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REVIEW ARTICLE

TOWARDS ALGAL BIOFUEL PRODUCTION: A CONCEPT OF GREEN BIO ENERGY DEVELOPMENT

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Abstract

The use of fossil fuels is increasingly questioned due to depleting natural energy assets and the buildup of greenhouse gases (GHGs). Biofuels are an efficient substitute to existing fossil fuel based energy sources as they can be utilized for transport with little modification to existing techniques. They thus offer the prospect of ecological sustainability and reduced GHGs emission. Microalgae are unicellular autotrophic organisms that can convert atmospheric CO_2 into lipids which, after esterification, can be utilized as an energy source. Moreover, other bio-metabolites such as bioethanol and bio-hydrogen produced by algal cells are also potentially exploitable energy sources. On large scales, microalgae are cultivated either in open pond systems or in closed photo bioreactors. In this paper we review the prospects of micro-algae for the production of valuable lipid compounds and other byproduct that can be additionally used for biofuel production.

Keywords: microalgae, algal biofuels, ethanol, photo bioreactors and bio-methane.

Introduction

Continuous exploitation of petroleum fuels is unsustainable as they are inadequate and non-renewable resources of energy (Verma *et al.*, 2010) and their ignition results in buildup of the greenhouse gases (GHGs), such as CO_2, NO, CH_4, SO_2 and volatile organic compounds (VOCs) (Heather, 2003). Biodiesel from agricultural oil crops, and bioethanol from sugarcane bagasse and other agro industrial waste have been reported as renewable biofuels, but their production is still

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restricted to small scale (Moses *et al.*, 2010). Microphytes or microalgae are microscopic algae, naturally found in freshwater and marine water.

Microalgae are photosynthetic organisms which, compared to other sources of biomass, have comparatively simple nutrient requirements for growth. They are unicellular organisms which subsist independently, or in chains or groups. It has been estimated that about 200,000-800,000 species exist of which about 35,000 species have been characterized (Cardozo *et al.*, 2007). Over 15,000 novel compounds originating from algal biomass have been chemically resolved (Taskin *et al.*, 2010). The carbon source essential for the cultivation of microalgae represents up to 60% of the total cost of the nutrients. Microalgae can grow rapidly and convert solar energy to chemical energy via CO_2 fixation and are now a promising 4^{th} generation source for the commercial production of biofuel (Mata *et al.*, 2010).

Under optimal culture conditions, various microalgae species are capable of synthesizing up to 50-70% of oil/lipid per dry weight and produce up to 58,700 L_{oil}/ha, which is two orders of magnitudes higher than the nearest oil producing energy crop (Chisti, 2007).

Macroalgae surpass all other commercial oil yielding crops in terms of their lipid content which can account for up to 80% of the dry weight of algal biomass (Table 1). Agricultural oil crops such as soybean and palm oil, which are frequently being used to produce biodiesel, have relatively low oil content (< 5% of total biomass basis) compared with microalgae (Khan *et al.*, 2009).

Consequently such crops yield only small quantities of biodiesel for blending with petroleum and are, unable to meet worldwide transport and energy requirements. Research and development on enhancing biofuel production has been accelerating for reason of both environmental sustainability and cost effectiveness as they are seen as a viable alternative to petroleum- based energy (Prasad *et al.*, 2007).

Table 1. *Comparison of oil content in agricultural crops and microalgae*

S. No.	Comparison of oil content in algal biomass and other biodiesel feedstock (gallons $acre^{-1}$ $year^{-1}$)		Edible/Non Edible
1	Microalgae (70% lipid by weight)	136-900	Non edible
2	Microalgae (30% lipid by weight)	58-700	Non edible
3	Karanj	2,500	Non edible
4	Castor	1307	Non edible
5	Camelina	915	Non edible
6	Jatropha	741	Non edible
7	Palm oil	700	Edible
8	Hemp	363	Non edible
9	Coconut	230	Edible
10	Chinese Tallow	97	Non-edible
11	Peanut	90	Edible
12	Soy (Indiana)	59-98	Edible
13	Linseed	51	Non edible
14	Rapeseed oil	50-100	Edible
15	Sunflower oil	50-100	Edible
16	Soya oil	50-100	Edible
17	Corn oil	50-100	Edible
18	Cotton	35	Edible

Various government and non-government organizations are funded to reduce setup and operating expenses and accomplish the goal of commercially viable production. In the last few decades, cultivation of microalgae has been acknowledged a potential attention on account of their application as an appropriate CO_2 sequestration system and supplementary GHGs emission.

Various studies have been carried out for the determination of microalgal potential to sequester high quantities of CO_2 from the atmosphere (Lee *et al.*, 2002, Mata *et al.*, 2010). After lipid extraction, the residual biomass can further be used as a high protein feed for livestock and for the production of biogas in anaerobic digesters. It provides supplementary significance to the bioprocess and finishes with an overall cost effective achievability.

Large scale cultivation of microalgal biomass is a promising method of producing a renewable feedstock for a wide variety of high-value biofuels (Singh and Gu, 2010). It integrates methane produced by anaerobic digestion of biomass, biodiesel derived from oil, hydrogen and bioethanol produced by small microalgae and hydrogen and bioethanol by cell metabolism (Lee *et al.*, 2002; Li *et al.*, 2008 and Ergas *et al.*, 2010).

The large scale production of methane was the primary target goal of the majority of the early work on the large-scale cultivation of microalgae. Anaerobic digestion of algal biomass is a conventional practice for biogas production. However, their high lipid content, led to the consideration of microalgae as a potential source of fuel oil (Schenk *et al.*, 2008). A number of reports have been published in relation to production of starch by the cultivation of microalgae and its fermentation by yeasts and bacteria to yield ethanol (Harun *et al.*, 2010).

Biomass can be transformed by the use of thermo-chemical or biological methods. Thermo-chemical approaches consist of direct combustion that provides electricity, heat and mechanical power. Biological alteration includes the fermentation of the biomass to yield energy carriers such as hydrogen, ethanol and syngas, or the extraction of lipids from the biomass for biodiesel production. Fatty acids can be extracted and transformed into biodiesel, which is a renewable, biodegradable, non-toxic and environmentally friendly fuel. Thus, the cultivation of high carbohydrate-producing marine microalgae can generate an alternate biomass resource for bioethanol production.

