Recent Trends in Biodiesel Production from Microalgae

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Abstract: Petroleum is a non-renewable source of fuel. Its combustion produces Carbon, Nitrogen and Sulfur oxides which is a major cause of acid rain and green house effect resulting into global warming. Focusing on these problems a renewable source of biodiesel production is an important requirement as an alternative source of petroleum. Recent research has been focused on biofuel as an alternative source of petroleum fuel because bio-fuel is environment friendly and economical source of fuel. People have extensively studied biodiesel production from food and vegetable oil by transesterification reaction. But these sources of biodiesel production can't be used due to the scarcity and drought situation in India. Microalgae are rich source of fatty acids so they are the best source for biodiesel production. In this review, we have discussed the current status of different system of algae cultivation and different methods for biodiesel production.

Keywords: Petroleum, global warming, biofuel, microalgae, fatty acids, cultivation, transesterification and biodiesel.

1. Introduction

Energy is a global economy for development. Petroleum is the primary source of fuel energy, which is a non-renewable source of fuel and probable to be exhausted in near future. Petroleum consists of hydrocarbon and some other compounds (nitrogen, sulfur and aromatic compounds). The main product of combustion of petroleum fuel is carbon dioxide and other by products such as nitrogen dioxide, nitrogen oxide, carbon monoxide, sulfur dioxide. Carbon dioxide absorbs heat and increases the atmospheric temperature thus contributes to greenhouse effect, which is the ultimate cause of global warming. Sulfur dioxide reacts with water in atmosphere and form sulfuric acid which is resulting to acidic rain (Chmielewski, 1999; Innocent et al., 2013; Dowell et al., 2017). To concern about the depletion of petroleum source and their adverse effects on environment, we need to find out a renewable biodiesel source as an alternative of petroleum fuel.

In recent years, biodiesel has been focused for a new source of fuel production from microalgae. Biodiesel production from microalgae is a renewable source of fuel and is a good alternative for petroleum fuel (Oliveira and Silva, 2013; Blinova et al., 2015). Biodiesel contains fatty acid esters which combustion doesn't produce sulfur and other aromatics compounds like petroleum fuel, thus biodiesel is environment friendly as well as economically reasonable source of fuel. Initially, biodiesel was produced by transesterification reaction from vegetable oil or animal fat (Belarbi et al., 2000; Okoye et al., 2016; Kianimanesh et al., 2017). But these sources of biodiesel production cannot be used for the purpose due to the scarcity and drought situation in India (Shalini Rajvanshi and Mahendra Pal Sharma, 2012).

Microalgae are the good renewable source and is extensively using for biodiesel production in industries. Because, microalgae are rich in fatty acids thus they are the best source for biodiesel production (Oliveira and Silva, 2013; Lenka Blinova et al., 2015). Microalgae have a diverse range of habitat as well. They can survive in diverse environment such as sea water, fresh water and saline water (Rindi, 2007; Lee et al., 2014). Algae can grow in non potable and saline water sources that are not suitable for cooking, drinking and crop production, thus it is beneficial to use waste water for produce biodiesel. Most of the algal species are photoautotroph. Photoautotrophic algae convert solar energy into chemical energy through photosynthesis. They utilize inorganic carbon (such as carbon dioxide) for their food source thus helps in reduction of atmospheric carbon dioxide. Whereas, some algal species are photoheterotroph, they utilize organic carbon (such as sugar) as their food source (Liang et al., 2009; Tse-ShihLin and Jane-YiiWu, 2014).

1.1 Current status of biodiesel production from microalgae

In order to concern about the current situation of global warming, exhaustion of non- renewable energy source for biofuel production and increased air pollution due to the combustion of biofuel; extensive research has been going on both internationally as well as nationally in the area of biofuel production.

Biodiesel production is much explored at the international level as compared to India. Internationally various researcher groups are working on production of biofuel from microalgae (Al-lwayzy et al. 2014; Chen et al. 2018; El-Shimi et al. 2013; Hong-Seop 2017; Hu et al. 2008; Li et al. 2008; Liam Brennan and Philip Owende, 2010; Park et al. 2011; Xu et al. 2006; Halim et al. 2011; Koberg et al. 2011; Milano et al. 2016; Johnson and Wen 2009; Patil et al. 2008; Saddam and Yusaf, 2013; Schenk et al, 2008 Zheng et al. 2012) in large scale to produce biodiesel production and to fulfill the increased demand of sustainable renewable energy source.

Biodiesel production from microalgae is not much explored in India. Biodiesel production is an urgent requirement in India due to the limiting source of fuel. Different national organizations/institutes (Institute of Minerals and Materials Technology (IMMT), Bhubaneswar; Indian Institute of Chemical Technology, Hyderabad; Center for Jatropha Promotion and Biodiesel, Churu, Rajasthan; Ipcowala Santram Institute of Biotechnology & Emerging Sciences (ISIBES), Dharmaj-Anand, Gujarat; University of Madras, Chennai; Central Food Technological Research Institute (CFTRI), Mysore; Vivekananda Institute of Algal Technology (VIAT), Chennai; Synthetic Biology & biofuel Group (ICGEB, New Delhi) are currently working on microalgae cultivation and biodiesel production.

