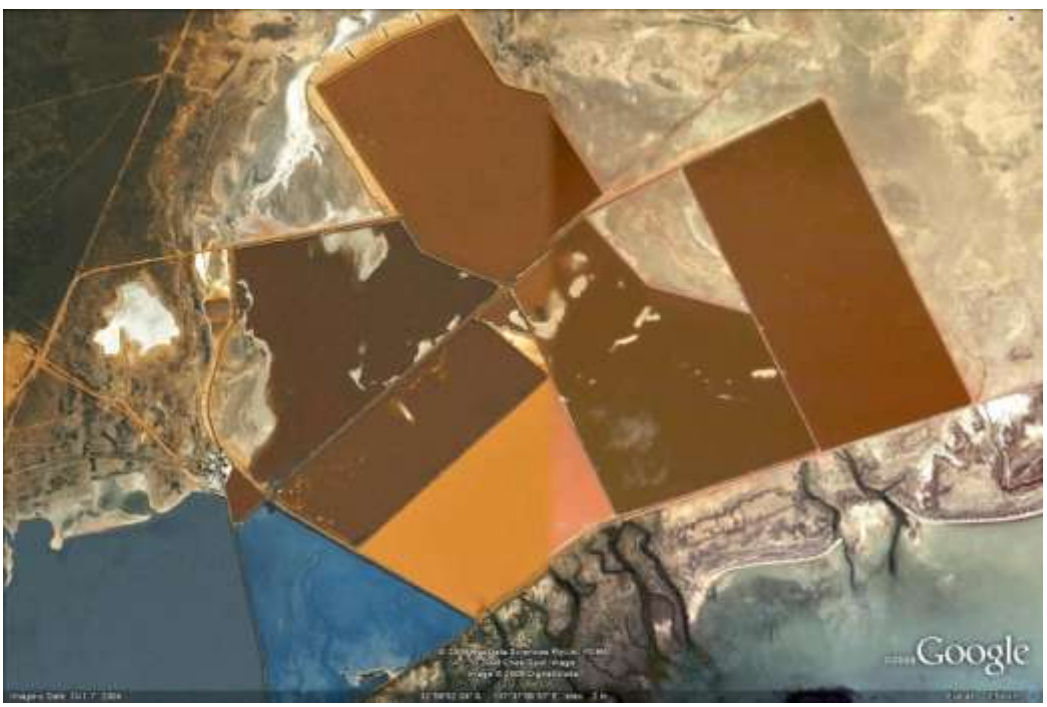

Figure 2-4 Extensive Pond Production of *Dunaliella salina*

Whyalla, Australia (Google Earth)

2.2.2 *Intensive Ponds*

Intensive or open, raceway ponds are shallow (typically 15 cm to 35 cm deep) circuits or raceways (see Figures 2-5) wherein the pond contents are cycled continuously around the pond circuit by the action of a paddlewheel. In this design, even mixing of inputs across the entire pond can be achieved by slow feeding at a single point as the pond contents move past the feed intake. Likewise, the contents can be harvested evenly across the pond from a single outtake. Algae production in these ponds can be as much as 10 times higher than in extensive ponds. Current installations are lined with plastic or cement, and typically between 0.2 ha and 0.5 ha in size. Larger ponds are envisioned for biofuels production and these ponds may be clay-based (unlined) to reduce capital cost.

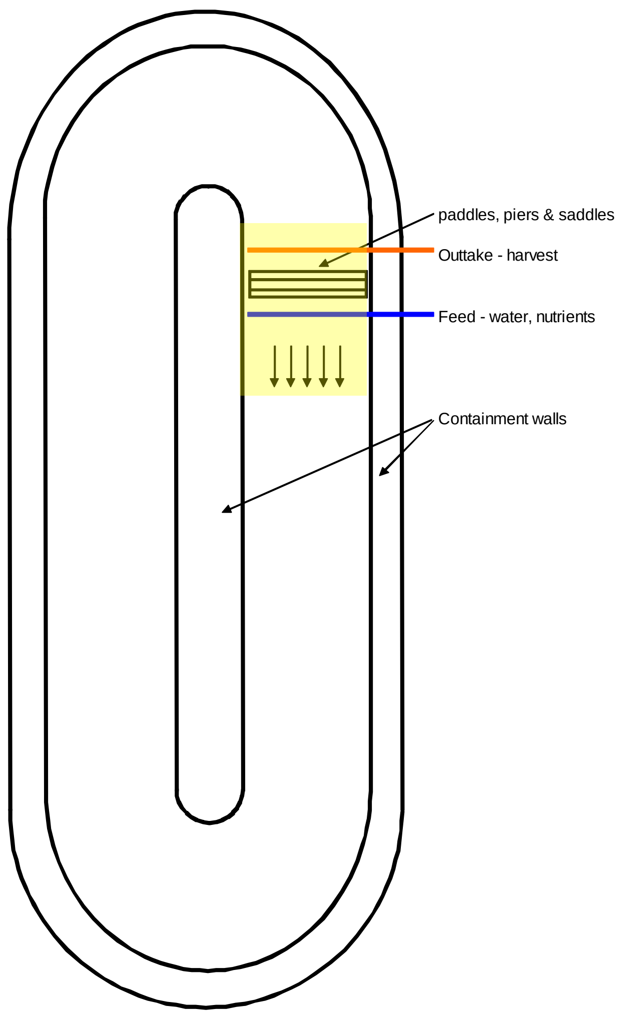

Figure 2-5 Typical Raceway Pond Design.

Raceway ponds are currently used for production of spirulina (*Arthrospira platensis* and *A. maxima*), *D. salina*, *Chlorella vulgaris*, and *Haematococcus pluvialis* (for astaxanthin). Some wastewater treatment facilities also use raceway ponds. Spirulina and *H. pluvialis* production at Earthrise Nutraceuticals LLC and Cyanotech Corp. are shown in Figures 2-6 and 2-7. Due to climate limitations, Earthrise Nutraceuticals LLC is in production for only 7 to 8 months of the year. The largest pond at the Earthrise facility (Figure 2-6) has a surface area of ca. 3.2 ha and its use appears to be preferred during periods of reduced production. Circular mixed ponds are still used in *Chlorella* production in the Far East, but are slowly being displaced by raceway-type designs. As a very general average figure the plant gate bulk selling prices is about $10/kg for spirulina, $20/kg for *Chlorella* and $100/kg for *Haematococcus* (with Chinese production costs lower than other countries).

Figure 2-6 Intensive Pond Production of Spirulina

Earthrise Nutraceuticals LLC, California (Google Earth)

Figure 2-7 Spirulina and Haematococcus pluvialis (red ponds, bottom middle) Production in Intensive Raceway Ponds

Cyanotech Corp., Hawaii (Google Earth)

2.2.3 Closed Photobioreactors

Photobioreactors are closed systems of transparent tubes, plates, bags or hemispherical domes. Photobioreactors improve yields (*cf.* intensive ponds) by protecting productive strains to some extent from contamination, pathogens, and predators, offer the benefits of some temperature control and eliminate climate related impacts of open ponds (viz. rainfall, evaporation, and diurnal and seasonal temperature fluctuations). While better mixing in photobioreactors may provide slight area productivity gains (*cf.* intensive ponds), claims of productivity, which refer to the area or footprint of the growth vessel, can be extremely high when the reactors are configured vertically and are misleading. Vertical photobioreactors must be situated far enough from each other so as to not shade, and consequently the basic limitation on productivity remains the same for both open ponds and closed photobioreactors.

Surface fouling due to bacteria, other organisms, and, in particular, algae, is a major problem with photobioreactors, and cleaning can be a major design and operational problem. Where CO_2 input and O_2 evolution must be optimized for maximum productivity, gas transfer, which is restricted to the surface area of gas-liquid interfaces, can limit scalability of photobioreactor designs.

Commercial photobioreactors facilities include the production of *H. pluvialis* in Israel (Figure 2-8) and Hawaii (Figure 2-9) and *C. vulgaris* in Germany. Typical plant gate selling prices/production costs are well above $100/kg from such systems. Consequently, biofuels production based entirely on photobioreactor systems is generally considered unlikely to be commercially viable.

Figure 2-8 ***H. pluvialis*** **Production in Tubular Photobioreactors**

(Algatech Corp., Israel)

Figure 2-9 **Production in Hemispherical Photobioreactors**

(Fuji Co., Hawaii)

2.2.4 Fermenters

Conventional closed fermenters are used for the production of heterotrophic algae where sugars (and other simple carbon sources) and O_2 rather than sunlight and CO_2 provide for growth. Open fermenters of the type used in the Brazilian sugar industry for ethanol manufacture are not suitable because algae growth is too slow by comparison to yeasts and bacteria, and consequently would be almost impossible to cultivate as a dominant species in an open sugar rich medium.

Commercial heterotrophic production of microalgae rivals autotrophic production in terms of volume and exceeds it in terms of value by a factor of two or three. For example, ω-3 fatty acids (specifically docosahexaenoic acid [DHA; 22:6 (n-3)] and eicosapentaenoic acid [EPA; 20:5 (n-3)]) are extracted from Thraustochytrids produced by Heterotrophic fermentation in the USA, China and India. Production takes place in conventional fermentors using sugars and O_2. DHA is sold for well over US$100/kg. Fermentations are also used to a minor extent in the production of *C. vulgaris*.

It has already been mentioned that Solarzyme Inc. proposes to manufacture biofuels form heterotrophic algae fermentations. This process like any fermentative process for the production of a commodity chemical or fuel has the advantage of well understood production methods at large scale but the disadvantage of requiring a high volume low cost source of carbohydrate (and in the case of fuel manufacture one that has low embodied emissions and is not a human food). This report focuses on the primary thermodynamic advantage of algal biofuel production process (viz. the harnessing of sunlight energy and CO_2 fixation). Heterotrophic production processes are not further considered.

2.2.5 Commercial Algal Production Systems

The commercial production of microalgae is presently a small industry world-wide, with little more than 6,000 $t \cdot yr^{-1}$ (dry mass) being produced autotrophically (with sunlight and CO_2). The number of companies producing algal-based products is modest, and the majority focus on cultivating and producing green and blue-green algae for food supplements, beta-carotene, and related pigments for the nutraceutical and food markets (Shadi, 2006; Olaizola, 2003; Cysewski and Thomason, 2002). In many of these operations, the final product is the algae itself, harvested, dried, and formulated into pellets or powders for direct consumption. The plant gate value of these microalgae crops is US$10,000 t^{-1} or greater.

In the case of *D. salina,* the algal biomass is harvested by adsorption of polymers and their oil. It is very high in beta carotene and is extracted and sold commercially. The algal biomass has a value of >$50/kg, based on the beta carotene content.

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.

2.2.6 Growth and Competition

Commercial extensive and intensive pond systems demonstrate large scale continuous algal cultures can be maintained for extended periods of time with a single dominant species. However, current production systems generally use specific algae strains that suited to extremes of pH or salinity. Production under these conditions limits the ability of contaminating species to thrive and take over. Very few oil producing algal species have been identified with this clear competitive advantage. Although rapidly growing oil producing algal strains have been identified, it has proven difficult to demonstrate that these strains can out compete native strains in open ponds (Sheehan et al., 1998).

Other strategies to favour growth of target species include use of a nitrogen-fixing cyanobacterium as the production strain and the genetic engineering of a suitable oil producer for fast growth, growth in extreme conditions or herbicide tolerance.

Nitrogen fixation is a characteristic of some species of cyanobacteria but is not found in any of the eukaryotic algae. It provides a potential growth advantage for nitrogen-fixing cyanobacteria, in that they are able to thrive in environments lacking readily available nitrogen sources such as ammonia, nitrate, or urea, and it provides a potential economic advantage because nitrogen supplements will not be needed for biomass production. Growth of a selected nitrogen-fixing cyanobacterium under nitrogen limiting will not prevent challenges from wild cyanobacteria. Furthermore, nitrogen fixation is an energy demanding process, and its requirement of photosynthetic energy will reduce biomass and oil production.

The use of any genetically modified microorganism, such as one with a herbicide resistance to make it the equivalent of "Roundup Ready Corn", will require careful consideration and mitigation of risks to gain regulatory approval, and management of the perception of risk to gain community approval. Herbicide can be added to the culture water to reduce the ability of natural "weed" algae to take over the population. Although the genetic manipulation of eukaryotic algae reduces the risk of lateral gene transfer into wild populations, the possibility of generating spontaneous herbicide tolerant mutant wild algae species argues against the use of this strategy. Notwithstanding this there are considerable environmental issues related to the use of large amounts of herbicide in open water systems.

In all open pond systems the amount of sunlight, temperature, nutrient level and water chemistry will change with the seasons and fluctuations in weather and impact on growth. In addition, new competing strains, pathogens, and predators constantly will be added to the culture from the air, makeup water and rain runoff. Consequently maintaining good growth of the target oil producing algae as a dominant species in an open pond is a considerably complex management challenge. Some areas of the world will provide more uniform environments that reduce the complexity of pond management (see section 4- Siting of Large Scale Algal Biofuels Production Facilities). However even in the most favourable climates, continuous operation of raceway ponds for 365 days of the year without significant intervention (i.e. draining, cleaning, refilling and inoculating) is unlikely to be achievable.

2.2.7 Algae Cultivation for Biofuel Production

One of the envisioned technologies for large scale algal biofuel production is based on very large intensive pond systems (e.g. ca. 5,200 ha at 20 $g{\cdot}m^{-2}{\cdot}d^{-1}$ productivity and 30% of dry algae mass as lipid for 100 $ML{\cdot}yr^{-1}$ production) with the production of sufficient inoculums (in smaller ponds and/or photobioreactors) to get an algal culture (re)started whenever required. While the rapid growth rate of algae will allow for brief and periodic shutdown of production for cleaning and maintenance to deal with challenges from climate, competitors, grazers, and pathogens, the frequency of such shutdowns will impact on productivity (or capital utilisation) and the economics of biofuel production. If frequent re-starts are required, as is the case for commercial Chlorella production, inoculum production will be too costly for biofuels.

Sustained growth in commercial algae mass culture (aside from spirulina or *Dunaliella*, which use selective culture medium) mostly is achieved by proprietary techniques but includes limiting nutrient supplies to reduce the ability of the contaminants to grow. Rotifers and other grazers require different approaches, including occasional excursions in pond environment to conditions inimical to these animals. Even if weeds and grazers are successfully combated, there remain the viruses and other "diseases" (lytic bacteria, fungal infections, and others) to which algae cultures will be prone, and about which almost nothing is known. The situation may be compared to early agriculture, only at a microscopic scale and at a very fast tempo. Indeed, the rapid rate at which algal growth, infection, and recovery can unfold in a mass culture is advantageous as it allows the study of these phenomena in weeks and months, instead of years and decades as was the case in early agriculture.

Until a large-scale system has been demonstrated, many uncertainties remain. Issues such as pond construction materials, mixing, optimal pond scale, and CO_2 provision have been considered, but definitive answers await detailed scale-up evaluation.

2.3 Harvesting and Concentrating

In continuous production the concentration of algae in a pond or photobioreactor is likely to be less than 0.1% mass on a dry basis. Notionally after harvesting and concentration, the algae will be in the form of a wet paste at 10% to 20% solids. Consequently the harvesting and concentrating processes requires the removal of between 100 t and 200 t of water for each tonne of algae wet paste produced and must be accomplished at low cost and with minimum energy input. There are a number of proposed and practiced harvesting and concentration methods that either are dependent on the properties of the chosen algae species (specific harvesting methods) or are generally applicable to most if not all algal species (general harvesting methods).

Examples of specific harvesting methods include;

- Filtration with >25 microns-opening screens, used for filamentous algae, e.g. spirulina.
- Simple settling (gravity thickeners without flocculants) used for *H. pluvialis* after it reaches the red cyst stage.
- Absorption to a hydrophobic material (polystyrene) coupled to an iron filament, which is then captured on a high gradient magnet. This is used for *Dunaliella* harvesting in Australia.

General harvesting methods include the use of gravitational (sedimentation) or dissolved air floatation (DAF) thickeners with the aid flocculants (viz. lime, alum ferric chloride and ionic polymers), centrifugal dewatering, membrane filtration and screening (Benemann and Oswald, 1996). With the exception of membrane filtration, all these general harvesting methods are used to some extent in sewerage treatment to remove suspended solids in treated water from oxidation ponds. Centrifugation is used for harvesting *Chlorella* in Japan and Taiwan. The cost of flocculants to aid sedimentation and DAF thickeners is considered to be too high for algal biofuel production.

Membrane filtration of microalgae has been suggested as an economical way of harvesting large quantities of algae, but it has not yet been demonstrated at any significant scale. A schematic of a submerged membrane filtration system in which the membrane filter is placed directly into a bioreactor is shown in Figure 2-

10. Despite advances in membrane technology such as pulsed backwashing and continuous air bubble scouring that slow fouling (maintain maximum flux rates for longer), when contacted about the application to algae harvesting, vendors for membrane technology were not optimistic, stating that fouling would be a major problem in such applications and would greatly increasing costs (I. Woertz, MicroBio Engineering, personal communication). Thirty years after membranes were first tested for low cost algae harvesting under a US DOE sponsored study (Gregor and Gregor, 1978), the technology is not yet up to this task.

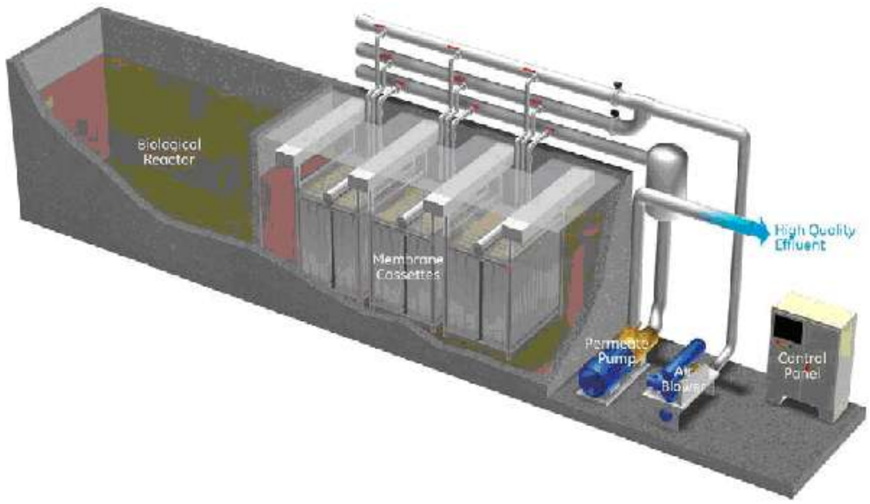

Figure 2-10 Submerged Membrane Wastewater Treatment Process

(GE Water Technologies Ltd)

In commercial production of high value algal products many of these harvesting methods have some application. For example, *Dunaliella* cells when grown under extreme salinity conditions acquire a hydrophobic surface and can be harvested by adhesion to a hydrophobic material (as practised in Australia). In India, the *Dunaliella* harvested with alum is extracted into vegetable oils for production of a carotenoid extract. In Israel, the *Dunaliella* product is used directly as a food supplement. The use of alum or other chemical flocculants is not possible so it is harvested by centrifugation (a more expensive process). In fact the centrifuges used in Israel cost several-fold more than the cultivation system itself, in part because the fragility of these cells (lacking a cell wall) requires a much slower flow rate than possible with Chlorella or other hardier algae. Clearly, the harvesting method of choice depends on not only the algal species, but also the cultivation conditions and the use of the product (and in the case of biofuels production the intended use of the co-products).

Bioflocculation refers to the characteristic of some algae to form clumps or spontaneously flocculate when grown to high cell densities. Since these clumps are much easier to harvest than unicellular algae, this characteristic could significantly reduce harvesting costs. For algal species with this characteristic, bioflocculation and sedimentation appears to take between 6 and 10 hours and produces a primary concentrate of between 2% and 4% solids. Further concentrating by centrifugation is required to yield a wet algal paste (10% to 20% solids). For example, Seambiotic (Israel) favour bioflocculation followed by basket and decanter centrifugation for harvesting *Nannochloropsis* for biofuel production and claim this is a low cost process.

A similar approach was adopted by Benemann *et al.* (1978, 1982) in their pilot-scale work was the basis for their much cited engineering and economic analyses of algae biofuels production. They argued that this was the only process that was of potentially low enough cost for biofuels production, and that bioflocculation was prevalent in natural ecosystems. However, not much is known about the mechanism of bioflocculation, and so enhancement of this trait or transfer to commercially interesting oil producing strains may require significant basic research.

Technology for low-cost harvesting of microalgae has not yet been demonstrated at any significant scale. If algae oil is to become a viable future energy source, this fundamental problem will need to be overcome. It is essential that harvesting process development takes place in an integrative manner with strain development and cultivation scale up because all three are interdependent. Harvesting processes still need to be more fully developed and engineered to demonstrate economic and technical feasibility.

2.4 Oil Extraction

Oil from algae cannot be extracted by the more conventional method used in oil seed processing (viz. pressing de-husked seeds). Algal lipids are stored inside the cell as storage droplets or in the cell membrane (Figure 2-11). The small size of the algal cell and the thickness of the cell wall prevent simple expelling to release the oil. Extraction of these oils requires disrupting the cell wall and cell membrane.

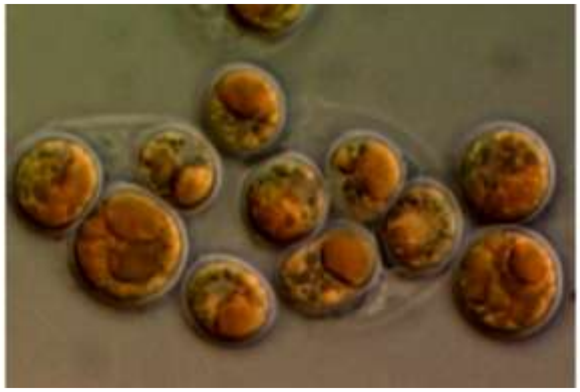

Figure 2-11 Light Micrograph of Palmellococcus Cells (a green alga) Accumulating Large Oil Droplets or Lipid/oil Bodies Under High Light Conditions.

Photo courtesy of Dr. Qiang Hu, Arizona State University.

Solvent extraction with conventional organic solvents (with and without in-situ transesterification), supercritical critical fluid fluids or heated oil, mechanical extraction and biological extractions have been considered for oil recovery from algae wet pastes.

2.4.1 Conventional Solvent Extraction

Solvents, such as hexane, have been used to extract and purify soybean seed oils, high-value fatty acids, and specialty nutraceutical products. These types of solvent-based processes are most effective with dried feedstocks or those with minimal free water. The cost of drying the feedstock significantly adds to the overall production cost and requires significant energy. A limited number of solvent have been evaluated for large scale extraction of algal biomass with some success, but at the time no effort was made to determine the process economics or material and energy balances of such processes (Nagel and Lemke, 1990). The drying of algae wet pastes for the large-scale organic solvent extraction may not be economically feasible or sound in terms of embodied energy for biofuels. Nevertheless, it is frequently used in economic assessments of algal biofuel production as it is known technology and at least for oil seeds, is practiced on a large scale with well established economics.

Another variation of organic solvent based processes process is extraction by in-situ transesterification. In this approach, the bound lipids are released as methyl esters produced by directly adding the catalyst and methanol to the dried algae. While this method has been used at larger scales, the approach works best using dried algae and is affected by the same cost and net energy issues associated with drying the biomass (Mendes et. al., 2003).

2.4.2 Supercritical Fluid Extraction (SFE)

Supercritical CO_2 has been used in manufacturing to remove caffeine from coffee, to separate high-value oils from plants, and in the laboratory to transesterify lipids into biodiesel from domestic sewage sludge (Dufreche *et al.*, 2007). Supercritical CO_2 has both liquid and gas properties, allowing the fluid to penetrate the biomass and act as an organic solvent, without the challenges and expense of separating the organic solvent from the final product. Literature describes successful extraction of algal lipids with Supercritical CO_2, albeit on a small-scale (Couto *et al*., 2010), and the resulting conversion into biodiesel. The ability of SFE to operate at low temperatures preserves the algal lipid quality during the extraction process and minimizes the need for additional solvent processing. However, the capital and operating costs for a high-pressure SFE operations currently limits its potential for biofuel production. Over time SFE applications have targeted lower value products, but not yet commodity chemicals. Technology development (e.g. gas antisolvent and subcritical fluid extractions) and further reductions in costs may lead to processes applicable to biofuel production.

2.4.3 Heated Oil Extraction

Benemann and Oswald (1996) proposed contacting algae wet paste from a gravitational thickener with heated oil and then combining centrifugal dewatering with oil extraction in a three phase centrifuge which could separate oil, water, and solids (i.e., residual biomass). In this extraction, a fraction of the oil is returned to the heater and then to extraction, and the remainder is fed forward to biofuel production. Three phase centrifuges (Figure 2-12) are commonly used in the oil and food industries and, recently in corn ethanol production for recovery of the oil fraction for biodiesel production. While this extraction method has never been demonstrated, preliminary analysis suggests it may be viable. However, extraction efficiencies are uncertain and will be species dependent and biomass pretreatments to make oil more available for extraction will need to be investigated.

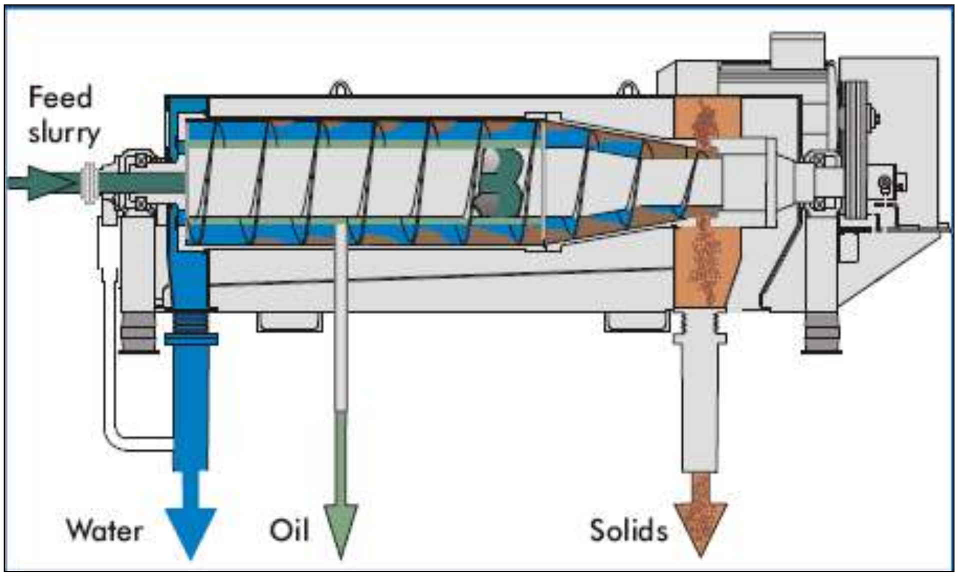

Figure 2-12 Schematic of a Three Phase Centrifuge

2.4.4 Mechanical Extraction

Mechanical treatments, such as ultra sonication (disruption with high-frequency sound waves) and homogenization (carried out by rapid pressure drops), may be used to disrupt cell walls and lead to enhanced oil recovery. For example Pursuit Dynamics Ltd (http://pursuitdynamics.com/) manufacture a device based on steam injection and supersonic disruption and claim homogenisation of plant material with very low energy input. Systems based on sonication process and centrifugation may provide economic solutions for algal lipid recover.

