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Review Paper

Microalgal Biodiesel - A Comprehensive Review on the Potential and Alternative Biofuel

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Abstract

Sustainable and renewable energy resources are highly essential to replace the vanishing petroleum fossil fuels. Biofuels play a vital role in mitigating CO_2 emission, reducing global warming and bringing down the hike in oil prices. Biodiesel has become a recent attraction since it is biodegradable, renewable and non toxic. The objective of the paper is to study the potential of microalgae as an alternative raw material for biodiesel generation. Microalga has been chosen as a biodiesel producer due to high mass productivity and faster lipid production. Production of biodiesel from microalgae could be a greater alternative to oil crops due to economical instability, jeopardizing agricultural lands and insufficient oil crops. This review article focusses on the technical improvements in cultivation of different microalgal species, lipid content in various algal species, modes and efficiency of harvesting and transesterification methods. This paper thus serves the researchers to further enhance the production and commercialization of biodiesel.

Keywords: Microalgae, photobioreactor, biodiesel, transesterification, FAMEs.

Introduction

Energy crisis: Burgeoning population and uncontrolled urbanization has created serious problems of energy requirement[1,2]. The world's oil production is expected to decline in between one and ten decades. As a result of this impending energy crisis, both government and private industries are examining alternative sources of energy[3]. Over 1.5 trillion barrels of oil equivalent have been produced since Edwin Drake drilled the world's first oil well in 1859. The world will need that same amount to meet demand in the next 25 years alone. The International Energy Agency (IEA) has reported in the reference scenario that world's primary energy need is projected to grow by 55% between 2005 and 2030, at an average annual rate of 1.8% per year[4]. The petroleum reserves are highly concentrated in certain regions of the world, therefore those countries not having these resources are facing energy/foreign exchange crisis, mainly due to import of crude petroleum[5].

Conventional Methods: The basic sources of this energy are petroleum, natural gas, coal, hydro and nuclear[6,7]. The major disadvantage of using petroleum based fuel is atmospheric pollution. Petroleum diesel combustion is also a major source of green house gas (GHG). Apart from these emissions, petroleum diesel combustion is also a major source of other air contaminants including NO_x, SO_x,COs, particulate matter and volatile organic compounds[7,8], which are adversely affecting the environment and causing air pollution[6].

Biodiesel production: In recent years, research has been directed to explore alternate to diesel fuel, which is produced from oils via transesterification[9]. Biodiesel is a potential substitute for conventional diesel fuel[10,11]. According to the US standard specification for biodiesel (American Society for Testing and materials (ASTM) 6751), biodiesel is defined as a fuel comprised of mono alkyl esters of long chain fatty acids from vegetable oils or animal fats[12]. Biodiesel has shown its ability to meet the energy demand of the world in the transportation, agriculture, commercial, industrial[13] and domestic sectors for the generation of power/ mechanical energy[11]. To distinguish from the biodiesel derived from conventional sources (vegetable oils or animal fats), we use a novel term 'microbio-diesel' to describe fatty acid methyl esters (FAMEs)[13,14,15], transesterified from microorganism oils[12].

Biodiesel from Edible sources: Currently, biodiesel is produced from different crops such as tobacco seed oil[16], Jojoba oil[17], palm oil, soybean oil, canola, rice bran, sunflower, coconut, corn oil, fish oil, chicken fat, algae[18,19], cotton seed, mustard seed, rapeseed, microalgae[20], soybean, pungam seed oil[21], groundnut oil [22],olive, peanut, safflower, beef tallow, lard oil, yellow grease, coconut, fried oils, animal fat[23], linseed[24], tall oil[25], soapnut, rubber, mahua[26]. Biodiesel production from edible oil also has negative environmental impact because it requires much available, arable land[24]. Moreover, current supplies from oil crop and animal fats account for only 0.3% of the current demand for transport fuels[27-32].

Biodiesel from Non-edible sources: Non edible oils like Jatropha, Pongamia[33], Argemone, Castor, Sal, etc., can be used for the production of biodiesel. Jatropha curcas has tremendous potential for biodiesel production[34,35]. However, due to increasing population and industrialization lead to serious land shortages and we cannot use agriculture land already used for food crops.

Biodiesel from waste oil: Several studies have been done on the production of biodiesel from waste oils or animal fats. The feasibility of making quality biodiesel from this feedstock can be described after identifying the problems with free fatty acids present in the raw materials.

Biodiesel from microalgae: Algal biomass is one of the emerging sources of sustainable energy. The US Department of Energy reports that biodiesel produced from algae could see yields greater than oilseed crops [36]. Recent research has proved that oil production from microalgae is clearly superior to that of terrestrial plants such as palm, rapeseed, soybean or jatropha. Microalgae commonly double their biomass within 24hr [6]. Biomass doubling time during exponential growth is commonly as short as 3.5hr[37,38] can double their biomass in mean times that range from 2-5 days, achieving large yields, without the need for the application of pesticides, herbicides or fungicides[39]. Microalgae are capable of synthesizing more oil per acre than the terrestrial plants which are currently used for the fabrication of biofuels and using microalgae to produce biodiesel will not compromise production of food, fodder and other products derived from crops [8] (table 1).

