Annex XXXIV Subtask 2

A Report from the IEA Advanced Motor Fuels Implementing Agreement

Algae as a Feedstock for Biofuels: An Assessment of the State of Technology and Opportunities

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DISCLAIMER

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About the Cover:

Photos from left to right: (1) microalgae *Haematococcus pluvialis*, vegetative (Palmella-) stage, are shown under light microscope (Image Source: Fraunhofer IGB: http://www.igb.fraunhofer.de/start.en.html); (2) cyanobacteria often grow in long filaments resembling algae (Image Source: biotechnologie.de); (3) macroalgae are mulitcellullar, photosynthetic marine organisms with plant-like features (Image Source: The Scottish Association for Marine Science); an aerial view shows Cyanotech's outdoor algae pond cultivation facility in Kailua Kona, Hawaii (Image Source: Cyanotech Corp.). For details, see Chapter 4 in text.

Notice:

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Preface

Recently, a worldwide reemergence of interest in pursuing research and development of algae as a feedstock for biofuels has occurred, and many R&D initiatives are underway around the world as researchers, governments, and policy-makers become aware of the considerable potential that algae possess. It can be expected that these various initiatives will go in many different directions as researchers look for answers to the challenges that algae-derived fuels face. Some pathways will be deemed unsuccessful for large scale production while others will produce high-quality results. It is important at this point to make an inventory and an assessment of the many various activities and to try to develop recommendations about the most promising pathways to success in making large quantities of transportation fuels from algae, which may help policy-makers reach wise decisions about which areas of effort to support.

Specifically, the project team has been tasked under the International Energy Agency (IEA) Implementing Agreement on Advanced Motors Fuels (AMF) with conducting a study that assesses the state of the technology and opportunities associated with algal fuels. Since the AMF Agreement focuses on end-use fuels, the focal point of this report will be on the downstream activities (e.g., dewatering, oil/biomass extraction, and conversion of algae and algal components to energy products). However, limited investigation of upstream activities (e.g., strain selection, cultivation) was also conducted to help identify promising lifecycle pathways. For more detail on upstream activities, the IEA Implementing Agreement on Bioenergy Task 39 report entitled “Current Status and Potential for Algal Biofuels Production” should be accessed (Darzins, Pienkos, & Edye, 2010).

The authors would like to acknowledge several individuals and organizations for their support throughout the preparation of this report. Specifically, the authors would like to extend gratitude to:

- Cost sharing provided by International Energy Agency Advanced Motor Fuels Implementing Agreement member countries Canada, Finland, Japan (LEVO), and United States.

- Stephen O’Leary (Research Officer) and Patrick McGinn (Scientific Leader) at the National Research Council (NRC) Canada Institute for Marine Biosciences for extensive property data on algal strains housed at the Institute’s Research Facilities and Ketch Harbour Research Facilities located in Nova Scotia, Canada.

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Acronyms and Abbreviations

AACT	Algae Aqua-Culture Technology
AFC	Algal Fuels Consortium
AMF	IEA Advanced Motor Fuels Implementing Agreement
ARPA-E	Advanced Research Projects Agency - Energy
ARRA	American Recovery and Reinvestment Act of 2009
ASP	Aquatic Species Program
ATF	Algae Task Force
AUD	Australian dollar
BAL	Bio-Architecture Lab
bbl	barrel
BMBF	German Federal Ministry of Education and Research
BP	British Petroleum
CAD	Canadian dollar
CEA	The French Alternative Energies and Atomic Energy Commission
CEO	Chief Executive Officer
CHG	Catalytic Hydrothermal Gasification
CHP	combined heat and power
CNG	compressed natural gas
CORFO	Chilean Economic Development Agency
cP	centipoise
CSIR	Council for Scientific and Industrial Research
DARPA	U.S. Defense Advanced Research Projects Agency
DOE	U.S. Department of Energy
EERC	The Energy & Environmental Research Center
EET	Economy, Ecology, Technology
EIA	U.S. Energy Information Administration
EU	European Union
EUR	euro
FAME	fatty acid methyl ester
FAO	The Food and Agriculture Organization of the United Nations
gal/acre/yr	gallon per acre per year
gal/yr	gallon per year
GBP	British pound
GHG	greenhouse gas
GJ/ha/yr	gigajoule per hectare per year

HC	hydrocarbon
ICE	internal combustion engine
IEA	International Energy Agency
IFREMER	French Research Institute for Exploitation of the Sea
JCU	James Cook University
JDA	joint development agreement
kg/m^3	kilogram per cubic meter
kJ/L/h	kilojoule per liter per hour
kW	kilowatt
L/ha/yr	liter per hectare per year
L/yr	liter per year
L3BM	The Laboratory of Microalgal and Bacterial Bioenergetics and Biotechnology
LANL	Los Alamos National Laboratory
LCA	life-cycle analysis
LEVO	Organization for the Promotion of Low Emission Vehicles
mg/kg	milligram per kilogram
m/m	by mass
mm^2/s	square millimeter per second
MPa	megapascal
Mtoe	megaton oil equivalent
MWe	megawatt electric
NCSU	North Carolina State University
NIST	National Institute of Standards and Technology
NIWA	National Institute of Water and Atmospheric Research
NRC	National Research Council of Canada
NREL	National Renewable Energy Laboratory
PBR	photobioreactor
R&D	research and development
RTP™	Rapid Thermal Process
SAIC	Science Applications International Corporation
SVO	straight vegetable oil
TJ/ha/yr	terajoule per hectare per year
US	United States
USD	US dollar

Executive Summary

Introduction

The pursuit of a stable, economically-sound, and environmentally-friendly source of transportation fuel has led to extensive research and development (R&D) efforts focused on the conversion of various feedstocks into biofuels. Some feedstocks, such as sugar cane, corn and woody biomass, are targeted because their structures can be broken down into sugars and fermented into alcohols. Other feedstocks, such as vegetable oils, are appealing because they contain considerable amounts of lipids, which can be extracted and converted into biodiesel or other fuels. While significant R&D and commercial strides have been made with each of these feedstocks, technical and market barriers (e.g., cost, scalability, infrastructure requirements, and "food vs. fuel" debates) currently limit the penetration of the resultant biofuels into the mainstream.

Because of algae's ability to potentially address several of these barriers, its use as a feedstock for biofuels has led to much excitement and initiative within the energy industry. Algae are highly diverse, single- or multi-cellular organisms comprised of mostly lipids, protein, and carbohydrates, which may be used to produce a wide variety of biofuels. Algae offer many competitive advantages over other feedstocks, including:

> *Given microalgae's high lipid content and rapid growth rates, maximum oil yields of 20,000-115,000 L/ha/yr (2,140-13,360 gal/ac/yr) have been estimated.*

- Higher potential lipid content than terrestrial plants, sometimes exceeding 50% of the cell's dry biomass (U.S. DOE, May '10; Tornabene et al., 1983)
- Rapid growth rates that are 20-30 times higher than terrestrial crops (McDill, 2009) and, in some cases, capable of doubling in size with 10 hours
- Diverse number of species that can collectively thrive in a wide range of environments throughout the world, presenting an overall high overall tolerance for climate, sunlight, nutrient levels, etc.
- Daily harvesting potential instead of seasonal harvest periods associated with terrestrial crops
- Potential to redirect CO_2 from industry operations to algal cultivation facilities to be used in an algal biofuel cycle before it is released into the atmosphere
- Ability to be cultivated on land that that is unsuitable for agriculture, so it does not directly compete with farmland

- Ability to thrive in seawater, wastewater, or other non-potable sources, so it does not directly compete with fresh water resources. In fact, wastewater can provide algae with some essential nutrients, such as nitrogen, so algae may contribute to cleaning up wastewater streams.

- Non-toxic and biodegradable

- Co-products that may present high value in other markets, including nutriceuticals and cosmetics

Given microalgae's high lipid content and rapid growth rate, maximum oil yields of 20,000 – 115,000 liters per hectare per year (L/ha/yr) (2,140 – 13,360 gallons per acre per year) (Baldos, 2009; Wijffels, 2008) have been estimated, which is considerably higher than any other competing feedstock.

Although algae species collectively present many strong advantages (although one specific species is unlikely to possess all of the advantages listed), a sustainable algal biofuel industry is at least one or two decades away from maturity, and no commercial scale operations currently exist. Several barriers must first be overcome before algal biofuels can compete with traditional petroleum-based fuels. Production chains with net energy output need to be identified, and continued R&D is needed to reduce the cost in all segments of the production spectrum (e.g., harvesting, dewatering, extracting of oil). Further research to identify strains with high production rates and/or oil yields may also improve competitiveness within the market. Initiatives to seamlessly integrate algal biofuels into the existing transportation infrastructure may increase their convenience level.

Industy Outlook

As interest in algal biofuels reemerges globally, many industry players are actively pursuing R&D ventures, operating pilot scale facilities, and seeking investment support. Over time, the market will likely experience some consolidation as various current technologies exit the market due to lack of cost-competitiveness and production efficiency. A status summary of the current and projected algae-to-biofuels industry follows.

ALGAE-TO-BIOFUELS MARKET

CURRENT INDUSTRY

- **SIZE:** By mid-2010, an estimated 200 companies were directly participating in algal biofuels production, rising from virtually no companies at the start of the decade.
- **VALUE:** In a span of approximately ten years, the algal biofuel industry grew from minuscule in value in 2000 to on track to reach an estimated market value of 271 million USD in 2010 (Algae, 2010).
- **PRODUCTION LEVEL:** Algal biofuel is not currently being produced at commercial scale, and no commercial scale plants are operational at this time due to early-technology high production costs. Instead, numerous companies have set up demonstration and pilot-scale plants that produce a variety of fuels in relatively small quantities for use by limited customers.
- **PRODUCTION COST:** Recent cost estimates for today's algae biofuel production range from 8 to 30 USD/gal (~2.11 to 7.93 USD/L)

PROJECTED INDUSTRY

- **SIZE:** Pilot facilities that demonstrate sustainable and economic solutions in the algae-to-biofuels industry are expected to transition into commercial-scale facilities in the next one to two decades.
- **VALUE:** SBI Energy estimates a total algal biofuels market worth of 1.6 billion USD in 2015 (Algae, 2010). This indicates a 43% annual growth rate between 2010 and 2015.
- **PRODUCTION LEVEL:** Announcements by Algenol Biofuels, Aurora Algae, PetroAlgae, Sapphire Energy, and Solazyme have resulted in production projections of between 100 million and 1 billion gallons (380 million and 3.8 billion L) of algal biofuels by 2015 (Emerging Markets Online). Pike Research, however, projects only 61 million gal (231 million L) of algae-based biofuels produced by 2020.
- **PRODUCTION COST:** Recent cost estimates for future algae biofuel production are as low as 1USD/gal (0.26 USD/L) and 60 USD/bbl.

Government Support

> *To date, over 500 million USD in government funding has been awarded worldwide to support the algae-to-biofuels industry.*

Worldwide government support for the algal biofuel industry is continually increasing with hopes of stimulating new industry ventures that will ultimately help drive down capital and operating costs, and accelerate the production of algal fuel. Governmental support for R&D related to algae as feedstock for biofuels is often embedded in more general programs for renewable energy or biofuels. Sometimes government support is provided to individual companies while other times it funds activities run by large consortiums or government laboratories. Furthermore, the direction of funds to date has ranged from upstream R&D initiatives to downstream pilot production facilities. To date, over 500 million USD in government funding has been awarded worldwide to support the algae-to-biofuels industry.

Algal Biofuel Production Spectrum

The algal biofuels production spectrum is currently comprised of a fairly complex set of steps that begins with upstream algal strain selection and concludes with the conversion of algal biomass into a finished energy product. Algae are highly versatile organisms, presenting many pathways for navigating from one end of the spectrum to the other. Most companies choose to perfect one or two steps in the spectrum and then form business relationships with other industry stakeholders to complete the supply chain. Other companies' business models instead attempt to conquer the entire spectrum.

This industry is still considered to be in its infancy, and algal biofuels are not currently being produced at the industry scale. Considerable amounts of R&D are underway, and pilot plants are up and running worldwide to test promising new methods for improving system efficiency and cost-competiveness with traditional fuel industries. As the industry matures and production ramps up, the portfolio of techniques is expected to naturally consolidate to address scalability, cost, and demand issues. The state of the technology is investigated in this report for the five key steps of the algal biofuels production spectrum: 1) Algal Strain Selection, 2) Algae Cultivation, 3) Harvesting and Dewatering, 4) Oil / Biomass Separation, and 5) End-Use Fuel Production. The major categories under each of these five steps are listed in the following graphic.

Algal Strain Selection	Algae Cultivation	Harvesting and Dewatering	Oil / Biomass Separation	End-Use Fuel Production
Microalgae **Cyanobacteria** **Macroalgae**	**Open Ponds / Raceways** **Photobioreactors** **Heterotrophic Fermenters** **Marine Systems**	**Filtration / Microscreening** **Centrifugation** **Flocculation** **Flotation** **Off-shore Methods** **Other**	**Physical** **Chemical / Solvent** **Enzymatic** **Wet / Single Step**	**Transesterification** **Hydroprocessing** **Fermentation** **Anaerobic Digestion** **Gasification** **Pyrolysis/ Liquefaction** **Biophotolysis** **Purification of SVO**

Brief descriptions of all five key steps are provided in the following paragraphs. Immediately following these descriptions, five summary charts are provided that discuss strengths, weaknesses, cost data, and/or industry activity associated with each individual technique that falls within each key step.

Algal Strain Selection: In order to optimize production and minimize cost, the search for "superalgae" is underway in the algal biofuels industry. Microalgal strains appear to have the strongest industry appeal due to their simple structure, rapid growth rate, and often high oil content. However, macroalgae (seaweeds) and cyanobacteria are also being studied as energy sources due to their rapid growth rates. These three groups of algae – microalgae, cyanobacteria, and macroalgae – are examined in this report to understand how their unique characteristics are being applied within the biofuels industry.

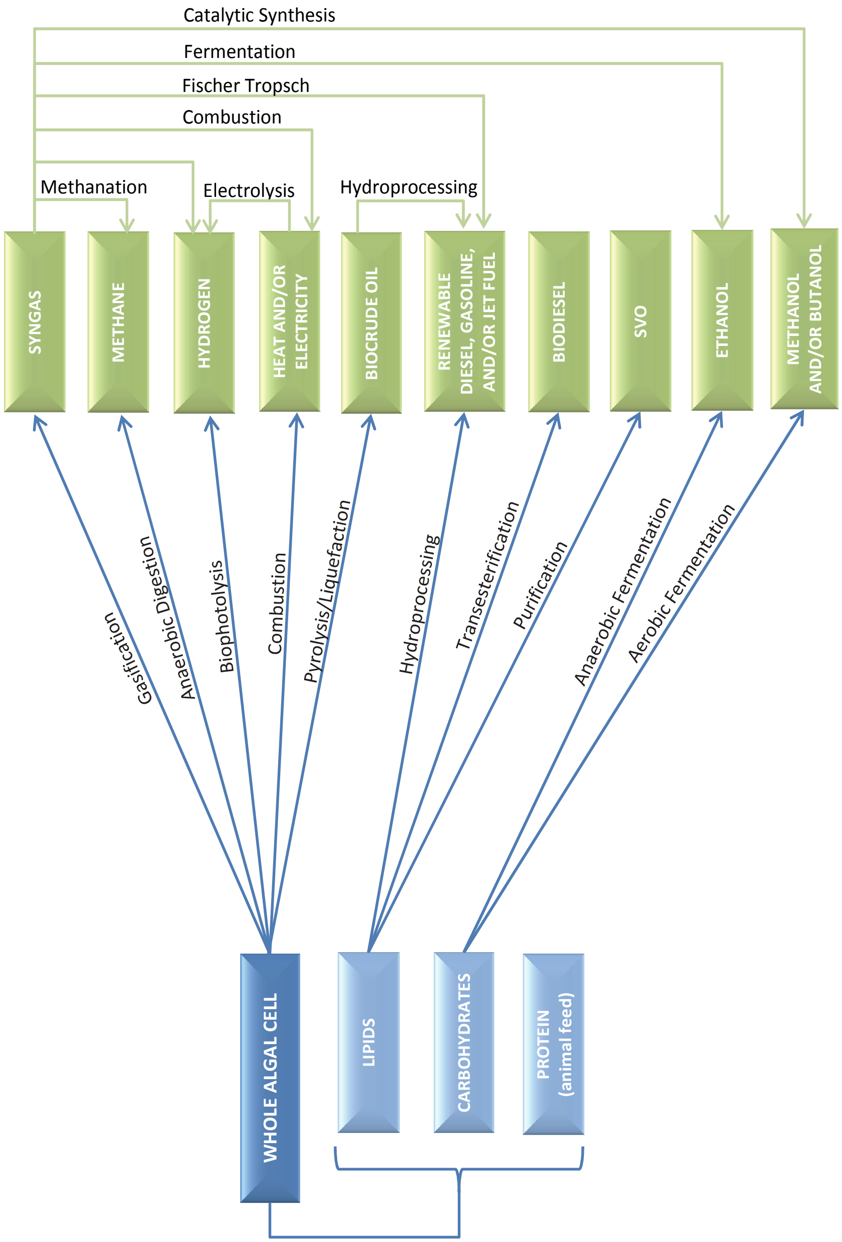
Algae Cultivation: Once an algal strain (or set of strains) has been selected for production, a growth environment must be chosen. For traditional microalgae cultivation, open ponds / raceways and closed photobioreactors, or PBRs, filled with water are the two most common designs. Each of these methods requires the same general inputs – light, nutrients, and CO_2 – and algae grown in these ponds or vessels are sent downstream to be harvested once they reach the desired level of maturity or lipid capacity. Heterotrophic fermentation is a less traditional approach, where algae thrive in vessels by feeding on sugar and nutrients (no light required) until they are ripe for harvesting. Finally, macroalgae are most often cultivated in marine settings where water and space are abundant. These four groups – open pond /raceways, PBRs, heterotrophic fermenters, and marine systems – are examined in this report to understand why each method may be chosen for certain situations.

Harvesting and Dewatering: Once algae are cultivated to the desired level, they must then, in most cases, be recovered from the water or other growing medium, dewatered to reach a certain moisture content in preparation for processing, and relocated to the processing site. While this may seem straightforward in theory, harvesting and dewatering are often collectively considered the greatest bottleneck to scaling up algal biofuel production. Such a large percentage of the energy and cost is expended in order to obtain sludge dry enough to conduct lipid extraction and/or fuel conversion. In fact, 30-50% of the total cost of algae cultivation is on average expended when trying to convert an algae culture to sufficiently dry algae cakes (U.S. DOE, n.d.), and energy costs rise substantially as moisture contents required for downstream processing get smaller. With that said, this step also presents large opportunities to decrease the overall cost of algal biofuel production. The major existing techniques for harvesting and dewatering algae – filtration / microscreening, centrifugation, flocculation, flotation, and off-shore methods – are examined in this report.

Oil Biomass / Separation: A large percentage of algal biofuels are produced by processing specific cellular components that must first be separated from the rest of the cell. Before oil-based biofuels (e.g., biodiesel) can be processed, for example, lipids housed within the algal cells must be isolated. Separating the various cell components can be achieved through physical, chemical, enzymatic, or other extraction methods. Some algal biofuel companies choose to use mature techniques already currently being used in other industries, while other companies invest in the R&D to find experimental "breakthrough" technologies. The most common methods for separating lipids from the rest of the cell biomass are characterized in this report as either physical, chemical, enzymatic, or wet / single step.

Other algal biofuels may be achieved by converting entire algal cells, eliminating the need for cell fractionation. For example, whole algal cells can be gasified to create syngas, or anaerobically digested to create methane. Also, seaweeds generally do not contain lipids, so separation of lipids from seaweeds is not necessary. This means that biomass separation for seaweeds will only consist of removing other elements such as stones (from seaweeds that grow on holdfasts), snails that may be present on the surface of the plant, debris, and sand (Bruton et al., 2009).

End-Use Fuel Production. Algae and its cellular components have been considered for feedstocks to be processed to create a variety of end-use energy products, which include a wide range of liquid and gaseous transportation fuels. Such fuels include biodiesel; renewable gasoline, diesel, and jet fuel; ethanol; methane; synthesis gas; hydrogen; and straight vegetable oil (SVO). In the biophotolysis fuel production route, algae (or cyanobacteria) are not used as feedstock, but they are the actual producers of the fuel (hydrogen), which means that the algae are not consumed in this process. In addition to transportation fuels, dry algal biomass can also be directly combusted to create heat or electricity. The combusted biomass may consist of the entire algal cell, algal oil, or de-oiled algal cake, depending on a company's business model. Since the focus of this report is advanced motor fuels, combustion is considered outside of the scope and will not be discussed in detail. The following diagram summarizes the multiple pathways for obtaining the various transportation fuels and other energy products.



Summary of Fuel Options Attainable with Algae

ALGAL STRAIN SELECTION

MICROALGAE

- **STRENGTHS:** Microalgae have high replication rates, high energy content, and have greater lipid yields than cyanobacteria and macroalgae.
- **WEAKNESSES:** Microalgae is difficult and costly to collect and harvest.
- **INDUSTRY ACTIVITY:** The large majority of algae production companies that exist today use microalgae.

CYANOBACTERIA

- **STRENGTHS:** Cyanobacteria have high replication rates and can store large quantities of carbohydrates. They are better positioned for genetic manipulation than other algal strains since bacterial genetics are more advanced than microalgae and macroalgae. Finally, cyanobacteria have higher light conversion rates than microalgae.
- **WEAKNESSES:** Like microalgae, cyanobacteria are difficult and costly to collect and harvest. Their lipid yields are generally low., and they possess durable membranes that are difficult to break down.
- **INDUSTRY ACTIVITY:** Companies that use cyanobacteria in their operations include Algenol Biofuels / Cyano Biofuels, Baltic EcoEnergy Cluster, Biolight Harvesting, Synthetic Genomics, and Targeted Growth.

MACROALGAE

- **STRENGTHS:** Macroalgae have rapid growth rates, are abundant in oceans and coastal waters, and can store large quantities of carbohydrates. They do not require arable land or potable water to grow, are easier to cultivate and harvest than microalgae and cyanobacteria, and have been grown at commercial scale for food for many years.
- **WEAKNESSES:** Lipid yields in macroalgae are generally low. Research for use in biofuels and energy industry is less advanced than for microalgae.
- **INDUSTRY ACTIVITY:** Companies that use macroalgae in their operations include Bio Architecture Lab, BioMara Project, Blue Sun Energy, Butamax Advanced Biofuels, Green Gold Algae and Seaweed Sciences Inc., Holmfjord, Oil Fox, POD Energy, and Seambiotic Ltd.

ALGAE CULTIVATION

OPEN PONDS / RACEWAYS

- **STRENGTHS:** Open ponds /raceways have low-to-moderate production costs and low maintenance costs. They permit high production volumes, can utilize undesirable land and space, are more conducive for incorporating wastewater, and are easy to clean and scale up.
- **WEAKNESSES:** Open ponds / raceways are susceptible to contamination by other algal strains or diseases, and the natural loss of water and CO_2 must be addressed. Inability to control certain conditions (e.g., temperature, pH, salinity) may affect productivity, and the growing timeframe may be limited due to seasons. Also, poor light utilization and inefficient stirring is common.
- **INDUSTRY ACTIVITY:** Companies that use open ponds or raceways in their operations include Aurora Biofuels, Blue Marble Energy, General Atomics, Kai Bioenergy, Kent Bioenergy, LiveFuels, PetroAlgae, PetroSun, Phycal LLC, Sapphire Energy, SBAE, Seambiotic, and SunEco Energy.

PHOTOBIOREACTORS

- **STRENGTHS:** PBRs offer controlled conditions (e.g., salinity, light, temperature, pH, CO_2) and are more conducive for growing genetically-modified strains and monocultures. They also accommodate higher concentrations and, therefore, yields.
- **WEAKNESSES:** PBRs have a high production cost, especially if artificial light is required. They are difficult to maintain, may need cooling during the daytime, and are not yet feasible for large volumes of algal mixtures. Fouling and buildup of algae on PBR walls may obstruct light. Finally, algae in PBRs are susceptible to high oxygen levels.
- **INDUSTRY ACTIVITY:** Companies that use PBRs in their operations include A2BE Carbon Capture, AlgaeLink, BARD LLC, Bionavitas, BioProcess Algae, Bodega Algae LLC, Global Green Solutions, Green Plains, Hezinger Algaetec, Plankton Power, Solix Biofuels, Subitec GmbH, Vertigro Energy, and W^2 Energy.

HETEROTROPHIC FERMENTERS

- **STRENGTHS:** Fermentation allows for high lipid yields and cell density, does not require artificial light, and uses significantly less water than other methods. The system does not require CO_2 and has easily controlled parameters. Operating costs are low, and it is a widely established process.
- **WEAKNESSES:** A high initial investment cost is associated with this process due to complex configuration and construction. The system is difficult to clean, and sufficient levels of oxygen must be maintained. It requires large amounts of sugar, which may contribute to the "food vs fuel" debate. Also, there is a limited ability to scale up operations.
- **INDUSTRY ACTIVITY:** Companies that use heterotrophic fermentation in their operations include Martek Biosciences (with BP) and Solazyme.

ALGAE CULTIVATION (CONT.)

MARINE SYSTEMS

- **STRENGTHS:** Marine systems can be adapted to near-shore or off-shore settings, have vast space for cultivation, and do not require fresh water. Modern structures have been successfully tested in various settings worldwide.
- **WEAKNESSES:** The design and stability of underwater structures could be improved, especially if it promotes attachment of macroalgae to structure. However, environmental regulations may inhibit aquaculture development in some countries.
- **INDUSTRY ACTIVITY:** Companies that use marine systems in their operations include Seaweed Energy Solutions and BAL (with StatOil).

HARVESTING AND DEWATERING

FILTRATION / MICROSCREENING

- **STRENGTHS:** Filtration is the most simple method of harvesting microalgae besides sedimentation and it is not an energy-intensive process.
- **WEAKNESSES:** Filtration is time consuming due to low flow rates (if suction is not applied), and limited yields can be expected. The potential for clogging also exists since mass sticks to the filter screen.
- **COST**: Very low (microstraining) or moderate (tangential flow filtration), relative to other methods

CENTRIFUGATION

- **STRENGTHS:** Centrifugation is highly efficient and can be applied to moderate volumes of algal culture at a time. It is best suited for cultures that are mostly liquid.
- **WEAKNESSES:** This process is highly energy intensive, which increases the operating cost. Also, a secondary watering step is still typically needed.
- **COST:** Very high, relative to other methods

FLOCCULATION

- **STRENGTHS:** Flocculation can be applied to large volumes of algal culture at a time and can be used on most algae strains. Less energy is required than with mechanical separation methods.
- **WEAKNESSES:** Flocculation is typically paired with a complementary harvesting technique (e.g., flotation), and a dewatering step is still needed. Flocculation also introduces new chemicals into the culture, which are difficult to remove. Finally, flocculants are often expensive and caustic.
- **COST:** Moderate to high (chemical flocculation) or very low (bioflocculation and autoflocculation), relative to other methods

FLOTATION

- **STRENGTHS:** Flotation is an efficient method for algae removal with low water losses and simple implementation.
- **WEAKNESSES:** Flotation must typically be paired with a complementary harvesting method (e.g., flocculation), which adds cost.
- **COST:** Moderate (if not combined with flocculation) or high (if combined with flocculation), relative to other methods

HARVESTING AND DEWATERING (CONT.)

OFF-SHORE METHODS

- **STRENGTHS:** Macroalgae are generally cheaper and easier to harvest than microalgae, and harvesting can occur on site before being sent ashore. Macroalgae that naturally washes ashore can also be diverted for fuel production.
- **WEAKNESSES:** A high water content is present in these methods so multiple dewatering techniques. may be required. Also, a lower replenishment rate is seen in macroalgae that with microalgae.
- **COST:** Low, relative to other methods

OIL / BIOMASS SEPARATION

PHYSICAL

- ***MECHANICAL PRESS***
  - **STRENGTHS:** Presses are widely used in industry and do not require the use of caustic chemicals. They are most useful for high-lipid algae strains and can extract up to 75% of lipids.
  - **WEAKNESSES:** Presses have high capital and maintenance costs, and are energy intensive. Oil and residual biomass do not easily separate, so secondary extraction techniques are typically required.
  - **INDUSTRY ACTIVITY:** PetroAlgae has used a mechnical press in its operations.
- ***ULTRASONICATION***
  - **STRENGTHS:** Dewatering is not needed beforehand, and the use of caustic chemicals in not required. This process is environmentally benign and has a relatively low cost.
  - **WEAKNESSES:** This process is energy intensive and typically requires a secondary extraction technique. It has not been demonstrated at industry scale.
  - **INDUSTRY ACTIVITY:** BARD LLC, Cavitation Technologies, OriginOil, and Solix Biofuels have used ultrasonication in their operations.
- ***OSMOTIC SHOCK***
  - **STRENGTHS:** Osmotic shock does not require dewatering beforehand or the use of caustic chemicals.
  - **WEAKNESSES:** Osmotic shock is not commonly used in industry.
  - **INDUSTRY ACTIVITY:** No companies identified.

CHEMICAL / SOLVENT

- ***ORGANIC SOLVENTS***
  - **STRENGTHS:** These solvents are relatively inexpensive and can release over 95% of oil.
  - **WEAKNESSES:** Precautions must be taken when working with chemicals, and the permitting process for chemical use may delay operations. They may also have a negative impact on the environment.
  - **INDUSTRY ACTIVITY:** No companies identified.
- ***SUPERCRITICAL FLUIDS***
  - **STRENGTHS:** This process is very efficient and results in high quality oil. No solvent residues remain in the extracted oil. It is widely used in other industries and is environmentally friendly.
  - **WEAKNESSES:** This process is energy intensive, has high capital costs, and a risk is associated with high pressure operations.
  - **INDUSTRY ACTIVITY:** Global Green Solutions uses supercritical fluids in its operations.

OIL / BIOMASS SEPARATION (CONT.)

ENZYMATIC

- **STRENGTHS:** Enzymatic extraction does not require dewatering, and caustic chemicals are not required.
- **WEAKNESSES:** Enzymatic extraction is very expensive compared to hexane extraction, and it is not commonly used in industry. Oil recovery is less than in conventional processes (e.g., pressing and hexane). Significant amounts of water and energy are also needed.
- **INDUSTRY ACTIVITY:** No companies identified.

WET / SINGLE STEP

- **STRENGTHS:** Using a wet or single step separation method eliminates the cost and time associated with dewatering. Neither hazardous chemicals nor heavy machinery are required. Cells may remain alive, and continuous extraction may be possible.
- **WEAKNESSES:** These are new techniques that are not commonly used in industry.
- **INDUSTRY ACTIVITY:** Companies that work with wet / single step separation methods include Catilin, OriginOil, Phycal, and Synthetic Genomics.

END-USE FUEL PRODUCTION

TRANSESTERIFICATION

- **STRENGTHS:** Transesterification is a well-understood process that has been used in industry for many years. Its feedstock does not interfere with the food industry, and the end-use product has many superior qualities relative to traditional diesel.
- **WEAKNESSES:** Byproducts (e.g., methanol, glycerol) are difficult to remove.
- **INDUSTRY ACTIVITY:** Companies that use transesterification in their operations include Aurora Biofuels, BARD LLC, Catilin, ENN Group, Green Star Products, Kuhmo Petrochemical, LiveFuels, LS9, and Solazyme (with Chevron).

HYDROPROCESSING

- **STRENGTHS:** Hydroprocessed fuels are indistinguishable from petroleum-based counterparts, and they meet existing fuel standards. Fuels have higher energy content than alcohols and biodiesel, and they are free of sulfur and nitrogen compounds. Finally, no infrastructure or engine adjustments are needed.
- **WEAKNESSES:** Hydroprocessing is a harsher process than transesterification.
- **INDUSTRY ACTIVITY:** Companies that use hydroprocessing in their operations include Aquaflow Bionomics (with UOP), Diversified Energy Corp, Emerging Fuels Technology, General Atomics, LS9, Neste Oil, SAIC, Sapphire Energy, Solazyme (with Chevron), and Solray Energy.

FERMENTATION

- **STRENGTHS:** With fermentation, the cost and time associated with dewatering, oil extraction, and oil processing may be avoided. If photosynthesis is unnecessary, then the cost of artificial light may be avoided and the depth/diameter of the tank is not an issue. Also, fermentation is widely used in other industries and is a well-understood process.
- **WEAKNESSES:** Fermentation may require large volumes (and cost) of sugar as an input, which may contribute to the "food vs. fuel" issues if sugar is not derived from non-food sources.
- **INDUSTRY ACTIVITY:** Algenol Biofuels uses fermentation in its operations.

ANAEROBIC DIGESTION

- **STRENGTHS:** In anaerobic digestion, dewatering of algae cultures and extraction of oils is unnecessary. It is less selective of algal strains and lipid contents compared to other methods. It is ideal for macroalgae processing and wastewater treatment plants. No emissions are released into the atmosphere during this process, and its byproducts are valuable.
- **WEAKNESSES:** Major capital and operating costs are associated with anaerobic digestion so may need to be integrated into a system that can utilize byproducts.
- **INDUSTRY ACTIVITY:** AACT and the Biomara Project are investigating the use of anaerobic digestion in their operations.

END-USE FUEL PRODUCTION (CONT.)

GASIFICATION

- **STRENGTHS:** Highly versatile end-products can be achieved with the resultant syngas created during gasification. A wide range of inputs can be used during this process, which is more efficient than combustion.
- **WEAKNESSES:** This process operates at extremely high temperatures, and a large-scale production is likely necessary to be cost-effective. Tailoring of the reactor operations and system inputs is needed to optimize syngas qualities. Also, tar and other byproduct buildup creates extra steps.
- **INDUSTRY ACTIVITY:** Genifuel Corporation and the Solena Group use gasification in their operations.

PYROLYSIS / LIQUEFACTION

- **STRENGTHS:** These two processes occurs relatively quickly. Liquefaction allows for biomass with high moisture content, so most of the cost of dewatering is avoided.
- **WEAKNESSES:** The resulting bio-oil is an intermediate product and must be converted into a final product in another process. Also, only dry biomass can be used in pyrolysis.
- **INDUSTRY ACTIVITY:** Envergent Technologies uses pyrolysis/ liquefaction in its operations.

BIOPHOTOLYSIS

- **STRENGTHS:** Biophotolysis is a clean and renewable method for producing hydrogen, which has very low emissions.
- **WEAKNESSES:** Biophotolysis is not yet used at commercial scale (only laboratory settings). Also, the storage of the end-use fuel presents challenges related to pressure, temperature, etc.
- **INDUSTRY ACTIVITY:** Karlsruhe Institute of Technology, the Solar Biofuels Consortium, and Solarvest BioEnergy are all investigating the use of biophotolysis.

PURIFICATION OF SVO

- **STRENGTHS:** Only simple purification of SVO is needed before it can be used in a modified diesel engine, so processing costs are very low.
- **WEAKNESSES:** SVO has high relative viscosity, so diesel engines must be modified to accommodate long-term operation.Viscosity drawbacks become worse in cold climates.
- **INDUSTRY ACTIVITY:** SunEco Energy purifies SVO as part of its operations.

Feasibility Assessment

Biofuels have the potential to increase transport fuel security by reducing the need for fossil fuels, and simultaneously reduce GHG emissions. In the long term, they may be produced without using fossil energy carriers and without net GHG (including CO_2) emissions over the well-to-wheel fuel chain. However, first generation biofuels have raised concerns regarding their sustainability on issues such as GHG balance, competition with food supply, biodiversity, the environment, and costs. Using algae as feedstock for biofuels production may mitigate or even eliminate these sustainability concerns. In an effort to estimate to which extent these issues may be addressed, potential of algae as feedstock for biofuels that are used in transportation is assessed in this report by investigating:

- Production capacity
- Total energy balance and GHG emissions
- Competition with food supply
- Environmental impacts
- Biodiversity and ecosystems
- Production cost
- Future state of the energy industry
- Adaptability among markets

Overall, it is concluded that algae do have strong potential as feedstock for biofuels. As previously mentioned the biomass productivity per hectare can be more than ten times higher than for terrestrial energy crops. Furthermore, when algae are cultivated on non-arable land, there is no competition with current food production. These benefits have led to much interest from industry, entrepreneurs, and governments, and increasing number of joint R&D projects are now underway. Besides use as feedstock in downstream fuels processing, under specific conditions some algae are able to naturally produce fuels such as hydrogen. This practice seems to be much further from commercialization and currently receives far less attention than using algae as feedstock for liquid biofuels.

Overall, it is concluded that algae do have strong potential as a feedstock for biofuels.

Algal biofuels are currently still in their infancy. Expectations are based on small-scale production for high-value products and on results of laboratory experiments. How these experiences translate to large-scale production is still largely unknown. So far upstream algae cultivation and harvesting receive the most attention from researchers, but experience with the conversion of algae into biofuels is still limited and needs further development. Uncertainties may be associated with getting a sufficient supply of CO_2 and fertilizer to the algae culture, the net energy balance of the total well-to-wheel chain, and the ecological impacts of large algae monocultures. Different options to deal with these issues have been proposed and more are under investigation. Given the current level of knowledge, preferred technologies cannot yet be selected.

To understand how large-scale algal biofuel systems operate, several pilot projects are underway and are expected to increase in number. Pilot projects should be adapted to local circumstances such as climatic conditions and native algae species, the availability of water, and potential markets. Real-world experience will help remove the uncertainties, and will also help clarify which practices are feasible and which are not.

Recommendations

The wealth of benefits that may be achievable with algae justify attention to algal biofuels from researchers, industries and (governmental) policy makers. The research that forms the basis of this report leads to the conclusion that the following issues are important for consideration in policymaking on algal biofuels:

1. Algal biofuels are in an early stage of development. Current expectations for the future are based on estimates and extrapolation of small-scale production and results of laboratory work. It seems appropriate to start pilot projects to obtain experience in scaling up the production process and to gain knowledge about the feasibility of different fuel production routes.

2. It is too early to select preferred algal fuel pathways and technologies. In practice there will not be one preferred production method. Different circumstances, such as climatic conditions and the availability of fresh or salt water, will have different optimum solutions.

