



## Constraints to large scale algae biomass production and utilization

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### Abstract

Microalgae offer great promise in the production of food supplements for humans, animal feed, oil extraction and its trans-esterification to produce biodiesel, electricity production upon combustion directly or by transforming the algae to methane anaerobically, CO<sub>2</sub> fixation ability, or fuel production via pyrolysis, gasification or anaerobic digestion. However, the development of algal biomass production technology is faced with numerous problems. In this review, we present an overview about the constraints militating against microalgae biomass production and utilisation. These challenges span the entire length of the algal production chain, from algal biology to algal cultivation to biomass harvesting to extraction of lipids and finally to the conversion of the algal oil to fuels. This perspective provides a brief overview of the potential technical and economic barriers that need to be overcome before production of microalgal-derived products can become a large-scale business.

**Keywords:** Microalgae; Triacylglycerols, Lipids, Biofuels; High energy density

### Introduction

Almost everything on this planet has been harnessed, farmed, or cultured for man's various needs. These resources have benefited man in many ways such as providing electricity, fuel for transportation and food. However, as population increases, these resources decreased proportionally. In some particular cases, resources have even dwindled much faster because of abuse. It is imperative that for a resource to be sustainable, the pace and amount of replenishment should be greater than the speed and volume of consumption, or at least, equal the rate of consumption. Humans, in their effort to survive, have designed various forms of intervention to ensure the sustainability of very important resources. Scientific studies and social researches have been advanced to find ways of developing sustainable projects, which can empower people to meet economic needs without relying on methods that can adversely affect the environment.

Micro-algae are one of the tiniest plants which alone produce about 60 percent of the Earth's oxygen. These organisms constitute a total of twenty-five to thirty thousand species, with a great diversity of forms and sizes, and that can exist from unicellular microscopic organisms (microalgae) to multicellular of great size (macroalgae). They can survive harsh conditions, are incredibly robust and in ideal cultivation conditions, produce protein and energy biomass from 30 to 100 times faster than land plants

while the variants which contain more carbohydrates and less oil it can be fermented to make ethanol or biogas. It is interesting to note however that some algae strains or variants contain up to 50 percent lipids making them very suitable for the production of liquid fuels. In this way, microalgae can be considered as genuine natural reactors being, in some cases, a good alternative to chemical synthesis for certain compounds.

However, the large scale algae biomass generation and utilisation face formidable challenges. The demand for algae biomass is increasing worldwide, yet the ability for producers to meet that demand requires development of technologies to reduce the cost of production while maintaining and improving product quality. Producers, processors and breeders are in need of systems that successfully identify, promote and harness algae biomass to maximize profits, secure supply, reduce environmental impacts, increase market competitiveness, sustain small and mid-sized scale producers and earn consumer confidence. In spite of the apparent potential usefulness of algal biomass and years of research centered around understanding algal growth in both laboratory and field culture, mass algal culture has not yet been commercially realized to any large extent due to the complexity of the production process. The present paper tends to highlight some physical and chemical constraints that may hinder large scale algae biomass generation and utilisation.

## SOME ECONOMIC STRAINS OF MICRO ALGAE

*Botryococcus braunii* is a green algae strain that is very unique in its ability to produce hydrocarbons. When colonies of the strain are grown, they show up as floating mass blooms allowing for easy skimming harvesting off of the top of the growth medium. According to researchers, optimal growth conditions to ensure maximum biomass and maximum hydrocarbon output have been achieved. However, Botryococcus are not able to sustain the agitation intensities of some of the growth techniques.

*Scenedesmus dimorphus* is usually categorized as a heavy bacterium that has a lipid content of 16-40 %. Scenedesmus is a very promising strain that should be further researched. The strain must be constantly agitated while grown because of the ease of sediment build-up which hinders growth. The optimal growth temperature falls between 30-35 degrees Celsius (86-95 degrees Fahrenheit). Scenedesmus will use any and all light it is given and should be further researched for use in mass production. Unlike botryococcus, scenedesmus can be acquired from a variety of public sources.

*Euglena gracilis* has a lipid content of 14-20 % by dry weight. The optimal temperature requirement is 27-31 degrees Celsius (80.6-87.8 degrees Fahrenheit). Euglena was one of the few strains that had information on the nutrient medium content for optimal photosynthesis. The strain enjoys a carbon dioxide concentration within the medium of 4 % and an oxygen concentration of 20 %. The lighting requirement for the strain is a photosynthetic photon flux of 100 micromoles  $m^{-2} s^{-1}$ . Euglena can be acquired easily from a variety of public sources.

*Prymnesium parvum* known as a golden alga, prymnesium has a lipid content on average of 22-38 %. The difficulty that comes with prymnesium is that it is considered toxic algae which could prove problematic when dealing with it in large quantities. If it were to be mass produced, various safety hazards would have to be taken into consideration when working with it. The strain optimally grows in salinity from 4 % to three times the salinity of regular water which is a constraint that should be further analyzed to narrow its window.

## PROCESSED ALGAL BIOMASS

### Algae Concentrates

Concentrated algae paste can be formed by concentrating algae from mass cultures and preserving the resultant paste through refrigeration, freezing or drying. The harvesting procedures to concentrate microalgae can be obtained by

centrifugation, flocculation, filtration, foam fractionation or photobioreactors.

Once the microalgae concentrate is obtained, it is preserved by adding additives or preservatives, freezing and refrigeration. The microalgae concentrate may be stored for several days, weeks or months. Generally, the maximum time the paste can be kept and still retain its nutritional value equivalent to fresh algae ranges from about 1 week to 4 weeks, depending upon the species of alga. Temperature and dark condition are important factors that can increase the period of viability of algal concentrates. Beside

temperature and light, oxygen can also be an important factor. In some algae, storage at low temperature with addition for air bubbling will increase viability. Although the production of concentrated microalgae is feasible, the issue is whether or not this concentrate is adequate for the culture of bivalve larvae.

### Dried algae

Another alternative to artificial diets that may overcome the costly and unpredictable production of fresh microalgae is the use of algae that has been preserved by drying. Heterotrophic production of dried algae is achieved by growing algae using organic carbon instead of light as an energy source in fermenters. The benefit of the heterotrophic technique is that microalgae production is at much higher densities and more cost effective than the photoautotrophic culture.

In spite of the promising results there are several disadvantages of using heterotrophic techniques, including the limited number of microalgae species that can be grown by this method. To date, just a few spray dried microalgae are grown commercially for use as aquaculture feeds such as *Cryptocodinium* sp. and *Schizochytrium* spp. Another disadvantage is that heterotrophic cultivation has potential to be contaminated by bacteria and growth inhibition when cultured in low organic substrate concentration. Overall, dried algae appear to be a good feed supplement but may not fully replace fresh microalgae due to their lower nutritional value.

## VALUABLE PRODUCTS FROM MICROALGAE BIOTECHNOLOGY

Microalgae are the bottom of the food chain in all aquatic ecosystems and comprise the greatest abundance of plant biomass in aquatic environments (Skjanes *et al.*, 2007). There are an estimated 25,000 microalgae species, with only around 15 in current commercial production (Raja *et al.*, 2008) for established markets for microalgal products. This almost untapped resource offers multiple commercial

development options (Kuda *et al.*, 2005). Commercial microalgae production can produce vitamins, minerals, proteins, fats, sugars, antioxidants, cosmetics, pharmaceuticals, soil conditioners, biomass, biochemicals, bioactive nutraceuticals, biofertilisers, natural dyes and colours, in addition to animal feeds. Some microalgae also produce useful carotenoids, phycobilins, polyketides, mycosporine-like amino acids, glycerol, steroids, tocopherol, lectins, astaxanthin, canthaxanthin, functional sulphated polysaccharides, zeaxanthin, halogenated compounds and some toxins. Alternative microalgal

applications include nitrogen fixation in rice cultivation, and erosion process suppression from assisted surface solidification in arid regions. A common commercial horticultural use of algae is also to stimulate plant germination, flowering, and as a stem and leaf growth promotent (Pulz and Gross, 2004). Microalgae can also be used as sensitive bio-indicators for lipophilic organic contaminants to detect the presence of pharmaceutical and personal care products in wastewater treatment plants (Skjanes *et al.*, 2007).

