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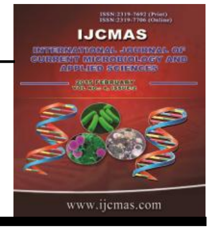

Review Article

Targeting cyanobacteria as a novel source of biofuel

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A B S T R A C T

Exploration of novel sources for the production of unconventional fuel i.e. biofuel such as biohydrogen, bioethanol, biodiesel and biogas is of utmost prerequisite in order to evade limited fuel assets, intensifying costs and of course, environmental issues due to rising emissions of harmful gases from fossil fuel. Therefore, significant considerations are given towards the biological conversion of carbon-di-oxide and solar energy to biofuel in order to facilitate an economically sustainable industrial society. Microalgae and cyanobacteria viz. *Botryococcus braunii*, *Chlorella*, *Dunaliella tertiolecta*, *Gracilaria, Pleurochrysis carterae, Sargassum* etc. are, in fact, matter of choice for biofuel production than that of plant biomass because of higher oil content, oxygenic photosynthesis, higher growth rates and per-acre productivity, fewer requirements for *in vitro* cultivation, non-food based feedstock, growth on non-productive land and on different water sources like freshwater, seawater, wastewater, production of valuable co-products and evidently algal biofuel is free of sulfur, is non-toxic, and is highly biodegradable. Microalgae contain approximately 2-40% lipids and fatty acids as membrane components, storage products, metabolites and sources of energy. It has been estimated that algae produces at least thirty times more energy per acre than land crops such as soybeans, and some estimate even higher yields up to 15000 gallons per acre. Moreover, these microbial fuel cells (MFCs) are relatively more convenient organisms to carry out genetic engineering in order to produce metabolites which are characteristically not produced by these organisms in nature. Producing biodiesel from algae provides the highest net energy because converting oil into biodiesel is much less energy-intensive than methods for conversion to other fuels such as ethanol, methane etc. This characteristic has made Biodiesel the favorite end-product from algae. Eventually, microalgae put forward the prospective to have an insightful impact for the welfare of earth and society on several vital concerns of alternative energy resources, global warming, human health and food security.

Keywords

Cyanobacteria, Biofuel, Photosynthesis, Oil Content, Microbial Fuel Cells.

Introduction

In the recent past, there has been a lot of discussion and interest around the viability of first generation biofuels as environment-friendly alternatives to foreign oil, primarily because of their possible competition with food crops and the use of non-sustainable practices for their production. Scientists and research groups have been searching for other sustainable sources for biofuel production, and microalgae seem to be one such alternative with a promising potential.

Microalgae are a diverse group of prokaryotic and eukaryotic photosynthetic microorganisms that can grow rapidly due to their simple structure (Kaushik and Chauhan, 2009). They have been investigated for the production of different biofuels including biodiesel, bio-oil and bio-hydrogen. Microalgal biofuel production is potentially sustainable. It is possible to produce adequate microalgal biofuels to satisfy the fast growing energy demand within the restraints of land and water resources.

Microalgae contain lipids and fatty acids as membrane components, storage products, metabolites and sources of energy. Algae contain anywhere between 2% and 40% of lipids/oils by weight. There is a greater possibiltiy to extract the oil/diesel from these natural resources. The advantages of deriving biodiesel from algae include rapid growth rates and high per-acre yield. Furthermore, algal biofuel contains no sulphur, is nontoxic, and is highly biodegradable. Some species of algae are ideally suited to biodiesel production due to their high oil content in some species, tapping out near 50%.

They are sunlight-driven cell factories that convert carbon dioxide to potential biofuels, foods, feeds and high-value bioactives (Metting and Pyne, 1986; Schwartz, 1990; Kay, 1991; Shimizu, 1996, 2003; Borowitzka, 1999; Ghirardi et al., 2000; Akkerman et al., 2002; Banerjee et al., 2002; Melis, 2002; Lorenz and Cysewski, 2003; Metzger and Largeau, 2005; Singh et al., 2005; Spolaore et al., 2006; Walter et al., 2005). In addition, these photosynthetic microorganisms are known to produce intracellular and extracellular metabolites with diverse biological activities such as antibacterial (Falch et al., 1995; Mundt et al., 2001; Rao et al., 2007; Kaushik and Chauhan; Kaushik et al., 2009), antifungal (MacMillan et al., 2002), cytotoxic (Luesch et al., 2000), algaecide (Papke et al., 1997), immunosuppressive (Koehn et al., 1992) and antiviral activities (Hayashi & Hayashi, 1996; Kaushik and Chauhan, 2009).