1.2 Microalgae using for biodiesel production:

Several microalgae are using to produce biodiesel such as Botryococcus braunii, Chlorella sps, Dunaliella, Spirogyra, Chlorococcum, Crypthecodinium cohnii, Cylindrotheca sps, Isochrysis sps, Nitzschia sps, Schizochytrium sps, Nannochloropsis sps and Nannochloris etc (Liandong Zhu, 2015). Microalgae are the best source of biodiesel production because, they are rich in lipids [such as palmitic acid (C16:0), oleic acid (C18:1), linoleic acid (C18:2) and linolenic acids (C18:3)], grow rapidly, do not compete for food, easy cultivation, tendency to adapt in

adverse conditions (Hannon et al., 2010; Sharma et al., 2012; Tse-ShihLin and Jane-YiiWu, 2014). Several studies have shown the lipid content of different algal species, such as Botryococcus braunii (25– 80%), Chlorella (18–57%), *Scenedesmum sps* (16-55 %) and *Spirogyra sp. (11-16 %)*, *Dunaliella sps* (6-25 %) and *Chlorococcum* (19-20 %) Euglena gracilis (14-20 %), Spirulina (4-16 %) (Chisti 2007; Khan et al., 2009; Mata et al., 2010; Wu et al., 2012; Han et al., 2014; Zhang et al., 2014). Different species have differential composition of lipid, biodiesel production highly depends on fatty acid content, length and branching of fatty acid chain and unsaturation of fatty acids (Sheehan J ; et al., 1998; Li Y et al., 2008; Muhammad Aminul Islam et al., 2013).

Algae convert atmospheric CO2 into glucose and use the glucose to form triglycerides. These triglycerides can be converted into fatty acid methyl esters (FAMEs) by transesterification reaction, which is the main component of biodiesel (Hossain et al., 2008, Tse-ShihLin and Jane-YiiWu, 2014; Jeon et al., 2017).

2. Process of biodiesel production from microalgae:

Multiple steps are involved in biodiesel production; Selection of microalgae, microalgae cultivation, harvesting of microalgae, extraction of microalgal content, biodiesel production from extract and further purification (Bligh and Dyer, 1959; Halim et al., 2011 ; Kale A, 2012; Chen et al., 2012; Gami et al., 2014; Chen et al., 2014).

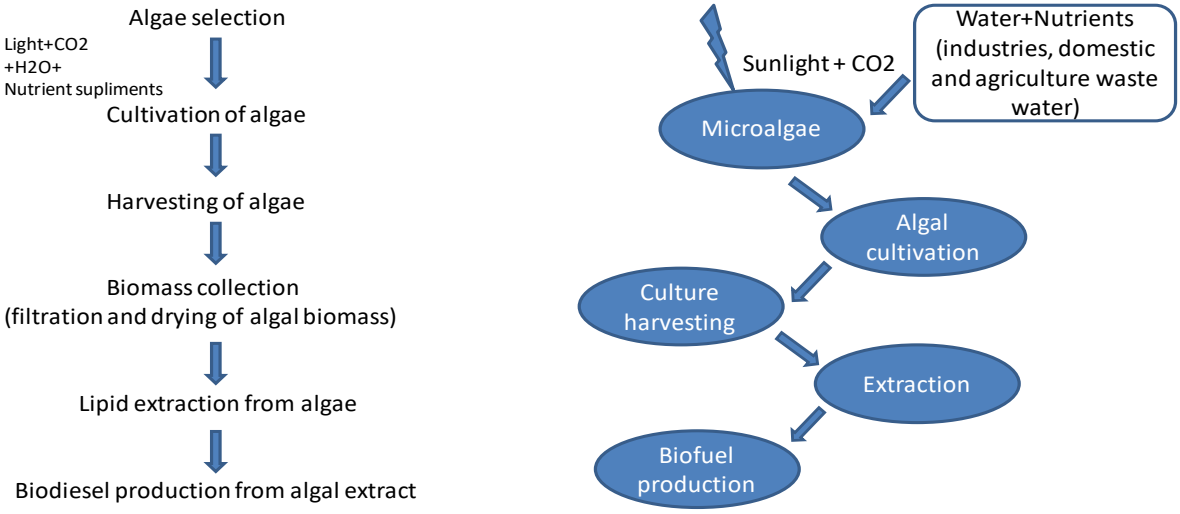


Figure 1: Represent flow chart of biodiesel production from algae.

2.1 Selection and cultivation of Microalgae:

Several parameters should keep in mind during algae selection such as water content, optimum salinity, nutrient requirement, optimum temperature, CO2 requirement and climate conditions (Maxwell EL et al., 1985). For the selection of algae several strategies should be considered (Muhammad Aminul Islam et al., 2013**)**-

- High biomass in natural condition with minimal requirement of nutrients.
- Lipid content must be high.
- Resistant to adverse climate conditions.
- Easy in biomass collection.

2.1.1 Microalgae culture condition

Stock culture is developed from isolated algae colony by incubating into 10 ml (A1 medium) into 50 ml flask (Table -1). For large scale culture, 15 % v/v of stock culture further inoculated in to 300 ml (A1 medium) into 500 ml Erlenmeyer flask, same culture is used as seed culture. For sterilization always autoclaved medium (autoclaved at 121 o C, 15 psi for 30 min) is used, pH 8-9, temperature is maintained at 30°C and rotation speed is kept at 125 rpm. Flasks are kept at 12 h light/dark period of photoperiod. The culture is continuously aerated with CO2 supply (Gami et al., 2014).

Ingradients	A1 medium
K2HPO4 (g/l)	1.0
KH2PO4 (g/l)	1.0
FeSO4 (mg/l)	5.0
Thiamine HCl (µg/l)	10.0
NH4 Cl (g/l)/ Urea (g/l)	0.1
Glucose (g/l)	12

Table1: Represent ingredients for algae culture media.