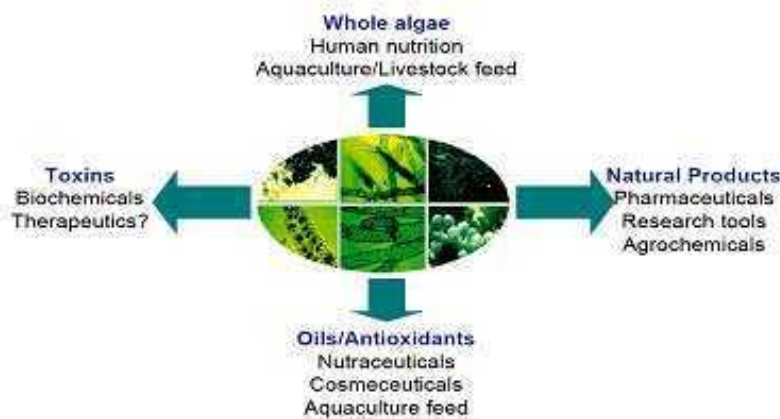


Figure 1 : Market sectors of micro algae (Jaspars, 2010)

Food fortification with microalgae products are potentially cheaper and safer supplies of fatty acids than conventional sources. Microalgal oils are also able to be consumed by vegetarians as they are considered plant sources, and can eliminate some concerns about potential fish product contaminants (Whelan *et al.*, 2006). In terms of animal feed, microalgal supplements at particular doses increase aquacultural fish feed efficiency and weight gain against control diets (Eufemia *et al.*, 2000).

The pursuit commercial algae production and expansion of new industries (such as advanced nutraceutical, protein therapeutic, and biofuels) merit further microalgae cross-disciplinary research and development (Rosenberg *et al.*, 2008). It is likely that the combination of continued technical innovation and market demand will ensure major advances and expansion of microalgal products, uses and

production technologies (Hankamer *et al.*, 2007). Whilst many of these products and synergies are divorced from the current mining and resource industries in the remote and arid areas, the industrial-scale supply chains inputs will require exploration of suitable locations to develop a secure and cost-effective system with robust production benefits.

#### ALGAE CULTURE AND SUSTAINABILITY

The degree of sustainability can be a determining factor of a product's success in society. Therefore for algae to be considered a sustainable engineering innovation, it must take into consideration the current problems in society as well as helping improve the future of the environment, economy and other aspects of society.



Figure 2: Aspects of algae Sustainability (Dinh *et al.*, 2009)

The most important issue when it comes to sustainability is the impact the biomass would have on the environment and society. When considering society, algae are the least invasive biomass compared to others. Algae can be grown in waste water plants and in enclosed ponds that do not interfere with human water use. Also, a major advantage of algae is the fact that it does not encumber the food supply. Socially, algae are a sustainable option because it decreases the dependence a state has on a foreign resource.

Algae production improves the environment by consuming the greenhouse gases, such as nitrogen and carbon dioxide, which the fossil fuels create. The production of algae is a natural process and does not harm the environment. Microalgae as a feedstock have many advantages over the other plant and animal biomass. The high oil content of algae and its fast growth rate is advantageous for mass production. Growing algae is spatially efficient and is not limited to certain growing seasons. The main resource needed to grow algae is water which is abundant and renewable. Large scale algal growth will benefit the environment since algae is a valuable carbon capture source and feeds on atmospheric nitrogen oxides, another prominent harmful greenhouse gas. Economically, algae are not a sustainable option because of the production costs. With several different manufacture options available for producing algae, making the transition from small scale ponds to mass production ponds is only a matter of figuring out how to enlarge the ponds while still keeping the process efficient.

Algae must be grown on a large scale to have a substantial impact. Naturally occurring algae are very low in density. In order to significantly increase the productivity, it is necessary to find ways to increase the growth rate and density of algae in the culture media. Algae species and strains vary greatly in terms of growth rate and

productivity, nutrient and light requirement, ability to accumulate lipids or other desirable compounds, ability to adapt to adverse conditions, etc. Therefore, the first step in mass cultivation of algae is to find or engineer right species and strains for specific purposes and cultivation systems. There are tens of thousands of algae species and strains in the world.

## LIMITATIONS ON ALGAL PRODUCTION TECHNOLOGY

### CULTIVATION LIMITATIONS

#### Strain Selection

The largest issue concerning growing microalgae for specific purposes is making the correct decision on a certain strain or strains to cultivate in the hopes of finding one that will work best for the purposes intended as well as provide the greatest returns. There are many species of micro algae with high potential value for biotechnology that are in early stages of development. Meanwhile, algal strain genetic improvement has lagged behind, hindered by a combination of the small numbers of families evaluated and lack of phenotypic information. Maintaining genetic diversity will be essential for providing a genetic basis for current and future breeding programs that will develop strains with efficient performance in different climates and production systems. Specifically, research is needed to characterize genetic diversity in domesticated and wild species, develop the means to identify and protect improved strains and conserve valuable germplasm. Selection of specific germplasm to be preserved requires phenotypic and genetic characterization for a wide array of phenotypic characters measured within appropriate production systems. The conditions of our specific system constraints must be considered for each strain to narrow the list down to a group that can be used on a mass production

basis. Characterization of genetic diversity should be performed using microsatellite and single nucleotide polymorphism (SNP) panels to ensure that the appropriate range and degree of variation is preserved. There is also a need to select, catalogue, and curate collections of germplasm for research and germplasm conservation purposes.

### Temperature

For effective growth of microalgae cultures an appropriate temperature range must be maintained depending upon the requirements of the strain. Every microalgae strain has a specific requirement for optimum temperature for maximum growth rates. Generally, temperatures between 15–25°C are acceptable for microalgae. Algae exhibit normal temperature to biological activity relationship with activity increasing with temperature until an optimum temperature is reached. Above the optimum temperature, biological activity declines, sometimes abruptly, to zero (Lowrey, 2011). Temperatures beyond the optimal temperature range will slow down the growth or kill the algae. Temperature tolerance may be found in some species or strains through screening, or may be trained through acclimation. In regions where winter is extremely cold, the algae production facility may be enclosed in a greenhouse to maintain a suitable temperature.

### Light Resource

Microalgae cell factories are driven by photosynthesis, their production therefore is dependent on the solar resource.

Across the globe, the solar resource varies. However, the maximum solar to chemical energy conversion potential is between 2.55-2.78 MJ m<sup>-2</sup> day<sup>-1</sup>. Therefore regions below this maximum chemical energy conversion potential will have to depend on artificial light source to host large proportion of micro algae in order to overcome the limitations posed by the low efficiency of photosynthesis.

Natural light is the first option in micro algal cultivation as light is critical to autotrophic growth of algae. Light is found to be a major limiting factor of productivity and growth when nutrition and temperature are satisfied (Cardozo *et al.*, 2007). Natural light fluctuates in either intensity or quality daily, seasonally and regionally. As well, the quality of light penetration is inhibitive to the surface layer of algae in the water column; however at the bottom of the water column the reduced light intensity might be fine or insufficient for photosynthesis due to, for example, blockage of upper layers of algae to light transmission. Artificial lighting may be necessary if algal growth 24-hour a day is desired and when natural lighting is inadequate. Both photo-inhibition and low light stress of photosynthesis causes decrease in biomass production (Kuda *et al.*, 2005). Furthermore, photosynthetic pigments (chlorophylls) exhibit best light absorption at around 440 and 680 nm wavelengths.

