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Micro and macroalgal biomass: A renewable source for bioethanol

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ABSTRACT

Population outburst together with increased motorization has led to an overwhelming increase in the demand for fuel. In the milieu of economical and environmental concern, algae capable of accumulating high starch/cellulose can serve as an excellent alternative to food crops for bioethanol production, a green fuel for sustainable future. Certain species of algae can produce ethanol during dark-anaerobic fermentation and thus serve as a direct source for ethanol production. Of late, oleaginous microalgae generate high starch/cellulose biomass waste after oil extraction, which can be hydrolyzed to generate sugary syrup to be used as substrate for ethanol production. Macroalgae are also harnessed as renewable source of biomass intended for ethanol production. Currently there are very few studies on this issue, and intense research is required in future in this area for efficient utilization of algal biomass and their industrial wastes to produce environmentally friendly fuel bioethanol.

1. Introduction

Needless to say, the world population is increasing at an alarming rate and so is the liquid fuel demand in the transport sector. Global warming, depletion of fossil fuels and increasing price of petroleum-based fuels are gaining great concern and the exigency of the situation has forced the search for alternative, sustainable, renewable, efficient and cost-effective energy sources with lesser green house gas emissions (Nigam and Singh, 2010). Biomass can serve as an excellent alternative source to meet the present and future fuel demands. Any type of fuel generated from biomass is termed biofuel. The two most common and successful biofuels are biodiesel and bioethanol which are aimed at replacing mainly the conventional liquid fuels like diesel and petrol.

The biofuel that is expected to be most widely used around the globe is ethanol, which can be produced from abundant supplies of starch/cellulose biomass. The most important bioethanol production countries in the world are Brazil, US and Canada (Chiaramonti, 2007). Since biomass assimilation by feedstock crops utilize atmospheric carbon dioxide, their growth for bioethanol production can reduce green house gas levels. In addition, ethanol is less toxic, is readily biodegradable and its use produces fewer air-borne pollutants than petroleum fuel. Under the Kyoto Protocol, the Government of Canada has committed to reduce the greenhouse gas emissions by 6% from 1990 levels between 2008 and 2012 (Champagne, 2007). Ethanol blended gasoline has the potential to contribute significantly to reduce these emissions. It can also be used as a fuel for electric power generation, in fuel cells (thermo-chemical action) and in power co-generation systems, and as a raw material in chemical industry (Petrou and Pappis, 2009). Bioethanol can be employed to replace octane enhancers such as methylcyclopentadienyl manganese tricarbonyl (MMT) and aromatic hydrocarbons such as benzene or oxygenates such as methyl tertiary butyl ether (MTBE) (Champagne, 2007).

Although growth of feedstock crops for ethanol production can address the environmental issues, it has raised doubts about its possible impact on food supply and security. Around the world, an urgent demand for alternative, sustainable fuels and feedstocks is growing to replace food-based feedstocks. In comparison to other feedstocks, algae can provide a high-yield source of biofuels without compromising food supplies, rainforests or arable land (Subhadra and Edwards, 2010). Several species of algae with high starch content are now being tested to produce ethanol. It is expected that the next decade will witness a tremendous growth and expansion in the global market for algal biofuels. The aviation and petroleum companies have already diverted their investment and interest to algae biofuels. The increasing demand for biofuels will create new opportunities for algae and other non-food feedstocks to meet ambitious targets for renewable biofuels replacing fossil fuels. This review focuses on the potential of algal biomass as source for the

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production of bioethanol, an environmentally friendlier and renewable transportation fuel.

2. Challenges in bioethanol production

Bioethanol, an environmentally friendly renewable liquid biofuel, can be produced from several different biomass feedstocks such as (i) sugar or starch crops (as sugar cane, sugar beet, corn and wheat), and from (ii) lignocellulosic biomass. Sugar cane is the main feedstock for bioethanol production in Brazil, while corn and sugar beet are the major resources in United States and European Union, respectively (Chiaramonti, 2007). Based on the type of feedstock used, biofuels are generally classified into "First generation fuels" and "Second generation fuels" (Nigam and Singh, 2010). Bioethanol from sugar/starch crops through traditional production technologies is included in the group of "First generation biofuels" while bioethanol from lignocellulosic biomass is considered as a "Second generation biofuels" (Chiaramonti, 2007). Industrial processes for the production of ethanol by fermentation of molasses, beet or cane sugars and sugars from grains such as corn and wheat are well established. The direct fermentation of these sugar containing biomass feedstocks is the least complex method for the production of bioethanol. Although the cost of preparation of bioethanol from sugars is low, the cost of raw materials is considerably high.

The production of grains and oil crop-based biofuels is limited due to unavailability of sufficient cultivable land on earth. Moreover, the replacement of food crops for cultivation of energy crops also result in an increase in food price levying burden on the poor. In the present scenario of population explosion, the most important question that arises is whether to use food crops for the production of bioethanol or to meet the nutritional demands of the increasing population. With increasing utilization of food materials for ethanol production, it can lead to problem of food scarcity. In addition, extensive cultivation of energy crops also raises concerns regarding pollution of agricultural land with fertilizers and pesticides, soil erosion, reduced crop biodiversity, biocontrol ecosystem service losses and green house gas emissions (Subhadra and Edwards, 2010; Donner and Kucharik, 2008; Fargione et al., 2008; Hill et al., 2009; Landis et al., 2008; Searchinger et al., 2008; Tilman et al., 2006).