Preference over first and second generation biofuels

The definition of "microalgae" as a feedstock for biofuels production is here interpreted to include multicellular as well as unicellular entities, and prokaryotic (*i.e.* cyanobacteria) as well as eukaryotic organisms. The potential advantages of microalgae over traditional agricultural oil crop as a source of biofuel are as follows:

- Seasonal growth of agricultural crops used for biofuel production such as corn, soybean, peanut, rapeseed and oil palm reduce their availability as a substrate for biofuel production. By contrast, microalgae can be cultivated all year thus maximizing their oil yield (Khan *et al.*, 2009).

- Sequestration of carbon dioxide from the atmosphere by algal cells. Microalgae utilized environmental CO_2 as carbon source which ultimately leads to reduction in GHGs (Schenk *et al.*, 2008). For example, Hanagata *et al.* (1992) reported that *Chlorella* sp. can be grown under 20% CO_2 conditions, whereas maize and cowpeas crops sequestrate 10 to 20 and 0.5 to 1 kg/ha, respectively.

- Several microalgae species produce valuable byproducts such as fats, polysaccharides, biopolymers, proteins, dyes and pigments (Hallmann, 2007).

- Microalgae are able to grow in a liquid culture medium, with better handling, and can utilize salts, *e.g.* from saline/brackish water/coastal sweater and nutrients from waste water streams **(**Chinnasamy *et al.*, 2010**).**

- Microalgae can be cultivated in habitats that inappropriate for agricultural practices (*e.g.* deserts and coastal land), or require a smaller footprint,

and thus mitigate the debate on food vs. fuel production.

- During photosynthesis sunlight is transformed to chemical energy which can be used to produce a wide range of fuel such as: bio-hydrogen fuels, polysaccharides (for bioethanol) and lipids (Wackett, 2007).

- Microalgae can grow rapidly and synthesizes high amount of lipids up to 20-70% dry weight of biomass (Kumar *et al.*, 2011).

- Microalgae utilize nitrate, phosphorus and other nutrients which are responsible for the eutrophication in water bodies, *e.g.* when polluted with wastewater (Gouveia, 2011). Microalgae can therefore be used simultaneously as a bioremediation agent or wastewater treatment and a feedstock for biofuel production.

- After lipid extraction, microalgal biomass can be utilized as a source of proteins and polysaccharides for conversion into bio-ethanol (John *et al.*, 2011).

Third generation biofuels

A number of biofuel options are being examined in order to minimize the use of petroleum fuels and mitigate the potential shortage.

Biodiesel and other biofuels from agricultural crops have attracted the attention of the petroleum sector as a possible substitute or supplement for the fossil fuels. However, debates on food vs. fuel and the need for increasing areas of land for the cultivation has cast doubt on the sustainability of fuel crops (Singh *et al.*, 2011). Cultivation of microalgae in marine water, in industrial effluents and in domestic waste-waters provides a potential solution to this energy debate (Gouveia, 2011). Microalgal species accumulate more than 50-70% lipids (triacylglycerides) of their mass (Chisti, 2007). Lipid content varies according to the microalgae species (Table 2).

Microalgae can be cultivated easily in open ponds or closed photo bioreactors. Under optimal culture conditions, these photosynthetic cells can produce lipids for biofuel in concentration that are, significantly higher than the conventional fuel crops (Rittman, 2008).

Table 2. *Lipid content in the dry biomass of various species of microalgae (Becker, 2004 and Chisti, 2007)*

Microalgae Strains	Habitat (water)	Lipid Content (% dry weight)	Reference(s)
Anabaena cylindrica	Fresh	4-7	Dunn and Wolk, 1970
Ankistrodesmus sp.	Fresh	24-40	Ben-Amotz and Tornabene, 1985
Botryococcus braunii	Fresh	25-80	Metzger and Largeau, 2005
Chlamydomonas sp.	Fresh	25-30	Vieler *et al.*, 2007
Chlorella emersonii	Fresh	28-32	Scragg *et al.*, 2002
Chlorella minutissima	Fresh	7-23	Yang *et al.*, 2011
Chlorella protothecoides	Fresh	40-55	Shen *et al.*, 2009
Chlorella sorokiana	Fresh	20-25	Hongjin *et al.*, 2009
Chlorella vulgaris	Fresh	14-22	Sheehan *et al.*, 1998
Chlorella pyrenoidosa	Fresh	40-45	Singh and Gu, 2010
Cyclotella sp.	Fresh	23-35	Round *et al.*, 1990
Euglena gracilis	Fresh	14-20	Regnault *et al.*, 1995
Hantzschia sp.	Fresh	66	Sheehan et al., 1998
Isochrysis galbana	Fresh	25-33	Valenzuela-Espinoza *et al.*, 2002
Nannochloropsis sp.	Fresh	31-68	Negoro *et al.*, 1991
Scenedesmus dimorphus	Fresh	16 - 40	Sheehan *et al.*, 1998
Scenedesmus obliquus	Fresh	12-14	Mandal and Mallick, 2009
Scenedesmus quadricauda	Fresh	1.9	Choi and Chung, 1990
Selenastrum sp.	Fresh	25-30	Verma *et al.*, 2010
Spirulina maxima	Fresh	6-7	Oliveira *et al.*, 1999
Spirulina plantensis	Fresh	4-9	Oliveira *et al.*, 1999
Spirogyra sp.	Fresh	11-21	Hossain, 2008
Stichococcus sp.	Fresh	33	Sheehan *et al.*, 1998

Chaetoceros muelleri	Marine	19-39	Pernet *et al.*, 2003
Chaetoceros calcitrans	Marine	35-40	Banerjee *et al.*, 2011
Chlorella emersonii	Marine	28-32	Scragg *et al.*, 2002
Crypthecodinium cohnii	Marine	20	Jara, 2003
Cylindrotheca sp.	Marine	16-37	Khan *et al.*, 2009
Dunaliella bioculata	Marine	8	Verma *et al.*, 2010
Dunaliella salina	Marine	6	Verma *et al.*, 2010
Dunaliella tertiolecta	Marine	40	Tsukahara and Sawayama, 2005
Monallantus salina	Marine	20	Shifrin and Chisholm, 1981
Nannochloropsis sp.	Marine	31-68	Negoro *et al.*, 1991
Neochloris oleoabundans	Marine	35-54	Pruvost, 2009
Nitschia closterium	Marine	45-47	Verma *et al.*, 2010
Phaeodactylum tricornutum	Marine	20-30	Chisti, 2007
Prymnesium parvum	Marine	22-38	Griffiths and Susan, 2009
Porphyridium cruentum	Marine	28-39	Oh, 2009
Skeletonema costatum	Marine	30	Yang *et al.*, 2002
Schizochytrium sp.	Marine	50-77	Song *et al.*, 2007
Tetraselmis suecia	Marine	15-23	Sheehan *et al.*, 1998
Tetraselmis maculata	Marine	3	Huntley and Redalje, 2007