3. Specialized scientists should be involved in the determination of ecological impacts of large-scale algae cultures.

4. Sustainability criteria should be developed for algal biofuels. Besides the energy, environmental, and ecological issues that are addressed in this report, criteria should be defined on issues not addressed in this report such as economic prosperity and social well-being.

5. It has been shown that under specific conditions, the algal biofuel production and distribution chain may have a net energy output, but further energy analysis of many different algae fuel chains is needed.

6. Algal biofuel policies and projects should aim to reduce fossil energy consumption and the environmental burden compared to conventional fuels. In parallel, these efforts should result in minimal impacts on ecosystems, which can originate from potential GHG emissions, fresh water consumption, effects of large monocultures and invasive species, etc. Therefore, sustainability analyses prior to construction and operations that examine all relevant stages of the fuel chain may be valuable.

7. Based on the high level of innovation demonstrated within the algal biofuels industry in just the past decade, it is likely that new, refined, or even breakthrough technologies will continue to be introduced in the future. It is important that industry stakeholders and policymakers remain open to new algal species, processes, and fuels besides the ones that are being considered today.

Chapter 1. Allure of Algae as Feedstock for Biofuels

Due to global energy market activities in recent decades, the pursuit of a stable, economically-sound, and environmentally-friendly source of transportation fuel has led to extensive research and development (R&D) efforts focused on the conversion of various feedstocks into biofuels. Some feedstocks, such as sugar cane, corn and woody biomass, are targeted because their structures can be broken down into sugars and fermented into alcohols. Other feedstocks, such as vegetable oils, are appealing because they contain considerable amounts of lipids, which can be extracted and converted into biodiesel or other fuels. While significant R&D and commercial strides have been made with each of these feedstocks, technical and market barriers (e.g., cost, scalability, infrastructure requirements, and “food vs. fuel” debates) currently limit the penetration of the resultant biofuels into the mainstream.

Because of algae’s ability to potentially address these barriers, its use as a feedstock for biofuels has led to much excitement and initiative within the energy industry. Algae are highly diverse, single- or multi-cellular organisms comprised of mostly lipids, protein and carbohydrates, which may be used to produce a wide variety of biofuels. Algae generally have higher lipid content than terrestrial plants sometimes exceeding 50% of the cell’s dry biomass (U.S. DOE, May ’10; Tornabene et al., 1983). Algae also benefit from growth rates that are 20-30 times higher than terrestrial crops (McDill, 2009) and, in some cases, capable of doubling in size with 10 hours. When these two qualities are combined, the result is maximum oil yields of 20,000 – 115,000 liters per hectare per year (L/ha/yr) (2,140 – 13,360 gallons per acre per year) (Baldos, 2009; Wijffels, 2008), which is considerably higher than any other competing feedstock (Table 1).

Table 1: Oil yields from common feedstocks.

Feedstock	Oil Yield (L/ha/yr)	References
Corn	172	Chisti, 2007
Soybean	446	Chisti, 2007
Canola/rapeseed	1,190-1,500	Chisti, 2007; Baldos, 2009; Ballerini, 2006
Coconut	2,689	Chisti, 2007
Palm Oil	5,940-6,000	Chisti, 2007; Baldos, 2009; Wijffels, 2008
Microalgae	20,000-115,000	Baldos, 2009; Wijffels, 2008

Beside rapid growth rates and high lipid contents, other competitive advantages that algae offer over other feedstocks include:

- The diverse range of algae species can thrive in a wide range of environments throughout the world, presenting an overall high overall tolerance for climate, sunlight, nutrient levels, etc. However, one species by itself will only tolerate a limited range of environments.

- Many algae species can be harvested daily as opposed the seasonal harvest periods associated with terrestrial crops.

- Carbon dioxide (CO_2) is a key ingredient for algae survival (along with water, nutrients, and sunlight), and CO_2 originating from industry operations could potentially be redirected to the algal cultivation facilities to be used in an algal biofuel cycle before it is released into the atmosphere.

- Algae cultivation can take place on land that is unsuitable for agriculture, so it does not directly compete with farmland.

- Algae can thrive in seawater, wastewater, or other non-potable sources, so it does not directly compete with fresh water resources. In fact, wastewater can provide algae with some essential nutrients, such as nitrogen, and as such algae may contribute to cleaning up wastewater streams.

- Algal biofuels are non-toxic and biodegradable.

- Co-products of algae biofuels may present high value in other markets, including nutriceuticals and cosmetics.

The potential of algae as a feedstock for biofuels was first extensively researched under the Aquatic Species Program (ASP) that operated at the National Renewable Energy Laboratory (NREL) in the United States from 1978 through 1996. The program, funded by the U.S. Department of Energy's (DOE) Office of Fuels Development, focused primarily on cultivating algae in open ponds and using algal lipids to produce biodiesel while mitigating CO_2 emissions from coal-fired power plants. Besides optimizing oil yields, researchers also investigated ways to improve production rates, hardiness, and resistance to contamination. Often, researchers realized that one strain cannot boast all of the desired characteristics but they are instead usually mutually exclusive (e.g., increased oil yields usually imply decreased growth rates).

Significant accomplishments in algal characterization and manipulation were made under the ASP. Over the course of the nearly two decades that the program existed, an extensive collection of approximately 3,000 algal strains was screened, isolated, and characterized by researchers. A portion of this original collection is currently housed at the University of Hawaii and is accessible to researchers. The program ended in 1996 as gasoline prices fell to approximately 0.26 USD/L (1.00 USD/gal) and algal biofuels were considered too costly to ever compete with inexpensive petroleum-based fuels.

Although algae species collectively present many strong advantages (although one specific species is unlikely to possess all of the advantages listed previously), a sustainable algal biofuel industry is at least one or two decades away from maturity, and no commercial scale operations currently exist. Several barriers must first be overcome before algal biofuels can compete with traditional petroleum-based fuels. Production chains with net energy output need to be identified, and continued R&D is needed to reduce the cost in all segments of the production spectrum (e.g., harvesting, dewatering, extracting of oil). Further research to identify strains with high production rates and/or oil yields may also improve competitiveness within the market. Initiatives to seamlessly integrate algal biofuels into the existing transportation infrastructure may increase their convenience level.

Chapter 2. Algal Fuel Industry Overview

2.1. Existing Industry

As interest in algal biofuels reemerges globally, many industry players are actively pursuing R&D ventures, operating pilot scale facilities, and seeking investment support. This section summarizes the current state of the market as it relates to industry participation, production volumes, and fuel prices.

2.1.1. Current Market Size

By mid-2010, an estimated 200 companies were directly participating in algal biofuels production, rising from virtually no companies at the start of the decade. Based on Oilgae's industry concentration estimate in Table 2, a 75% average annual growth is seen between 2001 and mid-2010. Perhaps the most significant ramp-up of company participation is seen between the beginning of 2008 (25 companies) and the close of 2009 (150 companies), accounting for an increase of six-fold during this time period. This boost in industry participation is partially driven by surging oil prices during this time. Lux Research reported a similar trend, noting that the number of companies in this industry would double between 2009 and 2010. Lux also stated that private investment in algae biofuel ventures has consistently doubled at a minimum each year since 2006 (Mollman, 2009).

Table 2: Estimated growth of algal biofuel companies between 2001 and 2010 (Oilgae Estimates)

Year	2001	2002	2003	2004	2005	2006	2007	2008	Mid 2009	End 2009	Mid 2010
Number of Companies	1	2	4	5	10	15	25	50	100	150	200

In a span of approximately ten years, the algal biofuel industry grew from minuscule in value in 2000 to on track to reach an estimated market value of 271 million USD in 2010 (Algae, 2010).

2.1.2 Current Production Volumes

Algal biofuel is not currently being produced at commercial scale, and no commercial scale plants are operational at this time due to early-technology high production costs. Instead, numerous companies have set up demonstration and pilot-scale plants that produce a variety of fuels in relatively small quantities for use by limited customers. For example, Solazyme has produced and delivered 1,500 gal (approximately 5,700 L) of 100% algae-based jet fuel to the U.S. Navy‘s testing and certification program to date (Solazyme, 2010).

Total annual biofuel consumption (e.g., biodiesel, ethanol) in 2008 only accounted for approximately 10 billon gal (40 billion L) in the United States, or less than 5% of total U.S. transportation fuel consumption (U.S. EIA, Apr ‘10), and approximately 21 billion gal (80 billion L) across the world (World, 2009). Despite recent industry plans for production ramp up, algal biofuels account for a negligible percentage of these volumes. However, the vastness of the transportation fuels industry presents a substantial potential market for non-traditional fuels to further displace traditional fuels.

2.1.3. Current Algal Biofuel Prices

With the algal biofuel industry still in its infancy, it is challenging to pin down an estimated price of algal-derived fuels. The variety of methods for upstream operations (e.g., cultivating and harvesting algae, extracting lipids) adds to the range of uncertainty. Many organizations have provided cost estimates for today’s algal biofuel production. These include:

- In 2009, Rodney Andrews, Director of the University of Kentucky’s Center for Applied Energy Research, suggested that algal biofuels range in cost from 18 to 30 USD/gal (approximately 4.75 to 7.93 USD/L) (Bruggers, 2009).

- In March 2010, a team of University of Nebraska-Lincoln researchers estimated that algal biodiesel currently costs between 10 and 30 USD/gal (approximately 2.64 to 7.93 USD/L) to produce (Moser, 2010).

- In 2009, Solix Biofuels was capable of producing biofuel for just under 33 USD/gal (approximately 8.72 USD/L) (Kanellos, Feb ’09).

- In their *Algae 2020* market outlook, Emerging Markets estimates the production of algal-derived biodiesel to cost 9 to 25 USD/gal (~ 2.38 to 6.60 USD/L) in ponds and 15 to 40 USD/gal (~ 3.96 to 10.57 USD/L) in photobioreactors (PBR) (Piccolo, 2009).

- The U.S. DOE agrees that algal biofuels would cost over 8 USD/gal (~ 2.11 USD/L) if produced at large volumes with current technology (U.S. DOE, Oct ’08).

- In a recent market study, the Prometheus Institute stated that production costs of third-generation algae biofuels[1] are 8 to 20 USD/gal (~2.11 to 5.28 USD/L) (Kagan and Bradford, 2009).

- European researchers appear to be more hesitant to give price estimates for algal fuels, because of all the uncertainties that still exist. However, with current technology, production costs of about 4 Euros per kg dry microalgae biomass have been mentioned (Ripplinger, 2009; Wijffels, 2008).

Assuming these estimates are accurate, companies have much cost improvements to achieve before algal biofuels are considered a feasible substitute for traditional transportation fuels (Emerging, 2009).

2.2. Projected Industry

Based on industry activities to date, projections for the next 10 to 15 years have been made for the algal biofuels market. This section summarizes the anticipated state of the market during this timeframe as it relates to industry participation, production volumes, and fuel prices.

2.2.1. Projected Market Size

The algal biofuels industry stakeholders will likely continue to demonstrate, commercialize, and implement new methods in the markets for 1) cultivation technologies, 2) harvesting and extraction technologies, and 3) algae biofuels production technologies. SBI Energy anticipates cultivation technology sales to comprise the majority of the market through 2015. When combined with the other market segments, SBI Energy estimates a total algal biofuels market worth of 1.6 billion USD in 2015 (Algae, 2010). This indicates a 43% annual growth rate between 2010 and 2015, based on their present day market value estimate of 271 million USD. During this time, the market will likely experience some consolidation as various current technologies exit the market due to lack of cost-competitiveness and production efficiency.

To put this value in perspective, the total market value for traditional biofuels is projected to be 123 billion USD in 2014, which accounts for less than 5% of total fossil fuel production (Oilgae, n.d.). While market growth will also be constrained by production capacity and cost relative to other fuels, the growth potential is expected to be quite large into the foreseeable future (SBI, 2010).

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[1] The Prometheus Institute defines third-generation algae biofuels as “biofuels that are either created using petroleum-like hydroprocessing, advanced bio-chemistry, or revolutionary processes…”

2.2.2. Projected Production Volumes

Announcements by major U.S. algal biofuel producers of new plant construction or plant expansion have contributed to aggressive production projections over the next couple of decades. Such announcements have been made by:

- Algenol Biofuels: Algenol is building a pilot plant at Dow Chemical's plant in Freeport, TX, designed to produce 100,000 gal (approximately 378,500 L) of ethanol per year. In addition, Algenol is moving into a facility capable of producing 300,000 gal (1.135 million L) of ethanol per year (D. Glass, 2010).

- Aurora Algae: Aurora is currently building a 50-acre (20.25 ha) pond and has plans for a 2,000-acre (810 ha) pond by 2011 or 2012. With production rate claims of 5,000 gal per acre per year (46,770 L/ha/yr), a 50-acre pond translates to a capacity of 250,000 gal/yr (946,000 L/yr) of biodiesel while a 2,000-acre pond translates to a capacity of 10 million gal/yr (37.8 million L/yr) (Aurora, 2009).

- PetroAlgae: Following a successful pilot test facility, PetroAlgae has plans to, in the long term, design a commercial production facility with a capacity of 200,000 metric tons per year (approximately 60 million gal/yr) of biodiesel (Lombardi, 2009).[2]

- Sapphire Energy: Sapphire is building a biorefinery to produce algal-based renewable gasoline, diesel, and jet fuel at 1 million gal/yr (3.8 million L/yr) (Fehrenbacher, 2010).

- Solazyme: Recent American Recovery and Reinvestment Act (ARRA) awards will enable Solazyme to ramp up production of "drop in" algal oil to over 500,000 gal/yr (1.9 million L/yr). Long term, Solazyme plans to build a large-scale production facility and refinery capable of producing millions of gallons of algal-based oil annually (Graff, 2010).

In Figure 1, commercial scale up of algal biofuel production between now and 2025 has been projected by Emerging Markets Online based primarily on the estimated scale up of the five major algal biofuels producers listed previously. As a result, between 100 million and 1 billion gal (380 million and 3.8 billion L) of algal biofuels are expected to be commercially produced and made available around 2015. A recent report published by Pike Research, on the contrary, projects only 61 million gal (231 million L) of algal-based biofuels to be produced by 2020 (Pike, 2010).

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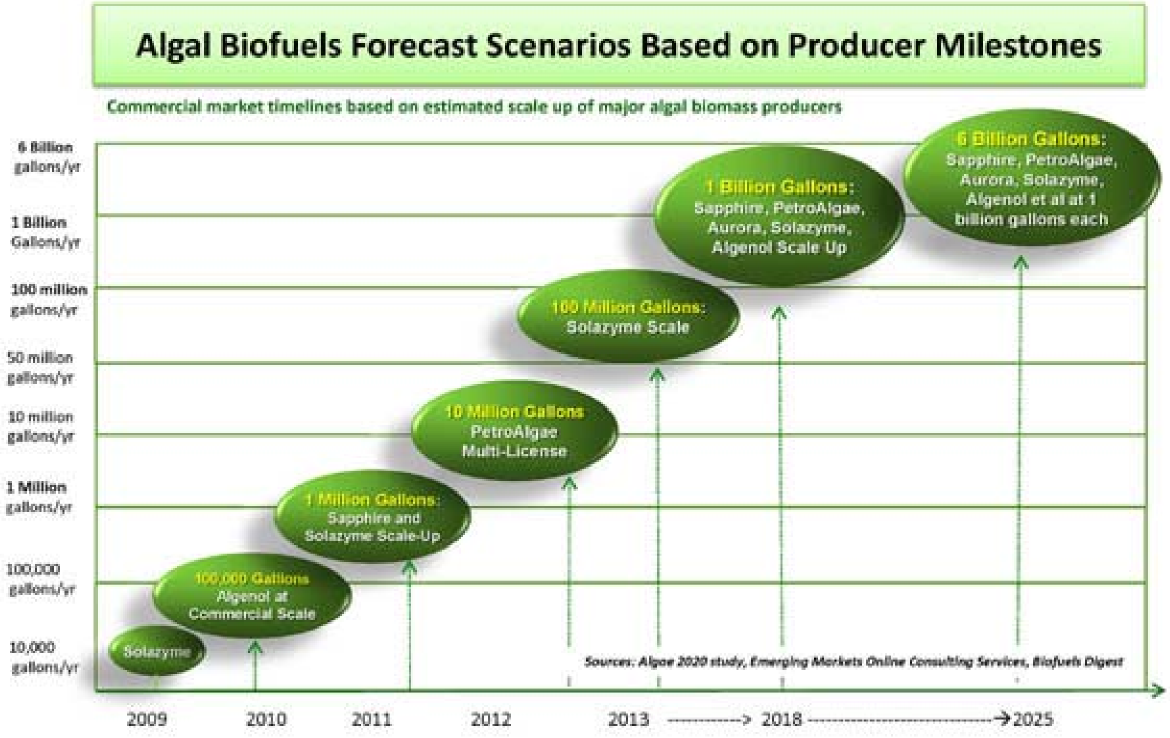
[2] Assumes density of biodiesel (B100) to be 0.88 kg/L.



Sources: Algae 2020 study, Emerging Markets Online Consulting Services, Biofuels Digest

Figure 1: Forecasted algal biofuel production through 2025 based on the anticipated commercial scale-up by five major algal biomass producers (Emerging, 2009).

2.2.3. Projected Algal Biofuel Prices

To be considered cost competitive with traditional fuels, algal biofuel producers must drive down cost to approximately 85 USD/bbl, which is competitive with current oil prices and is expected to remain competitive (or increase in competitiveness) as oil supplies become more scarce. Many organizations have provided cost projections or goals for future algal biofuel production. These include:

- In February 2010, special assistant for energy at the U.S. Defense Advanced Research Projects Agency (DARPA) stated that “oil from algae is projected at 2 USD/gal (0.53 USD/L), headed towards 1 USD/gal (0.26 USD/L)” (Lane, Feb ’10).

- Science Applications International Corporation (SAIC) aims to develop an algae-derived fuel for military jets at 3 USD/gal (0.80 USD/L) (Garthwaite, 2009).

- In June 2010, Solazyme’s Chief Executive Officer (CEO) Jonathan Wolfson predicted their algal oil to cost 60-80 USD/bbl within 12 to 24 months (Foroohar, 7 June ‘10).

- In July 2010, president of Sapphire Energy and former British Petroleum (BP) executive C.J. Warner projected commercial algae-based oil to eventually be available at 80 USD/bbl (Siegel, 2010).

Chapter 3. Government Support

Worldwide government support for the algal biofuel industry is continually increasing with hopes of stimulating new industry ventures that will ultimately help drive down capital and operating costs, and accelerate the production of algal fuel. Governmental support for R&D related to algae as feedstock for biofuels is often embedded in more general programs for renewable energy or biofuels. Sometimes government support is provided to individual companies while other times it funds activities run by large consortiums or government laboratories. Furthermore, the direction of funds to date has ranged from upstream R&D initiatives to downstream pilot production facilities. This chapter presents a selection of governmental support measures in support of algal biofuels, broken down by continent and country. It would be outside the scope of this report to present a complete listing of all governmental support efforts in every country.

3.1. North America

3.1.1. United States

Funding from the U.S. Federal Government in recent years has demonstrated significant support for algae-to-biofuel R&D and business growth for commercial scale algal biofuel production. In June 2010, for example, three research groups comprised of universities, national laboratories, and private industry were awarded up to 24 million USD by DOE to boost R&D efforts related to growing, harvesting, and processing algae for biofuels, which, in turn, will help accelerate commercialization (U.S. DOE, June ’10). Each project has an anticipated duration of three years. This funding is divides as follows:

- The Sustainable Algal Biofuels Consortium based in Mesa, Arizona, is awarded up to 6 million USD to investigate the biochemical conversion of algae to end-use products and to analyze physical properties of algal fuels and fuel intermediates. The team is led by Arizona State University.

- The Consortium for Algal Biofuels Commercialization based in San Diego, CA, is awarded up to 9 million USD to focus on developing algae as a robust biofuels feedstock through improved crop protection, nutrient optimization, and application of genetic tools.

- Cellana, LLC Consortium based in Kailua-Kona, Hawaii, is awarded up to 9 million USD to investigate large-scale algae production and harvesting in seawater test beds.

Other algae-related investments in private industry made by the U.S. government include:

- 44 million USD in American Recovery and Reinvestment Act (ARRA) funds awarded by DOE to the Donald Danforth Plant Science Center, which leads the National Alliance for Advanced Biofuels and Byproducts, to systematically achieve sustainable commercialization of algal biofuel (U.S. DOE, Jan ‘10).

- 1.5 million USD awarded by DOE to Aquaflow Bionomic Corporation (based in New Zealand) to partner with UOP Honeywell to demonstrate carbon capture from Honeywell’s manufacturing facility in Hopewell, Virginia (Williams, 2010).

- Sapphire Energy received 50 million USD from DOE to grow algae in open ponds and convert it into green fuels (Sapphire, 2009).

- Solazyme received over 21 million USD from DOE to validate the economics behind a multi-biofuel commercial scale biorefinery (U.S. DOE, Mar ’10). Prior to this funding, Solazyme received 2 million USD from the Commerce Department’s National Institute of Standards and Technology (NIST) to produce high quality biocrude oil (Solazyme, n.d.). Additionally, the U.S. Navy has contracted with Solazyme (worth 8.5 million USD) to develop algal jet fuel and an algal F-76 naval distillate (Donald, 2010).

- SAIC was awarded up to 24 million USD by DARPA, the R&D office for the U.S. Department of Defense, to create algal-based military jet fuel JP-8 at 3 USD/gal (Lane, Jan ’09).

- The Washington State Algae Alliance – comprised of Targeted Growth, Inc., Inventure Chemical, and Washington State University – received 2 million USD in the 2010 Senate Energy and Water Development appropriations bill to help develop new production systems for sustainable and renewable fuels and related products ($2 million, 2009).

3.1.2. Canada

To date, the Canadian government has actively supported domestic algal biofuel industry activities through various project funding. Examples of such funding follow.

- In June 2010, the Canadian government announced awards of approximately 5 million Canadian dollars (CAD) to the National Research Council (NRC) Institute for Marine Biosciences for a project to produce algal fuels on a large scale using strains native to Nova Scotia (Canadian, 2010).

- The Canadian Federal government invested over 377,000 CAD in November 2010 in R&D conducted by Solarvest (PEI), Inc., a subsidiary of Solarvest Bioenergy Inc., to generate hydrogen through algae production. Solarvest is investing 235,000 CAD in this venture (Governments, 2010).

3.2. Europe

3.2.1. European Union

BioMara (sustainable fuels from marine biomass) is a UK and Irish joint project that aims to demonstrate the feasibility and viability of producing third generation biofuels from marine biomass. It investigates the potential of both microalgae and macroalgae as alternatives to terrestrial biofuel crops. Universities and research institutes from the United Kingdom and Ireland collaborate in the project, which is coordinated by the Scottish Association for Marine Science. The total budget for the BioMara project is approximately 12 million Euros (EUR) of public funding (Euro. Union, 2010). In 2008, the European Union (EU) granted 75% of these funds from the Interreg IVA programme for Northern Ireland, the border region of Ireland and Western Scotland. This EU programme supports strategic cross-border cooperation aiming for a more prosperous and sustainable region (Special, 2010). The remaining 25% of the BioMara budget is jointly funded by the Scottish government's economic and community development agency ‘Highlands and Islands Enterprise’, the UK Crown Estate, the Scottish government, the Department of Enterprise, Trade and Investment of Northern Ireland, and the Irish Department of Communications, Energy and Natural Resources.

Under the Seventh Framework Programme for Research and Development, the European Commission has granted €747,152 EUR to the AquaFUELS (algae and aquatic biomass for a sustainable production of 2nd generation biofuels) project. In this project a consortium of twelve organizations – mostly European universities, some industry and one university from Israel – aims to establish the state of the art on research, technological development and demonstration activities regarding algal and other non-food aquatic biomass for biofuel production. AquaFUELS surveys and assessments on environmental, economic and social sustainability will be based on full lifecycle analyses of the fuels. By involving major stakeholders, the consortium aims to paint a realistic perspective for the future of ‘aquafuels’. The project started in January 2010 and is scheduled to run for 18 months (AquaFUELS, 2010).

There are also indirect forms of EU support. The secretariat of the European Biofuels Technology Platform is partly financed by a grant from the European Commission through the Seventh Framework Programme, for example (Euro. Biofuels, 2010). This platform focuses on all biofuels, including fuels from algae. It created an Algae Task Force (ATF) in October 2009.

3.2.2. France

The Shamash integrated research project in France started in December 2006. It aims to produce biofuels from autotrophic microalgae. Seven public research teams and a private company collaborate in the Shamash project. The total budget is €2.8 million EUR, including €0.8 million EUR support from the French National Program on Bioenergies Research (Shamash, n.d.).

The 2011 programme of the French National Research Agency (ANR Agence Nationale de la Recherche) mentions microalgae in some of the areas of the Bio-ME (Bio-Materials and Energies) research programme. It concerns improving the yield per hectare of biofuels, direct hydrogen production by algae (biophotolysis), and optimization of triglycerides production from microalgae. The document does not mention the budgets that are available for this research work (Agence, 2010).

3.2.3. Germany

Interdisciplinary collaboration of experts from science, industry, energy companies and politics is necessary to realize the potential of microalgae as an energy source. Therefore, the German Federal Ministry of Education and Research (BMBF) initiated in 2008 an ‘algae table for regulars’ to enhance information exchange between industry and research (BMBF, 2008).

The Karlsruhe Institute of Technology is coordinating the HydroMicPro (Hydrogen from Microalgae: With cell and reactor design to economic production) project that is conducted by a group of universities, research institutions and enterprises. The aim of this project is to develop highly efficient processes for the extraction of hydrogen from microalgae. BMBF is funding this project with a total of €2.1 million EUR from the ‘Fundamental Energy Research 2020+’ programme (Karlsruhe, 2009).

3.2.4. The Netherlands

In the period 1998-2003, the Dutch governmental programme EET (Economy, Ecology, Technology) funded research projects on sustainable co-production of chemicals and energy from microalgae (Reith, 2004).

In 2009, the Dutch government made €25 million EUR available for research on energy from plants and algae in a programme called ‘Towards Biosolar Cells’ (Netherlands, 2009). The programme focuses on three areas:

1. Increasing the photosynthetic efficiency of plants, aiming to increase the energy production per hectare.

2. Direct production of biofuels without harvesting the plants. Photosynthetic cyanobacteria or algae that produce butanol are mentioned as possible outcomes in this area.

3. Combining natural and technical processes into solar panels that produce fuel instead of electricity.

3.2.5. United Kingdom

To accelerate the move to a low carbon economy, the UK government has established the Carbon Trust. The Carbon Trust is a not-for-profit company providing support for business and the public sector. Developing technology for mass production of algae oil for biofuel use is currently one of the focus areas of the Carbon Trust. Therefore, it launched the Algae Biofuels Challenge in October 2008, aiming to commercialize the use of algae biofuel by 2020. In March 2010, the Carbon Trust had selected the universities and institutions that will work on five key challenges:

1. Isolating and screening algae strains.
2. Maximizing solar conversion efficiency.
3. Achieving both high oil content and high productivity.
4. Sustained algae cultivation in open ponds.
5. Design and engineering of cost effective production systems.

Via the Carbon Trust, the Department for Transport and the Department for Energy and Climate Change are investing a total amount of 8 million British pounds (GBP) over three years into this project (Carbon, Nov 2010; Carbon, Mar 2010).

3.3. Australia and New Zealand

The Australian government is considered a leader in providing financial support to domestic algal biofuel organizations and businesses. Specific examples of such funding opportunities include:

- In late 2009, Murdoch University received 1.89 million Australian dollars (AUD) (approximately 1.86 million USD) from the Australian government to lead an algae project under the Asia-Pacific Partnership on Clean Development and Climate to grow algal biomass in open saline ponds. The University of Adelaide is a research partner in this project (Murdoch, 2008).

- A 2.724 million AUD (2.26 million USD) development grant was awarded to the Algal Fuels Consortium (AFC) in 2009 by the Australian government to design and build a pilot-scale biorefinery for producing algal-based biofuels and valuable byproducts. This grant was available through the Department of

Resources Energy and Tourism's Second Generation Biofuels program in Australia (Sancon, 2009).

- In 2008, Queensland Premier Anna Bligh announced that the government would provide 166,000 AUD (160,000 USD) in funding towards a project to convert marine algae to biodiesel. James Cook University (JCU) and MBD Biodiesel Ltd. (part of MBD Energy), both located in Australia, are co-leading this project (Millikin, 2008). In a partnership with the government-funded Advanced Manufacturing Co-operative Research Center, MBD Energy has since been awarded 5 million AUD (~ 4.9 million USD), which it will match, to develop a system that utilizes wastewater and CO_2 from fossil-based power plants. Trials will be conducted at JCU (Bellona, 2010).

- The Western Australian Government has allocated 2 million AUD for Aurora Algae Pty Ltd to lead a project that will redirect CO_2 from a major industrial plant in the Karratha region for use in the cultivation of algae. Once harvested, the algae would be used to make various biofuels (Government, 2010).

- In 2010, the Queensland government and the University of Queensland created a global research consortium focused on green fuels, particularly aviation fuels. Projects using algae as a feedstock are receiving a total of 3.48 million AUD (~ 3.42 million USD) in state government funding (ABC Carbon, n.d.).

New Zealand's Foundation for Research Science and Technology funded the world's largest demonstration project for converting wastewater algae into biocrude oil, which opened in November 2009 in New Zealand. This project was conducted by the National Institute of Water and Atmospheric Research (NIWA) using Solray Energy's biocrude oil conversion technology (Garcia, 2009).

3.4. Asia

3.4.1. Thailand

Thailand's Alternative Energy Development and Efficiency Department has announced financial support for algal biofuel R&D with hopes of algae displacing significant volumes of crude palm oil. Marine algae R&D activities will take place at Burapha University while freshwater activities will take place at Kasetsart University. Information sharing is expected to occur frequently between these two universities, JCU and the Queensland University of Technology in Australia (Real, 2010).

3.4.2. Philippines

The Philippines national government has allocated 23 crore (5 million USD) to help construct a 250-acre (101.2 ha) ethanol plant and aqua farm cluster using macroalgae as a feedstock. Ethanol extraction technology developed at the Korean Institute for Industrial Technology will be used at this plant (Lane, May ’10).

3.4.3. South Korea

The South Korean National Energy Ministry is investing 275 million USD over a ten year period to accelerate production of ethanol from macroalgae, or seaweed. An off-shore seaweed forest of approximately 86,000 acres (~34,800 ha) will be constructed with a portion of these funds. The Ministry has set an annual production goal of 400 million gallons by 2020 (Thurmond, 2010).

3.4.4. Indonesia

Indonesia’s Ministry of Fishery and Marine Resources will collaborate with the Korea Institute of Industrial Technology to develop biofuels from macroalgae. Indonesia harvests extensive amounts of seaweed but lacks the needed technology to convert this feedstock into fuel. Korea, in contrast, has sufficient technology but low volumes of macroalgae, presenting a logical pairing (Ritch, Nov ’08).

3.5. South America

3.5.1. Brazil

The Brazilian government has shown interest in funding both microalgae and seaweed-based research projects that could promote biofuel production. Specifically, Brazil’s Ministries of Environment, and Science and Technology signed a decree in 2008 to finance 4.5 million Brazilian reals (~2.8 million USD) in non-refundable credits to support microalgae and other aquaculture projects that will lead to biodiesel production (TheBioenergySite, 2008).

3.5.2. Chile

In 2010, the Chilean Economic Development Agency (CORFO) invested 7 million USD in an ethanol project using macroalgae as the feedstock. The project is led by U.S.-based

Bio-Architecture Lab (BAL), and Chilean oil company ENAP and the Universidad de Los Lagos are collaborating (Go, 2010).

3.6. Africa

Within Africa, the South African government appears to currently extend the strongest support for algal biofuel R&D activities. Since 2006, South Africa’s Council for Scientific and Industrial Research (CSIR), which operates partially on government funding, has supported the investigation of biodiesel production using indigenous South African algae strains. As of late 2007, CSIR had invested approximately 1.4 million South African rand (approximately 200,000 USD) in this project (Swanepoel, 2007).

Chapter 4. Algal Biofuels Production Spectrum

The algal biofuels production spectrum is currently comprised of a fairly complex set of steps that begins with upstream algal strain selection and concludes with the conversion of algal biomass into a finished energy product. Algae are highly versatile organisms, presenting many pathways for navigating from one end of the spectrum to the other. Most companies choose to perfect one or two steps in the spectrum and then form business relationships with other industry stakeholders to complete the supply chain. Other companies' business models instead attempt to conquer the entire spectrum.

This industry is still considered to be in its infancy, and algal biofuels are not currently being produced at the industry scale. Considerable amounts of R&D are underway, and pilot plants are up and running worldwide to test promising new methods for improving system efficiency and cost-competiveness with traditional fuel industries. As the industry matures and production ramps up, the portfolio of techniques is expected to naturally consolidate to address scalability, cost, and demand issues. In this chapter, the state of the technology will be investigated for the five key steps of the algal biofuels production spectrum: 1) Algal Strain Selection, 2) Algae Cultivation, 3) Harvesting and Dewatering, 4) Oil / Biomass Separation, and 5) End-Use Fuel Production.

4.1. Algal Strain Selection

Algal Strain Selection	Algae Cultivation	Harvesting and Dewatering	Oil / Biomass Separation	End-Use Fuel Production
• **Microalgae** • **Cyanobacteria** • **Macroalgae**	• Open Ponds / Raceways • Photobioreactors • Heterotrophic Fermenters • Marine Systems	• Filtration / Microscreening • Centrifugation • Flocculation • Flotation • Off-shore Methods • Other	• Physical • Chemical / Solvent • Enzymatic • Wet / Single Step	• Transesterification • Hydroprocessing • Fermentation • Anaerobic Digestion • Gasification • Pyrolysis/ Liquefaction • Biophotolysis • Purification of SVO

In order to optimize production and minimize cost, the search for "superalgae" is underway in the algal biofuels industry. Microalgal strains appear to have the strongest industry appeal due to their simple structure, rapid growth rate, and often high oil content. However, macroalgae (seaweeds) and cyanobacteria are also being studied as energy sources due to their rapid growth rates. In this section, these three groups of algae – microalgae, cyanobacteria, and macroalgae – are examined to understand how their unique characteristics are being applied within the biofuels industry.

4.1.1. Microalgae

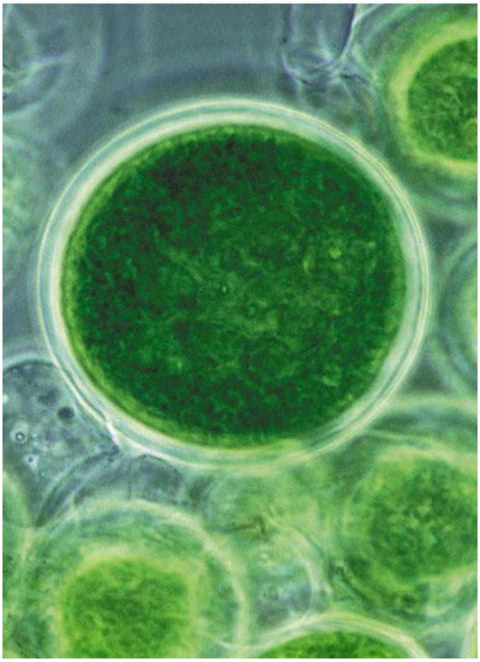


Figure 2: Microalgae *Haematococcus pluvialis*, vegetative (Palmella-) stage, under light microscope (Image Source: Fraunhofer IGB: http://www.igb.fraunhofer.de/start.en.html).

General Description. Microalgae refer to a diverse group of unicellular, eukaryotic[3] organisms that thrive in a wide range of freshwater and marine environments (Figure 2). Like most types of algae, microalgae use solar energy to convert CO_2 and water into carbohydrates, lipids, proteins, and oxygen. In fact, microalgae are responsible for producing about half of all atmospheric oxygen. To date, approximately 35,000 species of microalgae have been identified, although several hundreds of thousands are estimated to exist (Wageningen, 2010).

A large variety of microalgae strains have been considered for use in biofuel production primarily due to their ability to efficiently produce lipids, which can be converted into biodiesel and other oil-based fuels. Researchers are continuously investigating microalgae to learn which strains may offer competitive edges over alternate strains. Specifically, properties of interest include high lipid yield; fast growth/replication rate; resistance to disease and varying growth conditions (e.g., temperature, light, nutrients); salinity tolerance; CO_2 absorption rates; and others. Once a particular strain is selected, techniques (e.g., nutrient limitation) may be used to enhance the desired properties previously mentioned.

Several microalgal species that are often considered for use in biofuel production throughout the world due to their ability to produce high lipid and carbohydrate yields are listed in Table 3 (Edwards, 2010).

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[3] Eukaryotic organisms contain membrane-bound cells with complex structures including nuclei. Cells within eukaryotic organisms are much larger and more complex than prokaryotic cells, which have no nuclei.

Table 3: Oil content as a percent of cell's dry weight (dw) for select microalgal species.

Microalgal species	Lipids (% dw)	Carbohydrates (% dw)
Chlamydomonas rheinhar.	21	17
Chlorella ellipsoidea	84	16
Chorella pyrenoidosa	2	26
Chlorella vulgaris	14-22	12-17
Dunaliella salina	6	32
Porphyridium cruentum	9-14	40-57
Scenedesmus obliquus	12-14	10-17

While most companies focus on optimizing the characteristics of natural/wild strains, experimentation and manipulation of algal genes is becoming more common within the algal biofuels industry. Specifically, researchers are strategically tailoring natural lipid pathways in microalgae to optimize the cells' fatty acid mixtures, since these will ultimately be converted to biodiesel or other end-use fuels. Researchers are also investigating ways for cells to more efficiently absorb light (decreasing competition between neighboring cells), enhance algal cell growth rates, improve efficiency of photosynthesis by manipulating cell components to synthesize high amounts of photoreceptor molecules, and teach cells to thrive on alternative food sources (e.g., fermentation of sugars).

Strengths / Weaknesses. In their natural environment, wild strains of microalgae typically grow at a fast rate, but lipid yield is limited since the cell only produces the amount needed for its normal functions. Genetically-modified microalgal strains can, however, provide a boost in oil content to up to 70% of the cell's weight (Oilgae, 2009). High oil content is ideal for oil-based fuels (e.g., biodiesel, green fuels), but the value of other cell components (e.g., carbohydrates for ethanol production) should be taken into account when selecting a particular strain.