Microalgae can provide several different types of renewable biofuels. These include methane produced by anaerobic digestion of the algal biomass (Spolaore et al., 2006); biodiesel derived from microalgal oil (Roessler et al., 1994; Sawayama et al., 1995; Dunahay et al., 1996; Sheehan et al., 1998; Banerjee et al., 2002; Gavrilescu and Chisti, 2005); and photobiologically produced biohydrogen (Ghirardi et al., 2000; Akkerman et al., 2002; Melis, 2002; Fedorov et al., 2005; Kapdan and Kargi, 2006). The idea of using microalgae as a source of fuel is not new (Sawayama et al., 1995), but it is now being taken seriously because of the escalating price of petroleum and, more significantly, the emerging concern about global warming that is associated with burning fossil fuels (Gavrilescu and Chisti, 2005).

Between 1978 and 1996, the Aquatic Species Program (ASP) focused on the production of biodiesel from high lipid-content algae growing in outdoor ponds and using CO_2 from coal-fired power plants to increase the rate of algal growth and reduce carbon emissions (Sheehan, 1998). Under optimum growing conditions micro-algae will produce up to 4 lbs./sq. ft./year or 15,000 gallons of oil/acre/year. Microalgae are the fastest growing photosynthesizing organisms. They can complete an entire growing cycle every few days.

Green fuel Technologies in Cambridge, MA is field testing a closed system that uses the CO_2 in power plant flue gases (13% of flue gases in the test) to feed algae (Novakovic, 2005). Greenhouses can be modified to produce algae all year round. The surface area limitation which applies to ponds could be overcome in a greenhouse by adding a third layer of plastic inside the other two layers over which the pond water could flow in a thin enough film that it would receive enough solar radiation to grow algae. Microalgae is, by a factor of 8 to 25 for palm oil and a factor of 40 to 120 for rapeseed, the highest potential energy yield temperate vegetable oil crop.

Algae cultivation

Algae can produce 15-300 times more oil per acre than conventional crops, such as rapeseed, palms, soybeans, or jatropha, and they have a harvesting cycle of 1-10 days, which permits several harvests in a very short time frame, increasing the total yield (Chisti 2007). Algae can also be grown on land that is not suitable for other established crops, for instance, arid land, land with excessively saline soil, and drought-stricken land. This minimizes the issue of taking away pieces of land from the cultivation of food crops (Schenk et al. 2008). Water, carbon dioxide, minerals and light are all important factors in cultivation, and different algae have different requirements. The basic reaction in water is

Carbon dioxide + Light energy $\rightarrow$ Glucose + Oxygen

Temperature must remain generally within 20 to 30 °C. Microalgal biomass contains approximately 50% carbon by dry weight (Sanchez Miron et al., 2003). All of this carbon is typically derived from carbon dioxide. Producing 100 t of algal biomass fixes roughly 183 t of carbon dioxide. Carbon dioxide must be fed continually during daylight hours. Feeding controlled in response to signals from pH sensors minimizes loss of carbon dioxide and pH variations. Biodiesel production can potentially use some of the carbon dioxide that is released in power plants by burning fossil fuels (Sawayama et al., 1995; Yun et al., 1997). They can grow 20 to 30 times faster than food crops. Algae can be cultivated in open pond system and closed loop system

Use of bioreactors

Provide fast growth of algae with the high contents of oil. Photobioreactors have been successfully used for producing large quantities of microalgal biomass (Molina Grima et al., 1999; Tredici, 1999; Pulz, 2001; Carvalho et al., 2006). The term is more commonly used to define a closed system, as opposed to an open tank or pond. Because it is a closed system, the cultivator must provide all nutrients, including CO2. A photo bioreactor can operate in "batch mode", which involves restocking the reactor after each harvest, but it is also possible to grow and harvest continuously.