Table- 1
Comparison of some sources of biodiesel

Crop	Oil Yield (L/ha)	Land Area needed (M ha)[a]	Percent of existing US cropping area[a]
Corn	172	1540	846
Soybean	446	594	326
Canola	1190	223	122
Jatropha	1892	140	77
Coconut	2689	99	54
Oil palm	5950	45	24
Microalgae[b]	1,36,900	2	1.1
Microalgae[c]	58,700	4.5	2.5

[a]For meeting 50% of all transport fuel needs of the US; [b]70% oil (by weight) in biomass; [c]30% oil (by weight) in biomasss (Adapted from Chisti Y., 2007)[37]

Significance of Microalgae: Algae are very important from an ecological point of view. Algae are the food sources for many animals and belonging to the bottom of the food chain. Moreover, they are the principal producers of oxygen on earth[40]. Algae are one of the best sources of biodiesel[41]. Algae are the highest yielding feedstock for biodiesel. It can produce upto 250 times the amount of oil per acre as soybeans and 7-31 time greater oil than palm oil. It is very simple to extract oil from algae. The best algae for biodiesel would be microalgae. Microalgae have much more oil than macroalgae and it is much faster and easier to grow and yields high amount of lipids[41, 42] (table 2).

Table-2
Lipid content of different microalgae

Organisms	Lipid Content (% by dry weight)	References
*Ankistrodesmus*TR-87	28-40	[49]
Botryococcusbraunii	25-75	[14, 30, 38, 49]
Chaetoceros muelleri	33.6	[68]
Chlorella emersonii	25-63	[68]
Chlorella vulgaris	14-22	[26]
Chlorella protothecoides	15-55	[30]
Dunaliella tertiolecta	36-42	[52, 65]
Euglinagracilis	14-20	[68]
Monallanthus salina	20	[30]
Nannochloropsis sp.	31-68	[37, 38]
Nannochloris sp.	20-35	[38]
Neochloris oleoabundans	35-54	[30, 37, 69]
Nitzschia sp.	45-47	[38]
Schizochytrium sp.	50–77	[37,38]

Microalgae are prokaryotic or eukaryotic photosynthetic microorganisms having simple structure, which can grow rapidly and live under harsh conditions due to their unicellular or simple multicellular structure[43]. More than 50,000 microalgae species exist in the world, but only 30,000 species have been studied and analysed[44]. During the past decades extensive collections of microalgae have been created by researchers in different countries. An example is the fresh water microalgae collection of university of Coimbra, Portugal, considered as one of the world's largest, having more than 4000 strains and 1000 species. Other algae collection centers are Goettingen University, Germany (SAG) has about 2213 strains and 1273 species, University of Texas algal culture collection has 2300 different strains of fresh water algae. The National Institute for Environmental Studies collection (NIES), in Ibaraki (2150, 700 species), Japan and CSIRO collection of living Microalgae (CCLM), Australia has about 800 strains of different algae[39].

Microalgae, also called 'miniature sunlight-driven'[37], biochemical factories[45] are capable of producing several different types of renewable biofuels and byproducts. These include renewable biofuel[7,10], biohydrogen[37,46-48], hydrocarbon[6,49,50], methane[43], ethanol[43,51], carotenoids and phycocolloids[26], minerals, vitamins, polysaturated fatty acids (PUFAs), α-linolenic, eicosapentanoic and doco succinic acids, belong to ω-3 group[40,52], propylene glycol, acetol, butanol[45], biogas[39,54,55], neutral lipid[56], polar lipid, carbohydrates[57], sterols, tocopherols, carotenoids, terpenes, quinones and phytylated pyrrole derivatives such as the chlorophylls[5], β carotene[58], antioxidants, antibiotics,astaxanthin and pigment[59].

The advantages of culturing microalgae as a source of transportation biodiesel include the following: i. Enhanced efficiencies or reduction in cost. The costs associated with the harvesting and transportations of microalgae are relatively low compared to those of other biomass materials such as trees and crops. In addition, they do not directly affect the human food supply chain, eliminating the food vs. food dispute[24]. ii. Microalgae synthesize and accumulate a larger quantity of neutral lipids/oil (20-50% dry cell weight (DCW)) and grow at high rates (Eg. 1-3 doubling/day)[40]. iii. Microalgae could have significant social and environmental benefits because they do not compete for arable land with food crops and microalgal cultivation consumes less water than other crops[60,61]. iv. Microalgae can be grown in number of environments that are unsuitable for growing other crops, such as fresh, brackish or saline water or non-arable land[24,38,60,62]. v. Microalgal based biofuels do not interfere with food security concern[23]. vi. Microalgae tolerate marginal lands (e.g. desert, arid and semi arid lands) that are not suitable for conventional agricultural[40]. vii. Microalgae utilize nitrogen and phosphorus from variety of waste water sources and providing the additional benefit of domestic and industrial waste water bioremediation[62-66]. viii. Microalgae sequester CO_2 from fuel gases emitted from fossil fuel, fired power plants and other sources, thereby reducing emission of a major GHG. One kg of algal biomass requiring about 1-8kg of CO_2[24,37,40,67]. ix. Microalgae commonly double their biomass within 24hr but exponential growth rate can result in a doubling of their biomass in periods as short as 3.5hr[24]. x. Microalgae can be harvested daily[23]. xi. Microalgae produce value added co-products or byproducts (e.g. proteins, biopolymers, polysaccharides, pigment, animal feed and fertilizer) and does not need herbicide and pesticide[40]. xii. Microalgae lipids are mostly neutral lipids due to their high degree of saturation depending on the strain, synthesizing lipids as a potential diesel fuel substitute[24].

