

## Biodiesel from microalgae as a promising strategy for renewable bioenergy production - A review

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REVIEW ARTICLE

### ABSTRACT

Fossil fuels have provided the primary source of energy to humanity for centuries. However, the emerging concerns related to fossil fuel depletion, associated price rise and environmental pollution have increased the interest and demand for bioenergy through biomass. Microalgae are emerging as a potential source of renewable energy that can be converted into biofuel. Microalgae have the ability to mitigate carbon dioxide (CO<sub>2</sub>) emissions and produce oil with a higher productivity that could be potentially used as fuel in engines. However, the issues associated with the energy and costs involved in algal biofuel technologies act as a barrier in its large-scale industrial applications. This review presents a brief overview of the strategies for cultivation to increase the biomass productivity along with the related bioprocesses involved in oil extraction and thereby conversion of the extracted oil/lipids to suitable fuel for engine applications. The purpose of this work is to aid in the development of efficient and commercially viable technology for microalgae based biodiesel production.

### KEYWORDS

Algae; Biodiesel; Bioenergy; Biomass Productivity; Transesterification

## 1. INTRODUCTION

Today about 80% of the global primary energy is produced from fossil fuels. However, extensive utilization of fossil fuels has led to global climate change, environmental pollution, and health problems (Hallenbeck and Benemann, 2002; Mohr et al., 2015). Instability in the geographical regions from which the fossil fuels are sourced and high oil price threatens the independence and security of different nations especially in the developing countries. The threat of being held to ransom is unacceptable and hence the frantic search for more stable alternative sources of energy is on the horizon (Achara, 2012; Mansson et al., 2014). Due to the above mentioned economic and environmental jeopardies, most of the countries have diverted their central attention towards the development of new, clean, and sustainable energy

sources. Among the various available potential sources of renewable energy, biofuels are one of the promising alternatives for creating a stable and carbon neutral economy (Smith et al., 2010). At this critical juncture, the third generation biofuels from microalgal biomass has emerged as the green and economically viable sustainable energy option due to the presence of significant amount of lipids (upto 60%), ease of cultivation, faster growth rate and higher photosynthetic efficiency than other terrestrial plants (Chisti, 2007; Sharma and Singh, 2017). In spite of the projected benefits, the bottlenecks associated with the biomass productivity at the field scale, along with the problems associated with recovery and extraction of lipids and thereby their conversion to biodiesel limits their application.

The production of fuel from photosynthetic organisms is a renewable process as these organisms act as solar cell factories converting solar energy into useful

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Received: 15-09-2017  
Revised: 23-09-2017  
Accepted: 28-09-2017  
Available online: 29-09-2017

chemical energy stored as biomass. Therefore, biomass is a highly promising resource. The major attraction of using microalgal oil for biodiesel is the tremendous oil production capacity by microalgae, as they could produce up to 58,700 L oil per hectare, which is 1 - 2 magnitudes higher than that of any other energy crops (Chisti, 2007; Singh et al., 2011). Microalgae contain lipids and fatty acids as membrane components, storage products and metabolites (Bhatnagar et al., 2011). Microalgal oil can be produced through either biological conversion of the stored lipids/hydrocarbons or via thermochemical liquefaction of algal biomass. Besides the capability of producing energy or oil, algae can also be used as a source for the production of nutraceuticals, pharmaceuticals, biofertilizers and bioplastics (Chisti, 2007). This paper is an attempt to outline the different biochemical processes or methods thereof, for increasing the biomass productivity and lipid accumulation thereby converting microalgae to a liquid fuel like biodiesel. Hence, this mini-review is directed towards the critical examination of the literature on the cultivation and oil extraction from microalgae for bioenergy production.

