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A Brief Review on Algal Lipid

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Abstract: *Algae are gaining importance for the tremendous applications as fuel, food and feed. Due to increasing population in India, the question remains debatable whether food or fuel? The conventional fuel resources are depleting day today. In this contest alternative fuels are the answer for the question. Algae could be the better alternate source as it is not competing with agricultural crops. The major breakthrough in Algal biofuels still needs to be achieved, especially on enhancement of the lipid in algae. This is possible only when promising strains are developed with high lipid content and improvement in the downstream processing for economical feasibility. This paper briefs about algal potential, lipid enhancement and quantification techniques.*

Keywords: *Algae, lipid, Quantification, strain improvement*

1. INTRODUCTION

Biomass is one of the better sources of energy. Large-scale introduction of biomass energy could contribute to sustainable development, environmentally, socially and economically. The most common biofuels are biodiesel and bio-ethanol, which can replace diesel and gasoline. They are mainly produced from biomass or renewable energy sources and contribute to lower combustion emissions than fossil fuels per equivalent power output. Although biofuels are still more expensive than fossil fuels their production is increasing in countries around the world.The global production is estimated to be over 35 billion liters. The main alternative to diesel fuel is biodiesel, representing 82% of total biofuels production.[1]

Biodiesel (monoalkyl esters) is one of such alternative fuel, which is obtained by the transesterification of triglyceride oil with monohydric alcohols. Biodiesel is produced from vegetable oils (edible or non-edible) or animal fats. Since vegetable oils may also be used for human consumption, it can lead to an increase in price of food-grade oils, causing the cost of biodiesel to increase and preventing its usage, even if it has advantages comparing with diesel fuel.

2. POTENTIAL OF MICROALGAL BIODIESEL

The enormous amount of burning of fossil fuel has increased the CO_2 level in the atmosphere, causing global warming. Biomass is focused as an alternative energy source, as it's a renewable resource and it can fix atmospheric CO_2 through photosynthesis. Among biomass, algae (macro and microalgae) usually have a higher photosynthetic efficiency than other biomass producing plants. Biodiesel from microalgae appears to be a feasible solution to India, for replacing petro-diesel. The estimated annual consumption of petroleum product in India is nearly about 120 million tonnes per year, and no other feedstock except microalgae has the capacity to replace this large volume of oil. To elaborate, it has been calculated that, in order for a crop such as soybean or palm to yield enough oil capable of replacing petro-diesel completely, a very large percentage of current land available need to be utilized only for biodiesel crop production, which is quite infeasible[2]. For small countries, in fact it implies that all land available in the country be dedicated to biodiesel crop production. However, if the feedstock were to be algae, owing to its very high yield of oil per acre of cultivation, it has been estimated that less than 2-3 percent of total Indian cropping land is sufficient to produce enough biodiesel to replace all petrodiesel currently used in country. Clearly microalgae are superior alternative as a feedstock for large scale biodiesel production.

Table 1.1. *Comparison of some sources of biodiesel*

Crops	**Oil Yield** Gallons/acre
Corn	18
Soybean	48
Safflower	83
Sunflower	102
Rapeseed	127
Oil Palm	635
Microalgae	5000-15000

Microalgae appear to be an emerging source of biomass for biodiesel that has the potential to completely displace fossil diesel. Microalgal strains with high oil content are of great interest in search for sustainable feedstock for the production of biodiesel [3][4]. Algae can have anywhere between 20-80% of oil by weight of dry mass (Table 1.2). Lipid accumulation in algae typically occurs during period of environmental stress, including nutrient deficient conditions. Biochemical studies have suggested that acetyl-CoA carboxylase (ACCase), a biotin containing enzyme that catalyzes an early step in fatty acid biosynthesis, involved in the control of this lipid accumulation process. Therefore, it may be possible to enhance lipid production rates by increasing its activity

2.1. Advantages of Microalgae over Other Biofuel for Biodiesel Production

Microalgae are promising alternative source of lipid for biodiesel production. Due to their simple cellular structure, algae have higher rate of biomass and oil production than conventional crops [5]. Hence, algae have been claimed to be up to 20 times more productive per unit area than the best oil seed crop[6]. Microalgal species upon exposure to sunlight are capable of fixing CO_2 to produce biofuel and other chemical components. These are the miniature sunlight driven biochemical factories and some of the most efficient CO_2 fixers on this planet. The CO_2 fixation in these cells leads to the formation of lipids which after esterification with methanol produce biodiesel. After extracting oil from microalgae, the remaining biomass portion can also be used as a high protein feed for livestock [7][8].