Lignocellulosic biomass and starchy wastes, including crop residues, grasses, sawdust, woodchips, sludge and livestock manure, are alternative low-cost feedstocks, which can be enzymatically hydrolyzed to fermentable sugars for subsequent biofuel production. There are several research reports on bioethanol production from lignocellulosic waste materials such as crop residues (Kim and Dale, 2004), municipal solid waste (Mtui and Nakamura, 2005), forest products industry wastes (Kadar et al., 2004; Fan et al., 2003), leaf and yard waste (Lissens et al., 2004), as well as a few studies involving dairy and cattle manures (Wen et al., 2004; Chen et al., 2003, 2004). Nonetheless, the feasibility of using these materials as a feedstock is often limited by the low yield and the high cost of the hydrolysis process based on current technologies. The other carbohydrates must be hydrolyzed to sugars before they can be metabolized. The cost of cellulase enzymes is a major factor in the enzymatic saccharification of agricultural biomass, which contains lignin. Therefore, although starchy or cellulosic materials are cheaper than sugar-containing raw materials, the requirement of converting the starch or cellulosic materials to fermentable sugars is a disadvantage of these substrates. Moreover, lignin, a component of lignocellulosic feedstocks is very difficult to be degraded biologically and cannot be fermented (Harun et al., 2010). While considering abundance, trees and grasses are far more than agro-industrial or municipal residues, but cultivating them for biofuels may compete with agriculture intended to supply food, feed and fibre to an expanding world population (Sheehan, 2009).

3. Algae as potential source for bioethanol

In the perspective of above-mentioned issues, algae are gaining wide attention as an alternative renewable source of biomass for production of bioethanol, which is grouped under "Third generation biofuels" (Nigam and Singh, 2010). The major drawbacks of first and second generation biofuels are overcome to a greater extent by third generation biofuels. The concept of using algae as energy feedstock dates back to the late 1950s (Chen et al., 2009) but a concerted effort began with the oil crisis in 1970s. Over the last three decades there has been extensive research on algal biofuels production and the use of algae for CO_2 bioremediation (Borowitzka, 2008). The US Department of Energy (DOE) devoted $25 million to algal fuels research in its aquatic species program at the National Renewable Energy Lab (NREL) in Golden, Colorado from 1978 to 1996. The program gave way to mile stone advances that set the stage for algal biofuel research today (Waltz, 2009).

Algae represent a vast variety of photosynthetic species dwelling in diverse environments (Mata et al., 2010; Nigam and Singh, 2010). They may be autotrophic or heterotrophic. The autotrophic algae use photosynthesis to harness sunlight and fix the inorganic carbon from atmospheric CO_2 which is then assimilated in the form of reserve food materials such as carbohydrate. There are many algal species which are heterotrophic and they are able to take up small organic molecules in the environments and turn them into the building blocks of their own which are mainly fat or oil and proteins. There are certain algal species which can use either inorganic carbon (CO_2) from atmosphere or organic carbon from the environment and this process is called mixotrophy. Through any of the three processes, algae can produce carbohydrates, lipids and proteins over a short period of time, which can then be processed to generate biofuels. Some algae can even serve as self biorefinary for ethanol production during anaerobic dark condition by utilizing their photosynthates. There are several reports documenting the potential of algal biomass to generate biofuels (Vunjak-Novakovic et al., 2005; de Morais and Costa, 2007; Ratledge and Cohen, 2008). While considering algal biofuels, the first point that comes to view is about the biodiesel, as many of the algae are oleaginous in nature and are exploited for the production of biodiesel. Besides biodiesel, algae can be cultivated and can be used as a feedstock for the production of bioethanol. The algal starch, cellulose or other accumulating carbohydrates can be used for the production of ethanol after hydrolysis.

Generally, algae are grouped into two categories – microalgae and macroalgae – based on their morphology and size. As the name indicates, microalgae are microscopic photosynthetic organisms, many of which are unicellular. On the contrary, macroalgae, for example kelps, are composed of multiple cells which organize to structures resembling roots, stems, and leaves of higher plants (Chen et al., 2009).

There are several salient features which make algae excellent candidates for renewable bioethanol applications. Algae have higher photon conversion efficiency and can synthesize and accumulate large quantities of carbohydrate biomass for bioethanol production, from inexpensive raw materials (Subhadra and Edwards, 2010; Packer, 2009). Microalgae can tolerate and utilize substantially higher levels of CO_2. Hence they can utilize CO_2 emitted from petroleum-based power stations or other industrial sources which in turn can reduce emission of green house gas (Nigam and Singh, 2010). Aquatic algal cells are buoyant, evading