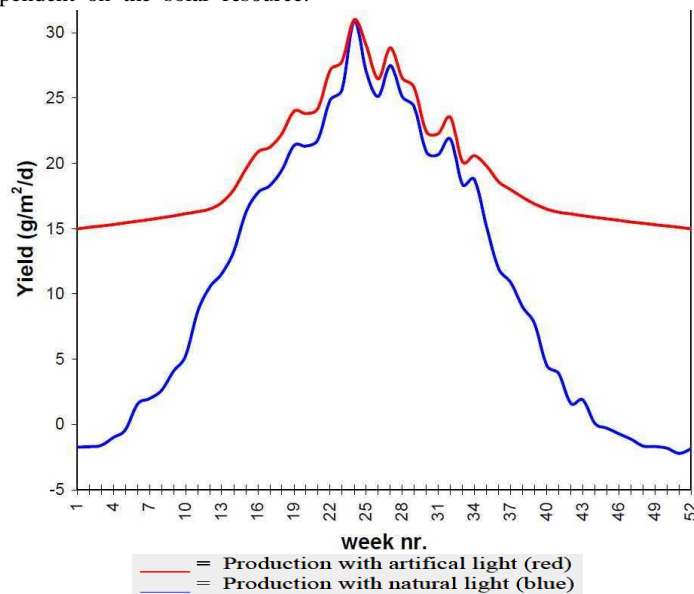


Figure 3: Estimated production period with and without artificial light (Berg-Nilsen, 2006)

White light with full spectral coverage cannot be fully absorbed by microalgae. Part of the light will be reflected or transmitted as wasted energy. However, studies had

shown that red LED (an artificial light source) (figure 2) is very attractive for photosynthesis because its emission spectrum fits with the photon energy needed to reach the first excited state of chlorophylls a and b. Blue light, of

which photons contain about 40 % more energy than the red light, can be absorbed by chlorophyll as well. Several studies utilized flashed LED light to simulate the light/dark cycle to prevent photo-inhibition. Both the flux density and time frequency can affect algae growth rate. There is a positive relationship between light intensity and productivity in which the maximal mixing-enhanced cell concentrations and productivity of biomass were obtained at the highest light intensity used. Photo-inhibition occurs during prolonged exposure to high irradiance

Photon absorption is affected by many factors, such as pigmentation in the algae cells, density of the culture, and the specific position of the cell (Richmond, 2004). Maximizing light utilization through design and operations is critical. The rate of mixing required careful optimization: when too low, maximal productivity resulting from the most efficient utilization of light could not be obtained. Too high a rate of mixing resulted in cell damage and reduced output rate. In open pond system, self-shading is affected by the cell density and depth of the pond. Due to irradiance variation throughout the year, the pond needs to be operated at different depths and cell densities in different seasons. For multi-stack and multi-row enclosed PBRs, the structure, orientation, and arrangement of the PBRs must be optimized to receive direct and diffuse light.

### **Bacterial Control**

Although in laboratory research pure or unialgal species in the culture system are possible, in reality contamination of algae with other aquatic organisms and lower metazoan is unavoidable, and is sometimes beneficial but often times disastrous. Bacteria are one of the major sources of contamination. Laboratory work showed that many bacteria if co-cultured with algae inhibit growth of algae probably by secreting toxic factors and interfering with algal metabolisms. However, some bacteria when introduced into the algal culture system could promote algal growth (De-Bashan, 2004). They are thus called growth-promoting bacteria. It is believed that these growth promoting bacteria are capable of generating and releasing some beneficial biofactors, but the identities of these biofactors remain unknown in most situations.

### **Microscopic Nature**

Micro-algal strains due to their microscopic nature (typically 3-20 microns) and their low concentrations in which they can be grown (typically less than 2 g algae/L water) are difficult to harvest in a conventional way. Microalgae which are about 5-50 micro-metre in size form stable suspensions due to their negatively charged surfaces. Suspensions tend to be relatively dilute, adding to the difficulty in harvesting algae. A compounding problem is the sensitivity of the cell walls in many species to damage

in high shear processes (e.g., centrifuging), which can result in leaching of the cell contents.

Methods for the harvesting of algae include concentration through centrifugation, foam fractionation, flocculation, membrane filtration and ultrasonic separation. All these harvesting methods contribute to the total cost of algae biomass. Filtration is normally performed using a cellulose membrane and a vacuum being applied in order to draw the liquid through the filter. Although this method is simple, the membrane tends to become clogged, rendering the process extremely time consuming. Centrifuging, in a continuous or semi-continuous process, appears to be more efficient in this regard; however, it is extremely energy intensive and cannot readily be scaled to very large applications. The third option, flotation, uses a bubble column. Gas is bubbled through the algae suspension, creating a froth of algae that can be skimmed off.

### **Limitations from water requirements**

Water is a limited resource and a shortage of it can lead to heavy impact on well-being, possible forced migration and episodes of famine. Furthermore, climate change is likely to exacerbate existing issues. As small scale systems will likely be open, shallow and located in sunny regions, a large amount of water will be lost through evaporation. This severely restricts the possibilities in arid regions, unless an alternative water source is available, but also regions with high annual rainfall may experience dry and wet seasons. Alternative water sources may be found, like wastewater streams from urban areas, or in some cases seawater or (saline) groundwater is available, but the cost of pumping the water to the cultivation system may be too high.

## **ENGINEERING CHALLENGES**

### **Mathematical models**

Most mathematical models describing algal growth revolve around nutrient concentration, light levels and temperature. These sub-models attempt to define the growth of a particular alga as a function of these parameters. These models attempt to simulate the response of algal cell production as a function of these three factors. Although simple in concept, because of the many possible limiting nutrients, multitudes of possible dominating algal species, combined with interaction between the three central variables, the resultant models become extremely detailed and complex. However, in the situation of high density algae culture, it is often the case that two factors, in particular, become most important in controlling production. The supply of inorganic carbon at a sufficient rate and concentration to meet algal carbon uptake rates and the availability of sufficient light intensity to supply the energy needs of the growing culture, are often suggested as controlling net cell productivities (King, 1990). Techno-

economic (TE) modelling and life cycle assessment (LCA) are necessary to provide the emerging industry with the required insights as it moves along the critical path to eventual commercialisation. TE analysis will help to specifically identify critical path elements that offer the best opportunities for cost reduction while allowing the industry to measure progress towards its R&D goals.

**Cultivation Techniques**

Algal producers are continually challenged to produce efficiently and economically. Production technologies must be developed to culture new species and to optimally culture existing species in existing and new environments. Production systems can be improved through the development and application of innovative biological and engineering approaches. Although production intensity varies widely among systems, optimal production efficiency is required for profitability. Optimal utilization of production inputs, including water, feed and mechanical energy and minimization of waste outputs requires knowledge of the interactions among inputs, culture species, production environment and economics. Deciding on a method of cultivation that not only will allow for the algae to grow in significant amounts but will be feasible for the specific goals must be ascertained before cultivation. There are three ways to grow and harvest micro-algae for mass production.

**Photo Bio-Reactors (PBR)** is man made machine designed to grow algae in optimum conditions. Photo-bioreactors provide solution of evaporation and contamination that is

associated with open pond cultivation. A photo-bioreactor contains a series of transparent tubes that contain the algae mixture that undergoes photosynthesis while continually flowing through the tubes as seen below. The transparent tubes enable sunlight to penetrate to the interior and react with the algae. Compared to open ponds, photo-bioreactors can produce up to thirteen times more biomass and harvesting the mature algae is less expensive (Campo *et al.*, 2009). Although closed photo-bioreactors are more suited for the cultivation of most microalgae, they are not without their imperfections. These reactors are very difficult to maintain, and effort needs to be put forth in order for continued success. During photosynthesis, plants produce oxygen. This same process occurs in algae. Because the photo-bioreactor is enclosed, oxygen would continue to rise until it poisons the algae. Because of this, the algae mixture has to pass through a degassing zone where air bubbles through the mixture in order to remove the oxygen. Since this is a closed system, more carbon dioxide is used than the amount produced. Carbon dioxide needs to be filtered through the system in order for there to be enough carbon dioxide for the algae to go through photosynthesis. Photo-bioreactors still need to be improved to the point where the carbon dioxide losses are minimized and oxygen is efficiently removed. Overall, the advantages of these reactors outweigh the disadvantages. For this reason, photo-bioreactors are one of the most common methods used for growing algae (Campo *et al.*, 2009).

