



Exploration of Indigenous Biodiesel Potential Microalgae from the Wetlands of Muzaffarpur District in the North Bihar

Sanjay Prasad, Research Scholar, University Department of Botany
Babasaheb Bhimrao Ambedkar Bihar University Muzaffarpur, Bihar, India

Abstract: - The current fossil fuel reserves are not sufficient to meet the increasing demand and very soon will become exhausted. Pollution, global warming, and inflated oil prices have led the quest for renewable energy sources. Algal biofuels represent a potential source of renewable energy. Algae, as the third-generation feedstock, are suitable for biodiesel and bioethanol production due to their quick growth, excellent biomass yield, and high lipid and carbohydrate contents. With their huge potential, algae are expected to surpass the first- and second-generation feedstocks. Only a few thousand algal species have been investigated as possible biofuel sources, and none of them was ideal. This review summarizes the current status of algal biofuels, important steps of algal biofuel production, and the major commercial production challenges. My Research Topic is Exploration of Indigenous Biodiesel Potential Microalgae from the Wetlands of Muzaffarpur District in the North Bihar.

Keywords: Biofuels, Microalgae, Renewable Energy, Algal Cultivation, Biofuel Conversion.

LITRODUCTION

The demand for fossil fuels is anticipated to grow 40% from 2015 to 2040. Therefore, to satisfy our energy needs, alternative energy sources have been, and are being, explored. Solar, wind and biomass are the major renewable energy sources. Biomass, derived from a biological precursor, has been used to produce biofuels and bioproducts over the last few decades. Depending on type of biomass, there are first to fourth biofuel generations. Biofuels include biodiesel, bioethanol, biohydrogen, and bio ethers biodimethyl ether, bioethyltetrabutyl ether and bioethyltetrabutyl ether. According to the Department of Energy, India the major biofuels are bioethanol and biodiesel, both of which represent the first generation of biofuel technology. Several biofuel projects have been funded by U.S.A., Australia and European Union. The United States funded projects in Arizona (2008), New Mexico (2009), Massachusetts (2011) and Florida (2013) while the European Union funded four pilot projects; three of which were from 2011 to 2015/16 and the fourth was from 2012 till 2017. Agrofuel is the first biofuel generation that used specific cultivated plants including sugar beet, sugarcane, maize, palm, soybean, and sweet sorghum as feedstocks for production. Agrofuel is produced through yeast fermentation of plant sugars or starch to give bio-ethanol and the extracted plant oils to produce biodiesel. These processes greatly negatively impact both the food and water sectors. Second generation biofuels depended on non-food plants like Jatropha, grass, switchgrass, silver grass and non-edible parts of current crops. In order to reduce land and water utilization and excessive use of harmful pesticides, algal biofuel emerged as the third generation of biofuels with no competition. The different types of algal biofuels are summarized. The fourth biofuel generation is focused on metabolic engineering of the microalgal genome to maximize the biofuel yields or minimize the cost. The leaders in biofuel development and consumption are Brazil, United States, France, Sweden and Germany. Recently, several studies have shown promising results in increasing carbon capture capacity, biomass production, and lipid enhancement in genetically modified microalgae.

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.

Muzaffarpur is the largest city as well as the economic and financial capital of North Bihar. It the headquarters of Muzaffarpur District and Tirhut Division. All the important roadways (like NH 27, NH 28, NH 57, NH 77, NH 102) and railways (like Muzaffarpur-Hajipur, Muzaffarpur-Motihari, Muzaffarpur-Sitamarhi, Muzaffarpur-Samastipur) have their junction in the city. This makes Muzaffarpur-the city with best connectivity in North Bihar.

Review of Literature

Advantage of using microalgae for biodiesel production has been reported by a number of workers. 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. The variation in fatty acid composition of oil from different algae species is reported by several authors. In fact, several studies have reported the use of microalgae for the production of biodiesel and other by products.

Moheimani studied the effect of pH on algal growth in a plate photobioreactor. Kaewpintong found better growth of microalgae in an airlift bioreactor due to better mixing of the microalgal culture. Thomas et al. 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 studied mass production of algae and have been done to develop a photobioreactor for algal culture. Weissman and Goebel studied primary harvesting methods for biofuels production. Samson and Leduy developed a flat reactor equipped with fluorescence lamps for the growth of micro algal oil Further, Ortega and Roux developed an 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.