Microalgae cultivation

Strain selection

Microalgae utilize sunlight for photosynthesis and carbon source as a chemical energy, which eventually results in the synthesis of lipids stored within the cellular compartments. The lipid content of the microalgae depends upon a combination of the cell potential to synthesize lipids and environmental conditions *e.g.* intensity of light, availability of carbon and other trace nutrients (Hu *et al.*, 2008).

The most productive strain of microalgae can be selected by screening large numbers naturally occurring algae. Their productivity may be further enhanced by advanced methods such as gene cloning, gene manipulation, mutagenesis, protein profiling, metabolic engineering and lipid characterization. Algae can be isolated from a variety of natural habitats including freshwater, brackish water, marine, hyper-saline aqueous environments, and soils (Kirkwood, 2006).

Additionally, within an aqueous habitat, algae are characteristically found in planktonic (free-floating) and benthic (surface-associated) environments. Planktonic algae may be grown in liquid culture (either batch or continuous), while benthic algae may grown in biofilm-based cultures (Table 3).

Table 3. *Outlines of algal biomass production systems*

System Input	Production system	Harvesting methods	Extraction methods	Output
Algal strains	Open ponds	Sedimentation	Solvent extraction	Biodiesel
Sunlight	Photo bioreactors	Pressing	Hydrolysis	Gasoline
Water source	Lab equipment	Centrifugation	Microwave	Feed
Nutrients (N,P,K)	Energy	Filtration	Ultrasonication	Additives
Land	Laboratory	Flocculation	Cavitation	Fertilizers
Fertilizers	System monitors	Fractionation		Omega-3
Finance				Ethanol
CO_2				Bio-methane
				DHA

Isolation techniques

Isolation of microalgal strains from natural habitats includes the application of comprised conventional cultivation protocols such as enrichment (Andersen and Kawachi, 2005). For large-scale sampling and isolation, high throughput automated isolation

techniques have proven to be extremely useful (Mutanda, 2011). Because of their morphological similarities, it is a skilled and time-consuming task to identify large numbers of algal species. The problems can be mitigated by the application of automated methods; such as those based on rRNA sequencing and lipid profiling.

Screening criteria and methods

The method of harvesting the microalgae is critical because injured or dead cells may result failure to recover the target metabolites. Primary screening for metabolites involves the determination of the cellular composition of proteins, lipids, and carbohydrates (Verma *et al.*, 2010). For instance, an appropriate screening would provide fatty acid profiles along with other characteristics. In addition, several algal strains secrete metabolites into the growth medium. For mass production of biomass from a specified algal strain, it is important to predict the strain's suitability, which includes number of parameters such as the consistency and long-term viability of cultures, and their vulnerability to predators (Scott *et al.*, 2010). A variety of culture media have been developed for isolation and cultivation of microalgae (Anderson, 2005 and Day *et al.*, 2009). Numerous conventional and modern techniques are used for the successful isolation of microalgae (Table 4).

Table 4. *Techniques of isolation and identification of microalgae*

Technique(s)	Reference(s)
Conventional methods	
Pringsheim's micropipette method	Melkonian, 1990
Culture media	
Single cell isolation	Andersen, 2005
Isolation using agar plates	Andersen, 2005
Atomized cell spray technique	Andersen, 2005
Dilution techniques	Andersen, 2005
Gravimetric separation	Andersen, 2005
Media enrichment	Andersen, 2005
Advanced methods	
Micromanipulation	Richmond, 2004
Flow cytometry	Richmond, 2004
Molecular tools	
18s rDNA	Tinti *et al.*, 2007
Mitochondria cytochrome locus	Linacre and Shanan, 2011
DNA barcoding	Moniz and Kaczmarska, 2010

Large scale production of algal biomass

Presently there are at least 35,000 known species of microalgae. So far only a handful is recognized as having potential applications for industry. In recent years, screening of microalgae has focused on high lipid productivity and consequent esterification. However, their fermentation potential for the production of bioethanol and biogas production should also be considered. Conventional commercial scale production of algal biomass can be achieved by two feasible techniques: (i) conventional open ponds systems, and; (ii) closed photo-bioreactors (PBRs) (Table 5; Fig 1).

Although open pond systems have comparatively low capital and operating cost, but they result in lower productivity and they are more difficult for the cultivation of specific algae. PBRs are more expensive both to construct and operate but offer higher productivity and better control in practice.

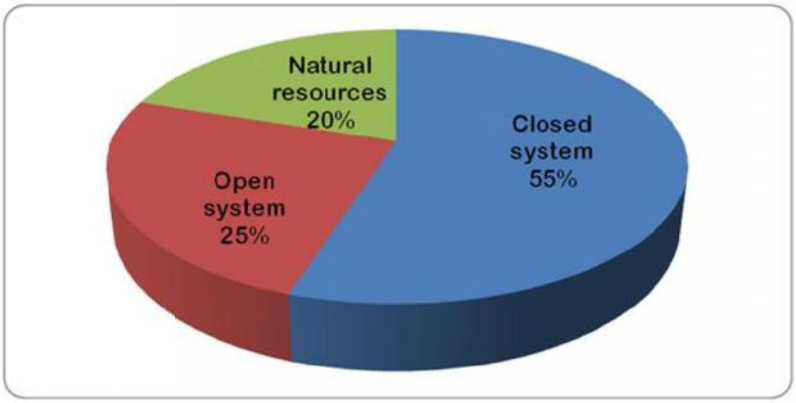


Figure 1. *Global microalgal biofuels production by different approaches*

Table 5. *Desirable characteristics of microalgae for large scale production*

Desirable characteristics	Advantages	Reference
Rapid growth rate	Competitive benefit	Singh and Gu, 2010
High lipid content	Large scale production	Singh and Gu, 2010
Cell size/ morphology*	Decrease in total process cost	Harun *et al.*, 2010
CO_2 sequestration	Reduction in atmospheric CO_2 level	Francisco *et al.*, 2010
Ability to metabolize contaminants	Grow in polluted water	Singh and Gu, 2010
Auto inhibitors	Reduce auto-inhibition of growth	Stephens *et al.*, 2010

* Small cell size and relatively low biomass levels of microalgal cultures which leads to low lipid contents

Conventional open ponds systems

The large-scale cultivation of microalgae is typically carried out in open pond systems and depends on natural sun-light as an illumination source. The operating and maintenance costs are extremely low compared to those of closed system bioreactors. Commonly, algae cultivated in open pond system include *Anabaena*, *Nostoc* (both of which are cyanobacteria), *Chlorella*, *Dunaliella*, *Haematococcus*, *Nostoc* (Blue green algae) and *Spirulina* (Verma *et al.*, 2010).