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the need for structural biopolymers such as hemicellulose and lignin which, other wise, are essential for higher plant growth in terrestrial environment. This in turn simplifies the process of bioethanol production by eliminating the chemical and enzymatic pre-treatment steps to breakdown these biopolymers into fermentable sugars. Algae are such a vast group comprising several thousand diverse species that enables the choice of desired species according to the working environment. Due to structural differences between algae and terrestrial plants, algae are capable of producing high yields of stored material when compared to most productive land plants. Kelp forests in shallow sub-tidal regions are amongst the most productive communities on earth, generating large amounts of organic carbon (Adams et al., 2009). Marine algae can provide huge amount of carbohydrate year around (Matsumoto et al., 2003). Moreover, algal cells can be harvested within a short span of time as compared to other feedstocks and hence can meet the increasing demand of feestocks for ethanol production (Harun et al., 2010). Algae have simple growth requirement, can grow to high densities, and use light, carbon dioxide, and other inorganic nutrients efficiently (Dismukes et al., 2008). Companies such as, Algenol Biofuels Inc. had developed technology to utilize sunlight trapping microalgal cells as a tiny biorefinary for ethanol production using specialized bioreactor. The technology can utilize marginal or desert land instead of agricultural land. They claimed that the system can produce 6000 gallons of ethanol per acre per year, far greater than the ethanol from corn which is only at a rate of 400 gallons of ethanol per acre per year (http://www.algenolbiofuels.com/Algenol%20101%20PUBLIC%20WEBSITE.pdf). Algae can be easily grown in various aquatic environments such as fresh water, saline water or municipal waste water (Shilton et al., 2008; Sheehan, 2009). Approximately 50% of global biomass is thought to be generated in marine environment (Carlsson et al., 2007). In fact, algae capable of growing in saline or municipal waste water are crucial for sustainable bioethanol production, since they have non-competing demands with food crops which require fresh water for irrigation. In addition to all, algae can provide a sustainable bioremediation of waste water through utilization of growth nutrients such as nitrogen and phosphorous from a variety of wastewater sources such as agricultural run-off, concentrated animal feed operations, and industrial and municipal wastewater (Subhadra and Edwards, 2010; Shilton et al., 2008). Furthermore, in addition to fuels, valuable co-products such as biopolymers, proteins and animal feed can also be made during the fuel generation process.

3.1. Bioethanol from microalgae

Microalgae are thought to be one of the earliest life forms on earth (Falkowski et al., 2004) and they are the fastest growing plants in the world. Since they can inhabit diverse ecological habitats ranging from freshwater, brackish water, or seawater, they are equipped to thrive in various extreme temperatures and pH conditions. These peculiarities make microalgae the most abundant organisms on earth. There has been a remarkable surge in research to investigate the utilization of microalgae as an advanced energy feedstock for bioethanol production (Huntley and Redalje, 2007; Rosenberg et al., 2008; Subhadra and Edwards, 2010). Microalgae like *Chlorella*, *Dunaliella*, *Chlamydomonas*, *Scenedesmus*, *Spirulina* are known to contain a large amount (>50% of the dry weight) of starch and glycogen, useful as raw materials for ethanol production (Ueda et al., 1996). Microalgae can also assimilate cellulose which can also be fermented to bioethanol (Chen et al., 2009). Table 1 summarises the starch or fermentable biomass content of some microalgae. Most of the microalgae fall under algal groups like dinoflagellates, Chlorophyceae, Chryosophyceae and diatoms (Packer, 2009). It is easy to provide optimal nutrient levels for culturing of microalgae. This is due to the well-mixed aqueous environment as compared to soil and requirement of only fewer nutrients. Absence of non-photosynthetic supporting structures (roots, stems, etc.) also favors the microalgal cultivation in aquaculture. Most of the microalgae under consideration are single-celled organisms that are self-contained and are productive. Microalgae do not have to spend energy towards distribution and transportation of storage molecules like starch between tissues. In addition, many microalgae show rapid growth under optimal conditions. For example the doubling time of some *Chlamydomanos* species is as short as 6 h (Chen et al., 2009). Asexual reproduction of microalgae like fragmentation helps to obtain biomass from very low levels to maximum under optimal conditions during continuous production. In the continuous culture systems such as raceway ponds and bioreactors, harvesting efforts can be controlled to match productivity. Due to their high cell division rate, handling is often simpler in research application and it can be performed several times faster with microalgae than that of the terrestrial crop species (Packer, 2009). There is evidence that small-scale experiments can be effectively translated into a large-scale facility for carbon dioxide capturing and biofuel production (Sheehan, 2009). In regard to strain improvement through genetic engineering, these species are acquiescent to firstly, nuclear transformation for control of metabolic pathways; secondly, chloroplastic transformation for high levels of protein expression; and thirdly, more clear-cut approaches to genetic alteration compared to higher plants (Rosenberg et al., 2008).

3.2. Bioethanol from macroalgae

Like microalgae, macroalgae, the large sized algae, also can be utilized for ethanol fermentation by converting their storage material to fermentable sugars (Adams et al., 2009). The absolute absence or near absence of lignin makes the enzymatic hydrolysis of algal cellulose simple. Macroalgal genera, such as, *Laminaria*, *Saccorhiza*, *Alaria* are belonging to brown algal group and grows up to meters and their main reserved food material is laminarin and mannitol (Nobe et al., 2003; Adams et al., 2009; Horn et al., 2000a). The red algae such as *Gelidium amansii*, which is composed of cellulose, glucan and galactan, also can serve as a potential feedstock for bioconversion to ethanol (Wi et al., 2009; Kim et al., 2010; Yoon et al., 2010). Akin to microalgae, macroalgae also have the ability to grow at a fast rate and yield huge amounts of biomass. The high yields are due to the fact that macroalgae require less energy for the production of supporting tissue than land plants, and they have the capability to take up nutrients over their entire surface. In fact, the amount of bioenergy produced by the biomass of red algae is greater than any other source of biomass (Wi et al., 2009). The surrounding water provides buoyancy and certain macroalgae have gas-filled bladders (Adams et al., 2009). Macroalgae may be cultivated in three dimensions rather than in two as on land. Macroalgae can be grown on nets or string, and can be seeded onto thin weighed strings suspended over a larger horizontal rope (Adams et al., 2009). Oleaginous algal residue after extraction of oil also can be used for obtaining fermentable sugar for bioethanol synthesis.

