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Recent approaches and Advances in Algal Biodiesel Technology

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ABSTRACT
Energy crisis has augmented due to fast depleting conventional sources of energy. There is imperative demand to investigate the renewable, eco-friendly and cost-effective non-conventional sources of energy. Algal biofuel is carbon neutral, biodegradable and nontoxic to the environment. Further, there is no need of arable land for cultivation along with short generation period, unlike other biofuel-yielding crops. This review investigates the recent advancement in the algal biodiesel technology and documents all those algal species with higher biomass growth rate and lipid content which are currently being used for biodiesel production. Recent developments in the techniques for dewatering of biomass and extraction of lipid and trans-esterification are discussed. Policy status of different countries using algae-based biodiesel is examined and recommendations are made from research perspective for economic viability of algal biodiesel technology.
Keywords: *Algal biofuel, biodiesel, carbon sequestration, conventional, flocculation*

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INTRODUCTION

In the current scenario, to meet the growing energy demand of increasing population and industries in developing countries, the exploration of newer energy source is the prime requirement. It is expected that only India and China will going to need up to 31% of global energy by 2035 [1]. Depletion of conventional sources of energy at unprecedented rate demands availability of new green biochemical technologies capable of converting biomass to fuel. Moreover, conventional modes of energy are causing environmental pollution. Hence, it is very much imperative to explore such alternative resource of energy which can fulfil the energy demands and helps in sustainable development. Biofuel (biodiesel, bioalcohols, biohydrogen and certain others) could be a promising option to serve the world energy demands without creating any problems to the environment [2, 3]. Primarily, soybeans, palm oil, cottonseeds, rapeseeds, corn etc. were used as first generation biofuel feedstock. But they pose food security challenges resulting into increase cost value of edible oil as well as biofuel. To solve these challenges focused shifted on non-edible plants like jojoba, castor, jatropha and karnja and these are termed as the second generation biofuels feedstock. But they also failed to meet the growing demands and competition for arable land remained as such [4]. Recently, microalgae have attracted the attention of scientific community and industrialist as third generation feedstock [5]. Biofuels based on algal biomass (micro- and macroalgae) are gaining substantial interest because there is no competition with food crops and algae can be cultivated in saltwater or wastewater ponds on marginal land, does not cause deforestation, environment friendly as it involves CO_2 sequestration, have rapid growth rate, require less water than terrestrial crops and along with the production of high-value compounds as by-products, makes the system economically viable [6, 7, 4]. It has been estimated that algae could yield 61,000 litres biofuel per hectare, compared with 200 litres to 450 litres from crops such as soya and canola [8]. Certain microalgae such as *Chlorella* sp. are rich source for biofuels and can accumulate lipid more than 50% of their biomass [9]. Previously published reviews dealt mainly with one or two areas of algal biodiesel technology, however, present communication provide a comprehensive overview with holistic approach and deal with significant practical advancements in every step of algae derived biodiesel. Moreover, this review has given importance on newly explored strains and genetic advancement in wild strains, with the purpose of increasing biofuel production. It is essential to understand the knowledge gaps at the current stage for further improvement and commercialization of algal biofuel technology.

EXPLORATION OF ALGAL BIODIVERSITY IN TERMS OF BIOMASS AND LIPID YIELD

Around 30,000 to 1 million algal species have been expected to be present on earth [10]. Among them, only few have been explored and utilized for the industrial purposes (Table 1). So there is opportunity to investigate and explore the biodiversity for more industrially useful strains. For biofuel productions from algae, selection criteria are growth rate (biomass yielding capacity per unit time) and lipid producing efficiency. There are many freshwater as well as marine macro/microalgae reported which have sufficient amount of lipid for biodiesel production. *Chlorococcum oleofaciens* and *Pseudokirchneriella subcapitata*, both microalgae were reported to have high lipid content (180 mgL^{-1} d^{-1}) and fatty acid content (160 mg L^{-1} d^{-1}) respectively under nitrate deficient continuous culture condition in comparison with the control culture [11]. A new microalgae strain, *Monoraphidium* sp. SDEC-17 was also identified with high biomass production, high lipid content and protein apart from its ability as a good bioremediator. In this strain, protein content was found to be increased by 44% when algae were grown in industrial effluent having the high content of ammonia, whereas only 34% protein content was reported in the BG11 medium. However, biomass yield and lipid content were not found significantly influenced under ammonia [12]. High lipid production (1184 mg L^{-1}) was observed in *Chlorella ellipsoidea* when subcultured into Wright's Cryptophyte medium (WC medium) from the BG11 medium at the late exponential phase when biomass yield was 3.2 g L^{-1} [13]. Improved cell density and biomass of *Chlorella sorokiniana* was observed in BBM medium with kelp extract and acetate [14]whereas another species *C. vulgaris* performed well with respect to the growth rate in domestic wastewater [15]. Recently, [16] found that algae grown in the effluent of anaerobic digester system are not only suitable for the higher amount of lipid accumulation but also capable of nitrogen and phosphorous removal simultaneously. Among *Scenedesmus* sp., *Chlorella sorokiniana* HS (KCTC12171BP), *C. vulgaris, Micractinium inermum* NLP-F014 (KCTC 12491BP) tested, *M. inermum* was found most efficient as it produced highest biomass and fatty acid methyl esters (FAME). Among marine water species, *Padina tetrastromatica* was found useful for biodiesel and bioethanol production [17]. *Thalassiosira* sp. was also examined under agricultural fertilizer based media (NPK-115, Na_2SiO_3 -6, $FeCl_3$ -3.3, Na_2EDTA -70 in mg L^{-1}) and 8.1 tons ha^{-1} (dry season) and 23.9 tons ha^{-1} (rainy season) algal biomass was obtained for biodiesel production [18].