Continuous operation requires precise control of all elements to prevent immediate collapse. The grower provides sterilized water, nutrients, air, and carbon dioxide at the correct rates. This allows the reactor to operate for long periods. An advantage is that algae that grows in the "log phase" is generally of higher nutrient content than old "senescent" algae. Maximum productivity occurs when the "exchange rate" (time to exchange one volume of liquid) is equal to the "doubling time" (in mass or volume) of the algae.

Harvesting

Algae can be harvested using microscreens, by centrifugation, by flocculation (Bilanovic et al., 1998) and by froth flotation. Interrupting the carbon dioxide supply can cause algae to flocculate on its own, which is called "auto-flocculation". "Chitosan", a commercial flocculent, more commonly used for water purification, is far more expensive. The powdered shells of crustaceans are processed to acquire chitin, a polysaccharide found in the shells, from which chitosan is derived via de-acetylation. Water that is more brackish, or saline requires larger amounts of flocculent. Flocculation is often too expensive for large operations. Alum and ferric chloride are other chemical flocculants. In froth flotation, the cultivator aerates the water into a froth, and then skims the algae from the top (Gilbert et al., 1961) Ultrasound and other harvesting methods are currently under development (Bosna et al., 2003; US Patent No. 6524486)

Extraction methods

1. Mechanical methods: The simplest method is mechanical crushing. Since different strains of algae vary widely in their physical attributes, various press configurations (screw, expeller, piston, etc) work better for specific algae types. Often, mechanical crushing is used in conjunction with chemicals. Mechanicals methods are of two types:

(a) Expression/Expeller press: Algae is dried it retains its oil content, which then can be "pressed" out with an oil press. Since different strains of algae vary widely in their physical attributes, various press configurations (screw, expeller, piston, etc) work better for specific algae types. Many commercial manufacturers of vegetable oil use a combination of mechanical pressing and chemical Solvents in extracting oil.

(b) Ultrasonic-assisted extraction: Ultrasonic extraction, a branch of sonochemistry, can greatly accelerate extraction processes. Using an ultrasonic reactor, ultrasonic waves are used to create cavitation bubbles in a solvent material, when these bubbles collapse near the cell walls, it creates shock waves and liquid jets that causes those cells walls to break and release their contents into the solvent.

2. Chemical Methods: Algal oil can be extracted using chemicals. Chemical methods include:

(a) Hexane Solvent Method: Hexane solvent extraction can be used in isolation or it can be used along with the oil press/expeller method. After the oil has been extracted using an expeller, the remaining pulp can be mixed with cyclo-hexane to extract the remaining oil content. The oil dissolves in the cyclohexane, and the pulp is filtered out from the solution. The oil and cyclohexane are separated by means of distillation.

(b) Soxhlet extraction: Soxhlet extraction is an extraction method that uses chemical solvents. Oils from the algae are extracted through repeated washing, or percolation, with an organic solvent such as hexane or petroleum ether, under reflux in special glassware.

3. Supercritical fluid Extraction: In supercritical fluid/ CO_2 extraction, CO_2 is liquefied under pressure and heated to the point that it has the properties of both a liquid and a gas, this liquified fluid then acts as the solvent in extracting the oil.

Algal fuels

The algae product can then be harvested and converted into biodiesel; the algae's carbohydrate content can be fermented into Biofuel.

Biodiesel

Microalgae have a large capacity for producing lipids for biodiesel and carbohydrates for bioethanol. The potential for microalgae as biofuel feedstocks is high because of their high rate of productivity, the potentially high percentage of biomass composed of lipids or carbohydrates, and because they lack lignin. The absence of lignin production in most algae is a benefit because processing lignin is currently a major impediment for bioethanol production (Moore, 2009).

Producing biodiesel from algae provides the highest net energy because converting oil into biodiesel is much less energy-intensive than methods for conversion to other fuels (such as Ethanol methane etc). This characteristic has made Biodiesel the favorite end-product from algae. Producing biodiesel from algae requires selecting high-oil content strains, and devising cost effective methods of harvesting, oil extraction and conversion of oil to biodiesel.