2.4.5 Biological Extraction

Biological methods used to capture and extract lipids offer low-tech and low-cost methods of harvesting and lipid extraction. Demonstrations in large open ponds of brine shrimp feeding on microalgae to concentrate the algae, followed by harvesting, crushing and homogenizing the larger brine shrimp to recover oil have been successful (Brune and Beecher, 2007). Using crustaceans to capture and concentrate microalgae would appear to be a promising solution for algae oil recovery. The use of enzymes to degrade algal cell walls and reduce the energy needed for mechanical disruption has also been investigated.

2.4.6 Fractionation

Low costs and high yield lipid extraction processes are unlikely to generate a feedstock clean enough to be converted directly to fuel. A crude lipid extract is likely to have a mixture of TAGs, free fatty acids, phospholipids, glycolipids, chlorophyll and other pigments, and perhaps hydrophobic proteins. Free fatty acids, phospholipids and glycolipids can interfere with transesterification by causing formation of soaps and gums. Pigments and proteins are also likely to interfere with the transesterification process. Phosphorous and metal ions (e.g. magnesium from chlorophyll) can interfere with hydrotreating (for conversion to green diesel or jet fuels) by poisoning catalyst (with consequent increase processing costs). In either case, it will be necessary to consider process options that result in feedstock streams sufficiently clean to generate biofuels.

2.5 Conversion of Algal Oil to Biofuels

The transesterification of biomass derived lipids to fatty acid methyl ester (FAME) liquid fuels is well established and practised on large scales. The alternative hydroprocessing (or hydrotreating) process has wide spread use in petroleum refining (e.g. for desulphurisation and heavy oil upgrading), has been demonstrated for biomass derived oil processing at the ca. 10 $Ml.yr^{-1}$ scale, and is currently at the commissioning or early production stages in larger capacity facilities. These processes are introduced here and discussed in terms of suitability for algal lipid processing.

2.5.1 Transesterification

The principal method of converting biomass derived lipids into biodiesel is transesterification. In this process, the relatively viscous TAGs are reacted with methanol in the presence of a catalyst to produce FAME, which more closely resemble petroleum-based diesel fuel and glycerol as a co-product (see Figure 2-13). High conversions are achieved in this reversible reaction by either adding an excess of methanol of removing glycerol as it is formed; both strategies have been used in commercial processes (van Gerpen et al., 2004).

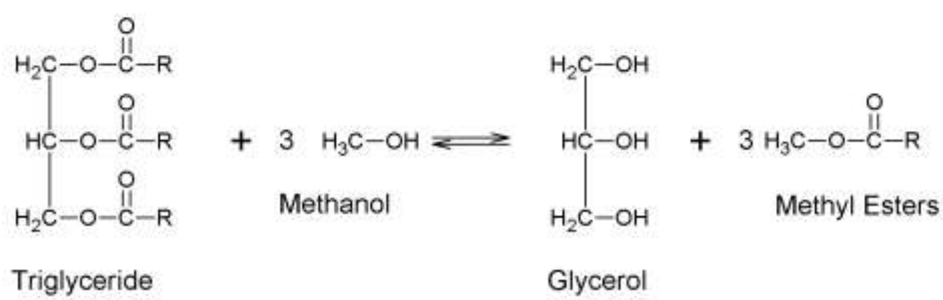

Figure 2-13 The Transesterification Reaction

Microalgae oil composition will be dependent on the organism, growing conditions and the extraction method. In addition to TAGs, the oil may also contain free fatty acids (FFAs), phospholipids, glycolipids and sulfolipids. The phospholipids, glycolipids and sulfolipids must be removed from the oil before processing. Depending upon the amount present, FFAs may be either converted to FAMEs in an acid catalyzed process or removed by caustic washing (which forms soaps by the saponification reaction of FFAs with base) prior to the base catalyzed transesterification reaction. Water can also cause undesirable saponification reactions during cleavage of the TAG ester bonds, so it must be removed. These extra processing steps with associated extra costs are required to avoid saponification in the main reaction vessel.

There are a number of variations of the transesterification process and biodiesel manufacturers will optimize the process for the each feedstock by balancing yields against equipment, catalyst, methanol and energy costs. In the case of algal biofuels, the feedstock composition is uncertain and will likely vary over time since changes in production temperature, light intensity and nutrient levels all affect algal lipid composition. Consequently, process optimization (albeit a known art) will need continuous attention in a production environment with the flexibility to deal with varying feedstock composition.

2.5.2 Hydroprocessing

The alternative path from biomass derived lipids to liquid fuels is hydrotreating or hydroprocessing, where the oil is reacted with hydrogen over a catalyst and then isomerised to produce a targeted mixture of alkanes, water, CO_2 and CO (see Figures 2-14 & 2-15). The alkane mixture can be fractionated to produce a synthetic kerosene jet fuel and hydrogenation-derived renewable diesel (HDRD) or green diesel. HDRD is compatible with petroleum processes and existing fuel infrastructure, and can be blended with petroleum products in any proportion. The glycerol moiety of the TAG is converted to propane, which can be combusted to provide process heat or liquefied and sold as LPG.

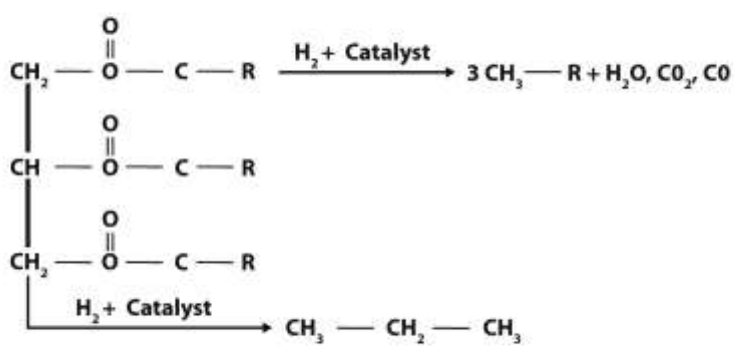

Figure 2-14 The Hydroprocessing Reaction

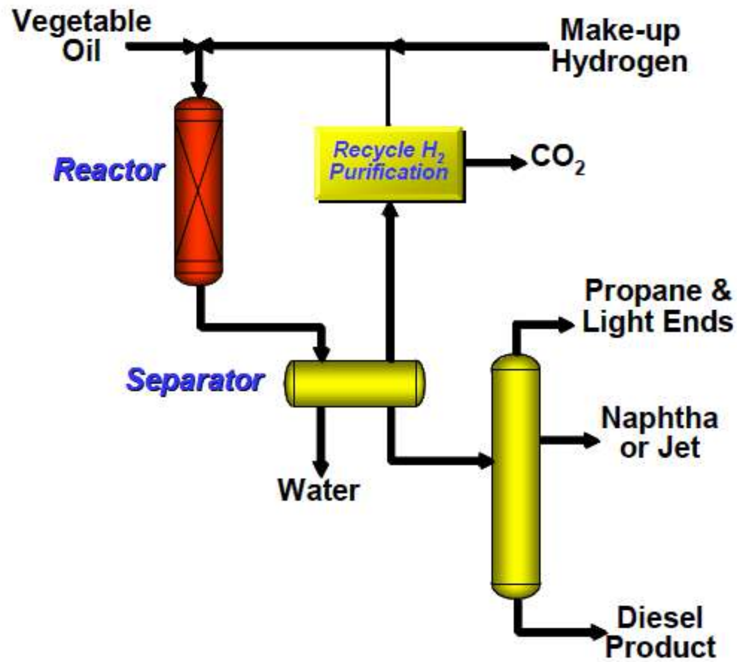

Figure 2-15 Simplified Hydroprocessing Process Flow Diagram

Vegetable oils and waste animal fats are being processed in a limited number of petroleum refineries to make HDRD. ConocoPhillips can produce up to ca. 160,000 L·day^{-1} of HDRD at the Whitegate refinery in Cork, Ireland primarily using soybean oil as feedstock. Dynamic Fuels LLC (a Syntroleum Corp. and Tyson Foods joint venture) are about to commission a 280 ML·yr^{-1} HDRD plant in Geismar, La. that will process animal oil and fat feedstocks from Tyson Foods' facilities. UOP and Eni S.p.A has installed HDRD production capacity (ca. 380 ML·yr^{-1}) at Eni's Livorno (Italy) Refinery and Galp Energia (Portugal's largest oil refiner) has licensed the UOP/ENI Ecofining process with the intention of installing production capacity at its Sines (Portugal) refinery. Cetane Energy LLC has constructed a small scale (11 ML·yr^{-1}) plant in Carlsbad, NM (USA). Neste oil operates two ca. 200 ML·yr^{-1} NExBTL plants in Finland and began early production trials at its ca. 930 ML·yr^{-1} Singapore plant in May 2010. Petrobras has adapted hydroprocessing reactors at four of its refineries to process mixtures of biomass derived and mineral oil (combined capacity ca. 256 ML·yr^{-1} biomass derived oil, primarily from soybean). Many of these projects are based on modifications to existing hydroprocessing reactors at refineries with surplus (or idle) hydrogenation capacity. In all cases the manufacturers and technology providers claim the HDRD has very low sulphur content, a higher cetane number than petroleum diesel and superior cold flow properties (cloud point). Furthermore almost any animal or plant derived oil can be processed to HDRD.

The conversion of algal oil to synthetic kerosene jet fuel has been demonstrated and the fuel has been tested by a commercial airline. In January 2009, Continental Airlines conducted the first test flight of a commercial jet using synthetic kerosene produced from Jatropha and algal oil using UOP/ENI hydroprocessing & isomerisation process. Jatropha oil was the major feedstock, but 2.5% (ca. 2,270 l) was from Sapphire Energy algal strains grown at Cyanotech in Hawaii (http://www.scientificamerican.com/article.cfm?id=air-algae-us-biofuel-flight-on-weeds-and-pond-scum). Additional test flights using fuel blends that include algal oils were conducted by Air New Zealand, Japan Air Lines, Virgin Atlantic, and KLM.

2.6 Conclusions

All of the elements for the production of lipid-based fuels from algae have been demonstrated. Algae can be grown in large outdoor cultures and harvested. The algal biomass will contain a certain percentage of lipids (though not necessarily all in the form of TAGs). Preferred or even viable technologies for algal oil extraction are at this point in time somewhat uncertain and likely to be algal species specific. However, algal oil can be obtained from harvested biomass by known means, albeit with sub optimal yield, cost and thermodynamic efficiencies. Biodiesel, HDRD and synthetic jet fuel production from algal oil have been demonstrated at non-commercial scales. The production of liquid transportation fuels from algal biomass is technically feasible.

2.6.1 The Need for Innovation

Reported favourable economic feasibility assessments are often based on biomass production rates and oil yields that are two to three times those achievable in existing production systems. These high growth rates and oil yields are yet to be demonstrated at any scale and over periods of time sufficient to provide assurance for investment in full scale production. Bioprospecting (isolation of algal species from nature), selective breeding and molecular biological strategies all offer potential to improve growth rate and oil yield. Likewise, further R&D is needed to identify and demonstrate high yield, low cost and energy efficient oil extraction. Success in these R&D endeavours has obvious benefit to the economic viability of an algal biofuels industry.

There is a need for innovation in all elements of algal biofuels production to address technical inefficiencies, which appear to represent significant challenges to the development of economically viable algal biofuel enterprises. Research needs, funding sources and current activities in the USA are reviewed in greater detail in Appendix A. The review clearly indicates the interest at all levels of government and the in private sector in the development of algal biofuels technologies and enterprises.

3. Sustainability

Sustainability is the subject of much discussion at international scientific and governmental forums on biofuels. Emerging from this discussion is a consensus that sustainability is of foremost importance as an overarching principle for the development of biomass-to-energy agro-industrial enterprises. While sustainability criteria that are agreeable to all nations are still being expounded, the generally accepted principles of sustainability include that;

- the greenhouse gas balance of the production chain is positive;
- the biomass production is not at the expense of carbon sinks in existing vegetation and soil;
- the biomass production does not endanger the food supply and existing local business activity (i.e. local supply of energy, medicines and building materials);
- the biomass production has no impact on biodiversity (protected or vulnerable biodiversity is not affected or if possible strengthened);
- soil and soil quality are retained or improved;
- ground water and surface water are not depleted and water quality is maintained or improved;
- air quality is maintained or improved; and
- the production and processing of biomass contributes to local prosperity and to the social well being of employees and the local population.

It is self evident that where there is a natural abundance of freshwater, it is likely on arable land (that may be under agriculture and may have multiple competing uses for the water resource), or on land in its natural state with considerable biodiversity value. With few exceptions where the abundance of freshwater is the consequence of human intervention, the water has multiple competing uses. Consequently, from the perspective of sustainability it seems obvious that algal production systems should target water resources other than freshwater. In fact, the proponents of algal biofuel claim that the production system is superior to biofuels based on terrestrial biomass because it can utilise non-arable land and waste water resources.

While the literature on the sustainability of algal biofuels is sparse, recent analyses appear to dispute the claims of superiority of algal production systems when compared to terrestrial crops. Two examples are provided here, namely articles published in the Environmental Science and Technology by Clarens *et al.* (2010) titled *Environmental Life Cycle Comparison of Algae to Other Bioenergy Feedstocks* and by Lardon *et al.* (2009) titled *Life-Cycle Assessment of Biodiesel Production from Microaglae*.

Clarens *et al.* (2010) compared the environmental life cycle impacts of algal biomass production to corn, switchgrass and canola production. The LCA was based on a 'cradle to farm gate' boundary (i.e. all products and processes upstream of delivered dry biomass). The functional unit was 317 GJ of biomass-derived energy or the amount of energy consumed by one American citizen in one year (i.e. the study sort to inform on the life cycle impacts associated with the production of 317 GJ of biomass based on the higher heating value of the material on a dry basis). Biomass production was modelled for three locations in the USA, and for algae was based on fresh water and municipal sewerage effluents from conventional activated sludge and biological nitrogen removal treatment plants. Algae production in raceway ponds varied from 0 $g \cdot m^{-2} \cdot d^{-1}$ (seasonal shut down) to 20 $g \cdot m^{-2} \cdot d^{-1}$ depending on site location and climate. All four biomass production systems had net positive energy (i.e. more energy produced than consumed in the biomass production). Algae cultivation had better land use and eutrophication LCA outputs than terrestrial crops, but the terrestrial crops were found to have lower energy use, greenhouse gas emissions and water use than algae production based on fresh water or municipal sewerage effluents. When industrial grade CO_2 was used in algal biomass production the system emitted more greenhouse gases (GHG) than it sequestered. Even when flue gas was used, the algal production system consumed more energy and emitted more GHG than the terrestrial plant production systems (mostly as a consequence of high mineral fertilizer use).

Lardon *et al.* (2009) compared the environmental life cycle impacts of microalgae biodiesel production to the impacts of palm, rape and soybean oil biodiesel and petroleum diesel production. The LCA was based on a 'cradle to combustion' boundary (i.e. all products and processes upstream of fuel combustion in a diesel engine). The functional unit was 1 MJ of fuel in a diesel engine. The study considered four algae biofuel production scenarios, viz. production under nitrogen fertilizer rich and starved conditions and with oil extraction from wet and dry biomass (perfect efficiency of nitrogen use was assumed in all cases). Algae production in raceway ponds varied from 19.25 $g \cdot m^{-2} \cdot d^{-1}$ (in the nitrogen starved case) to 24.75 $g \cdot m^{-2} \cdot d^{-1}$ (in the nitrogen rich case). Of the four algae biofuel production scenarios, only growth under starved nitrogen conditions with oil extraction from wet biomass had a positive net energy. In the three other algal biofuel scenarios, the energy consumed in the production was greater than the energy in the delivered biofuel. These balances assumed 100% recovery of energy from the algae cake residue after oil extraction. Fertilizer (nitrogen) consumption had a far greater impact on cumulative energy demand than drying biomass for extraction. Algae biofuel had better land use and eutrophication LCA outputs than biofuels from the terrestrial crops, but petroleum diesel had better land use and eutrophication impacts than all biofuels. In all other assessed metrics, one or all of the terrestrial crop biofuels had lower LCA impacts than all algal biofuel scenarios (again mostly as a consequence of high mineral fertilizer use).

It should be stressed that these LCA studies are based on hypothetical operating scenarios, not real production systems. The purpose of the studies is to highlight inefficiencies in the production systems that need to be addressed to create sustainable microalgae-to-biofuel enterprises. Nevertheless, these studies created debate in the scientific community and the exchange of comments published in subsequent editions of the journal. Principal among the criticisms form algae biofuel proponents are that the authors of LCA studies that report negative outcomes use too low growth rates and too high mineral fertilizer consumption figures.

In contrast, Christi (2008), a proponent of algal biofuels, provides an opinion in *Trends in Biotechnology* titled *Biodiesel from microalgae beats bioethanol*. The claimed superiority of algal biofuel over sugarcane ethanol is based solely on land use efficiencies. In this article, Christi claims algal biofuel can sustainably and completely replace all petroleum derived transport fuels, and quotes average annual algal biomass production in tropical regions as high as 1.535 $kg \cdot m^{-3} \cdot d^{-1}$ in photobioreactors (a productivity/reactor volume measurement). This report has already noted that claims of extremely high growth in vertically configured photobioreactors are misleading. Vertical photobioreactors must be situated far enough from each other so as to not shade, and consequently the basic limitations on land use and productivity remains the same for both open ponds and closed photobioreactors. Christi (2007) had previously claimed very high land use efficiencies in raceway ponds (viz. 136,000 L/ha of oil for algal biomass with an oil mass fraction of 70% and 58,700 L/ha of oil for algal biomass with an oil mass fraction of 30%). Such yields are only achievable with production of greater than 340 days in a year and at a pond productivity of ca. 50 $g \cdot m^{-2} \cdot d^{-1}$ (unrealistically high at the current state of technology). Christi also assumes that CO_2 is available at little or no cost (presumably in these same tropical regions); this is a challengeable assumption. Despite the liberal use of the word 'sustainable', Christi provides no other LCA metric than land use efficiency.

Reijnders (2008) in a rejoinder notes that Christi did not consider fossil fuel inputs during the biofuel life cycle, that previous LCA studies on *Dunaliella* and spirulina production showed little or no net energy benefit, and that by comparison terrestrial plant production systems are characterized by much lower fossil fuel inputs. The studies of Clarens *et al.* and Lardon *et al.* support Reijnders views. It would seem probable that while the assumptions imbedded in hypothetical production scenarios do have significant impacts on LCA outcomes (see examples in Table 3-1) algal biofuel production faces significant challenges to meet sustainability criteria. Limited LCA studies indicate that significant advances need to be made in reducing fossil fuel inputs associated with nutrient use, harvesting and extraction.

Table 3-1 Impacts of Assumptions in Hypothetical Production System on LCA Outcomes

Assumptions/Impacts	Energy balance	Land use
Productivity ($g \cdot m^{-2} \cdot d^{-1}$)	none	high
Concentration in ponds ($g \cdot L^{-1}$)	high	low
Biomass oil content (% mass)[a]	high	high
Extraction method	high	low
Production cycle (days/year)	very low	high

a – directly related to C:N ratios and fertilizer use

4. Siting of Large Scale Intensive Pond Algal Biofuels Production Facilities

Climate conditions, availability of CO_2, other nutrients (nitrogen and phosphorous), and water resources greatly affect algae productivity. In addition, land considerations, such as topography, use, and stewardship help define the land available for algae production. The perceived availability of water (of low quality with few competing uses), CO_2 and non-arable land resources in suitable climates is a significant driver for the development of algal biofuels. This section reviews these resources in terms of requirements for large scale algal biofuels production.

4.1 Climate

While algae's diverse nature has made them ubiquitous on the earth, the growth of any individual species (like all plants) is constrained by climate. Figure 4-1 illustrates the climate parameters affecting open pond algal production systems.

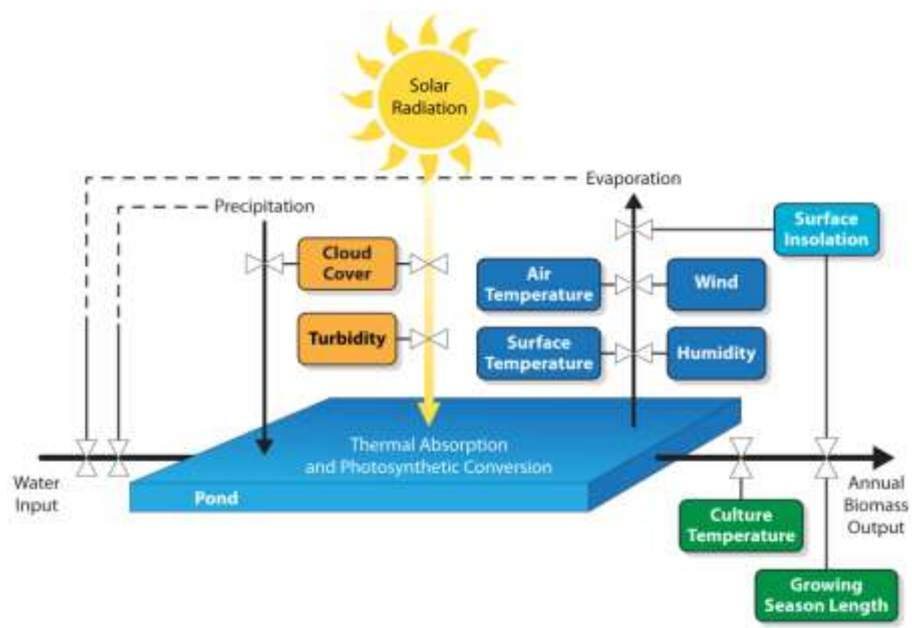

Figure 4-1 Climate Parameters Affecting Open Pond Algae Production Systems

(Maxwell et al. 1985)

Autotrophic algae, like terrestrial plants, depend upon sunlight for growth. However, algae evolved to thrive in a low light environment and have maximized their photosynthetic apparatus accordingly. While crop plants grow optimally in full sunlight, high solar radiation can inhibit algal growth and even cause cell death. Figure 4-2 illustrates the yearly sum of global solar irradiance averages over the period of 1981 to 2000 (Meteotest; database Meteonorm (www.meteonorm.com)). Solar radiation of ca. 1,500 kWh·m^{-2}·yr^{-1} is considered adequate for algae production, which means the majority of the earth's land surface would appear to be potentially suitable for algae production.

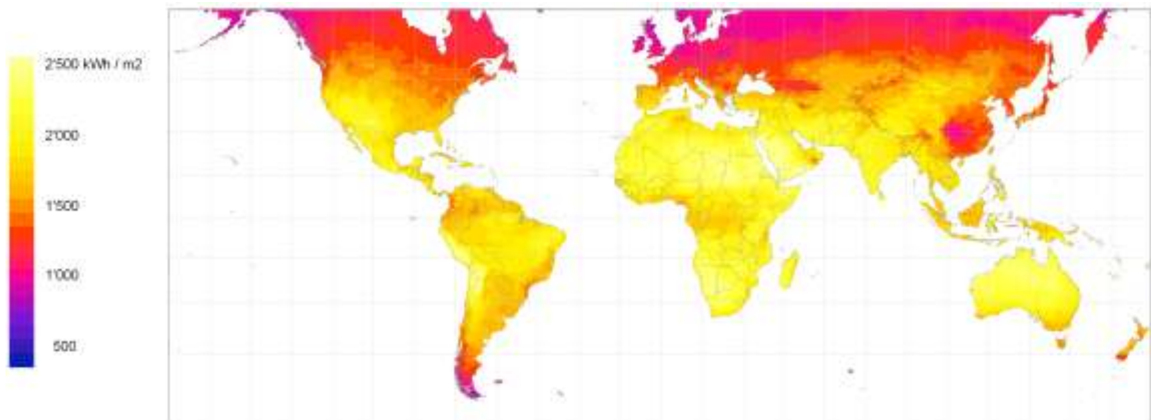

Figure 4-2   Global Solar Irradiance

(Meteotest; database Meteonorm (www.meteonorm.com))

Generally, all plant species grow well when maintained in a narrow temperature range (defined by adaptation to the ecosystem from which they are derived). Due to the differences in heat capacitance and thermal conductivity of air and water, variations in ambient temperature have slightly different affects on algae and terrestrial plants. Terrestrial plants that grow in air are immediately responsive to fluctuation in air temperature and sometimes with disastrous consequences for agriculture (e.g. frost intolerance of sugarcane). Algae suspended in water are less responsive to fluctuation in air temperature since the water temperature will be a function of solar heating, evaporative cooling, and other process factors in open ponds. However, once climatic conditions drive the temperature of the water in open ponds outside of the physiological range for the algal strain production will cease or be challenged by invasive algal species. Consequently, while temperature has a direct affect on growth rate, ambient temperature range or climate defines the effective growing season for an algal production system.

