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Microalgae: A Potential Source of Biodiesel

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ABSTRACT

The economic development of the world is highly dependent on fossil fuel supplies which are constrained not only by limited availability but also generate high levels of pollution. Looking at the limited fossil fuel associated with problems, concerted efforts have been started to search for alternative bio fuels like bio ethanol and biodiesel. Since the diesel is being used massively in industrial commercial, agriculture and other sectors. Therefore, the production and utilization of biodiesel from oil seeds crops has been getting renewed interest in recent years in the India to overcome the demerits of oil from oil seed crops. The production of biodiesel from microalgae has several advantages over the above resources due to higher algal biomass and oil productivities and the need of non-arable land for its growth. Industrial and municipal wastewaters can be potentially utilized for cultivation of micro algal oil that can be used for the production of biodiesel to completely displace petro diesel. The micro algal biomass has been reported to yield high oil contents and have the diesel production. Accordingly, lot of R & D work has been initiated for the growth, harvesting, oil extraction and conversion to biodiesel.

Keywords: Microalgal Species; Cultivation; Harvesting; Oil Extraction and Biodiesel Production

1. Introduction

Owing to the limited availability and associated environmental problems with fossil fuel utilization, the renewable energy based biofuel viz biodiesel and bioethanol are viewed as future substitute fuels for diesel and gasoline respectively. Sugar based fuel alcohol production is not feasible unless methods are developed to convert lingo-cellulosic biomass into ethanol and there is no competition with food supplies. The biodiesel from edible oils is also not a sustainable option due to heavy competition with seed plants and accordingly, non-edible oils like *Jatropha*, *Pongamia*, *Neam* oils etc. are accorded top priority for biodiesel production in India. The plantations of *Jatropha curcas* are under cultivation on large land area in the country and hopes are raised for sustainable availability of oil for conversion to biodiesel. Apart from these non-edible oil resources, microalgae is also becoming the focus as future source of biodiesel as these are found exceedingly rich in oil that can be converted to biodiesel using existing technology. Microalgae are prokaryotic (e.g. *Cyanobacteria*, *Cyanophyceae*) or eukaryotic (e.g. green algae) and diatoms (*Bacillariophyta*) that can grow rapidly and live in harsh conditions due to their unicellular or simple multicellular structure [1,2]. A study has estimated that more than 50,000 micro algal species exist, but only 30,000 are studied and analyzed as yet [3]. If grown properly, the microalgal based biodiesel has potential to completely substitute diesel without competing with the food and other supplies of agricultural products.

The oil yield from some microalgae is reported to exceed 80% (on dry weight basis) compared to 40% - 50% from edible/non-edible oil seeds. An average annual productivity of micro algal biomass in a well designed production system located in a tropical zone may be about 1.535 $kg \cdot m^{-2} d^{-1}$ with biodiesel yield of 98.4 m^3 per hectare. The other added advantage of microalgae is that unlike other oil crops, they grow rapidly and double their biomass within 24 h which could be as short as 3.5 h contrary to the time for oil crops (months together).

The present paper reviews the possibilities of using different types of micro algal species as source of oil, techniques for algal growth, harvesting, oil extraction and conversion to biodiesel and its future scope in India.

2. Literature Review

Advantage of using microalgae for biodiesel production has been reported by a number of workers [4-11]. The interest in microalgae for biodiesel started in 1970s during the first oil crisis due to high oil yields. The average oil yield is reported between 1% and 70% but under certain conditions, some species can yield up to 90% of dry biomass weight [12]. The variation in fatty acid composition of oil from different algae species is reported by several authors [13-17]. In fact, several studies have reported the use of microalgae for the production of biodiesel and other by products [18-22].