4. Culturing and harvesting of algae

Increased awareness of the various biosynthetic and metabolic pathways of algae has not only kindled interest in researchers to improve the available strains but also to search for better and more efficient algal strains for bioethanol production. The absolute and relative amounts of the bioethanol that can be generated vary markedly between algal species and even between strains of the

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Table 1
Algal sources for bioethanol production.

Algal source	% starch or biomass after oil extraction (g/dry weight)	Reference
Saccharina latissima	~50.0 (reserve food material)	Adams et al. (2009)
Green alga NKG 121701	>50.0 (starch)	Matsumoto et al. (2003)
Laminaria hyperborea	55.0 (reserve food material)	Horn et al. (2000a,b)
Spirogyra sp.	43.3 (biomass after oil extraction)	Hossain et al. (2008)
Oedigonium sp.	33.6 (biomass after oil extraction)	Hossain et al. (2008)
Chlamydomonas reinhardtii UTEX 90	53.0 (starch)	Kim et al. (2006)
C. reinhardtii (UTEX2247)	45.0 (starch)	Hirano et al. (1997)
C. reinhardtii	17.0 (starch)	Spolaore et al. (2006)
Chlorella vulgaris	12.0–17.0 (starch)	Spolaore et al. (2006)
C. vulgaris	37.0 (starch)	Hirano et al. (1997)
Chlorella sp. TISTR 8262	21.5 (starch)	Rodjaroen et al. (2007)
Chlorella sp. TISTR 8485	27.0 (starch)	Rodjaroen et al. (2007)
Chlorella sp. TISTR8593	22.0 (starch)	Rodjaroen et al. (2007)
Synechococcus sp.	15.0 (starch)	Spolaore et al. (2006)
Chlorococcum sp. TISTR8583	26.0 (starch)	Rodjaroen et al. (2007)
Chlorococcum sp. TISTR 8973	16.8 (starch)	Rodjaroen et al. (2007)
Chlorococcum sp.		Harun et al. (2010)
Scenedesmus sp. TISTR 8579	20.4 (starch)	Rodjaroen et al. (2007)
Scenedesmus sp. TISTR 8982	13.3 (starch)	Rodjaroen et al. (2007)
S. acuminatus TISTR 8457	7.3 (starch)	Rodjaroen et al. (2007)
S. acutiformis TISTR 8495	16.4 (starch)	Rodjaroen et al. (2007)
S. acutus TISTR 8447	18.6 (starch)	Rodjaroen et al. (2007)
S. arcuatus TISTR 8587	12.9 (starch)	Rodjaroen et al. (2007)
S. armatus TISTR 8591	15.4 (starch)	Rodjaroen et al. (2007)
S. obliquus TISTR 8522	23.7 (starch)	Rodjaroen et al. (2007)
S. obliquus TISTR 8546	23.4 (starch)	Rodjaroen et al. (2007)
Nostoc sp. TISTR 8872	30.7 (starch)	Rodjaroen et al. (2007)
Nostoc sp. TISTR 8873	32.9 (starch)	Rodjaroen et al. (2007)
N. maculiforme TISTR 8406	30.1 (starch)	Rodjaroen et al. (2007)
N. muscorum TISTR 8871	33.5 (starch)	Rodjaroen et al. (2007)
N. paludosum TISTR 8978	32.1 (starch)	Rodjaroen et al. (2007)
N. piscinale TISTR 8874	17.4 (starch)	Rodjaroen et al. (2007)
Oscillatoria sp. TISTR 8869	19.3 (starch)	Rodjaroen et al. (2007)
O. jasorvensis TISTR 8980	9.7 (starch)	Rodjaroen et al. (2007)
O. obscura TISTR 8245	12.6 (starch)	Rodjaroen et al. (2007)
O. okeni TISTR 8549	8.1 (starch)	Rodjaroen et al. (2007)
Phormidium angustissimum TISTR 8979	28.5 (starch)	Rodjaroen et al. (2007)
Spirulina fusiformis	37.3–56.1 (starch)	Rafiqul et al. (2003)

same species. This is because, algal 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. (Chen et al., 2009). Hence, it is customary to select strains which have the potential for producing highest amounts of bioethanol either directly or through biomass accumulation. Selection of a particular strain, however, is a tedious task, especially when commercially competent bioethanol yields are to be achieved. Brennan and Owende (2010) has listed the desirable characteristics of algal strains to be considered as candidates for biofuel production, such as (1) robust and able to survive the shear stresses common in photobioreactors; (2) able to dominate wild strains in open pond production systems; (3) high CO_2 sinking capacity; (4) limited nutrient requirements; (5) tolerant to a wide range in temperatures resulting from the diurnal cycle and seasonal variations; (6) potential to provide valuable co-products; (7) fast productivity cycle; (8) high photosynthetic efficiency, and (9) display self-flocculation characteristics. As far as bioethanol production is considered, while screening algal strains, high biomass with high starch/cellulose content should also be considered as a desirable characteristic since starch/cellulose can serve as substrate for ethanol fermentation. The total dry weight can be determined as a measure of biomass and the starch/cellulose content of the biomass can be determined by biochemical tests for the same (Rodjaroen et al., 2007).