Microalgal Biomass Production

Microalgae cultivation can be done in open culture systems such as lakes or ponds and in highly controlled, closed- culture systems called photobioreactors (PBRs)[43]. The photosynthetic growth of microalgal biomass require light, CO_2, water, organic salts and temperature of 20-30^{0}C[6]. The microalgal biomass can be achieved by different cultivating methods.

Raceway ponds: The pond in which the algae, cultivated are usually those are called the raceway ponds. In this pond, the algae, water and nutrients circulate around a race track. A raceway pond is made of a closed loop recirculation channel that is typically about 0.3m deep and paddlewheel[37]. Figure 1 illustrates the working principle of raceway pond[40] (figure 1).

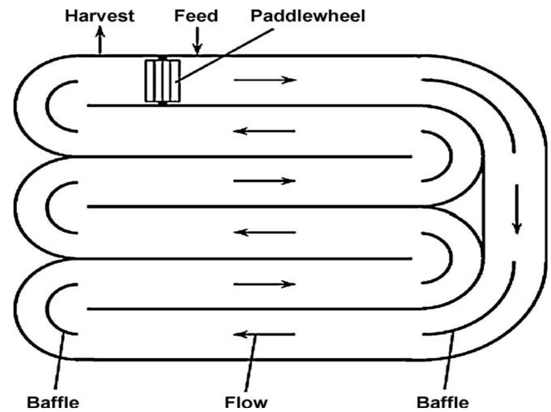


Figure-1
Schematic diagram of raceway pond

There is a paddlewheel used to mix, circulate the algal biomass and prevent the sedimentation. Raceway channel are built in concrete or compacted earth, it can be of different length and diameter and generally lined with white plastic. During daylight, the culture is fed continuously in front of the paddle wheel where the flow begins[36,40]. Open raceway pond system is a cost effective microalgae cultivation method and in commercial raceway ponds, the biomass production is in excess of 0.5g/l [64]. Raceways are perceived to be less expensive than PBRs, because they cost less to build and operate. Economically it is 10 times costly in comparison to PBRs[36]. In addition it adds minimal power consumption[33,66]. The advantage of this method is that readily available domestic municipal waste water can be used as medium for cultivation with the added benefit of bioremediation. If the system is located near a power plant, cheaply available flue gas can be used to speed up the photosynthetic rates in the pond or pure CO_2 can be bubbled into the pond[64,71]. Meanwhile it has some drawbacks that the environment in and around the pond is not completely understood. Another drawback is the uneven light intensity and difficult to maintain the certain temperature and atmospheric evaporation.

Raceway ponds for mass culture of microalgae have been used since the 1950s. The largest raceway based biomass production facility occupies an area of 440,000m[4]. This facility owned by Earthwise Nutritionals (www.earthwise.com), is used to produce cyanobacterial biomass for food[64].

Photobioreactor (PBR): A bioreactor which is used for cultivating algae and purpose to fix CO_2 producing biomass is called an algae bioreactor or an algae photobioreactor. The main advantage of the PBR is that they can produce a large amount of biomass[37,64]. PBRs are relatively safe, offer closed culture environment, safe from contamination and temperature can be controlled[38]. Photobioreactors permits the monoculture growth of microalgae for extended period[64]. Most closed bioreactors are designed as tubular reactors, plate reactors or bubbled column reactors[40]. A plate reactor simply consists of vertically arranged or inclined rectangular boxes which are often divided in two parts to effect an agitation of the reactor fluid. A bubble column photobioreactor consist of vertical arranged cylindrical column made out of transparent material (www.wikipedia.org)[65].

The most common type of closed bioreactor is the tubular photobioreactor[36]. A tubular photobioreactor consists of an array of straight transparent tubes that are usually made of plastic or glass. This tubular array or the solar collector, where the sunlight is captured, is generally 0.1m or less in diameter. Tube diameter is limited because light does not penetrate too deeply in the dense culture broth that is necessary for ensuring a high biomass productivity of the photobioreactor[33]. Microalgal broth is circulated from a reservoir (i.e. degassing column in figure) to the solar collector and back to the reservoir[36,62].

According to Bajhaiya et al[6], a photobioreactor is typically operated as a continuous culture during daylight. In a continuous culture, fresh culture medium is fed at a constant rate and the same quantity of microalgal broth is withdrawn continuously.

Photobioreactors require cooling during daylight hours. The loss of biomass at night due to respiration can be reduced by lowering the temperature at night. Outdoor tubular photobioreactors are effectively and inexpensively cooled using heat exchangers[69]. A heat exchange coil may be located in degassing column which shown in figure 2 figure 2. The biomass recovery from photobioreactor cultured broth costs only a fraction of the recovery cost for broth produced in raceways. This is because the typical biomass concentration that is produced in photobioreactors is nearly 30 times the biomass concentration that is generally obtained in raceways[37].