Moheimani [23] studied the effect of pH on algal growth in a plate photobioreactor. Kaewpintong [24] found better growth of microalgae in an airlift bioreactor due to better mixing of the microalgal culture. Thomas *et al*. [25] studied the growth of microalgal species that grow well in this medium containing carbon dioxide as a carbon source and nitrate as a nitrogen source and determined the effect of nitrogen as well as the salt on the chemical compositions of the algae. Ugwu and Aoyagi [26] studied mass production of algae and have been done to develop a photobioreactor for algal culture. Weissman and Goebel [27] studied primary harvesting methods for biofuels production.

Samson and Leduy [28] developed a flat reactor equipped with fluorescence lamps for the growth of micro algal oil Further, Ortega and Roux [29] developed a outdoor flat panel reactor using thick transparent PVC materials. The design of vertical alveolar panels and flat plate reactors for mass cultivation of different algae was reported by Tredici and Materassi, Zhang *et al.*, and Hoekema *et al*. [30-32]. Hu *et al*., Eriksen and Wang found that high dissolved oxygen (DO) levels can be reached in tubular photobioreactors [33-35].

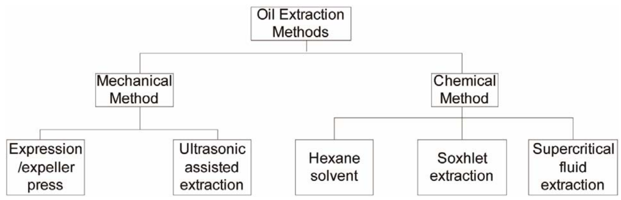
3. Classification of Microalgae

Photosynthetic organisms growing in aquatic environments include macroalgae, microalgae and emergents [36]. These primitive organisms with simple cellular structure and large surface to volume ratio are able to uptake large amount of nutrients. The photosynthesis in microalgae is similar to higher plants but is more efficient due to their simple cellular structure [37]. The microalgae can be classified on the basis of their pigmentation, life cycle and basic cellular structure as given in **Table 1**.

The mass production of oil is focused mainly on microalgae of 0.4 mm dia of diatoms and cyanobacteria rather than macroalgae e.g. Seaweed and is preferred for biodiesel production due to its less complex structure, fast growth and high oil content. Research & Development is also carried out to use the seaweeds for bio-energy, perhaps, due to higher resource availability [39,40]. *Botryococcus braunii*, *Chlorella*, Dunaliella tertiolecta, Gracilaria, Pleurochrysis carterae, Sargassum, are some of the microalgal species currently studied for their suitability for biodiesel production [41-43].

4. Algae Oil Extraction Techniques

Oil extraction from algae is one of the costly processes that can determine the sustainability of algae-based biodiesel. Oil extraction methods can be broadly classified as:



Each of these methods has drawbacks:

- The mechanical press generally requires drying of the algae, which is an energy intensive step.
- The use of chemical solvents poses safety and health issues.
- Supercritical extraction requires high pressure equipment that is both expensive and energy intensive.

Table 2 compares oil yields of microalgae with other oil feedstocks. It is seen from that there are significant variations in biomass productivity, oil yield and biodiesel productivity. Microalgae are more advantageous due to higher biomass productivity, oil and biodiesel yield.

The table shows that low, medium and high oil content micro-algae have high oil yield/ha/year and hence higher biodiesel productivities (l/ha/yr) which is much more than the productivities of oil seed crops. This is one of the most important reasons that microalgae have attracted the attention of researchers in India to scientifically grow, harvest, extract oil and convert it to biodiesel.

5. Technology for Growing Algae

The following technologies are used for the production of algae:

Table 1. Classification of microalgae [38].

S No	Name of microalgae	Known species	Storage material	Habitat
1	Diatoms (*Bacillariophyceae*)	100,000	Chyrsolaminarin (polymer of carbohydrates) and TAGs	Oceans, fresh and brackish water
2	Green algae (*Chlorophyceae*)	8000	Starch and TAGs	Freshwater
3	Blue-green algae (*Cyanophyceae*)	2000	Starch and TAGs	Different habitats
4	Golden algae (*Chrysophyceae*)	1000	TAGs and carbohydrates	Freshwater

Table 2. Comparison of microalgae with other biodiesel feedstocks [44-53].