2.1.2 Systems for Algae cultivation:

Algae can be cultivated in both open system as well as closed system. Open pond cultivation is cheaper, low coast, easy maintenance and handling compared to closed chamber or closed photo bioreactors. Open pond could be of two types; Natural open ponds and artificial open ponds. Artificial open ponds are maintained by paddle wheel, rotating arm and pumps for the proper agitation. Open ponds are cost effective but not effective for higher biomass production due to the lack in sustainability of light and dark cycle. Along with that contamination by other algal species is also a major drawback of open ponds; these contaminations may affect biomass production (P.M. Schenk et al., 2008; I. Rawat et al., 2011; A. Piasecka et al., 2014; L. Zhu, 2015). For the higher biomass production, open ponds should be covered to prevent the contamination and water loss. Open ponds are made maximum with 30 cm depth to reach proper sunlight that is suitable for maximum biomass production (G. Dragone et al., 2010).

On other hand photobioreactors are the close chambered reactors, they catalyze photobiological reaction in the presence of sun light. These bioreactors are used to produce large biomass in a controlled manner (Mata et al., 2010; Chisti Y, 2007). Bioreactores are costly as compared to open pond system but they are beneficial for bulk biomass production, required small land area for microalgae culturing and help to prevent contamination with other microalgae, thus preferred for culturing the monospecies of algae (L. Brennan and P. Owende, 2010; A. Demirba and M.F. Demirbas, 2011). Based on their shape, photobioreactors are of different types (P.M. Schenk et al., 2008).

2.1.2.a Tubular photobioreactor:

Tubular bioreactors are made up of glass or transparent plastic tubes with only 0.1 m in diameter. These tubes may be arranged in horizontal or vertical manner. Transparency and very thin tubes are preferred to enhance the accessibility of sunlight for photobiologcal reaction (L. Brennan and P. Owende, 2010). Tubular photoreactors are arranged in a fence like manner to increase the surface area for maximum growth of microalgae. These fence like glass tubes are arranged towards north and south direction to avoid the direct penetration of sunlight (P.M. Schenk et al., 2008). These transparent tubes are connected with a reservoir and a degassing chamber. Reservoir are used to circulate culture medium into glass tubes and then returned back. Whereas, degassing chamber is used to remove oxygen produced during photosynthesis (M. Hannon et al., 2010).

2.1.2.b Flate plate photobioreactor:

Flate plate photobioreactors are made up of very thin, transparent material arranged in flate plates. Thickning and the flate plate structure are very critical for the maximum reach of sunlight and to increase the surface area (Y.K. Lee, 2001). Flate plate photoreactors are highly preferred due to higher photosynthesis and low oxygen dissolution during cultivation (L. Brennan and P. Owende, 2010).

2.1.2.c Column photobioreactor:

Column bioreactors are arranged vertically in which gases are passes from bottom to top through column. The gas bubbles helps to circulate the culture within reactor. Column photobioreactors are highly efficient for mass production of microalgae. Column photobioreactors are easy in handling as well as highly used for biomass production at high controlled conditions, but these reactors are more costly (J. Merchuk et al., 2007; N. Chavada, 2012).

2.1.2.d Hybrid system: Hybrid system is a combination of both open pond and close system for biomass production. Open pond are good to produce high biomass but more prone to contamination by other microalgal species, where as close system is specific for the growth of one species to produce biomass but highly expensive technique. To keep in hybrid system was developed to produce the high biomass from single species culture in a low cost effective manner but require a trained operator. In this system closed photobioreactors are used prior to avoid contamination by other algal species. Then the large inoculum is transferred into the open ponds and allows to producing large biomass (P.M. Schenk et al., 2008; V.O. Adesanya et al., 2014).

2.2 Methods of algal cultivation:

There are three types of culturing methods are using now a days; Batch culture, continuous culture and semi continuous culture (R.A. Andersen, 2005; H.C. Lim and H.S. Shin, 2013).

2.2.1 Batch cultivation: Batch culture is carried out in a close photoreactor system. In this method, temperature, Oxygen and pH remained constant to avoid their effects on biomass production. So, batch cultivation is performed under controlled conditions. In batch culture the materials such as microalgae, nutrients media and water are provided only once at the initial of the process of biomass production but the nutrients are consuming up continuously during the process which is the limitation of batch culture of biomass production from microalgae.

2.2.2 Continuous cultivation: Like batch culture continuous culture is also carried out in close photoreactors. In continuous culture the nutrient medium added after a particular time interval for the constant growth of microalgae. Continuous culturing is effective for biomass production but the culture may get contaminated so this methods is not generally preferred for commercial purpose (P.A. Hoskisson and G. Hobbs, 2005; T. Egli, 2015).

2.2.3 Semi-continuous cultivation: Semi-continuous cultivation of microalgae is more preferred compared to batch or continuous cultivation. In this system culture is removed after reaching to saturation phase and again refill in a large inoculums of previous culture. The higher amount of inoculum ensures the single species culture for biomass production but there the chances of contamination by other microalgae. This culturing system is preferable for growing multiple cycles to produce large biomass of microalgae (T. Egli, 2015; C.D.C. Reichert et al., 2006).

2.2.4 Harvesting of microalgae: Several harvesting techniques are available for algal biomass; chemical-coagulation and flocculation, sedimentation, centrifugation, electro-coagulation, flotation and filtration (C.Y. Chen et al., 2011; S.O. Gultom and B. Hu, 2013). The harvesting methods are selected on the basis of density and size of microalgae since, algae are highly rich in water content so after collecting, biomass is preceded for thickening to reduce the water content. The thickening of slurry of biomass is done by filtration and centrifugation techniques. (C.Y. Chen et al., 2011).