The interaction between native microalgae strains and their environment are relatively well known, and their hardiness allows them to be better prepared for changing variables (e.g., temperature variation) relative to genetically-modified strains. Furthermore, uncertainty of how genetically-modified strains will interact with and potentially disrupt established ecosystems exists. Some experts have expressed concern that genetically-modified algae with rapid growth rates could escape into the environment and lead to overgrowths. As a result, the overgrowths could displace other species, steal oxygen away from native fish and marine life, and even enter the human food chain. Others say that, if they escape, these species would likely not be able to compete with native strains without proper nourishment and pest control; furthermore, some insist that multiple modifications to a single organism only weaken it, and, therefore, it could not thrive in natural environments (Pollack, 2010).

If a company decides to genetically tailor a microalgal strain, the necessary R&D investments that accompany advanced laboratory testing of such enhanced strains should be taken into consideration. Also, since the U.S. Environmental Protection Agency currently regulates genetically engineered microbes under the Toxic Substances Control Act (Pollack, 2010), it is likely that all new strains of genetically-modified algae will also be regulated by the Agency, the Department of Agriculture, the Food and Drug Administration, or a combination of the agencies. Under this process, environmental and health assessments would likely be conducted to ensure minimal impacts of these species.

Industry Activities. The large majority of companies choose to work with microalgal strains instead of cyanobacteria or macroalgae primarily because they yield the greatest volume of lipids, which are considered by most to hold the greatest value within the biofuels industry. Within microalgae, most companies choose to work with natural, or wild, strains, but approximately ten companies are either 1) actively researching genetically-modified algal strains for use in biofuels production or 2) integrating genetically-modified algal strains into their current operations. Often, these companies are either a biotechnology firm partnered with a big oil company, or vice versa. For example, Targeted Growth, a crop biotechnology company, has partnered with refining technology developer UOP LLC to increase yields in jet fuel production (Targeted, 2009). Similarly, Synthetic Genomics Inc., a genetic technology company, has partnered with ExxonMobil in hopes of manipulating algae to expurgate their oil while floating in the water (Kanellos, Aug ’09). Other companies pursuing breakthroughs with genetically-modified microalgal strains include Sapphire Energy (who has engineered over 4,000 strains) (Pollack, 2010) and Aurora Algae (Zimmerman, 2009).

4.1.2. Cyanobacteria

General Description. Like microalgae, cyanobacteria (shown in Figure 3) are rapidly-growing microscopic organisms that efficiently convert sunlight into key cellular components (lipids, carbohydrates, and proteins). However, cyanobacteria lack a nucleus and membrane-bound organelles, conduct photosynthesis in its cytoplasm as opposed to its chloroplasts, and have a different gene structure. Therefore, they are categorized as prokaryotes that most closely resemble bacteria although they are traditionally referred to as “blue-green algae” because of their similarities to microalgae. Cyanobacteria also play an important role in fixing nitrogen and emitting hydrogen within the atmosphere. Approximately 2,000 species of cyanobacteria are known (Komárek , 2003).

Strengths / Weaknesses. Cyanobacteria are known for their ability to rapidly produce and store a large quantity of sugars (carbohydrates), particularly glycogen. Some strains are capable of doubling in size in less than 10 hours (U.S. DOE, May ’10). Lipid production, however, in cyanobacteria is naturally very low. Because of its poor lipid yields, cyanobacteria were initially overlooked as a feedstock for biofuels, although R&D

endeavors have been successful at boosting lipid yields in some cyanobacteria strains. In addition, cyanobacteria membranes can be quite durable, presenting a challenge during oil extraction.

As strides are made in ethanol fermentation, cyanobacteria are also viewed as strong candidates for alcohol-based fuels. In addition, certain strains of wild cyanobacteria have been known to autoflocculate and drop out of solution (Lane, Dec ’09), which may significantly reduce the cost of harvesting and dewatering the culture, if applicable.

Figure 3: Cyanobacteria often grow in long filaments resembling algae (Image Source: biotechnologie.de).

Since bacterial genetics are much more advanced than with eukaryotes, cyanobacteria are better positioned for genetic manipulation than microalgae. In fact, some companies are focusing research on optimizing lipid contents of cyanobacteria while minimizing system inputs and cost. One company claims to have increased natural lipid growth in cyanobacteria by 400% (Business Wire, 2009).

Industry Activities. Algenol Biofuels and its German-based research subsidiary, Cyano Biofuels, manipulate cyanobacteria to produce and ferment energy-rich sugars into ethanol with its DIRECT TO ETHANOL® process (Algenol, n.d.). Algenol and Dow Chemical are in the process of building a 50 million USD pilot-scale biorefinery to employ Algenol’s technology (Business, 2009). In addition to Algenol, BioLight Harvesting (Lane, Aug ’09), Targeted Growth, and Synthetic Genomics are companies conducting research with cyanobacteria for biofuel purposes (D. Glass, 2010). Finally, the Baltic EcoEnergy Cluster is conducting a project to produce methane and hydrogen from cyanobacteria and other algae strains (Koszarek and Kubacka, 2009).

4.1.3. Macroalgae

Figure 4: Macroalgae are mulitcellullar, photosynthetic marine organisms with plant-like features (Image Source: The Scottish Association for Marine Science).

General Description. Macroalgae, commonly known as seaweeds, are a diverse group of multicellular marine plant organisms that have a ranging in length anywhere from a few millimeters to tens of meters (Reith et al., 2005), as shown in Figure 4. Macroalgae are most often cultivated and harvested in marine environments where sufficient volumes of water are available for growth. Many different types of seaweeds have been collected for human consumption (predominantly in Asia) and for industrial applications for a long time (Bruton et al., 2009).

Broadly, seaweeds can be classified in three categories according to their pigments: brown seaweeds, red seaweeds and green seaweeds (Bruton et al., 2009; Reith et al., 2005). Naturally, brown seaweeds dominate in cold waters and reds dominate in warmer waters (Bruton et al., 2009). There is no established large-scale energy-from-seaweed system established yet, and all three categories are currently under consideration for bioenergy production. A large number of detailed criteria for the selection of seaweeds that are suitable for large-scale production (farming) are mentioned in literature (Bruton et al., 2009; Chynoweth, 2002; Reith et al., 2005).

Recently, an interest in seaweeds as a potential source of energy has emerged. Macroalgae typically lack lipids, which limits its contribution to oil-based fuels. However, its energy yield per hectare of seaweeds is substantially higher than for terrestrial energy crops, so macroalgae are being considered as a feedstock for methane production via anaerobic digestion and ethanol production via fermentation of sugar. For the production of biofuels criteria such as the yield per hectare, well-to-wheel energy balance and emissions, and sustainability of the production chain are important.

Different seaweeds are considered suitable for biofuel production (see Table 4). The participants in a marine biomass workshop in Florida, July 1990, for example, came up with a list of eleven genera of marine macroalgae that seem suitable for marine farms (Chynoweth, 2002). More recently (in 2009), the brown seaweed *Laminaria sp.* and the green *Ulva sp.* are considered good candidates for the seas around Ireland (Bruton, 2009). A group of Dutch scientific researchers proposes a system for combined growth of *Laminaria sp., Ulva sp.* and the red seaweed *Palmaria sp.* in the North Sea (Reith et al., 2005).

Table 4: Selection of seaweeds that are suitable for marine farms and feedstock for biofuels.

Seaweed genera	Remarks
Alaria	*A. fistulosa* is float-bearing, arctic (Chynoweth, 2002)
Corallina	Calcareous, widely distributed, small, may be cultured with other large species (Chynoweth, 2002)
Cystoseira	Temperate, has float-bearing reproduction structure (Chynoweth, 2002)
Ecklonia	Subtropic and temperate, one float-bearing sp (Chynoweth, 2002)
Egregia	Temperate, float-bearing, very durable (Chynoweth, 2002)
Eucheuma	Tropic, cultivated, moderate size (Chynoweth, 2002)
Gracillaria	Widely distributed, cultivated, high productivity (Chynoweth, 2002)
Laminaria	Intensively cultivated, temperate (Chynoweth, 2002; Bruton et al., 2009; Reith et al., 2005)
Macrocystis	Semi-cultivated, harvested, temperate (Chynoweth, 2002). Known as ‘giant kelp’.
Palmaria	Temperate (Reith et al., 2005)
Pterygophora	Temperate, very durable (Chynoweth, 2002)
Sargassum	Widely distributed, many sp, float-bearing, temperate and tropic (Chynoweth, 2002)
Ulva	Temperate (Bruton et al., 2009; Reith et al., 2005)

Strengths / Weaknesses. Macroalgae are abundant in oceans and coastal waters, and, because macroalgae are usually cultivated in marine settings, securing a water source and acreage on land is not necessary for its cultivation. In addition, seaweeds can potentially improve local biodiversity in their marine settings and intake many nutrients that could otherwise lead to eutrophication. In some areas of the world, wild seaweed washed onshore becomes a burden for local coastal communities, but, if collected, it can instead be used as an energy source. In general, macroalgae are also much simpler to cultivate and harvest than microalgae since the large masses can be collected more easily than unicellular organisms.

Relative to microalgae, less research has been conducted to date on macroalgae for application in the biofuels and energy industry. The high content of carbohydrates found in macroalgae are very appealing for methane or ethanol production. Oil yields, as previously mentioned, are very low in macroalgae, likely ruling out its contribution to oil-based biofuels. There is consensus in the literature that using seaweeds only for energy purposes will generally not be economically feasible in the coming years. Nevertheless, combinations with other applications might be feasible and are under consideration. Seaweed can be used as feedstock for (marine) biorefineries with multiple output products such as chemicals, value-added products like omega fatty acids, biodiesel, ethanol, fertilizer, and nutrients. Also residues can be upgraded to valuable products. Some biorefinery concepts include a CHP (combined heat and power) process that uses a share of produced biogas to generate electricity and process heat (Bruton et al., 2009; Carlsson et al., 2007; Reith et al., 2005; van Ree and Annevelink, 2007).

Industry Activities. Today, seaweed is predominantly collected for human consumption and to produce hydrocolloids. The majority of seaweeds that are harvested in Asia are cultivated (Bruton et al., 2009). In Japan, for example, seaweed is cultivated for human consumption. In Europe, mostly natural stocks are harvested, but in Scotland cultivation is now an established process (Bruton et al., 2009; Biomara, n.d.). Related to the biofuels industry, E.I. du Pont de Nemours and Company (DuPont) was awarded a 9 million USD grant from DOE in October 2009 to produce bio-butanol from macroalgae. Bio-Architecture Lab, or BAL, will collaborate with DuPont in this venture (Millikin, Mar ’10). Also, the BioMara Project (a collaborative project between Scottish and Irish scientists) includes the creation of methane gas from macroalgae grown off-shore (UK, 2010).

4.1.4. Summary of Algal Strains

Table 5 summarizes the strengths, weaknesses, and industry activity related to algal strain selection.

Table 5: Summary of the three primary algae strain categories reviewed in this report.

ALGAL STRAIN SELECTION

MICROALGAE

- **STRENGTHS:** Microalgae have high replication rates, high energy content, and have greater lipid yields than cyanobacteria and macroalgae.
- **WEAKNESSES:** Microalgae is difficult and costly to collect and harvest.
- **INDUSTRY ACTIVITY:** The large majority of algae production companies use microalgae.

CYANOBACTERIA

- **STRENGTHS:** Cyanobacteria have high replication rates and can store large quantities of carbohydrates. They are better positioned for genetic manipulation since bacterial genetics are more advanced than microalgae and macroalgae. Finally, cyanobacteria have higher light conversion rates than microalgae.
- **WEAKNESSES:** Like microalgae, cyanobacteria are difficult and costly to collect and harvest. Their lipid yields are generally low. They also possess durable membranes that are difficult to break down.
- **INDUSTRY ACTIVITY:** Companies that use cyanobacteria in their operations include Algenol Biofuels / Cyano Biofuels, Baltic EcoEnergy Cluster, Biolight Harvesting, Synthetic Genomics, and Targeted Growth.

MACROALGAE

- **STRENGTHS:** Macroalgae have rapid growth rates, are abundant in oceans and coastal waters, can store large quantities of carbohydrates, do not require arable land or potable water to grow, are easier to cultivate and harvest than microalgae and cyanobacteria, and have been grown at commercial scale for food for many years.
- **WEAKNESSES:** Lipid yields in macroalgae are generally low. Research for use in biofuels and energy industry is less advanced than for microalgae.
- **INDUSTRY ACTIVITY:** Companies that use macroalgae in their operations include Bio Architecture Lab, BioMara Project, Blue Sun Energy, Butamax Advanced Biofuels, Green Gold Algae and Seaweed Sciences Inc., Holmfjord, Oil Fox, POD Energy, and Seambiotic Ltd.

4.2. Algae Cultivation

- Microalgae
- Cyanobacteria
- Macroalgae

- **Open Ponds / Raceways**
- **Photobioreactors**
- **Heterotrophic Fermenters**
- **Marine Systems**

- Filtration / Microscreening
- Centrifugation
- Flocculation
- Flotation
- Off-shore Methods
- Other

- Physical
- Chemical / Solvent
- Enzymatic
- Wet / Single Step

- Transesterification
- Hydroprocessing
- Fermentation
- Anaerobic Digestion
- Gasification
- Pyrolysis/Liquefaction
- Biophotolysis
- Purification of SVO

Once an algal strain (or set of strains) has been selected for production, a growth environment must be chosen. For traditional microalgae cultivation, open ponds / raceways and closed photobioreactors, or PBRs, filled with water are the two most common designs. Each of these methods requires the same general inputs – light, nutrients, and CO_2 – and algae grown in these ponds or vessels are sent downstream to be harvested once they reach the desired level of maturity or lipid capacity. Heterotrophic fermentation is a less traditional approach, where algae thrive in vessels by feeding on sugar and nutrients (no light required) until they are ripe for harvesting. Finally, macroalgae are most often cultivated in marine settings where water and space are abundant.

4.2.1. Open Ponds / Raceways

General Description. Shallow, artificial ponds of water have been used for cultivating algae for decades and have been subject to extensive study. They can be designed in a multitude of shapes and sizes (e.g., tanks, circular ponds), but “raceway ponds” have become the most popular open model for efficiently growing algae since motorized paddlewheels can be used to continuously circulate the culture and keep algae suspended in the water. A basic schematic of a raceway pond is provided in Figure 5. Ponds are usually no more than 30 cm in depth to ensure sufficient exposure to sunlight. CO_2 and nutrients must be fed into the system on a regular basis (Wageningen, 2010). Often, single raceways and ponds are aligned side-by-side to create expansive algae farms, such as Cyanotech’s outdoor pond facility in Kailua Kona, Hawaii that is shown in Figure 6. An outlet for algae to be harvested is also usually incorporated into the design. Commercial scale open ponds typically produce approximately 20 tons of biomass/ha/year (Wageningen, 2010).

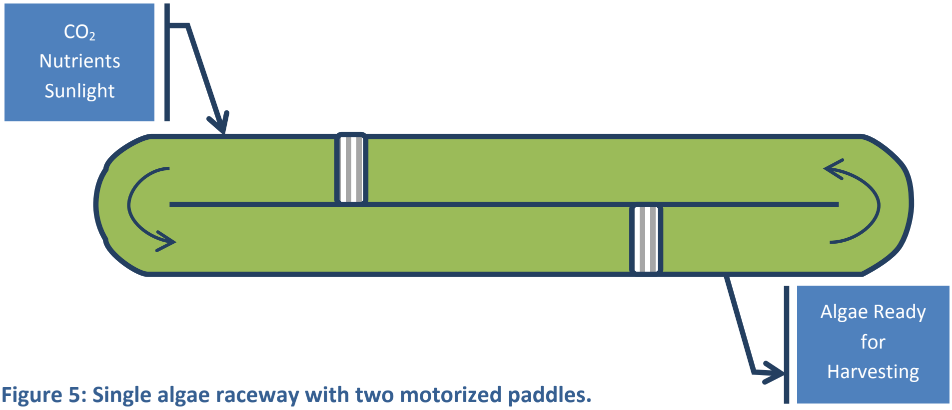

Figure 5: Single algae raceway with two motorized paddles.

Strengths / Weaknesses. The most distinguishing characteristic of ponds and raceways is that they are open to the elements. As a result, operators benefit from free sunlight and some nutrients or salt from the earth. However, they must continuously battle water evaporation, possible contamination by bacteria or invasive algae strains, potentially large swings in pH and temperature, severe weather, and loss of CO_2 into the atmosphere. Furthermore, paddlewheels are generally less efficient at stirring contents than PBR systems, leading to uneven light exposure, lower mass transfer rates, and hence, inferior algae productivity.

Figure 6: Aerial view of Cyanotech's outdoor algae pond cultivation facility in Kailua Kona, Hawaii (Image Source: Cyanotech Corp.)

This uncontrolled environment is not suitable for all algal strains, including monocultures and genetically-modified strains that cannot tolerate contamination. Furthermore, strains with high oil yields tend to be less hardy than those with higher amounts of proteins and carbohydrates and, therefore, do not thrive in uncontrolled open systems.

While the simplistic design of open ponds and raceways leads to low production and operating costs, extensive acreage may be needed to accommodate commercial scale systems. Site selection should take the length of the growing season into consideration, among several other factors, since the growing season may be limited to warmer months, unless the pond is artificially heated.

Cost Information. Many factors must be taken into consideration when estimating the cost of open pond operations, and these factors tend to be inconsistent across facilities. Besides the initial investment, labor and capital cost must be included. Operational costs, such as fertilizer, electricity, and maintenance, should also be factored in. Major sources of variation in cost estimates include total annual biomass production, biomass concentration in the medium, percent of lipids per cell, cost of CO_2, and potential revenue from byproducts (e.g., algae cake).

Relative to PBRs, the initial investment needed to build an open pond facility and bring it online is considered to be significantly less expensive. While much analysis has been conducted on whether open ponds or PBRs are most cost-effective, experts are still split on their overall conclusions. A brief literature review of recent algal biofuel analyses indicates that the theoretical cost of algal biomass achievable with large open pond raceways (1 ha or larger) will range from 0.20 – 2.80 USD/kg (Benemann and Oswald, 1996; Chisti, 2007; BCIC, 2009; Barclay et al., 1987; Bruton, 2009). Seambiotic in Israel recently estimated the cost per kilogram of algal biomass grown in open ponds or raceways to be 0.34 USD assuming a 10 ha area and a production rate of 20 g /m^2/day (Ben-Amotz, 2008). Much of this range overlaps with the estimated cost of large scale PBR systems, which are discussed in the next section. Until open-pond and raceway algal biofuel facilities reach commercial levels, the high cost per kg of algae is expected to result in algal oil that is too expensive to compete with petroleum-based oil.

Industry Activities. Approximately half of all companies that produce algae for biofuel purposes use open ponds and raceways. While the majority of companies on this list uses robust natural strains, some (e.g., Aurora Algae) choose to grow genetically-enhanced strains in the open.

4.2.2. Photobioreactors

General Description. A photobioreactor, or PBR, is a translucent, enclosed device used to grow algae in water or other medium. They are often used as an alternative to open ponds due to their ability to maintain a controlled environment, which is needed for certain strains. All PBRs use a light source (either sunlight or artificial light) to facilitate photosynthesis, and the PBR is designed to optimize light intensity. Other critical inputs, such as CO_2 and nutrients, must also be entered into the system. Common biomass production rates achievable with PBRs are estimated at 20-60 ton/ha/yr (Wageningen, 2010).

PBRs typically exist as tanks; flexible plastic bags or sleeves; or sturdy glass or plastic tubes. The latter option can be positioned vertically, horizontally, or coiled (Algae Coil, n.d.; Bioreactor, n.d.; Biofuels, n.d.). A variety of PBR designs is shown in Figure 7. Individual tubes generally range from 3-10 cm in diameter, allowing sufficient light to reach the center of the tubes, and are often 25-100 meters in length (Wageningen, 2010).

Most PBR systems operate in batch mode, where the algal culture is replenished following each harvest. Continuous operation mode is possible but requires great attention to the system's equilibrium to ensure a suitable environment for an extended period of time. As algae levels exceed the PBR's capacity, cells can overflow into a harvesting tank.

Strengths / Weaknesses. The closed design of PBRs provides excellent control within a system. Temperature, CO_2 intake, nutrient levels, pH, water level, and light intensity can all be fine-tuned to help optimize algal growth rates and lipid yields. This design also mitigates possible contamination by bacteria or invasive strains and are, therefore, ideal for cultivating monocultures and vulnerable, genetically-modified strains. Proper mass transfer is needed in a PBR system to avoid oxygen buildup that can inhibit growth of algae (Wageningen, 2010).

Unlike most open ponds and raceways, PBRs can operate throughout the entire year and even 24 hours per day if desired, although cooling systems may be necessary during daytime operating hours to maintain a constant temperature. This eliminates any issues related to limited growing seasons, severe weather, or downtime at night. Due to this and other design characteristics, volumetric productivity in PBRs can be up to ten times higher than volumetric productivity in open ponds (Wageningen, 2010).

Cost Information. The added benefits of PBRs come with increased costs. Relative to open pond systems, the initial investment needed to build a PBR facility and bring it online is generally much more expensive than for open ponds. However, less property area is typically required since biomass production concentrations and lipid yields are often higher in PBRs than in open pond systems. Furthermore, PBR systems can maximize space with vertical designs. Fewer personnel are generally needed to operate PBR systems, but salaries may be higher due to the increased complexity of the system relative to open pond systems.

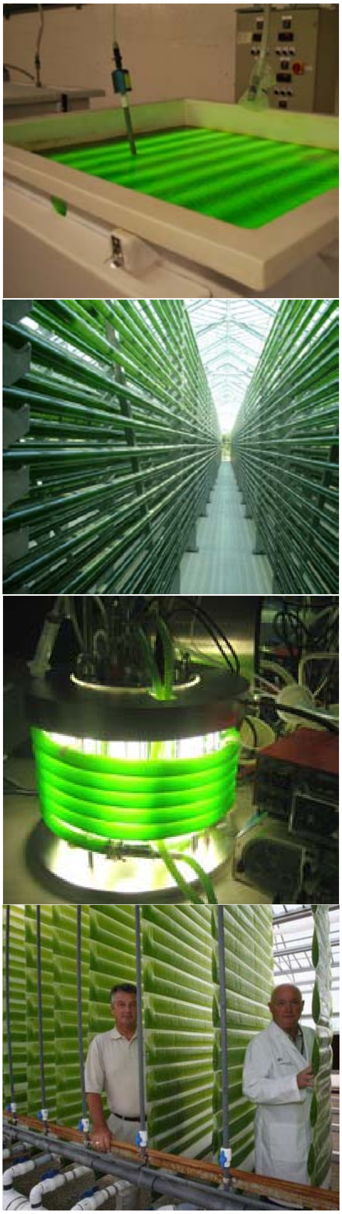

Figure 7: Various designs of PBRs used in industry. From top to bottom: Brite Box PBR (Source: Natural Resources Canada); Tubular PBR (Image Source: Bioprodukte Prof. Steinberg); Wrapped PBR (Source: The University of Georgia); High Density Vertical Bioreactor (Source: Vertigro Products, Inc.).

Operating costs are also generally higher for PBRs relative to open ponds. For example, artificial light is required if the system is set up indoors. Extensive monitoring and controls are needed to adjust mixing rates, prevent fouling (buildup of algae on PBR walls), and regulate temperature since some PBR systems require continuous cooling.

A brief literature review of recent algal biofuel analyses indicates that the average capital cost of PBR systems is currently between 100-190 USD/m^2 (Benemann, 2008; U.S. DOE, Oct '08), and, for commercial production capacities, the estimated cost to produce 1 kg of dry algal biomass ranges from 0.47- 7.32 USD/kg (Chisti, 2007; BCIC, 2009). While some of this range overlaps with the estimated cost of large scale open pond systems, it appears that PBRs stand to benefit the most from economies of scale. Like open pond systems, the cost per kg is expected to result in algal oil that cannot compete with petroleum-based oil until economies of scale are realized.

Industry Activities. Roughly half of all companies that produce algae for biofuel purposes use PBR systems. As the costs associated with PBRs continue to drop, more companies are expected to adopt this method for algae cultivation.

4.2.3. Heterotrophic Fermenters

General Description. In heterotrophic fermentation, algae are generally contained in a dark vessel where they ingest and metabolize sugars (e.g., glucose) or other organic substances, which are then converted to triglycerides (Figure 8). These high-quality triglycerides almost identically resemble the composition of common vegetable oil, which can then be converted into a variety of end-use biofuels. In this process, the sugar provides the carbon and energy requirements for the cell, substituting for photosynthesis. At least one company has claimed to reach as much as 75% of the dry weight of the cells in the form of oil (U.S. DOE, Mar '10), which leads to larger quantities of oil produced per day per liter than open pond or PBR systems. Other research suggests more conservative estimates of up to 50% (ITTC, 2008). Certain algae strains are capable of producing end-use alcohols (e.g., ethanol) through fermentation of the cell's carbohydrates, which is discussed in more detail in section 4.5.3.

Heterotrophic fermentation can be carried out in either batch or continuous mode. For batch mode, a fermenter is filled with a medium, inoculated, and emptied once the algae culture is ready for harvesting. In continuous mode, new growth medium is constantly input into the tank as mature cultures are removed for harvesting, maintaining a constant volume. The fermenter only requires emptying if it becomes contaminated or needs cleaning. Tanks typically range from 80-200 m^3 in volume and are usually capable of regulating temperature, pH, and oxygen levels (Wageningen, 2010). Impellers are sometimes incorporated to ensure an evenly mixed medium. Heterotrophic fermentation of algae typically yields at least 20 g of dry biomass per L/day, although 302 g/L/day have been achieved in laboratory settings (Theriault, 1965; BCIC, 2009).

Figure 8: Heterotrophic fermenter (Source: Wageningen University, 2010).

Strengths / Weaknesses. The high level of productivity achievable through heterotrophic fermentation helps to outweigh its investment and operating costs, which can be substantial. The fermentation process is well-controlled and results are reproducible between batches, and many of the cultivation variables presented with open ponds are avoided (e.g., inclement weather, evaporative losses). Ability to grow in the dark eliminates the cost and energy associated with artificial light, and a deep, high-volume vessel can be used since light penetration depth is not an issue. Only a fraction of the water used in a PBR or open pond is needed in heterotrophic fermentation. Finally, fermenters are widely used in the brewing industry, so the design is well understood and highly mature (Wageningen, 2010).

Since fermentation requires sugar, concern has been voiced that this process may add to the “food vs. fuel” debate where this process could be displacing food resources for the purposes of fuel production. Therefore, operators should consider using sugar derived from cellulose that will not interfere with the food supply.

Cost Information. Of the cultivation methods discussed in this section, heterotrophic fermentation is the most mature from a technology and cost standpoint. Besides the initial investment cost of a fermenter, which can be significant, major cost drivers include the system inputs (mainly sugar), electricity, steam, CO_2 (in some systems), and labor. A brief literature review of recent algal biofuel analyses indicates that for large-scale production capacities, the estimated cost to produce 1 kg of dry algal biomass from heterotrophic fermentation ranges from less than 1.00 USD/kg to 2.01 USD/kg (Behrens, 2005; Radmer and Parker, 1994; BCIC, 2009).

Industry Activities. Solazyme has become the most popular player in the area of heterotrophic fermentation using genetically-engineered algae that do not require light. They are building a pilot-scale biorefinery capable of processing 13 metric tons of dry feedstock per day. End use products derived from this oil are expected to include biodiesel and renewable diesel (U.S. DOE, Mar ’10).

In August 2009, Martek Biosciences Corporation, a leader in high-value algal oil production, entered a Joint Development Agreement (JDA) with BP to establish a proof-of-concept for cost-effective microbial biodiesel production through fermentation at the commercial-scale. BP has committed to contribute up to 10 million USD in the initial phase of this JDA, and Martek will lead the biotechnology R&D in this phase (BP, 2009).

4.2.4. Marine Systems

General Description. Macroalgae are typically cultivated in off-shore or near-shore marine environments. For cultivating large volumes of macroalgae, the seaweeds need to be attached to a support structure, like an anchored rope or a net, to keep them from drifting away. The foundations of off-shore wind farms are being considered to serve as anchoring points for these support structures. The concept of combining seaweed cultivation with off-shore wind farms already has support in Denmark, Germany, the Netherlands, and the United States (IEA BIA, 2010).

Besides cultivating seaweeds on off-shore or near-shore structures, it is also possible to harvest wild seaweed stocks and seaweeds that are washed ashore. However, the quantities of seaweed that could be harvested these ways may be too small for a significant biofuel production, and the impact on natural ecosystems needs to first be clarified for harvesting wild seaweeds. Cultivated seaweed would therefore be the most likely source for biofuels from seaweed.

Strengths / Weaknesses. Energy consumption of road transport is large, and only supplying a small share by biofuels from seaweed already requires large areas of cultivation. It was for example estimated that 700 ha of off-shore cultivation would be needed to supply 0.2% of road fuel demand in Ireland (Bruton et al., 2009). The Scottish Association for Marine Science estimates that a seaweed farm of 1.2 times the size of the United Kingdom would be necessary to meet the complete UK transport fuel needs (Stanley, 2009).

The large scale means that sustainability issues require special attention, as learned from recent discussions on sustainability of other biofuels. Risks of large-scale off-shore seaweed cultivation are for example related to vulnerabilities of monocultures, sedimentation of seaweed fragments with a negative effect on the amount of oxygen in the water, eutrophication, and possible negative effects on the migration of sea animals. On the other hand there might be positive effects such as the uptake of nutrients (stemming from human activities and carried by rivers to the sea) by the seaweeds, and an enhancement of marine biodiversity because the cultivated seaweed may offer a shelter for other marine life such as fish. In general it can be recommended to cultivate seaweed species that are native to the production region.

Cost Information. Because macroalgae cultivation is largely performed manually, the cost of obtaining dry macroalgal biomass may vary significantly worldwide depending on the regional cost of labor. In developing countries where labor rates are low, macroalgae can be cultivated for under 100 USD per dry ton whereas the rate in more developed countries is 100-300 USD per dry ton. As operations ramp up and cultivating techniques become more mechanized, the cost per dry ton is expected to drop (Oilgae, Apr '10).

Industry Activities. Traditionally, macroalgae systems have been based on ropes or nets. Norwegian-based Seaweed Energy Solutions, however, has developed and patented the first ever macroalgae cultivation structure called "the Seaweed Carrier" whose shape mimics a large seaweed plant attached to the sea floor. This design, shown in Figure 9, allows for cultivation at greater ocean depths (Seaweed, n.d.).

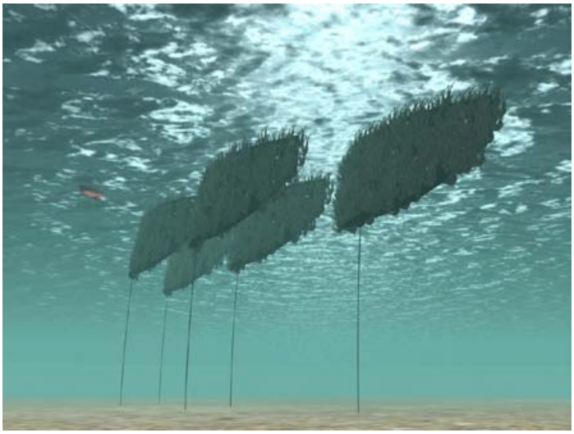

Figure 9: Schematic of a full-grown Seaweed Carrier designed by Seaweed Energy Solution (Seaweed, n.d.).

4.2.5. Summary of Algae Cultivation Methods

Table 6 summarizes strengths, weaknesses, and industry activity related to algal cultivation methods.

Table 6: Summary of the four primary algae cultivation settings reviewed in this report.

ALGAE CULTIVATION

OPEN PONDS / RACEWAYS

- **STRENGTHS:** Open ponds /raceways have low-to-moderate production costs and low maintenance costs. They permit high production volumes, can utilize undesirable land and space, are more conducive for incorporating wastewater, and are easy to clean and scale up.
- **WEAKNESSES:** Open ponds / raceways are susceptible to contamination by other algal strains or diseases, and natural loss of water and CO_2 must be addressed. Inability to control certain conditions (e.g., temperature, pH, salinity) may affect productivity, and growing timeframe may be limited due to seasons. Also, poor light utilization and inefficient stirring is common.
- **INDUSTRY ACTIVITY:** Companies that use open ponds or raceways in their operations include Aurora Biofuels, Blue Marble Energy, General Atomics, Kai Bioenergy, Kent Bioenergy, LiveFuels, PetroAlgae, PetroSun, Phycal LLC, Sapphire Energy, SBAE, Seambiotic, SunEco Energy.

PHOTOBIOREACTORS

- **STRENGTHS:** PBRs offer controlled conditions (e.g., salinity, light, temperature, pH, CO_2) and are more conducive for growing genetically-modified strains and monocultures. They also accommodate higher concentrations and, therefore, yields.
- **WEAKNESSES:** PBRs have a high production cost, especially if artificial light is required. They are difficult to maintain, may need cooling during the daytime, and are not yet feasible for large volumes of algal mixtures. Fouling and buildup of algae on PBR walls may obstruct light. Algae in PBRs are susceptible to high oxygen levels.
- **INDUSTRY ACTIVITY:** Companies that manufacture PBRs or use PBRs in their operations include A2BE Carbon Capture, AlgaeLink, BARD LLC, Bionavitas, BioProcess Algae, Bodega Algae LLC, Ecoduna, Global Green Solutions, Green Plains, Hezinger Algaetec, Plankton Power, Solix Biofuels, Subitec GmbH, Vertigro Energy, and W^2 Energy.

Table 6: (Cont.)

ALGAE CULTIVATION (CONT.)

HETEROTROPHIC FERMENTERS

- **STRENGTHS:** Fermentation allows for high lipid yields and cell density, does not require artificial light, and uses significantly less water than other methods. The system does not require CO_2 and has easily controlled parameters. Operating costs are low, and it is a widely established process.
- **WEAKNESSES:** A high initial investment cost is associated with this process due to complex configuration and construction. The system is difficult to clean , and sufficient levels of oxygen must be maintained. It requires large amounts of sugar, which may contribute to the "food vs fuel" debate. There is a limited ability to scale up operations.
- **INDUSTRY ACTIVITY:** Companies that use heterotrophic fermentation in their operations include Martek Biosciences (with BP) and Solazyme.

MARINE SYSTEMS

- **STRENGTHS:** Marine systems can be adapted to near-shore or off-shore settings, have vast space for cultivation, and do not need fresh water. Modern structures have been successfully tested in various settings worldwide.
- **WEAKNESSES:** The design and stability of underwater structures could be improved, especially if it promotes attachment of macroalgae to structure. Finally, environmental regulations may inhibit aquaculture development in some countries.
- **INDUSTRY ACTIVITY:** Companies that use marine systems in their operations include Seaweed Energy Solutions and BAL (with StatOil).

4.3. Harvesting and Dewatering

- Microalgae
- Cyanobacteria
- Macroalgae

- Open Ponds / Raceways
- Photobioreactors
- Heterotrophic Fermenters
- Marine Systems

- **Filtration / Microscreening**
- **Centrifugation**
- **Flocculation**
- **Flotation**
- **Off-shore Methods**
- **Other**

- Physical
- Chemical / Solvent
- Enzymatic
- Wet / Single Step

- Transesterification
- Hydroprocessing
- Fermentation
- Anaerobic Digestion
- Gasification
- Pyrolysis/Liquefaction
- Biophotolysis
- Purification of SVO

Once algae are cultivated to the desired level, it must then, in most cases, be recovered from the water or other growing medium, dewatered to reach a certain moisture content in preparation for processing, and relocated to the processing site. (In cases where dry matter content of over 30% is required, additional thermal drying techniques are usually needed (Braun and Reith, 1993)). While this may seem straightforward in theory, harvesting and dewatering are often considered the greatest bottleneck to scaling up algal biofuel production. Such a large percentage of the energy and cost is expended in order to obtain sludge dry enough to conduct lipid extraction and/or fuel conversion. In fact, 30-50% of the total cost of algae cultivation is expended when trying to convert an algae culture to sufficiently dry algae cakes (U.S. DOE, n.d.), and energy costs rise substantially as moisture contents required for downstream processing get smaller. With that said, this step also presents large opportunities to decrease the overall cost of algal biofuel production.

Several companies, universities, and research groups have developed a variety of methods to improve efficiency and/or cost of harvesting and dewatering algal biomass.[4] While each method presents both strengths and weaknesses, it is difficult to declare a superior technique since various algal properties, such as cell size, specific gravity (relative to the growing medium), presence of a cell wall, and whether or not flocculation is administered, at least partially affect cost competitiveness. Overall, a cost-competitive method to harvest and dewater algae cultures has not yet been developed at the commercial scale.

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[4] It should be noted that many researchers are attempting to completely leapfrog the harvesting/dewatering step by designing algae capable of excreting oil or alcohol within its medium.

The major existing techniques for harvesting and dewatering algae are summarized in this section. They include: 1) Filtration / Microscreening; 2) Centrifugation; 3) Flocculation; 4) Flotation; 5) Off-shore Methods; and 6) Other.

4.3.1. Filtration / Microscreening

General Description. One of the simplest and most economic methods for separating algae cultures from their medium is by membrane filtering or microscreening. As a suspension passes through a filter, algal cells with diameters less than the filter pore size continue through while cells with diameters exceeding the pore size are retained. The flow-through rate of a filter increases with pore size and, as a result, the amount of algae that is collected (until the pore diameter exceeds the average algal cell diameter).

Filter membranes or microscreens are commonly cellulose-based, and pore sizes are usually based on the size of the algal species being cultivated. Since operators often prefer to filter the most mature cells, filter pore diameters are usually selected to let the small, immature cells pass through the filters and return to the culture while larger cells are captured and sent to downstream processing. This logic tends to work best with a monoculture to maintain a consistent range of cell diameters.