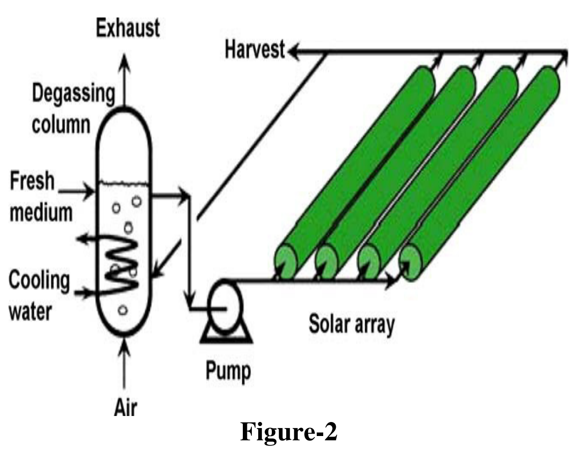


Figure-2
Schematic diagram of tubular photobioreactor with parallel run horizontal tubes

Harvesting Techniques of Microalgal Biomass

Harvesting biomass represents one of the significant cost factors in the production of biomass. Efficient harvesting biomass from cultivation broth is essential for mass production of biodiesel from microalgae. The selection of harvesting techniques is dependent on the properties of microalgae such as density, size, and value of the desired products. The major techniques presently applied to the harvesting of microalgae include centrifugation, flocculation, filtration, screening, gravity sedimentation, immobilization, flotation and electrophoresis[23,56].

Centrifugation: Most microalgae can be recovered from the liquid broth using centrifugation, because it is rapid, efficient and universal[56,64]. A report was given that the laboratory centrifugation tests were conducted on pond effluent at 500-1000g recovered about 80-90% microalgae within 2-5min [56]. Exposure of microalgal cells to high gravitational and shear forces can damage cell structure. In addition this is not economically feasible for large scale harvesting because it is energy intensive and time consuming[64].

Flocculation: Flocculation is the process in which dispersal particles are aggregated together to form large particles for setting. There are two types of flocculation such as autoflocculation, it occurs as a result of precipitation of carbonate salts with algal cells and chemical flocculation in which adding chemicals to microalgae culture to induce flocculation[56]. Flocculation is the first stage in the bulk harvesting process and is used to aggregate the cells, so increasing their effective particle size and thus easing subsequent centrifugation, filtration or sedimentation steps. Alum ($Al_2(SO_4)_3$) in particular has been widely used in the waste water industry for this purpose. The auto flocculation also termed as bio flocculation[62]. Flocculation may be achieved by the use of cationic polymers or the addition of alkali substance to increase the pH[64].

Gravity sedimentation: Gravity sedimentation is a common method of harvesting biomass. The process is rudimentary but works for various types algae and is highly energy efficient [64]. Gravity sedimentation can be effective for separating larger and smaller organisms[23]. Enhanced microalgal harvesting by sedimentation can be achieved through lamella separators and sedimentation tanks[56].

Filtration: Filtration is a method commonly used for solid liquid separation[64]. Vacuum filtration is effective in the recovery of larger algae (greater 70μm), when used with the aid of filters. For small cells, membrane microfiltration and ultra-filtration are alternative methods[62]. Microstrainer and vibrating screen filters are one of the two of the primary screening devices in microalgae harvesting. Microstrainers can be realized as rotating filter with fine mesh screens with frequent backwash. In addition, microstrainers have several advantages such as simplicity in function and construction, easy operation, low investment, energy intensive and having high filtration ratios[56].

Floatation: A further method of harvesting is floatation. Floatation is a gravity separation process in which air or gas bubbles attach to solid particles and then carry them to the liquid surface. Floatation is more beneficial and effective than sedimentation with regard to removing microalgae. Flotation can capture particle with a diameter of less than 500µm by collision between a bubble and a particle and the subsequent adhesion of the bubble and particle[56]. The mechanism of action is interaction with the negatively charged hydrophilic surfaces of algal cells[64]. Ozonation dispersed flotation of cells grown in open pond culture may prove challenging due to contamination. Lipid content of *C. vulgaris*, harvested by ozonation dispersed flotation should increase from 31% to 55% in the flotation stage. An advantage of the use of this ozone is its ability to cause cell lysis. Lysis of cells would release biopolymers which act as coagulating agents thus enabling more effective separation. Algal cell lysis may also serve to enhance the extraction of lipid. A disadvantage of this method is that it is an expensive process.

Electrolytic Method: The electrolytic method is another potential approach to separate algae without the need to add any chemicals. In this method, an electric field drives changed algae to move out of the solution. Water electrolysis generates hydrogen which adheres to the microalgal floc and carries them to the surface. There are several benefits to using electrochemical methods, including environmental compatibility, versatility, energy efficiency, safety, selectivity and effectiveness[56].

Immobilization: Many microorganisms (including some groups of microalgae) have a natural tendency to attach to surfaces and grow on them[57]. This character is used for immobilized cells on immobilizing agents. Immobilization of the microalgal cultures provides a ready-to-retrieve alternative for biomass retrieval [64]. Immobilization is the artificial attachment or encapsulation in alginates or similar substances. Immobilized biomass can be used for biofuel conversion by thermal or fermentative means. For example, immobilization in alginate beads of hydrocarbon-rich microalgae, *Botryococcus braunii, Botryococcus protuberance,* yielded a significant increase in chlorophyll, carotenoids, dry weight and lipids during the stationary and resting growth phases compared to free living cells. In addition, the immobilized cells are more stable than free cells.

Ultrasonic Aggregation: Ultrasonic aggregation is another method for harvesting algal biomass. Ultrasound had beensuccessfully used to optimize the aggregation efficiency and concentration factorand achieved 92% separation efficiency and a concentration factor of 20 times[71].