Hundreds of Microalgal strains capable of producing high content of lipid have been screened and their lipid production metabolisms have been characterized and reported [9]. Lipid productivity is a key characteristic for choosing algal species for biodiesel production [10]. Several studies have shown that the quantity and quality of lipids within the cell can vary as a result of changes in growth conditions, such as temperature and light intensity, nutrient media characteristics, concentration of nitrogen, phosphates and iron [11][12][13][14].

Production of biodiesel from algae is technically, but not yet economically feasible [6]. The major economic bottleneck cited in the literature is algal productivity, followed by labor and harvesting costs [15]. Laboratory yields are reportedly rarely reached in large scale culture, due to issues such as contamination , evaporation, flooding and lack of control over temperature and light provision in open ponds, as well as difficulties with fouling limiting light intensity and oxygen build up in closed photo bioreactors [16][17]. Harvesting unicellular algae from solution remains a major challenge and the dilute biomass produced further aggravates the need for an integrated approach to minimizing consumption of water and energy as well as downstream processing cost [18].

2.2. Microalgal Species Considered for Biodiesel Production

Table 1.2. *Microalgal species considered for biodiesel production*

Algal Strains	% lipid	References
Anabaena cylindrical	4-7	[5]
Ankistrodesmus species	28-40	[18]
Botryococcus braunii	25-86	[19]
Chaetoceros muelleri	24.4	[20]
Chlamydomonas	23	[21]
Chlorella emersonii	63	[22]
Chlorella minutissima	57	[23]
Chlorella protothecoides	15-55	[24]
Chlorella sorokiana	22	[23]
Chlorella vulgaris	14-56	[23]
Cyclotella species	42	[9]
Dunaliella bioculata	8	[5]
Dunaliella salina	28.1	[21]
Dunaliella tertiolecta	36-42	[25]
Hantzschia species	66	[9]
Isochrysis galbana	21.2	[21]
Monallantus salina	72	[26]
Nannochloropsis species	28.7	[23]
Neochloris oleoabundans	35-65	[27]
Nitschia closterium	27.8	[21]
Nitschia frustulum	25.9	[21]
Phaeodactylum tricornutum	30	[4][28]
Scenedesmus dimorphus	16-40	[5]
Scenedesmus obliques	12-14	[5]
Scenedesmus quadricauda	19.9	[21]
Selenastrum species	21.7	[21]
Skeletonema costatum	19.7	[21]
Spirulina maxima	6-7	[5]
Spirulina plantensis	16.6	[22]
Stichococcus species	33	[9]
Tetraselmis maculate	3	[5]
*Tetraselmis suecia*s	15-23	[4][29]

3. STRAIN IMPROVEMENT FOR ENHANCED LIPID

The major breakthrough in algal biofuels need to be achieved for enhanced lipid content without compromising biomass. Many reports have stated that lipid enhancement can be done through environmental stress like nitrogen starvation but limitation lies in decreased biomass. Strain improvement should be done without affecting other quantitative traits. As lipid is also one of the quantitative trait where mere change in one or two traits will not yield better results. Genetic Engineering for improved biofuels traits is been done[30] and Transgenics algal studies are done, but due to genetic instability, environmental safety and ethical issues are major constraints need to be addressed.

Other method to improve the strain is random mutagenesis. Screening mutagenized populations is a more preferable alternative, which could provide strains with large cell size and rapid cell cycle. Therefore, over expressing a single gene in lipid biosynthesis is unlikely to increase the lipid yield. A study on mutagenesis was done on *Chlamydomonas* to increase lipid production. Analysis of *Chlamydomonas* strains with the starchless phenotype was due to defective in ADP-glucose pyrophospho- rylase, an enzyme involved in starch biosynthesis, increased the total lipid content over the wildtype [31]. This finding demonstrates the possibility in obtaining higher lipid producing strains and improvement in the growth of the algae in photobioreactoprs[32] that are resulted from alterations in molecular processes by random mutagenesis. .

The major disadvantage is screening process, which is highly laborious. Methods for identifying strains with ability to grow faster or having large cell by analyzing individual cell among the mutant population are extremely laborious.

4. EXTRACTION AND QUANTIFICATION OF LIPID FROM ALGAL BIOMASS

For biodiesel production, lipids and fatty acids have to be extracted from the microalgal biomass. Various methods are available for the extraction of algal oil, such as mechanical extraction using hydraulic or screw, enzymatic extraction, chemical extraction through different organic solvents, Ultrasonic extraction, and supercritical extraction using carbon dioxide above its Standard temperature and pressure. For lipids a solvent extraction is normally done directly from the lyophilized biomass, being a quick and efficient extraction method that slightly reduces the degradation. The lipid in the algal cells can be extracted according to the protocol described by Bligh and Dyer [33]. In Nano chloropsis sp., CCMP1776, lipid was extracted by microwave method further it was transesterified and later it was quantified by GC-MS and TLC analysis [34]. In *Chlorella vulgaris* mechanical method was associated with petroleum ether and later it was transesterified with NaOH and methanol and further it was quantified with GC-MS. GC-MS, HPLC and TLC are widely used but most of them are not economical and they are suited for experimental purposes but not for commercialization. Spectrophotometer quantification is also done, a cheaper way of quantification [42].