Filters can either be integrated into an algae cultivation system as a separate downstream step or as a component of the bioreactor itself. A vacuum system is often incorporated to assist in filtration, but, when suction is applied, membrane clogging is nearly inevitable. Additional mechanisms, such as pulsed backwashing or air scouring, are sometimes incorporated to inhibit membrane fouling and obstruction. Tangential, or cross, flow filtration is also being considered for algal biomass recovery due to its ability to keep cells in suspension and minimize fouling, although this technique requires more energy than other filtration methods (Uduman et al., 2010).

Resulting harvested concentrations of algae solids achievable with basic filtering or microscreening is relatively low at 2-4%. This increases to up to 6% with tangential flow filtration (Benemann and Oswald, 1996). Improvements are continuously being made to maximize water concentration over a given time period while minimizing the occurrences of fouling or clogging. Filtration has been demonstrated for small-scale algae cultivation systems but has yet to be scaled up to commercial levels.

Strengths / Weaknesses. Filtration technologies are widely used in industry and are relatively mature. The use of filters and microscreens to harvest algae allows for the collection of cells with very low density, which is often the case with microalgae and cyanobacteria. Furthermore, small immature algal cells within a monoculture may pass through a filter, allowing for a longer growth period, while larger, more mature, cells are collected. Filtration does not require the use of chemicals or any pretreating of water, which contributes to the simplistic nature of this process.

Disadvantages to filters and microscreens include eventual buildup of algal cells that will require routine maintenance and cleaning. The cost and time needed to address fouling issues significantly limit the scalability of filtration. In addition, a 2003 study indicated that filtering of microalgae under pressure or vacuum may only be effective in recovering cells with large diameters and not those with diameters similar to bacteria (Grima et al., 2003).

Cost Information. Relative to other harvesting/dewatering techniques, basic filtering or microscreening has very low cost and a moderate level of required energy, but, as previously mentioned, the resulting concentration of algae is only 2-4%. If tangential flow filtration is incorporated into a harvesting system, which increases the resulting harvested algal culture solids concentration to up to 6%, a greater amount of energy is required, and the cost increases to an average level, relative to other harvesting/dewatering techniques.

Industry Activities. Univenture, Inc. and AlgaeVenture LLC have developed a harvesting, dewatering, and drying model that uses membrane technology to promote natural liquid flow characteristics – surface tension, adhesion, cohesion, capillary action, etc. – as opposed to forcing movement of substances (e.g., with centrifugation, differential pressure). The resultant system (Figure 10) uses a porous conveyor belt to continuously draw algae from the culture, pulls water through the bottom of the conveyor belt (via liquid adhesion) with a second superabsorbent belt that passes directly underneath the primary belt, and delivers dried flakes of algae (AlgaeVenture, 2009).

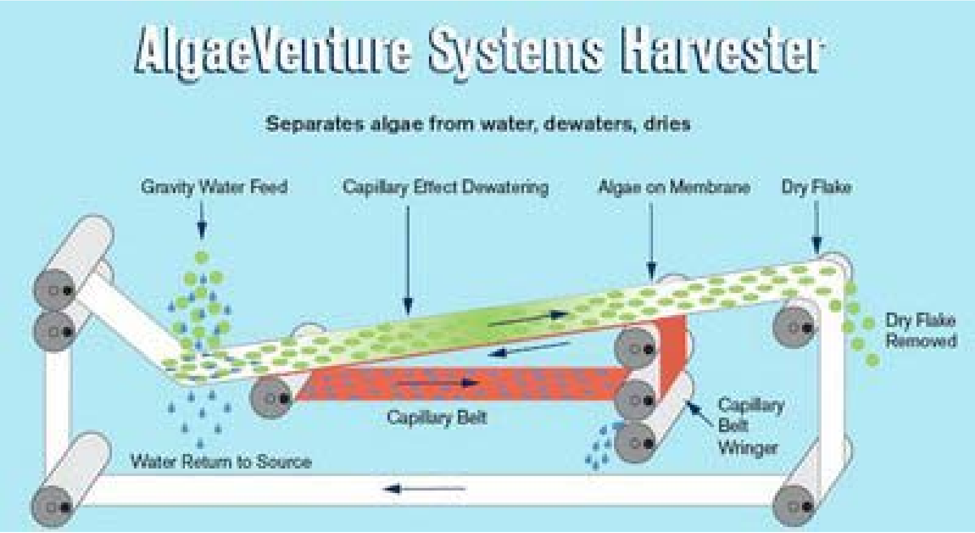


Figure 10: Schematic of AlgaeVenture's harvesting model (AlgaeVenture, 2009).

Florida-based PetroAlgae removes water from their harvested algae cultures through vacuuming skimming to screen filters. 98% of the water is then recycled within the system (PetroAlgae, 2010).

4.3.2. Centrifugation

General Description. Centrifuges can be used to separate water from solids (in this case, algal biomass) through centrifugal force created by a motor. At several thousand rpms, water is typically pushed out of the centrifuge through a filtered wall, leaving a thick algal paste comprised of approximately 15-25% algae (with water accounting for the remaining percentage) (Shelef, Sukenik, & Green, 1984).

To reach such high rotational speed, centrifugation requires a relatively large amount of energy and may not be a practical primary method for dewatering algal cultures. It may instead be better suited as a secondary method to others techniques (e.g., flotation) to achieve further moisture removal as needed for downstream processing.

In addition to simple dewatering, three-phase centrifuges can be used to separate algal cultures into water, oil, and solids. This process combines harvesting, dewatering, and oil extraction into one step, which can significantly reduce production cost. To weaken cell walls, pretreatments are sometimes applied to algal cultures before entering a three-phase centrifuge to promote easy cell rupture for escaping of oil.

Strengths / Weaknesses. Centrifuges have been used in industry for many years and are continuously improved and redesigned to meet the objectives of potential markets, such as algae harvesting. Furthermore, centrifugation is rapid and effective, and it is considered the preferred dewatering method by many.

The greatest barriers to using centrifuges in commercial settings are the large initial capital costs and energy-driven operating costs, which make the technology cost-prohibitive at this time. Furthermore, centrifuges can only process limited volumes at a time, which can create a bottleneck in production. Also, the strength of cell walls should be considered before subjecting certain strains to the shear forces of centrifugation since structural damage can result.

Cost Information. Centrifugation is currently considered the most expensive method for harvesting/dewatering algal cultures. In fact, DOE's Aquatic Species Program estimated centrifugation to account for 40% of production cost and 50% of investment cost if used as a primary harvesting method (van Iersel et al., 2009). However, if employed as a secondary harvesting method to increase dry solids concentrations from 1-5% to 15-20%, the cost of centrifugation may drop by at least 50 times (Benemann and Oswald, 1996).

Industry Activities. Companies that design and produce centrifuges to help separate algal biomass from water include Lavin Centrifuge, Alfa Laval, Flottweg Separation, GEA Westfalia, and U.S. Centrifuge Systems.

4.3.3. Flocculation

General Description. Flocculation refers to the aggregation of individual, free-floating, suspended algal cells (1 to 30 µm each) (Ryan, 2009) into easily harvestable masses of algae, known as flocs or flakes (Figure 11), which allow for easier sedimentation or skimming. It is most commonly initiated with chemicals comprised of either inorganic materials (e.g., calcium carbonate, crushed crustacean shells, alum, and ferric chloride) or organic anionic polymers. Flocculation can also occur in the absence of chemical additives. For example, adjusting pH and CO_2 levels can cause algae to spontaneously "autoflocculate" and settle. Algae can also naturally "bioflocculate" if in the presence of certain natural polymeric or microbial flocculants. Electricity and metal ion flocculants may also be used to accelerate aggregation of cells.

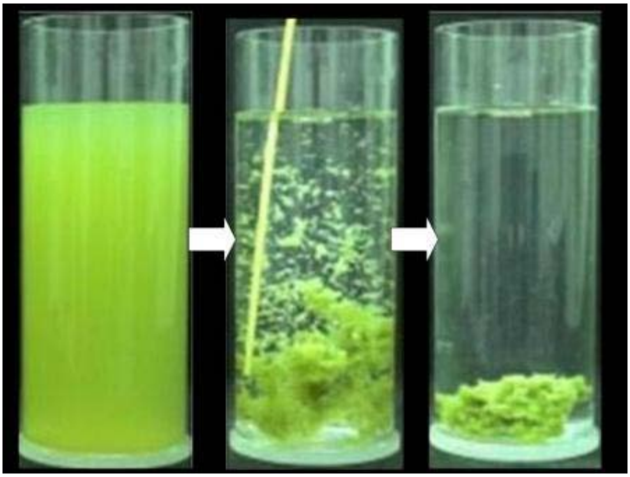


Figure 11: In flocculation, free-floating cells clump to form easily harvestable algal masses (Wageningen, 2010).

Strengths / Weaknesses. The greatest advantage of using flocculants is that flocs of algae are much easier to filter or settle compared to unicellular algae in suspension, resulting in as much as 95% removal of microalgae (Bilanovic and Shelef, 1988). Because of this feature, flocculants are often used to aid in other harvesting techniques, such as flotation, to enhance skimming practices. Flocculants can easily be applied to very large volumes of algal cultures, and additional tanks or equipment is not needed to do so.

Researchers are pursuing genetically-modified strains of algae that can autoflocculate under specific conditions, potentially increasing process efficiency. Furthermore, continuing to understand the drivers of bioflocculation may allow researchers to identify and optimize conditions for cell aggregation without the use of toxic chemicals.

Flocculation alone may not be sufficient to harvest and dewater algae. Therefore, a complementary method may be necessary, which would add to overall cost and time. Additionally, the cost to administer, remove, and dispose of high volumes of chemical flocculants presents a large barrier to using flocculation at a commercial scale. To become economical, methods to 1) minimize the cost of recovery and disposal; 2) reduce or eliminate the need for additives; and/or 3) increase recovery ratio of algae to water should be investigated further. In addition to economics, the effects of synthetic flocculants on the environment and human health are currently not fully understood. Therefore, residual biomass contaminated with flocculant agents may require washing prior to reuse or release.

Cost Information. Although it achieves 8-10% of solid algal biomass concentration (Benemann and Oswald, 1996), the use of chemical flocculants for algae harvesting/dewatering is currently considered the second most expensive method (behind centrifugation), and it accounts for a large percentage of algae production cost. Not including disposal of flocculants, the operating cost of incorporating chemical flocculation into an open pond system is estimated at 1,100 USD/ha/yr (U.S. DOE, Mar '08), suggesting that chemical flocculation is presently too costly for commercial scale systems.

Bioflocculation and autoflocculation, in contrast, have far lower relative costs partially due to reduced energy inputs needed. However, these methods result in an algal culture with a solids concentration of only 1-3% (Benemann and Oswald, 1996), and further R&D is needed in this area to fully understand its optimal conditions and mechanisms.

Industry Activities. Companies and research organizations that have incorporated flocculation into their algal harvesting and dewatering processes include the National Technology University in Argentina, Seambiotic in Israel (Bruton et al., 2009), and XL Renewables.

4.3.4. Flotation

General Description. If algal cells float on the surface of a medium (e.g., water), they can be skimmed off into a separate vessel with relative ease. Sometimes, algal cells are naturally buoyant due to high oil contents. Other times, the culture suspends throughout the medium, requiring additional means to bring algae to the surface. Various flotation techniques are used to drive cells to the surface with air or gas bubbles. Chemical flocculants are often used in combination with flotation to increase the algal mass that adheres to bubbles and allow for easier skimming along the surface.

The two primary types of flotation that are discussed in this section are dissolved air flotation and dispersed air flotation:

- **Dissolved Air Flotation –** Also known as froth flotation, dissolved air flotation is the most popular flotation technique used in industry. Microscopic pressurized air bubbles enter the pond or bioreactor through nozzles or needle valves as part of a water stream, and algae adhere to the bubbles as they rise to the surface. The bubbles congregate on the surface to form a foamy consistency that can be easily and efficiently skimmed or suctioned off. The resulting sludge often has a solids concentration of 2-5% (Farmerie, 2005).

- **Dispersed Air Flotation –**In dispersed air flotation, bubbles with diameters ranging from 700 to 1500 μm are generated and dispersed with a high speed mechanical agitator and air injection system. Bubbles are often coated with electrically-charged surfactants or collectors to enhance bonding with flocs (Uduman et al., 2010).

Strengths / Weaknesses. Flotation techniques can quickly separate the bulk of algal biomass from its medium, and it can be applied to the primary bioreactor so no additional tanks or large pieces of equipment are needed. If flocculants are used to aid flotation, algae will be much easier to filter or settle than if not used. Overall, this process appears to be one of the most cost effective ways to treat water with high algal cell count.

Cost Information. Dissolved air flotation has been successfully implemented into several other industries and is viewed as potentially economical for large-scale algae harvesting/dewatering operations. The cost to administer, remove, and dispose of chemical flocculants still applies in addition to the cost to install and operate the flotation system. Operational costs for dissolved air flotation are moderate to high relative to other harvesting/dewatering methods. Also, since flotation is most effective when combined with chemical flocculants, the additional cost of chemicals should be considered before applying flotation techniques to a system (Lassing et al., 2008).

Industry Activities. Dissolved air flotation has commonly been used by companies worldwide, such as DAF Corporation and Aquatec-Maxicon Pty. Ltd., to thicken sludge in wastewater treatment plants, paper plants, and/or meat processing facilities. It is a well-understood process and is considered economically viable in these industries.

4.3.5. Off-shore Methods

General Description. Off-shore macroalgae can be harvested manually (e.g., hand cutting methods) or mechanically. Generally, a mechanized approach to harvesting seaweeds is preferred, because manual harvesting is likely too costly for biofuel production. Mechanized methods include mowing, dredging, and suction, and most of these methods use dedicated ships. Naturally occurring giant kelp (*Macrocystis*) floating at the surface is, for example, harvested along the Californian coast with vessels that can collect up to 600 tons of kelp per day (Ryan and Patyten, 2004). Mechanical harvesting is also possible for non-floating species that are growing on support structures such as rocks and ropes. The most common techniques to harvest these seaweeds are trawlers that are used in Norway and ships with a hook system that are used in France to harvest the brown seaweed *Laminaria* (Bruton, 2009). The quantities that are harvested today are small compared to the quantities that would be needed for a significant share in the automotive fuel market, so R&D is required to determine the optimal harvesting technology.

Fresh seaweed contains 85-90% water, and pumping it as slurry is easy, so it can be transported by pipeline to a processing site. For transport by ship, dewatering at sea is recommended before the seaweed is taken to a (onshore) processing plant (Reith et al., 2005). Local circumstances will determine which method is best. Besides harvesting at sea, seaweeds that seasonably wash ashore can be collected and converted to biomethanol, bioethanol, or other vehicle fuels. The quantities of fuel from stranded seaweed might be limited (for example, about 70,000 tons of the green seaweed *Ulva* are annually collected from the Brittany coast (Dupont, 2010; Mortureux, 2010), but because the excess of seaweeds has to be removed anyway, this might be a sensible niche market in applicable regions.

Water content of seaweeds is high, so dewatering before further processing should be considered. Pressing, sometimes combined with filtering, or drying are existing options for dewatering. All dewatering processes require energy, but they may reduce the energy consumption of the next steps in biofuel production. Together with the options for removing sand and other contaminants, these are parameters that should be optimized on a case-by-case basis, taking local circumstances into account.

Strengths / Weaknesses. A variety of manual and mechanized techniques have been developed for macroalgae harvesting based on the local environment. Mechanized techniques have resulted in decreased labor intensity. Because of their size, macroalgae are generally easier and less expensive to collect than microalgae although it cannot be harvested daily. Instead, macroalgae typically need several months to replenish its supply.

In the region of Brittany at the West coast of France, and in Japan, large quantities of seaweeds on the shore are causing problems because they start decomposing, and large quantities of annoying gases such as hydrogen sulfide (H_2S) are emitted in that process (Bruton et al., 2009; Dupont, 2010; van Es and Stroeks, 2010). H_2S at moderate concentrations irritates the eyes and respiratory system, and, at higher concentrations, it is lethal for humans and animals. To avoid such problems, excessive quantities of seaweeds are being removed, if necessary, on a daily basis. In July 2010, France published a set of safety measures for people who are working with the seaweeds and for people living on the coast (Mortureux, 2010). This kind of risk also holds for storing seaweeds and, therefore, should be taken into account when setting up a biofuel industry.

Cost Information. When macroalgae are harvested manually, the large majority of cost is labor, which may vary significantly depending on the country. As previously mentioned in section 4.2, macroalgae can be cultivated and harvested in developing countries for under 100 USD per dry ton on a continuous basis whereas the rate in more developed countries is in the range of 100-300 USD per dry ton on a continuous basis. As operations ramp up and harvesting techniques become more mechanized, the cost per dry ton is expected to drop significantly to approximately 75 USD per dry ton (Oilgae, Apr '10). Based on these cost estimations, macroalgae harvesting is easily cost competitive with microalgal harvesting.

Industry Activities. While manual harvesting methods are still prevalent throughout the world, the use of mechanized harvesting is increasing in a handful of countries, such as Norway, Iceland, and France.

4.3.6. Other

Several additional innovative approaches have been developed in hopes of achieving an algal harvesting technique that is less expensive and/or more effective than existing techniques. Most of these techniques are still in the development phase and have not been demonstrated at commercial levels. These approaches include:

- **Electrophoresis** - Electrochemical processes can be used to initiate or aid the separation of biomass and water. For example, in electrolytic coagulation, reactive electrodes such as iron or aluminum produce metal ions that lead to the aggregation of algal cells, which can then be easily collected. In electrolytic flotation, hydrogen bubbles from an inactive metal cathode are created from water electrolysis, and the bubbles carry algal flocs to the surface for collection. Finally, an electrolytic flocculation technique that pulls negatively charged microalgae to the anode has been studied (Uduman et al., 2010).

- **Biomagnetic Separation –** Siemens AG has demonstrated a method for using magnets to harvest algae. Specifically, iron oxide (Fe_2O_3) ground into a fine powder is added to the algal culture, and the algae attach to the magnetic particles. Then, a permanent magnet applied to the outer surface can be used to pull magnetized algae to an isolated area for collection. Figure 12 demonstrates this process by showing algae settled at the bottom of a flask being pulled to the side by a magnet via Fe_2O_3. After harvesting, the Fe_2O_3 would have to be removed prior to downstream processing of the biomass (Hatch, 2009).

Figure 12: Biomagnetic separation of algae from water using finely ground iron oxide, at the bottom of a flask (Image Source: Siemens AG).

- **Acoustic Waves / Ultrasonication –** Solix Biofuels, in partnership with Los Alamos National Laboratory (LANL), announced intentions of using LANL sound wave technology to create ultrasonic fields that "concentrate algal cells into a dense sludge and extract oil" (Moresco, 2009). This method, while energy-intensive and expensive, would consolidate harvesting and oil extraction into a single step process while eliminating the need for chemical additives. (This process is also covered in section 4.4 Oil / Biomass Separation.)

- **Bioharvesting –** Live grazers (e.g., shrimp or fish) can be used to harvest (eat) and metabolize algae, and store the oil. Then, when the fish are processed, oil can be extracted in addition to a variety of valuable byproducts, such as omega-3 fatty acids. The tilapia used by LiveFuels eat over one-third of their body weight in wet algae daily. Concern over the economics of growing and maintaining two separate organisms – algae and fish – was initially raised, but the LiveFuels business model benefits from the lack of need for expensive bioreactors, machinery, or chemicals (Sibley, 2009). Furthermore, questions related to the ethics of harvesting fish for transportation fuels have been raised (McDermott, 2009).

4.3.7. Summary of Algae Harvesting and Dewatering Techniques

Table 7 summarizes the strengths and weaknesses related to algal harvesting and dewatering techniques.

Table 7: Summary of the five primary algae harvesting and dewatering techniques reviewed in this report.

Harvesting and Dewatering

Filtration / Microscreening

- **STRENGTHS:** Filtration is the most simple method of harvesting microalgae besides sedimentation. It is not an energy-intensive process.
- **WEAKNESSES:** Filtration is time consuming due to low flow rates (if suction is not applied) and limited yields can be expected. The potential for clogging exists since mass sticks to filter screen.
- **COST**: Very low (microstraining) or moderate (tangential flow filtration), relative to other methods

Centrifugation

- **STRENGTHS:** Centrifugation is highly efficient and can be applied to moderate volumes of algal culture at a time. It is best suited for cultures that are mostly liquid.
- **WEAKNESSES:** This process is highly energy intensive, which increases the operating cost. Also, a secondary watering step is still typically needed, which adds cost.
- **COST:** Very high, relative to other methods

Flocculation

- **STRENGTHS:** Flocculation can be applied to large volumes of culture at a time and can be used on most algae strains. Less energy is required that with mechanical separation methods.
- **WEAKNESSES:** Flocculation is typically paired with a complementary harvesting technique (e.g., flotation), and a dewatering step is still needed. Flocculation introduces new chemicals into the culture, which are difficult to remove. Finally, flocculants are often expensive and caustic.
- **COST:** Moderate to high (chemical flocculation) or very low (bioflocculation and autoflocculation), relative to other methods

Table 7: (Cont.)

HARVESTING AND DEWATERING (CONT.)

FLOTATION

- **STRENGTHS:** Flotation is an efficient method for algae removal with low water losses and simple implementation.
- **WEAKNESSES:** Flotation must typically be paired with a complementary harvesting method (e.g., flocculation), which adds cost.
- **COST:** Moderate (if not combined with flocculation) or high (if combined with flocculation), relative to other methods

OFF-SHORE METHODS

- **STRENGTHS:** Macroalgae are generally cheaper and easier to harvest than microalgae, and harvesting can occur on site before being sent ashore. Macroalgae that naturally washes ashore can also be diverted for fuel production.
- **WEAKNESSES:** A high water content is present in these methods so multiple dewatering techniques may be required. Also, a lower replenishment rate is seen in macroalgae that with microalgae.
- **COST:** Low, relative to other methods

4.4. Oil / Biomass Separation

- Microalgae
- Cyanobacteria
- Macroalgae

- Open Ponds / Raceways
- Photobioreactors
- Heterotrophic Fermenters
- Marine Systems

- Filtration / Microscreening
- Centrifugation
- Flocculation
- Flotation
- Off-shore Methods
- Other

- **Physical**
- **Chemical / Solvent**
- **Enzymatic**
- **Wet / Single Step**

- Transesterification
- Hydroprocessing
- Fermentation
- Anaerobic Digestion
- Gasification
- Pyrolysis/ Liquefaction
- Biophotolysis
- Purification of SVO

Most algal biofuels are produced by processing specific cellular components that must first be separated from the rest of the cell. Before oil-based biofuels (e.g., biodiesel) can be processed, for example, lipids housed within the algal cells must be isolated. Separating the various cell components can be achieved through physical, chemical, enzymatic, or other extraction methods. Some algal biofuel companies choose to use mature techniques already currently being used in other industries, while other companies invest in the R&D to find experimental "breakthrough" technologies. This section will identify and describe the most common methods for separating lipids from the rest of the cell biomass. Since most methods are new and still in the experimental phase, sufficient cost data is not publicly available and, therefore, is not addressed in this section.

Other algal biofuels may be achieved by converting entire algal cells, eliminating the need for cell fractionation, and will therefore not be addressed in this section. For example, whole algal cells can be gasified to create syngas, or anaerobically digested to create methane. Also, seaweeds generally do not contain lipids, so separation of lipids from seaweeds is not addressed in this section. This means that biomass separation for seaweeds will only consist of removing other elements such as stones (from seaweeds that grow on holdfasts), snails that may be present on the surface of the plant, debris, and sand (Bruton et al., 2009).

4.4.1. Physical Extraction

Mechanical Press. The most straightforward method for extracting oil from microalgal cells is by continuously feeding the culture through a mechanical press. Such presses have been used by commercial manufacturers of vegetable and other oils for many years to crush seeds, nuts, etc., releasing the cells' contents. Typically, the configuration of the press (e.g., screw, expeller, piston) is chosen based on the type of algal strains since physical attributes, such as cell wall thicknesses, can vary significantly.

Presses are typically capable of extracting up to 75% of oil from algae cultures (Global, n.d.). To increase this percentage, manufacturers often combine mechanical pressing with chemical solvents, which are further discussed in section 4.4.2. Use of genetically-modified algae could also help increase yield since their cell wall can be designed to more easily fracture.

Ultrasonication. The use of ultrasonic reactors, or sonicators, to extract cell components (e.g., trace minerals, nutrients) has been employed in the nutraceutical industry for years, and now biofuel manufacturers are integrating this method into their lipid extraction process. Typically, algal cells are suspended in a liquid medium within an ultrasonic reactor and are subjected to alternating low-pressure and high-pressure ultrasonic waves that create small vacuum bubbles throughout the medium. During the high-pressure cycles, bubbles of a certain size violently collapse (a process known as cavitation). If in close proximity of algal cells, the resulting shock waves and liquid jets can fracture the cell walls, allowing the cell contents to outflow, collect on top of the medium, and be skimmed off (Prince, n.d.).

Similar to mechanical pressing, sonication is commonly used in conjunction with another extraction methods to maximize yields and accelerate reaction time. Ultrasonic waves help solvents or enzymes more efficiently infiltrate cells. When used with solvents, the solution must be distilled to separate the oil from the solvent, adding an additional step to the extraction process. When used with enzymes, water can serve as the solvent, simplifying the overall process (Hielscher, n.d.). Solvent and enzymatic extraction methods are discussed in further detail in sections 4.4.2 and 4.4.3, respectively.

Overall, sonication considerably accelerates the diffusion process that would occur if only solvent extraction conditions were used. It is also environmentally nonthreatening and can be performed at a relatively low cost. However, the need for a secondary extraction technique adds cost and complexity to the entire extraction process.

BARD LLC, Cavitation Technologies, OriginOil, and Solix Biofuels are among the companies that have announced incorporation of ultrasonication into their lipid extraction process. Prior to transesterification of oil into biodiesel, BARD plans to extract lipids “by ultrasound disintegration of the algal cells” (Bard, 2010). Cavitation Technologies has patents pending for a reactor that creates cavitation bubbles in a solvent material that, upon collapse, result in the “sudden disintegration of unicellular and/or multi-cellular algal microorganisms and their intracellular organelles to release oil and other cell contents, while leaving the shell intact” (Cavitation, n.d.). In its novel “Quantum Fracturing” process, OriginOil uses a combination of microwaves and ultrasound to break down cell walls and extract key cell components (OriginOil, n.d.). Finally, Solix Biofuels (in partnership with LANL) announced intentions of using LANL sound wave technology to create ultrasonic fields that “concentrate algal cells into a dense sludge and extract oil” (Moresco, 2009).

In addition to lipid extraction applications, ultrasonic power has also been demonstrated as an aid in the transesterification of oil to biodiesel. Specifically, reaction times can be decreased, catalyst requirements can be reduced, and reaction temperatures can be lowered (Prince, n.d.).

Osmotic Shock. Although not yet commercially popular, the rupturing of algal cells through osmotic shock, or a sudden shift in solute concentration, has been demonstrated as a method for effectively extracting lipids. Osmotic shock is usually accomplished one of two ways (Lang et al., 2005).

1. Cultivate algae in a high saline medium (i.e., very salty water), harvest into a sludge, and then dump the sludge into salt-free water. Water rapidly enters through the cell walls to reach osmotic equilibrium, causing the cells to swell and rupture. Oil can then be skimmed off the surface.

2. Suddenly add large amounts of salt into the algae growth medium. Water will rapidly exit through the cell wall to reach osmotic equilibrium and prevents entry of salts and cofactors through the cell walls, essentially “shocking” the cells.

4.4.2. Chemical (Solvent) Extraction

As an alternative to physical extraction methods, chemicals are frequently used to extract lipids from within algal cells. Specifically, the use of solvents to extract algal oil is considered the most common of all extraction techniques due to its high percentage of recovered oil and low cost. In this process, the harvested algae are treated with a solvent, oils are released into the solvent, and the resulting mixture is then distilled to separate the oil from the solvent. The recovered solvent can then be reused in the process. Generally, this method recovers over 99% of the oil from harvested algal biomass.

The largest drawback to working with chemical solvents is the potential effects of handling hazardous materials. In fact, benzene, a common solvent, is a known carcinogen. In addition to the precautions that must be taken to avoid exposure to these chemicals, chemical solvents are often flammable and can trigger explosions.

Organic Solvents. Hexane is the most commonly used chemical solvent for oil extraction because of its low cost, but benzene and ether can also be used. Hexane solvent treatments are commonly used in combination with other extraction techniques, such as the mechanical press. Once the majority of oil is extracted with a press, for example, the remaining pulp can be treated with hexane, or a similar solvent, to dissolve excess oil. Hexane’s low boiling point (67°C / 152°F) and high oil solubility also support its use in oil extraction.

In some cases, a Soxhlet extractor is used to remove lipids from the algae through repeated washing with hexane or other organic solvents under reflux. As shown in Figure 13, the organic solvent heats in a flask, vaporizes, and flows through an extraction tube into a condenser tube where it returns to a liquid state. The solvent then drips down into a thimble harboring the sample in solution and dissolves the lipids. Once the solution rises to a certain level, it is repeatedly siphoned back into the flask with fresh, heated solvent. Each cycle is referred to as a reflux event. With each reflux event, the extraction rate is greatly increased (Wild and de Koning, 1997).

Figure 13: Schematic of a Soxhlet extractor (Image Source: Alex Tan).

Supercritical Fluids. As a substitute for organic solvents, CO_2 (or certain other compounds) may act as a solvent when it exceeds its critical point, at which point it possesses the properties of both a liquid and gas. In this energy-intensive process, the compound reaches this supercritical state under high levels of pressure and heat (see Figure 14). At this state, CO_2 can flow through solids like a gas and break down substances like a liquid (Supercritical, n.d.). Specifically, in this process, liquefied CO_2 is pumped into a chamber containing the algae mixture, dissolves the oil, and is then pumped into a separation chamber where the CO_2 is depressurized and completely evaporated, leaving no solvent residue in the extracted oil. The CO_2 is then captured and recycled for use in subsequent runs (Ghisalberti, 2008).

This extraction process is highly efficient with an oil extraction yield of nearly 100% (Ryan, 2009). The process is faster than most others because of the low viscosities and high diffusivity rates of supercritical fluids. Furthermore, supercritical fluids can cover a wide density range (Table 8) with small variations in pressure or temperature, which allows for simple manipulation of its properties by adjusting the pressure and temperature levels (Supercritical, n.d.). It also allows operators to pinpoint the materials that they would like to extract (in

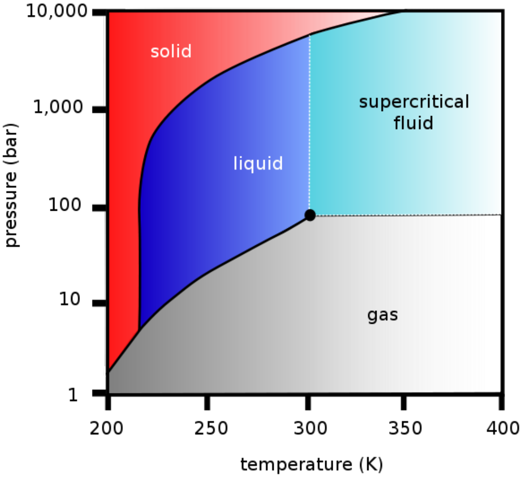


Figure 14: Carbon dioxide pressure-temperature phase diagram

this case, lipids from algal cells). However, this method is much more expensive and energy intensive than most other extraction techniques.

Table 8: Comparison of common values for gases, liquids, and supercritical fluids.

Comparison of Gases, Supercritical Fluids and Liquids (Supercritical, 2010)			
Property	**Density (kg/m^3)**	**Viscosity (cP)**	**Diffusivity (mm^2/s)**
Gas	1	0.01	1-10
Supercritical Fluid	100-800	0.05-0.1	0.01-0.1
Liquid	1,000	0.5-1.0	0.001

Supercritical CO_2 is currently used to decaffeinate green coffee beans and teas, extract hops for beer brewing, and aid in various nutraceutical applications (Eden, n.d.). For algal biofuels applications, supercritical fluid extraction is primarily used in bench-scale laboratory settings.

4.4.3. Enzymatic Extraction

As an alternative to more traditional approaches, algal cell walls can be broken down by enzymes, releasing the various cellular components into a solvent. In this process, water usually serves as the solvent, which has clear advantages over hexane. Not only are the potential chemical dangers removed, but oil and other cell contents are simpler to separate from water than from an organic solvent such as hexane. However, enzymatic extraction is estimated to be much more expensive than hexane solvent extraction and may not result in higher oil recovery rates than conventional methods (e.g., combination of pressing and hexane solvents) (North, 1994).

Like most other extraction techniques, enzymes are often used in conjunction with other complementary methods. While enzymes are capable of degrading cell walls, the addition of ultrasonication (referred to as sonoenzymatic treatment), for example, may accelerate this step and increase yield. Of course, additional methods typically increase overall production cost.

At the time of this report, no companies were identified as using enzymatic extraction in their continuous algal biofuel production process.

4.4.4. Wet Extraction

Wet, or “single step,” extraction, for example, allows producers to bypass the costly and energy-intensive dewatering step by extracting the oils while algae remains in water, without using hazardous chemicals or heavy machinery. Extraction in this manner has been demonstrated through pH modification, genetic optimization, electromagnetic fields, or a combination of these.

Among the advantages of this method, the cell cultures do not require dewatering, which saves considerable amounts of energy, cost, and time. Furthermore, since the algae are not killed, they can be used for multiple events. This characteristic allows companies to scale down the amount of biomass it must produce to maintain operations. As with other new and innovative extraction approaches, live extraction will likely undergo considerable amounts of R&D before it can be scaled up to commercial applications; therefore, large R&D funds will likely be needed by companies that pursue this route.

Multiple companies are currently pursuing wet extraction techniques that manipulate algal cells into continuously excreting oil into the ponds while keeping the cells alive. To date, this efficient process has been demonstrated by:

1. Soaking algae in solvents capable of sucking out the oil;
2. Using electrical modulations to stimulate algae to excrete oil; or
3. “Nanofarming” with mesoporous nanoparticles to extract oil.

Limited information is currently available on wet extraction methods used in industry. OriginOil has announced their new patent-pending Single-Step Extraction™ method that uses microbubbles, pulsed electromagnetic fields, and pH modification to accelerate the fracture of cell walls. After this process is completed, the processed culture moves to a settling tank where gravity fully separates the oil, water, and biomass. A schematic of this process is shown in Figure 15.

Synthetic Genomics is working on a genetically-modified algal strain that will continuously secrete oil into the medium (Synthetic, n.d.). Aurora Algae has also announced that it is experimenting with a “wet extraction” method to eliminate the dewatering step (Wesoff, 2009).

U.S.-based Phycal LLC accomplishes wet extraction that keeps the cells alive through its Olexal™ process, which continuously “milks” oil from algae without dewatering. In fact, some strains are capable of being reused up to four time or more (Kanellos, Feb ’09). In its first generation process using Olexal™, Phycal has set a cost target of 4 USD/gal (~160 USD/bbl) using natural algae grown in open ponds. For its future process, however, Phycal aims to reach 1-2 USD/gal using transgenic, biosecure algae in open ponds (Bargiel, 2009).

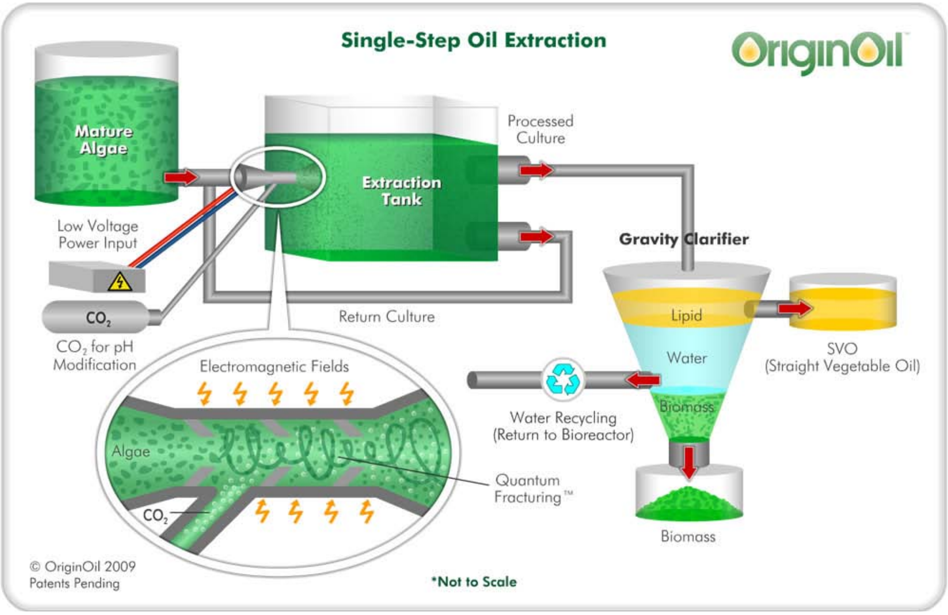


Figure 15: OriginOil's Single-Step Oil Extraction combines microbubbles, pulsed electromagnetic waves, and pH modification in their patent-pending extraction process (OriginOil, n.d.)

Similarly, OriginOil uses specific electrical modulations to stimulate algae cells in their patent-pending Live Extraction™ process, causing them to excrete oil without permanently damaging the cells (OriginOil, n.d.). Finally, a joint venture between the Ames Laboratory, Iowa State University, and nanotechnology company Catilin has led to R&D of nanoparticles that extract and capture oil from algae without killing the cells (Ames, 2009).

The Laboratory of Microalgal and Bacterial Bioenergetics and Biotechnology (L3BM) of the French Alternative Energies and Atomic Energy Commission (CEA) are exploring the possibility of having lipids migrate to the surface of the microalgae, where it would be relatively easy to harvest the lipids (Le Hir, 2010).

4.4.5. Summary of Oil / Biomass Separation Methods

Table 9 summarizes strengths, weaknesses, and industry activity for oil/biomass separation techniques.

Table 9: Summary of the primary techniques used to separate oil and biomass within algal cultures.