Studies show that algae can produce up to 60% of their biomass in the form of oil. Because the cells grow in aqueous suspension where they have more efficient access to water, CO2 and dissolved nutrients, microalgae are capable of producing large amounts of biomass and usable oil in either high rate algal ponds or photobioreactors. This oil can then be turned into biodiesel which could be sold for use in automobiles. The more efficient this process becomes the larger the profit that is turned by the company. Regional production of microalgae and processing into biofuels will provide economic benefits to rural communities. Other sources of commercial biodiesel include canola oil, animal fat, palm oil, corn oil, waste cooking oil (Felizardo et al., 2006; Kulkarni and Dalai, 2006), and jatropha oil (Barnwal and Sharma, 2005).

Lipids from various sources can be converted to biodiesel through the process of transesterification (Chisti 2007). Microalgae provide an excellent source of lipids for two major reasons. First, microalgae productivity can be an order of magnitude greater than terrestrial vegetation used for biofuel feedstocks. Second, the lipid content of microalgae can exceed 70% of their dry mass, although algae with lipid content of around 30% is more common (Chisti 2007). High productivity combined with high lipid content results in a large amount of lipid that can be harvested annually for biodiesel production.

In addition to utilizing the lipids and carbohydrates from microalgal biomass for biofuel production, it is also possible to make use of the other compounds present in microalgal biomass. Microalgal proteins can be use for fish feeds in aquaculture or animal feeds for livestock and poultry (Meng et al. 2009). Algae are currently grown commercially to produce profitable chemicals, such as antioxidants and dietary supplements.

These chemicals comprise only a minor component of the microalgae cells, but have a high enough value to make their production profitable (Rosenberg et al. 2008). The cost of producing bioethanol and biodiesel from microalgae can be reduced by exploiting every component of microalgae biomass.

Transesterification of Oil into Biodiesel

According to Y. Chisti (2007) parent oil used in making biodiesel consists of triglycerides (Fig.2) in which three fatty acid molecules are esterified with a molecule of glycerol. In making biodiesel, triglycerides are reacted with methanol in a reaction known as transesterification or alcoholysis. Transestrification produces methyl esters of fatty acids, that are biodiesel, and glycerol (Fig. 2). The reaction occurs stepwise: triglycerides are first converted to diglycerides, then to monoglycerides and finally to glycerol.

Transesterification of Oil into Biodiesel

Transesterification is catalyzed by acids, alkalis (Fukuda et al., 2001; Meher et al., 2006) and lipase enzymes (Sharma et al., 2001). Alkali-catalyzed transesterification is about 4000 times faster than the acid catalyzed reaction (Fukuda et al., 2001). Biodiesel is recovered by repeated washing with water to remove glycerol and methanol.

Ethanol from algae

Ethanol from algae is possible by converting the starch (the storage component) and Cellulose (the cell wall component). Put simply, lipids in algae oil can be made into Biodiesel while the carbohydrates can be converted to ethanol.

Algae are the optimal source for second generation Bioethanol due to the fact that they are high in carbohydrates/polysaccharides and thin Cellulose walls. Some prominent strains of algae that have a high carbohydrate content and hence are promising candidates for ethanol production are *Sargassum*, *Glacilaria*, *Prymnesium parvum*, *Euglena gracilis.*

Fermentation process: Fermentation process to produce ethanol include the following stages:

1. Growing starch-accumulating, filament-forming, or colony-forming algae in an aqua culture environment;
2. Harvesting the grown algae to form a biomass;
3. Initiating decay of the biomass;
4. Contacting the decaying biomass with a yeast capable of fermenting it to form a fermentation solution; and,
5. Separating the resulting ethanol from the fermentation solution.

Other algae fules: Biogasoline, Methane Straight Vegetable Oil, Hydrocracking to traditional transport fuels and Jet Fuel are produced from algae.

Oil yields: The table-4 below presents indicative oil yields from various oilseeds and algae.