There are various types of systems comprising large shallow ponds, tanks, circular ponds and raceway ponds. The ponds are operated continuously, with the constant removal of algae being balanced by the growth and reproduction of the remaining cells.

Growth rates and productivity of microalgae in open pond systems are usually is lower than those grown in photo-bioreactors (Satyanarayana *et al.*, 2011).

Closed systems

An alternative to open pond is a closed pond system where the control over the environment variables are better. Covering the pond is usually achieved by means of greenhouse materials such as Plexiglas (Tredici and Materassi, 1992).

While this typically results in a smaller system, it does resolve many of the problems related with an open system, *e.g.* it allows a wide range of species

to be grown, and target species remain dominant and the growing season to be extended.

Closed photo bioreactors (PBRs)

A photo bioreactor is a closed container which provides a restricted environment and enables high productivity of algae. PBRs allows for better control of the culture environment, for example by optimization CO_2 concentration, water supplies, temperature, optimal exposure to light, culture density, pH levels, *etc*. Large scale cultivation of microalgal in PBRs for biomass production is more common than as a source of biofuel. Several species are grown commercially in closed PBRs including *Cyclotella cryptica*, *Monoraphidium minutum* and *Tetraselmis suecica* (Huntley and Redalje, 2007).

Several modifications of photo bioreactors existing for the cultivation of algal biomass (Singh and Gu, 2010). The main types of reactors are tubular PBRs, plate reactors, bubble column reactors and semi-hollow spheres (Verma *et al.*, 2010). Tubular photo bioreactors consist of transparent collection of tubes made up of glass and plastics and have strong and stable transmission of light in the photo synthetically active range (Kumar *et al.*, 2011).

These solar collector tubes are typically 0.01 to 0.1 m in diameter and fixed normally in the north-south direction to facilitate the maximum light penetration (Verma *et al.*, 2010). Furthermore, the floor beneath the solar collector is usually either dyed white or enclosed with white sheets of plastic to increase the amount of reflected light. The inflow of microalgal inoculums and fresh culture medium is maintained at a steady growth rate. Mixing in the reactor is necessary in order to avoid sedimentation of cells and even distribute gases. The main advantages of closed PBRs are that they remain free from contamination for extended periods while providing optimal conditions for growth (Schenk *et al.*, 2008).

Hybrid systems

In hybrid systems, open ponds and closed bioreactor are used in combination to get enhance productivity. Open ponds are an outstandingly proficient and profitable technique of cultivating algae, but they often contaminated with unwanted species.

Table 6. *Comparison of open pond systems and photo bioreactors for the cultivation of algae (Harun et al., 2010)*

Parameters	Photo bioreactors	Open pond system
Contamination control	Easy	Difficult
Contamination risk	Low	High
Production control	Easy	Difficult
Culture conditions control	Easy	Difficult
Space required	Small	Large
Setup cost	High	Low
Operation cost	High	Low
Light utilization	Optimum	Sub-optimum
Productivity of the process	High	Low
Seasonal effect	Insignificant	Significant
Biomass productivity	High	Low
Scale-up	Difficult	Easy
Extraction of product	Easy	Laborious
CO_2 sequestration rate	Limited	High
Loss of growth medium	High	Low
Recovery of by-products	Simple	Difficult
Economics	Expensive	Cost-effective
Cultivation cost	High	Low
Sterilizing conditions	Adequate	Inadequate
Cleaning	Required	None

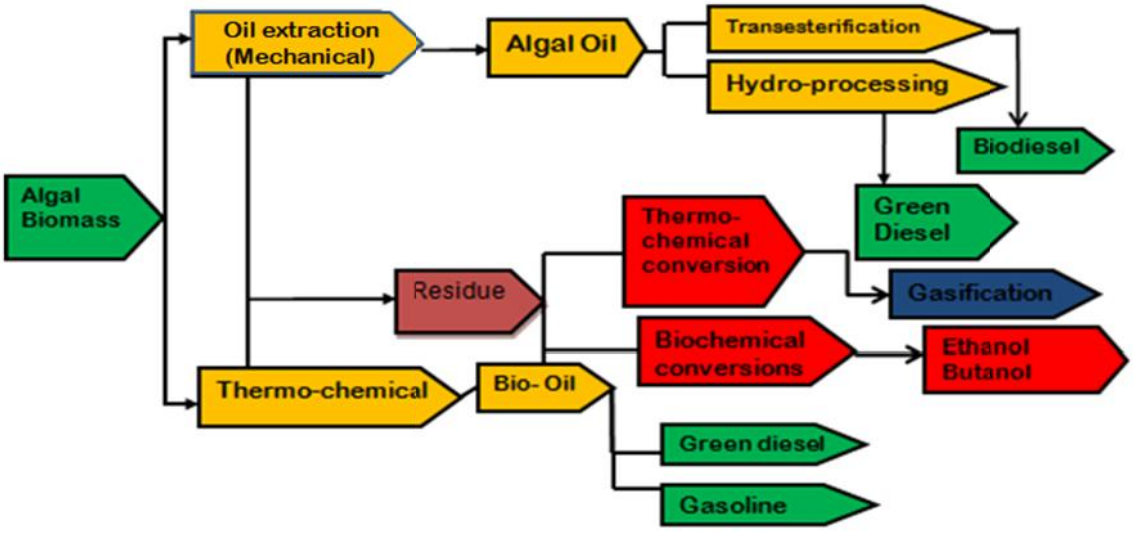


Figure 2. *Flow chart for an outline of algal biomass processing system*

A combination of both systems is potentially the most efficient and cost-effective method for the cultivation of high-yielding strains for biofuels. In such a system, open ponds are inoculated with a preferred strain cultivated in a bioreactor (Schenk *et al.*, 2008).