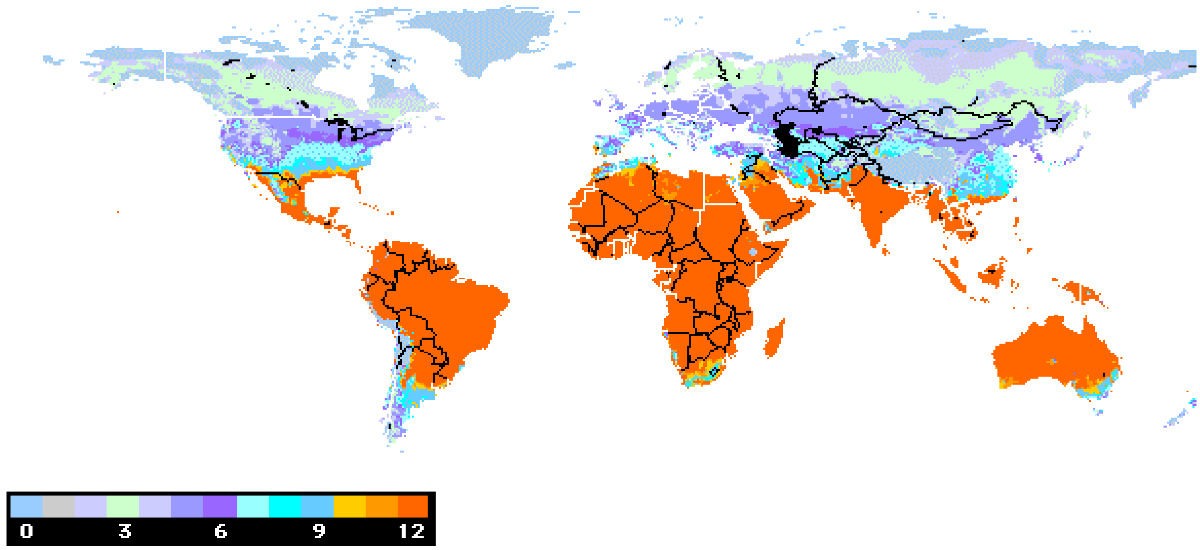

Figure 4-3 Global climate - Number of Months with Average Temperatures Exceeding 10 °C

(FAO-SDRN, 1997a)

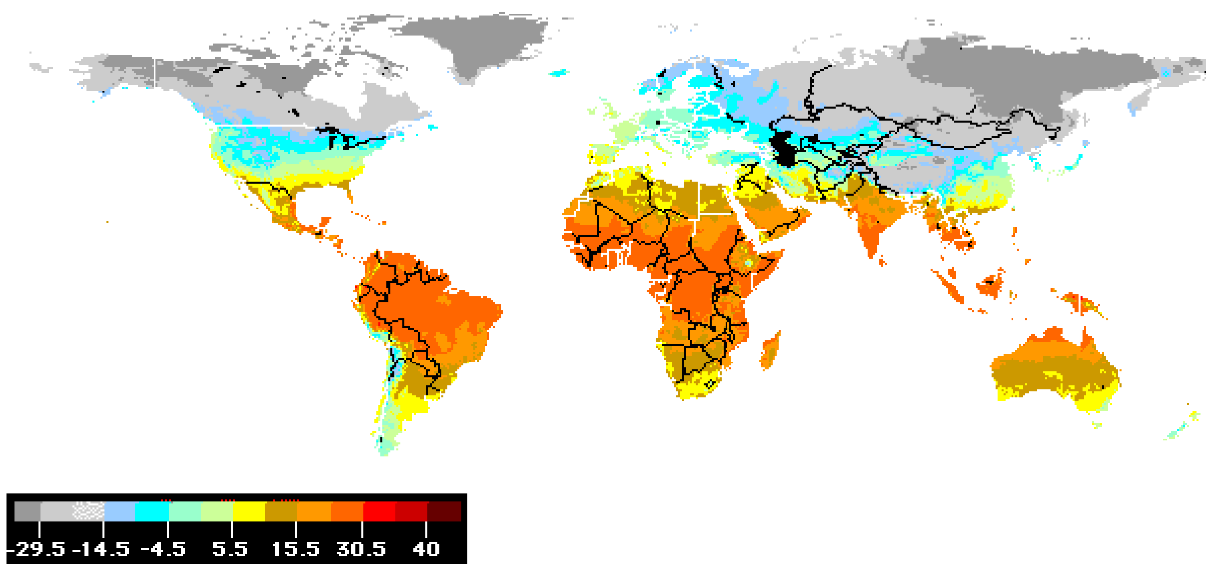

Figure 4-4 Global Climate – Average Monthly Temperature of Coldest Month in °C

(FAO-SDRN, 1997b)

Figures 4-3 and 4-4 show global temperature patterns. Ambient temperature is strongly linked to economic feasibility. It is generally considered that year round production in open ponds may be achievable where average monthly temperatures of the coldest month exceed 15 °C. There will likely be seasonal production in more temperate climates, with a consequent requirement to shut down and re-establish production on an annual basis. Re-establishing algal production in large scale facilities will have significant operating and capital costs and in some cases may be technically difficult. As production scale increases, these costs and technical difficulties may become prohibitive. Consequently, climates with ambient temperatures suitable for continuous year-round cultivation have the advantages of higher annual productivities, better capital utilisation and avoidance of seasonal re-establishment costs. While solar irradiance averages would indicate that the majority of the earth's land surface appears to be suitable for algae production, significantly less of this land would be suitable for year round cultivation.

Other climate elements that impact on the suitability of land for large scale intensive pond algal biofuels production facilities are precipitation, evaporation, and severe weather. Since sustainable open pond algal production systems are likely to be based on seawater or brackish water resources, precipitation constitutes significant challenge to the production system. Rainfall in the wet tropics can reach 30 $mm \cdot h^{-1}$ and be sustained for days. Such precipitation into a 20-30 cm deep pond reduces salinity, causes osmotic shock and death in the algal culture. While evaporation increases water requirements for an algae growth system, it is relatively consistent and the impact on salinity is manageable by dilution (especially in the case of hypersaline systems fed by seawater and systems fed by brackish water). Figure 4-5 shows the amount of effluent produced in maintaining a stable pond salt concentration with seawater and 3590 mm annual evaporation. Hypersaline systems fed by seawater produce less effluent (& use less make up water) than systems operating close to seawater salt concentrations. This reduced water demand also reduces pumping energy requirements.

Severe weather, such as hail, tornadoes, and cyclonic storms damage infrastructure. Consequently dry tropical environments, with low probability of severe weather are the most suitable to year round algal production. Such a climatic requirement places further limitations on land area most suitable for year round, large scale open pond production systems.

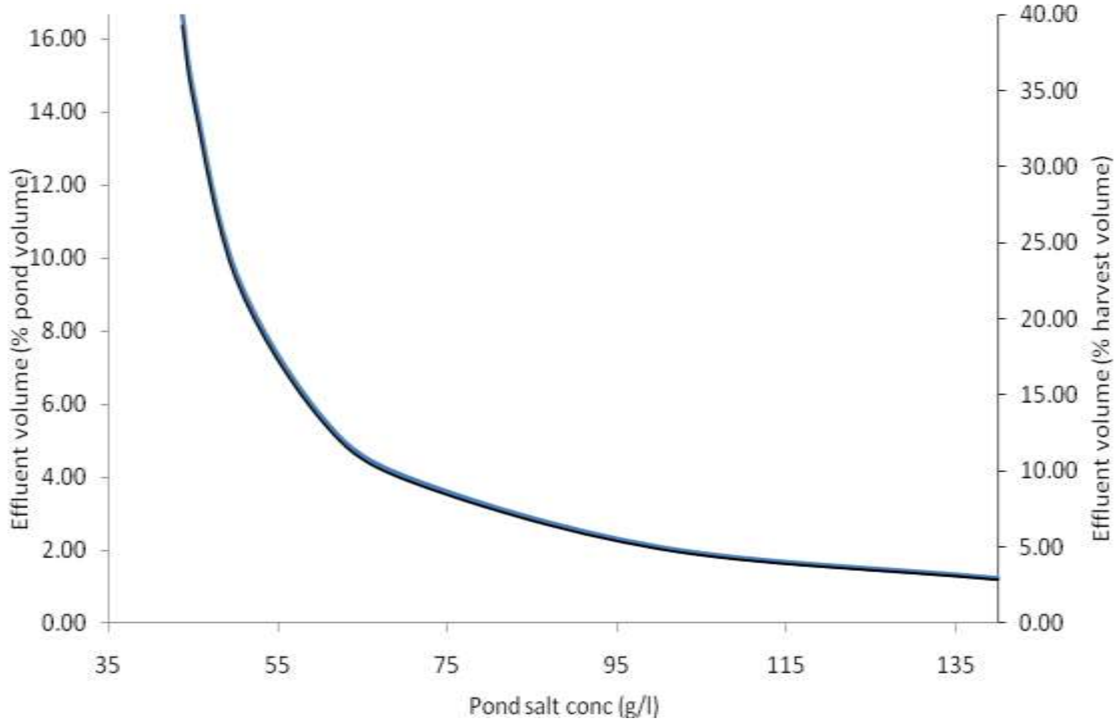

Figure 4-5 Effluent Production in a Continuously Harvested Saline Pond

4.2 Water Resources

One of the major benefits of growing algae is that unlike terrestrial agriculture, algal culture can utilize water with few competing uses, such as seawater and brackish water from aquifers, oil and natural gas wells, and coal seams. While coastal land is obviously suitable for algae production, relatively little is known about saline groundwater resources. An improved knowledge base is needed to better define global distribution, quantity, physical and chemical characteristics of groundwater resources and to predict the effects of its extraction on the environment (Alley, 2003). In the absence of this information, coastal regions are more obviously suitable for sustainable large scale algal production systems.

Some countries, for example the USA, have conducted surveys that map location and depth of saline aquifers as shown in the following figure (Feth 1965 – date of most recent survey). Depth to groundwater and geological data are pertinent to the economics and LCA of algal production based on subterranean water resources as this information influences the cost of drilling a well and the energy required to pump the water to the surface.

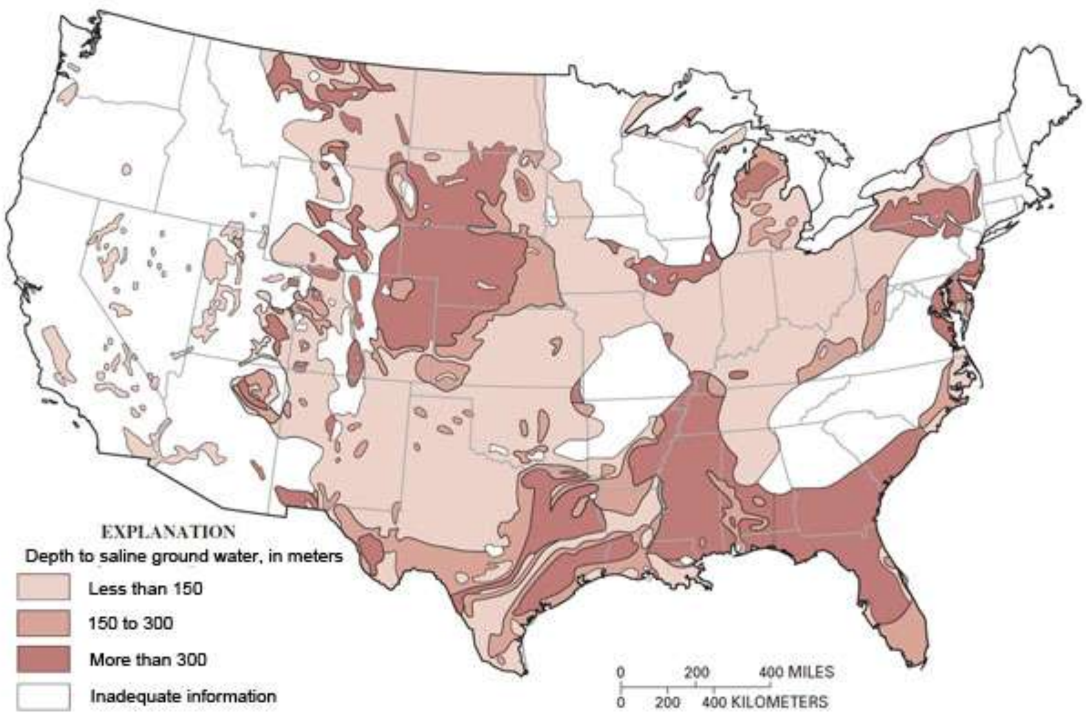

Figure 4-6 Depth to Saline Groundwater

(Feth. 1965)

Waste water, especially municipal sewage, is another possible water resource. In many large cities, the size of this resource and the suitability of the existing treatment infrastructure to modification for algal biomass production will constitute a niche opportunity. However, the algal biomass produced at these modified waste water treatment facilities may not be sufficient to justify investment in oil extraction and liquid fuel production infrastructure. Other options such as anaerobic digestion or animal feed production may be more attractive. As more sewage treatment facilities are converted to algal biomass production, stabilizing the biomass (most likely by drying) and transport to process facilities may become economically feasible. The contribution of such productions value chains to the global fuel supply is difficult to assess.

4.3 Carbon Dioxide Resources

Optimal algae growth occurs in a CO_2 enriched environment. Since CO_2 capture and geo-sequestration (or carbon capture & storage, CCS) is likely to become a necessary activity for stationary energy providers and other large volume industrial CO_2 emitters, it would seem that algae production plants could provide an alternative CCS.

Not all CO_2 emissions are suitable for capture. CO_2 could be captured from large stationary emission sources, such as power plants and industrial facilities, with high concentration of CO_2. Currently, the vast majority of large emission sources have CO_2 concentrations of less than 15 % (in some cases, substantially less). However, a small portion of the fossil fuel-based industrial sources has CO_2 concentrations in excess of 95 %. These high-concentration sources could be potential candidates for the early implementation of CO_2 capture. Applications separating CO_2 in large industrial plants, including natural gas treatment plants and ammonia production facilities, are already in operation today (Rubin, 2005). Large-scale biomass conversion facilities, such as ethanol biorefineries, also generate emissions with high CO_2 content and could be suitable for CO_2 capture.

Power plants with CCS and access to geological or ocean storage require 10 to 40% more energy than plants without CCS (depending on type, i.e. for Natural Gas Combined Cycle plants, the range is 11–22%, for Pulverized Coal plants, 24–40% and for Integrated Gasification Combined Cycle plants, 14–25%) to provide the same power to the electricity network. Therefore, there is an economic driver for power generators to consider other lower energy alternatives to CCS.

Figure 4-7 depicts the location of large stationary sources of CO_2. The most obvious characteristic of this distribution is that very few of these large CO_2 emitters are in close proximity to regions identified as being most suitable for year round large scale open pond production systems (dry tropical coastal regions). Large point sources of CO_2 are concentrated in proximity to major industrial and urban areas. Preliminary research on CCS conducted by the IPCC suggests that, globally, a small proportion of large point sources are close to oceans (potential storage locations for CO_2 in the case of CCS analysis and the water resource in the case of algal biofuel production). Of the larger proportion not near the ocean, the proportion near to sufficient and suitable brackish aquifers or waste water is unknown.

Figure 4-7 Point Source CO_2 Emissions

In circumstances where algae production plants appear to be an attractive alternative to CCS, the capacity to match scale of emissions to scale of algal production will be an important consideration. Solar energy conversion efficiencies in algae production are in the order of 1 % to 2% (300 $GJ{\cdot}ha^{-1}{\cdot}yr^{-1}$ to 600 $GJ{\cdot}ha^{-1}{\cdot}yr^{-1}$ or 1 $W{\cdot}m^{-2}$ to 2 $W{\cdot}m^{-2}$ and the solar energy collection required for micro-algae to capture a power plant's CO_2 output is about one hundred times larger than the power plant's electricity output. (Larson, 1993; Melis *et al.*, 1999; Richmond and Zou, 1999). At an average of 200 $W{\cdot}m^{-2}$ solar irradiation, a small power plant (100 MW) would have a CO_2 production rate of 37 $t{\cdot}h^{-1}$ and 81 $t{\cdot}h^{-1}$ and require an algal solar collection area in the order of 50 km^2. At a pond depth of 30 cm, an algae concentration of 0.6 $g{\cdot}L^{-1}$, a productivity of 20 $g{\cdot}m^{-2}{\cdot}d^{-1}$ and an algal oil content of 30% mass a 50 km^2 facility would produce ca. 100 ML of algal biofuel. Since the power plant will produce CO_2 24 hours per day but the algae will only consume it during daylight, there will be a need for investment in gas storage. In the case where a power company is compelled by an emissions trading scheme (ETS), carbon tax or legislation to reduce CO_2 emissions, the algal production system will need to match the emission reduction requirement. If it is significantly smaller (e.g. due to the availability of other resources) then the investment decisions will be based on the relative costs of the algal production system and the incremental increase in CCS capacity (and economies of scale may favour the latter).

Table 4-1 is adapted from the IPCC Special Report on Carbon Dioxide Capture and Storage (2005), which shows a breakdown of costs associated with CCS (the alternative to the provision of CO_2 for algal production for a large emitter). There are many underlying assumptions in this table but the message is clear, the major cost of CCS is the physical capture and separation of gases at the point source. In the absence of an ETS or other forcing mechanism the cost of algal biofuel production will include cost of CO_2 capture and transport. If the particular industry's flue gas is suitable for the algal production then gas separation will not be necessary (and capture costs will be lower than those shown in Table 4-1). In the presence of an ETS that compels industry to capture CO_2, rather than purchase emissions credits, the extent to which algal biofuel production might be attractive to industries that produce large amounts of CO_2 will be influenced by many factors (viz. the cost of emissions credits, the avoided costs of available sequestration options, the profitability of algal biofuels production in itself and the industry's understanding of and access to liquid fuel markets). As a business separate to the CO_2 producing industry and in the presence of an ETS, algal biofuel production will avoid costs for CO_2 only if capture and transport costs are less than the cost of emissions credits for sources without CCS and only if the additional transport costs are less than the storage option for sources with CCS (and not considering expansions that increase emissions).

Table 4-1 Carbon Capture and Storage Costs

CCS system components	Cost range (US$·$tCO_2^{-1}$)	Remarks
Capture from a coal- or gas-fired power plant	15-75 (net captured)	Net costs of captured CO_2, compared to the same plant without capture.
Capture from hydrogen and ammonia production or gas processing	5-55 (net captured)	Applies to high-purity sources requiring simple drying and compression.
Capture from other industrial sources	25-115 (net captured)	Range reflects use of a number of different technologies and fuels.
Transportation (pipelines)	1-8 (transported)	Per 250 km pipeline or shipping for mass flow rates of 5 (high end) to 40 (low end) $MtCO_2\ yr^{-1}$
Transport (liquefaction and shipping)	Uncertain	
Geological storage[a]	0.5-8 (net injected)	Excluding potential revenues from EOR or ECBM[b].
Geological storage: monitoring and verification	0.1-0.3 (injected)	This covers pre-injection, injection, and post-injection monitoring, and depends on the regulatory requirements.
Ocean storage	5-30 (net injected)	Including offshore transportation of 100-500 km, excluding monitoring and verification.

[a] Over the long term, there may be additional costs for remediation and liabilities.
[b] EOR – enhanced oil recovery, ECBM – enhanced coal bed methane recovery

IPCC Special Report on Carbon Dioxide Capture and Storage (2005) concludes that for emitters that are not close to oceans or suitable geo-sequestration sites pipelines are preferred for transporting large amounts of CO_2 for distances up to 1,000 km. In fact, pipeline transport of CO_2 operates as a mature market technology in the southwest of the USA, where over 2,500 km of pipelines transport more than 40 $Mt \cdot yr^{-1}$ of CO_2 from production wells to inject underground at other sites for enhanced oil recovery. For amounts smaller than a few million tonnes of CO_2 per year and for larger distances overseas, liquefaction and shipping might be economically feasible for CCS, but the costs are uncertain. Such a transport system would also incur additional energy costs for liquefaction and shipping which would have implications for algal biofuel remote to CO_2 emitters.

The requirement of nearness to large point source CO_2 emitters places another constraint on the capacity of algal biofuel production. Even when all requirements of climate, water and nearness to emitters is met, CO_2 is not freely available.

4.4 Land

Large areas of relatively flat land are required for large scale algal biofuel production (see Figure 4-7). Land availability is influenced by many physical, social, economic, legal, and political factors. Land use could constrain the installation of algal biomass production systems due to high cost, value of agricultural activity, or intrinsic environmental or cultural value (e.g. parks, wildlife areas, archaeological sites, and historical monuments). Land use change has often non-obvious life cycle impacts and sustainability issues. Physical characteristics, such as topography and soil, could also 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 five percent 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.

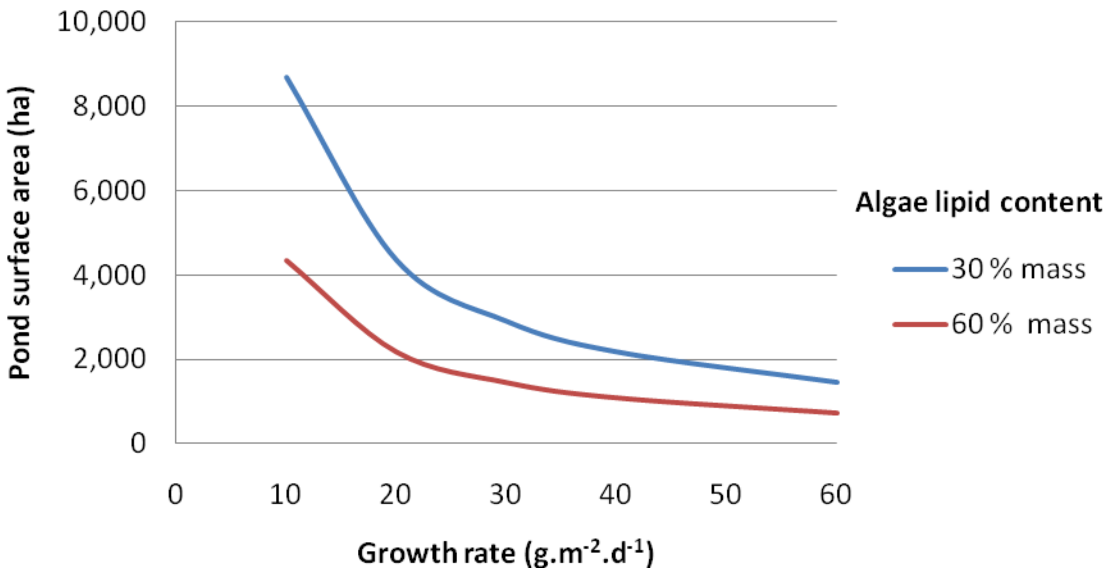

Figure 4-8 Pond Surface Area Requirement for a 100 ML Algal Biofuel Plant

4.5 Nutrients

The nutritional requirements for algae growth are similar to those for terrestrial plants. The main requirements are nitrogen, potassium and phosphorous. Algal strains differ in their ability to utilize different nitrogen sources though most can use nitrate, ammonia, or urea. The main nutrients can be supplied in the form of agricultural fertilizer, although there is a growing recognition that fertilizer production is dependent on fossil fuel, and this must be included in life cycle assessments. In addition, use of fertilizer calls to question the issue of food vs. fuel as fertilizer (especially phosphorous) is becoming increasingly in demand and wide scale use for algal cultivation could be seen as exacerbating world food production. One way to avoid this competition (and input cost) is to use wastewater, as discussed earlier, both as a source of nutrient and for cultivation. Siting large algal cultivation facilities near wastewater treatment facilities is an added complication, but it is widely held that this approach may be a means to allow for early stage development of algal biomass as it holds the potential both for fuel production and wastewater remediation.

In addition to the main inorganic nutrients, algae need a number of micronutrients used as part of the catalytic machinery needed for cell replication. These micronutrients include sulphur, iron, magnesium, manganese, calcium, potassium, and molybdenum. Sulphur in the form of sulphur dioxide may be supplied by the flue gas of a coal-fired power plant. Alternatively, sulphur as well as many of the other requirements can be found groundwater and so supplements will not be needed. Diatoms, which have a cell wall composed largely of silicon, will also require a silicon source for efficient growth.

Many processes proposed for production of algal biofuels envision energy-related co-products derived from the remaining biomass after lipid extractions. Anaerobic digestion or thermochemical conversion to syngas or pyrolysis fluid may allow for recapture of the inorganic nutrients for recycle to the cultivation medium.

4.6 Conclusions on Siting

Undoubtedly land and water in suitable climates for large scale algal biofuels production exist, but the economics of production, and the embodied energy and GHG mitigation of the biofuel will be influenced by the proximity of these resources. It is less obvious that the CO_2 is available in regions most suited to year round algal growth. Optimal siting of large scale algal biofuels production facilities will require that the resources exist in close proximity, or that there are drivers to ensure the provision of the missing resource (most likely CO_2). However, much more effort is required to develop a more complete picture of ideal locations for algae growth.

The impact of resource proximity on the economics of algal biofuels production is addressed in section 5 of this report. The availability of land in suitable climates and proximity to water and CO_2 resources may place physical limits on the contribution of algal biofuels to future liquid transportation markets; this is discussed in more detail in section 6 of this report.

5. Economics of Algal Biofuel Production

A detailed review of algae-to-fuels research, development, and commercialization would not be complete without an investigation of the potential costs for the technology. Like many aspects of algal biofuels production, there is great uncertainty with respect to the economics of future commercial scale algal production. Two distinct estimates of the economic are presented here, the first is an update of the techno-economic analysis completed for the US Aquatic Species Program employing new process information and current cost data, and the second is a ground up analysis based on large open pond systems in an Australian context. Finally, a comparison of the two analyses is provided.

5.1 NREL Updated Techno-economic Analysis

The results presented here are meant to be a building block for future analyses. Additional work is required to provide a complete, detailed picture of the algae potential from a resource and techno-economic basis. Further modeling of the algae to fuels concept, including the development of mass and energy balances around the system boundaries, are essential for complete understanding of the technology drivers.

The basis for the algae-to-lipids techno-economic analysis presented here is the assessment completed by Benemann and Oswald in 1996. The envisioned process uses an open "raceway" pond concept, a not yet demonstrated hot oil technology for extracting the oils from the algae, and hydrotreating to form green diesel or transesterification to form FAME biodiesel. Figure 5-1 shows a diagram of the production process.

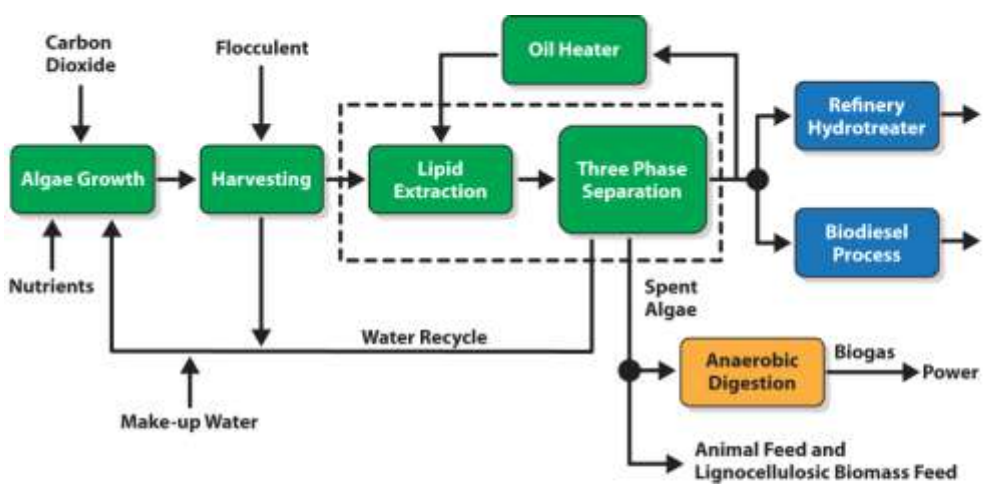

Figure 5-1 Algae Lipid Production Process

Open raceway ponds and closed photobioreactors are the only known practical methods of large scale production of microalgae. A raceway pond (see Figure 5-2 for a diagram of a typical raceway pond and Figures 2.8 and 2.9 for photographs of actual ponds) is an open system made of a closed loop recirculation channel that is typically about 0.2 - 0.3 m deep (Chisti, 2007). Raceway ponds for mass culture of microalgae have existed since the 1950s. Production of microalgae for making biodiesel has been extensively evaluated by DOE (Sheehan *et al*., 1998). Raceway ponds are less expensive than photobioreactors, but typically produce less biomass per litre of water (Chisti, 2007).

A photobioreactor can exist in a number of different forms and configurations. Figure 5-2 shows a tubular photobioreactor that consists of an array of straight transparent tubes. A photograph of a tubular photobioreactor can be seen in Figure 2.8. These tubes can be made from either plastic or glass. The tubular array is where the sunlight is collected. Microalgal broth is circulated from a reservoir to the solar collector and back to the reservoir.