2.2.4.a Chemical coagulation and flocculation: In this process some chemicals are used to aggregate the algal biomass which will cause flocculation. If the biomass is high, the algal cells could flocculate themselves by cellular interactions. Algae cells are negatively charged so they repels to each other to maintain the cell suspension. The coagulants could be organic or inorganic such as ferric, aluminum or chitosan. Cationic coagulants are used to aggregate the algal biomass such as ferric or aluminum, whereas the anionic coagulants are not used due to the repulsion of negatively charged algae and coagulants. A cationic coagulant interacts and neutralizes the charge of algae cell which leads to the aggregation of algal cells (C.Y. Chen et al., 2011; D. Vandamme, 2013).

2.2.4.b Sedimentation: Sedimentation process is very common to separate algal species from culture media. The rate of sedimentation is depends on the density and the size of algal species. Algae with higher density and large cellular size sediment first compared to low density and small size algal cells. Sedimentation method is used to enhance the harvesting (J. Hanotu et al., 2002; C.Y. Chen et al., 2011).

2.2.4.c Centrifugation: The separation of algae based on the centrifugal force generated by centrifuge. Centrifuge is very effective method for algae harvesting. By centrifugation approximately 95% of biomass can be isolated but it cannot be used on large scale and it is a high cost process (G. Shelef et al., 1984; T.M. Mata et al., 2010).

2.2.4.d Electrocoagulation: Electrocoagulation is costly process as compared to chemical coagulation of algae. In electrocoagulation electrode has to be sacrificed during the coagulation of algae. In electrocoagulation, microalgae migrate and coagulate on anode which neutralize the negative charge of algae which promote the coagulation (E. Poelman et al., 1997; G. Azarian et al,. 2007)

2.2.4.e Flotation: Flotation is process without the use of any chemical microalgae adheres with each other with the help of air bubbles. Depends on the bubbles size, flotation has been classified into two classes; dissolved air flotation and dispersed air flotation. In dissolved air flotation small size of air bubbles are generated and the flotation of algae depends on the air solubility in water. Some flocculants and can be used to enhanced the flotation on the surface of water. Whereas in dispersed air flotation there is continuous air bubbles are generated (J.H. Shah et al., 2014; J. Hanotu et al., 2012).

2.2.4.f Filtration: Filtration is used to isolate large sizes microalgae through filtration membranes. These membranes are replaced on regular basis so it is high cost process. The efficiency of filtration depends on the type of membrane, different types of algaeand the size of algae (T.M. Mata et al., 2010; J.K. Pittman et al., 2011; M. Al Hattab et al., 2015).

2.3 Lipid extraction from algae biomass

To proceed for lipid extraction, algal biomass should be dry and it should not contain high water content. For drying the algae biomass several methods could be used; use of direct sunlight, use heat produced by fossil fuel or natural gas (M.K. Lam, K.T. Lee, 2012). After collection and drying of algal biomass, it would be processed for lipid extraction. First of all the plasma membrane and cell wall should be ruptured for effective lipid extraction. Cells are ruptured by homogenizer, sonication and freeze and though treatment. The lipids are extracted from sonicated/ homogenized algae slurry by some solvents and these solvents should be non toxic, non volatile and low cost (I. Rawat et al., 2011; D. Ozçimen et al., 2012; Y. Wang, 2013). The modified Bligh and Dyer method or by Hexane-Isopropanol Extraction method using Soxhlet Apparatus is very effective for lipid extraction from algal biomass (Bligh and Dyer, 1959; Halim et al., 2011). The collected algae biomasses grind with mortar and pestle. Grinded algae dried at 80°C for 20 minutes in oven. 10 g of dried algae is incubated with 30 ml of chloroform and methanol at 1:2 ratio for 1 hour at 65°C. After 1 hour, mixture is centrifuged at 3000 rpm for 5 minutes. This extraction process carried out three times. The supernatant is separated and mixed with 10 ml chloroform and 20 ml of 1% sodium chloride solution to make the final concentration of 1:1:1 (chloroform/methanol/water). Then, the mixture allowed to settle down and transfer to the vial carefully and washed with 20 ml of 0.5 % NaCl solution then dried at 60°C. The total content of lipid is calculated as percentage of dry weight of algae (Gami et al., 2014). Fatty acid content of microalgae extract is identified using TLC.

There are several other methods are also available for the lipid extraction.

2.3.1 Enzymatic extraction: Enzymes are used in enzymatic extraction from algal biomass such as cellulases, hemicellulase and mannanase. This method could be useful for wet algal biomass also. In a report, it has been shown that upto 90 % of lipid extracted using pretreatment of cellulose and mannanase at 53°C and pH 4.4 (Y. Wang, 2013; K. Liang et al., 2012; A. Zuorro et al., 2016).

2.3.2 Chemical press extraction: This method is simpler than enzymatic extraction method. It is used to extract approximately 95 % of lipid content. This technique required enzyme for cell wall degradation but not required high temperature like enzymatic extraction method. Lipids are extracted by applying mechanical pressure on algal biomass mixed with non toxic or non volatile low cost enzymes (Y. Wang, 2013; A. Piasecka et al., 2014).