## 2. MICROALGAE CULTIVATION SYSTEMS

Algae are simple phytoplankton with diverse habitat that could range from the tiny microalgae to large macroalgal seaweeds such as giant kelp more than one hundred feet in length (Chisti, 2007). Algae can be grown under a wide variety of agroclimatic conditions using wide array of water resources such as brackish, sea, and low quality industrial process water and effluents of biological wastewater treatment that is unsuitable for cultivating agricultural crops. Thereby utilising the flue gases from industries results in sequestering and capturing of CO<sub>2</sub> (Milano et al., 2016). Apart from acting as a source of bioenergy, microalgae can also play a major role in marine ecosystems, providing aquatic food chains to support all fisheries in the oceans and islands, as well as contributes about 70-80% of oxygen in the atmosphere along with other marine plants (Badger et al., 2000).

Microalgae are regarded to have an extraordinary potential for cultivation as energy crops (Bhatnagar et al., 2011; Hamed, 2016). They contain significant quantities of lipids, carbohydrates as well as proteins. The biochemical content of microalgae varies with the strain and cultivation conditions (Mehrabi et al., 2015). The chemical composition of the selected algae

on a dry matter basis is given in Table 1. The proportions of energy precursors like lipids and carbohydrates can be increased by subjecting the microalgae to nutrient stress as suggested by several studies (Karemore et al., 2013; Chu et al., 2014). Alteration in the mode of nutrient uptake and operational parameters like pH, temperature and light availability also affects the ratio of the above mentioned components (Sutherland et al., 2015). Thus, bioprocess control and management for maintaining appropriate operating conditions is an essential strategy for improvising the quantity and quality of microalgal biofuels. Several predictive models have been proposed which have evaluated the influence of climatic and spatiotemporal variables on microalgal growth potential. Aly and Balasubramanian (2016; 2017) have evaluated the effect of photo inhibition and geographical coordinates on microalgal growth and carbon dioxide sequestration potential in hypothetical open ponds through mathematical modeling. Aly et al. (2017) further modeled the effect of photo inhibition on microalgal production potential in fixed and traceable photo bioreactors for the entire state of Odisha, India by discretizing the study area to 1195 locations by taking local atmospheric conditions into consideration.

Following isolation and characterization of appropriate strain, selection of optimized cultivation conditions for achieving desirable biomass and lipid productivity is an essential area of concern. The cultivation systems for producing algae are numerous and the complexity ranges from shallow unmixed open ponds to precisely controlled photo bioreactors (Mata et al., 2010; Kumar et al., 2015). Growth rate and lipid productivity of microalgae are significantly influenced by the mode of nutrient uptake and cultivation mode operational in the reactor. The different cultivation modes commonly used has been explained in subsequent sections.

### 2.1. Phototrophic cultivation

Most microalgae grow under phototrophic mode, using light, such as sunlight as the energy source and inorganic carbon (e.g., CO<sub>2</sub>) from the atmosphere as the sole carbon source to form the chemical energy through photosynthesis (Huang et al., 2010). It is the most commonly and naturally used cultivation condition for microalgal growth as the solar energy and CO<sub>2</sub> is converted into biomass without supplementing the media with additional carbon source (Gouveia et al., 2009; Gouveia and Oliveira, 2009; Singh and Dhar, 2011). Normally a nutrient-limiting condition,

**Table 1.** Chemical composition of some algae on a dry matter basis (%) (based on Brown, 1991; Becker, 1994; Demirbas, 2009).