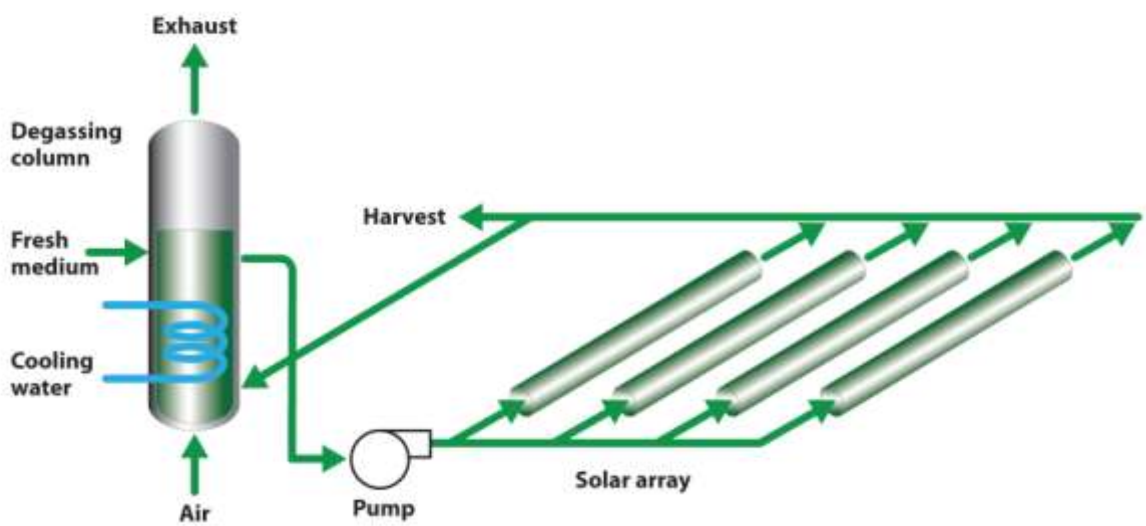

Figure 5-2 Tubular Photobioreactor with Parallel Run Horizontal Tubes

Closed photobioreactors provide some advantages over open pond systems and will be investigated in a sensitivity analysis later in this section.

The specifications for the raceway ponds used in the base analysis are included in the Appendix D. The process begins with algae growth, which is initiated and maintained by the addition of CO_2, nitrogen, and other nutrients. The algae are then harvested. During the harvesting step, a flocculent is added to the pond, which causes the algae to clump, though bioflocculation has been observed in some systems and could provide a lower cost alternative. The resulting mixture is sent to a settling tank where the water is removed and recycled. The concentrated algae stream from the settling tank is fed to a vessel, where it is contacted with hot oil. The hot oil extracts the lipids from the algae cells, producing a three-phase mixture (oil, "spent" algae cells, and water). In the Benemann and Oswald study in 1996, a centrifuge is used to separate the three-phase mixture into a lipid stream, a spent algal slurry, and a dilute water stream. A percentage of the water stream (determined both by the efficiency of the harvesting system and the quality of the recycled water) is recycled to the growth ponds. A portion of the lipid stream is sent to a heater, and then used as the hot oil extractant, while the remainder can be sent to a biodiesel or green diesel production process.

The spent algal biomass from the extraction process still contains valuable carbohydrates and proteins that can be processed and sold for byproduct credit. In this analysis, it is assumed that this residue stream either is sent to an anaerobic digester, where the resulting biogas is combusted to generate power, or is sold "as is" for animal feed and lignocellulosic biomass feed. In the base case analysis, it is assumed that an anaerobic digester is used. A sensitivity case is presented with the assumption that the residual algal biomass is used as animal feed.

Table 5-1 shows the three algae growth and lipid production scenarios analyzed in this section.

Table 5-1 Algae Production Analysis Scenarios

	Demonstrated at Roswell	Higher oil content	Higher productivity
Dry Algae			
Areal Productivity ($g/m^2/day$)	10	10	50
Volumetric Productivity ($g/m^3/day$)[a]	50	50	250
Lipid			
Mass Fraction (% of algae)	15%	40%	40%
Areal Productivity ($g/m^2/day$)	1.5	4	20
Volumetric Productivity ($g/m^3/day$)[a]	7.5	20	80
Carbon Dioxide			
Carbon Dioxide Consumption ($g/m^2/day$)	20	20	50

[a] Represents a pond that is 20 cm deep.

The Roswell case in Table 5-1 represents a conservative production rate based on data from the Aquatic Species Program. The higher oil case assumes identification of a strain with higher lipid productivity or a process that allows for nutrient limitation and concomitant higher lipid content. The higher productivity case represents the peak growth rates observed at Roswell maintained over the long term along with high lipid content. This latter case may require strain development work to realize productivities beyond those achievable with naturally occurring organisms.

The key parameter for determining the cost results is the annual fuel production rate. The base case production rate for this algae-to-fuels facility is 37.8 ML/yr. An Excel-based economic model was developed to calculate process and economic results for an algae-to-biofuels system. This model uses many processing and economic assumptions, and determines production cost of both lipids and final fuel, and resource requirements (i.e., water and CO_2 requirements). For this analysis, the model was programmed to look at the three different algae growth rate and lipid weight fraction scenarios, and determine the required land area, CO_2 usage, water usage, and production costs (capital and operating) for each case. The final production cost of lipids and fuels is calculated using a fixed charge rate methodology.

5.1.1 Techno-economic Analysis

The key cost and process assumptions used in calculating the results presented in this section are summarized in the appendix.

Figure 5-3 shows how these three different growth scenarios affect the land area required for open raceway ponds to produce 37.8 ML/yr of biodiesel.

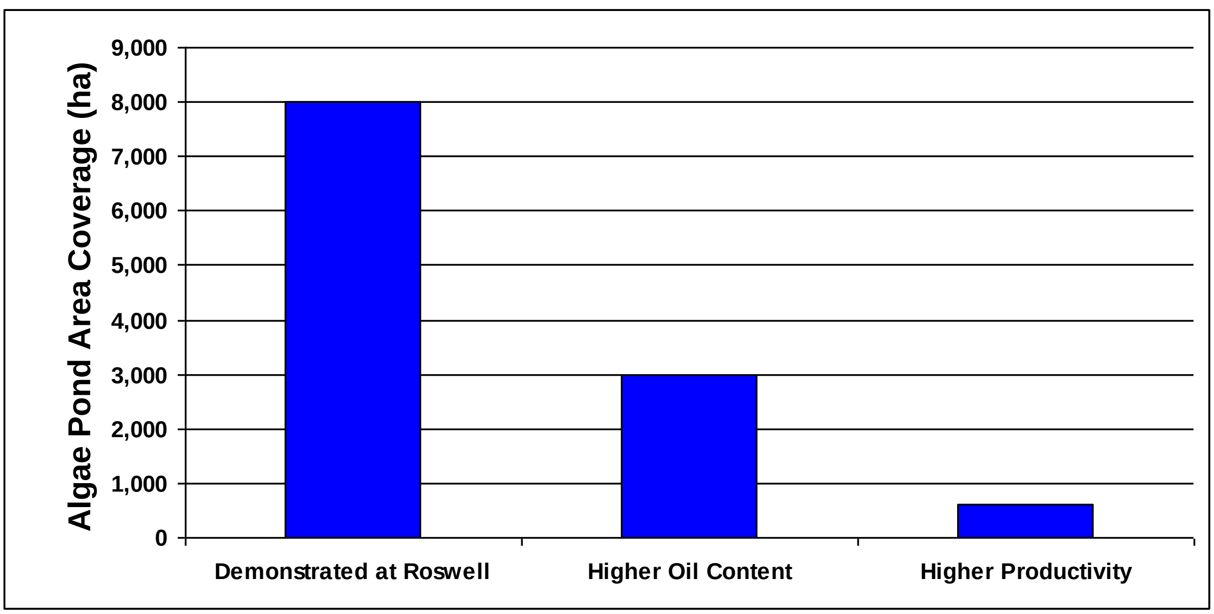

Figure 5-3 Land Required to produce 37.8ML/yr of Biodiesel at Different Algae Production Scenarios

The results in Figure 5-3 help illustrate the importance of identifying the right strain. The capital costs for the reactor system increase as the areal coverage increases, which means that not only will the current case strain cover a larger area (for reference, O'Hare International Airport in Chicago covers just over 3,000 hectares), its capital cost to produce the same amount of fuel will be higher.

Figure 5-4 shows the cost for producing 46.9 ML/yr of lipids at each algae growth scenario and the significance of the capital cost is shown. Approximately 46.9 ML/yr of lipids is required to produce 37.8 ML/yr of biodiesel.

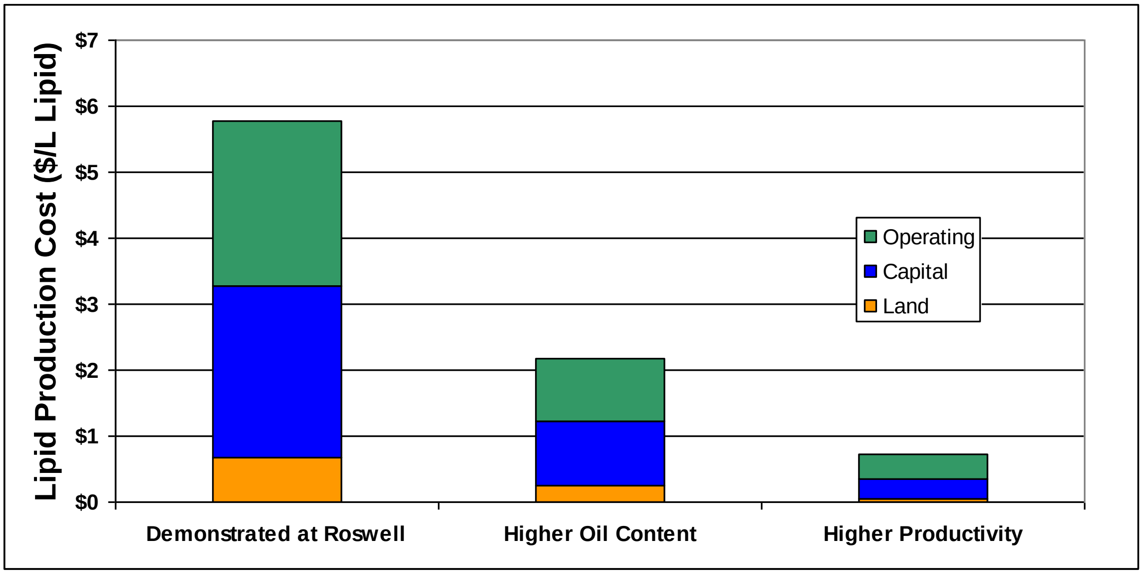

Figure 5-4 Cost to Produce 46.9 ML/yr of Algal Derived Lipids at the Three Different Algae Growth Scenarios

The cost of producing lipids ranges from approximately $5.80/litre of lipid on the high end to approximately $0.75/litre of lipid on the low end. Contrast this with the current selling cost of soybean oil (approximately $0.90/litre). The blue portion of the bar represents the capital cost, which are approximately equal to operating costs and represent the main cost elements. The cultivation system represents 25-30 percent of the total capital. The percentage contribution for the raceway pond decreases with increased productivity, because the model is established for a constant lipid output.

The technology for converting algal lipids into green diesel has only recently been developed, and the process costs are not well known. Production costs for biodiesel from vegetable oil, on the other hand is well-established. The total fuel production cost for 37.8 ML/yr of biodiesel from an algae process is shown in Figure 5-5. Land area and water requirements for each case are shown in Table 5-2. Conversion of algal lipid to biodiesel adds only a small increment to the final cost because the overall yield of biodiesel from algal lipids is 96 percent and because capital costs for a biodiesel plant are relatively low

Table 5-2 Requirements to Produce 37.8 ML/yr of Biodiesel

	Demonstrated at Roswell	Higher Oil Content	Higher Productivity
Land Area			
Hectares	9,914	3,718	744
Acres	24,480	9,180	1,836
Water Requirement			
Initial Charge (ML)	19,822	7,433	1,487
Annual Requirement (ML/yr)[a]	130,828	49,060	19,624

[a] Based on evaporation rate, harvesting rate, and water loss.

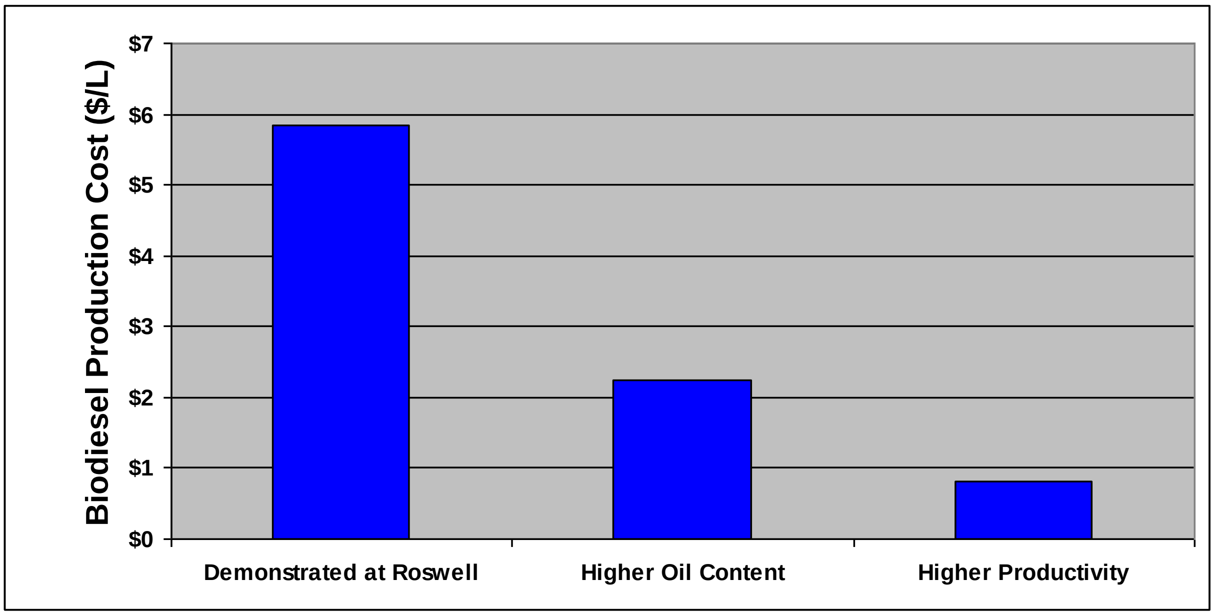

Figure 5-5 Comparison of Fuel Production Costs for Green Diesel and Biodiesel from Algae

The most apparent conclusion from the plots in Figure 5-5 is that it will take the high productivity case to produce algal biodiesel at a cost comparable to that of petroleum diesel which is hovering around $0.53/litre (EIA as of 6/02/2010). It must be noted that the biodiesel costs shown in Figure 5-5 do not include marketing and delivery costs, and it is clear that major challenges must be met to achieve the higher productivity under real process conditions.

5.1.2 Sensitivity Analysis

Photobioreactors have been discussed as a potential alternative to raceway ponds. A photobioreactor has the potential to produce significantly higher quantities of lipids per volume of water (Chisti, 2007). Higher volumetric productivity occurs because the photobioreactor has higher surface area per volume of algae/water mixture than typical raceway ponds. The result of this higher volumetric efficiency is a reduction in water consumption, which can have a significant impact on the technical and economic feasibility of the algae-to-fuels process.

A reasonable approximation for closed photobioreactor cost is $1 million/hectare (ha) ($400,000/acre) (Huntley and Redalje, 2007). The case was also run with a photobioreactor cost of $500,000/hectare ($200,000/acre). The results from this sensitivity analysis for the production of 37.8 ML/yr of biodiesel are shown in Figure 5-6.

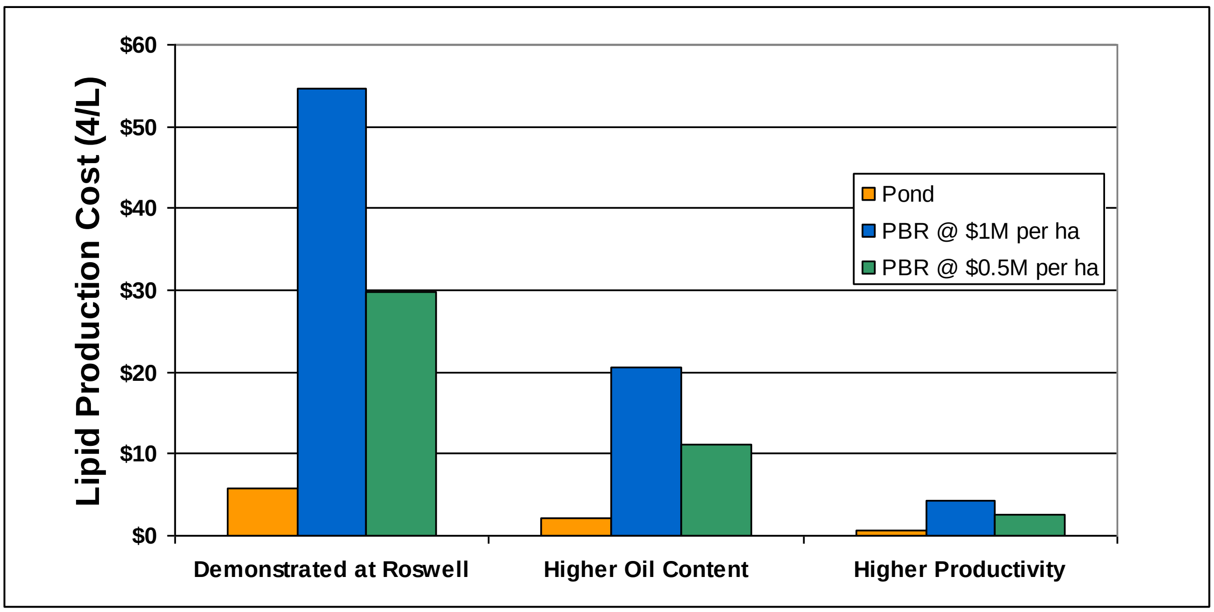

Figure 5-6 Comparison of Raceway Ponds and Photobioreactors, With Different Photobioreactor Cost

The use of photobioreactors causes the production cost of biodiesel to be significantly higher than when raceway ponds are used. At $500,000/ha, the higher productivity scenario produces biodiesel at $2.52/litre, while the raceway pond case is only $0.74/litre. In order to compete with raceway ponds, this analysis shows that the photobioreactor cost would have to be less than $100,000/hectare ($40,000/acre). This model, thus agrees with many other published analyses. Based on cost alone, the photobioreactor option does not look as appealing as the raceway pond concept, but this does not take into consideration other cultivation aspects such as maintenance of long-term growth of the desired algae strain without interference by competitors, grazers or pathogens. These results also indicate how strong the overall production cost depends on the reactor (open or closed) cost.

The base case analysis assumed that the CO_2 was available at $50/tonne. The price of CO_2 will vary, however, depending on the source and the proximity from the source to the algal cultivation facility. If the algal plant is located next to a power plant for example, it is entirely possible that the flue gas could be used as a CO_2 source at no cost. However, it is important to look at how the CO_2 cost changes the production cost. Figure 5-7 shows a plot of production cost for producing 37.8 ML/yr of biodiesel using open pond algal cultivation with different CO_2 prices. The negative CO_2 prices assume that reducing carbon emissions has market value (i.e., cap and trade mechanism).

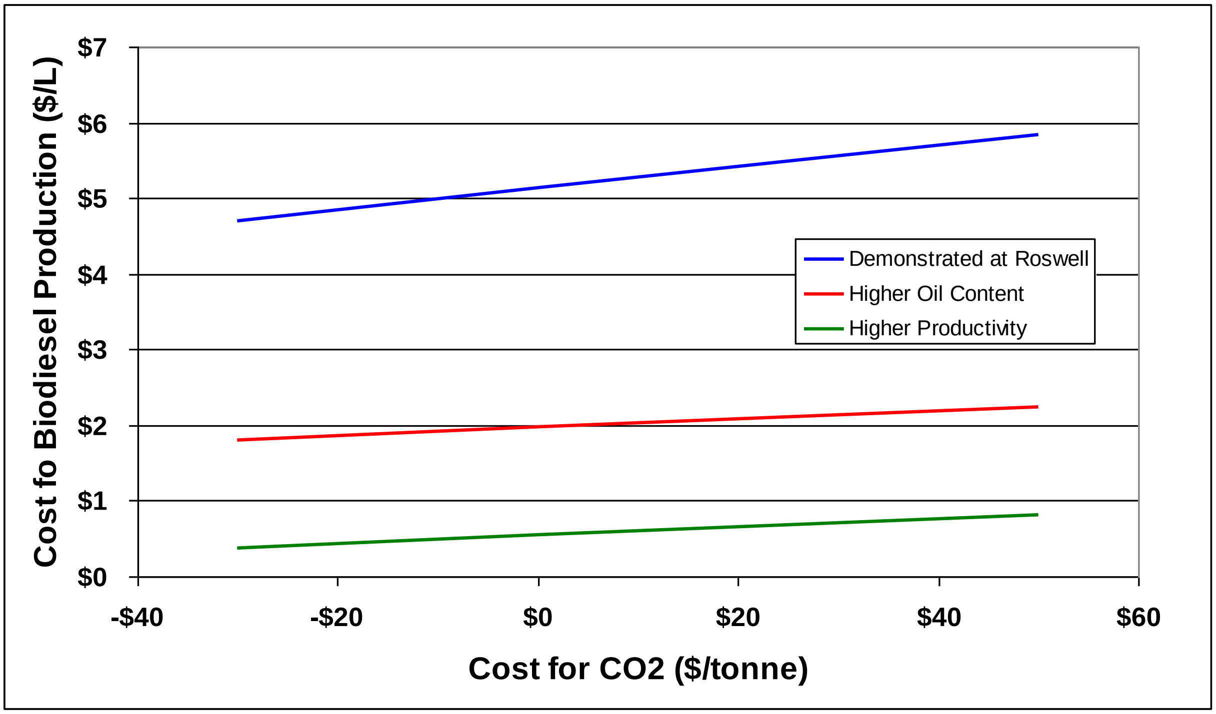

Figure 5-7 Effect of CO_2 Cost on the Total Biodiesel Production Cost from Algae

CO_2 has a fairly significant impact on the production cost. A $25/tonne decrease in price produces a reduction in the biodiesel production cost of approximately $0.36/litre for low productivity and $0.13 for high productivity.

As discussed earlier, in the base case analysis, the spent algal biomass was assumed to be sent to a digester, and the produced biogas converted into electricity. Another potential use for the spent algae is animal feed and biomass feed (protein and carbohydrates are the other primary components of algae). With the assumption that the spent algae contains 50 percent protein (animal feed) and 50 percent carbohydrate (biomass feed), and potential animal feed value of $90/tonne and biomass feed value of $9/tonne, a reduction is biodiesel production cost is recognized, especially at lower lipid productivity, where more of the algal biomass will become co-product. Figure 5-8 shows this result.

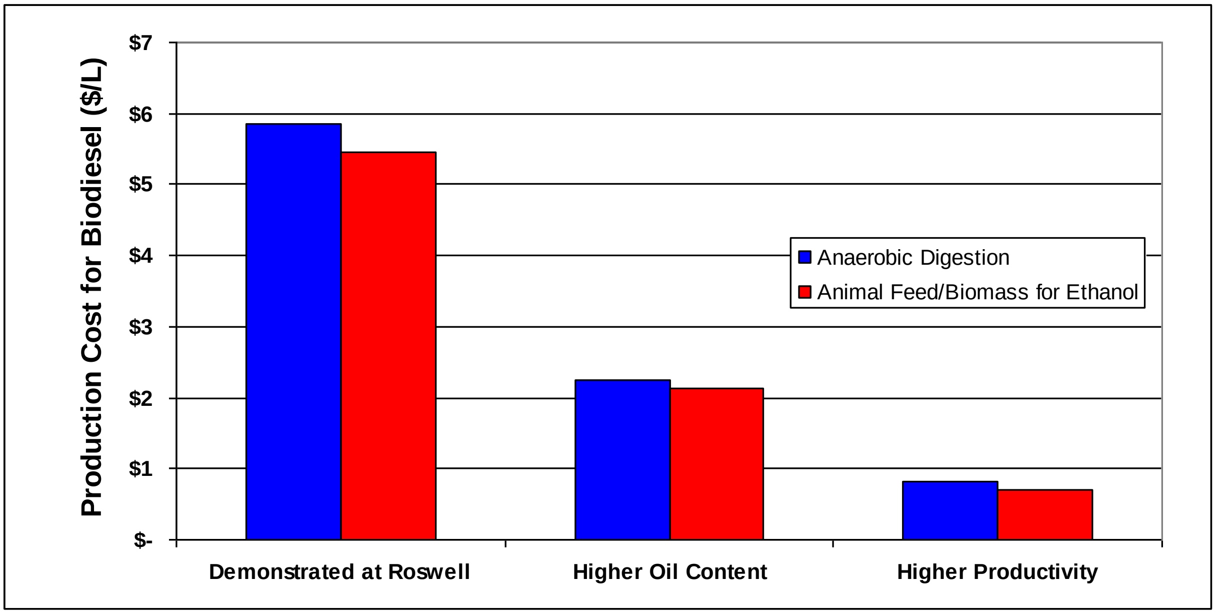

Figure 5-8 Difference In Production Cost If Spent Algae Is Used For Anaerobic Digester Feed, Or Animal Feed and Biomass Feed

Using the spent algae as biomass feed has attracted interest because it could then be converted into ethanol. The overall production cost is reduced by about 7-14 percent when the spent algae are valued as animal feed and biomass feed, rather than digester feed.

The cost of water for the lipid production process is another sensitivity that needs to be considered. The water required for the algae growth system depends on the size of the ponds (or photobioreactors), the depth of the ponds, and the evaporation rate. As discussed, one advantage of the photobioreactor system is that the water evaporation can be minimized. The sensitivity presented here looks at three different water cost scenarios. All of the results presented up to this point have considered a water cost of $0.00002/litre. Table 7-2, presented earlier in this section, shows the land and water requirements for the three different algae growth scenarios.

Water costs are extremely difficult to determine, as they depend on many variables, including location and source. To prepare this sensitivity analysis, four different references looking at various types of water were used:

- $0.00/m³
- $0.20/ m³ from a biochemical lignocellulosic ethanol production facility Aden et al. (2002)
- $0.02/ m³ for cooling water (Wang, 2002)
- $0.50/ m³ for process water (Rafelis, 2002)

Figure 5-9 shows the results of the sensitivity analysis for water, assuming that the current case algae growth scenario specifications are used to produce 37.8 ML/yr of biodiesel.