OIL / BIOMASS SEPARATION

PHYSICAL

- ***MECHANICAL PRESS***
  - **STRENGTHS:** Presses are widely used in industry and do not require the use of caustic chemicals. They are most useful for high-lipid algae strains and can extract up to 75% of lipids.
  - **WEAKNESSES:** Presses have high capital and maintenance costs, and are energy intensive. Oil and residual biomass do not easily separate, so secondary extraction techniques are typically required.
  - **INDUSTRY ACTIVITY:** PetroAlgae uses a mechnical press in its operations.
- ***ULTRASONICATION***
  - **STRENGTHS:** Dewatering is not needed beforehand, and the use of caustic chemicals is not required. This process is environmentally benign and has a relatively low cost.
  - **WEAKNESSES:** This process is energy intensive and typically requires a secondary extraction technique. It has not been demonstrated at industry scale.
  - **INDUSTRY ACTIVITY:** BARD LLC, Cavitation Technologies, OriginOil, and Solix Biofuels use ultrasonication in their operations.
- ***OSMOTIC SHOCK***
  - **STRENGTHS:** Osmotic shock does not require dewatering beforehand or the use of caustic chemicals.
  - **WEAKNESSES:** Osmotic shock is not commonly used in industry.
  - **INDUSTRY ACTIVITY:** No companies identified.

CHEMICAL / SOLVENT

- ***ORGANIC SOLVENTS***
  - **STRENGTHS:** These solvents are relatively inexpensive and can release over 95% of oil.
  - **WEAKNESSES:** Precautions must be taken when working with chemicals, and the permitting process for chemical use may delay operations. They may also have a negative impact on the environment.
  - **INDUSTRY ACTIVITY:** No companies identified.
- ***SUPERCRITICAL FLUIDS***
  - **STRENGTHS:** This process is very efficient and results in high quality oil. No solvent residues remain in the extracted oil. It is widely used in other industries and is environmentally friendly.
  - **WEAKNESSES:** This process is energy intensive, has high capital costs, and a risk is associated with high pressure operations.
  - **INDUSTRY ACTIVITY:** Global Green Solutions uses supercritical fluids in its operations.

Table 9: (Cont.)

OIL / BIOMASS SEPARATION (CONT.)

ENZYMATIC

- **STRENGTHS:** Enzymatic extraction does not require dewatering beforehand, and the use of caustic chemicals is not required.
- **WEAKNESSES:** Enzymatic extraction is very expensive compared to hexane extraction, and it is not commonly used in industry. Oil recovery is less than in conventional processes (e.g., pressing and hexane). Significant amounts of water and energy are also needed.
- **INDUSTRY ACTIVITY:** No companies identified.

WET / SINGLE STEP

- **STRENGTHS:** Using a wet or single step separation method eliminates the cost and time associated with dewatering. Neither hazardous chemicals nor heavy machinery are required. Cells may remain alive, and continuous extraction may be possible.
- **WEAKNESSES:** These are new techniques that are not commonly used in industry.
- **INDUSTRY ACTIVITY:** Companies that work with wet / single step separation methods include Catilin, OriginOil, Phycal, and Synthetic Genomics.

4.5. End-Use Fuel Production

Algal Strain Selection
- Cyanobacteria
- Microalgae
- Macroalgae

Algae Cultivation
- Open Ponds / Raceways
- Photobioreactors
- Heterotrophic Fermenters
- Marine Systems

Harvesting and Dewatering
- Filtration / Microscreening
- Centrifugation
- Flocculation
- Flotation
- Off-shore Methods
- Other

Oil / Biomass Separation
- Physical
- Chemical / Solvent
- Enzymatic
- Wet / Single Step

End-Use Fuel Production
- **Transesterification**
- **Hydroprocessing**
- **Fermentation**
- **Anaerobic Digestion**
- **Gasification**
- **Pyrolysis/ Liquefaction**
- **Biophotolysis**
- **Purification of SVO**

Algae and its cellular components have been considered as feedstocks to be processed to create a variety of end-use energy products, which include a wide range of liquid and gaseous transportation fuels. Such fuels include biodiesel; renewable gasoline, diesel, and jet fuel; ethanol; methane; synthesis gas; hydrogen; and straight vegetable oil (SVO). In the biophotolysis fuel production route, algae (or cyanobacteria) are not used as feedstock, but they are the actual producers of the fuel (hydrogen), which means that the algae are not consumed in this process. In addition to transportation fuels, dry algal biomass can also be directly combusted to create heat or electricity. The combusted biomass may consist of the entire algal cell, algal oil, or de-oiled algal cake, depending on a company's business model. Since the focus of this report is advanced motor fuels, combustion is considered outside of the scope and will not be discussed in detail.

This section investigates the advantages and disadvantages, technology status, and cost estimates for the major existing algae-to-biofuel conversion techniques. Figure 16 summarizes the multiple pathways for obtaining the various transportation fuels and other energy products.

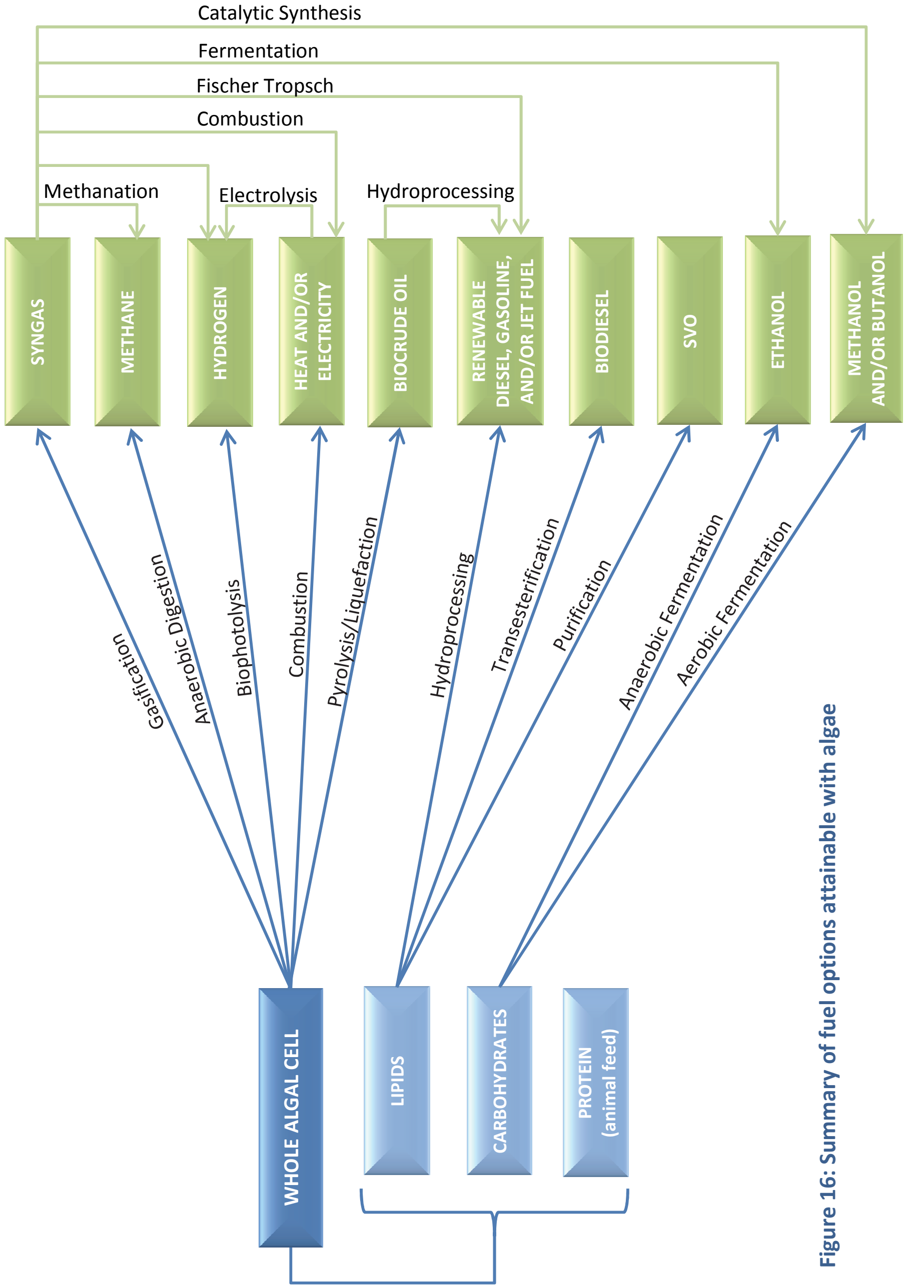
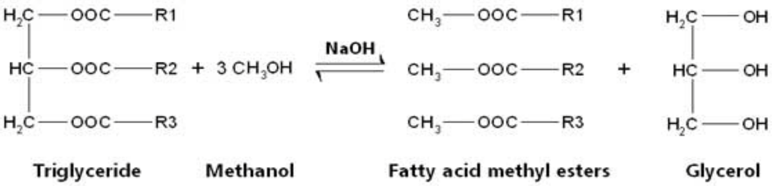


Figure 16: Summary of fuel options attainable with algae

4.5.1. Transesterification

General Description. Transesterification is a process commonly used to convert various vegetable or other land-based crop oils to biodiesel, since these oils are considered too viscous to directly run in a diesel engine. Algal lipids have similar makeup to land-based crop oils, and transesterification has been proven effective for converting these oils into biodiesel.

Biodiesel production via transesterification can be performed continuously or in batch; either way, the same series of steps applies. First, the oil is filtered of any dirt or contaminants, and water is completely removed. Then, triglycerides (three fatty acid molecules esterified with a glycerol molecule) are mixed with methanol or another alcohol in the presence of a catalyst. In the reaction, the triglycerides are converted to diglycerides, then monoglycerides, and finally into glycerol while three fatty acid methyl ester (FAME) molecules (biodiesel) remain, as shown in Equation 1. Since this is a reversible reaction, alcohol is usually added in excess to ensure all of the lipids have been converted to FAMEs (McIntyre, 2007).



(Eq. 1)

Careful observation to the amount of water and free fatty acids is taken to minimize soap formation and separation of glycerol. Other byproducts, such as water and excess alcohol, often occur and must be removed.

Transesterification catalysts are usually strong alkalis such as NaOH (as shown in Eq. 1), but acidic and enzymatic catalysts are sometimes used. An alkali catalyst can speed up the reaction by up to 4,000 times relative to natural conditions (Wörgetter et al., 2007). Assuming the alkali catalyst is used, the reaction can be completed in approximately 90 minutes at 60°C (140°F) under atmospheric pressure since methanol evaporates at 65°C under atmospheric pressure (Hussain, 2010). Variations of temperature, pressure, catalyst materials, and alcohols can be used in the transesterification process, but they usually add to overall cost of the operation.

In addition to traditional transesterification, supercritical alcohols (e.g., methanol, ethanol) are currently being researched to simultaneously act as mediums in oil extraction as well as catalysts in the transesterification process. Combining oil extraction and transesterification into one step has the potential to significantly improve production efficiency. To date, this process has only been demonstrated with vegetable oils but can be applied to other feedstocks, including algae (U.S. DOE, May ’10).

Strengths / Weaknesses. Transesterification is a well understood method for producing biodiesel and has been used for many decades. The end-use product (biodiesel) is non-toxic, biodegradable, and emits significantly less greenhouse gas (GHG) emissions than traditional diesel. Transesterification of oils results in biodiesel with a higher cetane number than traditional diesel, so it contributes to easy cold starting and minimal idle noise. The high lubricity of the biodiesel also enhances engine life (Joshi, 2006). Finally, use of algal oils as a feedstock does not directly compete with the food industry, like some vegetable oils (e.g., corn or soybean), and they can be cultivated and processed on non-arable land.

The difficult removal of byproducts that remain after transesterification is considered a major disadvantage for transesterification. Glycerol, a major byproduct, is much denser than biodiesel and can be separated with gravity (Figure 17) (Kyndt, 2010). Excess methanol remains in the glycerol and biodiesel, so this is commonly removed with flash evaporation or distillation and is sometimes reused. (If residual methanol remains in the biodiesel, engine failure can result.) Acid is added to the glycerol byproduct to neutralize the excess catalyst and soaps, and the biodiesel is washed with warm water to remove any remaining catalyst and soaps.

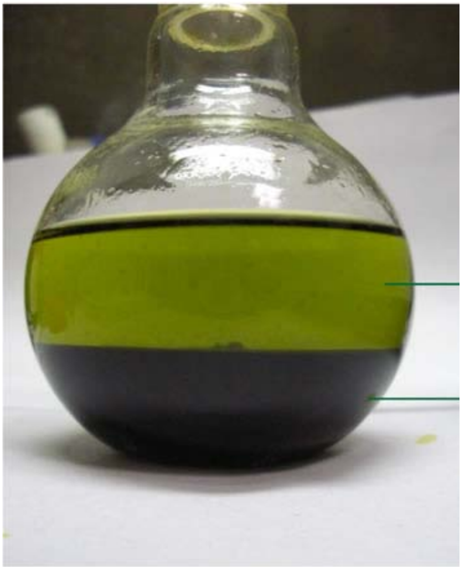


Figure 17: Primary byproducts of transesterification are biodiesel on the top half and glycerol on the bottom half (Image Source: www.AlgaeForBiofuels.com).

Cost Information. According to a techno-economic analysis performed by NREL researchers, the cost to transesterify algal oil in biodiesel based on current achievable production parameters is 2 to 3 times higher than the price of fossil diesel fuel (Darzins, Pienkos, & Edye, 2010), which has ranged between 2.70 and 3.20 USD/gal throughout 2010 (U.S. EIA, Nov ‘10).

As more productive algal strains are identified and the price of algal oil declines over the next decade, this estimated price may fall to a level that is competitive with traditional diesel prices. To provide a frame of reference, the price of biodiesel transesterified by other more mature alternative feedstocks is 3.45-3.95 USD/gal for animal fats, 4.00-4.50 USD/gal for soy oil, and 4.20-4.275 USD/gal for palm oil, as of December 2010 (Ferris, 2010).

Industry Activities. Several companies are attempting to scale algal biodiesel up to commercial production levels. Aurora Algae, BARD LLC, Catilin, Green Star Products, Inventure Chemical (with Seambiotic), and Solazyme (with Chevron) are among the private companies pursuing advancements in this market. Aurora Algae was among the first companies to scale up algae-based biodiesel production with a pilot biodiesel algae facility that has been in operation since August 2007 (Aurora, 2010).

4.5.2. Hydroprocessing

General Description. A rising trend in the algal biofuels market involves catalytic hydroprocessing of algal oil to produce "drop-in" hydrocarbon fuels with very similar chemical structures and energy contents as standard gasoline, diesel, and jet fuel. As a result, these green fuels can be blended with their petroleum-derived counterparts without any necessary adjustments to the vehicles, distribution system, or fueling infrastructure.

Hydrotreating and hydrocracking are the two main forms of hydroprocessing that can be used to upgrade oils to transportation fuel quality. Both methods remove undesired elements and contaminants (e.g., sulfur, nitrogen, metals), use similar hardware, and hydrogenate the oil. However, hydrocracking is considered to be a more severe process, using higher temperatures, pressures (Chorkendorff and Niemantsverdriet, 2007), and catalyst volumes to break carbon-to-carbon bonds and reshape molecules. In hydrotreating, only minimal molecular changes occur, and the process occurs at a lower temperature (approximately 300-390°C) and pressure (approximately 50-150 bar) (Gevert and Otterstedt, 1987). In recent decades, hydrotreating has become much more commonly used in this industry than traditional hydrocracking (Robinson and Dolbear, 2007).

In preparation for hydrotreating, algal lipids typically undergo hydrolysis to separate fatty acid chains from the glycerol backbone. Next, the free fatty acids are deoxygenated by adding large amounts of hydrogen to the oil, often in the presence of a catalyst, to reach desired energy density levels. The resulting products of this deoxygenation step are *n*-alkanes and CO_2. The *n*-alkanes can then be separated and processed into end-use products (e.g., renewable gasoline, diesel, jet fuel). A breakdown of the key steps of hydrotreating is provided in Figure 18 using a schematic of Diversified Energy's and North Carolina State University's (NCSU) Four Step Centia™ Biofuels Production Process (Diversified, n.d.).

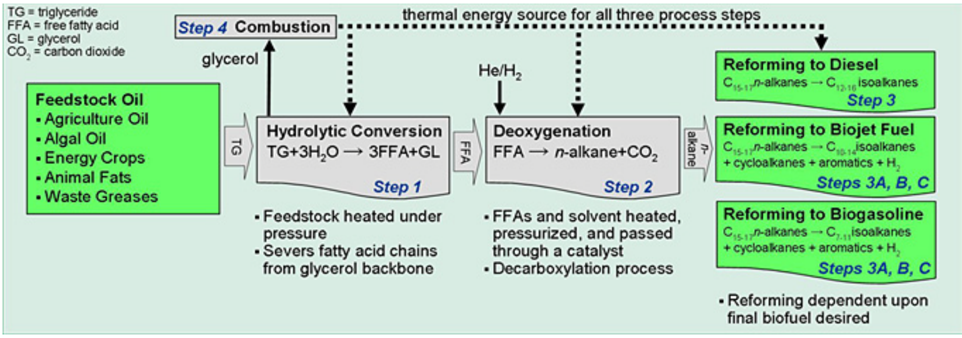


Figure 18: Four-Step Centia™ Biofuels Production Process developed by Diversified Energy Corporation and North Carolina State University (Diversified, n.d.)

Strengths / Weaknesses. The greatest advantage of hydroprocessing algal oil is that the resultant renewable fuel can simply be mixed with petroleum-based fuels since they essentially have the same chemical structure. No adjustments to existing vehicles or infrastructure are required. The fuels are also compatible with existing fuel standards. Hydroprocessed end-use fuels are actually higher quality than petroleum-based fuels because they are virtually free of sulfur and nitrogen compounds. They also have higher energy contents than biodiesel or alcohol.

Hydrotreating appears to be a more appropriate method for processing algal oils than hydrocracking since they are not very heavy oils and typically do not need reforming at the most basic molecular levels. Furthermore, hydrotreating occurs at much lower temperatures and pressures, which reduces energy costs.

Cost Information. Because hydroprocessing of algal oil is a relatively new practice, limited cost data is publicly available. According to a techno-economic analysis performed by NREL researchers, the cost to hydroprocess algal oil into green diesel based on current achievable production parameters is 2 to 3 times higher than the price of fossil diesel fuel (Darzins, Pienkos, & Edye, 2010), which has ranged between 2.70 and 3.20 USD/gal throughout 2010 (U.S. EIA, Nov ’10). However, according to Barbara McQuinton, special assistant to energy for U.S. Department of Defense’s DARPA, the Agency is on track to hydroprocess algal oil into jet fuel at a price below 3 USD/gal. This price projection includes the cost of extracting the algal oil to be processed (Kagan, 2010).

Industry Activities. Government funding has driven the launch of many projects and partnerships related to hydroprocessing of algae. In December 2009, for instance, Solazyme Inc. was awarded a 21.8 million USD federal grant to build its first integrated algae fuel refinery. At this refinery, Solazyme (who has partnered with Chevron Corp.) will enhance its technology portfolio that includes converting algal oil into renewable fuels that are fully compatible with current petroleum-based fuel infrastructure (Solazyme, 2009). Sapphire Energy also benefited from a DOE grant valued at 50 million

USD (plus 100 million USD contributed by private investors) (Foroohar, 3 June ‘10) to convert pond-grown algae into green fuels, including jet fuel and diesel, with their Dynamic Fuels refining process.

DARPA recently announced that it will be producing algal-based jet fuel via hydroprocessing activities that are cost-competitive with petroleum-based fuel in the near future. DARPA has played a substantial funding role in the research of algae-based jet fuel. Recipients of multi-million dollar funding opportunities have included SAIC, General Atomics, the Energy & Environmental Research Center (EERC), and UOP (a Honeywell company) (Ritch, Dec ’08).

In addition to government support, UOP has established multiple partnerships to help commercialize its Ecofining process that can produce renewable fuels from second generation, non-food feedstocks (e.g., algae, cellulosic waste). With the aid of a 25 million USD award from DOE, the company will begin construction on a demonstration unit at Tesoro Corp’s Hawaii-based refinery to upgrade pyrolysis oil produced by Ensyn Corp. to green fuel (Millikin, Jan ’10). The unit, expected to start up in 2014, is designed for a capacity of 232,400 L/yr (61,320 gal/yr). In addition, UOP has partnered with Boeing to demonstrate its renewable jet fuel in flights operated by Air New Zealand, Continental Airlines, Japan Airlines, and KLM Airlines (UOP, n.d.).

As shown in Figure 18, Diversified Energy Corporation and NCSU use their patent pending Centia™ Advanced Biorefinery Process to convert triglycerides into renewable fuels. Unlike traditional hydrotreating processes, Centia™ is differentiated by using a catalytic decarboxylation route to deoxygenate oil (in lieu of adding large amounts of hydrogen). Diversified Energy and NCSU received an award by DOE’s Advanced Research Projects Agency – Energy (ARPA-E) in November 2009 valued at 5.2 million USD (Diversified, 2009).

Finland-based Neste Oil has developed a hydrotreating technology referred to as NExBTL (a **NEx**T generation **B**iomass **T**o **L**iquid technology) that produces high-quality synthetic diesel fuel from vegetable oils and animal fats. Neste Oil has been researching the potential of algal oil as a feedstock for NExBTL and plans to produce its first trial batch of NExBTL renewable diesel from algal oil within the year (Flinkkila, 2010).

Finally, several companies have built their businesses by producing and selling biocrude, the renewable equivalent of petroleum, to refineries that are ready for hydroprocessing into renewable fuels. Such companies include PetroAlgae, Kai Bioenergy Corp., Solix Biofuels, and SunEco Energy.

4.5.3. Fermentation

General Description. The concept of fermentation can be applied to multiple algae-to-fuel pathways. For example, as previously discussed in section 4.2.3, certain strains of algae are grown in closed bioreactors by ingesting sugar fed into the system, reaching high volumes of oil to be processed into biodiesel and other end-use fuels. Other strains are instead selected for fermentation because their carbohydrates are capable of directly producing alcohols, such as ethanol, presenting one of the most direct pathways from algae cultivation to biofuel, which will be discussed in this section.

Alcohol can be produced through fermentation by one of two primary ways:

1. **With light,** where starch is created and stored through photosynthesis and fermented intracellularly. Resulting ethanol is excreted into the medium. The formula for this process is:

$$2CO_2 + 3H_2O \rightarrow C_2H_5OH + 3O_2 \qquad \text{(Eq. 2)}$$

2. **Without light,** where sugar is fed to the system, which is anaerobically fermented (in absence of oxygen) with the aid of microorganisms (e.g., bacteria or yeast). The fermented mixture is then processed into alcohol and CO_2. This formula for this process is :

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 \qquad \text{(Eq. 3)}$$

Aerobic fermentation, which occurs in the presence of oxygen, can be exercised to produce butanol and methanol. However, this is a more complicated process that entails glycolysis, a Krebs cycle, and electron transport. The alcohol yields associated with aerobic fermentation are often low, and recovery is considered a challenge. Additional R&D is likely needed before these products become cost competitive in the market.

Cyanobacteria and macroalgae are strong candidates for fermentation to ethanol since they have high carbohydrate contents. However, seaweed composition differs from land plants so marine biorefineries should be adapted to the feedstock. The presence of salt, for example, should be carefully managed because it may be an inhibitor of fermentation processes. Also the sugars present in seaweeds may differ from land plants. The sugars present in the brown seaweed *Laminaria* are not easily fermented, so they require a biochemical or thermo-mechanical process to break them down prior to fermentation, or a new direct fermentation process has to be developed. Green seaweeds such as *Ulva* contain more easily accessible sugars such as starch and cellulose (Bruton et al., 2009).

Strengths / Weaknesses. The greatest advantage of using heterotrophic fermentation to produce ethanol is that it eliminates the need to dewater the algal culture, extract oil, and process the oils. Without the costs that each of these steps represent, fermentation becomes more cost competitive. Also, if photosynthesis is used to drive fermentation ("**with light**" option), large volumes of CO_2 can be redirected from industry sources and fed into the system as opposed to being released into the atmosphere, although it will be released later when the biofuel is combusted in a motor vehicle engine.

Furthermore, as mentioned in section 4.2.3, if the process occurs "**without light**," the cost and energy associated with artificial light is eliminated, and a deep, high-volume vessel can be used since light penetration is not an issue. If light is not used, relatively small amounts of water are needed in heterotrophic fermentation, and the algae that grow in this process do not require CO_2 absorption. Finally, fermenters are widely used in the brewing industry, so the design is highly mature (Wageningen, 2010).

Again, if sugar must be fed into the system (see "**without light**" option described previously), concern has been voiced that this process may add to the "food vs. fuel" debate where this process could be displacing food for the purposes of fuel production. Therefore, operators should consider using cellulose-derived sugar that will not interfere with the food supply.

Cost Information. With only one major company (Algenol Biofuels) pursuing ethanol production via fermentation of algae, insufficient cost information is publicly available at the time of this report's publication.

Industry Activities. Algenol Biofuels incorporates fermentation into its Direct to Ethanol™ process. As described in the "**with light**" option described previously, Algenol's cyanobacteria is genetically enhanced to ferment photosynthetically-produced sugar intracellularly, and then the resultant ethanol diffuses into the seawater medium. This process takes place outdoors in flexible film PBR tubes. The water/ethanol mix evaporates, forms condensation on the inner surface of the roof, and eventually runs down the sides of the tube, where it is collected in troughs that span the distance of the PBR (Figure 19). Algenol claims that its Direct to Ethanol™ process can yield 6,000 US gal/acre/yr (approximately 56,000 L/ha/yr), which is significantly higher than corn and sugar cane yields (Algenol, n.d.).

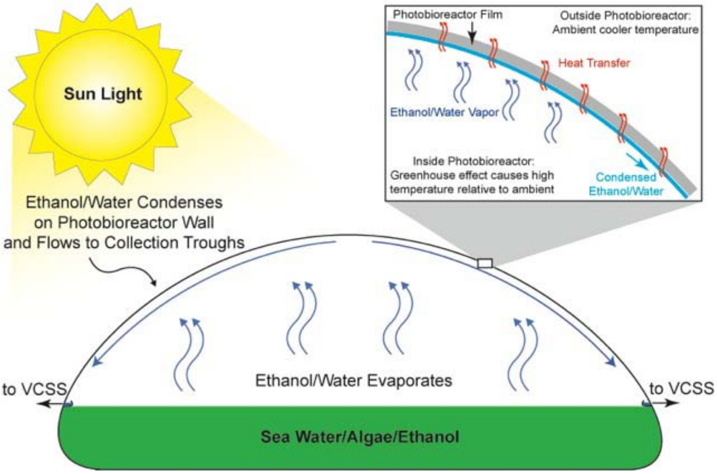


Figure 19: Algenol's proprietary PBRs do not require harvesting or dewatering of algal cultures (Algenol, n.d.).

4.5.4. Anaerobic Digestion

General Description. Anaerobic digestion is a biochemical reaction (Figure 20) that reduces complex organic compounds, such as algae, down to methane and CO_2 in the absence of oxygen. The resultant methane gas can be compressed and used as a motor fuel in the form of natural gas. Traditionally, three separate groups of bacteria are used during anaerobic digestion. Assuming whole algal cells enter a digester, lipids must first be broken down to fatty acids, carbohydrates to monosaccharides, and proteins to amino acids. This step, referred to as hydrolysis, usually entails a collection of enzymes excreted by hydrolytic and fermentative bacteria. Next, acetogenic bacteria are responsible for converting these acids and alcohols into acetate, CO_2, and hydrogen. Finally, methanogenic bacteria complete the conversion of these products into CO_2 and methane (Vergara-Fernandez et al., 2008).

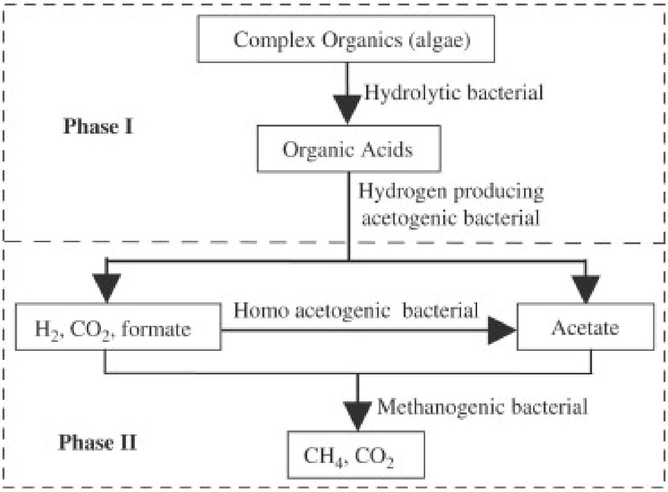


Figure 20: Common anaerobic digestion process (Vergara-Fernandez et al., 2008).

The natural sugars and other carbohydrates found in macroalgae can also be used to produce methane via anaerobic digestion. Many different parameters influence the actual energy yield from seaweeds. These include the presence of debris and sand, microbial degradation that may have occurred between harvesting and processing, water content, seaweed composition, digestion and fermentation process parameters, scale, etc. Table 10 presents possible methane yields from seaweeds. Little background information is available on the actual processes for which these yields have been obtained, and therefore, they should only be used as a first indication. These yields are likely obtained in small-scale installations or are estimates, because no large-scale facilities exist yet.

Table 10: Indications of methane yields of seaweeds.

Seaweed genera	Methane Yield (m^3/kg)	Remarks	Reference
Laminaria	0.26 – 0.28	--	Carlsson et al., 2007
Laminaria	0.3 – 0.48	Maximum, per kg organic matter	Reith et al., 2005
Miscellaneous	0.12 – 0.41	--	Carlsson et al., 2007

Strengths / Weaknesses. Anaerobic digestion removes certain barriers associated with other algal biofuel processes. For example, dewatering of algal cultures and extracting of oils are unnecessary in anaerobic digestion, which may reduce production costs. Furthermore, anaerobic digesters are less selective of algal strains compared to other methods, so measures to reach high lipid yields are not as important. Therefore, settings where various strains are growing uncontrolled, such as wastewater treatment plants, may be ideal for digestion.

Because methane gas has a greenhouse effect 21 times that of CO_2, the biogas created in anaerobic digestion needs to be controlled. Since the reaction occurs in totally enclosed systems, the level of control needed can be accommodated. Furthermore, methane can be combusted or otherwise converted into products with lower CO_2 intensities. Compost material and nutrient-rich water, the two primary byproducts of anaerobic digestion, hold considerable market value in the agricultural industry and can be reused as fertilizer materials (IEA, 2001). If algae and biomass (e.g., wood) are the only inputs, the fertilizer could even be certified as organic.

Cost Information. Anaerobic digestion of waste streams is well established throughout Europe; however, algae have not been substituted as a biomass on a commercial scale. Therefore, cost data is only available on traditional biomass inputs (e.g., municipal solid waste). Several factors must be considered when projecting the capital and operating costs of an anaerobic digestion facility. For capital costs, the plant size, location, type of feedstock, and potential pretreatment needs should be factored into the cost equation. Operating costs are primarily related to labor, transport of inputs and products, and pollution abatement and control measures. Finally, additional income originating from the sale of byproducts, electricity, or heat may be deducted from annual expenditures. Despite the numerous factors that determine overall costs, an anaerobic digestion facility designed to process 10,000-20,000 metric tons per year is generally expected, on average, to accrue approximately 3.25-4.25 million GBP in annual capital costs and approximately 100,000 GBP in annual operating costs (Seafish, 2005).

Industry Activities. Algae Aqua-Culture Technology (AACT) is developing a system to cultivate algae and convert it, along with wood waste, into methane, heat, electricity, and high-quality organic fertilizer. AACT refers to this system as a “Green Power House.” The company, based in Montana, uses a unique two-stage digester that separates the bacteria by using precise temperature controls during the various phases. AACT received a 350,000 USD grant under ARRA funding to build a Green Power House (Alternative, 2010).

BioMara, a joint project conducted by UK and Irish researchers, will be assessing the feasibility of converting microalgae and macroalgae into methane via anaerobic digestion. In addition to methane, the BioMara project will include similar assessments on biodiesel and ethanol (Biomara, n.d.).

4.5.5. Gasification

General Description. Algal biomass can be converted into a syngas in a thermochemical process called gasification. The versatile syngas that results is primarily comprised of carbon monoxide (CO), CO_2, and hydrogen, but can also include nitrogen, methane, water, tar, and ash particles. Several of these syngas components can act as intermediates in the production of transportation fuels, such as hydrogen, ethanol, and methanol. The gases can also be directly combusted in turbines to produce electricity, or they can be converted to liquid alkanes via Fischer-Tropsch synthesis. Product proportions vary with the characteristics of the feedstock (e.g., moisture content) and gasifier (e.g., temperature, pressure, catalyst) (Ryan, 2009).

In conventional gasification, dry algal biomass (less than 15-20% moisture content) is reacted at temperatures ranging from 800°C to 1,000°C with a controlled amount of oxygen or water (Ryan, 2009). Supercritical water, or hydrothermal, gasification is a process that allows the reaction to occur at lower temperatures – under 350°C using a metal catalyst and under 700°C with the aid of a carbonaceous or alkali catalyst (Ryan, 2009). Lower operating temperature may accommodate smaller gasifiers and reduced energy input. Supercritical water gasification may also permit biomass with higher moisture contents, which will help eliminate costs and energy expenditures associated with dewatering algae.

Strengths / Weaknesses. The greatest benefit of gasification is the wide variety of end-use fuels and valuable byproducts that can be derived from the syngas. The process is also widely used in industry, since almost any organic material can be fed into the system (e.g., biomass, wood, plastic). Gasification is also considered to be more efficient than direct combustion (Berinstein, 2001).

Opportunities for synergies exist when considering algae as a feedstock for gasification. For instance, excessive heat from the reactor can be redirected with a heat exchanger to help dry algae in preparation for gasification.

Several challenges of gasifying algae are present, at least in the near term. First, in order for the process to be cost-efficient, large-scale production is likely necessary, which would likely either require 1) a transition of feedstocks within an existing reactor or 2) significant capital expenditures to construct a new reactor. Second, reactor operations and system inputs would need to be tailored to optimize syngas characteristics. Finally, regardless of feedstock type, removal of tar accumulation and other unwanted byproducts in conventional gasification adds steps to the overall process.

Cost Information. Limited information is available on the operating costs associated with using algae as a feedstock for gasification. Operating costs including fuel of 1,000 USD/yr for biomass-based integrated gasification combined-cycle power systems with outputs ranging from 56-132 MWe are estimated by NREL to fall between 13.4 and

28.7 million USD/yr (Craig and Mann, 1996). Capital costs for the same power systems are estimated by NREL to fall between 1,100 and 1,700 USD/kW. The U.S. Energy Information Administration (EIA) estimations are very consistent, with the assumed overnight capital cost for a biomass integrated gasification combined cycle plant estimated to be approximately 1,500 USD/kW (in 2000 dollars) (U.S. EIA, 2001).

Generally, biomass has to be fairly dry to undergo gasification, so significant cost reductions in dewatering of algal biomass during the harvesting phase would be necessary in order for gasification to be a cost effective conversion method in the near term. However, some companies are working on ways to increase the allowable water content (read the following description of Genifuel Corporation's technology).

Industry Activities. Very few biomass gasification reactors are in operation on an industrial scale. One company named Genifuel Corporation uses their patent pending Catalytic Hydrothermal Gasification (CHG) technique that dramatically reduces the operating temperature and accommodates biomass (including algae) with higher moisture content. The Genifuel process, developed by researchers at the Pacific Northwest National Laboratory, outputs syngas composed of 60% methane and 40% CO_2 (Genifuel, 2010). In addition, the Solena Group uses a patented plasma gasification technology that operates at over 5,000°C to convert carbon-based feedstocks into syngas, which can be used in place of natural gas or for power production (Solena, n.d.).

4.5.6. Pyrolysis/Liquefaction

General Description. Pyrolysis and liquefaction are both thermochemical pretreatments that can be applied to organic material under high temperature and high pressure to produce an intermediary bio-oil of low viscosity, which can then be hydroprocessed to produce renewable diesel, gasoline, and jet fuel. Application of either method to algae is relatively new, and large-scale production is likely several years away.

In pyrolysis, dry biomass is thermally decomposed in the limited presence of oxygen. When cooled, bio-oil, wastewater, and CO are the key outputs (Ryan, 2009). Additional byproducts, including charcoal and phenol-formaldehyde, can be used in fertilizer and animal feed production. Flash, or "fast," pyrolysis can be performed between 350-500°C for less than 2 seconds if the feedstock is finely ground (U.S. DOE, May '10). This is more efficient than the conventional slow pyrolysis and results in higher quality bio-oil. Since fast pyrolysis of algae is a newly investigated process, literature on process yields, bio-oil compositions, and optimal operating conditions is limited and inconsistent.

Liquefaction is a newer process where wet biomass (≥60% moisture content) can be decomposed at approximately 300°C and pressures between 10-20 MPa, and reformed into simpler molecules with higher energy density and/or more market value than the original biomass (U.S. DOE, May '10; University, n.d.). The water from the biomass helps facilitate the breakdown of chemical bonds and reforming of new molecules. The process is not as rapid as fast pyrolysis, but it can still occur in a matter of only minutes or hours (Ryan, 2009). While the resultant bio-oil can be hydroprocessed for liquid fuels, remaining residue can also be directly combusted to create heat or electricity or fermented into animal feed or fertilizer.

Strengths / Weaknesses. Pyrolysis and liquefaction both occur very quickly compared to other conversion techniques, especially if fast pyrolysis is performed. Unfortunately, pyrolysis/liquefaction is only one of at least two steps required to achieve a compatible, end-use fuel. In the case of fast pyrolysis, dry algal biomass is appealing since the culture is a collection of single cells and would be simple to grind into fine particles.

A major barrier to cost-effective pyrolysis of algae lies in the required moisture content, which is very low. Therefore, the efficiency and cost of dewatering techniques will play a critical role in the commercial viability of pyrolysis. Most other aspects of the technology are relatively mature, so technology breakthroughs related to the actual pyrolysis process are not anticipated. Liquefaction, on the other hand, is void of costs associated with dewatering; the water instead plays an important role in the reaction process.