FACTORS AFFECTING ALGAL CULTIVATION

Cultivation methodology is one of the important aspects for optimizing the best productivity of lipid and biomass yield. Algae cultivation requires a plentiful supply of CO_2, water, and sunlight for desirable growth. The concentration of CO_2 directly influences the biomass growth rate and lipid yield [19]. Generally 0.04% v/v conc. of CO_2 is present in the culture media. It is reported that intracellular acetyl-CoA and lipid accumulation is enhanced in *Chlorella vulgaris* at >0.04% v/v conc. of CO_2 in medium [20]. However, >5% v/v conc. of CO_2 inhibit the microalgal growth as noticed in *Nannochloropsis occulata* [21, 22]. Cell counts of *Haematococcus pluvialis* were observed to be higher at low intensity of light (25±3 μmol photon m^{-2} s^{-1}) than high intensity (78±3 μmol photon m^{-2} s^{-1}) [23]. Photoperiods had been reported to play a key role in *Nannochloropsis gaditana* which produced the highest biomass of 1.25 g L^{-1} at the photoperiod of 16 h light and 8 h dark, resulting in enhanced lipid as well as protein content, over the control [24]. Cultivation system is another crucial aspect of biodiesel production. Generally, the culture of algae is carried out in open raceway ponds, outdoor photobioreactors and closed photobioreactors [25, 18, 26].Further, it is observed that position of photobioreactors against solar irradiation also influences the cell growth by altering the absorption of light within the reactor. *Scenedesmus obtusiusculus,* when illuminated with the light intensity of 1400 μmol photon $m^{-2}s^{-1}$ in a flatplate photobioreactor, yielded enhanced biomass (4.95 gL^{-1} cellular dry weight) within 3.5 d and beyond the optimum irradiances photo inhibitory effects occurred [27]. Closed photobioreactor with artificial lightning requires much energy [28] so in order to economize the system, various parameters such as temperature, light intensity, irradiance, and oxygen concentration within the photobioreactors needs to be standardize to obtain maximum growth rate and ease in transfer of gases [26,29]. Interestingly, mixotrophic condition or enrichment of carbon by any organic or inorganic source mark significant impact on lipid production and biomass concentration in algae [30].Another study reported that mixotrophically grown *Chlorella* sp. on liquid waste (containing optimized concentration of 2.70 gL^{-1} technical grade glycerol and 0.114 gL^{-1} of nitrogen) for biogas production showed increased biomass of 2.41 gL^{-1} [31].*Chlorella sorokiniana* was cultivated under heterotrophic conditions by adding 400 mgL^{-1} sodium nitrate in aquaculture wastewater and reported to yield 498.14 $mgL^{-1}d^{-1}$ of biomass with 150.19 $mgL^{-1}d^{-1}$ lipid for biodiesel production[32]. An elevated biomass was observed on addition of ribose and sodium acetate while culturing *Haematococcus pluvialis* [23]. These studies suggest that various factors influence the algal biomass productivity and lipid content. To minimize the cost of cultivation, algae can be grown on sludge domestic wastewater, pharmaceutical wastes. Moreover, algae can produce comparatively higher biomass in wastewater along with their potential application to remove toxicity from water [33, 34, 35, 36, 15].

HARVESTING ALGAL BIOMASS

Harvesting is the process of recovering raw or unprocessed biomass of microalgae for further retrieval of useful metabolites. To make biodiesel cost effective we ought to focus on minimizing the harvesting cost, therefore, it could be considered as a crucial step for biodiesel production. Natural sedimentation is not efficient to harvest the microalgal biomass [37]. Strategies like centrifugation, filtration, bio-flocculation, electro-flocculation, chemical-flocculation, floatation and co-cultivation are currently in use for harvesting purposes. Flocculation is considered as the best technique among them as best output can be obtained by it.

For large scale harvesting, use of flocculation showed the increase in efficiencies up to 60% and 75% for *Chlorella vulgaris* and *Scenedesmus obliquus* respectively [38, 39]. Some compounds like iron (III) chloride ($FeCl_3$), potassium aluminum sulfate ($KAl(SO_4)_2$) and chitosan have also been tested with 80, 95 and 92% flocculation efficiencies respectively. However, these compounds were not found suitable for viability of algal cells, no reuse in medium, subjected to water pollution and chitosan is a very expensive for large-scale use [40, 39]. It is also observed that change in pH is more feasible and eco-friendly than the addition of chemical flocculants [41]. The addition of CO_2, Mg^{+2} and NaOH results in altered pH of the medium and induced floc formation [42]. However, changing pH at large scale is not easy [43]. Seed powder of angiosperm trees like *Moringa oleifera* and *Strychnos potatorum* have proved as prospective bioflocculant with 87% and 99.68% flocculation efficiency, respectively, to harvest *Chlorella vulgaris* [40]. *Coelastrum* cf. *pseudomicroporum,* a green microalga was also reported as bioflocculant [44]. As a low-cost flocculant agent egg shells 100 mgL^{-1} is used to harvest *Chlorella* sp. and 98% efficiency at 40°C and 99% efficiency at 50°C were obtained within 30 min [45].