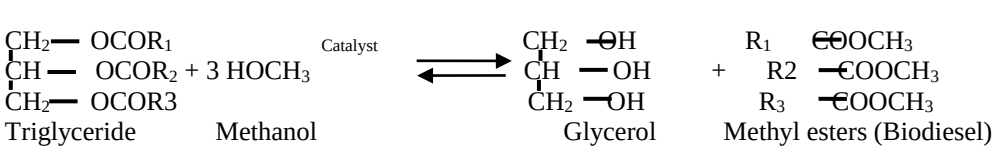
2.3.3 Critical extraction method: This method required high temperature and pressure (50 °C at 22 to 25 MPa) to rupture algal cell wall for lipid extraction. Liquefied Co2 is used as solvent and heated to reach critical stage for lipid extraction (G. Dragone et al., 2010; A. Demirbas, M.F. Demirbas, 2011; A. Santana et al., 2012).

2.4 Biodiesel production from algal extract:

Lipid content extracted from algal biomass further proceeds for the biodiesel production. Transesterification is the major process of biodiesel production. Apart from transesterification other methods are also available; biodiesel production by chemical catalysis and lipase catalysis.

2.4.1 Transesterification by alcohols:

Transesterification reaction is the most common procedure for biodiesel production. In transesterification, chloroform and methanol are used for the conversion of lipids into fatty acid methyl esters (biodiesel). The amount of production of biodiesel is dependent on the lipid content of different microalgae. In transesterification, algae extract is incubated with 1% phosphoric acid at 85°C for 1 hour at stirred condition to remove the non lipid impurities.



The algae extract is incubated with methanol (1:20) in the presence of catalyst (0.5 g KOH or NaOH per 10 ml of methanol) for 2-3 hours at 60°C, 300 rpm. After 2-3 hours of shaking, the mixture centrifuged at 6000 rpm to separate the product. After separation it is washed with pure water until it gets clear and pass through dehydrator to obtained pure biodiesel (Hossain et al., 2008; Chen et al., 2012; Chen et al., 2014).

2.4.2 Transesterification by alkali or base catalysis:

Acid or base catalysis required higher temperature as well as longer reaction time. Removal of alkaline catalyst needs to be removed after completion to reaction. To address these problems, several modifications has been made such as using different types of catalyst, fast heating using microwave and ultrasonic treatment (Alsalme et al., 2008; Cao et al., 2008; Refaat and El Sheltawy, 2008; Kalva et al., 2008).

2.4.3 Transesterification by lipase catalysis:

Lipases are considered as an biocatalysis for transesterification reaction with mild conditions. This method is not much used due to their high cost and short life time. But now a day's people are using immobilization technique, which enhance the stability and increase life time of enzymes (Xiong and Wu, 2008).

References:

1. A. Santanaa a*, S. Jesusa , M. A. Larrayozb , R. M. Filhoa (2012). Supercritical carbon dioxide extraction of algal lipids for the biodiesel production. Procedia Engineering 42, 1755 – 1761.
2. A.B.M. Sharif Hossain, Aishah Salleh, Amru Nasrulhaq Boyce, Partha chowdhury and Mohd Naqiuddin (2008). Biodiesel Fuel Production from Algae as Renewable Energy American Journal of Biochemistry and Biotechnology 4 (3):250-254.
3. Agata Piasecka*, Izabela Krzemińska, and Jerzy Tys (2014). Physical methods of microalgal biomass pretreatment. Int. Agrophys., 2014, 28, 341-348.
4. Al hattab M, Ghaly A, Hammoud A (2015) Microalgae Harvesting Methods for Industrial Production of Biodiesel: Critical Review and Comparative Analysis. J Fundam Renewable Energy Appl 5:154.
5. Alsalme, A., Kozhevnikova, E.F., Kozhevnikov, I.V. (2008). Heteropoly acids as catalysts for liquid-phase esterification and transesterification. Appl. Catal. A Gen. 349 (1-2), 170–176.
6. Andersen, R.A., Ed. (2005) Algal Culturing Techniques. Elsevier Academic Press, New York, 578 p.
7. Aniket Kale (2012). METHODS OF PRODUCING BOFUELS FROMANAL.GAL BOMASS. United States Patent, US 8,137,556 B2.
8. AntonioZuorro[a]SeleniaMiglietta[b]GiuseppeFamiliari[b]RobertoLavecchia (2016). Enhanced lipid recovery from *Nannochloropsis* microalgae by treatment with optimized cell wall degrading enzyme mixtures. Bioresource Technology, Volume 212, Pages 35-41.