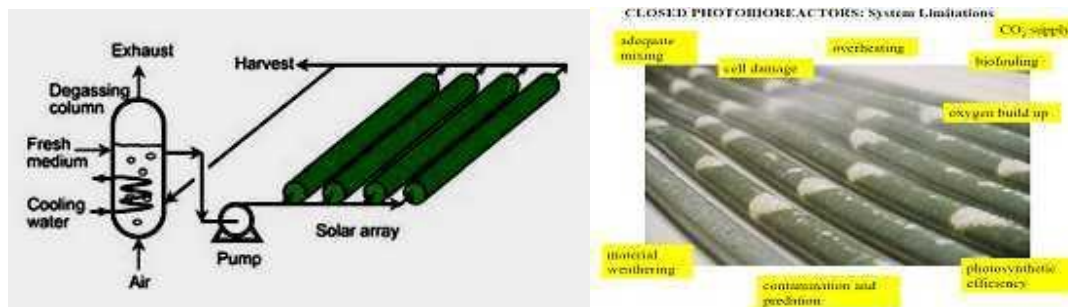


Figure 4: A Photo-bioreactor

Table 1: Factors impacting photo-bioreactor selection and design (Pulz, 2001)

PARAMETRE	OPEN SYSTEM	CLOSED SYSTEM
Risk of contamination	Extremely high	Low
Space requirements	High	Low
Water losses	Extremely high	Extremely low
CO <sub>2</sub> losses	High	Extremely low
Gas transfer efficiency	Low	Moderate

Light utilization efficiency	Low	Moderate-high
Suitability for culture variation	Low	High
Product quality	Low- moderate	Moderate-high
Controllability	Low	High
Weather/Climate issues	Extremely high	Low
Growth period relative to interruptions	High (6-8 weeks)	Moderate (2-4 weeks)
Biomass production concentration	Low (0.1-0.2 g/L)	High (2-8 g/L)
Downstream treatment efficiency	Low	High
Cost	Moderate	High

**Open Pond System** is a system like a small lake where algae are grown in open air with a moving paddle wheel in place to allow the algae to circulate. The simplest way to grow algae is by using an open pond system. Open ponds can be categorized as lakes, lagoons, ponds and artificial

containers. In open ponds, algae can absorb all the sunlight necessary for growth without artificial light provided. Open ponds are easy to make, although they are not easy to maintain. There are several advantages and disadvantages to growing algae in an open pond.

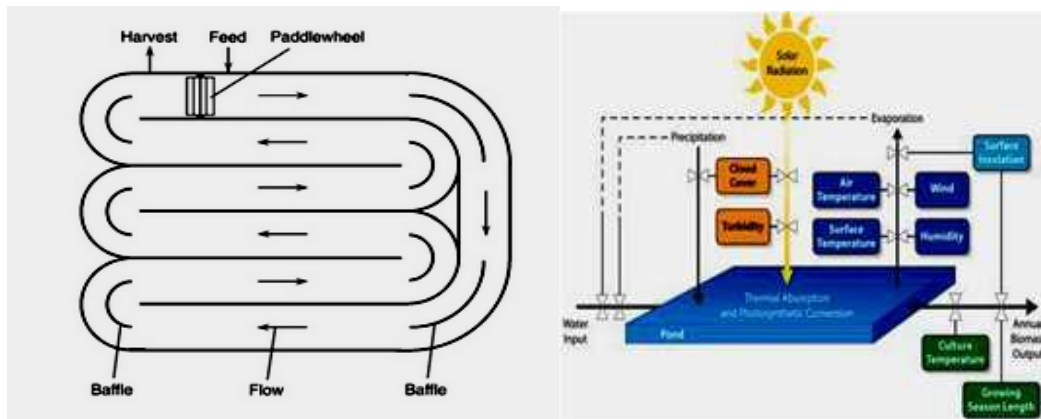


Figure 5: Climate Parameters Affecting Open Pond Algae Production Systems (Maxwell *et al.*, 1985)

The main advantage of open ponds is that they allow the algae to grow in their natural outdoor environment. Because the open ponds are not protected, open ponds are fairly easy to contaminate. Once the ponds become contaminated, it is difficult to harvest the algae from the pond and the process will become more expensive (Campo *et al.*, 2009). Another disadvantage of this system is that the pond will undergo water loss due to evaporation, which

limits the amount of algal biomass produced. With such limitations, it is necessary to search for a more efficient way to grow algae.

**Closed Pond System** is similar to small lake but have a cover to protect the algae from contamination and extreme weather patterns.

Table 2: Some advantages and limitations of algal culture systems

Culture Systems	Advantages	Limitations
Open systems	Relatively economical Easy to clean up Easy maintenance Utilization of non-agricultural	Little control of culture conditions Poor mixing, light and CO <sub>2</sub> utilization Difficult to grow algal cultures for long periods Poor productivity



	land Low energy inputs	Limited to few strains Cultures are easily contaminated
Tubular PBR	Relatively cheap Large illumination surface area Suitable for outdoor cultures Good biomass productivities	Gradients of pH, dissolved oxygen and CO <sub>2</sub> along the tubes Fouling Some degree of wall growth Requires large land space Photo-inhibition
Flat PBR	Relatively cheap Easy to clean up Large illumination surface area Suitable for outdoor cultures Low power consumption Good biomass productivities Good light path Readily tempered Low oxygen build-up Shortest oxygen path	Difficult scale-up Difficult temperature control Some degree of wall growth Hydrodynamic stress to some algal strains Low photosynthetic efficiency
Column PBR	Low energy consumption Readily tempered High mass transfer Good mixing Best exposure to light-dark cycles Low shear stress Easy to sterilize Reduced photo-inhibition Reduced photo-oxidation High photosynthetic efficiency	Small illumination surface area Sophisticated construction materials Shear stress to algal cultures Decrease of illumination surface area upon scale-up Expensive compared to open ponds Support costs Modest scalability

**Harvest and Post Harvest Techniques**

There are a number of complex challenges related to process control and monitoring of subsystems and at the interfaces between each subsystem. Similar to other industries, this will require the development optimization of a wide variety of pumps, mixing apparatus, thermal management systems, new instrumentation, control systems and process algorithms.

As with any large-scale technological development effort, advanced coatings and materials are needed to improve various component- and process-level functions. In certain cultivation systems, new polymers that enable the creation of super-hydrophobic coatings capable of reducing hydrodynamic drag and cleaning requirements are needed, as are low-cost, spectrally selective thin films used to reject infrared and ultraviolet solar radiation. In some cultivating environments, new optical components should be considered (e.g., planar waveguides) to improve areal and volumetric yield through enhanced sunlight distribution and utilization. In downstream processing systems, new materials and coatings will be necessary to address compatibility issues with energy distribution/storage

infrastructures, engine systems, and extreme operating environments.

There are two primary-systems integration issues related to algal energy systems. The first is within the algal cultivation facility itself where integration of such elements as sunlight transmission systems, nutrient delivery systems, harvesting systems and pH management systems is needed. This is complicated by higher-level system integration issues (i.e., the cultivation system coupling with a CO<sub>2</sub> source and the integration of the microalgal growth facility with downstream use/processing systems). For example, an algal-based system could be used to recycle the CO<sub>2</sub> emitted from a coal-to-liquids plant that, in turn, uses the residue from the algae as a gasification feedstock (with the coal) to produce liquid transportation fuels. Another example would be to use algal energy systems to recycle CO<sub>2</sub> from bio-digesters, use nutrient-rich digester sludge to fertilize algae, and use the waste matter, after processing high-value products from the algae, as an input to the bio-digester to make additional biogas. These examples point to integration issues of significant scale.