In order to meet the fuel demand of the exploding population it is inevitable that there should be year-round supply of algal biomass. Naturally occurring algae are very low in density (Chen et al., 2009) and it is pertinent that they should be mass cultured in controlled environments to ensure reliable high productivity and all factors which can have possible impact on biomass yield need to be optimized and efficiently integrated (Borowitzka, 2008). Analogous to any other industrial process, the major challenge in the production of bioethanol from algal biomass is that the process must be cost-effective.

The most common production systems employed for algal cultivation are outdoor open ponds and enclosed photobioreactors. Production systems vary in terms of growth parameters control, contamination, water evaporation, productivity, downstream processing characteristics, capital and operational costs, etc. Recently, Brennan and Owende (2010) have reviewed the technologies for the production, processing and extraction of biofuels and co-products from microalgae.

Open ponds are the most widely used system for large-scale outdoor microalgae cultivation since they are cheaper, easy to build and operate (Brennan and Owende, 2010). Open pond systems are of three types – raceway pond, circular pond and sloped pond – depending on their size, shape, type of agitation and inclination (Shen et al., 2009). While raceway ponds holds relatively low capital and maintenance costs (Borowitzka, 2005), circular ponds are less attractive because of expensive concrete construction, high energy consumption of stirring, mechanical complexity of supplying CO_2 and inefficient land use (Chen et al., 2009). Though open pond systems are economical, there are several disadvantages such as low productivity, high harvesting cost, water

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loss through evaporation, and lower carbon dioxide use efficiency (Lee, 2001; Chisti, 2007; Shen et al., 2009). Temperature fluctuations due to diurnal variations are difficult to control in open ponds (Chisti, 2007). Moreover, there are chances of contamination by other algae species and protozoa. Nonetheless, monoculture is possible by maintenance of extreme culture conditions for which only a few strains are suitable (Brennan and Owende, 2010).

The limitations of open pond systems led to the development of enclosed photobioreactors for mass cultivation of algae. There are two major types of enclosed photobioreactors – tubular and plate types. The enclosed structure, narrow light path, large illuminating area and relatively controllable environment, together with less contamination issues facilitate higher cell density in photobioreactors than in open pond system (Lee, 2001; Ugwu et al., 2008). Still there are certain disadvantages too which include gradients of pH, dissolved oxygen and CO_2 along the tubes, wall growth, fouling, hydrodynamic stress, and high expense to scale up (Lee, 2001; Ugwu et al., 2008; Chen et al., 2009; Borowitzka, 2008). Owing to the higher cell mass productivities attained harvesting cost can be significantly reduced. However, the over all cost of photobioreactors is substantially higher that open pond systems (Brennan and Owende, 2010).

Recently, hybrid systems combining the features of both open pond and enclosed PBR are up-coming. In hybrid systems the algae are first cultured in a photobioreactor to such cell quantities that there is no more chances of contamination by unwanted strains and then they are transferred to open ponds where the algae are subjected to controlled nutrient conditions which stimulate the production of the desired product (Brennan and Owende, 2010). Originoil company, Los Angeles, CA has developed an internally-illuminated photobioreactor, Helix PBR, in which light array rotates vertically allowing algal growth in deep media and providing agitation. Green Star Products, a US company, developed another hybrid system which is located in Montana (http://www.green.autoblog.com/2007/05/13/greenstar-completes-first-phase-of-algae-biodiesel-demonstratio/). The enclosed pond was able to maintain water temperatures at the optimum level for algal growth even when the outside temperature was far below the optimum. The efficiency of the photobioreactor is determined by the integration of: light capturing, light transportation, light distribution, and light usage (Chen et al., 2009).

In mass algal cultivation light can be the greatest limiting factor for scaling up, since the energy source for algal biomass production is light. As the autotrophic growth and productivity of algae is greatly dependent on light, the most ideal geographical areas for algae bioethanol production are those with high solar radiation for the whole year (Borowitzka, 2008; Brennan and Owende, 2010). There are diurnal and seasonal variations in the intensity and quality of natural light. Prolonged exposure to high intense light brings about photoinhibition of biomass assimilation, and on the other hand reduced light intensity might be insufficient for photosynthesis. Moreover, chlorophyll, the photosynthetic pigment exhibits best light absorption at around 440 and 680 nm wavelengths. Theoretically, better algal growth can be achieved by providing artificial light sources around these two wavelengths (Matthijs et al., 1996; Chen et al., 2009). When natural light is inadequate, artificial lighting may be indispensable to guarantee continuous and uninterrupted algal growth.

Once the desired algal strain is cultivated on a large scale, the next step is to harvest the algal biomass for bioethanol production. The algal cells have low specific gravity and hence, separating and collecting them from the bulk liquid is a tedious and expensive task (Chen et al., 2009). Several physical, chemical and mechanical harvest methods including membrane filtration, chemical flocculation, air flotation, centrifugation and ultrasound wave are being practiced either individually or in combination.