Extraction of lipids from Microalgal cells

Extraction of microalgal lipid is an important process for the production of biodiesel. Lipid extraction is performed by chemical methods in the form of solvent extractions, physical methods or a combination of the two. Extraction methods used should be fast, effective and non damaging to lipids extracted and easily scaled up[64]. The harvested biomass must be dried because intracellular elements such as oils are difficult to extract from wet biomass[70]. Methods that have been used include sun drying, low pressure, shelf drying, spray drying, drum drying, fluidized bed drying, freeze drying and RefractanceWindow™ technology drying[55,75] and freeze dried cells are preferable for biodiesel production.

Various methods are available for the extraction of algal oil, such as expeller/press, enzymatic extraction, chemical extraction through different organic solvents, ultrasonic extraction and supercritical extraction using carbon dioxide[6,25]. A simple process is to use a press to extract a large percentage (70-75%) of the oils out of algae [25]. In enzymatic extraction, cell wall degrading enzymes are used to release the intracellular protein and oil[6]. The lipids produced by algae are often accumulated intracellularly, which would require extraction of the lipids from crude pastes. Different cell disruption techniques such as autoclaving, osmotic stress, sonication, microwaves and bead beating, high pressure homogenization, addition of hydrochloric acid, sodium hydroxide or alkaline lysis have been evaluated in order to increase lipid extraction efficiency[43,64,75].

In chemical extraction, many methods for algal lipid extraction have been recommended; the most popular is slightly modified method of Bligh and Dyer (1959), Soxhlet method and Folch method[6,23,76]. The solvent extraction was still the main extraction procedure used by many researchers due to its simplicity and relatively in expensive requiring almost no investment for equipment[23]. The choice of solvent for lipid extraction, as with harvesting will depend on the type of the microalgae grown. Other preferred characteristics of the solvents are that, they should be inexpensive, volatile, non toxic and non polar and poor extractors of other cellular components [64]. The most popular chemical for solvent extraction is hexane, which is relatively inexpensive[6].

A further technique, ultrasonic extraction of algal oil involve intense sonication of liquid which generates sound waves that propagate into the liquid medium resulting in alternating high pressure and low pressure cycles. During the high pressure cycle, ultrasonic waves support the diffusion of solvents such as hexane into the cell structure. As ultrasound reaches the cell wall mechanically by the cavitations shear forces, it facilitates the transfer of lipids from the cell into the solvents[6]. The drawback of this method is cost effective for large scale application.

Supercritical carbondioxide ($SCCO_2$) extraction is promising green technology that can potentially displace the use of traditional organic solvents for lipid extractions[76]. Advantages associated with $SCCO_2$ extraction include lineable solvating power, low toxicity of the supercritical fluid, favourable mass transfer equilibrium due to the intermediate diffusion/viscosity properties of the fluid, and the production of solvent free extract. It has large number of advantages like the biomass residues that remains after extraction of the oil could be used as high protein animal feed. The algal biomass residue remains after oil extraction can also be used to produce biomass by anaerobic digestion[6]. In addition the remaining biomass fraction can be used as a protein feed for livestock. This gives further value to the process and reduces waste[25].

Conversion Microalgal oil to Microbiodiesel

Currently three methods are used for production of biodiesel include; thermal liquefication, pyrolysis and transesterification (chemical and enzymes).

Thermal liquefaction: Thermal liquefaction is a process that can be employed to convert wet algal biomass material into liquid fuel[23,75]. Thermal liquefaction is a low temperature (300-350^{0}C), high pressure (5-20MPa) process added by a catalyst in the presence of hydrogen to yield bio-oil. Hydrothermal liquefaction occurs by decomposition of biomass into smaller molecules with high energy by utilizing high water activity in sub-critical conditions. Liquefaction of microalgae has resulted in production of between 30% and 65% dry weight of oil depending on species used[76]. Added advantage of this technology is conversion of wet biomass into useful energy[23,64,72,73], there is no drying process involved. Disadvantages are reactors for thermochemical liquefaction and fuel-feed systems are complex and expensive[64].

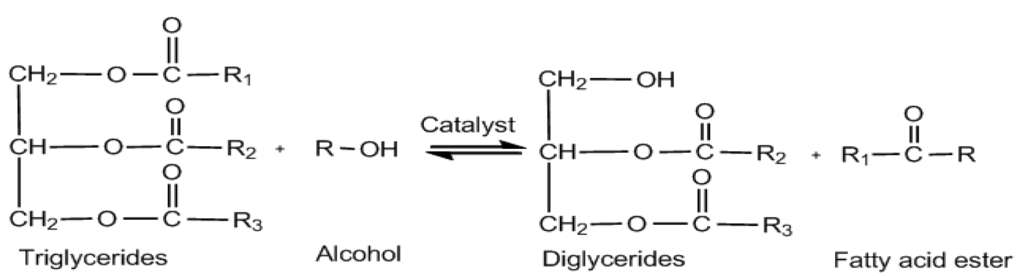
Thermochemical liquefaction of microalgae species like *Botryococcus braunii*, *Dunaniella tertiolecta* and *Spirullina platensis* yielded 64,42 and 30-80% dry weight basis of oil and fuel properties of bio crude oil (30-45.9 MJ/kg)[23]. It indicates that the thermal conversion of biomass to biofuel is an attractive method for liquid fuel production.