Floatation is another energy proficient approach for harvesting microalgae. Electroflotation for harvesting of *Scenedesmus obliquus* could be used as productive and cost effective method by adding 10% of seawater thereby enhancing electrical conductivity in media (BG-11 medium) and it was found that only 0.68 Wh (watt-hour) electric power was consumed so there is no need of coagulants [46]. Air floatation by the addition of cationic surfactant $C_{16}TAB$, also resulted in efficient harvesting, because it increased the hydrophobicity of microalgae cell surface [47]

LIPID EXTRACTION

Lipid production from oil yielding algae is not simple; it has obstacles when it comes to industrial implementation or large scale production [48]. In order to make lipid production more cost effective and efficient it is important to select suitable solvent for extraction. There are many solvent mixtures for lipid extraction such as chloroform:methanol, dichloromethane, hexane:diethyl ether, nitro hexane [49, 50] etc. in use. However, none of these solvents when used alone can result in high yield. To carry out extraction within the solvent, various thermal and mechanical methods are used for cell wall disruption like microwave assisted extraction pulse electric fields, bead milling, ultrasonication, high-pressure homogenization [51, 52, 53]. Further, chemical methods like alkaline treatment, enzymatic hydrolysis are also applied for oil extraction [54]. Among all methods, only microwave assisted extraction method can be carried out with or without solvents. Further, it is less time consuming and prevents dissipation of solvents. However, this method is still not used very frequently because it results into denaturation of desired products due to high radiation energy [55]. Bead milling causes no chemical modification of extract because it does not involve any chemical. However, there is a limitation in this method likes the feeding of biomass to the unit is not economically viable and seems suitable only for lab scale [56]. Microalgae can be disrupted rapidly with ultrasonication and homogenization [57]. Ultrasonication of microalgae is very efficient method over bead beating, microwave and autoclaving methods [58]. Ultrasonication is not only effective for cell wall disruption but it also enhances extraction efficiency of fatty acids. It does not alter fatty acid profile and biomass [53]. Further, ultrasonication assisted transesterification (*in situ* transesterification) not only maximize the lipid yield without drying of the biomass but it is also less time consuming (within 5 min) than two-step transesterification which takes 12 h [59]. The addition of solvents in ultrasonication like chloroform:methanol (2:1) enhance the lipid extraction yield and decrease the time of extraction as observed in brown microalgae *Padina tetrastromatica* [17]. Enzymatic hydrolysis of cell wall is also found effective as it avoids the risk of damage and maintains specificity of desired products [60]. Cellulase, pectinase, protease and lysozyme could be used for enzymatic treatment [54] but limitation factors like high cost and limited yield of lipid make it less economic [61, 62]. To overcome these problems combinations of different enzymes preceded by the alkaline pre-treatment is suggested for efficient extraction [54]. Supercritical CO_2 is also used to extract lipid from algal biomass as it result in biodiesel with better ignition and higher cetane number [63,64]. Supercritical CO_2 employs low-cost solvents, have lower critical points and pressure requirement, and could be easily optimized for industrial scale production along with environment friendly approach. Supercritical CO_2 facilitates bioactive compounds (solutes) of the cell to come out and dissolved with much ease. It has few drawbacks as it can be applied in the case of non-polar solutes only and requires costly equipment set-up [55, 65].

CONVERSION OF EXTRACTED LIPID INTO BIODIESEL

For biodiesel production, it is important to know the fatty acid profile of feedstock. It is desirable to have <1% of free fatty acid (FFA) level. If total lipid content has 70-80% polar lipid along with FFAs then it is considered unsuitable for biodiesel production because the higher amount of FFAs will tend to ruin all the triglycerides [66]. Various parameters like amount of catalyst, solvents, co-solvents, temperature and reaction time have the significant impact on biodiesel yield [54, 67]. These parameters were also found very crucial in direct transesterification under supercritical conditions and have the direct influence on cost [68]. Phosphoric acid (H_3PO_4), Hydrochloric acid (HCl) and acid sulfonates are able to perform esterification and transesterification as acid catalysts [69]. The high amounts of FFAs (11.4%) in *Padina tetrastromatica* were decreased up to 0.81% after the esterification with H_2SO_4 (1.5% v/v) [17]. However, catalyzed reactions are very slow [70] and obtained products have quality issues, along with the acid separation problem after reaction [71].1-Butyl-3-methylimidazolium hydrogen sulfate ([Bmim][HSO_4]) in addition with methanol showed significant reusability as a solvent for transesterification of *Nannochloropsis* sp. [72]. Alkali catalyzed transesterification was found more productive in terms of output and low corrosiveness [70]. Mixing of t-butanol in lipase catalyzed reaction system for biodiesel production reduce the viscosity of algal lipid and increase the interaction between lipase and algal lipid. Therefore, addition of t-butanol increases the efficiency of lipase and also helps in the dissolution of methanol and FAME in the downstream process reducing cost by reusability after many cycles [54]. Ethanol can also be used as a solvent for lipase to perform transesterification reaction with 92.3% efficiency. Yield can be enhanced further by extending the reaction time [67].

POLICY STATUS REGARDING PRODUCTION AND UTILIZATION OF BIOFUELS

Globally, developed and developing nations are actively looking for the alternative energy sources to meet the energy crisis. As biofuel has come up as the best option, various policies to set up it as an energy source are being formulated. It is expected that worldwide production of biofuel will approximately reach up to 35 billion liters by 2020 [73]. Brazil is the first country which formulated and implemented biofuel policy in order to enhance biofuel production and using it by blending 5% of biodiesel and 20-25% of ethanol in conventional fuel [74]. The United States is producing 3.7 billion gallons of biofuel at present with the target of 36 billion gallons of biofuel production by 2022. US government already proposed strategies to replace up to 17% of conventional transportation fuel with algal biofuel by 2019. In 2013, USA was on top among biofuel producing countries followed by Brazil, Germany, Argentina, France and China [4]. Biofuel industries are also well established in countries of European Union (EU) with the investment of up to €20-30 millions in algae biofuel research projects. EU put €6 million, in a joint venture of England and Ireland for application of algae strain having a potential for industrial production. China quickly emerged in the field of microalgae-derived energy and mainly investing in marine algae energy projects. In 2010 and 2012, China has invested in the National level program on microalgae-based energy for improvements like large scale cultivation and oil quality upgradations [75]. In order to enhance biodiesel production, India has also initiated "Bio-diesel Purchase Policy", in 2005. In 2015 ministry permitted producers to sell high-speed diesel blended with biodiesel commercially acronymized as 'B-5' to certified dealers and certain outlets [76]. Indian biofuel policy is mainly focused on the cost of unrefined oil, promotion of the climatic improvement by decreasing the greenhouse gases and encourages the biodiesel production with non-edible crops [77]. In 2014, Indian Oil Corporation (IOC) allocated 2.4 million dollars with Algae. Tec Ltd., an Australian-based company, for research in high biomass yield of algae [78]. Reliance Industries (India) also invested 116 million dollars for algae-derived energy in two enterprises, 22.5 million in Aurora Algae and 93.5 million in Algeno [79]. In 2013, Sapphire Energy has made collaboration with Phillips66 for blending ratio of algae oil with conventional crude oil.