9. Belarbi EH, Molina Grima E, Chisti Y (2000). A process for high yield and scaleable recovery of high purity eicosapentaenoic acid esters from microalgae and fish oil. Enzyme Microb. Technol. 26: 516-529.
10. Bharat Gamia*, J. P. Patelb, I.L.Kotharia Cultivation of Chlorella protothecoides under autotrophic and heterotrophic conditions for biofuel production (2014). Algal Biomass Utln. 5 (2): 20 – 29.
11. Bligh and Dyer (1959). A rapid method of total lipid extraction and purification1, the national research council of canada; 37(8): 911-917.
12. C. C. Reichert[I]; C. O. Reinehr[II]; J. A. V. Costa (2006). Semicontinuous cultivation of the cyanobacterium *Spirulina platensis* in a closed photobioreactor. Braz. J. Chem. Eng. vol.23.
13. Cao, F., Chen, Y., Zhai, F., Li, J., Wang, J., Wang, X., et al., 2008. Biodiesel production from high acid value waste frying oil catalyzed by superacid heteropolyacid. Biotechnol. Bioeng. 101 (1), 93–100.
14. Chavada, Nilesh (2012). OPTIMIZATION OF VERTICAL PHOTOBIOREACTORS. University of Dayton.
15. Chen CY[1], Yeh KL, Aisyah R, Lee DJ, Chang JS. (2011). Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: a critical review. Bioresour Technol. 2011 Jan;102(1):71-81
16. Chen X, Huang C, Liu T. 2012. Harvesting of microalgae Scenedesmus sp.using polyvinylidene fluoride microfiltration membrane. Desalin Wat Treat;45:177–81.
17. Chen, X., Hu, G., and Liu, L. (2018). Hacking an algal transcription factor for lipid biosynthesis. *Trends Plant Sci.* 23, 181–184. doi: 10.1016/j.tplants.2017.12.008.
18. Chisti, Y. Biodiesel from microalgae. Biotechnol. Adv. 2007, 25, 294–306.
19. Chmielewski, A.G. (1999). Environmental effects of fossil fuel combustion (INCT--4/B/99). Poland.
20. D. Özçimen, M. Ö. Gülyurt, and B. İnan,Algal Biorefinery for Biodiesel ProductionIn:Biodiesel - Feedstocks, Production and Applications, Prof. Zhen Fang Ed. Intech, 2012.
21. Demirbas A, Fatih Demirbas M. Importance of algae oil as a source of biodiesel. Energy Conversion and Management 2011;52:163–70.
22. Demshemino S. Innocent,O'Donnell P. Sylvester, Muhammad F. Yahaya,IsiomaNwadike, and Linus N. Okoro (2013). Comparative analysis of biodiesel and petroleum diesel. International Journal of Education and Research Vol. 1 No. 8.
23. Dowell, N.M., Fennell, P.S., Shah, N., Maitland, G.C., 2017. The role of CO2 capture and utilization in mitigating climate change. Nat. Clim. Change 7, 243–249.
24. Fabio Rindi (2007). Diversity, distribution and ecology of green algae and cyanobacteria in urban habitats. Algae and Cyanobacteria in Extreme Environments, 619–638.
25. G. Shelef, A. Sukenik, M. Green (1984). Shelef, G., Sukenik, A., and Green, M.. *Microalgae harvesting and processing: a literature review*. United States.
26. GH Azarian 1, AR Mesdaghinia 1,*F Vaezi 2, R Nabizadeh 2, D Nematollahi (2007). Algae Removal by Electro-coagulation Process, Application for Treatment of the Effluent from an Industrial Wastewater Treatment Plant. Iranian J Publ Health, Vol. 36, No.4, pp.57-64.
27. Giuliano Dragone ⇑ , Bruno D. Fernandes, Ana P. Abreu, António A. Vicente, José A. Teixeira (2010). Nutrient limitation as a strategy for increasing starch accumulation in microalgae. Applied Energy 88 (2011) 3331–3335.
28. GuanyiChen, LiuZhao, YunQi (2014). Enhancing the productivity of microalgae cultivated in wastewater toward biofuel production: A critical review. Applied Energy
29. H. I. El-Shimi1 , Nahed K. Attia2*, S. T. El-Sheltawy1 , G. I. El-Diwani2 (2013). Biodiesel Production from Spirulina-Platensis Microalgae by In-Situ Transesterification Process. Journal of Sustainable Bioenergy Systems, 2013, 3, 224-233.