Pyrolysis: Pyrolysis is the thermal conversion decomposition of materials in the absence of oxygen or when significantly less oxygen is present than required for complete combustion [23]. Pyrolysis involves chemically reducing triglyceride molecules to fatty acid alkyl esters (FAAEs) through the application of extreme heat[74,13]. Two types of pyrolysis process such as slow pyrolysis and fast pyrolysis. In slow pyrolysis, the biomass is associated with liquid fuels, at low temperature (675-775K) and/or gas, at high temperature[23]. Disadvantage of this process include high equipment for separation of various fractions. Also the product obtained was similar to gasoline containing sulfur which makes it less eco-friendly[5]. The product yields from pyrolysis for an average particle size of 0.8mm[75,76].

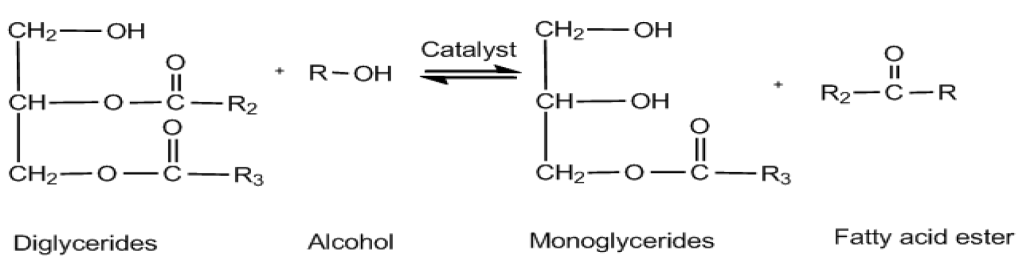
Transesterification: Biodiesel is a mixture of FAAEs obtained by transesterification (ester exchange reaction) of vegetable oil or animal fats[43]. Raw microalgal oil is high in viscosity, thus requiring conversion to lower molecular weight constituents in the form of fatty acid alkyl esters[64]. Transesterification has been demonstrated as the simplest and most efficient route for biodiesel production in large quantities, against less ecofriendly, costly and eventual low yield methods of pyrolysis and microemulsification. Therefore, transesterification has become popular and the production method of choice[13].

Transesterification is a multiple step reaction, including three reversible steps in series, where triglycerides are converted to diglyceride, then diglycerides are converted to monoglycerides, and monoglycerides are then converted to esters (biodiesel) and glycerol (byproduct)[43]. Each conversion steps yields one FAAE molecule, giving a total of three FAAEs per triglyceride molecules. The process describe by following equation[13].

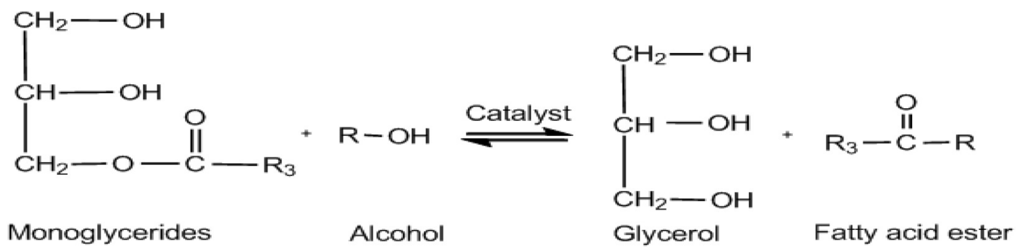
Transesterification stepwise reaction:
1. Conversion of triglycerides to diglycerides:



2. Conversion of diglycerides to monoglycerides:



3. Conversion of monoglycerides to glycerin molecules:



Transesterification is reversible reaction of fat or oil (which is composed of triglyceride) with an alcohol to form fatty acid alkyl esters and glycerol[6]. The stoichiometry ratio of alcohol and oil for the reaction is 3:1[6,64,77]. Among the alcohols that can be used in the transesterification process such as methanol, ethanol, propanol, butanol and amyl alcohol. Methanol and ethanol are frequently used. Methanol is preferred for the commercial development because of its low cost and its physical and chemical advantages (polar and shortest chain alcohol)[5].

The making of biodiesel through transesterification can be done in number of ways such as using an alkali catalyst, acid catalyst, enzyme catalyst, heterogenous catalyst or using alcohol in their supercritical state[6]. Catalysts that take part in the reaction are acids, bases or enzymes[64]. Transesterification reaction can be catalysed both homogenous and heterogenous catalysts. Homogenous catalysts include alkalis and acids.

Acid Catalyst: Acid catalysis is suitable for transesterification of oils containing high levels of fatty acids. The reaction however is slow. Speeding up the acid catalysed reaction requires an increase in temperature and pressure making it prohibitively expensive at large scale[64]. Sulfuric, sulfonic acid and hydrochloric are usually used as catalysts in the acid catalysed reaction.

Alkyl Catalyst: Base catalysis is a faster reaction but is limited by the free fatty acids content. Free fatty acids content in the region of 20-50% is responsible for saponification during the base catalysed transesterification. Saponification is responsible for consumption of the base catalyst as well as making downstream recovery difficult[78]. However, the use of alkali catalyst is 100% in commercial sector[13]. The most commonly used alkali catalysts are NaOH, KOH[63]. Other alkaline catalysts include carbonates, methoxides, sodium ethoxide, sodiumpropoxide and sodium butoxide[13]. An advantage of this method is higher conversion rate in short reaction time.