Table 1. Important algal strain recently suggested for biodiesel production.

Microalgae	Biomass growth rate	Lipid	Habitat	References
Tetraselmis elliptica	0.122 g CDW L^{-1} d^{-1}	12.96 mg g^{-1} CDW	Hypersaline	[83]
Chlorella ellipsoidea	3.2 g L^{-1}	1184 mg L^{-1}	Fresh	[13]
Pseudokirchneriella subcapitata	-	180 mg L^{-1} d^{-1}	Fresh	[11]
Scenedesmus obliquus CNW-N	245.8 mg L^{-1} d^{-1}	-	-	[84]
Chlorella vulgaris	-	212 mg L^{-1} d^{-1}	Fresh	[85]
Chlamydomonas reinhardtii (CCAP 11/32C)	62.3 mg L^{-1} d^{-1}	10.3625 mg L^{-1} d^{-1}	Fresh	[86]
Chlorella vulgaris	15.93 mg L^{-1} d^{-1}	4.09 mg L^{-1} d^{-1}	Fresh	[87]
Ankistrodesmus falcatus	198.46 mg L^{-1} d^{-1}	-	Fresh	[88]
Chlorella sorokiniana	157.04 mg L^{-1} d^{-1}	-	Fresh	[88]
Chlorella sp. Y8-1		0.01 g/L/d	Fresh	[89]
Scenedesmus obliquus		111.8 mg/(L·d)		[90]
Chlorella vulgaris		85.7 mg/(L·d)		[90]

Table 2. Genetically modified algal strains for enhanced biofuel production.

S.No.	Algae	Remarks	Reference
1.	*Synechococcus* sp. PCC 7942	Ethanol synthesis by genetically modified cyanobacteria	[91]
2.	*Anabaena variabilis*	Targeting hupSL (hydrogenase) gene for increased rates in H_2 accumulation resulting in three times more hydrogen	[92]
3.	*Synechocystis* sp. PCC6803	Fatty acid secretion yield was increased by adding codon-optimized thioesterase genes	[93]
4.	*Phaeodactylum tricornutum*	Overexpression of Acyl-ACP thioesterases leading to improve biofuel production	[94]
5.	*Synechocystis* sp. PCC 6803	Production of ethanol from genetically modified cyanobacteria	[95]
6.	*Phaeodactylum tricornutum*	Targeting malic enzyme producing gene (PtME) for increase of the total lipid content in transgenic cells by 2.5-fold	[96]
7.	*Chlamydomonas reinhardtii*	Over-expression of isoamylase leading to accumulation of much higher amounts of starch than unmodified algae. Patent US9290782 B2	[97]
8.	*Chlamydomonas reinhardtii*	Production of hydrogen biofuel five times higher than their normal yield through genetically modified algae	[98]
9.	*Acutodesmus dimorphus*	First EPA approved outdoor field trial of genetically modified algae for enhanced fatty acid biosynthesis	[82]

CONCLUSION

Algal biodiesel technology is still in its infancy with many limitations hindering its industrial level utility. Each and every step of utilizing algae for biodiesel; right from strain selection to growing algae, producing maximum lipid, harvesting, and conversion of oil into biodiesel have the scope for improvement. To ensure sustainability and economic viability of algal biodiesel based industry, one must standardize the production of good quality and quantity of biodiesel, while the growing biomass should yield high-value products in an eco-friendly manner. Future studies are required to combine phycoremediation, production of by-products with biodiesel at industrial scale, with further use of spent biomass in electricity or biogas generation (Fig. 1). Only few algal strains have yet been tested and currently in use for production of biodiesel, therefore, exploration of the algal biodiversity for higher growth rate and high amount of lipid content is required. Further, the genetic makeup of algal strain must be optimized in a manner that algal cell function as bio-factories for the production of lipid and high value compounds simultaneously. For this recent advance in genetic engineering technology like CRISPR-Cas9 [80] should be utilized for easy transformation of algae. Few studies have reported genetically engineered algae optimized for the production of biofuel (Table 2). One diatom species namely *Phaeodactylum tricornutum* is transformed by overexpressing diacylglycerol acyltransferase (DGAT2D) gene, resulting in double the amount of lipid content [81]. One study has reported no ecological risk to the native algal population in open lake body by genetically engineered algae optimized for the production of biofuel [82]. Possibilities of metabolic engineering for improving algae strains are endless right from improving fatty acid chain length, growth rate, production of high-value compounds, ease of harvesting/flocculation etc.

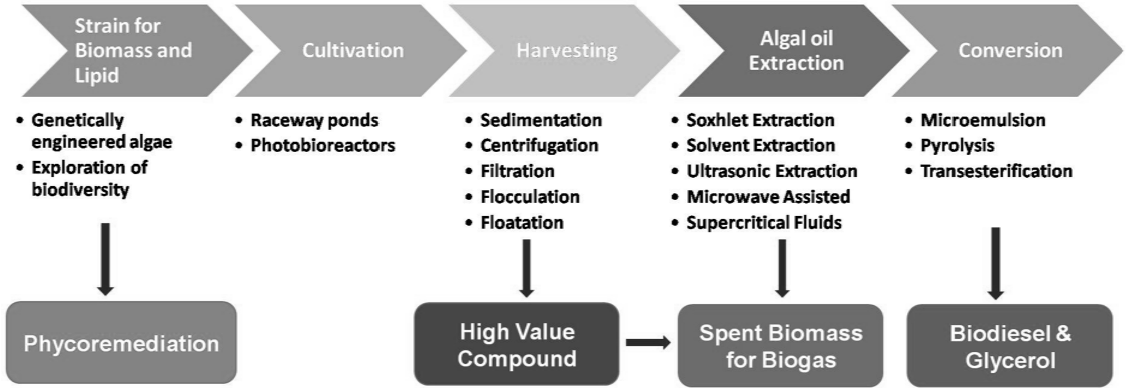

Fig. 1. Steps required for sustainable biodiesel production from algae.