Scientific name of algae	Proteins	Carbohydrates	Lipids	Nucleic acids
<i>Anabaena cylindrical</i>	50 to 56	10 to 17	12 to 14	3 to 6
<i>Aphanizomenon flos-aquae</i>	47	NA	1.9	NA
<i>Chaetoceros calcitrans</i>	8 to 18	21 to 52	16 to 40	NA
<i>Chaetoceros gracilis</i>	48	17	21	NA
<i>Chlamydomonas reinhardtii</i>	51 to 58	12 to 17	14 to 22	4 to 5
<i>Chlorella protothecoides (autotrophic)</i>	54.64	10.62	14.67	NA
<i>Chlorella protothecoides (heterotrophic)</i>	10.28	15.43	55.2	NA
<i>Chlorella pyrenoidosa</i>	57	26	2	NA
<i>Chlorella vulgaris</i>	6 to 20	33 to 64	11 to 21	NA
<i>Chroomonas salina</i>	49	4	8	NA
<i>Dunaliella bioculata</i>	57	32	6	NA
<i>Dunaliella salina</i>	39 to 61	14 to 18	14 to 20	NA
<i>Dunaliella tertiolecta</i>	28 to 45	25 to 33	22 to 38	NA
<i>Euglena gracilis</i>	52	15	3	NA
<i>Isochrysis galbana</i>	28 to 39	40 to 57	9 to 14	NA
<i>Isochrysis aff. Galbana (T-iso)</i>	46 to 63	8 to 14	4 to 9	2 to 5
<i>Nannochloris atomus</i>	60 to 71	13 to 16	6 to 7	3 to 4.5
<i>Nannochloropsis oculata</i>	63	15	11	5
<i>Nitzschia closterium</i>	43 to 56	25 to 30	4 to 7	NA
<i>Pavlova lutheri</i>	62	23	3	NA
<i>Pavlova salina</i>	34	6	16	NA
<i>Phaeodactylum tricornutum</i>	12	4.7	7.2	NA
<i>Porphyridium cruentum</i>	26	9.8	13	NA
<i>Prymnesium parvum</i>	30	8.4	14	NA
<i>Scenedesmus dimorphus</i>	25	4.6	10	NA
<i>Scenedesmus obliquus</i>	34	8.8	19	NA
<i>Scenedesmus quadricauda</i>	20	12.2	15	NA
<i>Skeletonema costatum</i>	30	23	21	NA
<i>Spirogyra</i> sp.	29	9.1	12	NA
<i>Spirulina maxima</i>	35	7.8	18	NA
<i>Spirulina platensis</i>	31	12.1	17	NA
<i>Synechococcus</i> sp.	31	12	10	NA
<i>Tetraselmis chui</i>	29	12.9	23	NA
<i>Tetraselmis maculata</i>	23	6	20	NA
<i>Tetraselmis suecica</i>	29	9	12	NA
<i>Thalassiosira pseudonana</i>	26	7.4	12	NA

i.e., nitrogen or phosphorous deficient condition has been used by several researchers to increase the lipid content in microalgae (Mata et al., 2010; Liang et al., 2013; Marcilhac et al., 2015). As a result, achieving higher lipid content is usually at the expense of lower

biomass productivity. However, lipid content is not the sole factor determining the oil-producing ability of microalgae. Instead, both lipid content and biomass production need to be considered simultaneously. The highest lipid productivity reported in the literature is

about 179 mg/L/d by *Chlorella* sp. under phototrophic cultivation using 2% of CO<sub>2</sub> with 0.25 vvm aeration (Chiu et al., 2008). The major advantage of using autotrophic cultivation to produce microalgal oil is the consumption of CO<sub>2</sub> as a carbon source for the cell growth and oil production thus making the process energetically and economically sustainable. It could also serve as an added strategy towards CO<sub>2</sub> sequestration (Mohan et al., 2015). Moreover, compared to other types of cultivation, the contamination problem is less severe when using autotrophic growth (Huang et al., 2010). However, low biomass and lipid productivity are a grave concern when the reactors are operated under photoautotrophic condition due to mutual shading of cells that leads to light attenuation (Liang et al., 2009).

## 2.2. Heterotrophic cultivation

In heterotrophic kind of cultivation, the algae use organic carbon under dark conditions for growth, just like bacteria (Chojnacka and Marquez, 2004; Cheirsilp and Torpee, 2012). This type of cultivation could avoid the problems associated with limited light that hinders high cell density in large-scale photobioreactors during phototrophic cultivation (Huang et al., 2010). Devi et al. (2012) had proposed that growing microalgae for wastewater treatment under heterotrophic mode can significantly increase the accumulation of polyunsaturated fatty acids (PUFAs) compared to phototrophic mode. For heterotrophic cultivation of microalgae, bioreactors are constructed in the form of large tanks by increasing the media volume along with increasing the concentration of substrate wherever feasible. Thus, the production of microalgae can be greatly enhanced by increasing the volume of the bioreactor rather than the surface area of the bioreactor in case of open ponds (Kumar et al., 2015).