Cost Information. Limited cost data is available on both pyrolysis and liquefaction of algal biomass to make renewable diesel, gasoline, and jet fuel since this application is relatively new; however, a few general conclusions can be drawn based on the process characteristics. Since biomass has to be fairly dry to undergo pyrolysis, major cost reductions must first be met regarding dewatering of algal biomass during the harvesting phase in order for pyrolysis to be a cost-effective conversion method in the near term. Liquefaction, on the other hand, accommodates biomass with greater water content, which may eliminate some upstream costs, and operates at lower temperatures, which may reduce operating costs relative to pyrolysis. However, the increased operating time may negate some or all of the savings achieved due to reduced temperature.

Industry Activities. Envergent Technologies, a joint venture between UOP (part of Honeywell) and Ensyn Corp., is using its Rapid Thermal Process (RTP™) to convert biomass to pyrolysis oil, and then upgrade the oil to transportation fuel quality with a hydroprocessing unit. Fast pyrolysis at approximately 500°C in the absence of oxygen is employed in this process (Envergent, 2010). The system can be used to convert algae residue into pyrolysis oil, which the company may choose to investigate in the future (Honeywell, 2010).

4.5.7. Biophotolysis

General Description. A number of microalgae and cyanobacteria are able to split water into hydrogen and oxygen, using light as the energy source, in a process called biophotolysis. This is different from other options of algae for biofuels, where the algae are converted into liquid fuel. In biophotolysis, the algae are naturally producing the fuel. Direct biophotolysis is the simplest form of photobiological hydrogen production (Reith, Wijffels, & Barten, 2003). It can be considered the biological equivalent of electrolysis of water.

The green microalgae *Chlamydomonas reinhardtii* has been studied extensively for its hydrogen generating capacities. The enzyme hydrogenase, which is present in the algal cells, is the catalyst for hydrogen formation. Unfortunately, the oxygen that is produced simultaneously deactivates the hydrogenase, and thus prevents sustained hydrogen production (Kruse et al., 2005; Reith, Wijffels, & Barten, 2003; U.S. DOE, May ’10; Yu and Takahashi, 2007). However, it has been shown that sustained photobiological hydrogen by *Chlamydomonas reinhardtii* is possible in a sulfur-deprived medium under anaerobic conditions (Reith, Wijffels, & Barten, 2003; Yu and Takahashi, 2007).

Cyanobacteria can also use the enzyme nitrogenase for hydrogen formation in air or another nitrogen-containing atmosphere, while hydrogen is a minor byproduct. Therefore the conversion efficiency from light to hydrogen by nitrogenase is quite low. However, nitrogen starvation is an efficient way to increase hydrogen productivity. This can be achieved by using a nitrogen-free gas such as argon plus CO_2 as the atmosphere in which the cyanobacteria live (Yu and Takahashi, 2007).

Table 11 presents an overview of the energy productivity in the form of hydrogen for biophotolysis by microalgae and cyanobacteria. It should be noted that all the values in this table have been obtained in laboratory scale experiments. There is no information available yet if these values can be achieved or maybe even exceeded in large-scale production.

To improve the hydrogen yield of biophotolysis, other options are under investigation or have been suggested as well:

- Preventing oxygen inhibition of the hydrogenase enzyme in green microalgae by indirect biophotolytic processes that separate the oxygen and hydrogen production stages in space or time. These processes are currently under development (Laboratory, 2009; Reith, Wijffels, & Barten, 2003; Yu and Takahashi, 2007).

- Removing oxygen immediately after it is formed to increase the photochemical efficiency of the biophotolysis process (Reith, 2003).

- Identifying the mechanisms behind the oxygen sensitivity of hydrogenase, to find ways to reduce this sensitivity (Laboratory, 2009). In other words: engineering hydrogenases with improved oxygen tolerance (U.S. DOE, May ’10).

Table 11: Energy productivity in the form of hydrogen for biophotolysis by microalgae and cyanobacteria, obtained in laboratory experiments. The energy is expressed per liter liquid volume of the culture in the bioreactor, and per hour.

	Organism	Maximum hydrogen productivity (kJ/L/h)	Reference
Direct biophotolysis			
Green microalgae	*Chlamydomonas reinhardtii Stm6*	0.02 (*)	Kruse et al., 2005
Green microalgae	*Chlamydomonas reinhardtii*	0.02 - 0.12	Yu and Takahashi, 2007
Cyanobacteria	*Anabaena*	0.02 - 0.22	Yu and Takahashi, 2007
Indirect biophotolysis			
Green microalgae	*Chlamydomonas reinhardtii*	0.03 - 0.05	Yu and Takahashi, 2007
Cyanobacteria	Miscellaneous	up to 0.38	Yu and Takahashi, 2007

(*) 540 ml H_2 (up to 98% pure) was generated in about 300 hours (Kruse et al., 2005). Calculating with an energy density of 10.3 kJ/L for gaseous hydrogen; this results in 0.02 kJ/L/h.

- Engineering the photosynthetic system to increase the efficiency of solar light utilization (Argonne, 2004; U.S. DOE, May ’10).

- Developing biohybrid (with both biological and synthetic components) and synthetic photosynthesis processes that mimic photosynthetic organisms (U.S. DOE, May ’10). Recently the universities of Bochum and Münster in Germany reported that they have succeeded in realizing in vitro hydrogen production via photosynthesis, using components of the green algae *Chlamydomonas reinhardtii* (Winkler et al., 2009).

- Genetic modification of the algae or the cyanobacteria (Kruse et al., 2005; Reith, Wijffels, & Barten, 2003).

- Find new organisms with higher light conversion efficiencies (Argonne, 2004).

Strengths / Weaknesses. Water biophotolysis by microalgae or cyanobacteria is a clean and renewable way of hydrogen production. When hydrogen is used for vehicle propulsion, in principle the vehicle only emits water vapor as exhaust product. This fully holds for fuel cell vehicles. When hydrogen is used in an internal combustion engine (ICE), also NO_x is also being formed, and a very small amount of hydrocarbons (HC) originating from the engine oil may be generated. However, with an appropriate engine and exhaust system design, ICE vehicular NO_x and HC emission levels will be very low. When in other stages of the well-to-wheel fuel chain, such as hydrogen transport and compression, if renewable energies are used, a sustainable closed loop without any CO_2 emissions is possible.

Biophotolysis produces an alternative automotive fuel with its own strengths and weaknesses. Hydrogen is a clean fuel when produced in a sustainable manner and it has a high energy density per kilogram. On the other hand its volumetric energy density is low, so on-board storage requires dedicated measures such as high-pressure cylinders or a well-isolated tank for storage at low temperature.

Cost Information. Regarding biophotolysis feedstocks, water and sunlight are widely available at low cost. The cost to acquire microalgae or cyanobacteria would need to be included. The equipment for cultivation and biophotolysis is expected to be the major cost factor for hydrogen production. Also transport and storage of hydrogen will have an impact on the final costs for the user. Scaling up laboratory size equipment to fuel production plants to estimate hydrogen costs may lead to unrealistically large hydrogen production facilities. Nevertheless, such an exercise indicates that, on the basis of hydrogen energy content that is produced, direct biophotolysis would be much more expensive than indirect biophotolysis. Because of the lower productivity that is assumed for direct biophotolysis the production plant is much bigger, with associated increases in production and labor costs (Resnick, 2004). Literature suggests that based on ‘favourable assumptions’, a preliminary cost indication of photobiohydrogen would be 10-15 EUR/GJ (Reith, Wijffels, & Barten, 2003). A timeframe or a production scale for this estimate is not mentioned.

Industry Activities. Biophotolysis is still mostly in the research phase, but the production of hydrogen by microalgae and cyanobacteria via biophotolysis has been demonstrated in laboratory settings. Some industries are teaming up with universities and other research institutes. One example is the Solar Biofuel Consortium, consisting of eight universities in Australia, Germany and the United Kingdom, and a number of companies that are active in similar sectors (Solar, 2010). In Canada, Solarvest BioEnergy uses genetically-modified microalgae to cyclically produce hydrogen under laboratory conditions. Unlike most algae-based hydrogen production systems, Solarvest has consolidated the process into a single bioreactor instead of two separate bioreactors (Solarvest, n.d.).

4.5.8. Purification of SVO

General Description. Most commonly, extracted algal oil is esterified to produce biodiesel. However, if left unrefined, algal oil can act as an SVO and, therefore be used in SVO applications. For example, SVO can directly be used as a fuel in diesel engines although modifications to the engine are required. Diesel engines, in contrast, do not have to be adapted to operate on biodiesel (refined from SVO) assuming the engine is designed to use ultra-low sulfur diesel (a requirement of new diesel engines since 2006). Prior to use as a fuel, algal oil should be purified to get rid of excess water, solvents, and/or impurities. Methods for oil purification include filtration and distillation (Pressman and Morris, 2007).

SVO varies significantly from biodiesel. For example, SVO has a much higher viscosity than biodiesel or other petroleum-derived diesel, and the additional thickness more rapidly results in wear and tear on an engine compared to the more “fluid” biodiesel. Adverse effects of using SVO in a standard diesel engine may include piston ring sticking; deposits in the injector, combustion chamber, and fuel system; degraded power and fuel economy; and increased exhaust emissions (EMA, 2006). SVO also has different combustion properties than biodiesel and petroleum-derived diesel, which result in different burn characteristics.

Strengths / Weaknesses. The greatest advantage of using algal oil as SVO is that no major additional steps (e.g., transesterification, hydrotreatment) are required once the oil is extracted from the algal cells. This reduces energy needs and associated costs. As previously mentioned, however, normal diesel engines must be modified to safely run on SVO for an extended period of time due to the consequences of relatively high viscosity. Cold climates amplify the negative effects of high viscosity on a diesel engine and potentially keep the fuel from being fed into the engine. To help negate the effects of high viscosity, diesel vehicle owners often install oil preheaters to enhance flow through the engine components. Other potential modifications include replacement of traditional fuel injectors with ones that can accommodate higher pressures, insertion of a second SVO-specific tank for dual-fuel use options, an additional fuel line, and various added controls (Neely, 2010).

Cost Information. Since no major processing is needed to use SVO as a fuel, direct costs are only associated with removing any debris and remaining water content by filtration or distillation. However, the indirect costs needed to modify a diesel engine to operate on SVO are considerable and should be taken into account.

Industry Activities. As one of their key revenue streams, SunEco Energy, Inc. currently sells SVO to biodiesel producers to be converted into motor fuel. According to the company, SunEco SVO can be combined up to 50% with low sulfur petroleum diesel with no reduction in energy density, cetane number, or cloud point. SunEco’s capacity for producing SVO is estimated at 33,000 gal/acre-ft/yr (~100 L/m^3/yr) (SunEco, n.d.).

4.5.9. Summary of Methods for Producing End-Use Fuels from Algal Biomass

Table 12 summarizes strengths, weaknesses, and industry activity for end-use fuel production from algal biomass.

Table 12: Summary of the primary techniques used to convert algal biomass into transportation fuels and energy products.

END-USE FUEL PRODUCTION

TRANSESTERIFICATION

- **STRENGTHS:** Transesterification is a well-understood process that has been used in industry for many years. Its feedstock does not interfere with the food industry, and the end-use product has many superior qualities relative to traditional diesel.
- **WEAKNESSES:** Byproducts (e.g., methanol, glycerol) are difficult to remove.
- **INDUSTRY ACTIVITY:** Companies that use transesterification in their operations include Aurora Biofuels, BARD LLC, Catilin, ENN Group, Green Star Products, Kuhmo Petrochemical, LiveFuels, LS9, and Solazyme (with Chevron).

HYDROPROCESSING

- **STRENGTHS:** Hydroprocessed fuels are indistinguishable from petroleum-based counterparts, and they meet existing fuel standards. Fuels have higher energy content than alcohols and biodiesel, and they are free of sulfur and nitrogen compounds. Finally, no infrastructure or engine adjustments are needed.
- **WEAKNESSES:** Hydroprocessing is a harsher process than transesterification.
- **INDUSTRY ACTIVITY:** Companies that use hydroprocessing in their operations include Aquaflow Bionomics (with UOP), Diversified Energy Corp, Emerging Fuels Technology, General Atomics, LS9, Neste Oil, SAIC, Sapphire Energy, Solazyme (with Chevron), and Solray Energy.

FERMENTATION

- **STRENGTHS:** With fermentation, the cost and time associated with dewatering, oil extraction, and oil processing may be avoided. If photosynthesis is unnecessary, then the cost of artificial light may be avoided and the depth/diameter of the tank is not an issue. Also, fermentation is widely used in other industries and is a well-understood process.
- **WEAKNESSES:** Fermentation may require large volumes (and cost) of sugar as an input, which may contribute to the "food vs. fuel" issues if sugar is not derived from non-food sources.
- **INDUSTRY ACTIVITY:** Algenol Biofuels uses fermentation in its operations.

ANAEROBIC DIGESTION

- **STRENGTHS:** In anaerobic digestion, dewatering of algae cultures and extraction of oils is unnecessary. It is less selective of algal strains and lipid contents compared to other methods. It is ideal for macroalgae processing and wastewater treatment plants. No emissions are released into the atmosphere during this process, and its byproducts are valuable.
- **WEAKNESSES:** Major capital and operating costs are associated with anaerobic digestion so may need to be integrated into a system that can utilize byproducts.
- **INDUSTRY ACTIVITY:** AACT and the Biomara Project are investigating the use of anaerobic digestion in their operations.

Table 12: (Cont.)

END-USE FUEL PRODUCTION (CONT.)

GASIFICATION

- **STRENGTHS:** Highly versatile end-products can be achieved with the resultant syngas created during gasification. A wide range of inputs can be used during this process, which is more efficient than combustion.
- **WEAKNESSES:** This process operates at extremely high temperatures, and a large-scale production is likely necessary to be cost effective. Tailoring of the reactor operations and system inputs is needed to optimize syngas qualities. Also, tar and other byproduct buildup creates extra steps.
- **INDUSTRY ACTIVITY:** Genifuel Corporation and the Solena Group use gasification in their operations.

PYROLYSIS / LIQUEFACTION

- **STRENGTHS:** These two processes occurs relatively quickly. Liquefaction allows for biomass with high moisture content, so most of the cost of dewatering is avoided.
- **WEAKNESSES:** The resulting bio-oil is an intermediate product and must be converted into a final product in another process. Also, only dry biomass can be used in pyrolysis.
- **INDUSTRY ACTIVITY:** Envergent Technologies uses pyrolysis/ liquefaction in its operations.

BIOPHOTOLYSIS

- **STRENGTHS:** Biophotolysis is a clean and renewable method for producing hydrogen, which has very low emissions.
- **WEAKNESSES:** Biophotolysis is not yet used at commercial scale (only laboratory settings). Also, the storage of the end-use fuel presents challenges related to pressure, temperature, etc.
- **INDUSTRY ACTIVITY:** Karlsruhe Institute of Technology, the Solar Biofuels Consortium, and Solarvest BioEnergy are all investigating the use of biophotolysis.

PURIFICATION OF SVO

- **STRENGTHS:** Only simple purification of SVO is needed before it can be used in a modified diesel engine, so processing costs are very low.
- **WEAKNESSES:** SVO has high relative viscosity, so diesel engines must be modified to accommodate long-term operation.Viscosity drawbacks become worse in cold climates.
- **INDUSTRY ACTIVITY:** SunEco Energy purifies SVO as part of its operations.

Chapter 5. Feasibility Assessment

Biofuels have the potential to increase transport fuel security by reducing the need for fossil fuels, and simultaneously reduce GHG emissions. In the long term, they may be produced without using fossil energy carriers and without net GHG (including CO_2) emissions over the well-to-wheel fuel chain. However, first generation biofuels have raised concerns regarding their sustainability on issues such as GHG balance, competition with food supply, biodiversity, the environment, and costs. Using algae as feedstock for biofuels production promises to mitigate or even eliminate these sustainability concerns.

In an effort to estimate to which extent these promises may be met, this chapter assesses the potential of algae as feedstock for biofuels that are used in transportation. The assessment is performed by using inputs related to:

- Production capacity
- Total energy balance and GHG emissions
- Competition with food supply
- Environmental impacts
- Biodiversity and ecosystems
- Production cost
- Future state of the energy industry
- Adaptability among markets

Although recently most attention is on algae production, the complete well-to-wheel fuel chain must be considered to assess the feasibility of algae as feedstock for biofuels. Five main stages can be distinguished in this chain: feedstock production, feedstock transportation, fuel production, fuel distribution, and fuel use in vehicles (Figure 21). In each of these stages energy is consumed, exhaust gases may be emitted, technology may need to be developed, safety issues may play a role, etc. Additionally, all five stages have an impact on the costs of a fuel. The well-to-wheel chain should also be used as the basis for any life-cycle analysis (LCA) of transportation fuels. Besides looking at the process parameters of fuel production and use, an LCA also includes aspects of construction and demolition of the equipment that is used in all stages of the process.

Figure 21: The five stages of the well-to-wheel chain for transportation fuels.

An enormous diversity in algae exists on earth, and they can be used to produce a number of different biofuels as documented in Chapter 4. To provide a simple comparison, the feasibility of three unique algal fuel pathways is addressed in this chapter:

- Using lipids from microalgae to produce biodiesel,
- Using macroalgae (seaweeds) to produce methane via anaerobic digestion and/or to produce ethanol by fermentation,
- Using microalgae and cyanobacteria that produce hydrogen by biophotolysis.

It should be mentioned here that the use of algae for biofuel production is still in its infancy. Therefore an assessment today is based on extrapolations of current small-scale production and on estimates resulting from experiments in laboratories.

5.1. Production Capacity

Many microalgae growing in open ponds or in PBRs have higher lipid content and higher growth rates than terrestrial crops, and therefore their annual biomass and bio-oil yield per hectare is higher. Table 13 presents an overview of possible yields for micro- and macroalgae in different regions. Because currently PBRs tend to have higher biomass production rates of microalgae than open ponds, the production system is also mentioned. Although not directly visible from the data in this table, the annual biomass yield per hectare of seaweeds is also higher than for terrestrial crops, but the difference is smaller than for oil-based algal fuels (Schiener, 2010). It should be stressed again here that commercial scale algal fuel production does not exist yet, and the yields in the table are estimates. It should also be noted that the oil yields in this table would still need to be converted to biodiesel before it could be practically used in most vehicles.

Can the possible yields of algal biomass lead to substantial quantities of transportation fuel? An arbitrary choice for ‘substantial’ could be 10%. This certainly represents more than a nice application. To get a grip on the answer without speculating about the future, the current European biofuel situation is used here as a reference. The share of biofuels consumed in road transport in the EU (27 countries) in 2009 was 4% (EurObserv’ER, 2010). On an energy basis, 80% of this amount was biodiesel, so that translates to 3.2% of the fuel consumed in road transport. European biodiesel production accounted for 83% of biodiesel consumption (the remainder was imported), and if it is assumed that all feedstock for European biodiesel production was grown in Europe, this means that for 2009, the 2.7% of total European road transport fuel that was biodiesel was grown in Europe, predominantly as rapeseed.

From Table 13 it can be seen that a conservative estimate for the bio-oil yield of algae per hectare is 13 times the yield of rapeseed. Assuming similar oil-to-fuel conversion efficiency for algae as for rapeseed, it can be concluded that if the same amount of space (ignoring any siting issues such as using valuable agricultural land for growing algae that could be cultivated at non-arable sites) that was used for rapeseed were to be used for growing algae, 35% of European road transport fuel could be algal fuel. This space could be considered not to compete with food production, because there was no food shortage in Europe in 2009. However, this kind of result must be handled with care for different reasons. It starts with the yield of algal biomass. The figures have not yet been confirmed by large-scale commercial production. Further, different assumptions have been made to obtain the biomass and oil yields.

Even though the assumptions are based on careful considerations, every deviation leads to an increase in the uncertainty margin of the end result. Also technical issues may affect the end result. One important question is whether the algae production facilities can be located close to CO_2 sources. CO_2 injection into an algae culture is a prerequisite to obtain the estimated biomass yields. One even can question the remark about absence of competition with food production because Europe is importing food from other continents which otherwise could be consumed near the production location. If Europe would have to produce more of its own food, the area available to produce biofuel would be reduced. However, in spite of all the uncertainties, and even if only half of the estimated amount of road transport fuel could be substituted by algal fuel for example, the potential algal biofuel quantity would still be substantial.

In case of hydrogen production by algae or cyanobacteria via biophotolysis, using average sunlight intensities in different locations and ‘favourable assumptions with regard to light conversion efficiency and productivity’, the hydrogen production potential is estimated between 3 and 5.3 TJ (10^{12} Joule) per hectare per year (Reith, Wijffels, & Barten, 2003). In energy terms, this would be 100 fold the energy yield of biodiesel from rapeseed per hectare (see Table 14). A large amount of R&D is still necessary to obtain these actual hydrogen yields from biophotolysis. Besides that, road transport is not ready yet for the use of hydrogen fuel. Hydrogen ICE and hydrogen fuel cell vehicles are not commercially available today, and a hydrogen distribution infrastructure would have to be built. Nevertheless, given the high energy yield compared to terrestrial crops, there is a long term potential for substantial hydrogen production via biophotolysis. For comparison, world transportation (all modes) energy consumption in 2008 was 2,299.37 Mtoe (Mega ton oil equivalent) (IEA, 2010). Calculating with 1 Mtoe = $41{,}868 \times 10^{12}$ Joule gives the equivalent to 96.27×10^{18} Joule (96.27 EJ).

Table 13: Estimates of biomass and/or oil yield from micro- and macroalgae, based on small-scale production data and extrapolation from results of experiments in laboratories.

Organism	Production system	Location	Year	Yield biomass [t/ha/y]	Yield oil [L/ha/y]	Remarks	Reference
MICROALGAE							
Microalgae	Pond	The Netherlands	2008	15-30	--	Estimate maximum in practice	Knip, 2008
Microalgae	PBR	The Netherlands	2008	25-40	--	Estimate maximum in practice	Knip, 2008
Chlorella	PBR	Germany	2007	50	--	Obtained	Carlsson et al., 2007
Microalgae	Raceway pond	n.s.	2007	50-60	--	Highest reproducible productivities	Carlsson et al., 2007
Microalgae	Flat-panel PBR	Germany	2009	75	--	Estimate	Ripplinger, 2009
Microalgae	Flat-panel PBR	Central Europe	2009	90	--	Estimate	Ripplinger, 2009
Microalgae	Flat-panel PBR	Mediterranean region	2009	113	--	Estimate	Ripplinger, 2009
Microalgae	High-rate raceway pond	n.s.	2007	127	--	Scenario	Sialve, Bernet, & Bernard, 2009 (quoting Chisti)
Microalgae	PBR	The Netherlands	2008	135	--	Theoretical maximum	Knip, 2008
Microalgae	Flat-panel PBR	Tropics	2009	137	--	Estimate	Ripplinger, 2009
Microalgae	PBR	France	2010	150-180	--	Expected maximum	Le Hir, 2010

Table 13: (Cont.)

Organism	Production system	Location	Year	Yield biomass [t/ha/y]	Yield oil [L/ha/y]	Remarks	Reference
MICROALGAE							
Algae	PBR	Spain	2008	250	--	Eventually achievable	Mees, 2008
Microalgae	n.s.	Spain	2008	--	20,000	State-of-the-art	Wijffels, 2008
Microalgae	PBR	France	2007	--	24,000	Expected feasible	Baldos, 2007
Microalgae	n.s.	Spain	2008	280	115,000	Theoretical maximum	Wijffels, 2008
MACROALGAE (SEAWEEDS)							
Laminaria	--	Ireland	2009	20-35	--	Indicative	Bruton, 2009
Ulva, Laminaria, Palmaria	--	North Sea	2005	20-50	--	Scenario	Reith et al., 2005
FOR COMPARISON							
Canola / rapeseed	--	Europe	2002	--	1,300 - 1,430	--	Ballerini, 2006
Canola / rapeseed	--	France	2007	--	1,500	--	Baldos, 2007
Palm oil	--	--	2007	--	6,000	--	Baldos, 2007
Palm oil	--	--	2008	--	6,000	--	Wijffels, 2008

L/ha/y = liters per hectare per year

n.s. = not specified

t/ha/y = tons per hectare per year

Table 14: Comparison of energy in crop and photovoltaic electricity production.

	Location	Energy production [GJ/ha/y]	Reference
Biodiesel from rapeseed	n.s.	30 - 50	IEA, 2008
Palm oil	Malaysia	142 - 180 (1)	Reijnders, 2008
Microalgae	n.s.	928 – 2,300^{+}	Chisti, 2008
Photovoltaic power plant	Portugal	1,339	IEA, 2009
Hydrogen via biophotolysis	The Netherlands	3,000	Reith, Wijffels, & Barten, 2003
Hydrogen via biophotolysis	Australia	5,300	Reith, Wijffels, & Barten, 2003
Photovoltaic modules	Brazil	7,600 - 8,700	Reijnders, 2008

GJ/ha/y = Giga Joules per hectare per year
1 GJ = 10^9 Joule
n.s. = not specified
(1) Net energy yield, after deduction of fossil energy input of the biofuel life cycle.

Solar photovoltaic electricity production is included in Table 14 for comparison. It should be noted that the yields in this table are the energy that is contained in the fuel. However, energy is used in different stages of the well-to-wheel fuel chain, and this energy consumption should be deducted from the yield to obtain the net energy balance. This issue is addressed in section 5.2.

5.2. Total Energy Balance and GHG Emissions

To determine if algal biofuels reduce fossil fuel use and if their GHG emissions are lower than for petroleum fuels, all five stages of the well-to-wheel fuel chain (Figure 21) should be taken into account, as previously mentioned. During algae growth in the feedstock production stage, solar energy is captured and CO_2 is absorbed, but process energy is required in all stages of the fuel chain and depending on the energy source this may come with emissions of CO_2 and other GHGs. A full LCA of the algal biofuel chain will give insights in the energy balance and global warming potential. It also enables comparison with other automotive fuels.

It has been mentioned earlier in this report that there is no production of algal biofuels on a commercial scale yet, and this means that many input values necessary to make an LCA are not yet available. Despite of this lack of data and even though technologies are still immature, researchers have started to publish LCA results for algal biofuels, for example from France, Italy, the United Kingdom and the United States (Aresta, Dibenedetto, & Barberio, 2005; Clarens et al., 2010; Lardon et al., 2009; Stephenson et al., 2010). The inputs for these LCAs have been obtained by extrapolation of laboratory experiences and data from small-scale algae production. The researchers were not aiming for detailed outcomes but they have used models of possible algal biofuel chains to assess different pathways, to determine the relative contribution of the different process steps, to identify bottlenecks, and to evaluate the sensitivity to different process parameters.

Besides looking at algal biofuel production in different climate zones, different production methods (such as nitrogen starvation or nutrient-sufficient growth of microalgae), consideration of open ponds and/or closed PBRs and so forth, researchers also used different system boundaries for their LCAs. Some studies were based on the energy content in the final fuel, while for example the study from the U.S. excluded the conversion of algal biomass into fuel from its scope because that kind of information was considered to be available elsewhere. It should be noted that most LCAs focus on biodiesel from microalgae, because of the expected high energy yields in algal lipids and because data is available on a number of microalgae species that have been examined extensively. Because of all these considerations and limitations, only general tendencies and qualitative results will be provided here. Microalgae for biodiesel are addressed first, followed by macroalgae and biophotolysis.

Biodiesel from Microalgae. Feedstock production includes algae cultivation, harvesting and dewatering, and oil/biomass separation. The main inputs that are required to grow microalgae are light, water, CO_2, nitrogen and phosphorus (Wijffels, 2010). Lamps may be used for PBR systems or small-scale algae production for high-value end products. CO_2 production, fertilizer (nitrogen and phosphorus supply) and pumping the algae culture around in the system are the three largest energy consumers of algae cultivation. When fossil energy is used, those three are also the largest contributors to the global warming potential.

To reduce the burdens from CO_2 production and fertilizer use, it is often recommended to use waste streams such as flue gas and wastewater effluent respectively. It should be noted that these are point sources of large quantities of CO_2 and fertilizer, which need to be distributed over vast areas of algae cultures. The construction of the distribution infrastructure and the actual distribution itself add to the energy consumption of the biofuel production chain. This issue receives little attention in literature and data on this kind of energy consumption have not been found. Another way to reduce fertilizer requirement is to grow algae under nitrogen constraints. This increases the lipid content in the algae but it reduces the productivity, so an optimum compromise should be sought. The energy needed to pump the algae culture around in raceway ponds is smaller than in PBRs but still significant. The LCA results are sensitive to the velocity of the culture, so optimization between algal productivity and velocity is recommended.

In the context of using flue gas as a CO_2 source for algae cultivation, the uptake by algae is sometimes considered as CO_2 sequestration or at least mitigating the CO_2 emissions from the source of the flue gas. This is misleading because the carbon of the CO_2 is only stored in the algae for a short time. When the fuel is burned in the vehicle ICE, this carbon is released to the atmosphere in the form of CO_2. So instead of being directly emitted into the atmosphere by an industrial plant, the same amount of CO_2 is now emitted from vehicle exhaust pipes, as shown on the left side of Figure 23. In this case, there is no net carbon sequestration, and algal biofuel production is made dependent on fossil fuels. Nevertheless, advantages of using flue gas for algal biofuels are that the concentration of CO_2 in flue gas is higher than in the atmosphere enabling higher yields per hectare in algae cultivation, and secondly the algal fuel replaces other (fossil) automotive fuels.

However, if society wants to move away from using fossil energy, the algal fuel cycle should be disconnected from fossil fuel use, as shown on the right side of Figure 22. The CO_2 loop of algal biofuels can be fully closed if renewable energy is used in the different production and transportation stages of the fuel chain. The right side of Figure 22 also makes clear that independently from the algal fuel cycle, stationary energy consumers should move away from fossil energy carriers by themselves. If they change to using biofuels the link with algae cultivation can be kept, else other CO_2 sources for high yields in algae cultivation may have to be found.”

Centrifugation is an effective way of harvesting microalgae from the culture, but because biomass concentration in the culture is generally low, energy consumption is relatively high and therefore unsuitable for large-scale biofuel production. To reduce the energy consumption of collecting the microalgae, flocculation followed by sedimentation or flotation is often assumed before centrifugation or filtration. Working this way the energy consumption of harvesting represents only a small share of the full fuel chain energy need. Successively extracting the oil from the algae cells does not have a great impact on the full fuel chain energy consumption either. The actual oil yield from extraction is more important, because the recovery percentage of the oil that is present in the algae cells has a linear correlation with the energy efficiency of the feedstock production stage.

Feedstock (algal oil) transportation to the fuel production site has a negligible impact on the full fuel chain energy consumption in the scenarios that were considered by the researchers previously mentioned.

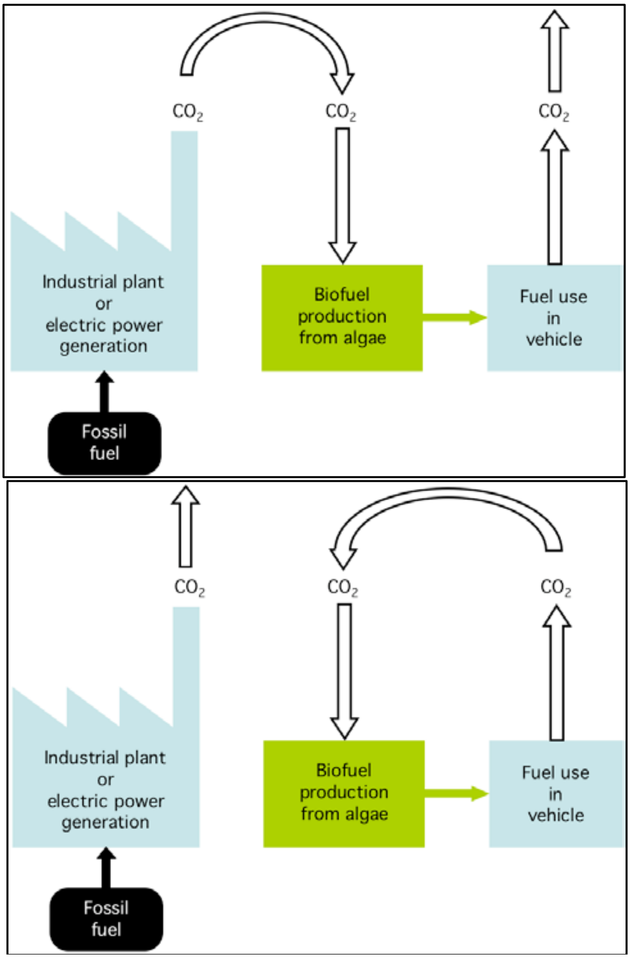


Figure 22: On left - CO$_2$ emissions from an industrial plant are released in the atmosphere via an algal biofuel cycle. There is no net carbon sequestration. On right - An industrial plant burning fossil fuels is a source of CO$_2$ emissions. An algal biofuel well-to-wheel chain can be an independent cycle by itself. If the CO$_2$ emissions from the biofuel chain process energy are ignored, the algae based well-to-wheel fuel chain is CO$_2$ neutral. The same amount of CO$_2$ that algae take from the atmosphere during their growth is emitted when the fuel is used in a vehicle.

In the quoted literature on LCAs, conversion of the algal oil into biofuel receives much less attention than algae cultivation. Glycerol is a by-product of transesterification of algal oil into biodiesel, and there is also residual biomass. In the case of algal biofuels, anaerobic digestion of residual biomass into methane is proposed. Depending on system parameters, the amount of methane produced may offset the heat and electricity requirements of the process plant, or even result in an excess of energy.

Large-scale biodiesel production from microalgae may result in saturation of the world glycerol market. In that case the surplus glycerol could also be used as energy source.

Like in other liquid biofuel chains and similar to gasoline and diesel, energy consumption of fuel distribution forms only a small share of the full fuel chain. Combustion of algal biodiesel in road vehicles will be very similar to using other diesel fuels.

In summary it was found that the cultivation stage has the largest share in energy consumption of the microalgae to biodiesel chain. The large energy consumers are CO_2 production, fertilizer, and pumping around the culture in the production system. Further, the lipid content of the algae has a large influence on the net energy balance; high lipid contents have a positive impact. The net energy balance of most systems was negative, with current knowledge it seems that only by careful fine-tuning a raceway pond based algal biodiesel chain may result in a positive energy balance.

The energy inputs over the whole fuel cycle in the LCAs identified for algal biofuels are based on the current situation. This means that mostly fossil energy carriers are used for process heat and electricity production. Consequently, GHG emissions associated with the algal biofuel chain are roughly proportional to energy consumption in the chain. However, if more and more renewable and sustainable energy sources are used in the future, the resulting GHG emissions should decrease.

Ethanol or Methane from Macroalgae. Interest for biofuels from macroalgae to date is not currently as strong as for microalgae, and only one publication concerning the energy balance for macroalgae LCAs has been found (Aresta, Dibenedetto, & Barberio, 2005). It shows that also for macroalgae it would be advantageous to use flue gas as CO_2 supply and wastewater effluent instead of fertilizer. A difference with microalgae is that macroalgae cultures do not have to be stirred or pumped around, which avoids energy consumption of such processes. The net energy gain of the macroalgae biofuel chain depends on the conversion technology for biomass into fuel, and a positive balance seems to be possible.

Hydrogen from Biophotolysis. Biophotolysis is in an early stage of development, and therefore it is not surprising that no LCA of this production method of algal biofuels has been found. The hydrogen-producing algae are not consumed, and nitrogen starvation increases hydrogen productivity, so it may be expected that less fertilizer is needed than for biodiesel from microalgae, with the accompanying advantages in energy needs. On the other hand, creating a nitrogen-free atmosphere for the algae and separating the hydrogen and oxygen that are produced will require energy consumption that does not exist in the microalgae and macroalgae chains. Conversion of the product (hydrogen) into an automotive fuel is not necessary, but it must be compressed or liquefied for use in road vehicles. All these issues have an impact on the well-to-wheel energy consumption of the biophotolysis, biofuel chain. Without thorough analysis it is not possible to predict if the net energy yield of the biophotolysis route will be higher or lower than for the other algal biofuels.

5.3. Competition with Food Supply

An important reason to consider algae as feedstock for biofuel production is the claim that it does not compete with food production. This statement is based on the fact that ponds and PBRs to produce microalgae may be placed on non-arable land. Macroalgae (seaweeds) are being harvested for human consumption (predominantly in Asia), but on a relatively small scale. Vast space is available in oceans and seas to cultivate seaweeds for biofuels, and ponds may be constructed on non-arable land.

Yield per hectare also plays a role in the competition with food production. The higher the biofuel yield per hectare, the lower the pressure on available space, so algal biofuels would have an advantage over biofuels from terrestrial crops. The Food and Agriculture Organization of the United Nations (FAO) estimates that food production must increase by 70% to feed the world population in 2050. In developing countries, 80% of this increase would come from increases in yields and cropping intensity, and 20% would come from expansion of arable land. Studies show that if the current degradation of the natural resource base is stopped or significantly slowed, the natural resource base should be adequate to meet the future demand at global level. However, the future total demand for agricultural commodities may exceed the demand for food, depending on the increase of demand for biofuels and the technology that will be used for the conversion of agricultural biomass into biofuels. This means that the development of the bioenergy market will influence whether the growing demand will be met at affordable prices (United Nations, 2009). The FAO does not mention the option of an increased use of seaweed or microalgae, not for food purposes or for biofuels, but it seems that algae might be able to relieve some pressure from future demand for biomass-based products.

Depletion of freshwater reserves is becoming problematic in some parts of the world. In these regions it seems wise to focus on seawater algae or algae that thrive in wastewater effluents for biofuel production. More on the water footprint of algal biofuels can be found in the next section on environmental impacts.

5.4. Environmental Impacts

Besides potential GHG emissions, formation of ozone and acidification are possible throughout the different stages of the algal biofuel lifecycle. Vehicle emissions when using algal biofuels may be expected to be roughly similar to vehicles using other biofuels. LCAs provide insights in this kind of phenomena, but available information is still limited so detailed information cannot be presented yet. Instead, this subsection focuses on two other environmental issues on which more information is available: fertilizer and water consumption, respectively.

Nitrogen and phosphorus are the most important minerals that algae require for their growth. The need for nitrogen is higher than for phosphorus, because algae biomass consists of 7% nitrogen and 1% phosphorus (Wijffels and Barbosa, 2010). To reduce net mineral consumption of the algal biofuel chain, recycling of minerals from residual biomass that is generated in the fuel production stage to the algae production phase is recommended. Already mentioned is that wastewater effluent could be used as mineral source for algae cultivation. This would eliminate the energy and environmental burdens of fertilizer production and it would help avoiding eutrophication. It has also been suggested that cultivation of seaweeds in open waters may reduce existing eutrophication in these waters.