### Algae biomass dewatering

Live microalgae are tiny particles (1 to 30 mm) suspended in the culture media. Therefore separating and collecting these fine particles with low specific gravity from the bulk liquid is challenging and costly. Cost-effective and efficient concentration processes have been identified as a key technical hurdle for the commercialization of algae. Several physical, chemical, and mechanical harvest methods, individually or in combination, have been tested.

Membrane filtration with the aid of a suction or vacuum pump is usually the preferred method. This method is simple and simultaneously removes water and collects algae. Membranes are usually made of modified fibres or cellulose. However, membrane fouling and clogging are major problems associated with cell penetration into the membrane structures and cell packing. Removing such large amounts of water through filtration can be very energy consuming. Innovation in new membrane materials that facilitate water removal and algae recovery could provide a solution to these problems. Chemical flocculation also appears to be a viable method. Microalgae have negative charges on their surfaces that keep individual cells separated in suspension. When adding coagulants (e.g., iron, alum, lime, cellulose, salts, polyacrylamide polymers, surfactants, chitosan, and other man-made fibres), the negative surface charges are disrupted, causing microalgae in suspensions to flocculate and settle.

Air flotation, dissolved air flotation, and suspended air flotation (Wiley *et al.*, 2009) are methods in which fine air bubbles are generated through air injection and adhered to algal cells, causing algal cells to float as foam to the top of a treatment column. The foam with concentrated algae is removed from the top, or the water below the foam is drained or siphoned off. Flotation methods are commonly combined with chemical flocculation. Flotation methods can be expensive to operate because they involve energy-intensive air compression.

Centrifugation is another widely tested method. It may be used alone or as a second step to further remove water from concentrated algae collected with other methods. Centrifugation of large volumes of algal culture may be carried out using large centrifuges such as a cream separator. Algal cells are deposited on the walls of the centrifuge head as a thick algal paste.

Ultrasound wave is a relatively new method in which algal cells experience low energy ultrasound waves and move to the low pressure nodes of ultrasound waves, causing the algal cells to agglomerate. The cell agglomeration is aided by the acoustic interaction forces and particle-particle

interaction forces. Algae aggregates grow to such a size that they settle due to gravity when the ultrasonic field is turned off (Bosma, 2003). The advantages of this technique are that it is non-fouling, causes no shear, and is free of mechanical failures because it does not involve moving parts and offers the possibility of continuous operation. Its major disadvantages are high power consumption and low concentration factors compared with traditional centrifugation and flocculation methods.

**Drying of Algal Biomass:** Drying of algal biomass also poses a major challenge towards commercialisation because of aquatic (rich water content) nature of algae. Drying to 50 % water content is required in order to produce a solid material that can be easily handled. Given the fact that algae paste, as obtained by centrifuging or filtering, typically consists of 90 % water, drying algae is an energy intensive proposition. Consequently, solar drying is the main approach that has been considered to date. Solar drying is used commercially for drying grains and timber, and is inherently inexpensive; however, drying large quantities of algae would necessitate the use of considerable areas of land. Considering the methods available for algae harvesting, it is clear that more research is needed in order to improve efficiency and to reduce the required energy input. Flocculation appears to be a promising alternative to the technologies described above, providing that the necessary flocculants are either very inexpensive or can be recycled. Rather than using solar energy for subsequent dewatering/drying of the algae, a better approach might be to develop processes that make use of low-grade waste heat from an existing CO<sub>2</sub> source (e.g., power plant).

## SCIOECONOMIC CHALLENGES

### Cost

Producing micro algal biomass is generally more expensive than growing crops. The cost of culturing *Botryococcus braunii* typically varies between \$5–10 per kilogram (Chisti, 2007). Production costs vary significantly with production scale and the system of production (open or closed systems). Harvesting of microalgae is expensive due to high energy requirements and the capital costs involved (Wijffels and Barbosa, 2010). The cost of a litre of microalgae biofuels is actually quite high, if compared to the average cost of gasoline, diesel and first-generation biofuels. Furthermore, significant reductions in cost will be difficult to achieve. Along these lines, experts agree that a future overall approach would adopt an integrated algal-based biorefinery model, where valuable coproducts such as oils, proteins and carbohydrates would complement biofuel production (Pienkos and Darzins, 2009). Based on a very preliminary cost analysis of algal oil production data obtained from the literature and several unpublished

contributions, it is currently estimated that the cost of producing a gallon of algal oil is in the range of US\$10-40 depending on whether open pond raceways or closed photobioreactors (PBRs) are used for cultivation. (The latter are more expensive to build and maintain than open raceway ponds). Cost estimates for large-scale microalgae production and carbon recycling show that there are little prospects for any alternatives to the open pond designs, given the low cost requirements associated with fuel production and limited knowledge of externalities related to extensive water use and wildlife impacts. The driving cost factors are biological, and not engineering or environmentally-related. High productive organisms capable of near-theoretical levels of conversion of sunlight to biomass are needed. By assessing the viability of algae projects from a market perspective, it is clear that total installation, operation and maintenance costs will be a major barrier to future commercialization.

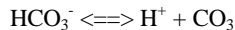
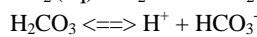
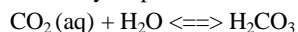
### Funding

Economics of algal energy systems is gradually becoming more complex and evolving rapidly. For example, new inputs to cost models including carbon recycling and environmental reclamation opportunities are being considered. However, regardless of technological and biological breakthroughs or carbon mandates, the fact remains that the commercial marketplace will not have an appetite for funding capital intensive algal energy projects unless the risk-return ratio is acceptable to debt and equity financiers. The capital costs are usually divided into costs associated with algal biomass growth, harvesting, dewatering, and algal oil extraction systems. In addition, there are more traditional project costs to include (e.g., engineering, permitting, infrastructure preparation, balance of plant, installation and integration and contractor fees). This lack of interest from financial institutions have been driven by the absence of large-scale commercial algal production centres with which to develop and substantiate data, the companies developing new technologies and architectures are very protective of their detailed financial data and because of the immaturity of the market, there are many unknowns coupled with a number of companies making aggressive claims.

The economics of continuous algal propagation can be severely challenged by the growth of contaminating algal species as well as the presence of grazers and pathogens. The rapid growth rate of algae and year-round cultivation will allow for opportunities briefly and will periodically shut down production for cleaning and maintenance to deal with competitors, grazers, and pathogens.

### Carbon Life-Cycle Assessment

Accurate estimation of carbon lifecycle impacts on human health, wildlife and the environment is critical for the development of algal production technologies. These impacts present significant hurdles for various approaches to the bio-fixation of CO<sub>2</sub> using algae. Algae require approximately 2g of CO<sub>2</sub> for every g biomass generated and thus have a tremendous potential to capture CO<sub>2</sub> emissions from power plant flue gases and other fixed sources. In photo-bioreactors, CO<sub>2</sub> can be used as both an input to photosynthesis, as well as a pH controller. As microalgae grow and uptake CO<sub>2</sub> within a closed system, the growth media exhibits an increasing pH. When CO<sub>2</sub> is added to the photo-bioreactor, it drives the formation of carbonic acid, thereby lowering the pH (Mehlitz, 2009). The following equations illustrate the role of carbonate chemistry on pH in water:



Aqueous carbon dioxide (CO<sub>2</sub> (aq)), carbonic acid (H<sub>2</sub>CO<sub>3</sub>), bicarbonate (HCO<sub>3</sub><sup>-</sup>), and carbonate (CO<sub>3</sub><sup>2-</sup>) are all in equilibrium depending on the concentration of hydrogen ions in solution (H<sup>+</sup>). Considering the preceding equations in continuity, all variables have a cascading effect on the others. In a biological system, CO<sub>2</sub> and pH are variable, with a clear effect on one another. Simply adding CO<sub>2</sub> to a system increases the concentration of hydrogen ions in solution, thus reducing the pH.