It is important that the size of the inoculum is large for the desired species to dominate the open pond system before an unwanted species can proliferate. In order to further minimize problems of contamination issues, regular cleaning or flushing of the ponds should be undertaken.

Open ponds operated in this way can thus be considered as batch cultures (Khan *et al.*, 2009). As an example of such a system *Haematococcus pluvialis* is grown continually in photo-bioreactors under nutrient rich conditions, a portion of which periodically is transferred to nutrient limited open ponds to thus stimulating astaxanthin production (Johnson and An, 1991). Different aspects of an open pond system are compared with PBRs in Table 6. A flow chart of a typical algal biomass processing system is shown in Figure 2.

Cultivation of algae in deserts

Algae can be grown economically in saltwater ponds of the desert or even more proficiently in PBRs. It is extremely promising that the PBRs can operate successfully in a desert environment, although one of the major challenges is to maintain a consistent temperature of around 70°F to maximize production in PBRs. The primary provisions for cultivating algae are water, CO_2, nutrients and sunlight. These conditions would be the optimally accomplished closer to the desert, where seasonal sunlight levels and temperatures do not vary as much. Algal strains suitable for include *Haematococcus pluvialis*, *Microcoleus vaginatus*, *Chlamydomonas perigranulata* and *Synechocystis* sp. (Gouveia, 2011).

Cultivation of algae in sewage and wastewater

Algae are significant bioremediation agents, and are utilized by many wastewater treatment plants worldwide. The advantage of algal-based treatment includes cost-effective treatment, low energy requirements, low sludge formation and the production of algal biomass.

Algae can be used to treat both municipal and industrial wastewaters (Tuefekci *et al.*, 2007) and algae play a major role in the aerobic treatment of waste in a number of secondary treatment processes. Algal-based system for the treatment of municipal wastewater are mostly used for removal of nutrients (nitrogen and phosphorous). In some cases, algae also play a role in the removal of pathogens during the tertiary treatment.

Conventional wastewater treatment such as the activated sludge process involves high energy costs, in particular for mechanical aeration in order to supply sufficient oxygen for the mineralization of organic compounds by aerobic microorganisms.

Aeration typically accounts for 45 % to 75 % of the total energy cost for operating wastewater treatment plants of this type. By contrast, in algae based wastewater treatment, algae provide the oxygen.

Algae consume nutrients while simultaneously providing oxygen to the aerobic through photosynthesis (Munoz, 2003) thus lowering treatment cost. Wastewater treatment using algae are usually implemented either using simple oxidation ponds or high density algal ponds. In a few cases, particularly where high productivity of algal biomass is preferred, companies are exploring the prospect of using closed systems such as photo bioreactors. Industrial effluents are enormously varied so a wide range of microorganisms are involved in the treatment processes.

Some of the algal strains which are used frequently are listed in Table 7. Additionally algae have the capability to accumulate heavy metals thereby removing them from the wastewater (Narasimhulu and Sreenivasa, 2009).

Table 7. *Application of selected microalgae in waste water bioremediation*

Microalgae	Application
Phormidium bohneri	Removal of nitrogen and phosphorus (Chevalier *et al.*, 2000)
Spirogyra condensate	Biosorption of chromium (Onyancha *et al.*, 2008)
Scenedesmus acutus	Removal of cadmium (Torricelli *et al.*, 2004)
Euglena gracilis	Removal of Zinc (Fukami *et al.*, 1968)
Pithophora odeogonia	Removal of Lead (Singh *et al.*, 2007)
Spirogyra neglecta	Removal of Lead (Singh *et al.*, 2007)

Downstream processing: harvesting and dewatering

Downstream processing costs may contribute 20-30% to the total cost of oil production through algal biomass (Jena *et al.*, 2011). The existing technology is not adequate to put forward such optimal harvesting processes, and further research and development work is required in order optimized product recovery. Planktonic microalgae can be considered as particles in suspension. Some strains have a tendency to agglomerate naturally and to settle at a well defined sedimentation rate (Brissaud *et al.*, 2003). Moreover, some algal strains are motile and will not settle naturally. The volume of water in such system is typically high. Efficient harvesting is often the key to maximizing the yield of the overall process.

The highest efficiencies for harvesting microbial biomass are often found at waste treatment plants. There are four basic methods of harvesting microalgae: sedimentation, filtration, flotation and centrifugation. In addition, methods for harvesting unicellular algae may employ ultra filtration (Mata *et al.*, 2010), and pre-treatment of the biomass may also be required (*e.g.* flocculation) to improve the yield. The use of algae grown in open ponds, PBRs and off-shore systems to produce liquid biofuel requires proficient downstream processing steps such as harvesting, removal of water, and successive recovery of fuel precursors (lipids and carbohydrates) and the final yield concentration depends largely on the recovery methods employed.

A feasible algal biomass to fuel planning must therefore, take account of energy costs and issues associated with harvesting and dewatering. Processing techniques depend on the type of algae utilized. Processes that are limited to unicellular microalgae are quite different from the technologies applicable to macro-algae.

Richmond (2004) defined efficiency criteria for selecting the most appropriate harvesting method, which depends upon the desired product quality. For low value products, sedimentation method may be used, usually enhanced by flocculation, whereas for high value product recovery centrifugation is usually the most appropriate approach.

Additionally, centrifuge can be easily handled and maintained to avoid bacterial cross contamination. After harvesting, the algal biomass requires rapid

processing in order to minimize spoilage which might result in lowering the lipid content.

Harvesting

An efficient harvesting process typically results in a slurry material with approximately 2-7 % algal concentration. Dewatering is therefore required in order to obtain a more concentrated algal biomass. Dewatering is usually carried out either by pressing or by centrifugal force. These steps are normally performed during the harvesting operation. Concentration by heating may also be used to reduce water content, but the operating cost is usually expensive unless cheap heating sources are available (Mata *et al.*, 2010).