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REFERENCES
1. U.S.E.I.A. (2011). International Energy Outlook, U.S. Energy Information Administration. International Energy Outlook.
2. Knothe G. (2005). Dependence of biodiesel fuel properties on the structure of fatty acid alkyl esters. Fuel Processing Technology. 86(10):1059-1070.
3. Kirrolia A., Bishnoi N.R. and Singh R. (2013). Microalgae as a boon for sustainable energy production and its future research & development aspects. Renewable and Sustainable Energy Reviews. 20: 642-656.
4. Sharma Y.C. and Singh V. (2017). Microalgal biodiesel: A possible solution for India's energy security(2017). Renewable and Sustainable Energy Review. ; 67: 72-88.
5. Phukan M.M., Chutia R.S., Konwar B.K. and Kataki R., (2011). Microalgae *Chlorella* as a potential bio-energy feedstock. Applied Energy.; 88(10): 3307-3312.
6. Hu Q., Sommerfeld M., Jarvis E., Ghirardi M., Posewitz M., Seibert M. and Darzins A. (2008). Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. The Plant Journal. 54 (4): 621-639.
7. Vassilev S.V. and Vassileva C.G. (2016). Composition, properties and challenges of algae biomass for biofuel application: An overview. Fuel. 181: 1-33.
8. Savage N. (2011). Algae: the scum solution. Nature*,* 474(7352): S15-16.
9. Sharma Y., Singh B. and Korstad J. (2011). A critical review of recent methods used for economically viable and eco-friendly development of microalgae as a potential feedstock for synthesis of biodiesel. Green Chemistry. 13: 2993-3006.
10. Guiry M.D. (2012). How many species of algae are there? Journal of Phycology. 48(5): 1057-1063.
11. Del Río E., García-Gómez E., Moreno J., Guerrero M.G. and García-González M. (2017). Microalgae for oil. Assessment of fatty acid productivity in continuous culture by two high-yield strains, *Chlorococcum oleofaciens* and *Pseudokirchneriella subcapitata*. Algal Research. 23: 37-42.
12. Jiang L., Pei H., Hu W., Hou Q., Han F. and Nie C. (2016). Biomass production and nutrient assimilation by a novel microalga, *Monoraphidium spp*. SDEC-17, cultivated in a high-ammonia wastewater. Energy Conversion and Management.**;** 23: 423-430.
13. Purkayastha J., Bora A., Gogoi H.K. and Singh L. (2017). Growth of high oil yielding green alga *Chlorella ellipsoidea* in diverse autotrophic media, effect on its constituents. Algal Research. 21: 81-88.
14. Zheng S., He M., Sui Y., Gebreluel T., Zou S., Kemuma N.D. and Wang C. (2017). Kelp waste extracts combined with acetate enhances the biofuel characteristics of *Chlorella sorokiniana*. Bioresource. Technology. ; 225: 142-150.
15. Lam M.K., Yusoff M.I., Uemura Y., Lim J.W., Khoo C.G., Lee K.T. and Ong H.C., (2017). Cultivation of *Chlorella vulgaris* using nutrients source from domestic wastewater for biodiesel production: Growth condition and kinetic studies. Renewable Energy. 103: 197-207.
16. Kim G.Y., Yun Y.M., Shin H.S. and Han J.I. (2017). Cultivation of four microalgae species in the effluent of anaerobic digester for biodiesel production. Bioresource Technology. 224: 738-742.