5. LIPID EXTRACTION AND QUANTIFICATION TECHNIQUES

Some of the oil extraction procedures and quantification techniques are listed in table 1.3. Limited numbers are chosen, to mention others is beyond the scope of this paper.

Sl.no	Species	Extraction of oil	Quantification method	Reference
1	Nannochloropsis sp.,CCMP1776	Microwave assisted extraction	GC-MS analysis and TLC	[34]
2	*Chlorella vulgaris*	Cell disruption- Manual grinding, Ultrasonication, Bead Milling, Enzymatic Lysis and Microwaves- followed by solvent extraction	GC-MS	[35]
3	Chaetoceros lauderi CCMP 193, Emiliana huxleyi CCMP372, Crypthecodinium cohnii CCMP316, Rhodomonas salina CS24, Nanochloropsis sp. CS 246, Pavlvova pinguis CS 375, Chlorella zofingiensis, C. vulgaris LARB#2, Palmelococcusb miniatus etc	Solvent extraction (Bligh-dyer method)	Nile red fluroscence method	[36]
4	Algal biomass	Solvent extraction with magnetic stirred agitation	GC-MS	[37]
5	Cladophora fracata, Chlorella protothecoids	Solvent extraction using soxhlet apparatus	TLC	[38]
6	Oedogonium and Spirogyra sps	Mechanical(pestle& mortar) and Solvent extraction(N-hexane)	-	[39]
7	Chlorella protothecoides	n-hexane solvent extraction	Wt/%	[40]
8	Chlorella vulgaris and Pseudokirchneriella subcapitata	Cell disruption by vortex mixture at 90^0c with saponification reagent	spectrophotometer	[41]

9	Phaeodactylum tricornutum and Chlorella vulgaris CM2	Cell disruption and extraction in saponification reagent	spectrophotometer	[42]

6. TRANSESTERIFICATION OF ALGAL OIL TO PRODUCE BIODIESEL

Biodiesel production from microalgae can be done using several well known industrial processes, the most common of which is base catalyzed transesterification with alcohol. The transesterification is the reversible reaction of fat or oil (which is composed of triglyceride) with an alcohol to form fatty acid alkyl ester and glycerol. Stoichio-metrically, the reaction requires a 3:1 molar alcohol to oil ratio, but excess alcohol is (usually methyl alcohol is used) added to drive the equilibrium toward the product side [43].The reaction occurs stepwise: triglycerides are first converted to diglycerides, then to monoglycerides and finally to glycerol [2]. Transesterfication can be catalyzed by acids, alkalis [43][44] and lipase enzymes [45]. However enzyme catalysts are rarely used as they are less effective [46]. The alkali-catalyzed transesterification is about 4000 times faster than the acid catalyzed reaction [47]. Consequently, alkalis such as sodium and potassium hydroxide are commonly used as commercial catalysts at a concentration of about 1% by weight of oil. Alkoxides such as sodium methoxide are even better catalysts than sodium hydroxide and are being increasingly used. Use of lipases offers important advantages [2][47].

Alkali-catalyzed transesterification is carried out at approximately 60°C under atmospheric pressure, as methanol boils off at 65 °C at atmospheric pressure. Under these conditions, reaction takes about 90 min to complete. A higher temperature can be used in combination with higher pressure. Methanol and oil do not mix; hence the reaction mixture contains two liquid phases. Other alcohols can be used, but methanol is the least expensive. To prevent yield loss due to saponification reactions (i.e. soap formation), the oil and alcohol must be dry and the oil should have a minimum of free fatty acids. Biodiesel is recovered by repeated washing with water to remove glycerol and methanol [2]. This process of biodiesel production is found to be most efficient and least corrosive of all the processes as the reaction rate is reasonably high even at a low temperature of 60°C.

7. CONCLUSION

Although various techniques are available for lipid extraction from algae, none of them are economically viable. Although the quantification are done through TLC, GC, Flow Cell cytometry and HPLC techniques , results are accurate but they are not economically feasible because of time consuming, costlier and laborious. In this regard a simple viable technique need to be achieved for industrial purpose. This is one of the major thrust area in algal lipid research and commercialization in private sectors. Research in Ggenetic Engineering of algae for enhanced lipid content without neglecting biomass and downstream processing need to be scaled up.

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