Oil feedstocks	Oil content (% dry wt. biomass)	Oil yield (L oil/ha/year)	Land use (m^2/year/L biodiesel)	Biodiesel productivity (L biodiesel/ha/year)
Oil Seeds				
Microalgae (low oil content)	30	58,700	0.2	61,091
Microalgae (medium oil content)	50	97,800	0.1	101,782
Microalgae (high oil content)	70	1,36,900	0.1	142,475
Corn/Maize (*Zea mays* L.)	44	172	56	179
Hemp (*Cannabis sativa* L.)	33	363	26	378
Soyabean (*Glycine max* L.)	18	636	15	661
Jatropha (*Jatropha curcas* L.)	28	741	13	772
Camelina (*Camelina sativa* L.)	42	915	10	952
Canola/Rapseed (*Brassica napus* L.)	41	974	10	1014
Sunflower (*Helianthus annuus* L.)	40	1070	9	1113
Caster (*Ricinus communis*)	48	1307	8	1360
Palm oil (*Elaeis guineensis*)	36	5366	2	5585

5.1. Open Pond System

Cultivation of algae in open ponds is studied extensively. Open ponds can be categorized into natural waters (lakes, lagoons, ponds) and artificial ponds (containers). The most commonly used system includes shallow big ponds, tanks circular and raceway ponds. The major advantage of open ponds is that they are easier to construct and operate than the closed systems. The major constraints are poor light utilization, large evaporative losses, diffusion of CO_2 to the atmosphere and requirement of large areas. The attack by predators and other fast growing heterotrophs restricts the commercial production of algae in these systems. The biomass productivities are lower due to lack of proper stirring.

The "raceway ponds" provide better circulation of algae, water and nutrients using paddlewheels on regular frequency. The shallow ponds are also used to allow the algae to be exposed to sunlight. Such ponds are operated in a continuous manner with CO_2 and other nutrients constantly fed to the pond with circulation of the remaining algae-containing water at the other end.

Their advantages are their simplicity, low production and operating costs. The contamination with bacterial strains and maintenance of optimum temperature are the main difficulty in large pond area.

5.2. Closed Ponds

Control of environment in closed ponds is much better but there are costlier and less efficient than open pond system. The closed system allows more species to grow, control the temp., increase the CO_2 resulting in increased algae growth.

5.3. Photo Bioreactor

Photobioreactor (PBR) is a translucent closed container making use of light source. A PBR can be operated in "batch mode", but with a continuous stream of sterilized water containing nutrients, air and carbon dioxide. As the algae grows, excess culture overflows and is harvested. Its advantage is that microalgae in the "log phase" are produced with higher nutrient content. The maximum productivity occurs when the "exchange rate" is equal to the "doubling time" of the algae. Such systems can be illuminated by artificial light, solar light or by both. Naturally illuminated systems with large illumination surface areas include open ponds, flat-plate, horizontal/serpentine tubular airlift and inclined tubular photobioreactors, while large scale photobioreactors are artificially illuminated (either internally or externally) using fluorescent lamps. Some other photobioreactors include bubble column, airlift column, stirred-tank, helical tubular, conical, type etc.

6. Harvesting of Algae

Algal harvesting consists of recovery of biomass from the culture medium that constitutes about 20% - 30% of the total biomass production cost. Most common harvesting methods include sedimentation, centrifugation, filtration, ultra-filtration or combination of flocculation-flotation. Flocculation is used to aggregate the microalgal cells to increase the effective particle size and hence ease the sedimentation, centrifugal recovery and filtration [54]. These techniques are summarized in **Table 3**.

High-density algal cultures can be concentrated by chemical flocculation or centrifugation using aluminum sulphate, ferric chloride etc to coagulate and precipitate the cells to settle down at the bottom or to float to the surface. Algal biomass is finally recovered by siphoning off the supernatant or skimming the cells off the surface.