Water consumption in the well-to-wheel chain of biofuel production is substantial. For biofuels from agricultural crops the water footprint is between 1,400 and 20,000 L of water per liter of biofuel (Gerbens-Leenes, Hoekstra, & van der Meer, 2009; Wijffels and Barbosa, 2010). Biofuels from algae will likely also result in a substantial water footprint. First, water is consumed in the photosynthesis that converts CO_2 into hydrocarbon constituents of the algae. Then, the water of the algae culture needs to be replenished at regular intervals, to limit the development of bacteria and to avoid accumulation of toxic compounds. Open ponds are more sensitive to water pollution than closed PBRs so the cleaning interval for open ponds might be shorter. Water replacement intervals of two months are mentioned for open ponds. Finally, water evaporates from open ponds. However, it is interesting to note that the annual rainfall in the UK is considered to be greater than the evaporation, but not much further south in the Mediterranean the average annual evaporation would be greater than the rainfall, significantly increasing the total requirement of process water (Stephenson et al., 2010). At the VITO research institute in Belgium, alongside costs, water management is considered one of the main issues of algae production, because once-through water systems are not sustainable, and process water should be recycled (Lemmens, 2010).

5.5. Biodiversity and Ecosystems

For algal biofuels to substantially contribute to the fueling of the transportation sector, large areas of algae cultivation are required. From agriculture it is known that large areas of monocultures come with risks such as diseases and plagues. This may be expected to also hold for large monocultures of algae. Because PBRs provide a closed environment, these risks may be lower than for algae cultures in open ponds. The ecological impacts of large areas of open ponds with algae culture are currently unknown.

Independent of using PBRs or open ponds, covering large areas of land with these systems replaces the ecosystem that was there before. Even if the systems are constructed on non-arable land, local ecosystems will be lost and species may disappear or be displaced. In general a loss of biodiversity may increase the vulnerability of the global ecosystem. The situation may be more complicated where large areas of coastal waters or seas are used for algae cultures. Besides replacing existing ecosystems, there will be an impact on the surroundings because the system borders are not as clear as with open ponds or PBRs. Additionally, when non-native algae species are introduced in open waters, there is a risk they become invasive and take over surrounding habitats. The use of local algal species at these sites may help reduce or negate this concern.

These concerns are difficult to quantify because scientific knowledge on ecosystems is in an early stage of development. So far, threats to biodiversity are often listed on a species-by-species basis, but it has been recognized that this should shift to an ecosystem-based approach (Hughes et al., 2009). According to Mr. Jean-Paul Cadoret of the French Research Institute for Exploitation of the Sea (IFREMER) it is difficult for scientists to estimate the potential effect of thousands of hectares of algae cultures on ecosystems: “It is the big unknown” (Mao, 2009).

5.6. Production Cost

Today, production costs of algal biofuels with current technology are estimated to be between 2.11 and 10.57 USD/L (8 - 40 USD/gal) (see section 2.1.3). This is much higher than the late 2010 crude oil price of 0.53 USD/L (85 USD/bbl), which results in automotive petroleum fuel production costs well below 1 USD/L. Future algal biofuel price estimates range between 0.37 USD/L (based on 60 USD/bbl) and 0.79 USD/L (3 USD/gal) (see section 2.2.3), which would be cost competitive with traditional petroleum-based fuels. As with any new technology, many parameters can influence the future costs of algal biofuels. These parameters include:

- Production volume levels,
- The algae species that is used and its production system,
- The harvesting and dewatering technology that is used,
- The technology used to extract the biomass (if applicable),
- The technology that is chosen to convert the biomass into a fuel, and
- How by-products are valued.

5.7. Future State of the Energy Industry

It seems that on a global level, several entities speculate that oil production is near or at its maximum (peak oil) and might start to decline in the coming decade; meanwhile, a further increasing demand is expected. Algal biofuels have the advantage – unlike electricity or hydrogen for example – of being able to feed directly into the existing vehicle fleet and its infrastructure. However, large-scale algal biofuels production is expected to be at least ten years away, so they may not be readily available in time to bridge some of the gap between declining crude oil production and ongoing demand. Mr. Carel Callenbach of Dutch algae manufacturer Ingrepro has an interesting vision related to this issue: “Road vehicles will not use algal biofuels. They will be electric and will not carry liquid fuels on-board. Batteries are too heavy for airplanes and they cannot use bio-ethanol either because of the extremely low temperatures at cruising height. By the time algae can be produced at very low costs, the price of kerosene becomes too high, and biofuel can be blended into kerosene, we might see airplanes using algal fuel" (Algen, 2009). This opinion can of course be discussed, but it highlights that it could be time to start thinking about the role that algae can play in future energy supply.

5.8. Adaptability among Markets

As demonstrated in section 4.5, algae can be converted into many different end-use fuels, and different species are better suited for different pathways, providing great adaptability to meet market demands. Given the large number of parameters in the algal biofuel production chain that impact the viability of algal biofuels, the variation in algal species with climate, and the diversity of circumstances at algae production sites, it becomes clear that no one algal biofuel pathway can be deemed the clear winner. Different algae species and cultivation systems, combined with different fuel production methods, will be optimal in different locations around the globe.

5.9. Overall Assessment

Algae do have potential as feedstock for biofuels. The biomass productivity per hectare can be more than ten times higher than for terrestrial energy crops. However, careful selection of the process parameters in the fuel production chain is necessary for a positive net energy balance. When algae are cultivated on non-arable land, there is no competition with current food production. These benefits have led to much interest from industry, entrepreneurs, and governments, and increasing number of joint R&D projects are underway. Besides use as feedstock in downstream fuels processing, under specific conditions some algae are able to naturally produce fuels such as hydrogen. This practice seems to be much further from commercialization and currently receives far less attention than the use of algae as feedstock for liquid biofuels.

Algal biofuels are currently still in their infancy. Expectations are based on small-scale production for high-value products and on results of laboratory experiments. How these experiences translate to large-scale production is still largely unknown. So far upstream algae cultivation and harvesting receive the most attention from researchers, but experience with the conversion of algae into biofuels is still limited and needs further development. Uncertainties may be associated with getting a sufficient supply of CO_2 and fertilizer to the algae culture, the net energy balance of the total well-to-wheel chain, and the ecological impacts of large algae monocultures. Different options to deal with these issues have been proposed and more are under investigation. Given the current level of knowledge preferred technologies cannot yet be selected.

To acquire knowledge about how large-scale algal biofuel systems operate, several pilot projects are underway and are expected to increase in number. Pilot projects should be adapted to local circumstances such as climatic conditions and native algae species, the availability of water, and potential markets. Real-world experience will help remove the uncertainties, and will also help clarify which practices are feasible and which are not.

On a final note, for algal biofuels to comprise a notable percentage of transportation fuels, it is important to remember that this industry would be based on massive volumes of feedstock based on living organisms. Consequently, environmental, ecological, and economic risks of large monocultures will be assumed. As society has learned with the introduction of large-scale technologies or methods based on living organisms, it is not always easy to see all the indirect and long-term impacts. For example, antibiotics may help cure illnesses, but some bacteria become resistant to them. Similarly, fertilizer significantly increases crop yields, but it can also wash out and result in eutrophication of seas. Finally, petroleum fuels are cheap and very practical in road vehicles, but many believe that the resultant GHG emissions contribute to climate change. To ensure investment in the most promising options, a holistic view and detailed LCAs are prerequisites for developing sustainable algal biofuels.

Chapter 6. Recommendations for Policy Makers

Algae have potential as a feedstock for biofuels. Depending on their composition, different algae species may be suitable for a range of biofuels. For example, lipids in microalgae may be a source for production of biodiesel and other oil-based transportation fuels. Macroalgae (seaweeds) may be fermented to produce ethanol, or anaerobically digested to create methane. In these processes algae take up CO_2 from the atmosphere during their growth, and the same amount of CO_2 is released when the biofuel is used in vehicles. Other microalgae and cyanobacteria are able to produce hydrogen in a process called biophotolysis where the algae are not consumed. All options show the potential for closed CO_2 cycles, excluding the fossil energy consumed in the total (well-to-wheel) fuel chain. Another advantage of these fuels is their compatibility with existing vehicles. For instance:

- Biodiesel can be used in diesel vehicles,
- Ethanol can be blended with gasoline (to an extent) for use in ICE vehicles,
- Methane can be used in compressed natural gas (CNG) vehicles,
- Renewable gasoline, diesel, and jet fuel can fuel traditional ICE vehicles, diesel vehicles, and jets, respectively, and
- Hydrogen can be used in fuel cell vehicles or hydrogen ICE vehicles.

Additionally, algal biomass productivity per hectare can be more than ten times higher than for terrestrial energy crops. Last but not least, algae can be cultivated at sea or on non-arable land, so there is no competition with current food production.

These reasons justify attention to algal biofuels from researchers, industries and (governmental) policy makers. The research that forms the basis of this report leads to the conclusion that the following issues are important for consideration in policymaking on algal biofuels:

1. Algal biofuels are in an early stage of development. Current expectations for the future are based on estimates and extrapolation of small-scale production and results of laboratory work. It seems appropriate to start pilot projects to obtain experience in scaling up the production process and to gain knowledge about the feasibility of different fuel production routes.
2. It is too early to select preferred algal fuel pathways and technologies. In practice there will not be one preferred production method. Different circumstances, such as climatic conditions and the availability of fresh or salt water, will have different optimum solutions.

3. Specialized scientists should be involved in the determination of ecological impacts of large-scale algae cultures.

4. Sustainability criteria should be developed for algal biofuels. Besides the energy, environmental, and ecological issues that are addressed in this report, criteria should be defined on issues not addressed in this report such as economic prosperity and social well-being.

5. It has been shown that under specific conditions, the algal biofuel production and distribution chain may have a net energy output, but further energy analysis of many different algae fuel chains is needed. On a related note, examination of whether it is ecologically and economically sustainable to base algae growth for biofuels on flue gas of fossil fuel combustion should be conducted.

6. Algal biofuel policies and projects should aim to reduce fossil energy consumption and the environmental burden compared to conventional fuels. In parallel, these efforts should result in acceptable impacts on ecosystems, which can originate from potential GHG emissions, fresh water consumption, effects of large monocultures and invasive species, etc. Therefore, some believe that government agencies that fund pilot projects should require a complete sustainability analysis prior to construction and operations that examines all stages of the fuel chain that apply to the pilot project. During the execution of the project, energy consumption and emissions should be measured to ensure that actual measurements are consistent with those in the sustainability analysis and to collect inputs for later LCA analyses.

7. Based on the high level of innovation demonstrated within the algal biofuels industry in just the past decade, it is likely that new, refined, or even breakthrough technologies will continue to be introduced in the future. It is important that industry stakeholders and policymakers remain open to new algal species, processes, and fuels besides the ones that are being considered today.

SPECIAL FOCUS: Investigation of How Algal Species-Specific Properties Drive End-Use Fuel Properties

Included in the scope of this study is the identification of a particular area of work in algal fuels which deserves special focus. If preliminary outcomes of this area appear promising, the topic may warrant a more in-depth follow-up study, or second phase of work, to be pursued by the IEA-AMF in the near future. As a result, the project team chose to investigate whether certain properties of end-use biodiesel can be forecasted based on unique characteristics of the strain of algal oil being processed. This chapter provides a background on feedstock attributes (including those of algal oil) that help define biodiesel quality, the general approach and collaborative efforts established, preliminary findings from the analysis, and recommended future steps by IEA-AMF members.

Background on Biodiesel Process Inputs

Today, SVO and animal fats are being used as feedstocks to produce what is commonly known as "biodiesel" fuel. The biodiesel that is processed from these oils and fats mimics many of the properties of petroleum-based diesel fuels, and can be used in existing diesel engines with little to no modification. SVOs themselves can theoretically be used as a diesel fuel, but the life of the engine may be greatly compromised. A number of properties of SVOs are out of range when considering usage in diesel engines. To start with, the viscosity of vegetable oils is typically more than ten-fold that of diesel fuel. Also, cetane number and distillation range are unsuitable. Therefore, SVOs need to be processed by transesterification (see section 4.5.1) or hydrotreatment (see section 4.5.2) to products which are more suitable for use in diesel engines or as blends with diesel fuel.

Overwhelmingly, the transesterification process is used today to make most biodiesel fuels from SVOs. This process is not severe since it runs at atmospheric pressure and quite low temperatures of approximately 60°C (140°F) (Hussain, 2010). As a result, many properties of the feedstock vegetable fuel are retained in the finished fuel in the sense that the vegetable oil properties still have a great impact on the properties of the fuel that emerges from the process.

Based on Chapter 4's assessment of technologies, it is very possible that the hydrotreatment process will be the process of choice for integrating vegetable oils into fuel supplies. In this process, SVO is introduced as a separate stream of feedstock in a petroleum refinery. It is hydrotreated and then mingled with the petroleum feedstock to produce a fully qualified diesel fuel, often with qualities superior to the fuel from petroleum by itself.

When reviewing what is known within the industry about vegetable oils and their influence on biodiesel fuel properties via the transesterification process, the properties of vegetable oils tend to vary with the type of vegetable. Freezing point, viscosity, and cetane number of finished fuels can vary greatly depending on the selection of plants from which the oils are derived. For example, oils from tropical plants, such as coconut and palm, have the highest cetane numbers but also the worst cold flow properties.

The viscosity of plant and animal oils varies widely from crystalline solids to light oils at room temperature. High melting points can cause problems in fuel systems such as partial or complete blockage as the triglyceride thickens with falling temperatures. To some extent, the same phenomenon can occur with diesel fuel, but it is much easier to control in the refining process, a common practice in the oil refineries, prior to distributing the fuel to the customer.

NREL's "Biodiesel Handling and Use Guidelines" (NREL, 2009) contains an excellent discussion of the differing fuel properties from various feedstocks, and the reader is encouraged to consult this reference. Information within these guidelines was used heavily from this source for the information provided in this section's assessment.

As previously mentioned, biodiesel can be made from a variety of vegetable oils and fats, including those mentioned in Table 15. The oils and fats in this table are made up of ten common types of fatty acids. All have between 12 and 22 carbon atoms with the great majority between 16 and 18 carbons. Some of these feedstocks are saturated (fully saturated with hydrogen – no double bonds between carbons), some monounsaturated (one double bond in the fatty acid chain), and some polyunsaturated (multiple double bonds in the fatty acid chain).

Table 15: Common feedstocks for biodiesel production

Animal Fats	Vegetable Oils	Recycled Greases
Edible tallow Inedible tallow Lard Yellow grease Poultry fats Fish oil	Soy Corn Canola (rapeseed) Sunflower Cottonseed	Used cooking oils Restaurant frying oils

The different feedstocks listed in Table 15 are made up of different proportions of saturated, monounsaturated, and polyunsaturated fatty acids. This is illustrated in Figure 23.

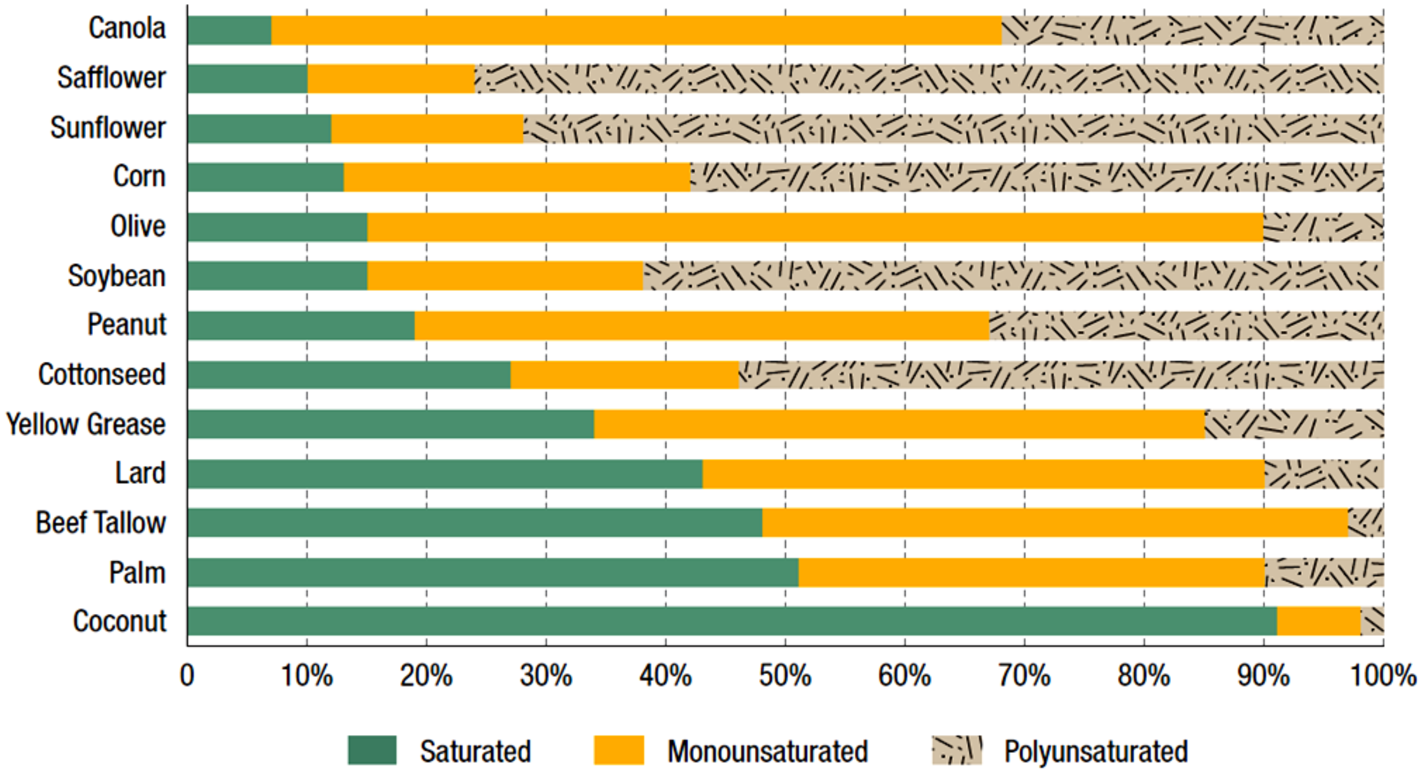


Figure 23: Compositions of types of fatty acids in various biodiesel feedstocks (NREL, 2009)

As previously discussed, the different levels of feedstock saturation can affect the finished biodiesel fuel's properties, and this proves to be a significant factor in selecting the best feedstock for a particular end-use application. This fact is illustrated in Table 16. In the table, general trends in three fuel properties – cetane number, cloud point, and stability – are shown to be related to the degree of saturation of the feedstock. Shown are typical fatty acids of different carbon content and different levels of saturation. The fuels from saturated fatty acids generally have better performance in cetane number and stability, which degrades as the number of double bonds increases.

Table 16: Variation of finished biofuel properties with feedstock composition (NREL, 2009)

	Saturated	Monounsaturated	Polyunsaturated
Fatty acid	12:0, 14:0, 16:0, 18:0, 20:0, 22:0	16:1; 18:1, 20:1, 22:1	18:2, 18:3
Cetane Number	High	Medium	Low
Cloud Point	High	Medium	Low
Stability	High	Medium	Low

General Approach and Collaborative Efforts

Since the properties of certain oils can often be used to forecast the properties of finished biofuels, this premise was used to assess whether the properties of end-use biodiesel fuels can be forecast when the feedstock is algae. The answer is yes, to a certain extent. Using samples of the lipids (oils) that are derived from algae, such a forecast is quite possible and could provide useful insight into selection of algae strains for use as biodiesel feedstocks.

To reach this conclusion, samples of algal oils were collected and analyzed to allow the opportunity to forecast their end-use performance in an engine when transformed (in the transesterification process) to biodiesel. Thus, samples of oils were sought from a number of commercial research efforts dealing with algae. No samples were forthcoming from private industry, so researchers at Canada's NRC that operates the Institute for Marine Biosciences were contacted, since the staff have very active ongoing R&D efforts in algae as a potential feedstock for biofuels.

Following execution of a memorandum of cooperation between NRC and the authors of this report, the Institute shared analyses of several algae strains. Data received to date from the Institute has been fatty acid profiles of several algae samples from which some of the potential properties of fuels that might result from these strains may be forecasted.

In particular, the Institute provided fatty acid profiles of the following algae strains:

- *Botryococcus braunii* (Race A)
- *Chlorella vulgaris*
- *Neochloris oleoabundans*
- *Phaeodactylum tricornutum*
- *Nannochloropsis granulata*
- *Isochrysis galbana*

Preliminary Analysis and Findings

Tables 17 through 22 show the distribution of fatty acids in each of the algae profiles. It is important to note that not all fatty acid peaks were identified, resulting in an "unknown" percentage in each fatty acid profile.

Table 17: Fatty acid profile for *Botryococcus braunii* (Race A)

***Botryococcus braunii* (Race A)**			
Peak ID	**Area %**		**Average area %**
	a	**b**	
	0.21	0.00	**0.11**
	2.68	2.66	**2.67**
C16:3	0.56	0.55	**0.55**
C18:1n9 (c&t)	65.93	66.36	**66.14**
C18:1n7	0.43	0.44	**0.44**
C18:2n6c	1.58	1.53	**1.56**
C18:3n3	4.52	4.45	**4.48**
C20:1n9	0.61	0.63	**0.62**
C20:3n3	0.31	0.30	**0.31**
C20:5n3	0.94	0.93	**0.94**
	0.38	0.39	**0.38**
C22:6n3	0.22	0.00	**0.11**
	0.59	0.62	**0.61**
	3.07	3.10	**3.09**
	1.17	1.18	**1.17**
	1.06	1.06	**1.06**
C28:1	6.69	6.79	**6.74**
C28:2	9.03	9.00	**9.02**

a,b = analytical replicates
c&t = cis and trans configuration of double bond

Table 18: Fatty acid profile for *Chlorella vulgaris*

Chlorella vulgaris			
Peak ID	**Area %**		**Average area %**
	a	**b**	
	0.76	0.76	**0.76**
	0.54	0.56	**0.55**
	0.87	0.87	**0.87**
	1.10	1.12	**1.11**
	0.45	0.43	**0.44**
C14:0	1.61	1.57	**1.59**
	0.66	0.67	**0.66**
	0.50	0.51	**0.50**
	0.50	0.49	**0.49**
C16:0	19.56	19.67	**19.62**
	0.84	0.84	**0.84**
C16:1n7	0.37	0.00	**0.18**
	0.87	0.88	**0.87**
	0.45	0.44	**0.45**
C17:1	2.71	2.76	**2.73**
	0.63	0.63	**0.63**
	13.23	13.52	**13.37**
	0.56	0.00	**0.28**
	6.33	6.37	**6.35**
C18:1n9 (c&t)	11.17	11.21	**11.19**
C18:2n6c	3.14	3.09	**3.11**
C18:3n3	27.93	28.29	**28.11**
C18:4n3	5.23	5.33	**5.28**

a,b = analytical replicates

c&t = cis and trans configuration of double bond

Table 19: Fatty acid profile for *Neochloris oleoabundans*

Neochloris oleoabundans			
Peak ID	Area %		Average area %
	a	b	
	0.34	0.34	**0.34**
	0.00	0.37	**0.18**
	0.00	0.24	**0.12**
	0.46	0.47	**0.46**
	0.00	0.50	**0.25**
	1.09	1.17	**1.13**
	1.43	1.42	**1.42**
	2.28	2.21	**2.24**
	0.89	0.89	**0.89**
	0.00	0.29	**0.15**
C14:0	2.09	2.07	**2.08**
	0.79	0.81	**0.80**
	1.22	1.22	**1.22**
	0.61	0.60	**0.60**
	1.07	1.02	**1.04**
	0.73	0.72	**0.73**
C16:0	16.53	16.01	**16.27**
	1.25	1.47	**1.36**
C16:1n7		0.32	**0.32**
	1.51	1.46	**1.48**
	4.87	4.75	**4.81**
C17:1	14.70	14.62	**14.66**
	1.07	1.03	**1.05**
C18:1n9 (c&t)	1.51	1.51	**1.51**
C18:1n7	1.08	1.12	**1.10**
C18:2n6c	16.31	15.87	**16.09**
C18:3n3	28.17	27.50	**27.84**

a,b = analytical replicates

c&t = cis and trans configuration of double bond

Table 20: Fatty acid profile for *Nannochloropsis granulata*

Nannochloropsis granulata				
Peak ID	**Area %**			**Average area %**
	a	**b**	**c**	
C12:0	0.40	0.39	0.41	**0.40**
	0.00	0.28	0.27	**0.19**
	0.58	0.92	0.29	**0.60**
	0.00	0.00	0.63	**0.21**
	0.84	0.79	0.81	**0.81**
	1.20	1.31	1.20	**1.23**
	0.48	0.51	0.49	**0.49**
C14:0	7.04	6.98	6.97	**7.00**
	0.00	0.42	0.46	**0.29**
	1.08	1.01	1.04	**1.04**
C15:0	0.00	0.26	0.28	**0.18**
	0.00	0.38	0.37	**0.25**
	0.56	0.60	0.55	**0.57**
	0.46	0.41	0.45	**0.44**
C16:0	13.81	13.49	13.48	**13.60**
	0.78	0.70	0.78	**0.75**
C16:1n7	23.98	23.45	23.40	**23.61**
	0.52	0.53	0.53	**0.53**
C16:3n4	0.66	0.65	0.65	**0.65**
	0.57	0.53	0.53	**0.55**
C18:1n9 (c&t)	4.99	5.00	4.98	**4.99**
C18:1n7	0.00	0.37	0.43	**0.26**
C18:2n6c	4.90	4.77	4.74	**4.81**
C18:3n3	0.00	0.00	0.24	**0.08**
C21:0	0.00	0.00	0.29	**0.10**
C20:3n6	0.00	0.32	0.24	**0.19**
C20:4n6	4.18	4.05	4.01	**4.08**
C20:5n3	32.95	31.87	31.49	**32.11**

a,b = analytical replicates

c&t = cis and trans configuration of double bond

Table 21: Fatty acid profile for *Phaeodactylum tricornutum*

Phaeodactylum tricornutum				
Peak ID	**Area %**			**Average area %**
	a	**b**	**c**	
	0.00	0.00	0.20	**0.07**
	0.00	0.00	0.16	**0.05**
	0.00	0.00	0.51	**0.17**
	0.00	0.69	0.66	**0.45**
	0.00	1.15	0.99	**0.71**
	0.00	0.00	0.39	**0.13**
C14:0	6.08	6.36	6.09	**6.18**
	0.00	0.00	0.70	**0.23**
	0.71	0.00	0.48	**0.40**
C15:0	0.41	0.00	0.00	**0.14**
	0.30	0.00	0.00	**0.10**
	0.50	0.00	0.00	**0.17**
	0.37	0.00	0.00	**0.12**
C16:0	14.69	15.47	14.70	**14.96**
	0.81	0.00	0.56	**0.46**
C16:1n7	19.48	20.17	19.24	**19.63**
	0.23	0.00	0.00	**0.08**
	0.99	1.06	0.99	**1.02**
	1.13	1.20	1.12	**1.15**
	2.95	3.14	2.98	**3.02**
	7.19	7.62	7.23	**7.35**
C17:1	1.31	1.40	1.32	**1.35**
	0.47	0.00	0.00	**0.16**
	1.11	1.17	1.11	**1.13**
C18:0	0.75	0.00	0.73	**0.49**
	1.68	1.65	1.61	**1.65**
C18:1n9 (c&t)	3.96	3.91	3.79	**3.89**
C18:2n6c	1.98	2.03	1.95	**1.98**
C18:3n3	0.60	0.00	0.58	**0.39**
C18:4n3	1.23	1.25	1.21	**1.23**
C20:3n3	0.26	0.00	0.00	**0.09**
C20:5n3	25.69	26.97	25.58	**26.08**
C22:0	0.61	0.00	0.59	**0.40**
C24:0	2.01	2.10	2.01	**2.04**
C22:6n3	2.51	2.65	2.49	**2.55**

a,b = analytical replicates
c&t = cis and trans configuration of double bond

Table 22: Fatty acid profile for *Isochrysis galbana*

Isochrysis galbana			
Peak ID	**Area %**		**Average area %**
	a	**b**	
	0.00	0.36	**0.18**
	0.00	0.76	**0.38**
C14:0	12.56	12.54	**12.55**
C16:0	19.30	19.36	**19.33**
C16:1n7	3.25	3.29	**3.27**
C18:1n9 (c&t)	23.22	23.22	**23.22**
C18:1n7	2.62	2.63	**2.62**
C18:2n6c	6.78	6.79	**6.79**
C18:3n3	3.88	3.86	**3.87**
C18:4n3	12.00	11.95	**11.98**
	2.32	2.34	**2.33**
C22:6n3	12.99	12.91	**12.95**
	1.08	0.00	**0.54**

a,b = analytical replicates
c&t = cis and trans configuration of double bond

The data in the preceding tables can be normalized according to fatty acid characterization as shown in Table 23 and Figure 24.

Table 23: Summaries of Fatty Acid Profiles Algal Strains provided by NRC Canada's Institute for Marine Biosciences

Algae Species	Saturated (%)	Mono-unsaturated (%)	Poly-unsaturated (%)	Unknown (%)
Botryococcus braunii (Race A)	0	73.94	7.94	18.12
Chlorella vulgaris	21.21	14.1	50.6	14.09
Neochloris oleoabundans	18.35	17.59	43.93	20.13
Phaeodactylum tricornutum	24.21	24.87	31.11	19.81
Nannochloropsis granulata	21.28	28.86	32.11	17.75
Isochrysis galbana	31.89	29.11	35.59	3.41

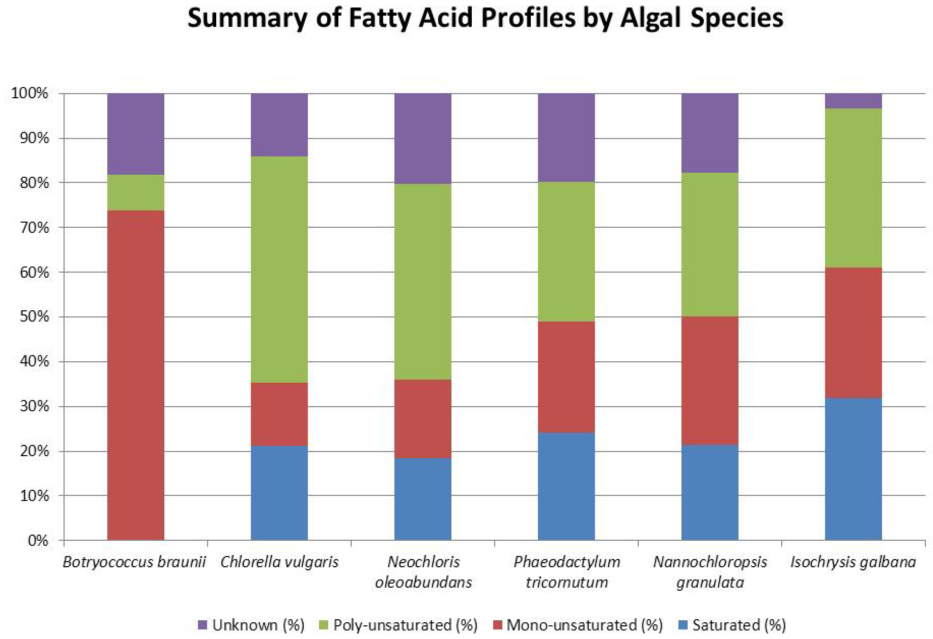


Figure 24: Normalized Fatty Acid Profiles

Referring back to Table 23, hints as to what kind of fuels these algae strains might produce begin to surface. For example, the *Botryococcus braunii* strain has nearly 70% of its content in mono-unsaturated fatty acids, and that would lead to medium values of cetane number, cloud point, and stability in the transesterified fuel. The *Isochrysis galbana* strain has about 30% of saturated fatty acid and another approximately 30% in mono-unsaturated fatty acid. This algae strain might produce a transesterified fuel with reasonably good properties, although the volume of poly-unsaturated fatty acid in the strain would tend to depress the properties somewhat. Of all strains investigated, the *Botryococcus braunii* strain probably has the most appealing set of end-use fuel qualities, although the volume of the unknown fatty acids in this strain is among the highest of all six strains investigated. This introduces uncertainty into the estimates of the fuel quality.

Algae-based biodiesel producers should be aware of the various property standards and test methods that exist worldwide for biodiesel (e.g., ASTM D6751, EN 14214). While ASTM D6751 is used in the US to regulate any form of biodiesel, Europe's EN 14214 applies only to fatty acid methyl ester, or FAME, biodiesel derived from biologically produced oils and fats (e.g., vegetable oils, animal fats, algal oils). Table 24 displays all properties that are regulated under EN 14214 along with the upper/lower limits and test methods.

Table 24: EN 14214 standards for FAME biodiesel (Stanhope, 2010).

Property	Units	Lower Limit	Upper Limit	Test Method
FAME content	% (m/m)	96,5	-	EN 14103
Density at 15°C	kg/m³	860	900	EN ISO 3675 / EN ISO 12185.
Viscosity at 40°C	mm²/s	3,5	5,0	EN ISO 3104
Flash point	°C	> 101	-	EN ISO 2719 / EN ISO 3679.
Sulfur content	mg/kg	-	10	- EN ISO 20846 / EN ISO 20884.
Carbon residue remnant (at 10% distillation remnant)	% (m/m)	-	0,3	EN ISO 10370
Cetane number	-	51,0	-	EN ISO 5165
Sulfated ash content	% (m/m)	-	0,02	ISO 3987
Water content	mg/kg	-	500	EN ISO 12937
Total contamination	mg/kg	-	24	EN 12662
Copper band corrosion (3 hours at 50 °C)	rating	Class 1	Class 1	EN ISO 2160
Oxidation stability, 110°C	hours	6	-	prEN 15751 / EN 14112
Acid value	mg KOH/g	-	0,5	EN 14104
Iodine value	-	-	120	EN 14111
Linolenic Acid Methylester	% (m/m)	-	12	EN 14103
Polyunsaturated (>= 4 Double bonds) Methylester	% (m/m)	-	1	EN 14103
Methanol content	% (m/m)	-	0,2	EN 14110l
Monoglyceride content	% (m/m)	-	0,8	EN 14105
Diglyceride content	% (m/m)	-	0,2	EN 14105
Triglyceride content	% (m/m)	-	0,2	EN 14105
Free Glycerine	% (m/m)	-	0,02	EN 14105 / EN 14106
Total Glycerine	% (m/m)	-	0,25	EN 14105
Group I metals (Na+K)	mg/kg	-	5	EN 14108 / EN 14109 / EN 14538
Group II metals (Ca+Mg)	mg/kg	-	5	EN 14538
Phosphorus content	mg/kg	-	4	EN14107

Anticipated Future Steps

Due to the promising findings of this preliminary analysis, more data exchange between the Institute for Marine Biosciences and the authors of this report is planned in upcoming months. Institute scientists are, at the time of this report, beginning to conduct some preliminary oil extractions from algal biomass using a supercritical CO_2 system. Data on these oils will be received at some point in the near future and analyzed. Shortly after, an addendum to this report will be written to provide a more comprehensive and detailed assessment of what biodiesel characteristics can be expected from various algal strains.

References

ABC Carbon. "Go Ahead for Algae/Aviation Fuel Research & New National Carbon Neutral Program." *ABC Carbon Blog Archive.* ABC Carbon, n.d. Web.

Agence Nationale de la Recherche. *Programmation 2011 de l'Agence nationale de la recherche.* Agence Nationale de la Recherche. 28 Oct. 2010. Web.

"Algae biofuel market forecast to hit $1.6bn in 2015." *NewEnergyWorldNetwork.com.* New Energy World Network, 2 Aug. 2010. Web.

Algae Coil. Digital Image. University of Georgia Engineering Outreach Service. Web. http://outreach.engineering.uga.edu/pictures/.

Algaeventure Systems Web Site. Univenture, Inc. and AlgaeVenture Systems, LLC. 2009. Web.

"Algen: Geen Groen Goud, maar Goed Zoeken naar Afzetmarkten." *GTT Glastuinbouwtechniek* 4.4 (2009) Web.

Algenol Web Site. Algenol Biofuels, n.d. Web.

Alternative Energy HQ. "Turning Algae and Wood Chips into Energy Jobs." Alternative Energy HQ, 8 Sep. 2010. Web.

"Ames Laboratory and Catilin Research Methods to Harvest Algal Oil for Biodiesel Production Without Killing Algae." *Biodiesel.* Biofuels Journal, 7 Apr. 2009. Web.

AquaFUELS "Algae and Aquatic Biomass for a Sustainable Production of 2nd Generation Biofuels." *AquaFUELS Home Page.* EU Commission Seventh Framework Programme for Research and Development. Web. 18 Nov. 2010. http://cordis.europa.eu

Aresta, Michele, Angela Dibenedetto and Grazia Barberio. "Utilization of Macro-Algae for Enhanced CO_2 Fixation and Biofuels Production: Development of a Computing Software for an LCA Study." *Fuel Processing Technology* 86.14-15 (2005): 1679-1693. Elsevier.

Argonne National Laboratory. "Basic Research Needs for the Hydrogen Economy - Report on the Basic Energy Sciences Workshop on Hydrogen Production, Storage, and Use." Argonne National Laboratory, Feb. 2004.

Aurora Algae. "Coming Soon: $2 a Gallon Diesel From Algae?" *Aurora Algae, Growing Natural Solutions.* Aurora Algae, 18 Aug. 2009. Web.

Aurora Algae. “The History of Biodiesel Algae.” *Aurora Algae Growing Natural Solutions.* Aurora Algae, May 2010. Web.

Baldos, Raphaël. “Des Microalgues pour Produire des Biocarburants.” *Planète \Environnement \Energie*. Novethic: Le Media Expert du Développement Durable, 25 July, 2007. Web.

Ballerini, Daniel. *Les Biocarburants. Etat des Lieux, Perspectives et Enjeux du Développement.* Paris: IFP Publications, 2006. Print.