### Lack of expertise

Lack of relevant expertise as algal industries is very young. Current microalgae production is based on relatively small systems, producing high value products for special niche markets. Only a handful of companies, in collaboration with research institutions, have started to generate the relevant know-how. There is as yet no 'turn-key solution', and even with continued extensive support any algal installation is likely to suffer repeated set-backs. It takes vision and a pioneering spirit to persevere. Lack of personnel trained in the installation, operation and maintenance of renewable resource cultivation and management equipment and personnel trained in financing mechanisms available to support renewable projects

### Nature of developing countries

Almost all developing countries are found at latitudes with high annual solar radiation, a key to a high productivity as well as the prospect for low wages in developing countries and lower costs for land and some required inputs and construction materials. However, some parts or materials may not be available locally and therefore require

expensive imports. Also introducing and publicizing a new product is more difficult in developing countries.

## LIMITATIONS TO ALGAL BIOMASS UTILISATION

### STORAGE TEMPERATURE

High temperatures cause water vapour capacity of the warm and humid air to be high. When this air cools, its maximum water vapour capacity drops. The excess of water vapour condenses. The exposed biomass temperatures can easily heat up, aggravating the condensation. Thus biomass is exposed both to high relative humidity and high temperature. As a result, insects easily develop in the biomass. In addition, when the moisture content increases, moulds and related aflatoxin easily develop. Eventually, heat build-up in the biomass is uncontrollable.

Condensation is the result of heat build-up at the headspace of the bulk, followed by cooling. Warm air has the capacity to hold more water than cold air and, aided by convection currents, warm headspace air in the biomass absorbs moisture from the biomass by adjusting its relative humidity to that of the biomass. This moisture is transferred to the headspace air from the biomass through diffusion and convection currents. At lower temperature, the air in the headspace which has become over-saturated with moisture then releases its excess of water. This is known as condensation drops or "sweating." The biomass becomes mouldy and black, heats up at high temperatures and can ignite due to spontaneous combustion.

Infestation generates more heat and thus creates favourable conditions for further insect and mould development. The temperature of biomass rises because of high moisture content or insect infestation but can be reduced by mechanical aeration - using fans that take advantage of the cold air. If well managed, aeration can control insect populations, which are suppressed at temperatures below 18°C.

### ALGAL OIL AND TRANSESTERIFICATION

Extraction of oil from algal biomass on a large scale for biofuel production is being uneconomical as regards the existing solvent based extraction (or other techniques) because of high cost, hindering the downstream processing and making the bi-product non-edible. Algal biomass is an interesting sustainable feedstock for biodiesel production. Various methods are available for the extraction of algal oil, such as mechanical extraction using hydraulic or screw, enzymatic extraction, chemical extraction through different organic solvents, ultrasonic extraction, and supercritical extraction using carbon dioxide above its standard temperature and pressure. The main problems associated with using triglycerides as a diesel replacement tend to be high viscosity, low volatility and polyunsaturated character. Transesterification is a method of reducing the viscosity of the triglycerides and enhancing the physical properties of the fuel. As a result, FAME biodiesel is the most common form of biodiesel used today.

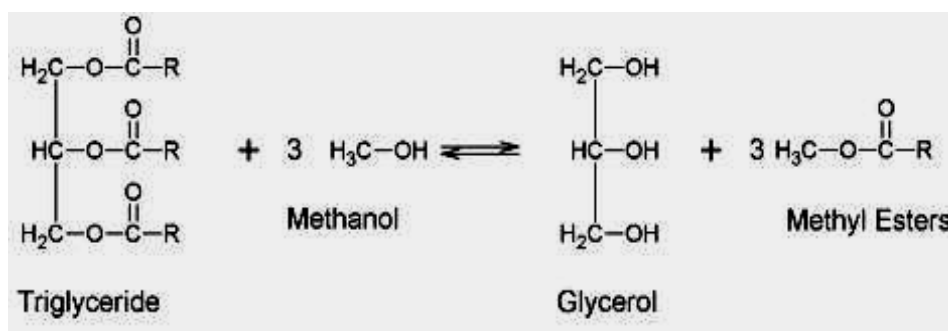


Figure 6: The Trans-esterification Reaction

FAME contains a relatively high amount of oxygen, compared to conventional fuel. For some biofuels with fatty acids of 18 carbon chain lengths (C18), the oxygen content of the neat biofuels is ~11% m/m while the ones with fatty acid chains predominantly in the C12-C14 range, the oxygen content is greater at ~14% m/m. Current technology for control of diesel combustion systems is open loop. The increase in oxygen content of the fuel with such control systems will give rise to reduced peak power

and torque, and increased volumetric fuel consumption, in line with the quantity of FAME blended into the diesel. The use of closed-loop control systems can help to mitigate this (Fussey *et al.*, 2009). The use of FAME-type biofuels in diesel engines can also affect the combustion system through the increase in deposits that foul the injectors, plug the filters and accumulate within the combustion chamber. The deposits are caused by the reduced overall back-end volatility of the fuel and the tendency of the heavier fuel

components to survive combustion. The FAME components that survive combustion condense more readily than petroleum diesel in lower temperature regions, and associate with existent carbon deposits on the combustion chamber walls. FAME may also polymerise to form large, stable complexes.

#### **Effect of FAME on lubrication**

The negative impact on lubrication from the use of FAME type biodiesels is a consequence of increased fuel in oil dilution. This increase is due to the properties of the biodiesel (Sinha and Agarwal, 2007; Dinh *et al.* 2005). Depending on the source, FAME usually has a higher molecular weight and lower volatility than conventional fossil diesel (C18 methyl esters boiling point ~345-360°C). A small amount of fuel in oil dilution occurs during normal engine running. Raising the biodiesel content increases the amount of oil dilution as the heavier hydrocarbons remain in the lubricant resulting in slower recovery. Biodiesel in oil dilution can cause problems with viscosity control. Excessive fuel in oil dilution over a typical oil drain interval can compromise the lubricant viscometrics and disturb oil film thickness, potentially opening up the possibility of increased wear. The increased content of FAME biodiesel in the sump can reduce the viscosity of the fuel. FAME in oil contamination might affect acidity control due to the introduction of more acids or substances that can oxidise more easily to form acidic components. This could contribute to increased corrosion of soft metals such as copper, lead, bronze and brass. The increase in the oxidation tendency of the lubricant could react to form polymers leading to deposits, gums and sediments. Advanced first generation products, such as HVO, and second generation options, such as BTL, offer respite from these problems.

#### **Effect of FAME on engine-out emissions**

Most studies show that using FAME contributes to reducing engine out particulates (PM), hydrocarbons (HC) and carbon monoxide (CO), but increases engine out nitrogen oxides (NO<sub>x</sub>), when compared to conventional fossil diesel. Some studies suggest the magnitude of these effects continue to increase with increased biodiesel content. There are many potential explanations for the mechanism which causes NO<sub>x</sub> emissions to increase with the use of FAME, such as those proposed by Bannister *et al.* (2010) and Mueller *et al.* (2009):

- The higher oxygen content of the fuel promotes a higher adiabatic flame temperature which reduces in-cylinder soot but lowers radiative heat loss resulting in higher flame temperatures and, therefore, more NO<sub>x</sub> production

- Fuel borne oxygen results in a pre-mix stage which is closer to stoichiometry, leading to higher local temperatures and increased NO<sub>x</sub>
- An increased pedal position is required to overcome the lower calorific value of the fuel. This changes the point of operation on the calibration map effecting EGR rates and injection timing, which ultimately effects emissions concentrations and fuel consumption.

It has been shown that FAME combustion also leads to an increase in some non-regulated emissions such as acetaldehyde and formaldehyde. FAME type biofuels have a lower calorific value than conventional fossil diesel. Therefore more fuel is required to provide the same energy. This increases the tank-to -wheel CO<sub>2</sub> emissions of the vehicle (Knothe *et al.*, 2005).

#### **Effect of FAME on after treatment systems**

Modern diesel engines use a combination of after treatment technologies to ensure the tailpipe emissions are within the legislative limits. These technologies include diesel oxidation catalysts (DOC), diesel particulate filters (DPF), lean NO<sub>x</sub> traps (LNT) and selective catalytic reduction (SCR). The use of FAME type biodiesels has the potential to reduce the efficiency of these systems. The use of FAME instead of fossil diesel can lead to a reduction in the exhaust gas temperature, making the DOC less effective and increasing tailpipe emissions of CO and HC (Wood and Ricardoor, 2010).