5. Algal biomass to bioethanol

Bioethanol from algae can be accomplished through any of the three following possible methods. Algae can assimilate considerable amounts of biomass in the form of starch/cellulose, which can be converted to fermentable sugars and these sugars can be converted to bioethanol by a suitable ethanol producer. Some algae can act as a mini factory for the production of ethanol during dark fermentation. Attempts were also done to generate genetic engineered microalgae for the direct production of ethanol.

5.1. Ethanol from algal metabolites

The microalgae store starch mainly in the cells and biomass can be harvested at regular intervals from photobioreactors or shallow raceway ponds for the extraction of starch. The starch can be extracted from the cells with the aid of mechanical means (e.g., ultrasonic, explosive disintegration, mechanical shear, etc.) or by dissolution of cell walls using enzymes. The starch is then separated by extraction with water or an organic solvent and used for fermentation to yield bioethanol.

Once the intracellular microalgal starch is extracted, the starch can be fermented to ethanol using the technology similar to other starch-based feedstocks, which involves two processes, saccharification and fermentation (Matsumoto et al., 2003; Rubin, 2008). The fermentation of starch to ethanol can be carried out in a single step or double step. Prior to fermentation the starch need to be hydrolyzed to simple sugars and this process is called saccharification. Acid or enzymatic (alpha- and glucoamylase) hydrolysis can be used for the conversion of starch to simple sugars. In the next step, the sugars are fermented to ethanol by a suitable yeast strain. Both these processes can be simultaneously carried out in a single step if an amylase producing strain can be used for ethanol fermentation. Utilization of starch degrading ethanol producers can preclude the cost incurred for acid or enzymatic saccharification of starch. Finally, the ethanol is purified by distillation to remove water and other impurities in the diluted alcohol product (10–15% ethanol). The concentrated ethanol (95% ethanol) is drawn off and condensed into liquid form, which can be blended with fossil fuels or directly used as fuel (Demirbas, 2001; Nigam and Singh, 2010; Brennan and Owende, 2010). The solid residue from the process can be used as cattle-feed (McKendry, 2002). This helps offset feedstock costs which typically make up 55–80% of the final alcohol selling price (Brennan and Owende, 2010).

Bush and Hall (2006) have reported the production of ethanol from starch accumulating and filament-forming or colony-forming algal biomass selected from Zygnemataceae, Cladophoraceae, Oedogoniales, or a combination (US Patent 7,135,308). The starch accumulating and filament-forming or colony-forming algae grown by aquaculture were harvested to form a biomass by flocculation, sedimentation, filtration or centrifugation. The algal biomass was subjected to decay by placing in a dark and anaerobic aqua environment. The digested algal biomass was fermented with *Saccharomyces cerevisiae* and *Saccharomyces uvarum* for production of ethanol which was then separated from the fermentation broth. This technology has been claimed superior to another patented technology (US Patent 5,578,472) which describes a different source of fermentable sugars, single-cell free floating algae. The latter technology is not industrially scalable due to the inherent limitations of single-cell free floating algae (Bush and Hall, 2006).

The residual biomass obtained after oil extraction may also be used as substrate for ethanol fermentation. Recently, Harun et al. (2010) investigated the suitability of the microalgae, *Chlorococum* sp. as a substrate for bioethanol production through fermentation by the yeast *Saccharomyces bayanus*. The lipid extracted microalgal

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debris was used for fermentation and the yield of bioethanol was about 3.8 g/L from 10 g/L of the substrate. They further confirmed the potential of microalgae to be used for bioethanol production on a commercial scale.

Besides starch, several algae, especially green algae can accumulate cellulose as the cell wall carbohydrate, which can also be used for ethanol production. Like the cellulosic biomass from other plant sources, the cellulosic biomass from the algae can also be enzymatic hydrolyzed using cellulase enzyme and converted to simple sugars which can then be easily fermented to ethanol. Similar to ethanol production from starch, single step conversion of cellulose to ethanol is possible if a suitable cellulase producing microorganism can be used for ethanol fermentation. Starch producing green algal biomass (both reserve starch and wall material cellulose) together after hydrolysis can be used for ethanol fermentation. Unlike higher plant cellulosic biomass which is seen in association with lignin, there is no need for complex and expensive pre-treatment processes and digestion process for simple and easily digestible algal cellulose. The red alga *Gelidium amansii* has rich content of carbohydrates such as cellulose, galactan and glucan (Kim et al., 2010; Yoon et al., 2010). The biomass from *G. amansii* can be depolymerised to yield mixed monosugars such as glucose and galactose. Kim et al. (2010) reported the production of bioethanol through conversion of cellulosic biomass from the red alga, *G. amansii*. Sulphuric acid was used for the pre-treatment of cellulose and at the optimum pre-treatment conditions, 88.8% of ethanol yield was obtained in 72 h.