Barclay, W.R., et al. “Potential of New Strains of Marine and Inland Saline-Adapted Microalgae for Aquaculture.” *Journal of the World Aquaculture Society* 18.4 (1987): 218-228. Web.

Bard Holding, Inc. “FORM S-1 /A Amendment No. 2.” *BARD HOLDING, INC. - FORM S-1/A - March 26, 2010.* Faq.org, 2010. Web.

Bargiel, Jeff. “Algal Oil for Biofuels.” OSU/OARDC Renewable Energy Workshop. 12 Nov. 2009. Presentation.

BCIC. Microalgae Technologies & Processes for Biofuels – Bioenergy. Production in British Columbia. 2009.

Behrens, Paul W. “Photobioreactors and Fermenters: The Light and Dark Sides of Growing Algae.” *Algal Culturing Techniques* Ed. Robert A. Andersen. Elsevier, 2005. 189-204. Web.

Bellona. “Australian Government Awards €3.5 Million to Algae.” *CCS News and Events, CO_2 Capture and Storage.* Bellona, 26 July 2010. Web.

Ben-Amotz, Ami. “Bio-Fuel and CO_2 Capture by Algae.” Organic Aquaculture Institute. Imperial, Texas. 24 June 2008. Presentation.

Benemann, John R., and William J. Oswald, P.I. “Systems and Economic Analysis of Microalgae Ponds for Conversion of CO_2 to Biomass.” Pittsburgh Energy Technology Center. U.S. Department of Energy, 1996. *Information Bridge DOE Scientific and Technical Information.* Web.

Benemann, J.R. “Open ponds and closed photobioreactors – Comparative economics (Slide presentation).” Paper presented at the 5th Annual World Congress on Industrial Biotechnology & Bioprocessing, 27-30 April 2008, Chicago, Illinois.

Berinstein, Paula. *Alternative Energy: Facts, Statistics, and Issues.* Westport, CT: Oryx Press; Greenwood, 2001. 87. Print.

Bilanovic, D., and G. Shelef. “Flocculation of Microalgae with Cationic Polymers — Effects of Medium Salinity.” *Biomass* 17.1 (1988): 65-76. Web.

Biofuels 3. California Polytechnic State University, Controlled Environment Agriculture & Energy Working Group (CEAE). Web. <http://brae.calpoly.edu/CEAE/images/ biofuels3.gif >

BioMara. *BioMara Website.* Web. Accessed 15 June 2009.

Bioreactor. Digital Image. The Greater Cincinnati Club Modern Efficient Diesel Technology (CintiTDI). Web. <http://www.cincitdi.com/system/files/2/bioreactor.jpg>

BMBF - German Federal Ministry of Education and Research. “Mikroalgen - die Energiequelle der Zukunft. Microalgae – Energy source of the future.” *Forschung.* BMBF, 16 Apr. 2008. Web. 23 Nov. 2011.

BP. “BP and Martek Biosciences Enter a Joint Development Agreement to Deliver Advanced Biodiesels.” *BP Alternative Energy News Press.* BP, 11 Aug. 2009. Web.

Braun, AR (Braun Consultants), and JH Reith (UVA). “Algen in de Nederlands Energiehuishouding.” Energiewinning uit Afval en Biomassa. Nederlandse Onderneming voor Energie en Milieu, Juli, 1993. Web.

Bruggers, James. “Cleaning up Pollution with Pond Scum: Algae's Ability to Soak up Carbon Dioxide Could Help Make Coal-Fired Kentucky Power Plants More Environmentally Friendly.” *Courier-Journal.com* 4 Jan. 2009. Web.

Bruton, Tom (BioXL), et al. “A Review of the Potential of Marine Algae as a Source of Biofuel in Ireland.” Sustainable Energy Ireland, Feb. 2009. Web.

Business Facilities Editorial Staff. “Algenol, Dow Partner to Develop $50-million Algae Biofuel Plant.” *Business Facilities Breaking News.* Business Facilities: The Location Advisor, 2 July 2009. Web.

Business Wire. “$2 Million ‘Shot in the Arm’ for Washington State Algae Alliance.” *Business Wire News.* Berkshire Hathaway, 7 Dec. 2009. Web.

Business Wire. “GreenFuel Algae CO_2 Recycling Project with Aurantia Enters Second Phase at Spanish Cement Plant.” AllBusiness.com, 21 Oct. 2008. Web.

Canadian Driver Communications, Inc “New biofuels could come from Nova Scotia algae.” *Canadian Driver, Green Scene*, 7 June 2010. Web.

Carbon Trust. *Carbon Trust Home Page.* Carbon Trust (UK), n.d. Web. 23 Nov. 2010.

Carbon Trust. “UK Takes on the World in Global Race to Commercialise Algae Biofuels.” *News, 2010 Press Releases.* Carbon Trust, 19 Mar. 2010. Web.

Carlsson, Anders, et al. Editor D. Bowles. “Micro- and Macro-algae: Utility for Industrial Applications: Outputs from the EPOBIO Project.” Ed. Dianna Bowles. EPOBIO, University of York, CLCPress. Sep. 2007. Web.

Cavitation Technologies. “CTI Nano Reactors Applications.” *CTI Technology.* Cavitation Technologies, n.d. Web.

Chisti, Yusuf. “Biodiesel from Microalgae.” *Biotechnology Advances* 25.3 (2007): 294-306. Web.

Chisti, Yusuf. “Response to Reijnders: Do biofuels from microalgae beat biofuels from terrestrial plants?” *Trends in Biotechnology* 26.7 (2008): 351-352. Web.

Chorkendorff, I., and J. W. Niemantsverdriet. *Concepts of Modern Catalysis and Kinetics.* Weinheim; Wiley-VCH, 2007. Web.

Chynoweth, D.P. “Review of biomethane from marine biomass. Review of history, results and conclusions of the ‘US marine biomass energy program (1968-1990).” Department of Agricultural and Biological Engineering, University of Florida, 2002. Web.

Clarens, Andres F., et al. “Environmental Life Cycle Comparison of Algae to Other Bioenergy Feedstocks.” *Environmental Science & Technology* 44.5 (2010): 1813-1819.

Craig, Kevin R., and Margaret K. Mann. “Cost and Performance Analysis of Biomass-Based Integrated Gasification Combined-Cycle (BIGCC) Power Systems.” U.S. Department of Energy National Renewable Energy Laboratory, Oct. 1996. Web.

D. Glass Associates, Inc. “Companies Reported to be Developing Genetically Modified Algae for Biofuels.” *Advanced Biotechnology for Biofuels.* Wordpress.com, 17 Mar. 2010. Web.

Darzins, Al, Philip Pienkos, and Les Edye. “Current Status and Potential for Algal Biofuels Production: A Report to IEA Bioenergy Task 39.” *Commercializing Liquid Biofuels from Biomass.* International Energy Agency Bioenergy Task 39, 6 Aug. 2010. Web.

Diversified Energy. “Centia™ - A "Renewable Petroleum" Fuels Production System.” *Diversified Energy Technologies.* Diversified Energy, n.d. Web.

Diversified Energy. “Diversified Energy Notified that Centia™ Advanced Biorefinery Process Selected for Department of Energy Award.” 4 Nov. 2009. Web.

Donald Danforth Plant Science Center. “Biofuels Consortium Led by the Danforth Plant Science Center Receives $44 Million Award from U.S. Department of Energy.” *Donald Danforth Plant Science Center News & Media.* Donald Danforth Plant Science Center. 13 Jan. 2010. Web.

Dupont, Gaëlle. “Une Nouvelle Panoplie Anti-Algues Vertes pour Protéger Touristes et Ramasseurs*. Le Monde*, 16 July 2010.

Eden Labs. “Extraction Methods.” *Eden Labs Welcome Page.* Eden Labs, n.d. Web.

Edwards, Mark. “Algal Species Selection.” *AlgaeIndustryMagazine.com.* 8 July, 2010. Web.

Emerging Markets. “Algae 2020: Biofuels Markets and Commercialization Outlook.” *Global Energy and Biofuels Market Intelligence.* Emerging Markets Online, 2009. Web.

Engine Manufacturers Association. “Use of Raw Vegetable Oil or Animal Fats in Diesel Engines.” CMO Engine Manufacturers Association, Mar. 2006. *National Biodiesel Board. Biodiesel.org.* Web.

Envergent Technologies. “The Practical, Proven Path to Green Energy. RTP™ Rapid Thermal Processing from Envergent Technologies.” Envergent Technologies, Mar. 2010. Web.

EurObserv’ER. “Biofuels Barometer.” *Systemes Solaires, Le Journal des Energies Renouvables* 198 (2010): 72-96. Web.

European Biofuels Technology Platform (EBTP). *Home Page.* European Biofuels Technology Platform, n.d. Web. 18 Nov. 2010.

European Union. “Successful Programmes.” European Regional Development Fund, European Union, n.d. Web. 24 Nov. 2010.

Farmerie, James A. “Dissolved Air Flotation as a Cost Effective Alternative for Potable Water Clarification.” FB Leopold Company. Alberta Water and Wastewater Operators Association, 16 Mar. 2005. Web.

Fehrenbacher, Katie. “15 Algae Fuel Startups, 2010 Edition.” *Sapphire Energy News and Media.* Sapphire Energy, Inc. 17 Oct. 2010. Web.

Ferris, John. “Projections of Biodiesel Gross Margins to the Year 2022: The Expanding Role of Corn Oil Extracted from DDGS.” *The Jacobsen Biodiesel Bulletin.* The Jacobsen, 1 Dec. 2010. Web.

Flinkkila, Johanna. “Searching out new raw materials for biofuel production.” Neste Oil website. 28 Oct. 2010.

Foroohar, Kambiz. “Algae Pools Spawning Biofuel, or Bio Fools?” *The Portland Press Herald.* Maine Today Media, Inc., 7 June 2010.

Foroohar, Kambiz. “Exxon Invests in Diesel Algae Fuel Formation.” *Interesting News 2010.* Wordpress.com, 3 June 2010.

Garcia, Patricia. “New Zealand to Reap Benefits of Pure Biocrude.” *EcoSeed, Algae Fuel.* EcoSeed, 1 Dec. 2009. Web.

Garthwaite, Josie. “SAIC Wins $25M Algae Jet Fuel Contract.” *Earth2Tech.* GigaOM, 28 Jan. 2009. Web.

Genifuel. “Clean Energy from Wet Biomass.” *Genifuel Home Page.* Genifuel, Oct. 2010. Web.

Gerbens-Leenes, Winnie, Arjen Y. Hoekstra, and Theo H. van der Meer. “The Water Footprint of Bioenergy.” *PNAS* 106.25 (2009): 10219 –10223.

Gevert, B.S., and J-E. Otterstedt. “Upgrading of directly liquefied biomass to transportation fuels – hydroprocessing.” *Biomass.* 13.2(1987).

Ghisalberti, Emilio L. “Detection and Isolation of Bioactive Natural Products.” Bioactive Natural Products: Detection, Isolation, and Structural Determination. Ed. Steven M. Colegate and Russell J. Molyneux. Boca Raton, Florida: CRC Press, Taylor & Francis Group, 2008.

Global Green Solutions. “Vertical Bioreactor FAQ's.” Global Green Solutions, n.d. Web.

Go, Kamille. “First Seaweed-Based Biofuel Plant Goes Ahead In Chile.” *MercoPress South Atlantic News Agency.* MercoPress, 22 Jan. 2010. Web.

Government of Western Australia. “State Government Invests in Low Emissions Technology.” Ministerial Media Statements. Government of Western Australia, 29 Sep. 2010. Web.

“Governments of Canada and PEI Provide Funding for Solarvest Algal Hydrogen Production Development.” *Biofuels Journal, Advanced Biofuels.* Country Journal, 17 Nov. 2010. Web.

Graff, Stephen. “Solazyme Developing Cheaper Algae Biofuels, Brings Jobs to Pennsylvania.” *Energy Empowers, the Nation’s Clean Energy Stories.* U.S. Department of Energy, Energy Efficiency and Renewable Energy, 6 Aug. 2010. Web.

Grima, E. Molina, et al. “Recovery of Microalgal Biomass and Metabolites: Process Options and Economics.” *Biotechnology Advances* 20.7-8 (2003): 491-515. Web.

Hatch, Gareth. “Magnets, Algae and Biofuels.” *Applications, Biofuels.* Terra Magnetica, 13 Dec. 2009. Web.

Hielscher Ultrasonics. “Biodiesel from Algae Using Ultrasonication.” *Ultrasound Technology Applications.* Hielscher Ultrasonics, n.d. Web.

“Honeywell’s UOP Awarded Funding for CO_2 Capture.” *Algae Industry Magazine.com Home, Process.* The Biofuel Media Group, 2010. Web.

Hughes A.R., et al. “Associations of Concern: Declining Seagrasses and Threatened Dependent Species.” The Ecological Society of America. *Frontiers in Ecology and the Environment* 7.5 (2009): 242-246. Text.

Hussain, Khalid. “Economically Effective Potential of Algae for Biofuel Production.” *World Applied Sciences Journal* 9.11 (2010): 1313-1323. Web.

International Energy Agency. “Annual Report 2008: Implementing Agreement on Photovoltaic Power Systems.” International Energy Agency Photovoltaic Power Systems, Apr. 2009. Text.

International Energy Agency (IEA). “Biogas and More! Systems and Markets Overview of Anaerobic digestion.” International Energy Agency Bioenergy. Culham, UK: AEA Technology Environment, July 2001. *BioRes.* Web.

International Energy Agency (IEA). “Energy Technology Perspectives 2008 - Scenarios & Strategies to 2050.” International Energy Agency, 2008. Text.

International Energy Agency (IEA) Bioenergy Implementing Agreement. “Algae – The Future for Bioenergy? Summary and Conclusions.” IEA Bioenergy ExCo64, Feb. 2010.

International Energy Agency (IEA). ”Key world energy statistics 2010”. IEA publication, Paris, France. 2010.

International Technology Transfer Center. “Second Generation Biodiesel Production by Algae Fermentation.” *ITTC, Opportunities.* International Technology Transfer Center, 13 May 2008. Web.

Joshi, D.D. “Manufacturing of Bio-Diesel from Jatropha Curcas (Ratanjyot): Extraction, Transesterification, Process Control, Quality Control and Quality Assurance.” Amity University School of Natural Resources and Sustainable Development. Noida, India. 2006. Presentation.

Kagan, Joshua. “UOP: An Unlikely Cleantech Leader.” *Greentech Media, Company Profile, Biofuels.* GTM Research, 12 Apr. 2010. Web.

Kagan, Joshua, and Travis Bradford. “Biofuels 2010: Spotting the Next Wave.” *GTM Research.* Greentech Media, Inc. 7 Dec. 2009. Web.

Kanellos, Michael. “Algae Biodiesel: It's $33 a Gallon.” GreenTechMedia. Greentech Media, Inc., 3 Feb. 2009. Web.

Kanellos, Michael. “BP Gives Nod to Algae Fermentation With Martek Deal.” *Greentech Media.* Greentech Media, Inc., 11 Aug. 2009. Web.

Karlsruhe Institute of Technology (KIT). “Hydrogen from Microalgae - Scientists Work on Highly Efficient Processes – KIT Coordinates Joint Project.” *Current Topics, Press Releases.* Karlsruhe Institute of Technology, 10 Dec. 2009. Web.

Knip, Karel. *Kweken van algen kost meer energie dan het oplevert. (Growing algae costs more energy than it yields.)* In Dutch. Newspaper nrc de week, 23 June 2008, p.12-13.

Komárek, J. “Coccoid and Colonial Cyanobacteria.” *FreshwaterAlgae of North America – Ecology and Classification*. Ed. John D. Wehr and Robert G. Sheath. Academic Press: Boston, 2003. 59-116. Le Hir, Pierre. “Les Microalgues, Usines a Biodiesel.” Le Monde. 12 Juin, 2010. Web.

Koszarek, Marita, and Maria Kubacka. “The Spatial Development Concept of the Polish Maritime Areas in the Context of the Approaching “Hydrogen Era”.” Future Use of the Polish Maritime Areas for Economic and Ecological Purposes. Gdynia, Poland. 21-22 Oct. 2009. Presentation.

Kruse, Olaf, et al. “Improved Photobiological H_2 Production in Engineered Green Algal Cells.” *The Journal of Biological Chemistry* 280.40 (2005): 34170–34177.

Kyndt, John. “Algae-based Biodiesel: Current Procedures and Innovations.” *Algae Based Biodiesel.* AlgaeforBiofuels, 9 May 2010. Web.

Laboratory of Microalgal and Bacterial Bioenergetics and Biotechnology (L3BM). “Photobiologic Production of Hydrogene.” *CEA Direction des Sciences Vivant – IBEB.* Institut de Biologie Environnementale et de Biotechnlogie, Commissariat à l'Énergie Atomique et aux Énergies Alternatives, n.d. Web. 17 Sep. 2009.

Lane, Jim. “50 Hottest Companies in Bioenergy Candidate Profile: Biolight Harvesting.” Biofuels Digest, 14 Aug. 2009. Web.

Lane, Jim. “Cyanobacteria: the New Biofuels Platform?” Biofuels Digest, 23 Dec. 2009. Web.

Lane, Jim. “DARPA Awards $25 Million to SAIC for Algae Jet Fuel Development: $3 Per Gallon Target.” Biofuels Digest, 28 Jan. 2009. Web.

Lane, Jim. “DARPA Official Says Teams at $2 Per Gallon Algal Fuel, Headed for $1; Commencing Commercial Scale by 2013.” Biofuels Digest, 15 Feb. 2010. Web.

Lane, Jim. “Philippines Government to Develop $5 Million, 250-Acre Seaweed Ethanol Aquafarm.” Biofuels Digest, 10 May 2010. Web.

Lang, K.S., et al. "Mechanisms of Suicidal Erythrocyte Death." *Cellular Physiology and Biochemistry* 15.5 (2005): 195–202. Web.

Lardon, Laurent; Arnaud Hélias; Bruno Sialve; Jean-Philippe Steyer; Olivier Bernard. *Life-cycle assessment of biodiesel production from microalgae.* Environmental Science & Technology, Vol.43, no.17, p.6475-6481. American Chemical Society, 2009.

Lassing, Merit, et al. “Final Report on Biodiesel Production from Microalgae A Feasibility Study.” Lund University, Department of Chemical Engineering. StatoilHydro ASA, 16 May 2008. Web.

Le Hir, Pierre. “Les microalgues, usines à biodiesel. (Microalgae, production plants of biodiesel.)” In French. Newspaper Le Monde, 12 June 2010.

Lemmens, Bert. “Water Recycling and Harvesting of Algae via Membrane Filtration.” Vision on Technology (VITO), AquaFUELS Roundtable. Brussels, Belgium. 21-22 Oct. 2010. Presentation.

Lombardi, Candace. “PetroAlgae Signs Deal with Indian Oil.” *Cnet News Greentech.* CBS Interactive, 5 Nov. 2009. Web.

Mao, Blaise. “Microalgues: Le Biocarburant du Futur?” *GEO Environment.* GEO, 22 Jan 2009. Web.

McDermott, Matthew. “LiveFuels to Farm Fish to Make Biofuel = Bad Idea.” *TreeHugger.com.* 13 Aug. 2009. Web.

McDill, Stuart. “Can Algae Save the World – Again?” *Reuters.com.* Thomson Reuters, 10 Feb. 2009. Web.

McIntyre, Craig. “Measurement Solutions for Biodiesel Renewable Fuels Industry.” Endress + Hauser, 12 Apr. 2007. Web.

Mees, S. *GreenFuel algae CO_2 recycling project with Aurantia enters second phase at Spanish cement plant.* Thomson Reuters, 21 October 2008. www.reuters.com viewed 17 September 2009.

Millikin, Mike, ed. “ARPA-E Awards $8.8M to DuPont and BAL for Macroalgae to BioButanol Work.” *Green Car Congress.* BioAge Group LLC, 4 Mar. 2010. Web.

Millikin, Mike, ed. “DOE to Award Honeywell’s UOP $25M for Pyrolysis Oil to Renewable Fuels Demo Unit.” *Green Car Congress.* BioAge Group, 12 Jan. 2010. Web.

Millikin, Mike, ed. “Queensland Government Puts A$166K Into Algae Biodiesel Project.” *Green Car Congress.* BioAge Group, LLC, 27 May 2008. Web.

Mollman, Steve. “’Green goo’ biofuel gets a boost.” *CNN.com.* Cable News Network, 24 Aug. 2009. Web.

Moresco, Justin. “Acoustic Algae? Solix & Los Alamos Lab Team Up.” *Earth2Tech.* GigaOM, 11 Sep. 2009.

Mortureux, Marc. “Avis - Relative aux Recommandations de Prévention des Risques Liés aux Algues Vertes pour la Santé des Populations Avoisinantes, des Promeneurs et des Travailleurs.” ANSES Agence Nationale de Sécurité Sanitaire de l’Alimentation, de l’Environnement et du Travail. 15 July 2010.Web.

Moser, Daniel. “UNL Hopes to Tap Into Algae as Biofuel Energy Source.” Institute of Agriculture and Natural Resources at the University of Nebraska-Lincoln. 16 Feb. 2010. Web.

Murdoch University. “$2 million to Turn Algae into Clean, Affordable Fuel.” *Murdoch University News.* Murdoch University, 2008. Web.

National Renewable Energy Laboratory. “Biodiesel Handling and Use Guide.” 4th ed. *Advanced Vehicles and Fuels Research.* National Renewable Energy Laboratory, Jan. 2009. Web.

Neely, Pamella. “Biodiesel or Straight Vegetable Oil.” *Articlesbase Automotive. Articlesbase,* 18 Apr. 2010. Web.

Netherlands Ministry of Economic Affairs, Agriculture and Innovation. “25 Million Euros for Unique Research into Photosynthetic Energy from Plants and Algae.” *Press Releases*. Netherlands Ministry of Economic Affairs, Agriculture and Innovation, 17 July 2009. Web.

North Carolina Department of Environment and Natural Resources, “Aqueous Enzymatic Extraction of Oil from Rapeseeds.” *Division of Pollution Prevention and Environmental Assistance.* North Carolina Department of Environment and Natural Resources, 1994. Web.

Oilgae Academic Edition. “Solix, Solazyme and Phycal’s Cost-Effective Harvesting Techniques!” Oilgae, 5 Feb. 2009. Web.

Oilgae. “Algae Fuels - Custom Research & Market Analysis.” *Oilgae.* Oilgae.com, n.d. Web.

Oilgae. “Oilgae Guide to Fuels From Macroalgae.” Oilgae, Apr. 2010.

OriginOil Web Site. *OriginOil Products.* n.d. Web.

PetroAlgae. “Transforming Sunlight into Fuel and Food for the World.” *PetroAlgae.* PetroAlgae, LLC, Sep. 2010. Web.

Piccolo, Tony. “Five Key Strategies for Algae Biofuels Commercialization.” *Aquatic Biofuels.* WordPress.com, 18 June 2009. Web.

Pike Research. “Algae-Based Biofuels Demand Drivers, Policy Issues, Emerging Technologies, Key Industry Players, and Global Market Forecasts.” *Research, Clean Transportation.* Pike Research, 2010. Web.

Pollack, Andrew. “Exploring Algae as Fuel.” *Business Day, Energy and Environment.* The New York Times, 26 July 2010. Web.

Pressman, Andrew, and Mike Morris. “Biodiesel, Use, Handling and Fuel Quality.” National Sustainable Agriculture Information Service (ATTRA), National Center for Appropriate Technology, Sep. 2007. Web.

Prince Edward Island. “Sonochemistry.” *Food Technology Centre, Natural Products and Bio-Technologies Services.* Prince Edward Island Canada, n.d. Web.

Radmer, R.J., & Parker, B.C. “Commercial applications of algae: opportunities and constraints.” *Journal of Applied Phycology.* 6(2), 93-98, 1994.

Real, Natalia. “Govt to Fund Algal Biofuel Research.” *FIS Worldnews.* Fish Information and Services, 3 Sep. 2010. Web.

Reijnders, Lucas. “Do Biofuels from Microalgae Beat Biofuels from Terrestrial Plants?” *Trends in Biotechnology* 26.7 (2008): 349-350.

Reith, J.H., et al. “*Bio-offshore - Grootschalige teelt van zeewieren in combinatie met offshore windparken in de Noordzee (Bio-offshore - Large scale cultivation of seaweeds in combination with offshore wind power farms in the North Sea.)* In Dutch, with an abstract in English. ECN Energy research Centre of the Netherlands, report no. ECN-C--05-008, March 2005.

Reith, J.H., ed. “Duurzame Co-Productie Van Fijnchemicaliën en Energie uit Micro-Algen.” ECN Energy Research Centre of the Netherlands, Apr. 2004. Web.

Reith, J.H., R.H. Wijffels, and H. Barten, ed. “Bio-Methane & Bio-Hydrogen - Status and Perspectives of Biological Methane and Hydrogen Production.” Dutch Biological Hydrogen Foundation, 2003. Web.

Resnick, Richard J. “The Economics of Biological Methods of Hydrogen Production.” Massachusetts Institute of Technology, June 2004.

Riis, Trygve, Elisabet F. Hagen, Preben J.S. Vie, and Oystein Ulleberg. “Hydrogen Production and Storage - R&D Priorities and Gaps.” International Energy Agency Hydrogen Implementing Agreement and IEA Hydrogen Co-ordination Group, Jan. 2006.

Ripplinger, Peter (Subitec GmbH). “Industrial Production of Microalgae Biomass with Flat-Panel Airlift Photobioreactors.” International Algae Congress 2009, Hamburg, Germany. 1-2 Dec. 2009. Presentation

Ritch, Emma. “Indonesia, S. Korea Partner for Seaweed Biodiesel.” *Cleantech Group News.* Cleantech Group, 6 Nov. 2008. Web.

Ritch, Emma. “US Military Funds $35M in Research of Algae-Based Jet Fuel.” *Cleantech Group News.* Cleantech Group, 22 Dec. 2008. Web.

Robinson, Paul R., and Geoffrey E. Dolbear. “Commercial Hydrotreating and Hydrocracking.” *Hydroprocessing of Heavy Oils and Residua.* Ed. Ancheyta, Jorge and James G. Speight. Boca Raton, Florida: CRC Press, Taylor and Francis Group, 2007. 281-312. Web.

Ryan, Catie (*Terrapin Bright Green LLC).* “Cultivating Clean Energy: The Promise of Algae Biofuels.” National Resources Defense Council, Oct. 2009. Web.

Ryan, Connie, and Mary Patyten ed. “Annual Status of the Fisheries Report through 2003.” California Department of Fish and Game, Marine Region. Fish and Game Commission, Dec. 2004.

Sancon Resources Recovery. “Algal Fuels Consortium (AFC) Won $2.724M in Australia Government’s Development Grant.” Sancon Resources Recovery, Inc., 20 Aug. 2009. Web.

Sapphire Energy. “Governor Bill Richardson Highlights More Than $100 Million to Be Invested in Alternative Fuels Project.” *Sapphire Energy News & Media.* Sapphire Energy, 4 Dec. 2009. Web.

Seaweed Energy Solutions. “Renewable Energy from the Sea. A Rapidly Growing Opportunity.” *Seaweed Energy Solutions.* Seaweed Energy Solutions AS, n.d. Web.

SBI. “Algae Biofuels Production Technologies Worldwide.” *ReportsandReports.* SBI, Aug. 2010. Web.

Schiener, Peter. “The Next Generation of Biofuels: Ocean – The Final Frontier.” BioMara Project. Scottish Association of Marine Science. Apr. 2010. Presentation.

Seafish. “Anaerobic Digestion.” *Seafish, the Authority on Seafood.* Seafish, 28 July 2005. Web.

“Shamash Project.” *Presentation.* Program National de Recherche sur les Bioenergies, Agence Nationale de la Recherche, n.d Web. 13 Oct. 2009 and 23 Nov. 2010.

Sheehan, John, et al. “A Look Back at the U.S. Department of Energy’s Aquatic Species Program—Biodiesel from Algae.” National Renewable Energy Laboratory. U.S. Department of Energy’s Office of Fuels Development, July 1998. Web.

Shelef, G., A. Sukenik, and M. Green. “Microalgae Harvesting and Processing: A Literature Review.” Technion Research and Development Foundation. Solar Energy Research Institute, Aug. 1984. Web.

Sialve, Bruno, Nicolas Bernet, and Olivier Bernard. “Anaerobic Digestion of Microalgae as a Necessary Step to Make Microalgal Biodiesel Sustainable.” *Biotechnology Advances* 27.4 (2009): 409-416.

Sibley, Lisa (Cleantech Group). “Algae Expert Calls Livefuels' Biofuel-from-Fish Approach Not 'Impossible'.” *Cleantech Group News.* Cleantech Group, 13 Aug. 2009. Web.

Siegel, RP. “Former BP Exec Sets Her Sights on Algae as the Fuel of the Future.” *Triple Pundit, People, Planet, Profit.* Triple Pundit, 14 July 2010. Web.

Solar BioFuel Consortium. “Home Page.” *Solar BioFuel Consortium*. BioFuels Australia, Sep. 2009. Web. 10 Oct. 2010.

Solarvest. “Solarvest Proposes the Creation of an Integrated Process with Three Main Components.” *Solarvest BioEnergy Technology.* Solarvest, n.d. Web.

“Solazyme Awarded US $21M Grant for Algae Refinery in Pennsylvania.” *RenewableEnergyWorld.com News.* RenewableEnergyWorld.com, 9 Dec. 2009. Web.

Solazyme. “Solazyme Delivers 100% Algal-Derived Renewable Jet Fuel to U.S. Navy: Biotechnology Company Showcases Solajet™HRJ-5 Jet Fuel at the World-famous

Farnborough International Air Show in UK." *Solazyme.com*. Solazyme, 19 July 2010. Web.

Solazyme Web Site. Solazyme, n.d. Web.

Solena Group. "NASA-based Technology and Algae System Eliminates CO_2 Emissions." *Solena Group Technology.* Solena Group, n.d. Web.

Special EU Programmes Body. "What is the INTERREG IVA Programme?" *2007-2013 Programmes, INTERREG IVA Programme.* Special EU Programmes Body, n.d. Web. 24 Nov. 2010.

Stanhope Seta Ltd. "Biofuel Specification." Stanhope Seta Ltd website. Accessed 4 Jan. 2011.

Stanley, Michele. "Fuel from the Sea?" IEA IA-Bioenergy ExCo64 workshop. Liege, Belgium. 1 October 2009. Presentation.

Stephenson, Anna L., et al. "Life-cycle Assessment of Potential Algal Biodiesel Production in the United Kingdom: A Comparison of Raceways and Air-lift Tubular Bioreactors." *Energy & Fuels* 24.7 (2010): 4062-4077.

SunEco Energy. "SunEco Biocrude and Straight Vegetable Oil (SVO)." *Products, BioCrude & SVO.* SunEcoEnergy, n.d. Web.

Supercritical Fluid Extraction Research Group. "Introduction of the Physico-Chemical Properties of the Supercritical Fluids." *Research Supercritical Fluid Extraction.* Budapest University of Technology and Economics, 2010. Web.

Supercritical Fluid Technologies Web Site. Supercritical Fluid Technologies, n.d. Web.

Swanepoel, Esmarie. "Algae – Fuel for Thought." *Algae Biodiesel Research in South Africa.* BioDieselNow, 2 Nov. 2007. Web.

Synthetic Genomics. "Next Generation Fuels and Chemicals." *What We Do.* Synthetic Genomics, n.d. Web.

Targeted Growth. "Targeted Growth Increases Algae Oil Yield." *Biodiesel Magazine.* BBI International Media, 29 July 2009. Web.

TheBioenergySite News Desk. "Brazil Fuels Algae Research with US$ 2.8 Million." *Global Bioindustry News.* The Bioenergy Site, 15 Aug. 2008. Web.

Theriault, R.I. "Heterotrophic growth and production of xanthophylls by *Chorella pyrenoidosa." Applied Microbiology.* 1965. *13,* 402-416.

Thurmond, Will. “Seaweed: A New Wave of Investment in Macro-Algae.” *Algae Biofuels, Biofuels International.* Emerging Markets Online, Oct. 2010. Web.

Tornabene, T.G., et al. “Lipid Composition of the Nitrogen Starved Green Alga Neochloris oleoabundans.” *Enzyme and Microbial Technology* 5.6 (1983): 435-440. Web.

Uduman, Nyomi, et al. “Dewatering of Microalgal Cultures: A Major Bottleneck to Algae-Based Fuels.” *Journal of Renewable and Sustainable Energy 2.1* (2010) Web.

“UK Scientists Collaborate on Macroalgae to Methane.” *Algae Industry Magazine, The Buzz.* The Biofuel Media Group, 11 Sep. 2010. Web.

United Nations, Food and Agriculture Organization (FAO). “How to Feed the World in 2050.” *Global Forum on Food Security and Nutrition.* United Nations Food and Agriculture Organization, 25 Sep. 2009. (date discussion published, but not of forum) Web.

University of Illinois at Urbana Champaign Department of Agricultural and Biological Engineering. “Illini Algae – Hydrothermal Liquefaction.” *UIUC Illini Algae.* University of Illinois at Urbana Champaign Department of Agricultural and Biological Engineering, n.d. Web.

UOP. “Green Jet Fuel.” *UOP Renewable Energy and Chemicals.* UOP, n.d. Web.

U.S. Department of Energy. “Department of Energy Announces $24 Million for Algal Biofuels Research Releases Final National Algal Biofuels Technology Roadmap.” *U.S. Department of Energy, Press Releases.* U.S. Department of Energy, 28 June 2010.

U.S. Department of Energy, Energy Efficiency and Renewable Energy. “Algal Biofuels: Growing America’s Energy Future.” U.S. Department of Energy, Energy Efficiency and Renewable Energy, 14 Oct. 2008. Web.

U.S. Department of Energy, Energy Efficiency and Renewable Energy. “Solazyme Integrated Biorefinery: Diesel Fuels from Heterotrophic Algae.” *Biomass Program.* U.S. Department of Energy, Energy Efficiency and Renewable Energy, Mar. 2010. Web.

U.S. Department of Energy. “National Algal Biofuels Technology Roadmap.” U.S. Department of Energy Office of Energy Efficiency and Renewable Energy Office of the Biomass Program, May 2010. Web.

U.S. Department of Energy. “Recovery Act Announcement: Secretary Chu Announces Nearly $80 Million Investment for Advanced Biofuels Research and Fueling Infrastructure.” U.S. Department of Energy Office of Energy Efficiency and Renewable Energy, 13 January 2010. Web.

U.S. Department of Energy. “Report to Congress: Microalgae Feedstocks for Biofuels Production (EISA 2007 – Section 228).” National Renewable Energy Laboratory, U.S. Department of Energy, 14 Mar. 2008. Print.

U.S. Department of Energy. “Univenture / Algaeventure Systems: Scaling and Commercialization of Algae Harvesting Technologies.” *Advanced Research Project Agency, Energy.* U.S. Department of Energy, n.d. Web.

U.S. Energy Information Administration. “Biomass for Electricity Generation.” *Independent Statistics and Analysis.* U.S. Energy Information Administration, 2001. Web.

U.S. Energy Information Administration: Independent Statistics and Analysis. “Alternatives to Traditional Transportation Fuels 2008.” U.S. Department of Energy, Apr. 2010. Web.

U.S. Energy Information Administration. “Weekly Retail On-Highway Diesel Prices.” *Independent Statistics and Analysis.* U.S. Energy Information Administration, Nov. 2010. Web.

van Es, Marjon, and Rob Stroeks. “De Status Van de Algenbiomassa in Japan.” *TWA Nieuws* 48.1 (2010): 33-34.

van Iersel, Sjors (Ecofys), et al. “Algae-Based Biofuels: A Review of Challenges and Opportunities for Developing Countries.” Food and Agriculture Organization of the United Nations (FAO) Environment, Climate Change and Bioenergy Division, May 2009. Web.

van Ree, René, and Bert Annevelink. “Status Report Biorefinery 2007.” SenterNovem. Wageningen UR (University & Research Centre), Agrotechnology and Food Sciences Group. Nov.2007.

Vergara-Fernández, Alberto, et al. “Evaluation of Marine Algae as a Source of Biogas in a Two-Stage Anaerobic Reactor System.” *Biomass and Bioenergy* 32.4 (2008): 338-344. Web.

Wageningen University & Research Centre. *Research on Microalgae within Wageningen UR.* Wageningen University, 2010. Web.

Wagner, Leonard. “Biodiesel from Algae Oil.” Mora Associates Ltd, 2007. Web.

Wesoff, Eric. “Growing Algae: Open Pond vs. Closed Bioreactors.” *Media.* Aurora Algae, 7 May 2009. Web.

Wijffels, Rene. “Biofuels from Microalgae.” Bioprocess Engineering Group, Wageningen UR (University & Research Centre), 15 Jan. 2008. Web.

Wijffels, René H., and Maria J. Barbosa. “An Outlook on Microalgal Biofuels.” *Science* 329.5993 (2010): 796-799. Web.

Wild, Geoff, and Jason de Koning. “Introduction.” *Contaminant Analysis Techniques.* Wheelock College, 1997. Web.

Williams, Jonathan. “U.S. Government's Investment in Algae.” *Celsias News and Opinions.* Celsias, 25 May 2010. Web.

Winkler, Martin, et al. “Characterization of the Key Step for Light-Driven Hydrogen Evolution in Green Algae.” *The Journal of Biological Chemistry* 284.52 (2009): 36620-36627.

Wörgetter, M., et al. “Biodiesel Production: Technologies and European Providers: A Report to IEA Bioenergy Task 39.” International Energy Agency Bioenergy Task 39, 6 July 2007. Web.

World Watch Institute . “World Watch Issue Brief U.S. Biofuels: Climate Change and Policies.” *WorldWatch.org.*, July 2009. Web.

Yu, J., and P. Takahashi. “Biophotolysis-Based Hydrogen Production by Cyanobacteria and Green Microalgae.” *Communicating Current Research and Educational Topics and Trends in Applied Microbiology.* Ed. A. Méndez-Vilas. Formatex, Spain, 2007.

Zimmerman, Eilene. “The Next Green Fuel Source: Algae.” *CNN Money.com Small Business.* Cable News Network, 30 Mar. 2009. Web.