30. Halim R[1], Gladman B, Danquah MK, Webley PA (2011). Oil extraction from microalgae for biodiesel production. Bioresour Technol. 102(1):178-85.
31. Hamid Reza Kianimanesh1 , Farzin Abbaspour-Aghdam1*, Mehrab Valizadeh Derakhshan (2017). Biodiesel production from vegetable oil: Process design, evaluation and optimization. Polish Journal of Chemical Technology, 19, 3, Pages 49—5.
32. Hong-Seop Jeon, So Eun Park, Bohye Ahn, and Young-Kee Kim (2017) Enhancement of Biodiesel Production in Chlorella vulgaris Cultivation using Silica Nanoparticles Biotechnology and Bioprocess Engineering 22: 136-141.
33. Hu Q[1], Sommerfeld M, Jarvis E, Ghirardi M, Posewitz M, Seibert M, Darzins A (2008). Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. Plant J. 2008 May;54(4):621-39.
34. I. Rawat, R. Ranjith Kumar, T. Mutanda, F. Bux (2011). Dual role of microalgae: Phycoremediation of domestic wastewater and biomass production for sustainable biofuels production. Applied Energy 88, 3411–3424.
35. J. C. Merchuk,* F. Garcia-Camacho, and E. Molina-Grima (2007). Photobioreactor Design and Fluid Dynamics. Chem. Biochem. Eng. Q. 21 (4) 345–355.
36. J.B.K.Park, R.J.Craggs[a]A.N.Shilton[b] (2011). J.B.K. Park, R.J. Craggs, A.N. ShiltonWastewater treatment high rate algal ponds for biofuel production. Bioresource Technology, 102 (1) (2011), pp. 35-42.
37. James Hanotu, H.C. Hemaka Bandulasena, William B. Zimmerman (2012). Microflotation performance for algal separation. Biotechnology and Bioengineering, Wiley Online Library.
38. Kalpesh K. Sharma, Holger Schuhmann and Peer M. Schenk (2010). High Lipid Induction in Microalgae for Biodiesel Production. Energies 2012, 5, 1532-1553.
39. Kalva, A., Sivasankar, T., Moholkar, V.S., 2008. Physical mechanism of ultrasound-assisted synthesis of biodiesel. Ind. Eng. Chem. Res. 48 (1), 534–544.
40. Keesoo Lee, Megan L. Eisterhold,[1] Fabio Rindi,[2] Swaminathan Palanisami,[1] and Paul K. Nam[3] (2014). Isolation and screening of microalgae from natural habitats in the Midwestern. United States of America for biomass and biodiesel sources, 5(2): 333–339.
41. Kianimanesh, H.R., Abbaspour-aghdam, F., Derakhshan, M.V., 2017. Biodiesel production from vegetable oil : Process design , evaluation and optimization. Polish J. Chem. Technol. 19, 49–55.
42. Koberg M[1], Cohen M, Ben-Amotz A, Gedanken A. (2011). Bio-diesel production directly from the microalgae biomass of Nannochloropsis by microwave and ultrasound radiation. Bioresour Technol. 102(5):4265-9.
43. Lam MK[1], Lee KT (2012). Microalgae biofuels: A critical review of issues, problems and the way forward. Biotechnol Adv.;30(3):673-90.
44. Lee YK (2001). Microalgal mass culture systems and methods: their limitation and potential. Journal of Applied Phycology;13(4):307–15.
45. Lenka blinova, Alica bartosova, Kristina gerulova (2015). Cultivation of microalgae (chlorella vulgaris) for biodiesel production, Volume 23, No. 36.
46. Li Y, Horsman M, Wu N, Lan CQ, Dubois-Calero N (2008) Biofuels from microalgae. Biotechnol Prog 24:815–820.
47. Liam Brennan, Philip Owende (2010). Biofuels from microalgae—A review of technologies for production, processing, and extractions of biofuels and co-products. Renewable and Sustainable Energy Reviews, Volume 14, Issue 2, Pages 557-577.
48. Liandong Zhu (2015). Microalgal culture strategies for biofuel production: a review. Wiley Online Library.
49. Liang Y[1], Sarkany N, Cui Y. (2009). Biomass and lipid productivities of Chlorella vulgaris under autotrophic, heterotrophic and mixotrophic growth conditions;31(7):1043-9.
50. Lim H. C., Shin H. S. Fed-batch cultures: Principles and applications of semi-batch bioreactors. England: Cambridge University Press, 2013
51. Maxwell, E. L., A. G. Folger, and S. E. Hogg (1985), Resource evaluation and site selection for microalgae production systems, Rep. SERI/TR-215- 2484, Solar Energy Res. Inst., Washington, D. C.
52. Michael Hannon,[1] Javier Gimpel,[1] Miller Tran,[1] Beth Rasala,[1] and Stephen Mayfield (2010). Biofuels from algae: challenges and potential. Biofuels. 1(5): 763–784.
53. Microalgae: A Potential Source of Biodiesel (2012). Shalini Rajvanshi, Mahendra Pal Sharma, Journal of Sustainable Bioenergy Systems, 2, 49-59.
54. Microalgal culture strategies for biofuel production: a review (2015). Biofuels, Bioprod. Bioref. 9:801–814.
55. Milano, J., H. C. Ong, H. H. Masjuki et al. 2016. Microalgae biofuels as an alternative to fossil fuel for power generation. Renewable and Sustainable Energy Reviews 58: 180–197.
56. Mohammed Abdul Raqeeb and Bhargavi R (2017). Biodiesel production from used cooking oil using controlled reactor plant. 7(12):670-681.
57. Muhammad Aminul Islam 1,*, Marie Magnusson 2,3, Richard J. Brown 1 , Godwin A. Ayoko 1 , Md. Nurun Nabi 1 and Kirsten Heimann (2014). Microalgal Species Selection for Biodiesel Production Based on Fuel Properties Derived from Fatty Acid Profiles. Energies 2013, 6, 5676-5702.
58. Oliveira L. E., Da Silva M. L. C. P. (2013). Comparative study of calorific value of rapeseed, soybean, jatropha curcas and crambe biodiesel. Renewable Energy and Power Quality Journal, 11, 1-4.
59. P.U. Okoye, B.H. Hameed (2016). Review on recent progress in catalytic carboxylation and acetylation of glycerol as a byproduct of biodiesel production. Renewable and Sustainable Energy Reviews 53 (2016) 558–574.
60. Paul A. Hoskisson1 and Glyn Hobbs (2005). Continuous culture – making a comeback? Microbiology, 151, 3153–3159
61. Peer M. Schenk & Skye R. Thomas-Hall & Evan Stephens & Ute C. Marx & Jan H. Mussgnug & Clemens Posten & Olaf Kruse & Ben Hankamer (2008). Second Generation Biofuels: High-Efficiency Microalgae for Biodiesel Production. Bioenerg. Res. (2008) 1:20–43
62. Pittman JK[1], Dean AP, Osundeko O. (2011). The potential of sustainable algal biofuel production using wastewater resources. Bioresour Technol. 102(1):17-25.
63. Poelman E, De pauw N, Jeurissen B (1997). Potential of electrolytic flocculation for recoveryofmicroalgae.Res ConservRecyc, 9:1-10.
64. Refaat, A.A., El Sheltawy, S.T., 2008. Time factor in microwave-enhanced biodiesel production. WSEAS Trans. Environ. Devel. 4 (4), 279–288.