17. Ashokkumar V., Salim M.R., Salam Z., Sivakumar P., Chong C.T., Elumalai S. and Ani F.N. (2017). Production of liquid biofuels (biodiesel and bioethanol) from brown marine macroalgae *Padina tetrastromatica.* Energy Conversion Management. 135: 351-361.
18. Kusumaningtyas P., Nurbaiti S., Suantika G., Amran M.B. and Nurachman Z. (2017). Enhanced oil production by the tropical marine diatom *Thalassiosira* sp. cultivated in outdoor photobioreactors. Applied Biochemistry and Biotechnology. 1-14 doi: 10.1007/s12010-017-2421-8
19. Mehrabadi A., Farid M.M. Craggs R. (2017). Effect of CO_2 addition on biomass energy yield in wastewater treatment high rate algal mesocosms. Algal Research. 22: 93-103.
20. Jose S. and Suraishkumar G.K. (2016). High carbon (CO_2) Supply leads to elevated intracellular acetyl CoA levels and increased lipid accumulation in *Chlorella vulgaris.* Algal Research. 19: 307-315.
21. Yun, Y.S., Lee S.B., Park J.M., Lee C.I., Yang J.W. (1997). Carbon dioxide fixation by algal cultivation using wastewater nutrients. Journal Chemical Technology and Biotechnology. 69(4):451-455.
22. Cheng L., Zhang L., Chen H. and Gao C., (2006). Carbon dioxide removal from air by microalgae cultured in a membrane-photobioreactor. Separation and Purification Technology. 50 (3): 324-329.
23. Pang N. and Chen S., (2017). Effects of C5 organic carbon and light on growth and cell activity of *Haematococcus pluvialis* under mixotrophic conditions. Algal Research. 21: 227-235.
24. Matos Â.P., Cavanholi M.G., Moecke E.H.S. and Sant'Anna E.S. (2017). Effects of different photoperiod and trophic conditions on biomass, protein and lipid production by the marine alga *Nannochloropsis gaditana* at optimal concentration of desalination concentrate. Bioresource Technology. 224: 490-497.
25. Hoffman J., Pate R.C., Drennen T. and Quinn J.C. (2017). Techno-economic assessment of open microalgae production systems. Algal Research. 23: 51-57.
26. Solimeno A., Gabriel F. and García J. (2017). Mechanistic model for design, analysis, operation and control of microalgae cultures: calibration and application to tubular photobioreactors. Algal Research. 21:236-246.
27. Koller A.P., Löwe H., Schmid V., Mundt S. and Weuster-Botz D. (2017). Model-supported phototrophic growth studies with *Scenedesmus obtusiusculus* in a flat-plate photobioreactor. Biotechnology and Bioengineering. 14(2): 308-320.
28. Chaudry S., Bahri P.A. and Moheimani. N.R. (2017). Superstructure optimization and energetic feasibility analysis of process of repetitive extraction of hydrocarbons from *Botryococcus braunii*–a species of microalgae. Computer and Chemical Engineering. 97: 36-46.
29. Béchet Q., Moussion P., Bernard O., (2017). Calibration of a productivity model for the microalgae *Dunaliella salina* accounting for light and temperature. Algal Research. ; 21: 156-160.
30. Andrade M.R. and Costa J.A. (2007). Mixotrophic cultivation of microalga *Spirulina platensis* using molasses as organic substrate. Aquaculture. 264(1):130-134.
31. Skorupskaite V., Makareviciene V. and Levisauskas D. (2015). Optimization of mixotrophic cultivation of microalgae *Chlorella* sp. for biofuel production using response surface methodology. Algal Research.; 7: 45-50.
32. Guldhe A., Ansari F.A., Singh P. and Bux F. (2017). Heterotrophic cultivation of microalgae using aquaculture wastewater: A biorefinery concept for biomass production and nutrient remediation. Ecological Engineering. 99: 47-53.
33. Pittman J.K., Dean A.P. and Osundeko O., (2011). The potential of sustainable algal biofuel production using wastewater resources. Bioresource Technology. 102(1): 17-25.
34. Gentili, F.G., Fick, J., (2016). Algal cultivation in urban wastewater: an efficient way to reduce pharmaceutical pollutants, *J. Appl. Phycol*., vol. 29, pp. 255-262.
35. Johnson T.J., Jahandideh A., Isaac I.C., Baldwin E.L., Muthukumarappan K., Zhou R. and Gibbons W.R. (2016). Determining the optimal nitrogen source for large-scale cultivation of filamentous cyanobacteria Journal Applied Phycology. 29: 1-13.
36. Wang L., Chen X., Wang H., Zhang Y., Tang Q. and Li J. (2017). *Chlorella vulgaris* cultivation in sludge extracts from 2, 4, 6-TCP wastewater treatment for toxicity removal and utilization. Journal of Environmental Management. 187: 146-153.
37. Şirin S., Trobajo R., Ibanez C. and Salvadó J. (2012).Harvesting the microalgae *Phaeodactylum tricornutum* with polyaluminum chloride, aluminium sulphate, chitosan and alkalinity-induced flocculation. Journal Applied Phycology. 24(5): 1067-1080.
38. Aléman-Nava G.S., Muylaert K., Bermudez S.P.C., Depraetere O., Rittmann B., Parra-Saldívar R., (2017). Vandamme D. Two-stage cultivation of *Nannochloropsis oculata* for lipid production using reversible alkaline flocculation. Bioresource Technology. 226:18-23.
39. Koley S., Prasad S., Bagchi S.K. and Mallick N. (2017). Development of a harvesting technique for large-scale microalgal harvesting for biodiesel production. RSC Advances. 7 (12): 7227-7237.
40. Razack S.A., Duraiarasan S., Shellomith A.S. and Muralikrishnan K. (2015). Statistical optimization of harvesting *Chlorella vulgaris* using a novel bio-source, *Strychnos potatorum*. Biotechnology Report. 7: 150-156.
41. Brady P.V., Pohl P.I. and Hewson J.C. (2014). A coordination chemistry model of algal autoflocculation. Algal Research. 5: 226-230.
42. Schlesinger A., Eisenstadt D., Bar-Gil A., Carmely H., Einbinder S. and Gressel J. (2012). Inexpensive non-toxic flocculation of microalgae contradicts theories; overcoming a major hurdle to bulk algal production. Biotechnology Advances. ; 30(5): 1023-1030.