## 2.3. Mixotrophic cultivation

Mixotrophic mode of cultivation in microalgae utilizes both organic and inorganic carbon sources as well as light energy to undergo photosynthesis. During the growth of microalgae, the process of respiration and photosynthesis co-occurs and could sustain under either phototrophic or heterotrophic conditions or both. Microalgae assimilate organic compounds and CO<sub>2</sub> as a carbon source, and the CO<sub>2</sub> released by microalgae via respiration is trapped and reused under phototrophic cultivation (Mata et al., 2010). Under mixotrophic conditions, the biomass and lipid productivity is increased with much shorter growth cycle (Adesanya

et al., 2014). Researchers have projected that there is an increase of 1.8-5.4 folds in biomass productivity and 2.4 - 5.2 folds in lipid productivity when reactors are operated under mixotrophic mode rather than phototrophic or heterotrophic mode (Ratha et al., 2013; Li et al., 2014). Wang et al. (2016) have reported an increase in lipid productivity of 23.9% with the increase in C/N ratio under mixotrophic mode.

## 2.4. Photoheterotrophic cultivation

Photoheterotrophic mode of cultivation uses light as the source of energy along with organic carbon compounds which are biochemically transformed into energy that is stored in the biomass. Hence, photoheterotrophic cultivation needs both sugars and light at the same time (Chojnacka and Marquez, 2004). Photoheterotrophic mode of cultivation provides better control over the growth regimes compared to photoautotrophic mode (Brennan and Owende, 2010). It reduces the lag phase witnessed in heterotrophic mode (Hena et al., 2015). Recently, photoautotrophic conditions were mostly used to produce some light-regulated metabolites and rarely for biofuel production. Very recently, Benavente-Valdes et al. (2017) have reported that a two-stage photoheterotrophic-photoautotrophic photobioreactor system produced 48% higher biomass productivity compared to only photoheterotrophic mode.

# 3. EXTRACTION OF OIL

Lipids and fatty acids in microalgae (generally expressed as dry cell weight [DCW] or in percentage) occur as membrane components, storage products, metabolites and as a form of stored energy (Mehrabadi et al., 2015). Their proportion varies by the culture conditions (Chisti, 2007). The algal oil contains both saturated and monounsaturated fatty acids. With the advancement of technology, many methods are available to extract oil from algae, yet, all processes can be broadly categorized into 2 main types such as mechanical and chemical methods:

- Mechanical extraction method include the use expeller press or bead beating and ultrasonic-assisted or microwave assisted extraction that consumes huge amount of energy and is suitable for dry algal biomass (Kumar et al., 2015).
- Chemical methods of extraction comprise extraction using organic solvents like hexane via soxhlet apparatus and supercritical fluid extraction which uses CO<sub>2</sub> at low pressure (72.9 bar) and

temperature (31 °C) (Santana et al., 2012). However, the problem of using chemical solvent is that they are cost intensive and environmentally toxic (Taher et al., 2014). Supercritical fluid extraction process has low flammability, requires higher energy and is unfeasible at large scale. The main advantage of this method is the possibility of recycling the solvent several times.

- Chemical extraction methods supplemented with mechanical methods like microwave or ultrasonic assisted oil extraction has been found to increase the extraction efficiency by 8 folds (Kumar et al., 2105).

The new non-conventional methods of lipid extraction include the following approaches:

- Isotonic based extraction methods use ionic solvents of specific polarity, hydrophobicity and conductivity to disrupt the algal cell wall. It is environmentally non-toxic but is economically

unfeasible at industrial scale (Pan et al., 2016).