Classification of Microalgae

Photosynthetic organisms growing in aquatic environments include macroalgae, microalgae and emergent. 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. 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. *Botryococcus braunii*, *Chlorella*, *Dunaliella tertiolecta*, *Gracilaria*, *Pleurochrysis carterae*, *Sargassum*, are some of the microalgal species currently studied for their suitability for biodiesel production.

Table 1. Classification of microalgae

S.N.	Name of Microalgae	Known Species	Known Species	Habitat
01.	Diatoms (<i>Bacillariophyceae</i>)	100,000	Chyrsolaminarin (polymer of carbohydrates) and TAGs	Oceans, fresh and brackish water
02.	Green algae (<i>Chlorophyceae</i>)	8000	Starch and TAGs	Freshwater
03.	Blue-green algae (<i>Cyanophyceae</i>)	2000	Starch and TAGs	Different habitats
04.	Golden algae (<i>Charophyceae</i>)	1000	TAGs and carbohydrates	Freshwater

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:

1. The mechanical press generally requires drying of the algae, which is an energy intensive step.
2. The use of chemical solvents poses safety and health issues.
3. 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.

Table 2. Comparison of microalgae with other biodiesel feedstocks

S.N.	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)
01.	Camelina (<i>Camelina sativa L.</i>)	42	915	10	952
02.	Canola/Rapeseed (<i>Brassica napus L.</i>)	41	974	10	1014
03.	Sunflower (<i>Helianthus annuus L.</i>)	40	1070	9	1113
04.	Caster (<i>Ricinus communis</i>)	48	1307	8	1360
05.	Palm oil (<i>Elaeis guineensis</i>)	36	5366	2	5585
06.	Microalgae (high oil content)	70	136900	0.1	142475
07.	Jatropha (<i>Jatropha curcas L.</i>)	28	741	13	772

Technology for Growing Algae

The following technologies are used for the production of algae:

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 CO₂ 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 CO₂ 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.

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 CO₂ resulting in increased algae growth.

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.

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. 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 are 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.

Table 3. Algal Harvesting Techniques

S.N.	Algae harvest method	Relative cost	Algal species
01.	Filtration	Low	Spirulina, Coelastrum
02.	Microstrainers	Very Low	Spirulina
03.	Tube settling	NA	Micractinium
04.	Diecrete sedimentation	High	Coelastrum
05.	Autoflocculation	Low	Micractinium
06.	Bioflocculation	Low	Micractinium

Table 4. Oil from different microalgal species

S.N.	Type of Microalgae	Oil Content (% dry wt. basis)
01.	<i>Monallanthus salina</i>	>20
02.	<i>Nannochloris sp.</i>	20 – 35
03.	<i>Nannochloropsis sp.</i>	31 – 68
04.	<i>Neochloris oleoabundans</i>	35 – 54
05.	<i>Nitzschia sp.</i>	45 – 47
06.	<i>Phaeodactylum tricornutum</i>	20 – 30

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. 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.

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 in 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. that the fatty acid profile of the oil influences the quality of the biodiesel considerably. 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. 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. whereas no polyunsaturated fuels, such as palm and olive methyl ester generally show good stability.

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.

Conclusions

Algae are attractive sources of feedstock for biofuel production. Biodiesel, biogas, bioethanol, pharmaceuticals, nutraceuticals and other valuable products can be obtained from algae. Biofuels are renewable, biodegradable, and environmentally-friendly. Algae possess many desirable features such as rapid-growth and high lipid content. Chlorophytes (including micro- and macro-algae) represent the biggest group of algae with applications in bioremediation, water treatment, food supply, pharmaceuticals and energy production. In this work, we shed light on different cultivation, harvesting and processing methods. The key challenges appear to be the high infrastructure, operation, and maintenance costs, selection of high lipid containing algal strains, harvesting on a commercial scale and water evaporation issues. Innovative and efficient techniques are necessary to make algal biofuel production preferable. Enhanced biofuel production will help in natural resources conservation and in turn saving the environment.

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