43. Wu Z., Zhu Y., Huang W., Zhang C., Li T., Zhang Y. and Li A. (2012). Evaluation of flocculation induced by pH increase for harvesting microalgae and reuse of flocculated medium. Bioresource Technology; 110: 496-502.
44. Úbeda B., Gálvez J.Á., Michel M. and Bartual A. (2017). Microalgae cultivation in urban wastewater: *Coelastrum cf. pseudomicroporum* as a novel carotenoid source and a potential microalgae harvesting tool. Bioresource Technology. 228: 210-217.
45. Kothari R., Pathak V.V., Pandey A., Ahmad S., Srivastava C. and Tyagi, V.V. (2017). A novel method to harvest *Chlorella* sp. via low cost bioflocculant: Influence of temperature with kinetic and thermodynamic functions. Bioresource Technology. ; 225: 84-89.
46. Shin H., Kim K., Jung J.Y., Bai S.C. and Chang Y.K., Han J.I. (2017). Harvesting of *Scenedesmus obliquus* cultivated in seawater using electro-flotation. Korean Journal of Chemical . Engineering. 34(1): 62-65.
47. Hao W., Yanpeng L., Zhou S., Xiangying. R, Wenjun Z. and Jun L. (2017). Surface characteristics of microalgae and their effects on harvesting performance by air flotation International Journal of Agricultural and Biological Engineering.; 10(1): 125-133.
48. Rathore D., Nizami A.S., Singh A. and Pant, D. (2016). Key issues in estimating energy and greenhouse gas savings of biofuels: challenges and perspectives. Biofuels Research journal. 3(2): 380-393.
49. Bligh E.G. and Dyer W.J. (1959). A rapid method of total lipid extraction and purification. Canadian Journal Biochemistry Physiology. 37(8): 911-917.
50. Hossain A.S., Salleh A., Boyce A.N., Chowdhury P. and Naqiuddin M. (2008). Biodiesel fuel production from algae as renewable energy, American Journal of Biochemistry and Biotechnology. 4 (3): 250-254.
51. Iqbal J. and Theegala C. (2013).Microwave assisted lipid extraction from microalgae using biodiesel as co-solvent. Algal Research. 2 (1): 34-42.
52. Postma P.R., Pataro G., Capitoli M., Barbosa M.J., Wijffels R.H., Eppin M.H.M. and Ferrari G. (2016).Selective extraction of intracellular components from the microalga *Chlorella vulgaris* by combined pulsed electric field–temperature treatment. Bioresource Technology. 203: 80-88.
53. Yang S., Mariappan V., Won D.C., Ann M., Lee S.H. (2016).Design of ultra-sonication pre-treatment system for microalgae cell wall degradation, Journal of Advance and Smart Convergence. 5(2): 18-23.
54. Wu S., Song L., Sommerfeld M., Hu Q. and Chen W. (2017).Optimization of an effective method for the conversion of crude algal lipids into biodiesel. Fuel.197: 467-473.
55. Esquivel-Hernández D.A., Ibarra-Garza I.P., Rodríguez-Rodríguez. J., Cuéllar-Bermúdez S.P., Rostro-Alanis M.D.J., Alemán-Nava G.S. and Parra-Saldívar R. (2016). Green extraction technologies for high-value metabolites from algae: a reviewBiofuels, Bioproducts and Biorefining. 11(1): 215-231.
56. Angles E., Jaouen P., Pruvost J. and Marchal L. (2017).Wet lipid extraction from the microalga *Nannochloropsis* sp.: Disruption, physiological effects and solvent screening. Algal Research.; 21: 27-34.
57. Halim R., Rupasinghe T.W., Tull D.L. and Webley P.A. (2013).Mechanical cell disruption for lipid extraction from microalgal biomass. Bioresource Technology. 140: 53-63.
58. Prabakaran P. and Ravindran A.D. (2011).A comparative study on effective cell disruption methods for lipid extraction from microalgae. Letters in Applied Microbiology. 53 (2) : 150-154.
59. Yellapu S.K., Kaur R. and Tyagi R.D. (2017).Detergent assisted ultrasonication aided in situ transesterification for biodiesel production from oleaginous yeast wet biomass. Bioresource Technology. 224: 365-372.
60. Fu C.C., Hung T.C., Chen J.Y., Su C.H. and Wu W.T. (2010).Hydrolysis of microalgae cell walls for production of reducing sugar and lipid extraction. Bioresource Technology. 101(22): 8750-8754.
61. Zuorro A., Miglietta S., Familiari G. and Lavecchia R. (2016).Enhanced lipid recovery from *Nannochloropsis* microalgae by treatment with optimized cell wall degrading enzyme mixtures. Bioresource .Technology. 212: 35-41.
62. Wang D., Li Y., Hu X., Su W., Zhong M. (2015).Combined enzymatic and mechanical cell disruption and lipid extraction of green alga *Neochloris oleoabundans*. International Journal of Molecular Sciences. 16(4): 7707-7722.
63. Herrero M., Mendiola J.A., Cifuentes A. and Ibáñez E. (2010). Supercritical fluid extraction: recent advances and applications Journal of Chromatography A. 1217(16): 2495-2511.
64. Patil P.D., Reddy H., Muppaneni T. and Deng S. (2017). Biodiesel fuel production from algal lipids using supercritical methyl acetate (glycerin-free) technology. Fuel. 195: 201-207.
65. Lorenzen J., Igl N., Tippelt M., Stege A., Qoura F., Sohling U. and Brück T. (2017).Extraction of microalgae derived lipids with supercritical carbon dioxide in an industrial relevant pilot plant. Bioprocess and Biosystems Engineering. 40 (6): 911-918.
66. Chisti Y. (2017). Biodiesel from microalgae. Biotechnology Advances. 25(3): 294-306.
67. Makareviciene V., Gumbyte M., Skorupskaite V. and Sendzikiene E. (2017).Biodiesel fuel production by enzymatic microalgae oil transesterification with ethanol. Journal of Renewable and Sustainable Energy. 9(2): 023101, doi: http://dx.doi.org/10.1063/1.4978369
68. Shirazi H.M., Karimi-Sabet J. and Ghotbi C. (2017).Biodiesel production from *Spirulina* microalgae feedstock using direct transesterification near supercritical methanol condition. Bioresource Technology. doi: https://doi.org/10.1016/j.biortech.2017.04.073
69. Cerveró J.M., Coca J. and Luque, S. (2008).Production of biodiesel from vegetable oils. Grasas Aceites.; 59(1): 76-83.
70. Marchetti J.M., Miguel V.U. and Errazu A.F. (2007). Possible methods for biodiesel production. Renewable and Sustainable Energy Review. 11(6): 1300-1311.