- Osmotic pressure method creates a pressure variation between the interior and exterior of the cell thus rupturing the algal cell wall exposing lipid (Kumar et al., 2015).
- Enzymatic extraction method uses enzymes like cellulase, trypsin, etc. that hydrolyzes the polysaccharides present in the recalcitrant algal cell wall, thus releasing lipids. However, the costs associated with the enzymes limits their use (Mishra et al., 2017).

Novel extraction methods which are under extensive current research like the use of mesoporous nanoparticles to extract fuel along with the use of green solvents like free nitrous acids which induces oxidative stress is expected to improvise the algal biofuel industries in near future (Zhang et al., 2013; Bai et al., 2016).

**Table 2.** Different applications of algal oil as source of energy via different routes

Methods	Advantages	Disadvantages	Practical problems	References
Direct use as fuel	Liquid nature and portability	Higher viscosity, contain free fatty acids, phospholipids, sterols, water, odorants and other impurities	Choking and soot formation	Tsaousis et al., 2014
Blending with diesel fuel	Higher heat content (80% of diesel fuel); readily available; Increase in cetane value	Lower volatility; reactivity of unsaturated hydrocarbon chains; lower CO and NO <sub>x</sub> emissions	Decrease in torque and power; carbon deposits; oil ring sticking; thickening and gelling of the lubricating oil	Tuccar and Aydin, 2013
Micro-emulsions: Colloidal equilibrium dispersion of optically isotropic fluid microstructures (1-150 nm range) formed spontaneously from two immiscible liquids and amphiphiles	Better spray patterns during combustion; lower fuel viscosities	Lower cetane number; lower energy content; lower NO <sub>x</sub> and particulate matter	Irregular injector needle sticking; incomplete combustion; heavy carbon deposits; increase lubrication oil viscosity	Reham et al., 2015; Xu et al., 2016
Thermal cracking (pyrolysis): The conversion of long-chain saturated hydrocarbons to biodiesel by heating	Similar to petroleum derived product	Energy intensive and hence higher cost	Low oxidative stability	Winayanuwattikun et al., 2008; Yang et al., 2015
Transesterification: The reaction of a fat or oil with an alcohol in the presence of a catalyst to form esters and glycerol	Renewability; higher cetane number; lower emissions; higher combustion efficiency	Waste disposal	Reduction in torque and brake thermal efficiency	Goodrum et al., 2003; Daroch et al., 2013

## 4. APPLICATIONS OF MICROALGAL OIL AS A SOURCE OF ENERGY VIA DIFFERENT ROUTES

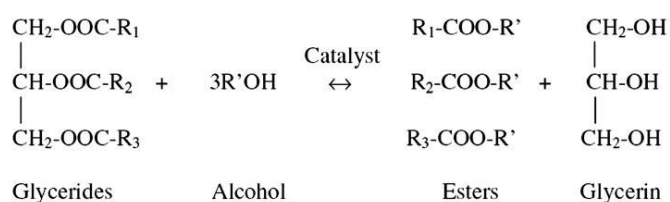
Oleaginous microalgae have the capacity to produce 5000 - 100000 L of oil/ha/day (Mehrabadi et al., 2015). With significant advancement in lipid extraction methodologies, the ability of oil extracted has been improved, and this has diversified the application arena of algal oil in engines through several strategies. The various routes along with their merits and demerits have been enlisted in Table 2.