#### **Effect of FAME on material compatibility**

The development of engine systems for biodiesel use must consider the long term chemical robustness of the component materials against the solvent character of the esters and the increased water content of the fuel. Consideration of engine seal materials outside of the fuel circuit is also important due to the potential for exposure to the biofuel through fuel in oil dilution effects. In some cases material coatings can be used for passivation protection, e.g. oxide coatings on diesel fuel pump casings. New materials and new applications of existing materials to fuel and lubricant require consideration of compatibility (Wood and Ricardoor, 2010).

#### **HYDROCARBON STRUCTURE**

Biomass is primarily hydrocarbons. The presence of particular groupings is associated with activities of varying kinds but not a guarantee of biological activity. However individual chemical groupings can be of significance in two ways. They may be essential for the manifestation of a particular type of biological activity, due perhaps to a special type of chemical reactivity or stereochemical arrangement. They may not be replaced by alternate groupings without loss of their characteristic function

unless the alternative groupings produce a molecule in which the chemical and physical properties are very close to those of the prototype. Secondly, individual groups may exert characteristic effects in modifying the intensity of a type of biological activity which is exhibited in compounds having a common molecular basis.

When a particular grouping is responsible through its chemical reactivity for producing biological activity, it is important to bear in mind the quantitative aspect of chemical reactivity. If the grouping is very reactive, the substance may react easily with a variety of cell constituents, perhaps even with water and in consequence it may not be able to reach a site within the organism where a specific cell constituent can be affected. At the other extreme, if the chemical reactivity is too low, biological activity will be diminished, perhaps to the point of disappearance.

Since biomass is generally associated with the presence of particular groupings essential for the manifestation of a particular type of activity, transformation into higher value products therefore must break C:H bonds and mediate the formation of new bonds of carbon with other heteroatoms, e.g.  $RH \rightarrow ROH$ . Nature functionalises biomass using heteroatoms in which one of the atoms is in a high oxidation state (i.e. electron deficient). Polar-head stereochemistry, including net charge and its distribution, the number and alkyl chain(s) length and the presence of various spacer groups that can change the hydrophobicity of a compound determined the functions of antioxidants. Phenol derivatives substituted at the C2 and C6 positions with large-size groups that constitute a steric hindrance operate as free radical scavengers. Compounds containing a metal and organic radical are amphiphilic. They interact with living organisms and exhibit toxic action (Przestalski *et al.*, 2000). However, the identification heteroatoms responsible for particular chemical reactivity in a molecule are usually difficult, especially oxygenated species. The tracking of hydrogen species is done through solid state measurements which involves expensive equipments like Nuclear Magnetic Resonance Spectroscopy and Fourier transform Infrared spectroscopy. Therefore transformations that may replicate such activities are usually limited. It requires expertise in bioorganic chemistry, heterogeneous and homogenous catalysis that provide more controlled hydrocarbon functionalisation chemistry. Therefore such transformations usually require insight from complementary backgrounds, including bioinorganic, organometallic, electrochemistry and computational chemistry to develop pathways for these transformations.

## BIO-PHOTOLYTIC HYDROGEN PRODUCTION

Green algae and cyanobacteria can use water-splitting photosynthetic processes to generate molecular hydrogen ( $H_2$ ) rather than fix carbon, the normal function of oxygenic photosynthesis. This natural process can produce hydrogen necessary to meet future energy demand especially, as the source of electrons to generate hydrogen is water, solar energy and engineered microbial systems (Melis and Happe, 2001).

Presently, there is a need to thoroughly examine the hydrogen metabolism in algae to provide new information about how hydrogen-production pathways are controlled, especially the buildup of a pH gradient across the photosynthetic membrane and variations in the concentrations of critical electron-transport carriers during the process. The bidirectional Fe hydrogenases that catalyze the hydrogen-evolution reaction in bio-photolytic systems are highly sensitive to oxygen. Oxygen sensitivity causes isolation of hydrogenase from cells and its subsequent analysis is a challenge which has to be met by new technologies. By understanding this mechanism of hydrogen-production, it will be possible to determine which metabolic pathways contribute, how eliminating hydrogen-consuming reactions affects hydrogen metabolism and other cellular processes, and how organisms can be adapted to increase hydrogen yields.

Key capabilities needed to address many of the gaps in current understanding of bio-photolytic hydrogen production include developing microbial hosts to produce hydrogenase enzymes, screening large numbers of enzymes for desired functionalities, large-scale molecular profiling to provide a global-view of hydrogen production, in vivo visualization of hydrogenase structure and activity, modeling of regulatory and metabolic networks, and metabolic engineering (Aebersold and Watts, 2002 and Brady *et al.*, 2002).

## SEVERAL ADVANTAGES OF MICRO ALGAE

### As Food Supplement

Various types of edible algae are used for human consumption the world over. Dried green (*Enteromorpha* spp.) and purple (*Porphyra* spp.) lavers (nori) are the most widely consumed among the edible algae and contain substantial amounts of vitamin B12. Some species of the cyanobacteria, including *Spirulina*, *Aphanizomenon* and *Nostoc*, are produced for food and pharmaceutical industries worldwide. Others include the under listed.

**Table 3: Some algae authorized for human consumption**

Brown Algae	Red Algae	Green Algae
<i>Ascophyllum nodosum</i> <i>Fucus vesiculosus</i> <i>Fucus serratus</i> <i>Himanthalia elongata</i> <i>Undaria pinnatifida</i>	<i>Porphyra umbilicalis</i> <i>Palmaria palmata</i> <i>Cracilaria verrucosa</i> <i>Chondrus crispus</i>	Ulva spp. Enteromorpha spp. Microalgae Spirulina spp. <i>Odontella aurita</i>

These algae must meet safety regulations in terms of toxicological and bacteriological criteria. This regulation, in addition to the potential nutritional properties of algae, allows the food industry to include algae as raw or semi-processed materials in the formulation of seafood products.

#### Zooplankton Enrichment

Microalgae have an important role in aquaculture as a means of enriching zooplankton for on-feeding to fish and other larvae. In addition to providing protein (essential amino acids) and energy, they provide other key nutrients such as vitamins, essential PUFAs, pigments and sterols, which are transferred through the food chain. However, often these do not provide the level of enrichment often sought for zooplankton, and commercial oil emulsions are often used.

The high costs associated with algal production, the risks for contamination, and temporal variations in the algal food value still pose problems for any aquaculture operation depending on the mass-cultures of unicellular algae. In order to overcome or reduce the problems and limitations associated with algal cultures, various investigators have attempted to replace algae by using artificial diets either as a supplement or as the main food source. Different approaches are being applied to reduce the need for on-site algal production, including the use of preserved algae, micro-encapsulated diets, and yeast-based feeds. There is further scope to develop the sector by introducing better quality products, since it is widely acknowledged that existing concentrated microalgae products still do not match live microalgae for hatchery applications.

#### Bio-fertilizer

Seaweeds like *Phymatolithon* spp., *Ecklonia* spp., and *Ascophyllum nodosum* are utilized to produce fertilizers and soil conditioners, especially for the horticultural industry (McHugh, 2003). The cyanobacterial symbiont *Anabaena-azollae* fixes atmospheric nitrogen estimated between 120 and 312 kg N<sub>2</sub> per hectare. *Azolla* supplies 150–300 tons per hectare per year of green manure, which supports growth of soil microorganisms including heterotrophic N<sub>2</sub> fixers. The use of algae and cyanobacteria in waste treatment is beneficial in different ways since they

can bring about oxygenation and mineralization, in addition to serving as food source for aquatic species.