71. Ishak Z.I., Sairi N.A., Alias Y., Aroua M.K.T. and Yusoff R. (2017).A review of ionic liquids as catalysts for transesterification reactions of biodiesel and glycerol carbonate production. Catalysis Reviews. 59(1): 44-93.
72. Sun Y., Cooke P., Reddy H.K., Muppaneni T., Wang J., Zeng Z. and Deng S. (2017).1-Butyl-3-methylimidazolium hydrogen sulfate catalyzed in-situ transesterification of *Nannochloropsis* to fatty acid methyl esters. Energy Conversion and Management. 132: 213-220.
73. COM 34 final. (2006).An EU strategy for biofuels, Commission of the European communities Brussels.
74. Sharma Y.C. and Singh B., (2009). Development of biodiesel: current scenario. Renewable and Sustainable Energy Review. 13(6):1646-1651
75. Su Y., Song K, Zhang P., Su Y., Cheng J. and Chen X. (2017). Progress of microalgae biofuel's commercialization, Renewable and Sustainable Energy Review. 74: 402-411.
76. Report of the Working Group on Petroleum & Natural Gas Sector for the XI Plan (2007–2012), MoPNG. (2006).
77. CII.(2010). Estimation of energy and carbon balance of biofuels in India. CII.
78. Mancheva M. (2014). Algae.Tec to back Indian biofuel project with stock sale.
79. Mookim B.S.S.(2012). Reliance Industries Credit Suisse.
80. Seth K. and Harish. (2016).Current status of potential applications of repurposed Cas9 for structural and functional genomics of plants. Biochemical and Biophysical Research Communications. 480 (4): 499-507.
81. Dinamarca J., Levitan O., Kumaraswamy G.K., Lun D.S. and Falkowski P. (2017).Overexpression of a diacylglycerol acyltransferase gene in *Phaeodactylum tricornutum* directs carbon towards lipid biosynthesis Journal of Phycology. 53(2): 405–414.
82. Szyjka S.J., Mandal S., Schoepp N.G., Tyler B.M., Yohn C.B., Poon Y.S. and Mayfield S.P., (2016).Evaluation of phenotype stability and ecological risk of a genetically engineered alga in open pond production. Algal Research. doi: https://doi.org/10.1016/j.algal.2017.04.006
83. Abomohra A.E.F., El-Sheekh M. and Hanelt, D. (2017).Screening of marine microalgae isolated from the hypersaline Bardawil lagoon for biodiesel feedstock. Renewable Energy. 101: 1266-1272.
84. Ho S.H., Chen Y.D., Chang C.Y., Lai Y.Y., Chen C.Y. , Kondo A. and Chang J.S., (2017).Feasibility of CO_2 mitigation and carbohydrate production by microalga *Scenedesmus obliquus* CNW-N used for bioethanol fermentation under outdoor conditions: effects of seasonal changes. Biotechnology for Biofuels. 10(1): 27.
85. Morschett H., Freier L., Rohde J., Wiechert W., Lieres E. and Oldiges M. (2017).A framework for accelerated phototrophic bioprocess development: integration of parallelized microscale cultivation, laboratory automation and kriging-assisted experimental design. Biotechnology for Biofuels. 10 (1): 26.
86. Bekirogullari M., Fragkopoulos I.S., Pittman J.K. and Theodoropoulos C. (2017).Production of lipid-based fuels and chemicals from microalgae: An integrated experimental and model-based optimization study, Algal Research., 23:78-87.
87. Tao Q., Gao F., Qian C.Y., Guo X.Z., Zheng Z. and Yang Z.H. (2017). Enhanced biomass/biofuel production and nutrient removal in an algal biofilm airlift photobioreactor. Algal Research. 21: 9-15.
88. Ansari F.A., Singh P., Guldhe A. and Bux, F. (2017). Microalgal cultivation using aquaculture wastewater: Integrated biomass generation and nutrient remediation. Algal Research. 21:169-177.
89. Lin T.S. and Wu J.Y. (2015).Effect of carbon sources on growth and lipid accumulation of newly isolated microalgae cultured under mixotrophic condition. Bioresource Technology. 184:100-107.
90. Deng M.D. and Coleman J.R. (1999). Ethanol synthesis by genetic engineering in cyanobacteria. Applied and Environmental Microbiology. 65: 523–528.
91. Haixing C., Qian F., Yun H., Ao X., Qiang L. and Xun Z. (2017).Improvement of microalgae lipid productivity and quality in an ion-exchange-membrane photobioreactor using real municipal wastewater. International Journal of Agricultural and Biological Engineering. 10(1): 97-106.
92. Happe T., Schutz K. and Bohme H. (2000).Transcriptional and mutational analysis of the uptake hydrogenase of the filamentous cyanobacterium *Anabaena variabilis* ATCC 29413 Journal of Bacteriology. 182: 1624-1631.
93. Liu X., Sheng J. and Curtiss III R. (2011),. Fatty acid production in genetically modified cyanobacteria. Proceedings of the National Academy of Sciences U.S.A. 108, (17): 6899-6904.
94. Radakovits R., Eduafo P.M. and Posewitz M.C. (2011).Genetic engineering of fatty acid chain length in *Phaeodactylum tricornutum*. Metabolic Engineering.; 13: 89-95.
95. Gao Z., Zhao H., Li Z., Tan X. and Lu X. (2012). Photosynthetic production of ethanol from carbon dioxide in genetically engineered cyanobacteria. Energy & Environmental Science. 5: 9857-9865.
96. Xue J., Niu Y.F., Huang T., Yang W.D., Liu J.S. and Li H.Y. (2015).Genetic improvement of the microalga *Phaeodactylum tricornutum* for boosting neutral lipid accumulation. Metabolic Engineering. 27: 1-9.
97. Work V.H., Radakovits R., Jinkerson R., Elliott L.G., Meuser J.E. and Posewitz M.C. (2016).Modified algae for improved biofuel productivity. Patent US 9290782 B2.
98. Eilenberg H., Weiner I., Ben-Zvi O., Pundak C., Marmari A., Liran O., Wecker M.S., Milrad Y. and Yacoby I. (2016).The dual effect of a ferredoxin-hydrogenase fusion protein in vivo: successful divergence of the photosynthetic electron flux towards hydrogen production and elevated oxygen tolerance. Biotechnology for biofuels. 9(1) 182, doi: 10.1186/s13068-016-0601-3.