Advantages of biofuel production

Biofuel production using microalgal farming offers the following advantages

(1) The high growth rate of microalgae makes it possible to satisfy the massive demand on biofuels using limited land resources without causing potential biomass deficit.
(2) Microalgal cultivation consumes less water than land crops.
(3) The tolerance of microalgae to high CO_2 content in gas streams allows high-efficiency CO_2 mitigation.
(4) Nitrous oxide release could be minimized when microalgae are used for biofuel production.
(5) Microalgal farming could be potentially more costeffective than conventional farming.

Table.1 Oil content of some microalgae (Adopted from Y. Chisti, 2007)

S. No.	Microalgae	Oil Content (% Dry Wt)
1	*Botryococcus braunii*	25–75
2	*Chlorella sp.*	28–32
3	*Crypthecodinium cohnii*	20
4	*Cylindrotheca sp.*	16–37
5	*Dunaliella primolecta*	23
6	*Isochrysis sp.*	25–33
7	*Monallanthus salina*	>20
8	*Nannochloris sp.*	20–35
9	*Nannochloropsis sp.*	31–68
10	*Neochloris oleoabundans*	35–54
11	*Nitzschia sp.*	45–47
12	*Phaeodactylum tricornutum*	20–30
13	*Schizochytrium sp.*	50–77
14	*Tetraselmis sueica*	15–23

Source: Department of Biological Sciences and Biotechnology, Tsinghua University, Beijing , China (2004)

Table.2 Lipid content of different algae

S. No.	Strains	% Lipid (on a Dry Basis)
1	*Chlorella* sp.	14 - 22
2	*Scenedesmus* sp.	12 - 40
3	*Chlamydomonas* sp.	21
4	*Euglena* sp.	14 - 20
5	*Spirogyra* sp.	11 - 21
6	*Dunaliella* sp.	6 - 8
7	*Synechoccus* sp.	11
8	*Prymnesium* sp.	22 - 38
9	*Porphyridium* sp.	9 - 14

Table.3 Comparison of biodiesel from microalgal oil and diesel fuel

S. No	Properties	Biodiesel from Microalgal Oil	Diesel Fuel
1	Density Kg l-1	0.864	0.838
2	Viscosity Pa s	5.2×10-4 (40 ºC)	1.9 - 4.1 ×10-4 (40 ºC)
3	Flash point ºC	65-115*	75
4	Solidifying point ºC	-12	-50 – 10
5	Cold filter plugging point ºC	-11	-3.0 (- 6.7 max)
6	Acid value mg KOH g-1	0.374	0.5 max
7	Heating value MJ kg-1	41	40 – 45
8	HC ratio	1.18	1.18

Based on data from multiple sources

Table.4 Comparison of average oil yields from algae with that from other oilseeds

S. No.	Oilseeds and Algae	Gallons of Oil per Acre per Year
1	Corn	18
2	Soybeans	48
3	Safflower	83
4	Sunflower	102
5	Rapeseed	127
6	Oil Palm	635
7	**Microalgae**	**5000-15000**