### 4.1. Direct use of algal oil for engine applications

Algal oil after extraction containing a significant amount of lipids about 60% can be directly used as a fuel in the combustion engine. The use of raw algal oil is expected to reduce the costs involved with the use of expensive chemicals for further processing the algal oil to fatty acid methyl esters (FAME). Tsaousis et al. (2014) have reported that the use of raw algal oil resulted in lower engine power output and NO<sub>x</sub> emissions but higher brake specific fuel consumption, particulate matter and CO<sub>2</sub> emissions. Since the viscosity of oil is high, with low cetane number and lower amount of polyunsaturated fatty acids as compared to the algal biodiesel produced via transesterification, the engine performance is significantly compromised. The algal oil is often blended in different proportions with conventional diesel in order to improve the performance of combustion engine. Blending reduces the viscosity of the algal oil and increases the level of unsaturation in the unprocessed algal oil. It results in improvement in torque and power output of engine compared to the use of only algal oil as fuel (Velapan et al., 2015). Another process for decreasing the level of saturation in algal oil involves thermal cracking/pyrolysis that converts the saturated hydrocarbons into PUFAs at 400-600 °C for 30-120 min, under ambient pressure (Yanik et al., 2013). Pyrolysis oil with a significantly higher degree of unsaturation though improves the engine performance but reduces the exhaust emissions. However, the low oxidative stability of the oil is a matter of concern in the course of their long-term use (Mehrabadi et al., 2015). Several strategies like hydrogenation, hydroxygenation, catalytic cracking and co-pyrolysis can be used to reduce the oxygen content making the oil more stable as engine fuel (Yang et al., 2015).

### 4.2. Use of microemulsion of algal oil

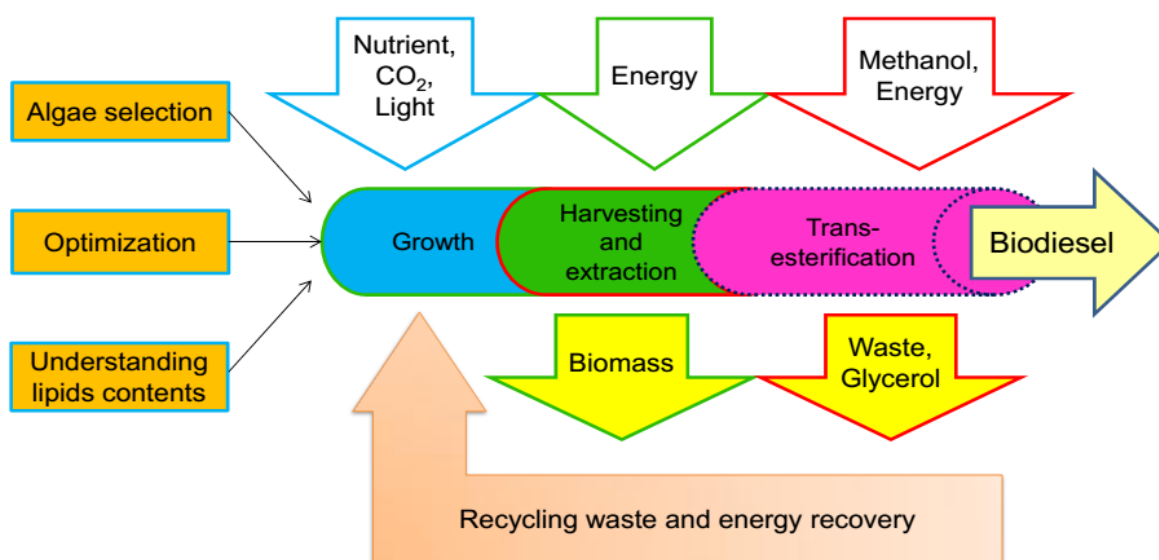
The use of water and algal oil or biofuel emulsion along with the surfactants or additives like organic enzymes reduces the interfacial tension between water and oil droplets forming a stable emulsion (Reham et al., 2015; Xu et al., 2016). The algal oil/water emulsion can be used in diesel engines with comparatively lower knocking during combustion thus improving the engine performance in terms of brake thermal efficiency, power output with comparatively lower emissions. Recently, Karthikeyan and Prathima (2017) used an emulsion of algal oil with nano-additives (doped with TiO<sub>2</sub> and SiO<sub>2</sub> nano-composites) that resulted in improvement in fuel properties, performance and a significant reduction in exhaust gas emissions.



**Figure 1.** Transesterification reaction. R<sub>1</sub>, R<sub>2</sub> and R<sub>3</sub> are long hydrocarbon chains, where R' is an alkyl group.