Once the algae is harvested and dried, several methods like mechanical solvent extraction and chemical methods can be applied for oil extraction, the choice of which depends upon the particle size of algal biomass. However, solvent extraction is usually applied to get high oil yields from algae.

The oil yields from different microalgae are given in **Table 4** which shows that *Nannochloropsis* species has highest while *Tetraselmis suecica* minimum oil yield.

7. Physicochemical Properties of Oil

To assess the potential of biodiesel as a substitute of diesel fuel, the properties of biodiesel such as density, viscosity, flash point, cold filter plugging point, solidifying point, and heating value were determined. A comparison of these properties of biodiesel from microalgal oil with diesel and ASTM biodiesel standard is given in **Table 5**. It can be seen that most of these parameters comply with the limits established by ASTM related to biodiesel quality. The microalgal biodiesel showed much lower cold filter plugging point of –11˚C in compared to than diesel while the viscosity and acid value is higher than diesel.

The **Table 5** shows that the fuel properties of microalgal biodiesel are comparable to diesel fuel.

8. Biodiesel Production from Algal Oil

Out of the four oil modification methods, the most promising method to overcome the problem of high viscosity is transesterification which is a multi step reaction consisting of three reversible steps, where triglycerides are converted to diglycerides, diglycerides to monoglycerides and monoglycerides to esters (biodiesel) and glycerol as by-product.

Transesterification of Microalgal Oil to Biodiesel

Transesterification does not alter the fatty acid composition of the feedstocks and hence the composition of biodiesel. The effect of FFA on biodiesel yield and adoption of suitable transesterification process is reviewed in **Table 6** which indicates that selection of base or acid-base catalyzed process and other conditions is based on the FFA contents of the oil and accordingly the time and other parameters of conversions are selected.

Table 3. Algal harvesting techniques.

S. No.	Algae harvest method	Relative cost	Algal species
1	Foam fractionation	Very high	*Scenedesmus*, *Chlorella*
2	Ozone flocculation	Very high	
3	Centrifugation	Very high	*Scenedesmus*, *Chlorella*
4	Electrofloatation	High	
5	Inorganic chemical flocculation	High	Oxidation ponds
6	Polyelectrolyte flocculation	High	*Dunaliella*
7	Filtration	High	*Spirulina*, *Coelastrum*
8	Microstrainers	Unknown	*Spirulina*
9	Tube settling	Low	*Micractinium*
10	Diecrete sedimentation	Low	*Coelastrum*
11	Phototactic autoconcentration	Very low	*Euglena*, *Dunaliella*
12	Autoflocculation	NA	*Micractinium*
13	Bioflocculation	NA	*Micractinium*
14	Tilapia-enhanced sedimentation	NA	*Scenedesmus*, *Chlorella*

Table 4. Oil from different microalgal species [7].

Type of microalgae	Oil content (% dry wt. basis)
Botryococcus braunii	25 - 75
Chlorella sp.	28 - 32
Crypthecodinium cohnii	20
Cylindrotheca sp.	16 - 37
Dunaliella primolecta	23
Isochrysis sp.	25 - 33
Monallanthus salina	>20
Nannochloris sp.	20 - 35
Nannochloropsis sp.	31 - 68
Neochloris oleoabundans	35 - 54
Nitzschia sp.	45 - 47
Phaeodactylum tricornutum	20 - 30
Schizochytrium sp.	50 - 77
Tetraselmis suecica	15 - 23

Table 5. Comparison of properties of micro algal biodiesel with diesel and ASTM biodiesel standard.