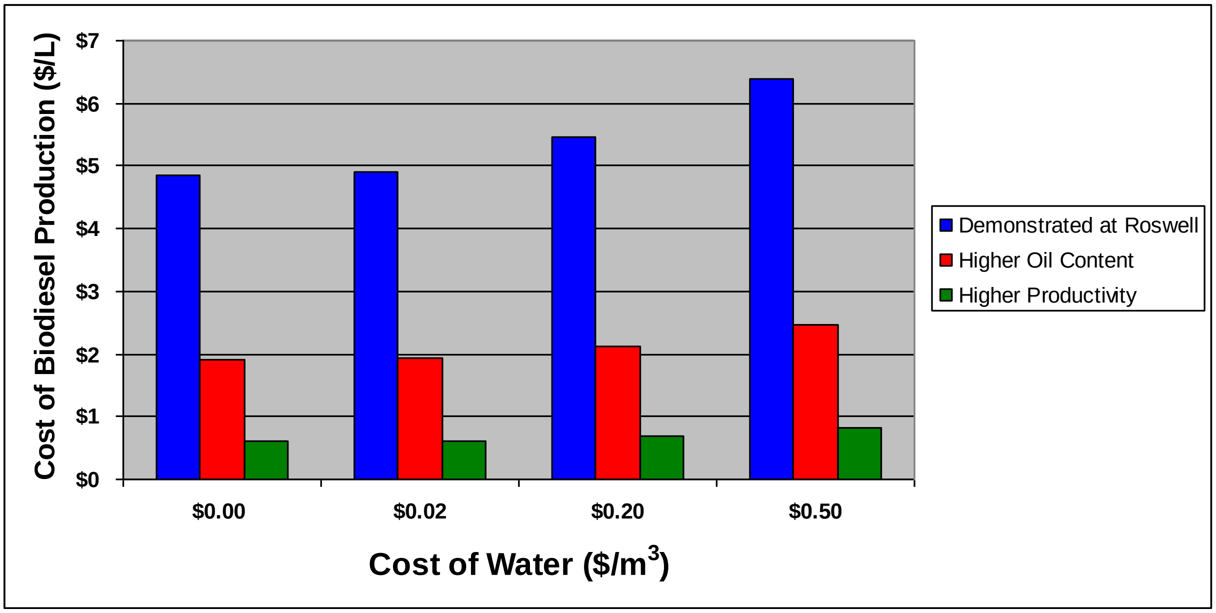

Figure 5-9 Cost to Produce 37.8 ML/Yr Of Biodiesel At Different Water Costs

As the results indicate, water use can have a significant impact on the final production cost especially at lower growth rates where the amount of water needed for cultivation and the amount to be processed is the highest. A reasonable water cost approximation, such as the cost of water used for cooling towers (Wang, 2002) added $0.09-0.60/litre to the cost of biodiesel production, depending on the productivity scenario. While that water cost might be reasonable, it assumes a certain level of purification and treatment prior to use. It is very difficult to determine what water treatment, if any, might be required prior to use in algae to fuels system. However, the potential for a significant cost impact exists, which means that a more detailed analysis of this issue should be undertaken.

The cost of water usage is only one of the areas where water will impact the feasibility of an algal biofuels process. A system of 1,000 hectares using the reactor design assumptions included in this report (depth of 30 cm, 1/3 per day harvest rate, and 0.3 cm/day evaporation rate), will process a billion litres of water. At least 90 percent of this water must be recycled back to the growth ponds. Lifting this much water more than a metre would require a significant amount of energy. Evaporation and blow-down will be at a minimum 10 percent of the daily harvest, or 100 M litres/day. Moving these amounts of water will represent a significant engineering challenge, as well as cost. In addition, it will be necessary to dispose of the 10 percent blow down water. This can likely be discharged to underground injections wells, much like the brine remaining from desalination plants, but there will be costs and regulatory issues involved. The overall cost of water (supply, movement, and disposal) is an area where closed photobioreactors have a clear advantage over open ponds because evaporation will be controlled (unless evaporative cooling is required) and water volumes will be reduced due to higher volumetric productivity. However, these differences are unlikely to reduce the cost sufficiently for photobioreactors to compete with open ponds. Much more research and analysis must be carried out in order to fully comprehend the magnitude of the water usage contribution to the overall cost of algal oil production.

5.1.3 Techno-economic Analysis Conclusions

The economic results presented in this section show that at high enough algae growth rates and lipid contents, biodiesel could be produced at prices that are comparable to but still higher than current petroleum derived diesel. Even with higher value co-products from the spent algal biomass, the costs for algae biodiesel production are higher than those of petroleum diesel. At Roswell-level productivities, the costs for production of fuels are prohibitive. An aggressive and perhaps long term, research effort will be necessary to achieve an economically viable process.

The data used in this analysis shows that open raceway ponds are significantly more attractive than closed photobioreactors. At current capital costs, photobioreactors cannot compete. However, these photobioreactors offer some advantages over closed ponds, and a more detailed analysis of their costs is required before specific conclusions can be drawn. Unfortunately, cost information is typically proprietary and not readily available. Additional sensitivity analyses show that CO_2 cost and usage of the spent algae cells can both impact the final production cost, but neither produce as much of a potential impact as water use and processing. Water use needs to be investigated more thoroughly to develop a better estimation of its overall effect on process feasibility. Systems dynamics provides a very powerful tool to evaluate scenarios for algal biofuel production that makes use of the variables described above as well as additional inputs such as current land usage, production costs, and subsidies to give a comprehensive view of the potential growth of a commercial algal biofuel industry to achieve federal mandates. The combination of multiple inputs and their interactions can provide information necessary for policy decisions to facilitate the growth of this industry.

5.2 Large Scale Open Ponds - Australia

As noted in the previous NREL analysis there is a need for detailed mass and energy balance models in order to further improve the cost estimates for algal biofuels. Accordingly, a dynamic material balance and economic model was developed to explore the cost of algal biofuel production under a number of production scenarios. The detailed assumptions that underpin the model and material balances for pond operation at 45 $g \cdot L^{-1}$ salt concentration (slightly above seawater concentration) and 140 $g \cdot L^{-1}$ salt concentration (hypersaline) are provided in Appendix C. In the hypothetical production system, algae (of an unspecified species) are grown in very large raceway ponds (see Figure 5-10) in Karratha, Western Australia (one of the few places in the world that meets all requirements for very large scale algae production, including nearness to a CO_2 emitter). Seawater is provided to the pond system by channels fed by tidal flow (in the lowest water cost scenario) and by pipes and pump stations over variable distances in a scenario than includes costs of infrastructure for the provision of water. Similarly, CO_2 is provided free of charge at the battery limits of the algal production facility (in the lowest CO_2 cost scenario) and provided by pipes over variable distances in a scenario than includes costs of infrastructure for the provision of CO_2. The algae are harvested and dewatered and extracted with organic solvents, prior to transesterification. The details of these processes and references to cost information are provided in Appendix C. A discounted cash flow method was used to determine the after-tax break-even cost of algal FAME production at a discount rate of 15% (i.e. the FAME price that gives an after-tax $0 NPV and 15% IRR).

The model provides the flexibility to include or exclude pond liners in the construction costs. The decision to include pond liners will be based on soil permeability. It is cheaper to use pond liners than to move clay to a site where the soil is permeable.

In the base case, it is assumed that the value of the algal cake produced from the solvent extraction process has no value other than its energy content. The value of the algal cake as a co-product is considered in more detail in section 5.2.1 of this report. Co-products are not defined, but rather given a value to show sensitivity of fuel production cost. It should be noted that while very high value co-products are possible for some algal species, the size of production would be far greater than existing markets for those products.

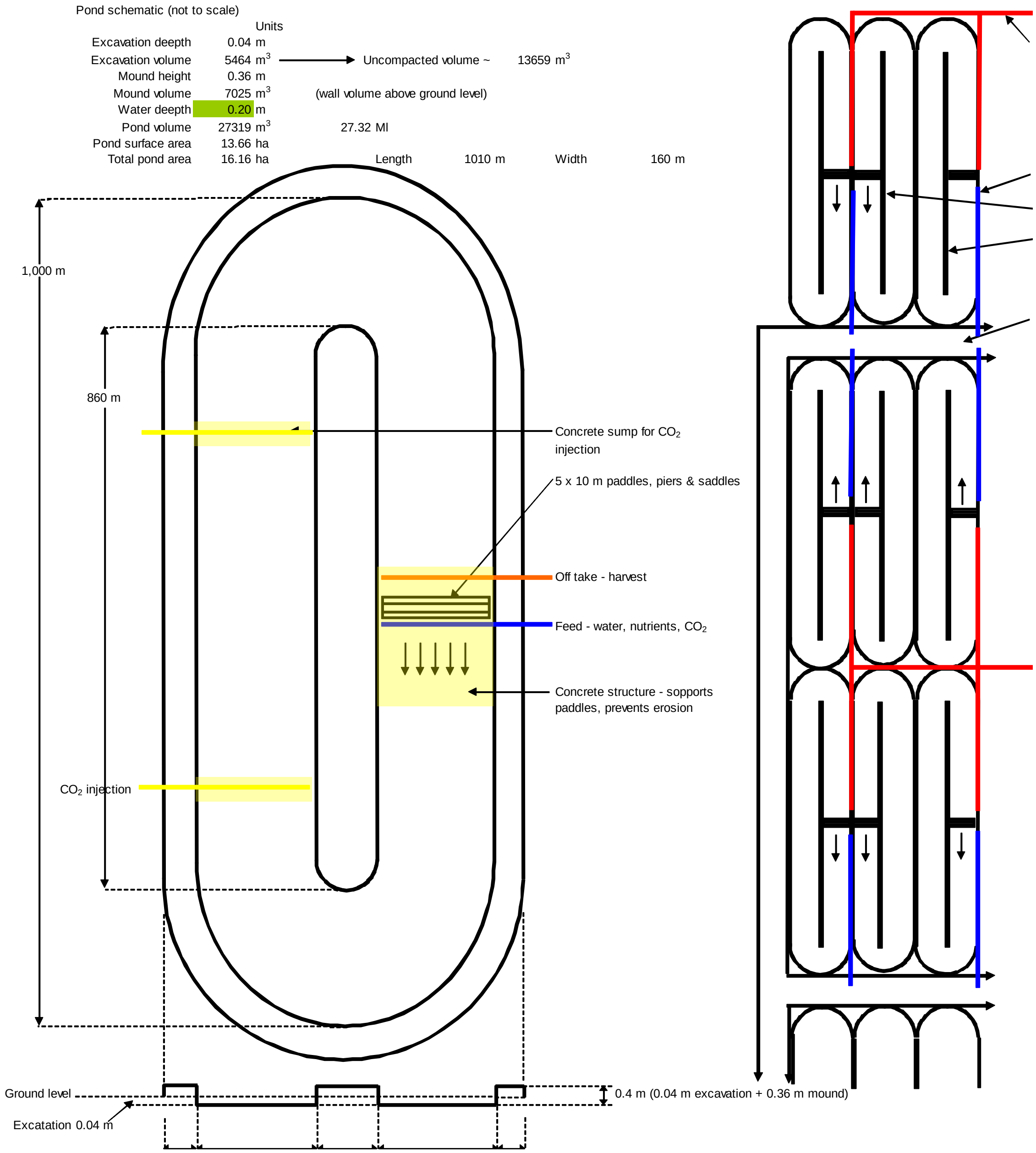

Figure 5-10 **Schematic of Raceway Pond System.**

The sensitivity of biodiesel cost to plant size is shown in Figure 5-11. For small plants 20 ML·yr^{-1} to 40 ML·yr^{-1} the economy of scale of the processing facility is evident. The pond construction costs scale up linearly and for larger plants 70 ML·yr^{-1} to 100 ML·yr^{-1} this is the biggest influence on the fuel production cost. Figure 5-2 also highlights the significant impact of the inclusion of pond liners on costs. For a 100 Ml plant operating ponds at 45 g·l^{-1} salt the extra cost of the inclusion of pond liners in the design (due to soil permeability) is equivalent to infrastructure costs for pumping water 13.7 km. For a plant the same size but operating at 140 $g \cdot l^{-1}$ salt ponds the inclusion of pond liners in the design is equivalent to infrastructure costs for pumping water 38.8 km.

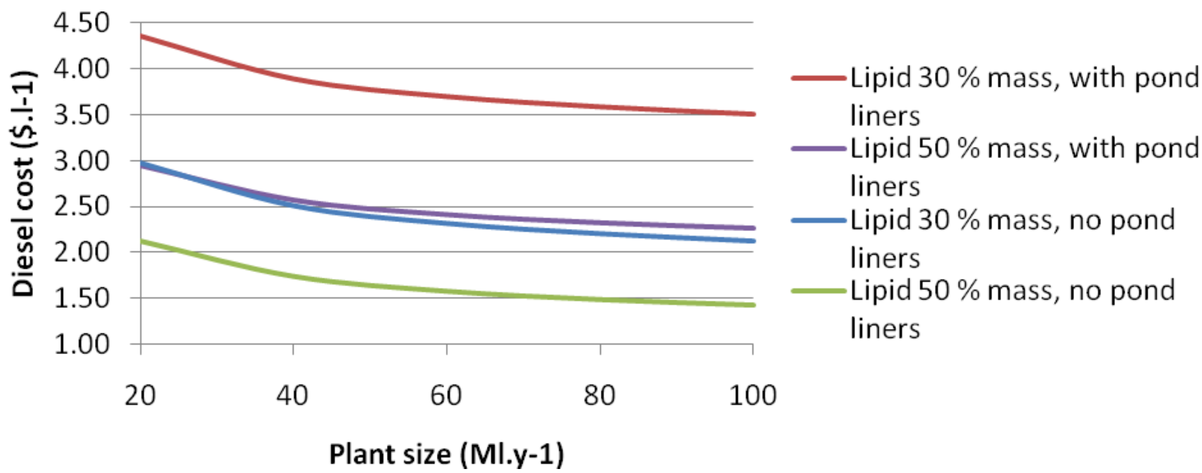

Figure 5-11 Diesel Production Cost Sensitivity to Plant Production Capacity

Figure 5-12 shows the sensitivity of fuel production cost to inclusion of infrastructure costs for the provision of water and CO_2. Infrastructure costs for water are high and have significant impact on fuel costs. By comparison costs for CO_2 provision are much lower. The figure clearly shows that, at least from the perspective of economic feasibility, proximity to the water resource is more important than proximity to CO_2.

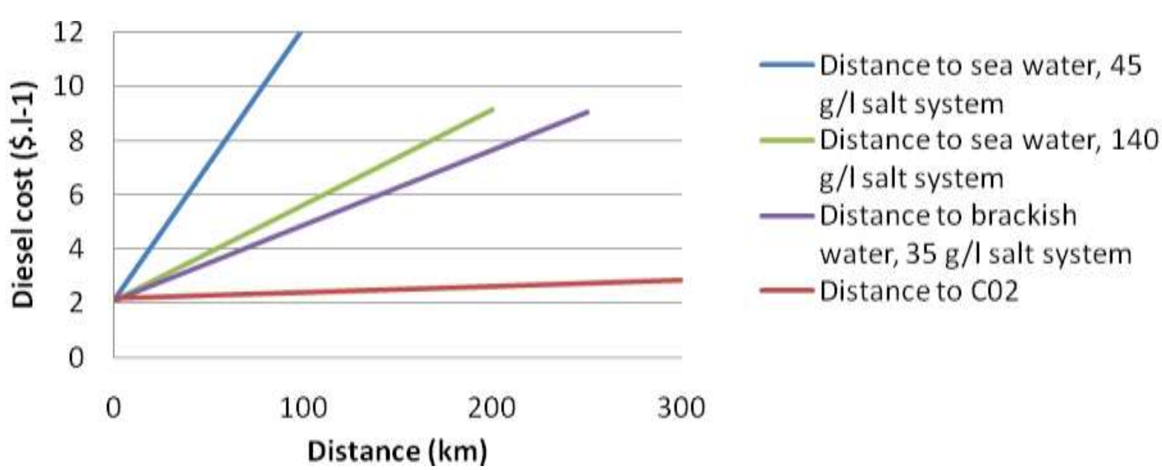

Figure 5-12 Impact of Water and CO_2 Infrastructure Costs on Diesel Production Cost

Figure 5-13 shows the effect of seasonal plant closures in climates unsuitable for year round production. Section 4-1 addresses climate requirements for large scale algal biofuel facilities and notes that tropical climates provide the opportunity for year round biomass production. Weather related shut downs incur re-establishment costs but also effect reduced capital utilisation (use of infrastructure) effectively lowering annual average productivities and increasing the land required to achieve the same production as facilities with year round operation.

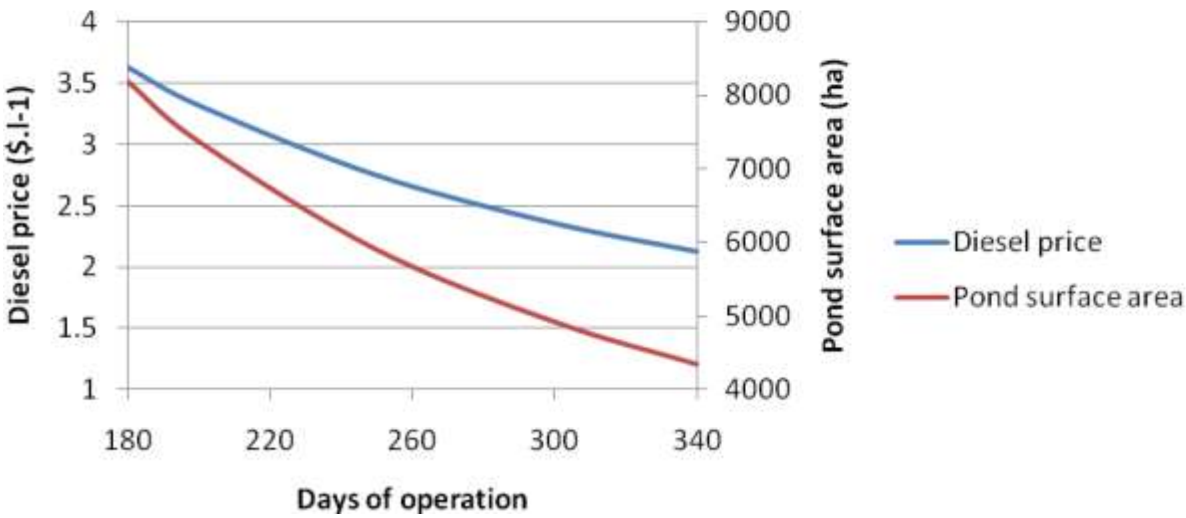

Figure 5-13 Impact of capital utilisation on diesel price and land use.

5.2.1 Impact of Co-product Value on Economics

Although most of the focus of algal biofuels centres on the production of lipids, other portions of algal biomass are likely to be important in the overall production process. Assuming that algal strains developed for commercial oil production can make more than 50 % of their cell mass as lipid, it will be important to generate value from the remaining biomass, which will be made up of approximately equal amounts of carbohydrate and protein.

The carbohydrate fraction is made up of cell wall material as well as carbohydrate storage material such as starch, glycogen, or chrysolaminarin (though the amount of carbohydrate storage material is likely to be quite low in cells producing 50 % of their weight as lipid storage material). Cell walls typically are made up of an amorphous component (mainly polysaccharides and a fibrillar component, commonly cellulose). Storage carbohydrates could easily be converted to ethanol just as cornstarch is easily fermented, and the cell wall material is likely to be more readily hydrolyzed and converted to ethanol than the lignin-containing cell walls of terrestrial plants.

The protein fraction could be recovered for animal feed provided the growth or lipid recovery processes do not render it unfit for consumption (due to accumulation of heavy metals, salinity of residue or contamination with lipid extraction solvents, for example). The current production level of protein feed as byproducts of the biofuels industry suggests that it may be difficult to penetrate this market with algal protein, but it may be possible to produce thickening agents (agar, carageenan), colouring agents (astaxanthin) and nutraceuticals (β-carotene, omega-3 fatty acids). In this analysis no particular product is assumed, but rather the impact of a notional value for algal cake on biofuel production cost is determined (see Figure 5-14). It appears that high value co-products may underpin the profitability of large plants. However it should be noted that Cognis Australia produces over 80% of the world's natural β-carotene from a total of ca. 960 ha of extensive ponds. A proposed 100 ML biofuels facility would comprise 4338 ha of ponds. There is likelihood that production of β-carotene at a single large facility would saturate a relatively small market.

For a 100 ML facility selling diesel at a price of \$0.61 l^{-1} the revenue from algal cake at \$305 t^{-1} db will exceed the revenue the biofuel.

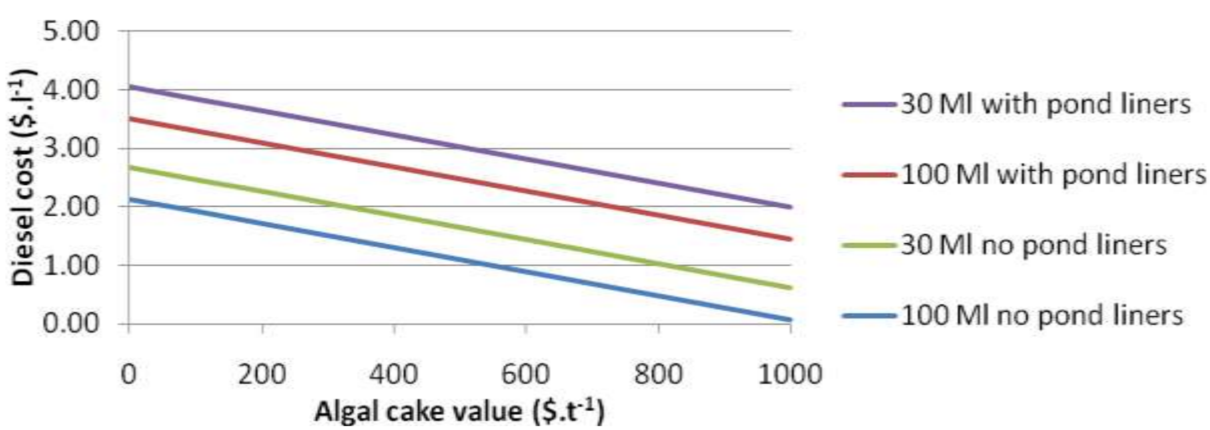

Figure 5-14 **Co-product Value Impact**

5.2.2 Impact of Technology Development on Economics

Figure 5-15 and 5-16 show the impact of innovation on the cost of algal biodiesel production. In the base case, algal productivity is assumed to be 20 $g{\cdot}m^{-2}{\cdot}d^{-1}$ and the algae is assumed to contain 30% oil. The biodiesel production cost for systems without pond liners is \$2.13 l-1 and with pond liners is \$3.51 l^{-1}. At an algal productivity of 60 $g \cdot m^{-2} \cdot d^{-1}$ and the algae oil content of 60% the biodiesel cost is \$0.88 l^{-1} (without pond liners) and \$1.10 l^{-1} (with pond liners). These increases in productivity and oil content also reduce Land use from 4338 ha to 723 ha of pond surface (for a 100 ML plant, see figure 5-17).

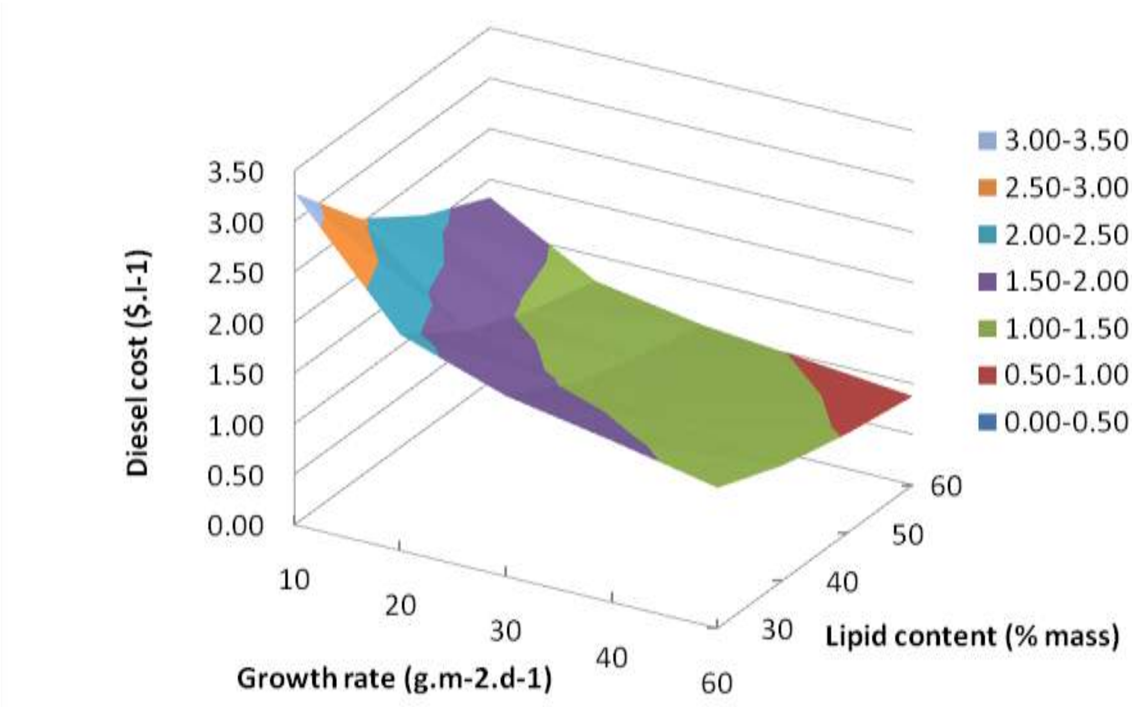

Figure 5-15 Effect of Growth Rate and Lipid Content of Algae in Clay-based Ponds on Diesel Production Cost

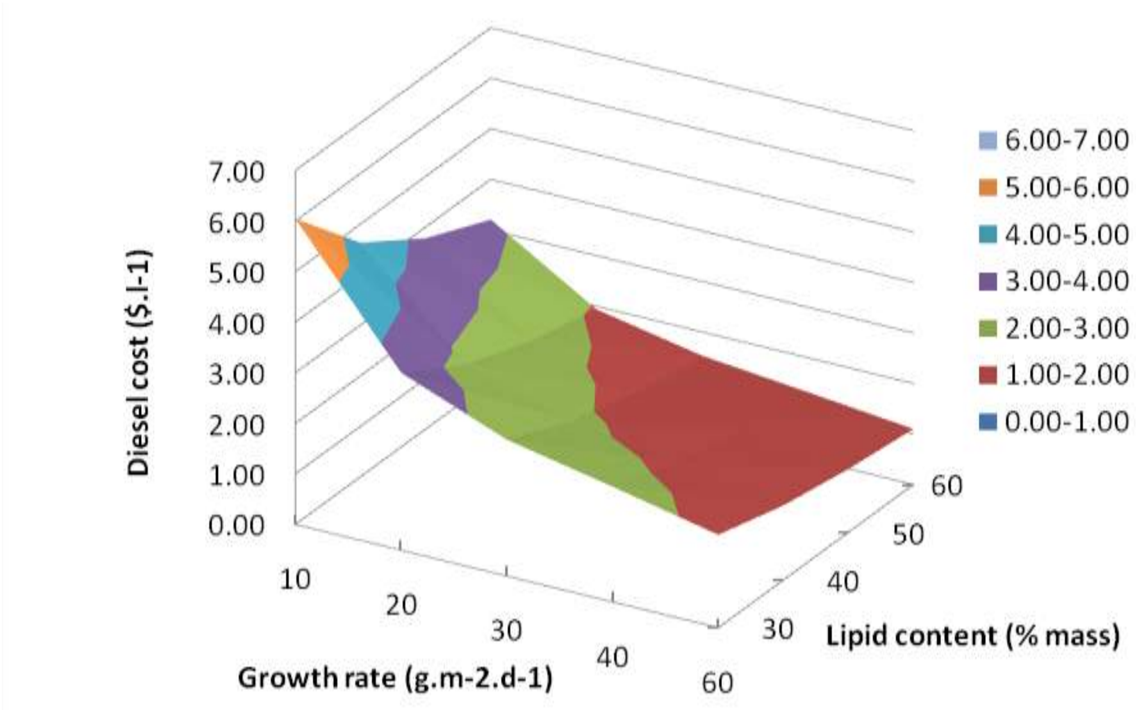

Figure 5-16 Effect of Growth Rate and Lipid Content of Algae in Lined Ponds on Diesel Production Cost

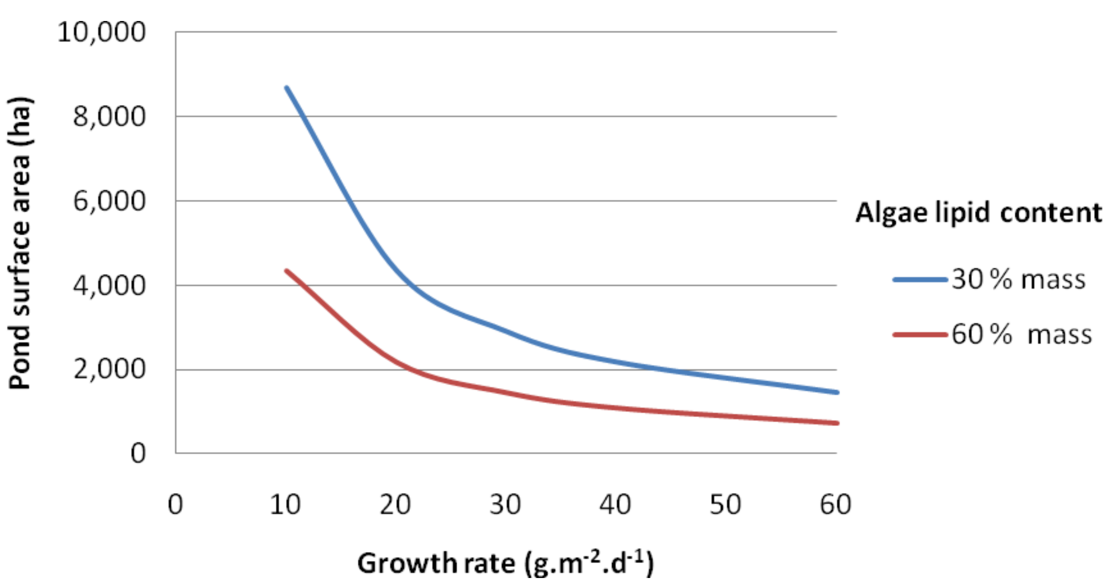

Figure 5-17 Pond Surface Area Requirement for 100Ml Plant

5.2.3 Impact of Carbon Price on Economics

Greenhouse gas emissions trading or taxation schemes are already in place in some countries and are likely to be adopted by most United Nations Framework Convention on Climate Change Annex 1 countries at some time in the future. The form of current and proposed schemes differ across countries, but presumably effective carbon price in cap and trade schemes will be driven by supply and demand. A worldwide cap and trade emissions scheme (ETS) is not beyond belief.