Whichever method is used, dewatering requires a lot of energy and is usually the economical bottleneck of the entire process. Dewatering can account for 70 % of the biofuel system cost. Modern methods of dewatering include spray drying, rotating drum drying and flash drying. All, however, are expensive. After harvesting, the biomass may be subject to the degradation by internal enzyme activity of the microalgal cells, for example, lipases that are involved in hydrolyze of cellular lipids to free fatty acids.

Flocculation and sedimentation

Unicellular microalgae (including cyanobacteria) remain suspended in the growth medium due to their small size and this is a hindrance to efficient harvesting. Flocculation is therefore carried out to in order to enhance sedimentation in older cultures because of nutrient depletion and CO_2 interruption. This involves the addition of flocculants (chemical additives) that bind the algae together or otherwise affect the physiochemical interactions between algae known to promote flocculation.

Commonly used flocculants include alum, lime, cellulose, salts, polyacrylamide polymers, surfactants, chitosan, and other synthetic fibers. Changing the pH, either with or without chemical additives is also an effective flocculation method. Physical flocculation methods such as electro-flocculation and electro-coagulation may be used in order to minimize the amount of chemicals needed (Chen, 2004). Cationic starch is a potentially useful flocculent for harvesting freshwater microalgae species. The advantages of cationic starch include its efficacy at lower doses compared to inorganic flocculants; it is non-toxic when ingested either in food or drinking water (Krentz *et al.*, 2006).

In this respect, cationic starch is analogous to chitosan, although chitosan is more costly than cationic starch; it is difficult to obtain in very large volumes and is more complex to apply due to its pH-dependence. Additional options to advance the flocculation effectiveness include the alteration of amylose to amylopectin proportion or modification of the polymer chain lengths.

Filtration

Filtration is technically simple but potentially very expensive due to the small cell size (< 10 µm) of the target cells. The pore size of filter is critical in order maximize the algal aggregation rate. While minimizing the blocking of filter pores (blinding) this reduces filtration rates (Satyanarayan *et al.*, 2011).

Culture purity is also important as a distribution of microorganism cell size will affect filtration efficiency and blinding rates (Rossi *et al.*, 2004). Ideally, the filter materials should both optimize filtration efficiency and be reused after the process.

Centrifugation

Centrifugation is widely used commercially for a range suspension separations application and has been investigated in algal biomass harvesting (Satyanarayan *et al.*, 2011). The efficiency of centrifugation is dependent on the cell size of the target species, smaller cells being more difficult to separate than larger ones. The cost of centrifugation, both capital and operational are relatively high compared to other methods, often prohibitively (Mercer and Armenta, 2011).

Drying

Drying is necessary in order to achieve high concentrations of biomass. Because drying generally involves heat, drum dryers and other oven-type dryers are usually required. The energy costs therefore depend on a combination of the temperature and the time at which the temperature is maintained. In order to minimize these costs,

renewable energy sources such as solar or wind may be used (Hossain *et al.*, 2008). Alternative drying methods includes air drying but this has the disadvantage of requiring more space and is time-consuming.

Current practices for lipid extraction

Mechanical disruption (cell rupture)

Algal biofuel schemes rely on the accumulation of intra-cellular lipids content. A successful extraction solvent must be able to: (1) penetrate through the cellular matrix containing the lipid molecules, (2) physically interact with the lipid material, and (3) solvate the lipid. The development of any extraction process must also account for the fact that the tissue structures and cell walls will likely impede lipid extraction.

Efficient mechanical disruption can help offset the need to use higher temperature and pressure processes that force the solvent into contact with desired biomolecules. Mechanical disintegration can include cell homogenizers, bead mills, ultrasound and autoclaving (Mata *et al.*, 2010).

Non-mechanical disruption methods

Non-mechanical approaches include techniques such as freezing, application of organic solvents, osmotic shock, and acid, base, enzyme reactions, microwave and ultrasonication (Mata *et al.*, 2010).

Although the use of microwave to disrupt cells and increase the efficiency of lipid extraction has been studied in the laboratory (Virot *et al.*, 2008), field applications have not been realized.

Direct transesterification of lipids into fatty acid methyl esters (FAMES)

Transesterification involves the addition of alcohol (*e.g.* methanol) and an acid catalyst (*e.g.* acetyl chloride) to the algal biomass in one-step reaction, followed by heating at 100 ^{o}C for an hour in a sealed container. This results in-relatively high recoveries of chain triglycerides while alleviating the need of antioxidants to protect unsaturated lipids. This approach has been applied to dried algal biomass in a modified method that to included hexane in the reaction phase in order to avoid a final purification step (Mata *et al.*, 2010).

Furthermore, it has been found that when applying direct transesterification using an acid catalyst, the efficiency of the reaction increased when a second less polar solvent such as toluene was mixed with the methanol to modify the polarity of the reaction medium (Carvalho and Malcata, 2005). In general, these findings suggest that the efficacy of the second co-solvent system depends upon its ability to solubilize the lipids coupled with its miscibility with methanol.

Direct production of biofuels from algae

The direct production of biofuels through heterotrophic fermentation has certain benefits. Heterotrophic fermentation has various benefits in terms of operational cost because it can remove several process steps (*e.g.* oil extraction). Heterotrophic fermentation also allows for maintaining extremely controlled conditions, which initially could be oriented in the direction of biomass production and afterwards oil production. Heterotrophic process can produce enormously high biomass and a high percentage of lipids (over 50%).

These methods are quite diverse from the typical algal biofuel processes that use algal biomass to produce biological oils which is subsequently extracted and blended for liquid fuel production, typically biodiesel. Heterotrophic growth also allows for maintaining optimum conditions, which first could be oriented toward algal biomass production and then lipid production.

Various types of biofuels can be produced directly from algal biomass, including alcohols, alkanes, and bio-hydrogen. Several algae, such as *Chlorella vulgaris* and *Chlamydomonas perigranulata*, are proficient of producing ethanol and other alcohols by the fermentation of starch (Hon-Nami, 2006).

This can be accomplished through the production and storage of starch via photosynthesis process within the cell, and subsequent anaerobic fermentation of carbon sources to produce ethanol under controlled and optimum conditions. This process is comparatively inexpensive as it involves less energy input, and allows alcohol to be extracted directly from the culture medium.

Metabolic pathway engineering within these algae is also an excellent approach involving genomic tools and biochemical engineering, may further help in producing a commercially viable organism (Verma *et al*., 2010).