Fig.1 Biodiesel making

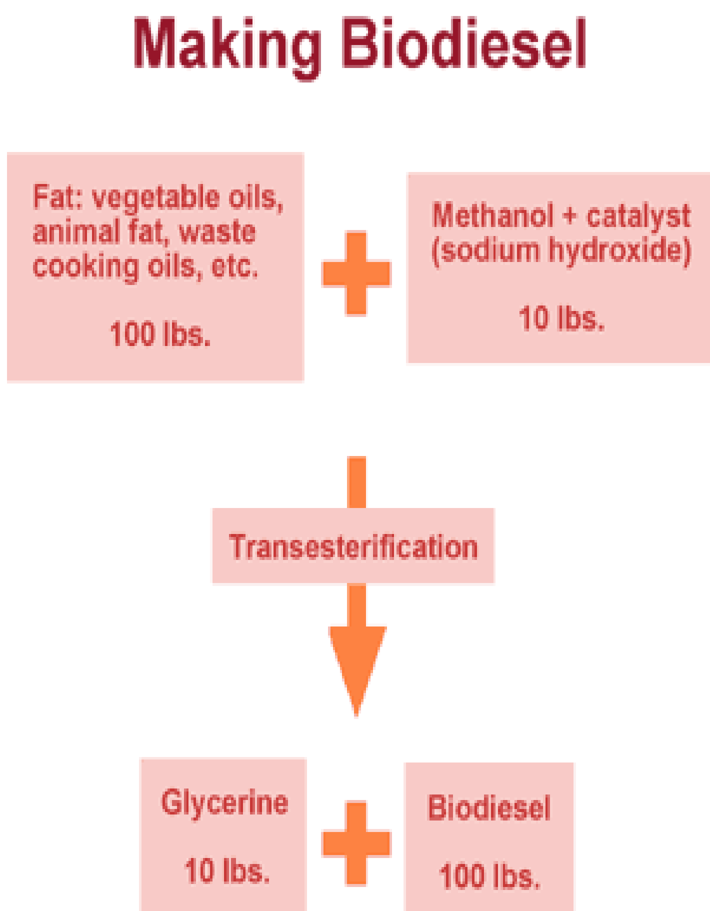


Fig.1 Technological circuit of installation of a bioreactor

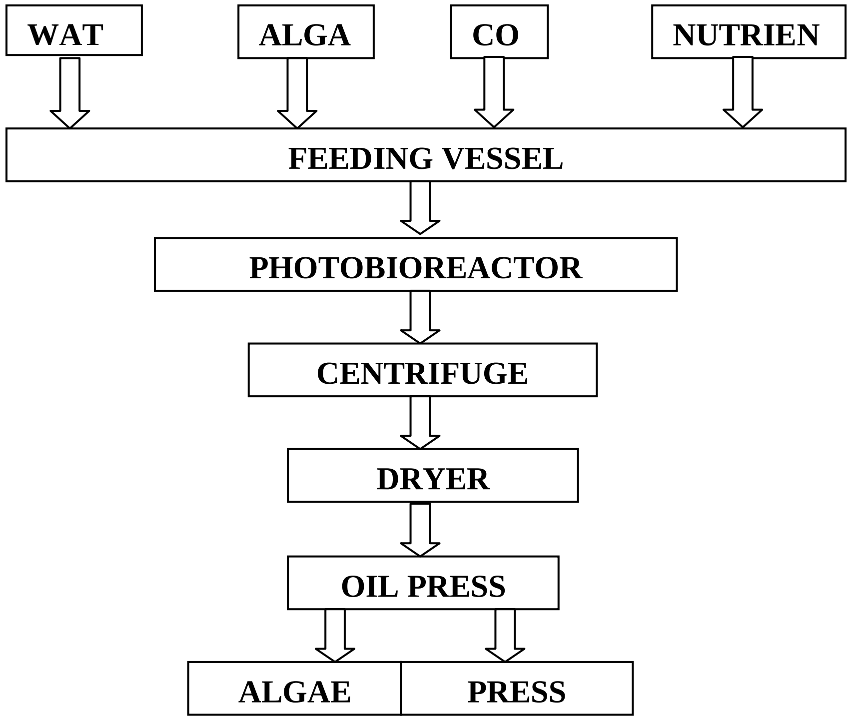
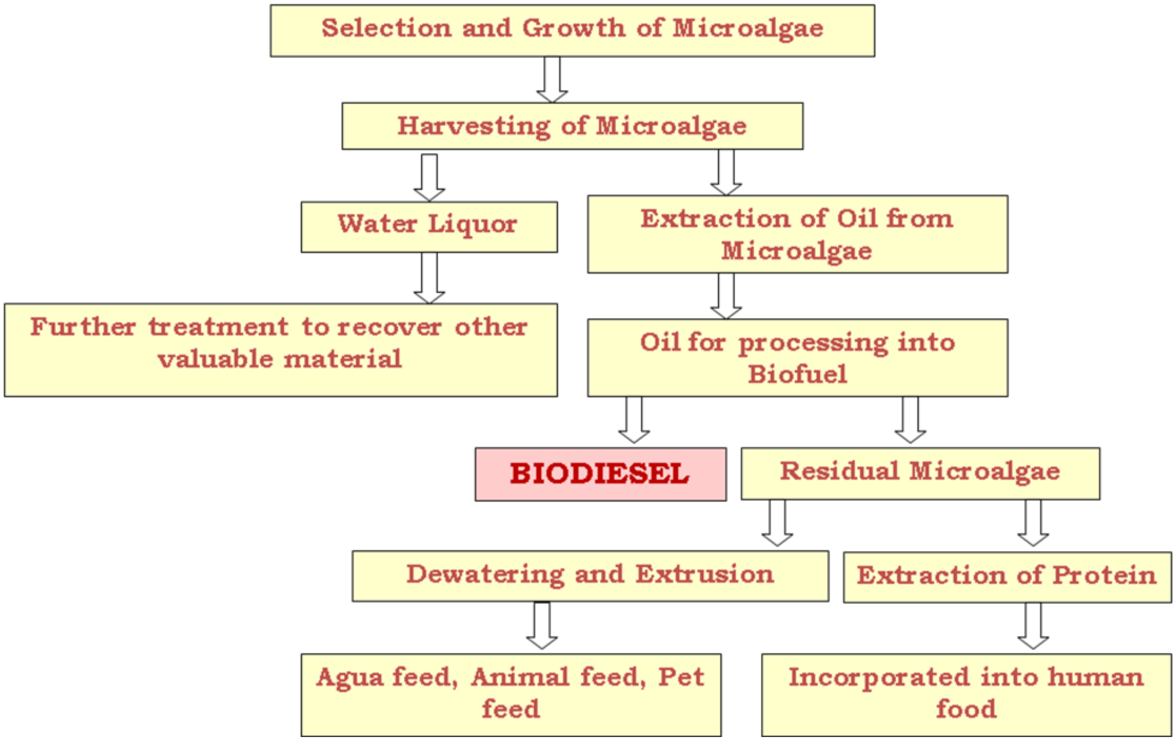