The IEA 2008 World Energy Outlook examines 3 scenarios for atmospheric CO_2 concentrations by the end of this century (viz. 770 ppm, 550 ppm and 450 ppm). The 770 ppm scenario is a reference case for a likely outcome in the absence of agreement among countries on limiting emissions. The 550 ppm scenario is based on policies that slow emissions growth, increase the share of renewables and effect rapid uptake of carbon capture and sequestration. In this scenario, a world ETS carbon price is predicted to be \$90 tCO_2equiv^{-1} by 2030. The 450 ppm scenario is based on much stronger policy settings and has a predicted ETS carbon price of \$180 t CO_2equiv^{-1} by 2030. It should be noted that the 2008 World Energy Outlook report expresses doubt that atmospheric CO_2 emissions can be haltered at 450 ppm. The required rate and scale of transformation in industry to effect a 450 ppm levelling is considered to be well beyond normal cycles of capital replacement and consequently would come at a disproportionately high cost, and it is uncertain if it is even technically achievable.

This analysis examines the effect of a carbon price on the economics of algal biofuel production. Ideally the methods of accounting for emissions in an ETS would follow the principles of life cycle assessment, but in practice legislation is likely to simplify and distort accounting methods. In the simplest (and most profitable) case, the CO_2 mitigation of algal fuel production might be credited with the amount of CO_2 (from the flue gases of industry) assimilated by the algae as they grow in the ponds (see Figure 5-18). In a more complex (and least profitable) case, CO_2 mitigation might be the difference of the algal biofuel and the petroleum fuel it replaces. Both methods of estimating the value of CO_2 mitigation are considered (see Figure 5-19). Figure 5-20 shows the impact of revenue from CO_2 mitigation (flue gas assimilation) and co-products required to meet a market prices for biodiesel from \$0.50 L^{-1} (\$80 bbl^{-1}) to \$0.84 l^{-1} (\$133 bbl^{-1}).

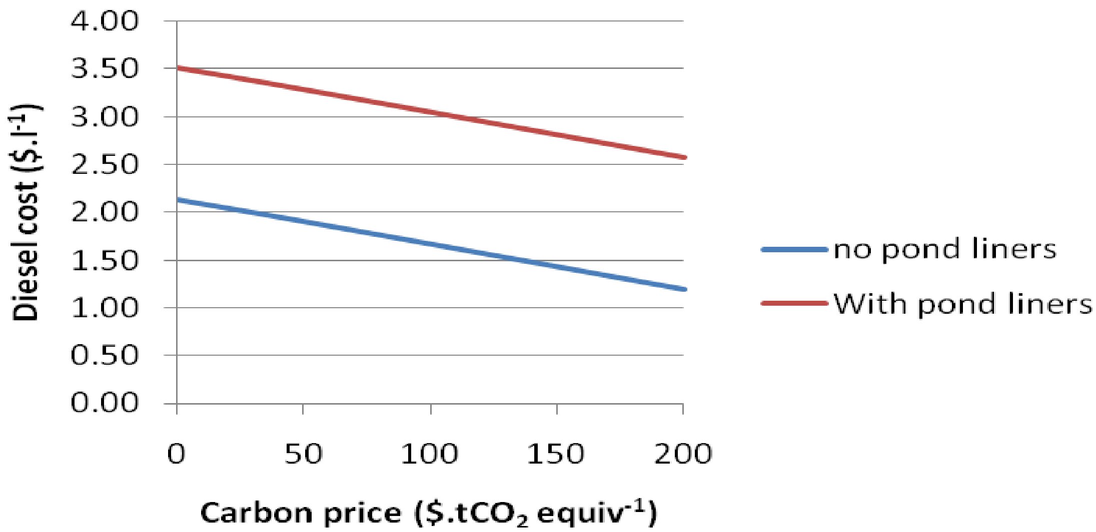

Figure 5-18 Effect of Carbon Price on Diesel Production Cost – Carbon Dioxide Capture

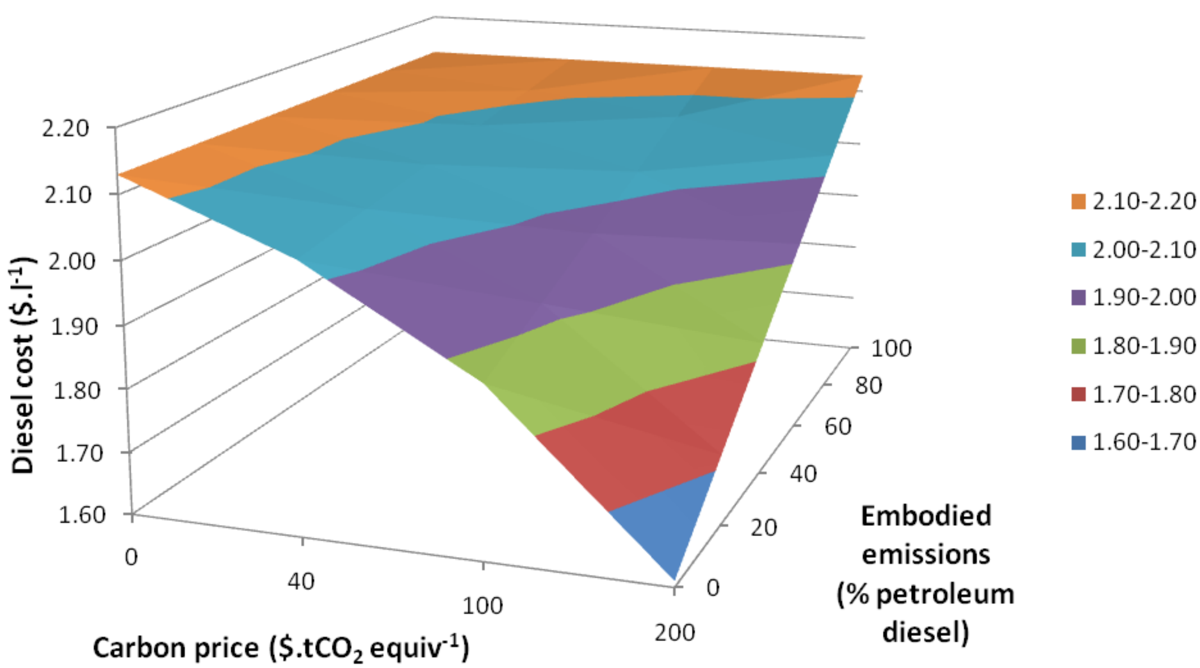

Figure 5-19 Effect of Carbon Price on Diesel Production Cost – Fuel Replacement

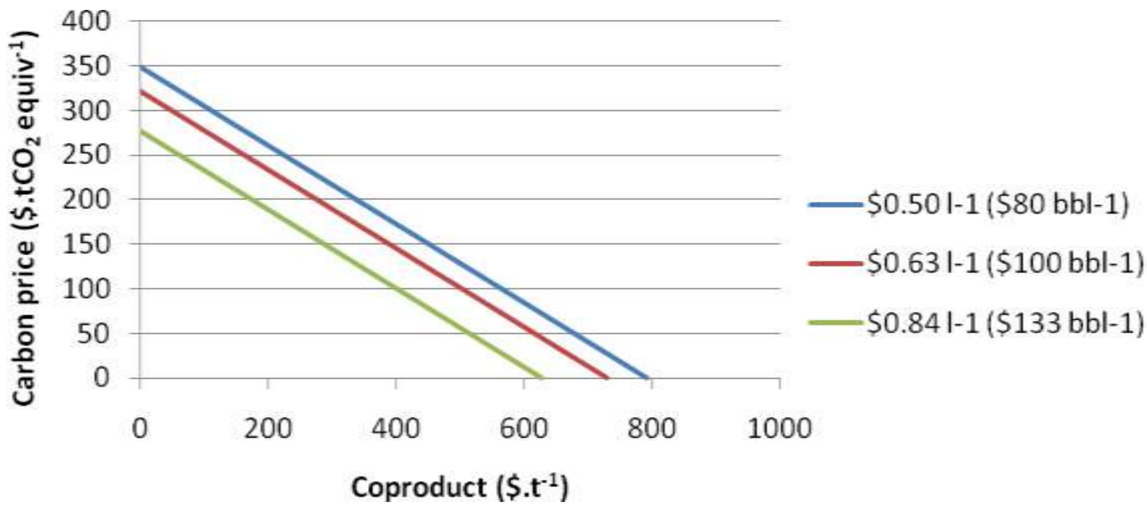

Figure 5-20 Carbon Revenue and Co-product Revenue Required to Achieve Acceptable Biodiesel Prices

5.2.4 Best Case Scenario

A best case scenario employs a maximum case where algal productivity is 60 $g \cdot m^{-2} \cdot d^{-1}$ and algal biomass is 60% lipid and these values are used in this scenario. The facility is situated in the dry tropics where low rainfall and high ambient temperatures allow for more than 340 days of operation in each pond. The land is flat and the soil is clay so pond liners can be avoided and capital costs are comparatively low. Seawater is provided to the pond system by tidal flow and the CO_2 source is close to the facility. An ETS (and a carbon emissions price of $100 tCO_2 equiv^{-1}) drives nearby industry to capture CO_2 and the algae are able to grow in ponds with direct feeding of flue gas. Algal cake is sold as an animal feed and returns $300 t^{-1} dry basis (effectively $600 t^{-1} protein content). Other higher value products are extracted and sold but markets for these products are no longer as attractive due to oversupply. The 100 ML facility is profitable at a diesel price of $0.63 l^{-1} ($100 bbl^{-1}) with after tax IRR between 20% and 23% depending on co-product values.

This best case scenario describes a production system that is achieved through significant research over an unknown timeframe. This analysis shows that research on algae biofuel production has potential benefit. It also shows that co-products and an ETS have significant impacts on profitability. However, the facility site is particularly advantageous for algal biofuels production.

5.3 Production Cost Summary

Both techno-economic analyses presented here reach similar conclusions. Algal biofuel production costs based on the currently achievable production parameters are higher than the price of fossil diesel fuel by a factor of 2 to 3. Both determined that this could be reduced, through the development of more productive strains, down to levels that are competitive with fossil diesel fuel prices.

The analyses also show that capital costs are an important parameter in determining the financial feasibility of algal oil production. Careful attention to detail with respect to site selection and construction techniques will be required for successful deployment of the technology. It is likely that capital costs can be reduced significantly as the industry develops through the “experience curve” that all new technologies experience.

Both of the analyses also demonstrated the importance of co-product credits and the positive potential impacts of monetizing the carbon reduction benefits of the fuels.

Finally, water costs will be an extremely important parameter in determining the financial feasibility of specific sites for algal biofuels production.

6. Contribution of Algal Biofuels to Future Liquid Transportation Markets

Hoogwijk (2004) and Antilla et al. (2009) describe different types of potentials for terrestrial agriculture, viz. theoretical, geographic, technical, economic and ecological potentials. Theoretical potential is the unconstrained potential of the production system. Florentinus et al. (2008) assess the theoretical potential of microalgae for biofuels production to be several hundred $EJ.yr^{-1}$, which interestingly less than similar assessments of terrestrial biomass (in the order of 1500 $EJ.yr^{-1}$) and much less than their assessment of macroalgae (>6000 $EJ.yr^{-1}$). The macroalgae potential is based on concepts of offshore algae farming that have not been demonstrated on any scale. Since algal photosynthesis accounts for 50% of the world's CO_2 fixation, the theoretical potential algal biofuels would appear to very large.

Proponents of algal biofuels make ambitious claims of the potential of this production system to contribute to the world's future fuel needs. For example, Christi (2007) claims that 50% of the US transportation fuel needs could be produced on 2 million ha of land if the algal biomass was 70% oil by mass and 4.5 million ha if 30 % oil by mass, and by comparison the same production would require 45 million ha of oil palm. Underlying this calculation is the assumption of ca. 50 $g \cdot m^{-2} \cdot d^{-1}$ algal growth and only the pond surface area is used the calculation. Christi's claims are based on a theoretical potential with optimistic assumptions. In the best case, where a more modest growth rate (i.e. 20 $g \cdot m^{-2} \cdot d^{-1}$) is assumed, and total land use is considered, algal oil production may be only marginally better than palm oil. In the worst case, where large evaporative ponds might be needed to dispose of waste water, palm oil may be a better choice in terms of land productivity.

When constraints are placed on all biomass productions systems, a lower potential is expected. Geographic potential takes into account available & suitable land. Technical potential includes consideration of likely productivity & efficiency within geographic potential. Economic potential includes cost competitive delivery to markets and ecological potential accounts for impact on biodiversity.

In the case of terrestrial biomass potentials, extensive assessments of land availability and suitability exist, technical and economic potentials are reasonably well established, and sustainability issues to some extent are understood. This is not the case for algal biomass production systems. Notwithstanding this, the potential of algal biofuels can be examined in terms of its likely contribution to projected liquid transportation supply.

The IEA and the US Energy Information Agency (EIA) provide data on energy supply, consumption and growth projections based on scenarios of population and mid-century atmospheric carbon dioxide concentrations resulting from international agreement on limiting further emissions (for example see http://www.eia.doe.gov/oiaf/ieo/liquid_fuels.html). The world currently produces and consumes ca. 4,900 Gigalitres of fossil oil per year (85 million bbl·d^{-1}). Approximately 52% of this oil is used for transportation. Current biofuels production comprises ethanol from starch and sucrose accumulating terrestrial plants, and fatty acid methyl esters from oil seed crops (and to a lesser extent from used cooking oil and tallow). Biofuels production is in the order of 90 Gigalitres per year, or ca. 3.5% of the transportation fuel supply.

By 2030, oil consumption is expected to increase to ca. 6.2 TL·yr^{-1} (106 million bbl·d^{-1}) with 66% of this growth likely to occur in non-OECD Asian countries (see figure 6-1). Transportation fuel use is expected to grow slightly to ca. 56% of total oil production (see figure 6-2). Over the same time period biofuels will maintain a relatively steady share of unconventional liquid fuel production and grow to between 277 GL·yr^{-1} and 416 GL·yr^{-1} (4.8 to 7.2 million Bbl·d^{-1}, or 8.0% to 12.0% of the liquid transportation fuel supply, see figure 6-3). The EIA uses a figure of ca. 340 GL·yr^{-1} as a reference case for total biofuel production in 2030. In this report algal biofuels contribution to future liquid transportation fuel supply is assessed against this figure.

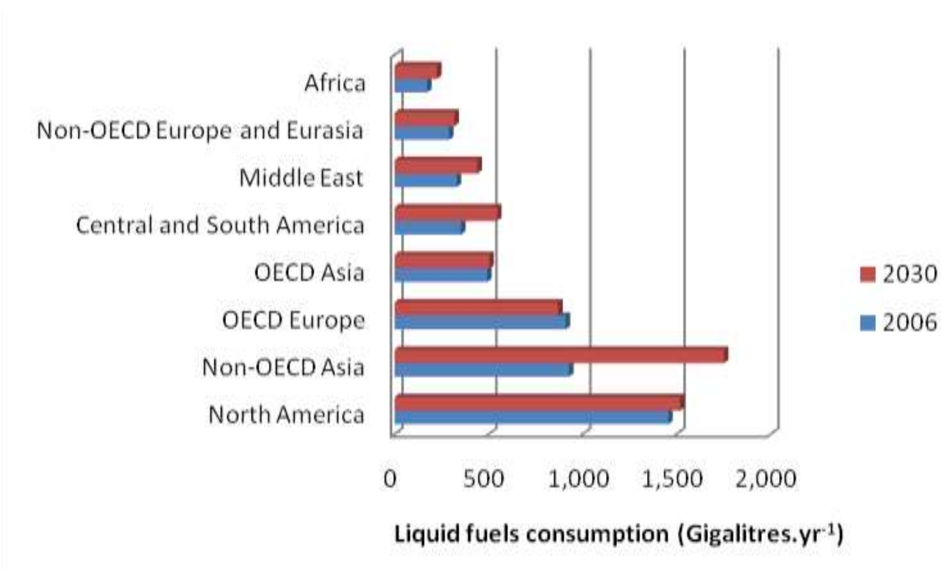

Figure 6-1 World Liquid Fuels Consumption, 2006 and 2030

(Source – EIA International Energy Outlook, 2009)

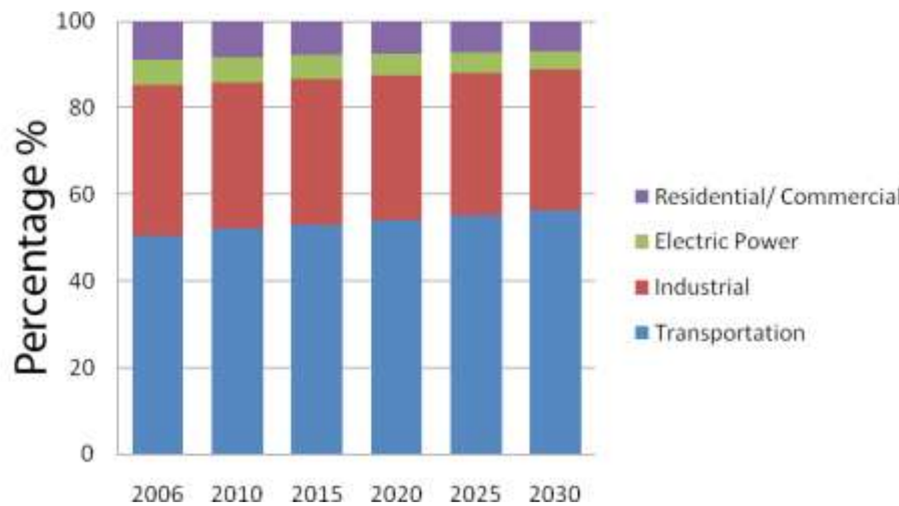

Figure 6-2 World Liquid Fuels Consumption by Sector, 2006-2030

(Source - EIA International Energy Outlook, 2009)

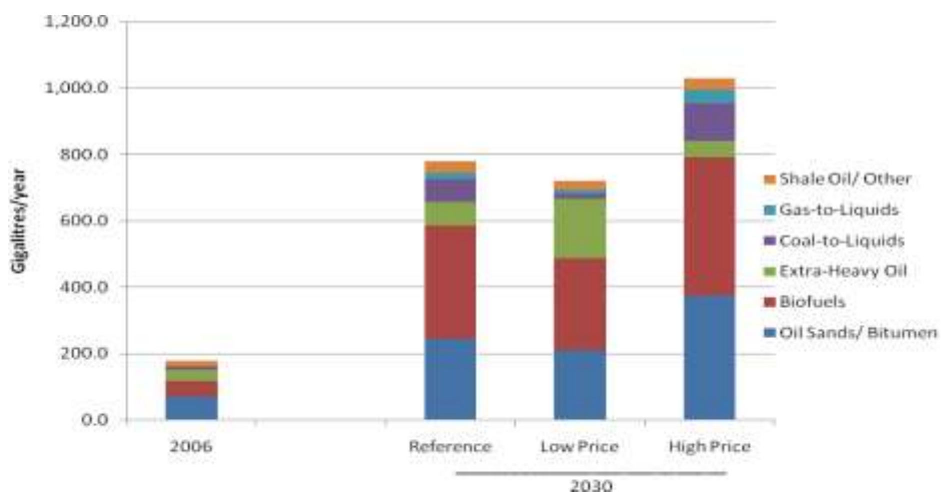

Figure 6-3 World Production of Unconventional Liquid Fuels

(Source - EIA International Energy Outlook, 2009)

A 5% contribution of algal biofuels to total biofuels supply by 2030 would require the construction of 170 100 ML facilities. When the technical uncertainty is considered, it seems unlikely that the first large scale plant would be commissioned before the middle of the coming decade, and even this would be ambitious. Approaches that rely on molecular biology to achieve breakthrough e.g. the Synthetic Genomics Inc. and ExxonMobil Corp. partnership are promising but may take more than a decade to reach commercial viability. Assuming success in the first commercial venture and accelerated rates of adoption beyond 2015-

2020, the construction of 170 facilities in a 15 year period is within the range of adoption already experienced with ethanol facilities in the United States and Brazil.

A 50% contribution of algal biofuels to predicted 2030 biofuels production would require the construction of 1700 100 ML facilities. Clearly, this is beyond the growth rates experienced by the biofuels industry in the past several decades and it would require very concerted effort industry and government to achieve by 2030. Beyond this timeframe, algal biofuels may make even greater contributions to liquid fuel supply. Certainly the analysis reported here suggests that large scale algal biofuel productions may eventually become economic viable. However, from the perspective of geographic potential, it is uncertain if there is sufficient suitable land near to water resources and CO_2 supply and in a climate conducive to management of large scale algae production. Land use, land suitability and resource spatial mapping data compiled for the purpose of assessing the geographic potential of algal biofuels does not currently exist.

7. Conclusions

The potential for production of algal biofuels has captured the attention of the nation and the world. It is written up in scientific literature and the popular press. It has stimulated activity in academic labs, start-up companies, large oil companies, and end users.

Algal biofuels have the potential to replace a significant portion of the total diesel used today with a smaller environmental footprint. In addition, algal biofuel production can be carried out using marginal land and saline water, placing no additional pressure on land needed for food production and freshwater supplies. Finally, algal biofuels have the potential to mitigate the impact of CO_2 released from point sources.

The potential of algal biofuels must be framed by the realization that virtually none of the technologies necessary for their production (with the exception of the conversion of the algal oils themselves to biodiesel or green diesel) have yet been demonstrated at scale or in an integrated fashion under conditions resembling a full-scale production facility. The potential of algal biofuels is based upon bench-scale observations, limited outdoor production data, extrapolation, assumption, and limited critical and economic analysis. The technical feasibility has been proven at small scale and, in fact, small samples of algal biodiesel have been produced, but economic feasibility is unknown. It is in recognition of the magnitude of its potential; however, this report has attempted to summarize the state of algae-to-fuels technology and document the economic challenges that must be met before algal biofuel can be produced commercially.

It is likely that a significant amount of research and a number of breakthroughs are needed to make algal biofuels a commercial reality. The economic analysis in this report indicates that the major cost for fuel production comes from the growth and harvesting of the algal biomass.

The current effort in algal biofuels research seems to follow that of the biotech industry in general, with basic research carried out mainly at academic labs, transitional work divided among academic labs, national labs, start-up companies, and scale-up split between the start-up companies and the larger commercial organizations that will likely play a major role in large-scale manufacturing.

As with other areas of biotechnology, it may become difficult to distinguish between purely academic labs and commercial start-ups working with algal biofuels. Additionally, it is likely that the future will bring a consolidation of start-ups looking to build upon the work of others. Commercialization will require R&D

efforts at both pilot and production scale—expensive efforts that require high-level financial support and can only be justified once technical and cost issues have been addressed.

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Appendix A. US Algal Biofuels Research, Development and Demonstration

The Aquatic Species Program funded by US DOE from 1978 to 1996 represents one of the most comprehensive research efforts to date on fuels from algae. DOE invested $22.1 million cumulatively over this time frame (nominal dollars) (Sheehan, 1998) to study a variety of aquatic species for use in renewable energy production, including microalgae, macroalgae, and cattails. The Aquatic Species Program demonstrated the feasibility of algal culture as a source of oil through algal strain isolation and characterization, studies of algal physiology and biochemistry, genetic engineering, engineering and process development, coupled with outdoor demonstration scale up of algal mass culture. Techno-economic analyses and resource assessments were important aspects of the program, and helped prioritize resources toward addressing the most important scientific and technical barriers. The program was discontinued when the potential cost of algal biofuel production was estimated in the $40-$60/barrel range, a factor of two or three times higher than the cost of petroleum at that time (less than $20/barrel in 1995). The program highlighted the need to understand and optimize the biological mechanisms of algal lipid accumulation and to find creative, cost-effective solutions for culture and process engineering to isolate lipids from very dilute biomass suspensions.