Enzymatic Catalyst: Enzymes are biological catalysts which allow many chemical reactions to occur within the homeostasis constraints of a living system[13]. Biocatalysts are becoming increasingly important in biodiesel preparation as it is believed that these catalysts will eventually have the ability to outperform chemical catalysts. Biocatalysts are naturally occurring enzymes like lipases which are used to catalyze some reaction such as hydrolysis of glycerol, alcoholysis and acidolysis, but it has been discovered that they can be used as catalyst for transesterification and esterification reactions too[5]. A number of lipases have been studied from microorganisms such as *Achromobacter, Alkaligenes, Arthrobacter, Bacillus, Burkholderia, Chromobacterium* and *Pseudomonas*[79]. Over the last two decades, substantial research has been performed on the use of enzymes in the synthesis of various organics. Enzyme catalyzed transesterification reactions have been extensively used in production of drug intermediates, biosurfactants and designer fats[13].

Sources of lipase: Lipases are found in all living organisms and are broadly classified as intracellular and extracellular and also classified based on its sources such as microorganisms, animal as pancreatic lipases, and plant as papaya latex, oat seed lipase, and castor seed lipase. The selection of a lipase for lipid modification is based on the nature of modification looked for like, position-specific modification of triacylglycerol, fatty acid specific modification, modification by hydrolysis, and modification by synthesis (direct synthesis and transesterification)[5]. Microorganisms, *Candida antarctica*, *Candida rugosa*, *Pseudomonas cepacia*, *Pseudomonas fluorescens*, *Rhizomucor miehei*, *Rhizopus chinensis*, *Rhizopus oryzae* and *Thermomyces lanuginosa* have produced the most effective lipases for transesterification[13].

Immobilized Enzyme Catalyst

Esterification using Immobilized extracellular enzyme: Microbial lipases are mostly intracellular, produced by submerged fermentation or solid state fermentation. The important purification step for producing extracellular lipase is a complex process and it depends on the origin and structure of the lipase. The large scale production of extracellular lipases should be economical, fast, easy and efficient. The majority of immobilized lipases that are commercially available are extracellular. The most commonly used ones are: Novozym 435 which is from *Candida antarctica*, Lipzyme RM IM, lipase produced by *Rhizomucor miehei* and Lipozyme TL IM, from *Thermomyces lanuginosus*[13].

Immobilized Whole cell biocatalyst (Intracellular lipases) mediated esterification: Challenge in generating biodiesel is the cost of enzymes. Hence, uses of whole cells are being implemented for the production. The utilization of lipase while still contained in the cells is referred to as intracellular lipases. This is advantageous for this process do not involve the purification of enzyme which is cost consuming. Direct use of compact cells for intracellular production of lipases or fungal cells immobilized within porous biomass support particles as a whole biocatalyst represents an attractive process for bulk production of biodiesel. Immobilization techniques can be classified under four general techniques: i. adsorption, ii. cross linking, iii. entrapment and iv. encapsulation. Using intracellular lipases as opposed to extracellular lipases slows down the transesterification process but increases the conversion efficiency. Immobilized lipases are advantageous due to its enhanced thermal and chemical stability, easy handling and reusability[80]. Certain microorganisms used as whole cell biocatalysts are: *Candida antarctica*, *Rhizopus chinensis*, *Rhizopus oryzae* and rarely *Saccharomyces cerevisiae.* Both whole cells and extracellular lipases should be immobilized so that they resemble ordinary solid phase catalysts that are conventionally used in chemical reactions[13].

Advantages of using lipase enzyme in biodiesel production: i. Possibility of regeneration and reuse of the immobilized residue, because it can be left in the reactor unaffected. ii. Use of enzymes in reactors allows use of high concentration of them and that makes for a longer activation of the lipases. iii. A bigger thermal stability of the enzyme due to the native state, iv. Immobilization of lipase protects it from the solvent that could be used in the reaction and that will prevent all the enzyme particles getting together v. Separation of product will be easier using this catalyst. vi. No soap formation and have ability to esterifies both FFA's and triglycerides in one step without the need of a washing step, vii. capitulate a higher quality glycerol, viii. ability to handle large variation in raw material quality, ix. a second generation raw materials like waste cooking oils, animal fat and similar waste fractions, with high FFA and water content, can be catalyzed with complete conversion to alkyl esters with significantly condensed amount of wastewater, x. works under milder conditions (which lead to less energy consumption) with lower alcohol to oil ratio than chemical catalysts, xi. ability to work in very different media which include biphasic systems, monophasic system (in the presence of hydrophilic or hydrophobic solvents), xii. they are robust and versatile enzymes that can be produce in bulk because of their extracellular nature in most producing system, xiii. many lipases show considerable activity to catalyze transesterification with long or branched chain alcohols, which can hardly be converted to fatty acid esters in the presence of conventional alkaline catalysts, xiv. products and byproduct separation in downstream process are extremely easier, xv. the immobilization of lipases on a carrier has facilitated the repeated use of enzymes after removal from the reaction mixture and when the lipase is in a packed bed reactor, no separation is necessary after transesterification and xvi. higher thermostability and short-chain alcohol-tolerant capabilities of lipase make it very convenient for use in biodiesel production[13].