### 4.3. Transesterification of algal oil to biodiesel

Transesterification (or) alcoholysis involves displacement of alcohol from an ester producing a triacylglycerol using a homogenous or heterogenous catalyst (Daroch et al., 2013). It reduces the viscosity of triglycerides producing fatty acid alkyl esters as the main product and glycerol as a by-product in the presence of a catalyst as shown in Figure 1. The technologies of the biodiesel production from vegetable oils can be applied to the biodiesel production of microalgal oils because of the similar physical and chemical properties. The overall stages involved in the life cycle of biodiesel production from microalgae are depicted in Figure 2. In the process of transesterification, alcohols are the key substrates. The commonly used alcohols include methanol, ethanol, propanol, butanol, and amyl alcohol. Out of these, methanol is applied more widely because of its low-cost and physical advantages. Conventional transesterification process uses an acid/alkali mediated reaction which due to the lower reaction rate and energy requirements results in increasing the overall cost of the process as well as greenhouse gas (GHG) emissions (Singh and Olsen, 2011). Enzymatic transesterification, on the other hand, uses an enzyme like lipase that catalyses the conversion of lipids in



**Figure 2.** Overall bioprocesses involved in the production of biodiesel from microalgae.

**Table 3.** Comparison of the chemical and enzymatic method for production of biodiesel via transesterification (source: Gog et al., 2012).

Parameter	Chemical method		Enzymatic method
	Acid process	Alkaline process	
Biodiesel yield	>90%	>96%	>96%
Free Fatty acid content in the substrate	Converted to biodiesel	Soap formation	Converted to biodiesel
Water in the substrate	Interference with reaction	Interference with reaction	No influence
Purification of methyl esters	Repeated washing	Repeated washing	None
Glycerol recovery	Complex, low grade glycerol	Complex, low grade glycerol	Easy, high grade glycerol
Reaction rate	Slow	High	Low
Reaction temperature	>100 °C	60-80 °C	20-50 °C
Catalyst recovery	Difficult, the catalyst ends up in the by-products	Difficult	Easy
Catalyst reuse	No reusability	Partially lost in post-processing steps	Reusable
Cost of catalyst	Low	Low	High
Wastewater generation	High	High	Low

algal oil to unsaturated fatty acids at moderately lower temperature. The process efficiency is higher with a significant reduction in downstream processing costs (Wang et al., 2014). However, the addition of short chain alcohols like methanol in the transesterification reaction mixture at moderate concentrations leads to inactivation of the enzyme and decreasing the yield of ester. Therefore, stepwise addition of methanol was

suggested as a solution (Mendes et al., 2012). Table 3 compares the chemical and enzymatic methods for production of biodiesel. Likewise, several other strategies have been adopted in recent times to enhance the productivity of biodiesel through transesterification processes as discussed by Daroch et al. (2013) and Park et al. (2015).

## 5. CONCLUSIONS

The energy yield from algae is more than terrestrial plants with no food versus fuel dilemma as in case of other energy crops drive the drift for sustainable energy production. The microalgal cultures need only nutrients, CO<sub>2</sub> and light which are available easily in nature, thus reducing the production costs. Hence, biofuel from microalgae is a great and greenest opportunity for partial replacement of fossil fuels, and it is highly indispensable for the prevention of environmental degradation with their potential for CO<sub>2</sub> fixation. The quantity of oil in microalgae varies, and some algae have oil concentration more than 60% of dry cell weight. Several methods are available for extraction of oil from algae (mechanical, chemical or combination thereof) and for using it in the combustion engine as a fuel following extraction. The biodiesel product has its key features quite similar to those of conventional diesel and/or compatible with conventional petroleum diesel, and it can also be blended in any proportion with petroleum diesel. More research should be directed towards overcoming the bottlenecks related to process energy and economics concerning cultivation, oil extraction and processing of crude algal oil. The emerging approaches are expected to delimit the challenges related to algal industry thereby resulting in scale-up of the production at mass levels making algal based biodiesel as a commercial reality in near future.

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