#### Bio-energy Technology

Algae can produce large amounts of oil all year long compared to most crops. There are many algae species which have quite high growth rate and oil content. Some studies have showed that algae can yield over 30 times more energy per unit area than any other crop. Therefore, microalgae appear to be the only source of bio-fuel that has the potential to completely displace fossil diesel. Another important advantage of micro-algae is that, unlike other oil crops, they grow extremely rapidly and commonly double their biomass within 24 hours and in the exponential phase of growth; the bio-mass doubling time is generally as short as 3.5 h (Hu *et al.*, 2006). Because of their high productivity and the accumulation of oils in their biomass, algae can be utilized as an alternative, renewable energy source that can reduce fossil fuel depletion, increasing air pollution, and global warming. One way is to use the biomass of macroalgae to produce methane. Another most promising way is to use algae to produce hydrogen. Hydrogen yields energy when it is either combusted or used with fuel cell technologies, leaves nothing behind but water. Many photosynthetic prokaryotic and eukaryotic microorganisms evolved the ability to reduce protons to hydrogen during light absorption by the photosynthetic apparatus.

#### Molecular Farming

The idea of molecular farming (also called molecular pharming, biopharming or gene pharming) in micro algae is to generate biomolecules valuable to medicine or industry that are difficult or even impossible to produce in another way, or which require prohibitively high production costs in other systems. Successful expression and assembly of a recombinant human monoclonal IgA antibody has already been demonstrated for *Chlamydomonas reinhardtii* (Mayfield *et al.*, 2003), while stable expression of the hepatitis B surface antigen gene has been shown in *Dunaliella salina* (Geng *et al.*, 2003; Sun *et al.*, 2003). In this way, antibody and vaccine production can become not only much more convenient, but also much cheaper than expression in other systems. Expression in an organism without an immune system allows expression of antibodies that would otherwise interfere with the immune system of

the host animal used in conventional antibody production. Since *Dunaliella* is otherwise used for nutrition, there is no need for purification of the antigen, so the intact algae could be used to deliver a vaccine. Microalgae have also been shown to be useful for expressing insecticidal proteins. Because the green alga *Chlorella* is one possible food for mosquito larvae, the mosquito hormone trypsin-modulating oostatic factor (TMOF) was heterologously expressed in *Chlorella*. TMOF causes termination of trypsin biosynthesis in the mosquito gut. After feeding mosquito larvae with these recombinant *Chlorella* cells the larvae died within 72 h (Borovsky, 2003). Because diseases such as malaria, dengue and west Nile fever are transmitted via mosquitoes, mosquito abatement is an expensive requirement in tropical countries. Use of such transgenic algae might be a much cheaper alternative. The utilization of algae as an expression system is not restricted to antibodies, antigens, or insecticidal proteins.

#### Bioremediation of Water and Soil

The huge amount of agricultural waste that is generated from confined animal feeding operations provides opportunities for both potential economic gain and also for benefits from an environmental perspective, if the nutrients in the wastes can be recycled appropriately, such as through the use of growing lipid-rich algae. The use of resources which can be grown or are a by-product of agricultural production, has the potential to be sustainable in the long term. Growing microalgae for biofuel is a promising approach, especially when this is combined with wastewater treatment. Algae can be used in wastewater treatment to reduce the content of nitrogen and phosphorus in sewage and certain agricultural wastes. Another application is the removal of toxic metals from industrial wastewater. Algae that are applicable to wastewater treatment must tolerate a wide variation in medium conditions (e.g. salinity). For reduction of nitrogen and phosphorus compounds, the green macroalgae *Ulva* spp. and *Monostroma* spp. could be suitable. The wild-type green alga *Chlamydomonas reinhardtii* tolerates noteworthy amounts of cadmium during its rapid reproduction, but a genetically altered *Chlamydomonas*, heterologously expressing the mothbean *P5CS* gene, grows in the presence of much higher heavy metal concentrations. Expression of the *P5CS* gene, which catalyzes the first dedicated step in proline synthesis, in the genetically engineered cells results in an 80 % higher free proline level and a four-fold increase in cadmium binding capacity relative to wild-type cells. Moreover, expression of this

gene results in rapid growth at otherwise deadly cadmium concentrations (Siripornadulsil *et al.*, 2002). One reason is that proline reduces the heavy metal stress for the cells by detoxification of free radicals produced as a result of heavy metal poisoning. Especially because this approach seems to be easily transferrable to other algae, generation of this transgenic *Chlamydomonas* is a significant step toward the use of algae for remediation of contaminated sites and waters.

Micro-algae can be cultured in sea water, blackish water or desert and other extreme environments (Hu *et al.*, 2006). Therefore, valuable farmland is not taken up by algal farms and threatens food supplies or the use of soil for other purposes. In addition, areas not suitable for planting crops can be utilized to cultivate algae. So, algae cultivation can significantly increase the utilization efficiency of the land and bring more wealth to human beings. Growing algae does not require the use of herbicides or pesticides (Rodolfi *et al.*, 2008);

Biochemical composition of algal biomass can be modulated by different growth conditions, so the oil yield can be significantly improved (Qin, 2005); and capability of performing the photobiological production of "biohydrogen" (Ghirardi *et al.*, 2000). With the development of metabolic engineering, the ability of producing other value-added chemicals also can be introduced into algal species.

Regarding air quality, production of microalgae biomass can fix carbon dioxide (1kg of algal biomass fixes roughly 1, 83 kg of CO<sub>2</sub>) (Chisti, 2007). The major advantage of algae as a feedstock is its massive consumption of carbon dioxide. Algae bio-fuel is carbon neutral; only emits CO<sub>2</sub> that it absorbs. Some latest studies have shown that the production of each gallon of oil from algae consumes 13 to 14 kilograms of the carbon dioxide. Other studies suggest that an algae-based system can capture about 80 % of the CO<sub>2</sub> emitted from a power plant during the day when sunlight is available. Economic development demands energy, yet energy consumption has historically led to increased environmental pollution. In the context of our modern society, the relationship of 'environment' and 'energy' are more often opponents rather than friendly co-existents. Environmental protection and energy production are among the greatest challenges facing mankind in the 21st century.



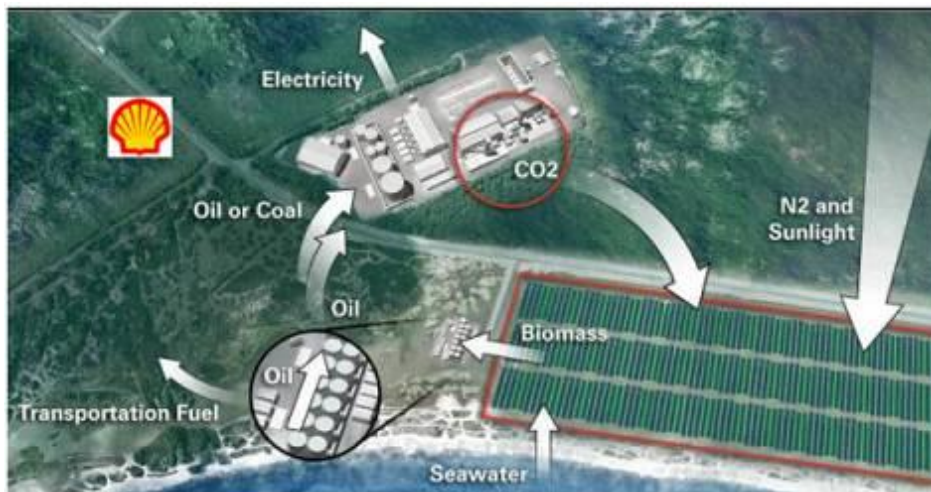


Figure 3: CO<sub>2</sub>-fixation in algal cultures from a coal or oil fired power plant (SYKE, 2008).

### Conclusion

While the technology for large scale algal biofuel production is not yet commercially viable, algal production systems may contribute to rural development, not only through their multiple environmental benefits but also through their contribution of diversification to integrated systems by efficiently co-producing energy with valuable nutrients, animal feed, fertilizers, biofuels and other products that can be customized on the basis of the local needs.

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