Improving Economics of Microbiodiesel: Commercialization of Microbiodiesel still is in challenging process.The estimated cost of producing a kilogram of microalgal biomass is $ 2.95 and $3.80 for photobioreactors and raceways, respectively [37]. If annual biomass production capacity is increased to 10,000 ton, the cost of production per kilogram reduces to roughly $ 0.47 and $ 0.60 for photobioreactors and raceways, respectively, because of economy scale. Assuming that the biomass contains 30 % oil by weight, the cost of biomass for providing a liter of oil would be something like $1.40 and $ 1.81 for photobioreactors and raceways respectively.

Beneficiary aspect of Microbiodiesel

Phycoremediation using algal technology: Phycoremediation may be defined in a broad sense as the use of macroalgae or microalgae for the removal or biotransformation of pollutants, including nutrients and xenobiotics from waste water and CO_2 from waste air with concomitant biomass propagation[73,81]. The term phycoremediation was introduced by John [64]. The release of free oxygen is of major significance in organically enriched waste water, promoting aerobic degradation process by and other microorganisms. Secondly, the role of microalgae is the accumulation and conversion of waste water, nutrients to biomass and lipids[64]. Microalgae are efficient in removing nitrogen, phosphorus and toxic metals from waste water and therefore, have potential to play an important remediation role, particularly, during the final (tertiary) treatment phase of waste water[62].

Global warming-CO_2 sequestration: Our reliance on fossil fuels has caused carbon dioxide (CO_2) enrichment of the atmosphere, and is the primary contributor to generally-accepted phenomenon called global warming. Because using coal produces even greater CO_2 emissions than oil, will be unlikely to improve this pattern of CO_2 enrichment[55]. The use of biodiesel will ultimately leads to reduction of harmful emission of carbon monoxide, hydrocarbons and particulate matter and to the elimination of SOx emission, which can also help in reducing the greenhouse effects and global warming[23,81].

Microalgae waste utilization: Depending on the microalgae species various high-value chemical compounds may be extracted such as pigments, antioxidants, β-carotenes, polysaccharides, triglycerides, fatty acids, vitamins, and biomass, which are largely used as bulk commodities in different industrial sectors (e.g. pharmaceuticals, cosmetics, nutraceuticals, functional foods, and biofuels). Microalgae are viewed as having a protein quality value greater than other vegetable sources, for example, wheat, rice, and legumes. *Dunaliella* sp. (especially *D. salina*) has become popular as foodgrade green microalgae. In particular, due to their lipids and protein contents, glycerol concentration, β-carotene content (up to 4% of dry weight) and their exceptional ability to grow under brackish conditions. These microalgae are currently being cultivated by several companies, in both Israel and Australia, as sources of these compounds and as dietary supplements and powders, containing vitamins A and C[43].

Long-chain polyunsaturated fatty acids (PUFAs), especially of ω-3 and ω-6 series such as eicosapentaenoic (EPA), docosahexaenoic (DHA), and arachidonic (AA) are considered pharmacologically important for dietetics and therapeutics. They have been used for prophylactic and therapeutic treatment of chronic inflammations (e.g. rheumatism, skin diseases, and inflammation of the mucosa of the gastrointestinal tract). Astaxanthin produced from *H. pluvialis* (1.5–3% of dry weight) is high-value carotenoids, being very good at protecting membranous phospholipids and other lipids against peroxidation. The U.S. FDA for marketing has cleared *H. pluvialis* as a dietary supplement and it has been also approved in several European countries for human consumption [43]. Microalgae can also be used for culturing several types of zooplankton (rotifers, cladocerans, brine shrimp or copepods) used as live food in crustacean and finfish farming. *Isochrysis galbana* and *Tetraselmis suecica* are considered as the best food for larval bivalves, growing much better in unfiltered seawater to which these algae have been added[43].

Conclusion

In the recent scenario there is a considerable increase in the need of domestic energy consumption, thus a rapid depletion of fossil fuel and hike in the price. To overcome the deterioration of petroleum- derived fuels, biodiesel, a third generation biofuel could an alternative energy source. Biodiesel productions are generally aimed from crops, plants, but found limited in availability of those substrates within the stipulated period and cultivable lands. Microalgae, thus can be an effective feedstock as it is readily available, possess comparatively a faster growth rate, require negligible nutrients, fresh water and most essentially, they contain higher lipid content than the plant feedstock. Algal biomass, to generate biodiesel, could be grown in photobioreactors that provide a controlled environment but needs a good study of construction that reduces its cost. Use of manmade open pond systems and natural water bodies might be cost effective and render a larger area for cultivation. Effluent tanks are good choices, for microalgae grown in them can generate biofuel and treat waste water. CO_2 emitted from the production from the production plants can be flowed into these tanks, aiding the growth, thus reducing the carbon dioxide emission and environmental pollution. Sewage lines yet another attractive option, could let microalgae, yield biofuel along with the removal of nitrogen, phosphorus and take up ammonia from them. The residual biomass procured after process could be used as an animal feed and for producing algal products. Researchers are yet carried out in elevating the biomass productivity by genetic and metabolic engineering, thereby increase the production of biodiesel. Another challenge in the generation of biofuels is how to lower the cost of production. Thus microalgal biodiesel, not only is an alternative and cheap energy source but also impart its significance as an ecofriendly green fuel.

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