Laminarin is yet another main storage carbohydrate extracted from the macroalgae Phaeophyta (brown algae) and it consists primarily of linear β-1,3-linked glucose residues with small amounts of β-1,6-linkages (Nobe et al., 2003; Adams et al., 2009). Laminarin can be hydrolyzed to glucose using laminarinase (Adams et al., 2009). The hydrolysis of laminarin in macroalgae can be enhanced by pre-treatments prior to fermentation. Mannitol, a sugar alcohol, is not readily fermentable by ethanol producers. It is oxidised to fructose by mannitol dehydrogenase, a reaction that generates NADH. Regeneration of NAD + requires oxygen (active electron transport chain) or transhydrogenase, which converts NADH to NADPH. Thus, many microorganisms are not able to carry out strictly anaerobic fermentation of mannitol. Nevertheless, *Zymobacter palmae*, a facultatively anaerobic bacterium is able to ferment sugar alcohols including mannitol from *Laminaria hyperborea* extracts (Horn et al., 2000a). Ethanol production and mannitol consumption were faster in the small volume when compared to large volume culture (Horn et al., 2000a). Horn et al. (2000b) screened organisms which can utilize *L. hyperborea* extracts and among the four tested microorganisms *Pichia anchophorae* could consume both mannitol and laminarin and yield ethanol.

5.2. Algal ethanol production under anaerobic condition (Dark fermentation)

Microalgae fix CO_2 during photosynthesis and accumulate starch in their cells. Some microalgae can also grow under dark conditions in the presence of organic nutrients such as sugars and thereby accumulate starch (heterotrophic nutrition) (Chen et al., 2009). It is necessary to separate intracellular starch by extraction as mentioned in Section 5.1. However, many algae have strong cell walls and significant power is consumed during cell disintegration. Furthermore, a large amount of organic solvent is required in the starch extraction step. Since the starch separated by extraction is raw, it must be subjected to heat treatment for gelatinization before being hydrolyzed to glucose. A large amount of heat energy is required for this purpose. Usually, this heat energy for gelatinization accounts for about 20–30% of the total energy consumed in the ethanol production process. In dark and in presence of oxygen, microalgae usually maintain their life by consuming starch or glycogen stored in the cells and decomposing them oxidatively to carbon dioxide. If dark and anaerobic conditions are established, the oxidative reaction of starch become incomplete and depending on the type of the microalga, hydrogen gas, carbon dioxide, ethanol, lactic acid, formic acid, acetic acid and other products are produced in varying proportions. Ueda et al. (1996) used microalgae as starting materials for the production of ethanol. The algal cells contained a large amount of polysaccharides composed of glucose in the cells, which were catabolized rapidly under dark and anaerobic conditions to ethanol. These microalgae fall under classes Chlorophyceae, Prasinophyceae, Cryptophyceae and Cyanophyceae. Typical genera belonging to the class Chlorophyceae include *Chlamydomonas* and *Chlorella*, and typical genera belonging to the class Cyanophyceae include *Spirulina*, *Oscillatoria* and *Microcystis* (Ueda et al., 1996).

Hirano et al. (1997) placed algal slurry into a light shielded tube under dark and anaerobic conditions. Conversion from intracellular starch to ethanol under dark and anaerobic conditions was then observed in almost all of the tested strains. But the levels of conversion to ethanol were significantly different from each other. Relatively high conversion rates of 30–40% (*vs.* a theoretical yield of 0.56 g of ethanol/1 g of starch) were observed in the two strains *Chlamydomonas reinhardtii* (UTEX2247) and Sak-1. The important findings were that there was no need of nitrogen flushing due to the complete utilization of oxygen and no need of agitation for ethanol production. The optimal pH for ethanol from *Chlamydomonas* cells were 7–8 at a temperature of 25–30 °C. Hirano et al. (1997) pointed out that ethanol production was directly proportional to the increase of biomass in slurry. This finding was backed up by Ueno et al. (1998). Ueno et al. (1998) also produced ethanol via dark fermentation of cellular starch of *Chlorococum littorale*, in which study, an increase in the incubation temperature affected the mode of cellular starch decomposition and brought about an increase in ethanol productivity up to 30 °C. The addition of methyl viologen to the reaction vial drastically decreased hydrogen formation, while the ethanol productivity increased. It might be due to the fact that more electrons were involved in ethanol formation in the presence of methyl viologen, although a proportion of the reducing equivalents might have been trapped by the added compound itself. Approximately 2.5-times higher ethanol productivity compared to that without methyl viologen was obtained at 30 °C.

5.3. Direct production of ethanol by engineered microalgae

A disadvantage of using starch/cellulosic biomass as feedstocks for biofuel production is that considerable energy is expended both in their synthesis and their destruction. Dark fermentation is less efficient if there is influence of light and oxygen, and at the same time ethanol production from hydrolyzed algae raises the cost of production. Direct conversion of CO_2 to biofuel by photosynthesis would avoid the unnecessary expenditure of energy to create and destroy biopolymers normally used for cell structure or energy storage (Sheehan, 2009). It is also certain that the dominant algal strains isolated from the local environmental conditions may not be the optimal for production of biofuel under controlled conditions, and hence, genetic engineering may be required (Brennan and Owende, 2010).