Bioethanol

Microalgae produce carbohydrates and proteins that can be used as carbon sources for ethanol fermentation. In addition, they exhibit greater sustainable and commercial advantages over conventional biofuel crops.

These include: (1) microalgae grow rapidly and in a wide range of locations, with or without soil (2) microalgae have the potential to absorb CO_2 and other GHGs for photosynthesis with using less agricultural land (3) microalgal cells have a very short harvesting period (1-10 days) compared to with other feedstock's and consequently provide adequate supplies to meet ethanol production demands. Microalgae biomass can be used for the production of bioethanol using either fermentation or thermo chemical conversions.

The main debate being put forth is the food vs. fuel necessities. Thus, both of these compete with food as well as land use. The recent practices for production of bioethanol are focused on microalgae as biomass for fermentation process. Microalgae are a rich source of polysaccharides (Table 8) and proteins that can be utilized as the carbon source during the fermentation.

Several industrially potent bacterial as well as yeast species are renowned for bioethanol fermentation (Petersson *et al.*, 2007 and Araque *et al.*, 2008). Although only a limited numbers of trials on fermentation have been reported, it is likely that bioethanol from microalgae will prove to a cost effective energy source in the future. Reported bioethanol production from microalgae is an efficient energy solution for the future.

Fermentation of microalgal biomass involves minimum input of energy and the whole process is less complicated compared to biodiesel production. Moreover, carbon dioxide produced during the process can be stored and a significantly proportional of it used as the carbon source for the cultivation of microalgae in closed PBRs.

In near future the potential of bioethanol production from microalgae is required to be additionally investigated (manipulated microalgae strains for higher sugar content) to fulfill demands for future energy requirements.

Table 8. *Carbohydrate content of some microalgae*

Microalgae	Carbohydrate content
Sargassum	~ 48% of dry wt.
Glacilaria	~ 45% of dry wt.
Kappaphycus	~ 35% of dry wt.
Eucheuma	~ 45% of dry wt.

Alkanes

Alkanes are saturated hydrocarbons, consist of hydrogen and carbon atoms, all bonds are single bonds, and the carbon atoms are not joined in cyclic structures but instead form a simple chain.

The algae are fed sugars, the cheap availability of which is a key consideration for cost-effective production of biofuels; these sugars are themselves available from renewable feed-stocks such as lignocellulosic biomass, in a pressure and heat-controlled environment.

This process can use different strains of algae to produce different types of alkanes (alkanes are saturated hydrocarbons); some algae produce a mix of hydrocarbons similar to light crude petroleum.

The production of different types of alkanes depends upon the strains of algae (Gouveia, 2011). Some algal strains produce a mixture of hydrocarbons similar to light crude petroleum. The process of cultivating the algae heterotrophically

may provide advantages over classical photoautotrophic-based technologies.

In this process, algal strains are cultivated in the dark which results in more production of alkanes than they do in the presence of sunlight. While their photosynthetic cycles are suppressed, the steps involved in converting sugar into alkanes can become active at higher rate.

Bio-hydrogen

Bio-hydrogen is considered as a secondary metabolite that is released by wide range of microorganisms (including microalgae) under particular conditions.

Different biological pathways of hydrogen production (bio-hydrogen) are reported from different types of microorganisms and these generation can be classified into four categories: (i) direct bio-photolysis by green microalgae and some cyanobacteria (Chader *et al.,* 2011) (ii) indirect bio-photolysis by other cyanobacteria and certain nitrogen-fixing bacteria, (iii) photo fermentation of waste and effluents by photo-heterotrophic bacteria, (iv) dark fermentation of rich sugar wastes (Many anaerobic organisms can produce hydrogen from carbohydrate containing organic wastes (such as *C. buytricum* (Yokoi *et al.,* 2001)). The large-scale utilization industrially produced bio-hydrogen has been difficult due to its low conversion rate. However, the generation of electricity via small fuel cells using bio-hydrogen as fuel seems to be a promising application.

Unfortunately, few studies report the practicality of coupling bio-hydrogen production to the operation of fuel cells. Electricity generated by the use of bio-hydrogen in fuel cell varies depending on the microorganism involved, the carbon source, and the, experimental and physiological conditions. Several strains of *Rhodobacter capsulatus* generate biogas containing hydrogen that has been successfully used as fuel for powering small Polymer Electrolyte Membrane fuel cell system (Chader *et al.,* 2011).

The production of hydrogen derived from algal cells has received significant attention in recent years. Nevertheless, there are several challenges that need to be overcome before bio-hydrogen production is considered a viable technology. These include the restriction of photosynthetic hydrogen production by accumulation of a proton gradient, the competitive inhibition of photosynthetic hydrogen production by CO_2, required bicarbonate binding at photo system II (PSII) for efficient photosynthetic activity and competitive drainage (photosynthetic hydrogen production by accretion of a proton gradient, competitive inhibition of photosynthetic hydrogen production by CO_2, necessity for bicarbonate binding at photo system II (PSII) for efficient photosynthetic action, and competitive drainage of electrons by oxygen in algal hydrogen production.) of electrons by oxygen in algal hydrogen production (Beer *et al.,* 2009).

The future of biological hydrogen production depends not only on advances in research, *e.g.* improvement in efficiency using genetic engineering techniques but also the improvements in PBRs design and efficiency.

Bio-methane production

After lipid extraction, the microalgal biomass can be used as the feedstock for biogas production during anaerobic digestion. The biogas produced mainly comprises methane and carbon dioxide and can be used for the production of electricity. The volume of bio-methane produced depends mainly on the retention time, temperature and quality of the substrate (algal biomass).

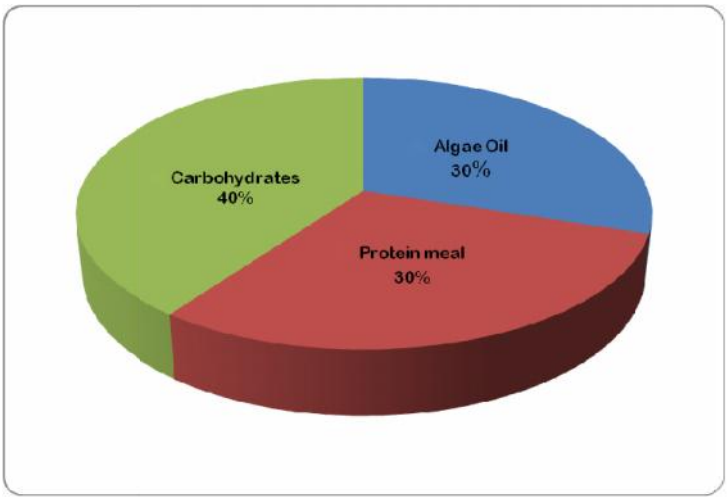
Generally the higher the organic content and longer the retention time, the greater the yield of biogas. Anaerobic digestion of microalgal biomass can be carried out in a wide range of temperature from mesophillic to thermophillic range (25-50 °C). The integrated processes that combine algae cultivation and wastewater treatment system for biogas production can be the most appropriate method to reduce cost and make it more advantageous (Schenk, 2008).