Fig.2 A detailed process of biodiesel from algae



Disadvantages of biofuel production

On the other hand, one of the major disadvantages of microalgae for biofuel production is the low biomass concentration in the microalgal culture due to the limit of light penetration, which in combination with the small size of algal cells makes the harvest of algal biomasses relatively costly. The large water content of harvested algal biomass also means its drying would be an energy-consuming process. The higher capital costs of and the rather intensive care required by a microalgal farming facility compared to a conventional agricultural farm is another factor that impedes the commercial implementation of the biofuels from microalgae strategy. Nevertheless, these problems are expected to be overcome or minimized by technology development. Given the vast potential of microalgae as the most efficient primary producers of biomass, there is little doubt that they will eventually become one of the most important alternative energy sources. Another problem that arises in microalgae production is that the algae-culture systems can easily and quickly be contaminated with other organisms (Zittelli et al. 2006). Species of algae other than the target species can be introduced to the production system and compete with the target species. This presents a problem because the overall productivity of the system can be reduced. One way to avoid the issue of contamination is to grow microalgae that flourish under extreme conditions. For example, Arthrospira maxima grow at very high pH (9.5-11), while Tertraselmis grow in extremely saline waters (Dismukes et al. 2008). Another method for avoiding contamination is to grow the microalgae in a closed system, under very controlled conditions. There, however, is no guarantee that contamination will not occur.

The potential benefits of large-scale production of microalgae for biofuels and other products far outweigh the existing and potential issues associated with microalgae production. At the moment, microalgae appear to be the best option for biofuel feedstocks because of their tremendous productivity, ability to use waste products in their production, and the valuable byproducts that can be produced. Research dealing with improving production systems, identifying ideal microalgae for biofuel production, and investigating the sustainability of microalgal biofuel is necessary before large-scale microalgal biofuel operations are established. Microalgal biotechnology appears to possess high potential for biodiesel production because a significant increase in lipid content of microalgae is now possible through heterotrophic cultivation and genetic engineering approaches. Keeping in view the advantages of microalgae, the study can be designed to develop the technologies for the production of biodiesel from microalgae, including the various modes of cultivation for the production of oil-rich microalgal biomass, as well as the subsequent downstream processing for biodiesel production.

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