Properties	Biodiesel from microalgae oil	Diesel fuel	ASTM biodiesel standard
Density ($kg \cdot L^{-1}$)	0.864	0.838	0.84 - 0.90
Viscosity ($mm^2 \cdot s^{-1}$, cP at 40˚C)	5.2	1.9 - 4.1	3.5 - 5.0
Flash Point (˚C)	155	60	Min 100
Solidifying point (˚C)	–12	–50 to 10	-
Cold filter plugging point (˚C)	–11	–3.0 (max –6.7)	Summer max 0, Winter max < –15
Acid value ($mg\ KOH \cdot g^{-1}$)	0.374	Max 0.5	Max 0.5
Heating value ($MJ \cdot kg^{-1}$)	41	40 - 45	-
H/C ratio	1.81	1.81	-

Table 6 reviews the different types of transesterification reactions depending on the presence of free fatty acids (FFA) contents in the oil.

Table shows that very little work is available on transesterification of microalgal oil to biodiesel. Depending upon the FFA contents in oil, the base catalysed process is applied to the oil with FFA < 1% while two step processes is applied to high FFA oils as per details reported in our paper [70,71].

It is reported that biodiesel from oils with a high FFA has higher cetane number and energy contents, but lower cloud and pour points and higher viscosity. These result shows that the fatty acid profile of the oil influences the quality of the biodiesel considerably. **Table 7** gives the fatty acid profile of some of the vegetable oil used for biodiesel production. The vegetable oil and their biodiesel with high content of oleic acid are the most suitable biofuel due to their greater stability and better fuel characteristics.

Oxidation stability, an important issue in the biodiesel due to the presence of polyunsaturated compounds, is influenced by factors such as presence of air, heat, traces of metal, peroxides, light, or structural features of the compounds, mainly, the presence of double bonds [74]. Biodiesel produced from oils with high concentrations of saturated fatty acid, has better stability. Therefore, vegetable oils rich in linoleic and linolenic acids such as soybean and sunflower tend to give methyl ester fuels with poor oxidation stability (**Table 7**) whereas nonpolyunsaturated fuels, such as palm and olive methyl ester generally show good stability [75].

9. Status of Biodiesel Production from Microalgal in India

As mentioned above, the microalgae have the highest oil yielding potential which is about 6 - 10 times more than vegetable oil. The algae production can be increased utilizing waste water from domestic and industrial sectors that contain considerable nutrients necessary for its growth. Further, the stability of biodiesel from microalgae is the added advantage of fuel characteristics which persist for longer period of time unlike biodiesel from oil seed crops.

Extensive work has been done by Indian scientists on utilization of microalgae for food and the pharmaceutical applications. The lists of organizations/institutions who are working on various aspects of microalgae such as microalgae collected from natural vegetation which is used for the production of biogas and biofuel in India are given in **Table 8**.

10. Conclusions

The microalgae are considered as one of the most promising feedstocks for future bio-diesel production in India. The advantages of microalgae are their widespread availability, higher oil yields and reduced pressure on cultivable land. The difficulty in efficient biodiesel production from algae lies not only in the extraction of the oil, but also developing an algal strain with a high lipid content and fast growth rate.

Once the microalgal oil starts to be unavailable the application of type of transesterification well established conversion process may be suitably used for converting to biodiesel which has fuel properties similar to petro diesel. Apart from technologies developed for algal cul-

Table 6. Effect of FFA on biodiesel yield and application of transesterification process.