The algal photosynthesis is mainly based on Calvin cycle in which ribulose-1,5-bisphosphate (RuBP) combines with CO_2 to produce two 3-phosphoglyceric acid (3-PGA) which is utilized for the synthesis of glucose and other metabolites. Attempts were carried out to redirect 3-PGA to ethanol by introducing ethanol producing genes (pyruvate decarboxylase and alcohol dehydrogenase). An ethanogenic recombinant of *Rhodobacter* sp. was developed for carbon redirection from the Calvin cycle to ethanol

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(Wahlund et al., 1996). The recombinant algal strain could produce ethanol in presence of light but required oxygen free condition as it was an anaerobe. Deng and Coleman (1999) reported another noteworthy result on ethanol production by genetic engineering methods, in which new genes were introduced into a cyanobacterium, *Synechococcus* sp. in order to create a novel pathway for fixed carbon utilization. The cyanobacterium *Synechococcus* sp. strain PCC 7942 was transformed by introducing the coding sequences of pyruvate decarboxylase (*pdc*) and alcohol dehydrogenase II (*adh*) from the bacterium *Zymomonas mobilis* through a shuttle vector pCB4. Both the genes were expressed at high levels under the control of the promoter from the *rbcLS* operon encoding the cyanobacterial ribulose-1,5-biphosphate carboxylase/oxygenase. The ethanol produced by the transformed cyanobacterium diffused from the cells into the culture medium. The simple growth requirements and ability to use light, CO_2 and inorganic elements efficiently, make cyanobacteria a potential system for bioconversion of solar energy and CO_2 into the valuable resource ethanol.

6. Future prospects of algal ethanol

Like any other industrial process, the challenge faced by algal bioethanol production is process economy. Albeit more than 100 algae-to-fuel companies have popped up worldwide mostly in the last couple of years, not a single commercial facility has been built so far (Waltz, 2009). Getting algae to produce bioethanol—in very large volumes, at very low cost—is the grand challenge that these young biotech firms has taken on (Sheridan, 2009). Economically feasible algal bioethanol can be turned out into a reality only through breakthrough technological innovations.

There are several bottlenecks in the algae-to-ethanol technology which need to be overcome before it can be successfully commercialized. Open pond cultivation exposes the desired algae to predators and other, stronger algal species. Hence, algal strains that not only have the potential for biofuel production, but also fend off other organisms are to be identified. Transgenic algae are particularly at risk because they may be less fit for open cultivation, though they have commercially important traits. It is theorized that transgenic extremophiles may have a better chance of survival than other types of transgenic algae since the competitors would be limited to minimum in extreme conditions (Waltz, 2009).

Many unicellular, colonial and filamentous microalgae can accumulate starch/cellulose as a major portion of their biomass. The high cost of starch/cellulose depolymerising enzymes for pre-treatment of algal biomass makes the cost of algal bioethanol several folds higher. At present, these enzymes are produced in microbial bioreactors for commercial use. Exploiting the microalgal strains to accumulate starch/cellulose and directly utilize their enzymatic or anaerobic digestion systems to produce ethanol can provide a cost-effective bioethanol production process. Further research is required on screening of high starch accumulating microalgae from corresponding water bodies or to generate efficient microalgae with conventional mutagenesis or using modern tools like genetic engineering. Genetic engineering methods should be triggered to produce all necessary enzymes such as amylases and cellulases within the algae so there would be no need, or only minimal need, for producing these enzymes in bioreactors. Furthermore, future research on the upregulation of starch/cellulose biosynthesis pathway enzymes for increased polysaccharides will also have the potential to increase algal biofuel production.

The possibility of competition between different pathways for carbon metabolism, including carbohydrate biosynthesis and storage, may limit ethanol production (Deng and Coleman, 1999). There must be a condition to produce ethanol simultaneously with photosynthesis and avoid the steps of accumulation of starch and conversion back to sugar for ethanol. In this approach, there may be an inhibition by accumulated ethanol towards the metabolic activity of algae and hence could decrease the productivity. This necessitates the need of ethanol tolerant algae for effective ethanol production.

Further, energy production using algal biomass may use large amounts of freshwater, which would compete with crops and cities (US DOE, 2006). Globally, commercial bioenergy production is projected to consume 18–46% of the current use of water by the year 2050. Already, the agricultural sector in the United States uses roughly 80% of the available freshwater and several regions face serious water shortages (US DOE, 2006). Development of high salt and temperature tolerant microalgae is necessary for a better utilization of marine water and trapping sunlight in elevated temperature area for getting higher growth and productivity. Akin to Algenol microalgae, the ethanol release in the form of vapour can reduce the risk of ethanol concentration and inhibition in the medium. Therefore, it is necessary to use process engineering approach for the development of effective bioreactor for the simultaneous production and recovery of ethanol.

Metabolic engineering, or extensive reprogramming the physiology of the producing organisms, is the answer to most of the above mentioned problems. Synthetic biology puts forward the means to achieve this goal. Although it is evident that synthetic biology is central to the development of advanced biofuels, it is uncertain whether a fully synthetic genome will ever be deployed in a live production environment. It is suspected that a fully synthetic microorganism may not have the robustness which is needed for large-scale industrial bioprocesses (Sheridan, 2009).

7. Conclusion

The utilization of algal biomass for bioethanol production is undoubtedly a sustainable and eco-friendly approach for renewable biofuel production. As the importance of microalgae in biodiesel production is growing, an equal or more attention is needed for the efficient use of these easily cultivable microorganisms to generate the green fuel bioethanol. There are possibilities to culture the algal strains even in marine or other waste waters for bioethanol production. Genetic engineering of selected strains to survive in adverse conditions and development of new bioreactor for effective production and recovery of ethanol are necessary to generate fuel for the exploding consumption.

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