65. Rindi F. (2007) Diversity, Distribution and Ecology of Green Algae and Cyanobacteria in Urban Habitats. In: Seckbach J. (eds) Algae and Cyanobacteria in Extreme Environments. Cellular Origin, Life in Extreme Habitats and Astrobiology, vol 11. Springer, Dordrecht.

66. Ronald Halim ⁎, Michael K. Danquah, Paul A. Webley (2012). Extraction of oil from microalgae for biodiesel production: A review; Volume 30, Issue 3, Pages 709-732.

67. Saddam H. Al-lwayzy 1,2,*, Talal Yusaf 1 and Raed A. Al-Juboori (2014). Biofuels from the Fresh Water Microalgae Chlorella vulgaris (FWM-CV) for Diesel Engines. Energies 2014, 7, 1829-1851; doi:10.3390/en7031829.

68. Sarman Oktovianus Gultom and Bo Hu (2013). Review of Microalgae Harvesting via Co-Pelletization with Filamentous Fungus. Energies 2013, 6, 5921-5939.

69. Seungjib Jeon1,2, Jong-Min Lim3 , Hyung-Gwan Lee4 , Sung-Eun Shin5 , Nam Kyu Kang1 , Youn-Il Park6 , Hee-Mock Oh4 , Won-Joong Jeong3 , Byeong-ryool Jeong1,2* and Yong Keun Chang1 (2017). Current status and perspectives of genome editing technology for microalgae. Biotechnol Biofuels) 10:267.

70. Shah, J.H., A. Deokar, K. Patel, K. Panchal, and A.V. Mehta. 2014. A comprehensive overview on various method of harvesting microalgae according to indian perspective, in International Conference on Multidisciplinary Research & Practice. International Conference on Multidisciplinary Research & Practice 1: 313–17.

71. Shakeel A.Khan[a]Rashmi[b]Mir Z.Hussain[a]S.Prasad[a]U.C.Banerjee[b] (2009). Prospects of biodiesel production from microalgae in India, Renewable and Sustainable Energy Reviews, Volume 13, Issue 9, Pages 2361-2372.

72. Shalini Rajvanshi, Mahendra Pal Sharma (2012). Microalgae: A Potential Source of Biodiesel. Journal of Sustainable Bioenergy Systems, 2012, 2, 49-59.

73. Sheehan, J.; Cambreco, J.; Graboski, M.; Shapouri, H. (1998). An overview of biodiesel and petroleum diesel life cycles. US Department of agriculture and Energy Report, p. 1–35.

74. Simoni M. Plentz Meneghetti, Mario R. Meneghetti, Carlos R. Wolf, Eid C. Silva, Gilvan E. S. Lima, Laelson de Lira Silva, Tatiana M. Serra, Fernanda Cauduro, and Lenise G. de Oliveira (2006). Biodiesel Production from Vegetable Oil Mixtures: Cottonseed, Soybean, and Castor Oils; Fuels, 21 (6), pp 3746–3747.

75. Song-Fang Han1, Wen-Biao Jin1, Ren-Jie Tu1, and Wei-Min Wu2 (2014). Biofuel production from microalgae as feedstock: current status and potential; 35(2):255-68.

76. Teresa M.Mata, António A.Martins, Nidia. S.Caetano (2009). Microalgae for biodiesel production and other applications: A review. Renewable and Sustainable Energy Reviews

77. Thomas Egli (2015). Microbial growth and physiology: A call for better craftsmanship. Frontiers in Microbiology, Volume 6 | Article 287.

78. Tse-Shih Lin, Jane-Yii Wu (2014). Effect of carbon sources on growth and lipid accumulation of newly isolated microalgae cultured under mixotrophic condition. Volume 184, May 2015, Pages 100-107.

79. Vandamme D[1], Foubert I, Muylaert K. (2013). Flocculation as a low-cost method for harvesting microalgae for bulk biomass production. Trends Biotechnol. 2013 Apr;31(4):233-9.

80. Victoria O.Adesanya, ErasmoCadena, [b]Stuart A.Scott, [a]Alison G.Smith (2014). Life cycle assessment on microalgal biodiesel production using a hybrid cultivation system. Bioresource Technology, Volume 163, July 2014, Pages 343-355.

81. Vishwanath Patil 1,*, Khanh-Quang Tran 2 and Hans Ragnar Giselrød (2008). Towards Sustainable Production of Biofuels from Microalgae. Int. J. Mol. Sci. 2008, 9, 1188-1195.

82. Wang Q., Ye L., Jiang G., Jensen P.D., Batstone D.J., and Yuan Z. (2013) Free nitrous acid (FNA)-based pretreatment enhances methane production from waste activated sludge. Environ Sci Technol, 47: 11897–904.

83. Wei-HsinChen, Bo-JhihLin[a], Ming-YuehHuang[a], Jo-ShuChang (2014). Thermochemical conversion of microalgal biomass into biofuels: A review. Bioresource Technology,

84. Xiaodan Wu 1,2, Rongsheng Ruan 1,2,3, Zhenyi Du 3 and Yuhuan Liu (2012). Current Status and Prospects of Biodiesel Production from Microalgae, Energies, Volume 5, Pages 2667-2682.

85. Xin Zhang1,2, Junfeng Rong3, Hui Chen1, Chenliu He1 and QiangWang1 (2014). Current status and outlook in the apploication of microalgae in biodiesel production and environmental protection, Front. Energy Res., Volume 2, Article 32.

86. Xiong, W., Wu, Q., 2008. High-density fermentation of microalga Chlorella protothecoides in bioreactor for microbiodiesel production. Appl. Microbiol. Biotechnol. 78, 29–36.

87. Xu H[1], Miao X, Wu Q. (2006). High quality biodiesel production from a microalga Chlorella protothecoides by heterotrophic growth in fermenters. J Biotechnol. 2006 Dec 1;126(4):499-507.Zheng H, Gao Z, Yin J, Tang X, Ji X, Huang H. (2012). Harvesting of microalgae by flocculation with poly (γ-glutamic acid). Bioresour Technol;112:212–20.

88. Zhiyou Wen and Michael B. Johnson (2009). Microalgae as a Feedstock for Biofuel Production. Virginia Polytechnic Institute and State University.