Other valuable products from microalgae

Food supplements and products of medicinal importance

Microalgae are an important source of food supplements and biomolecules such as Omega-3 fatty acids and chlorophyll. Omega-3 fatty acids

are traditionally obtained from fish oil but the taste is regarded by many as objectionable. Microalgae offer an alternative source of omega-3 fatty acids that have a more acceptable taste (Doughman *et al.*, 2007)



***Source:** Algae 2020 study, Emerging Markets Online Consulting Services.

Figure 3. *Algal biomass: oil, protein and carbohydrate fractions*

Animal feed

Microalgae can be used as livestock feed for farmhouse animals to aquaculture (Patil *et al.*, 2010). A large number of dietetic and toxicological evaluations verified the suitability of algal biomass as a precious food additive or an alternative to conventional protein sources (soybean meal, fish meal, rice bran, *etc*.). The greatest potential identified to date for algal biomass as animal feed is for poultry (Gouveia, 2011). Another growing market is the exploitation of micro algae in aquaculture. It is estimated that about 30% of the global production is used sold as animal feed.

Microalgae for human consumption

Regardless of its elevated content of protein, dried micro algae have not gained approval as a food additive or food replacement for humans. The most significant obstacles are the powder-like uniformity of the dried biomass, its dark green color and its somewhat fishy aroma (Becker *et al.*, 2004). Various attempts have been made to incorporate algal material with conventional food stuff as bread, noodle preparations and ravioli like food items, but none has met with wide success. To date, the most important sales of microalgal preparations for human consumption can be found in the health food market, although any medicinal benefits remain unproven.

Future strategies for commercialization of microalgae biofuels

- The main priority for microalgal biofuel production is to select or engineering algal strains that can be cultivated easily, grow rapidly and have high lipid content. Numerous algal varieties such as *Botryococcus braunii* synthesize elevated quantity of lipid throughout the cultivation. If the algae (with high lipid content) grow rapidly, the cost of production can be reduced.

- The large-scale production of algal biofuel is also reliant on the economics of the process. Developing novel technologies and optimizing all stages of the process should help to minimize biofuel production.

- Microalgae production system is the combination of a number of associated systems (cultivation, harvesting, product recovery and drying systems) that decrease in the number of steps in algal fuel processing leads to the noteworthy low cost systems. A fundamental economic challenge for algae producers is to discover low cost lipid extraction and harvesting methods.

- Finally the coproduction of some other valuable fractions and their marketing is also important for the success of algal biofuels. Biomass recovered after oil extraction contains valuable protein that can be used as feed for livestock, poultry and in aquaculture systems. Therefore, it may be concluded that a hybrid refinery concept can be implemented profitably for microalgae-based biofuels.

Challenges in commercialization of algal fuel

Commencing the enormous number of acknowledged marine and freshwater species, merely a handful are presently of commercial importance. These include *Chlorella*, *Spirulina, Dunaliella* and *Haematococcus*.

These are usually cultivated for extracting high value components such as pigments or proteins (Singh *et al.*, 2011). In current years, microalgae has drawn attention for producing valuable molecules ranging from remedial proteins to biofuels, outstanding distinctiveness as they merge the renewable energy capturing ability of photosynthesis with the elevated yields of controlled microalgae cultivation, making them potentially precious organisms for cost effective, industrial scale production processes in the upcoming decade (Mata *et al.*, 2010). A range of large scale systems are also required to be compared on their fundamental properties such as their light utilization effectiveness, capability to organize temperature, the ability to sustain the culture axenic and robustness of the culture to scale up from Research and development scale to industrial scale. The concluding preference of system is more or less always cooperation linking all of these considerations to accomplish a cost effective satisfactory product (Walker, 2009).

The future objective of microalgal technology is to get better productivity of these organisms in organized to meet the demands of a swiftly increasing market (Spolaore *et al.*, 2006).

Optimizing stress circumstances to achieve the maximum achievable yields of lipids in the cells is significant. An additional alternative is to choose wild local species that are previously modified to adapt to local growth conditions. Genetic modification (GM) is another option to advance production efficiency. Lipid extraction prior to esterification is an area demanding additional research. It would be a significant progress if methods could be developed that exclude drying or solvent extraction of the algae slurry as it would extensively decrease the cost of biomass pretreatment (Bruton *et al.*, 2009). The exploitation of accessible biodiesel production processes requires a lipid material free of both water and free fatty acids. This leads to elevated dispensation costs to dry the microalgae material. Improvement of lipase enzymes by mutational approaches for direct esterification or other extraction methods could get rid of the drying step.

Conclusion

The atmospheric variations due to increase in atmospheric CO_2 concentration coupled with depletion of fossil fuel oil reserves is constantly producing troublesome situations. Exploitation of microalgal biomass cultivation is not only beneficial for CO_2 fixation but also results in the biological production of triglycerides which after further modification with methanol get converted into biodiesel.

The technical and economic feasibility in microalgal biodiesel production makes this process suitable for wide acceptance. The genomics and metabolic engineering approach for metabolic flux management are the emerging concept that can show considerable potential in this area. Research on microalgae based biodiesel production is continuing and commercial scale use of microalgae for biodiesel would require massive investments in production facilities. This is also necessary to sustain our future needs for energy as well as to enable us to earn the carbon credit by adopting the green clean technology for biodiesel production.

Global atmospheric CO_2 increases and depletion of mineral oil reserves require the rapid development of carbon neutral renewable alternatives. Microalgal biofuels are also likely to have much lower impacts on the environment and the world's food supply than conventional biofuel producing crops. The main reasons for this are high yields, a near continuous harvest stream, and the potential to site the algal bioreactors on non-arable land.

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