Since the end of DOE's Aquatic Species Program, US Federal funding for algal research has been limited and intermittent. Federal funding in the United States is split mainly between the Department of Defense (DOD) and DOE. The Defense Advanced Research Projects Agency (DARPA) issued a solicitation for approximately $15 million in FY 2006 and another $42.6 million in a separate Broad Agency Announcement (BAA) in FY08 to develop a biological surrogate for the petroleum based military jet fuel (JP-8). The Air Force Office of Scientific Research (AFOSR) issued a BAA in 2008 with the primary objective to understand and improve the facility of certain algal species to produce lipids for jet fuel production. DOE recently invested $4.4 million in six university advanced biofuels projects. Two of the projects involve the development of algal biofuels. DOE has over the last years begun to ramp up its funding of algal biofuels research. For example, it recently announced the awards for its biorefinery (pilot and demonstration scale) solicitation. Several of the winning awards went to algal biofuels companies. In mid-2009, DOE released a Funding Opportunity Announcement (FOA) for an algal biofuels consortium, which would be funded at a level of $50M over a three year period. The award of this competition is expected to be announced at the beginning of 2010. DOE also issued a Small Business Innovative Research (SBIR) request under the topic of "Algae for Biodiesel" for FY 2008 and three Phase I awards of $100,000 were ultimately announced. A DOE

FY09 SBIR solicitation (DOE's Office of Energy Efficiency and Renewable Energy and the Office of Fossil Energy) included three subtopics on algal biofuels. The National Science Foundation has also made a few awards on behalf of algal biofuels research, and the 2008 Farm Bill under the U.S. Department of Agriculture has the potential to fund algal feedstock producers under Title IX (sections 9005 and 9001).

State funding programs have generated approximately $10 million for algal biofuels research while a similar amount has been allocated through the Laboratory Directed Research and Development (LDRD) program, DOE and work for others funding over the past few years for research on algal biofuels at a number of national labs including the National Renewable Energy Laboratory (NREL), Sandia National Laboratories (SNL), the National Energy Technology Laboratory (NETL), Los Alamos National Laboratory (LANL), the Pacific Northwest National Laboratory (PNNL), and Oak Ridge National Laboratory (ORNL).

Private investment to support algal biofuel commercialization comes from both the investment community and the petrochemical industry. Approximately 200 algae companies have formed in the last few years ranging from the virtual company to the well capitalized. Major investments include, Algenol Biofuels announcement that Biofields will invest $850 million to make ethanol from microalgae, and Exxon-Mobil's decision to invest $600 million in Synthetic Genomics. Recent awards by DOE for algal-based integrated biorefinery proposals and for a national algal biofuels consortium (described below) have also done much to stimulate growth in algal biofuel research.

DOE's Aquatic Species Program: From Cattails to Microalgae.

The Aquatic Species Program was funded by DOE from 1978-1996 with a total budget of $22.1 million (Sheehan, 1998) and took place primarily at NREL (formerly known as the Solar Energy Research Institute), though much work was carried out by academic subcontractors. As the name of the program indicates, the Aquatic Species Program initially involved more than microalgae. Macroalgae, freshwater plants like water hyacinth and duckweed, and wetland emergents such as cattails, reeds, and rushes were also evaluated (Pratt et al. 1984 http://www.nrel.gov/docs/legosti//old/2383.pdf and Ryther, 1982 http://www.nrel.gov/docs/legosti/old/98133-1A.pdf). The biomass from these organisms was considered as a potential feedstock for both ethanol fermentation and anaerobic digestion to methane. Ultimately, the decision was made to focus on microalgae, first as a source of hydrogen production, but later as a source of lipids for biodiesel production. In 1998, Sheehan et al. completed the Aquatic Species Closeout Report, which summarized the microalgal portion of the Aquatic Species Program. A brief summary of that work is described below.

Microalgae Strain Collection and Screening

The Aquatic Species Program included a bioprospecting component designed to collect novel algal strains from diverse saline habitats in the inland US, with environmental samples collected from sites chosen based on assumptions of ideal locations (in terms of temperature, sunlight, and land availability) for large scale cultivation facilities. Special attention was paid to the isolation of diatoms because of reports of high lipid accumulation characteristics. The objectives of the culture collection and screening effort were to:

- Isolate and purify algal strains from arid regions of Colorado, Utah, and New Mexico.
- Assemble a large number of unialgal cultures and maintain them under conditions designed to maximize the retention of characteristics essential for lipid productivity, including genetic variability and environmental adaptability.
- Develop media formulations suitable for growth and lipid production.
- Screen strains for growth and productivity over a range of culture conditions relevant to anticipated production facilities including pH, temperature and salinity.

This work led to the isolation and characterization of over 3,000 algal strains, and the 300 best production candidates (mainly green algae and diatoms) were transferred to the Center for Marine Microbial Ecology and Diversity (CMMED) at the University of Hawaii in 1996, with the understanding that they would be shared with any lab interested in algal research.

Oil Accumulation by Microalgae

Several of the strains isolated by ASP scientists were tested for lipid production under nutrient (mainly nitrogen or phosphorous) limitation conditions. Some strains were shown to accumulate as much as 60% lipids, primarily TAGS, during nutrient starvation. Although nutrient starvation led to an overall drop in lipid productivity because of the concomitant cessation of growth, it was believed (and it is still largely believed) that the study of the physiological response to nutrient limitation could lead to the identification of a metabolic trigger. This finding would provide substantial insights for strain improvement efforts.

In addition to nitrogen and phosphorous starvation, it was shown that silicon limitation in diatoms could also lead to increased lipid accumulation. In studies with Cyclotella cryptica, a diatom with high lipid content, insufficient silicon was shown to lead to increased levels of acetyl-CoA carboxylase (ACCase), which catalyzes the first step in the biosynthesis of fatty acids used for TAG synthesis

(Roessler, 1988). This led to the hypothesis that this step represented a key branch point for carbon flux to TAG synthesis and led to a program to characterize this enzyme in detail. Ultimately, Roessler and co-workers cloned the ACCase gene from C. cryptica (Roessler and Ohlrogge, 1993) and expressed it in both the host strain and in a second diatom, Navicula saprophila (Dunahay et al., 1995). This was the first example of metabolic engineering in microalgae for increased biofuel production. Although enhanced gene expression was reported, no impact on TAG production was observed.

The approach described above was meant to drive more carbon into TAG biosynthesis; a second approach, diverting carbon away from competing pathways, was also investigated. UDP Glucose pyrophosphorylase and chrysolaminarin synthase, enzymes catalyzing steps in the biosynthesis of the storage carbohydrate chrysolaminarin in C. cryptica (Roessler, 1987; 1988) were chosen as potential targets for downregulation to reduce carbon flow to carbohydrate storage. The UGPase gene was cloned and overexpressed in C. cryptica but attempts to down regulate gene expression were unsuccessful (Jarvis and Roessler, 1999).

Process Engineering

Although techno-economic analyses carried out during the ASP indicated that biological productivity was the most critical element in lipid production costs, it was also clear that the cost contributions of process steps including cultivation, dewatering, and lipid extraction were also prohibitively high (Sheehan et al., 1998). Cultivation process development focused on open pond technologies. Outdoor demonstration units were built and operated in California, Hawaii, and New Mexico. The largest of these (utilizing two 1,000 m^2 raceway ponds with paddle-wheel mixing, plus several smaller ponds for experimental purposes and inocula preparation) was the Outdoor Test Facility (OTF) in Roswell, N.M., operated by Microbial Products, Inc. (Weissman et al., 1989). The large ponds were operated in continuous mode for extended periods and were able to demonstrate the feasibility of long-term stable algal cultivation with a high efficiency (>90%) of CO_2 capture. Productivity was limited during winter months (with temperatures falling below freezing) but annual biomass productivity was measured at $g/m^2/day$ with peak performance at 50 $g/m^2/day$. A critically important point made during this period was the observation that lab strains could not necessarily compete with indigenous contaminating strains and lipid productivity fell off as these came to dominate the culture.

Flocculation was shown to have promise for a low cost dewatering step, and solvent extraction was also explored, but little progress was made in development of extraction methodology that would be scalable, cost effective and environmentally acceptable. The transesterification of algal TAGS for the production of biodiesel was shown to be straightforward, and other conversion to fuel processes (e.g., hydrotreating of lipids to hydrocarbons) were evaluated (Milne et al., 1990), but only on a small scale with limited product analysis.

Analysis of Resources and Techno-economics

Resource assessment were carried out during the ASP to determine the availability of suitable land, saline water, and fixed CO_2 resources with a focus on the U.S. Southwest. It was determined that sufficient resources were available for the production of sufficient fuel to displace a significant percentage of US diesel usage (currently 166 billion litres per year). These analyses did not take into consideration variations in such factors as land characteristics (slope, soil quality, etc.), depth of aquifers, distance from CO_2 point sources, and other issues. The techno-economic analyses demonstrated how the contributions of these and other details to the calculated price of lipid production needed to be minimized, and confirmed the need to restrict large scale cultivation to unlined open ponds. As noted above, calculated fuel costs were highly sensitive to the overall biological productivity (growth rate and lipid content). Because much of the techno-economic modeling was based on assumptions rather than data, a range of costs was calculated ($0.37-$1.16 per litre), which led to the conclusion that algal biofuels would never be able to compete with petroleum which was sold for <$20/barrel in 1995 and was expected to remain at that level over the next 20 years.

Conclusions of the ASP

The ASP successfully demonstrated that algal biomass could be produced at scale and be used as a source for biofuel, greatly advancing technology. The low cost of petroleum and the high calculated cost of algal oil highlighted the technical barriers to be addressed for a commercially viable process. It was clear that the entire value chain from basic biology to large scale cultivation, harvest, and extraction required advances to reduce both capital and operating costs. The curtailment of the ASP in 1996, and the subsequent limitation in funding for this work until recently, meant that many years would pass before a significant effort would once again be brought to bear to address these barriers.

A large gap exists between the current state of commercial microalgae production technology and the goal of producing a microalgae biomass with high oil content suitable for conversion to biofuels. Several government agencies are providing funding opportunities to research organizations and partners that are working to perfect microalgae production. The worldwide interest in clean technologies has prompted interest in microalgae production from the private sector. Energy companies, both large and small, are investing in demonstration plants, feedstock development, and process improvement. Some details on recent public and private involvement are provided below.

Department of Defense

Defense Advanced Research Projects Agency (DARPA)

DoD has been directed to explore a wide range of energy alternatives and fuel efficiency efforts to reduce the military's reliance on petroleum oil to power its aircraft, ground vehicles, and non-nuclear ships. The goal of the biofuels program is to develop an affordable and highly efficient alternative process for converting crop oil to a Jet Propellant 8 (JP-8) surrogate. In 2006 DARPA announced a Broad Agency Announcement (BAA) soliciting innovative research proposals to develop a process that efficiently produces a surrogate for JP-8 from oil rich crops produced either by agriculture or aquaculture (including but not limited to plants, algae, fungi, and bacteria) within an 18-month timeframe. The major deliverable included the production of a minimum of 100 litres of JP-8 surrogate biofuel for testing in a suitable DoD test facility. In 2007 DARPA awarded three teams, one led by UOP [(Honeywell Space, Cargill, Arizona State University (ASU), Sandia National Laboratories (SNL) and the Southwest Research Institute; $6.7 million]; the second led by the Energy & Environmental Research Center (EERC) at the University of North Dakota ($5 million); and the third led by General Electric ($3 million).

DARPA followed up on the initial biofuels program with the release of a second related announcement in late 2007 Biofuels—Cellulosic and Algal Feedstocks (BAA08-07). The key objectives of the second solicitation were limited to two technical areas: 1) Technologies and processes for the conversion of cellulosic materials to affordable JP-8 surrogates, and, 2) Processes for the affordable manufacture ($0.25-0.50/litre) of algal-derived JP-8 surrogate. Awards for this solicitation were made to two teams led by General Atomics and Science Applications International Corporation (SAIC).

United States Air Force (USAF)

The Air Force funded a cooperative agreement between Arizona Public Service (APS) and DOE-Fossil Energy (FE)/NETL to develop and demonstrate a coal hydrogasification process for co-production of substitute natural gas and electricity with near-zero emissions. In phase I, the initial hydrogasification reactor concept design and process model were developed. In phase II, a high-pressure, high-temperature, bench-scale hydrogasifier is being constructed. Field assessments of an algae farming technique to utilize CO_2 will be evaluated and a process for converting the algae into various liquid fuels will be developed.

Air Force Office of Scientific Research (AFOSR)

The AFOSR manages the basic research investment for the USAF. As a part of the Air Force Research Laboratory, AFOSR's technical experts foster and fund research within the AFRL, universities, and industry laboratories to ensure the transition of research results to support USAF needs. The AFOSR collaborated with NREL to help identify academic labs to perform basic research on algae-to-jet fuel. As a result, the AFOSR is currently funding four academic laboratories focusing on the basic biological science on developing jet fuel from algae. The AFOSR and NREL also hosted an algae-to-jet-fuel workshop (Feb. 19-21, 2008; Arlington, VA) that brought together a panel of outside experts in the microalgae field, mainly from academia and the national laboratory system, to discuss a variety of basic science research issues related to microalgal oil production. Major topics covered included a historical look at previous algae-to-biofuels efforts, algae bioprospecting from diverse aquatic environments, maintenance of algae culture collections and cryopreservation, algal genomics, use of algal model systems, algal growth and physiology, photosynthesis, general lipid metabolism in algae, triacylglyceride synthesis pathways, development of algal genetic tools, techno-economic analysis, cultivation of algae in open pond and closed photobioreactors, and biosafety, environmental, and regulatory issues.

The overall goal of the workshop was to identify specific hurdles that must be overcome in order to ultimately achieve cost-effective production of algal oil for jet fuel conversion. The workshop also addressed the basic science research requirements needed to overcome these hurdles as well as to elucidate various novel scientific approaches that will be needed for developing a fundamental understanding of algal lipid biosynthesis and biomass cultivation principles. The major outcome from this workshop will be a research "roadmap" from which recommendations will be made for future basic scientific funding opportunities for AFOSR and other federal agencies. This roadmap was used to identify key areas of fundamental algal research, and a number of projects were funded in FY09.

Defense Energy Support Center (DESC)

The DESC is the largest purchaser of biodiesel in the country. This agency deals with all aspects of the fuel distribution and any quality issues that occur in the field. A division within DESC actively works with the approval and certification of fuels for the military by working to resolve issues that are encountered with biodiesel in the field—cold temperature, storage stability, and combustion. DESC works closely with developing the B20 commercial specification at the American

Society for Testing and Materials (ASTM). It has recently certified that an algal oil-based biodiesel produced by Solazyme meets or exceeds the D6751 biodiesel blendstock specification and has superior cold temperature characteristics. In 2009, DESC announced that it would purchase 600,000 gallons of bio jet fuel from a number of producers including 1500 gallons of algal fuel produced by Solazyme and converted to jet fuel by UOP. In addition to this Solazyme entered into a an agreement with the US Navy to research, develop, and demonstrate commercial scale production of algae-derived F-76 Naval Distillate fuel (http://www.biofuelsdigest.com/blog2/2009/10/02/sustainable-oils-solazyme-cargill-to-supply-600000-gallons-of-jet-biofuel-to-us-military/).

Department of Energy (DOE)

Energy Efficiency and Renewable Energy (EERE) and Office of Biomass Program (OBP)

The microalgal production of diesel fuel substitutes will require overcoming technical challenges in various stages of the process—algal cultivation, oil (lipid) recovery, and fuel production. As diesel fuel represents roughly 1/3 of the U.S. transportation fuels market, "Algae for Biodiesel" was selected as one of the four subtopics under the Small Business Innovative Research (SBIR) program solicitation from OBP for potential funding in May/June of FY08. Grant applications were sought to develop innovative technologies for addressing one or more of the above technical challenges. Three projects were awarded Phase I funding of $100,000/project.

Algae are a feedstock included under the natural oils platform for the multi-year plan being developed by OBP. Microalgae have been included under the natural oils platform because of their lipid potential to produce biofuels. The feedstock team will also be considering this report for algae as it pertains to microalgae feedstock development. In addition, DOE hosted an Algal Biofuels Technology Roadmapping Workshop in December 2008 to outline a path forward to overcome barriers that are preventing the establishment of a robust commercial algal biofuels industry. This led to the publication of the National Algal Biofuels Technology Roadmap (http://www1.eere.energy.gov/biomass/pdfs/algal_biofuels_roadmap.pdf) in June, 2010.

Integrated Biorefinery Solicitation.

As part of the ongoing effort to increase the use of domestic renewable fuels, U.S. Secretary of Energy Steven Chu announced in May of 2009 plans to provide $786.5 million from the American Recovery and Reinvestment Act (ARRA) to accelerate advanced biofuels research and development and to provide additional funding for commercial-scale biorefinery demonstration projects. On December 4, 2009 U.S. DOE Secretary Steven Chu and Agriculture Secretary Tom Vilsack announced the selection of 19 integrated biorefinery projects to receive up to $564 million from the American Recovery and Reinvestment Act to accelerate the construction and operation of pilot, demonstration, and commercial scale facilities. Of the nineteen projects selected, four were related to algal biofuels development including awards to Algenol, Sapphire Energy, Solazyme and UOP.

Algal Biofuels Consortium

In July of 2009 the DOE's Office of Energy Efficiency and Renewable Energy (EERE), through the Office of the Biomass Program (OBP) announced the availability of funding for establishing a Consortium for the Development of Algal Based Biofuels. The primary objective of this topic area was to develop cost effective algal based biofuels that are competitive with their petroleum counterparts. The research and development was intended to focus on the following five key barriers as identified in DOE's National Algal Biofuels Roadmap:

- Feedstock Supply: Strain development and cultivation;
- Feedstock Logistics: Harvesting and extraction;
- Conversion/Production: Accumulation of intermediate and synthesis of fuels and co-products;
- Infrastructure: Fuel testing and standardization; and
- Sustainable Practices: Life-cycle and economic analyses, siting, and resources management.

In January 2010, DOE announced that funding ($44 million) would go to the National Alliance for Advanced Biofuels and Bioproducts (NAABB), led by the Donald Danforth Plant Science Center (St. Louis, MO). The NAABB will develop a systems approach for sustainable commercialization of algal biofuel and Bioproducts, by integrating resources from companies, universities, and national laboratories to overcome the critical barriers of outlined in the Algal Biofuels Roadmap: cost, resource use and efficiency, greenhouse gas emissions, and commercial viability.

Later 1n 2010, DOE announced the investment of up to $24 million for three additional research consortia to tackle key hurdles in the commercialization of algae-based biofuels. The three consortia selected for funding are Sustainable Algal Biofuels Consortium, led by Arizona State University; the Consortium for Algal Biofuels Commercialization, led by the University of California, San Diego; and the Cellana, LLC Consortium, led by Cellana, LLC.

Office of Science (SC)

The Photosynthetic Systems program, housed in the Office of Basic Energy Sciences (BES) supports fundamental research on the biological conversion of solar energy to chemical stored forms of energy. Organisms of study include plants, algae, cyanobacteria, purple and green bacteria, and some bacteria and archea that do not obtain energy from light. Topics of study include light harvesting, exciton transfer, charge separation, transfer of reductant to CO_2, and the biochemistry of carbon fixation and carbon storage. Areas where biological sciences intersect heavily with energy-relevant chemical sciences and physics are accentuated.

Joint Genome Institute

The Joint Genome Institute (JGI) is a DOE user facility providing access to high-throughput DNA sequencing in support of projects in alternative fuels, carbon cycling, and bioremediation. JGI recently published the genome sequence of C. reinhardii (Merchant et al., 2007) and completed the sequencing of an additional nine algal genomes. Currently, several additional algal genome sequencing and Expression Sequence Tag (EST) projects are in the pipeline resulting from user-initiated submissions to JGI's Community Sequencing Program. JGI-collaborating scientists are interested in engineering algae for biofuels production.

U.S. National Laboratories

National Renewable Energy Laboratory (NREL)

NREL is the nation's primary laboratory for renewable energy and energy efficiency research and development. Since 2006, NREL used internal funds of more than $6 million to restart its algal biofuels research program. This included providing funding for a Strategic Initiative (Defining an Algae Biofuels Portfolio) and a Laboratory Directed Research and Development project (Development of a Comprehensive High-Throughput Technique for Assessing Lipid Production in Algae), as well as the purchase of a Fluorescence Activated Cell Sorter. Externally funded algae-related activities included a collaboration with the AFOSR (algae oil-to-jet fuel), a Colorado Center for Biorefining and Biofuels seed grant (Establishment of a Bioenergy-Focused Microalgae Strain Collection Using Rapid, High-Throughput Methodologies), and a Cooperative Research and Development Agreement with Chevron to study and advance technology to produce liquid transportation fuels using algae. In addition to these, NREL algal biofuels R&D is supported by the DOE funding as a core partner in the Sustainable Algal Biofuels Consortium (see above) as well as by DOE funds for international collaborations with algal researchers in Canada and Israel. NREL also serves as a partner on DOE-funded IBR programs with Algenol-Dow and Solazyme.

Sandia National Laboratories (SNL)

The biofuels program at SNL takes a systems engineering and optimization approach to these challenges. Sandia is a multi-program U.S. DOE National Nuclear Security Administration laboratory that is noted for its science and engineering applied to national security challenges including energy security. Sandia's biofuels program includes a focus on algae-based production of biofuels and co-products using impaired waters (e.g., brackish, saline, water from oil and gas well extraction) as well as cellulosic ethanol. Nearly $5 million has been invested at Sandia over the last 5 years from various sources (e.g., LDRD, DARPA, U.S. industry, and other sources). SNL expertise ranges from fundamental bioscience to utilization, and helping annotate the DNA sequence for one of the two marine diatoms that produce lipids, to operating the DOE-funded Combustion Research Institute for more than 25 years. Other work includes biomass optimization, systems analysis, and intersections with water utilization. Sandia has applied both technical and economic modeling methods to understand phenomena relevant to algal biofuels form cellular response to economic viability. Like NREL, SNL is a core partner in the Sustainable Algal Biofuels Consortium (see above) and is also a partner on the DOE-funded international collaborations with algal researchers in Canada and Israel.

National Energy Technology Laboratory (NETL)

NETL is the DOE Office of Fossil Energy's lead laboratory for carbon management and guides the RD&D efforts for the main programs in CO_2 Capture & Sequestration, Advanced Integrated Gasification Combined Cycle, Coal-to-Hydrogen, and Innovations for Existing Plants. There is no distinct program for the conversion of algae to biofuels; such research is conducted under the auspices of NETL's main technology programs.

One ongoing Cooperative Agreement includes evaluation of algae cultivation for the biofixation of CO_2 from coal-fired power producing plants. Under joint sponsorship of the Coal-to-Hydrogen Program and the Gasification Program, the Cooperative Agreement DE-FC26-06NT42759 between DOE-FE/NETL and Arizona Public Service, Arizona Public Service is to develop and demonstrate a process for co-producing substitute natural gas (SNG) and electric power via a coal gasification pathway. While the co-production of SNG (a hydrogen-carrier) and electricity is the foremost objective of the Cooperative Agreement, a required feature for the process is the inclusion of a methodology for management of CO_2 emissions. After analysis of several more conventional options for CO_2 capture, the Arizona Public Service team evaluated a concept that "recycles" the CO_2 emissions from fossil fuelled plants. In this recycle concept, CO_2 is fixed by the photosynthesis of microalgae using the emissions from fossil-fuelled power plants, and the resulting algal oil is converted to various products including jet fuel. Field assessments of an algae farming technique to utilize CO_2 is being evaluated, and a process for converting the algal oil into various liquid fuels developed.

Pacific Northwest National Laboratory (PNNL)

PNNL, one of DOE Office of Science's multi-program laboratories, focuses on advancing science and technology in energy, environment, and national security. PNNL performs both basic and applied research in the area of microalgal biofuels (hydrogen, lipids, and hydrocarbons). These activities are funded primarily from three sources: internal LDRD funds ($150,000 over 3 years; completed), DOE funding from the NETL bioprocessing program ($180,000/year for 2 years; completed), and DOE funding from the Office of Science GTL program for metabolic modeling of H_2 production in cyanobacteria ($100,000/year for three years, subject to appropriations; current). Recently, DOE has funded siting and resource analysis for algal cultivation at PNNL as well as macroalgal biofuels R&D as part of the international collaboration with Canada. PNNL also serves as a core partner with the NAABB.

Los Alamos National Laboratory (LANL)

LANL is a national security research institution, delivering scientific and engineering solutions to the nation's most crucial and complex problems. LANL has been pursuing applied research in the algal biofuels area. Funding from the State of New Mexico aided a group attempting to establish an algal biodiesel business. LANL has recently become a leading US organization in algal biofuels research as a core partner in the NAABB. Dr. Jose Olivares, who serves as Deputy Division Leader for the Bioscience Division at LANL, has been named the Executive Director of the NAABB.

United States Environmental Protection Agency

EPA is working to get funding to validate the utility of large-scale application of the Algal Turf Scrubber (ATS) technology, developed by Dr. Walter Adey of the Smithsonian Institute, to remove nitrogen and phosphorus from polluted rivers, and use the algal biomass grown on the algal turf scrubber (ATS) as a source for biofuel development. At mid-latitude, the ATS system is estimated to produce an average of 3,500 gal/acre/year of biodiesel/biobutanol. Work is currently ongoing with researchers at Western Michigan University to refine and finalize this process. More recently, EPA commissioned work at NREL on techno-economic analysis of algal biofuels production using both open pond and close photobioreactor cultivations systems. This work was used to establish algal biofuels standards for the Renewable Fuel Standards II program.

United States Department of Agriculture

USDA and DOE participate in the annual Joint Bioenergy Solicitation to fund R&D for biofuels, bioenergy and high-value bio-based products. In 2010, funding levels have been set at $33 million. Also in 2010, USDA announced a loan guarantee of $54.5 million to Sapphire Energy for build out of a demonstration facility in New Mexico.

Appendix B. Algal Culture Collections

Culture collections identified by the World Federation for Culture Collections as containing significant numbers of algae species are listed below.