Feedstock	FFA content, %	Transesterification process used	Operating conditions				Yield %	References
			Alcohol to oil ratio	Catalyst to oil (%)	Time (hrs)	Temp. (˚C)		
Rapeseed		Alkali catalyzed	50% excess ethanol with NaOCH3 or 100% excess with KOH	0.5% sodium ethoxide or 1% KOH	2	Room temp.	98	[55]
Sunflower	NA	Alkali catalyzed	3.1	0.28% (w/w) KOH		70	96	[56]
Soybean	NA	Lipase catalyzed			2		94	[57]
Palm	NA	Alkali catalyzed	6.1	1% KOH	1	50	75	[58]
Peanut	2	Alkali catalyzed	6.1		1	65	82	
Jatropha	15	1. Alkali catalyzed	0.70 (w/w)	3.3% w/w NaOH to oil	2	60	88	[59]
		2. two-step process			1	65	55	[60]
		i. Acid pretreatment (FFA reduction to 1%)	i. 0.60 (w/w)	i. 1% (w/w)H_2SO_4	2	i. 50		
		ii. Alkali catalyzed process	ii. 0.24 (w/w)	ii. 1.4%NaOH to oil	1.26	ii. 65	90	
Mahua	17	Two-step process			0.5			
		i. Acid pretreatment (FFA reduction to 1%)	i. 0.32 (v/v)	i. 1.24 (v/v) H2So4	3	i. 60		
		ii. Alkali catalyzed	ii. 0.25 (v/v)	ii. 0.7% (w/v) KOH to oil	6.5 - 8	ii. 60	98	[61]
Pongamia	0.3	Alkali catalyzed	6-10:1	1% KOH	8	60 - 65	92 - 98	[62]
Neem		Alkali catalyzed	6:1 (methanol), 10:1 (ethanol)	0.7% NaOH	24	60 - 75	88 - 94	[63,64]
Cottonseed		Alkali catalyzed	12; 10.135/25 (w/w) methanol	2% (w/w) 0.017 g enzyme/g of oil. 32.5% t-butanol	-	60	90	[65,66]
		Enzyme catalyzed				50	97	
Sesame		Alkali catalyzed	6:1	0.5% NaOH	-	60	74	[67]
Scendesmus sp.		Two step catalyzed	Methanol to oil molar ratio of 12:1	2 % KOH	1 h	65	98	[68]
Hetrotrophic microalgae	8.97	Acid catalyzed	-	H_2SO_4	-	-	90	[69]

Table 7. % FFA in vegetable and micro algal oils [72,73].

S. No.	FFA Contents	Micro-algal (*P. tricornutum*) Oil %	*Jatropha curcas* Oil %	Palm Oil %
1	C16:0	15.5	16.4	42.6
2	C16:1	17.3	1.0	0.3
3	C18:0	0.3	6.2	4.4
4	C18:1	1.3	37.0	40.5
5	C18:2	2.2	39.2	10.1
6	C18:3	0.9	-	0.2
7	C20:0	-	0.2	-
8	C20:1	-	-	-
9	Others	62.5	-	0.7
10	SFA*	21.2	22.8	4.4
11	UFA**	78.8	77.2	94.9

*SFA—Saturated Fatty Acid, **UFA—Unsaturated Fatty Acid.

Table 8. Status of R & D work on microalgae in India.

S. No	Institution/Organization	Work on microalgae specific species	R & D area	Reference
1	University of Madras, Chennai	*Sargassum*	Cultivation	[76]
3	University of Madras, Chennai	*Seaweeds*	Biogas production	[77]
4	University of Madras, Chennai	*Botryococcus braunii*	Cultivation in open raceway pond	[78]
5	Central Food Technological Research Institute (CFTRI), Mysore	*Botryococcus braunii*	Isolation and characterization of hydrocarbon	[79-81]
6	Vivekananda Institute of Algal Technology (VIAT), Chennai	*Microalgae a*	Development of technology to treat industrial waste water	[82-84]
7	Central Rice Research Institute (CRRI), Cuttack, Orissa	*Chlorella vulgaris*	Production	[85]
8	Vivekananda Institute of Algal Technology (VIAT), Chennai	*Micro algae a*	Biofuel production from diatom species	[86]
9	Alternate Hydro Energy Centre, Indian Institute of Technology, Roorkee	*Microalgae*	Conversion of Microalgal oil to biodiesel	[87]

tivation, harvesting and oil extract. The transesterification processes are reviewed. Algal biodiesel stability is relatively better than seed oil based biodiesel. The status of R & D on microalgae biodiesel is also covered in this paper.

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