Australia

CSIRO Collection of Living Micro-algae

http://www.cmar.csiro.au/microalgae/supply.html

Murdoch University Algal Culture Collection

Brazil

Marine Microalgae Culture Collection

Freshwater Microalgae Collection Cultures

Canada

University of Toronto Culture Collection of Algae and Cyanobacteria

http://www.botany.utoronto.ca/utcc/

Canadian Centre for the Culture of Microorganisms

http://www.botany.ubc.ca/cccm/

China

Freshwater Algae Culture Collection at the Institute of Hydrobiology, Chinese Academy of science

http://algae.ihb.ac.cn

Czech Republic

Culture Collection of Algae of Charles University in Prague

http://botany.natur.cuni.cz/algo/caup.html

Culture Collection of Algal Laboratory (CCALA) Institute of Botany, Academy of Sciences of the Czech Republic

http://www.butbn.cas.cz/ccala/index.php

Denmark

Scandinavian Culture Collection of Algae & Protozoa

http://www.sccap.bot.ku.dk/

France

Roscoff Culture Collection – marine phytoplankton

http://www.sb-roscoff.fr/Phyto/RCC

The Biological Resource Center of Institute Pasteur

http://www.pasteur.fr/ip/portal/action/WebdriveActionEvent/

Germany

Culture Collection of Algae at the University of Cologne

http://www.ccac.uni-koeln.de

CCCryo Culture Collection of Cryophilic Algae at the Fraunhofer Institute for Biomedical Engineering

http://cccryo.fraunhofer.de/web/infos/welcome

Alfred Wegener Institute - Hustedt´s herbarium diatom collection

http://www.awi.de/en/research/research_divisions/biosciences/biological_oceanography/diatom_centre/collection/

India

Visva-Bharati Culture Collection of Algae

http://www.visva-bharati.ac.in/

Japan

Microbial Culture Collection at the National Institute for Environmental Studies

http://mcc.nies.go.jp/top.jsp;jsessionid=F5AEF93875D069AA046271EC6452036C

Marine Biotechnology Institute Culture Collection

http://www.mbio.jp/mbic/

Institute of Molecular and Cellular Biosciences Culture Collection at the University of Tokyo

http://www.iam.u-tokyo.ac.jp/misyst/ColleBOX/IAMcollection.html

Korea

Korea Marine Microalgae Culture Center at Pukyong National University

http://www.kmcc.re.kr

Norway

Culture Collection of Algae (NIVA) at the Norwegian Institute for Water Research

Philippines

Algal Culture Collection at the Museum of Natural History

Poland

Culture Collection of Baltic Algae at the University of Gdansk Institute of Oceanography

http://www.ocean.univ.gda.pl/~ccba/

Russia

Algae Culture Collection of Siberia

Collection of Algae in Leningrad, St. Petersburg, State University

Culture Collection of Microalgae IPPAS at the Institute of Plant Physiology, Russian Academy of Science

http://www.ippas.ru/

Peterhof Genetic Collection of Microalgae

Spain

National Bank of Algae at the Marine Biotechnology Center, University of Las Palmas de Gran Canaria

http://www.ulpgc/es/webs/cbm/

Turkey

Ege - Microalgae Culture Collection at Ege University

http://ebiltem.ege.edu.tr/ege-macc/

United Kingdom

Culture Collection of Algae and Protozoa at the Scottish Association for Marine Science

http://www.ccap.ac.uk/

USA

ATCC Protozoa and Algae Collection

http://www.atcc.org/CulturesandProducts/Microbiology/ProtozoaandAlgae/tabid/179/Default.aspx

The Culture Collection of Algae at the University of Texas at Austin, Texas

http://www.sbs.utexas.edu/utex/default.aspx

Provasoli-Guillard National Center for Culture of Marine Phytoplankton at the Bigelow Laboratory for Ocean Sciences in West Boothbay Harbor, Maine

http://ccmp.bigelow.org/

Ukraine

Herbarium of Kharkov University (CWU) - MicroAlgae Cultures Collection

The World Register of Marine Species (WoRMS) provides information and links for marine algae (http://www.marinespecies.org/aphia.php?p=webservice). Common and unaccepted taxonomic names of marine algae can be resolved with accepted taxonomic names, sources of these organisms can be obtained and geographic distributions for some species are available through a link to the Ocean Biogeographic Information System (http://www.iobis.org/). The site also contains a link to the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) which provides information on genome sequencing.

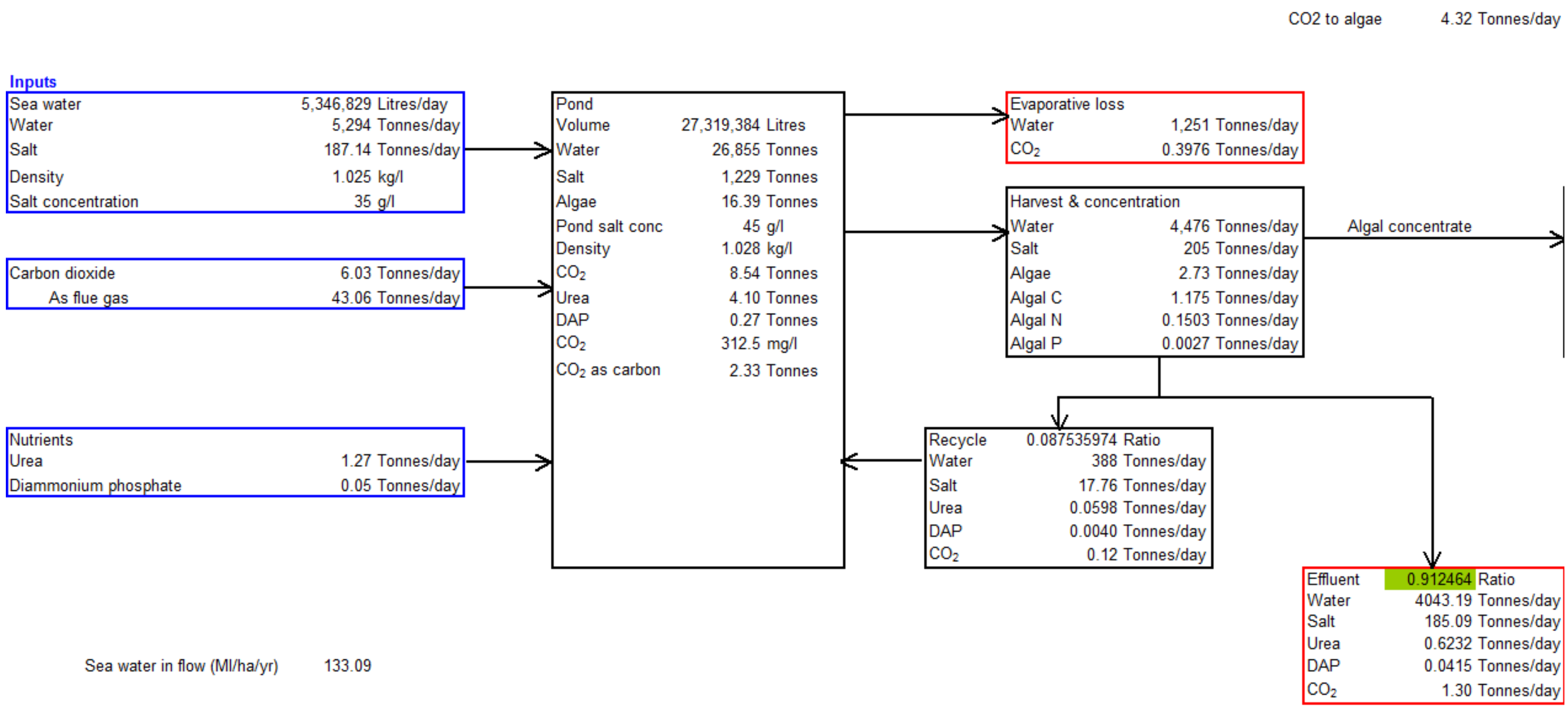
Appendix C. Economic Model and Material Balance Data

Parameters used in the material balances (Figures C1 & C2) and the economic model are shown in the following table along with ranges used in sensitivity analyses.

Table C-1 Parameters for Material Balances and Economic Model

Parameter	Value (range)	Units
Target Biofuel production	100 (20 to 100)	$Ml\cdot yr^{-1}$
Days of production	340	$Days\cdot yr^{-1}$
Production rate	20 (10 to 60)	$g\cdot m^{-2}\cdot day^{-1}$
Algal concentration in ponds	0.6	$g\cdot l^{-1}$
Algal concentration after separation	60	$g\cdot l^{-1}$
Algal dry weight after dewatering	35	% mass
Algal cell carbon	43	% mass
Algal cell nitrogen	5.5	% mass
Algal cell phosphorous	0.1	% mass
Extractable oil content	30 (30 to 60)	% mass
Sea water salt concentration	35	$g\cdot l^{-1}$
Pond salt concentration	45 (45 & 140)	$g\cdot l^{-1}$
Pond urea concentration	0.15	$g\cdot l^{-1}$
Pond DAP concentration	0.01	$g\cdot l^{-1}$
Pond CO_2 concentration	25	% saturation
Precipitation	250	$mm\cdot yr^{-1}$
Evaporation	3590	$mm\cdot yr^{-1}$
Mains CO_2 pipe velocity	3	$m\cdot s^{-1}$
Mains CO_2 pipe pressure	10	MPa
Mains sea water pipe velocity	1.5	$m\cdot s^{-1}$
Extraction solvent:algal concentrate ratio	0.5	mass ratio
Water wash:solvent ratio	0.25	mass ratio
Solvent loss	2	%
Esterification alcohol:oil ratio	6	molar ratio
Esterification catalyst	1.5	% oil
Phosphoric acid (glycerol clean up)	1.5	% oil
Soaps yield	1	% oil
FAME density	0.88	$g\cdot cm^{-3}$
Electricity cost	0.08	$\$\cdot kWh^{-1}$
Glycerol value	210	$\$\cdot tonne^{-1}$
Fertilizer value	26	$\$\cdot tonne^{-1}$ (as is)
Algal cake value	0 (0 to 1000)	$\$\cdot tonne^{-1}$ (as is)
Carbon credit value	0 (0 to 180)	$\$\cdot$tonne CO_2 equiv^{-1}
Discount rate	15	%
Tax rate	30	%
Inflation	2	%
Depreciation (linear)	4	%
Maintenance (other than labour)	1	% cap ex

Algae Production



Biodiesel Production

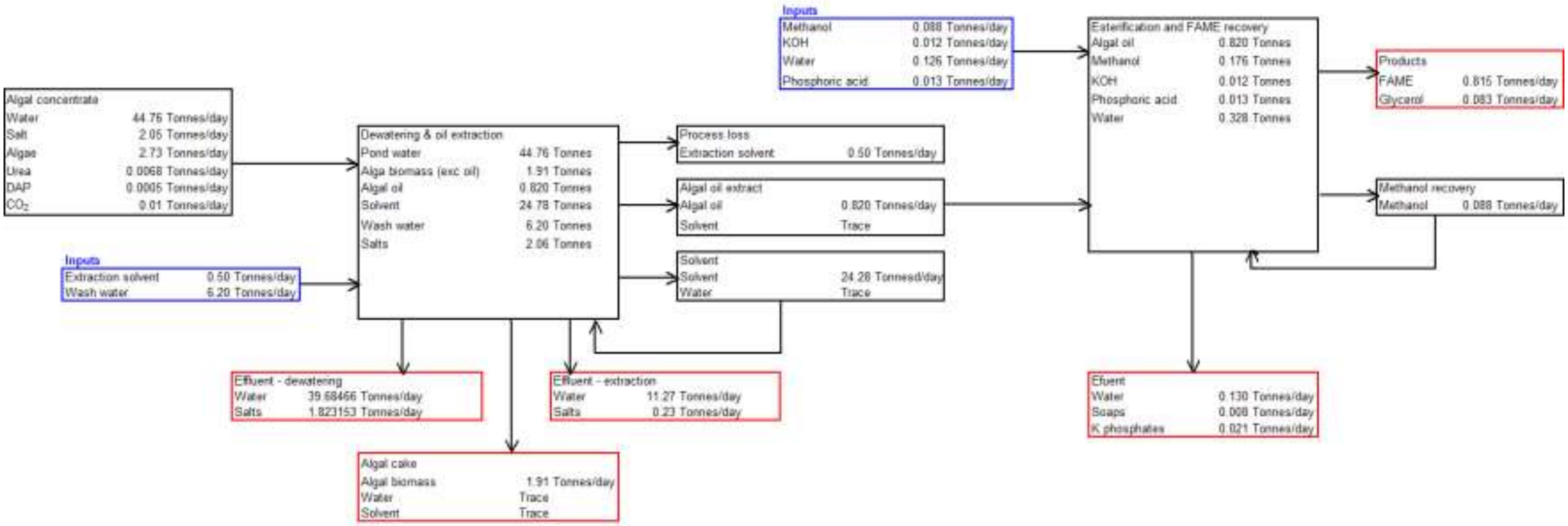
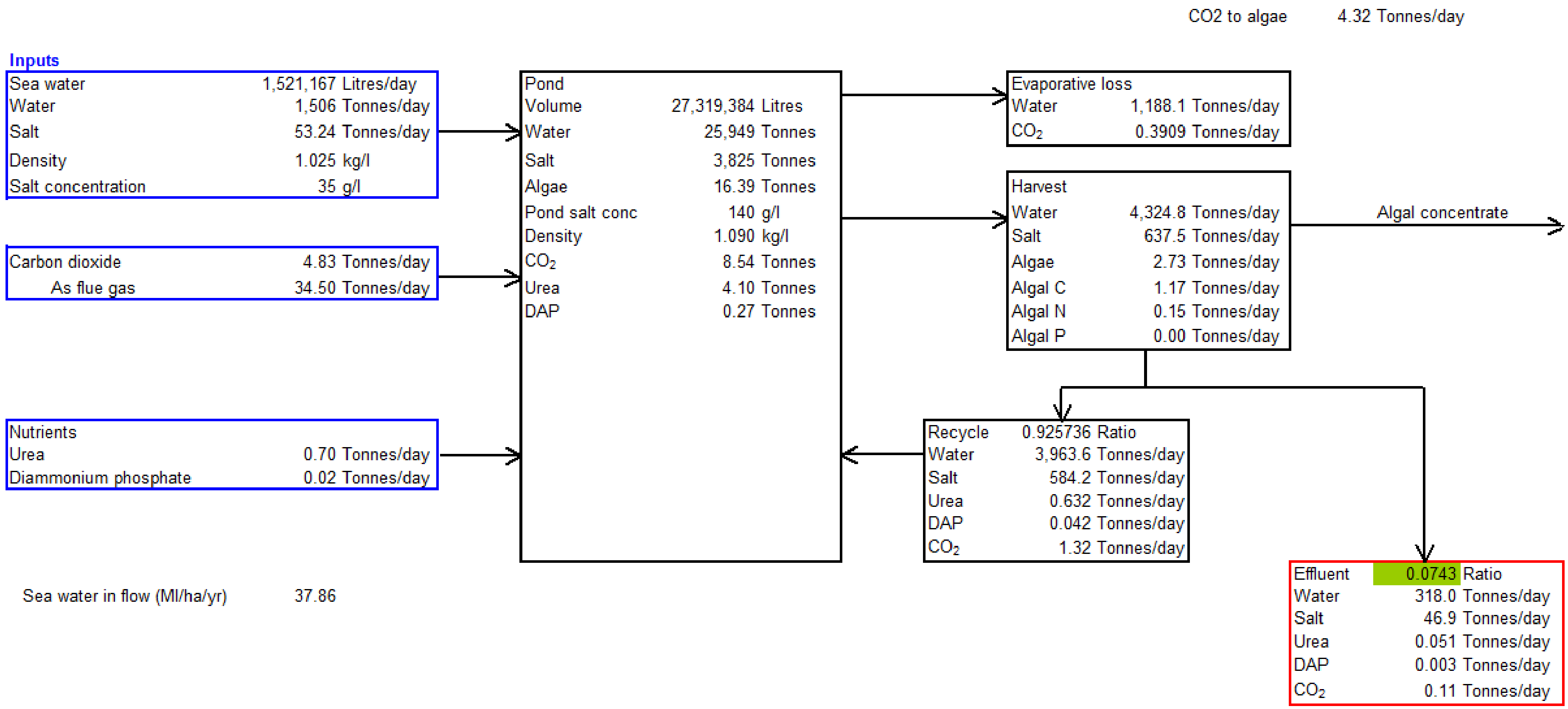


Figure C-8-1 **Material Balance for a Pond at 45 g·l^{-1} Salt**

Algae Production



Biodiesel Production

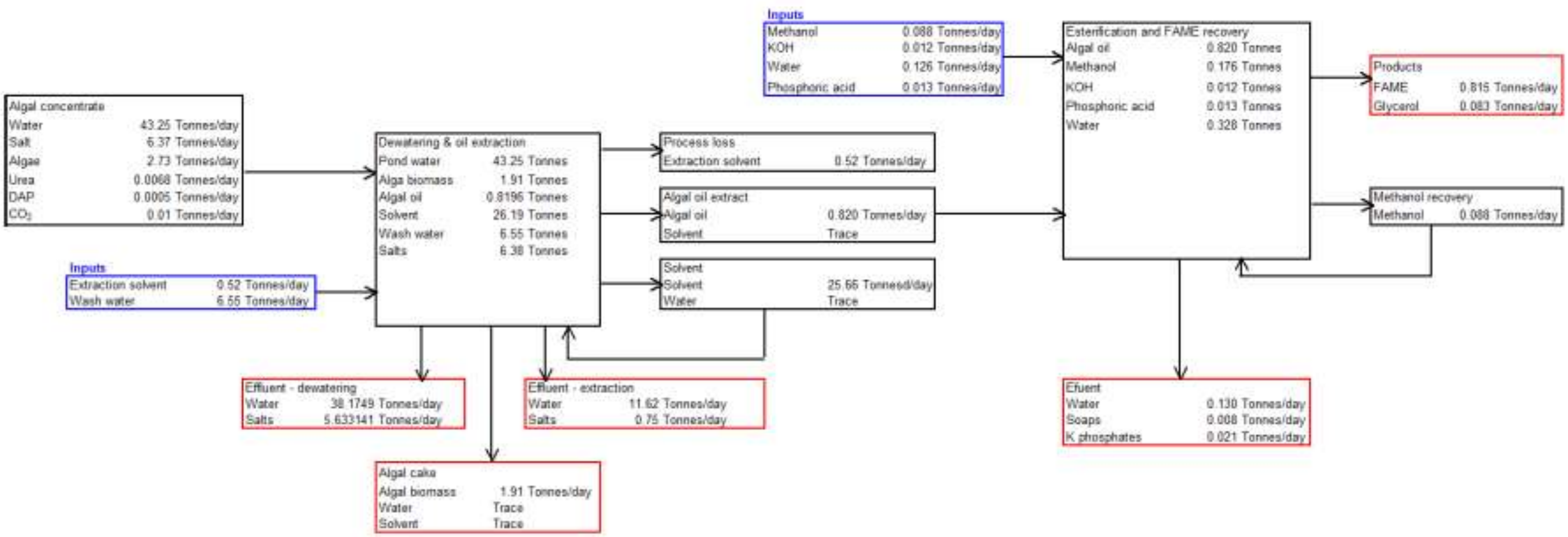


Figure C-8-2 **Material Balance for a Pond at 140 g·l^{-1} Salt**

Appendix D. NREL Techno-economic Parameters

Financial Assumptions	
	Value
Financial	
Discount Rate	10%
Depreciation Period (yrs)	15
Analysis Period (yrs)	20
Tax Rate	35%
Indirect Costs (% of Installed Depreciable Capital)[1]	
Site Development[2]	9%
Prorateable Costs[2]	10%
Field Expenses[2]	10%
Home Office and Construction[2]	25%
Contingency[3]	8%
Other Costs[2]	10%
Working Capital (% of Operating Costs)	25%

[1] Because of the detail that the Benemann and Oswald 1996 study analyzed the pond construction, site development is not applied to that capital cost.

[2] Aden et al. (2002)

[3] Peters and Timmerhaus (2004)

Technical and Process Assumptions – Algal Lipid Production		
	Value	**Source**
Algae Growth		
Pond Depth (cm)	20	Benemann and Oswald (1996)
Water Evaporation Rate (cm/day)	0.3	Engineering Estimate
CO_2 Requirement (kg/ha/yr)[1]	82,500	Benemann and Oswald (1996)
Harvesting Rate (% of total pond volume/day)	5-25%	Benemann and Oswald (1996)
Water Loss per Complete Harvest Cycle (%)	10%	Benemann and Oswald (1996)
Lipid Extraction/Three-Phase Separation		
Algae Lost (%)	20%	Chisti (2007)
Lipid Conversion Process		
Biodiesel		
Overall Yield (%)	96%	Bain (2007)
Spent Algae Conversion		
Anaerobic Digestion		
Net Power Generation (kWh/ha/yr)	26,500[3]	Benemann and Oswald (1996)
Animal Feed/Biomass Feed	50%	
Carbohydrate (wt%)	50%	Engineering Estimate
Protein (wt%)		Engineering Estimate

[1] Based on a 10 g/m^2/day growth rate.

[2] Based on pure CO_2 feed, and 30 g/m^2/day growth rate.

Capital and Operating Cost Assumptions – Algae Lipid Production[1]	
Capital Costs ($/ha)	**Value**
<u>Algae Growth</u>	
Ponds	$8,300
Mixing Paddle	$6,900
CO_2 Feed System	$5,900
Water/Nutrient/Waste System	$8,500
<u>Harvesting</u>	
Settling Tanks/Flocculation	$12,000
<u>Extraction Process</u>	
Three Phase Centrifuge/Hot Oil	$17,000
<u>Spent Algae Conversion</u>	
Anaerobic Digestion	
Digester	$4,000
Generator	$11,000
Operating Costs ($/ha/yr)	
<u>Algae Growth</u>	
Nutrients	$1,000
Waste Disposal	$1,100
<u>Harvesting</u>	
Flocculant	$1,100
<u>Spent Algae Conversion</u>	
Anaerobic Digestion	
Credit for Power[2]	$2,100
Animal Feed/Biomass Feed	
Credit for Animal Feed ($/tonne)	$50
Credit for Biomass Feed ($/tonne)	$10
<u>Miscellaneous</u>	
Power[3]	$2,200
Labour and Overheads	$3,900
CO_2 (base case, $/tonne)	$50
Water (base case, $/m^3)	$0.20
Land ($/ha)[3]	$12,500

[1] All capital and operating cost assumptions based on Benemann et al. (1996), except for CO_2 cost and water cost.
[2] Based on value of produced net power of $0.045/kWh.
[3] Based on purchased power cost of $0.065/kWh.

Capital and Operation Cost Assumptions – Biodiesel Production Facility, 10M gal/yr Production[1]	
Biodiesel Capital Costs	**Value**
Material Storage Equipment	$1,388,296
Processing Equipment	$2,869,676
Utility Equipment	$534,368
Biodiesel Operating Costs ($/yr)	**Value**
Raw Materials	
Methanol	$913,364
Sodium Methoxide Solution	$395,106
Hydrochloric Acid Solution 33%	$30,392
Sodium Hydroxide	$8,441
Water	$448
Utilities	
Natural Gas	$535,336
Wastewater Treatment	$50,909
Chilled Water	$0
Electric Power	$48,337
Labor	
Operating Labor	$215,519
Maintenance Labor	$48,982
Supervision	$137,149
Supplies	
Operating Supplies	$43,539
Maintenance Supplies	$122,998
General Works	
General and Administrative	$57,527
Property Taxes	$11,505
Property Insurance	$58,036
Co-product Credit	
80% Glycerine	$862,827

[1] Values taken from Bain (2007)

Appendix E. Photographic Collection

Figure E-1 Earthrise Nutraceuticals LLC, California at Full Production

Figure E-2 Typical Oxidation Pond

Figure E-3 Cyanotech at Full Production

Figure E-4 Seambiotic *Nannochloropsis sp.* Production, Israel

Appendix F. Glossary

Anaerobic digestion	Process in which microorganisms break down biodegradable material in the absence of oxygen. Often used in wastewater treatment.
Astaxanthan	Photosynthetic pigment known as a carotenoid. It's used as a food colouring agent and food supplement. Found in fish, shellfish, and algae.
Autotroph	An organism that builds complex organic compounds using inorganic molecules and light or inorganic chemical reactions.
Biodiesel	A renewable biofuel produced from vegetable or waste oils, fats, or grease that can be used in a diesel engine.
Bioreactor	Refers to any device or system that supports a biologically active environment.
Biorefinery	An integrated facility combining different biomass conversion processes and equipment to produce fuels, power, and value-added chemicals from biomass.
Biosynthesis	Production of chemical compounds from simpler substances, reactions usually carried out in living cells.
Carageenan	A polysaccharides extracted from red seaweeds. Gelatinous extracts of the seaweed have been used as food additives for hundreds of years.
Carotene	Carotene is an orange photosynthetic pigment important for photosynthesis.
Cellulose	Polymer of glucose, most abundant polymer on Earth.
Chrysolaminarin	Carbohydrate storage product in microalgae, similar to cellulose.
Contamination	Growth of unwanted biological organisms in a biological process.

Cross-flow filtration	Method of harvesting microalgae. Liquid flows across the surface of a membrane filtration and liquids and particles smaller than the pores in the filter are removed from the bottom of the filter.
Delipidated	Removal of lipids from an organism.
Dewatering	Removal of excess water to concentrate the solid fraction of a solution.
Diatomaceous	Diatomaceous earth consists of fossilized remains of diatoms.
Diatoms	Most diatoms are unicellular. A characteristic feature of diatom cells is that they are encased within a unique cell wall made of silica (hydrated silicon dioxide).
Feedstocks	A raw material going into a chemical process or plant as input to be converted into a product.
Flocculation	Process where a solute comes out of solution. The term is also used in colloid chemistry to refer to the process by which fine particulates are caused to clump together into floc. The floc may then float to the top of the liquid, settle to the bottom of the liquid, or can be readily filtered.
Flue gases	Refers to the combustion exhaust gas, such as carbon dioxide, produced at power plants.
Fouling	Refers to the accumulation and deposition of living organisms and certain non-living material on hard surfaces, most often in an aquatic environment.
Homogenization	Process that makes a mixture the same throughout the entire substance.
Hydrocracking	Process whereby complex organic molecules such as kerogens or heavy hydrocarbons are broken down into simpler molecules by the breaking of carbon-carbon bonds.
Hydrotreating	An oil refinery based process of hydrogen to crude oil to remove oxygen and increase the Btu content.

Inocula	Addition of microorganisms to a bioreactor to start the growth phase.
Intermediates	Compounds formed during a chemical reaction that are not the final product but are part of the overall reaction pathway.
Jatropha	Succulent seed bearing plant that has become naturalized in many tropical and subtropical areas. May be used for biodiesel production.
Lipid extraction	Process of removing lipids from a plant or microorganism. May be accomplished using a mechanical or solvent based process.
Lipids	Broadly defined as any fat-soluble, naturally-occurring compounds, such as fats, oils, waxes, cholesterol, sterols, fat-soluble vitamins such as A, D, E, and K, monoglycerides, diglycerides, or phospholipids.
Methyl-esters	Hydrolysis of triglycerides into fatty acids, followed by the addition of an alcohol group on the end of the fatty acid. These are produced during transesterification. The methyl esters from the conversion of vegetable oil are called biodiesel.
Microstrainers	Rotating screened drums with screen fronts used to harvest filamentous microalgae from ponds.
Monounsaturated	Having one single double bond in the fatty acid chain. By contrast, polyunsaturated fatty acids have more than one double bond.
Nutraceutical	Food, food additive, or extract of a food marketed to have a physiological benefit or provide protection against a chronic disease.
Oil palm	The oil extracted from a tropical palm tree.
Photosynthesis	The conversion of light energy into chemical energy by living organisms.

Photosynthetic efficiency The amount of solar energy used in photosynthesis as a percentage of the total available solar energy.

Productivity Measurement of algal growth expressed as weight of dried algae per square meter per day.

Saponification Commonly used to refer to the reaction of a metallic alkali (base) with a fat or oil to form soap.

Silicon Growth element for diatoms, a component of their cell wall.

Solvent A liquid that dissolves a solid, liquid, or gaseous solute, resulting in a solution.

Sparging Intermittent addition of gaseous carbon dioxide to a algal growth culture through fine bubbles.

Technoeconomic An analysis that combines both the technical portions of a study and the economic costs for the individual processes that reflect a final cost for the overall process.

Transesterification Process of exchanging the alkoxy group of an fatty acid by an alcohol. The primary method for producing biodiesel using vegetable oils or waste grease.

Triacylglycerols; Triacylglycerides; Triglycerides Simple fats or lipids consisting of glycerol esterified to three fatty acids.

Ultra sonication Method of cell disruption using high-frequency oscillation causing a localized high pressure region resulting in breaking open the cells.

Viscosity The measure of the resistance of a fluid to being deformed by either shear stress or extensional stress. It is commonly perceived as "thickness," or resistance to flow.

Zooplankton Heterotrophic plankton that consume organic matter; often found as a contaminate in algal ponds.