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# **Review Article**

# Values and Drawbacks of Biofuel Production From Microalgae

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#### Abstract

Increased energy consumption leads to a reduction in limited nonrenewable resources called fossil fuels. Due to this fact, researchers look for alternative energy sources to satisfy the need of the current generation without compromising the ability of future generations to meet their needs. As a result, nowadays the production of energy from biological sources is highly applicable and takes advantage of fossil fuel due to the limited impact on the environment. On the other hand, food cost, land use for some other purposes, and carbon emissions have risen due to the increased production of first-generation bioethanol. Even though the second-generation bioethanol from lignocellulose agricultural waste solved this problem, it again faced difficulties-to-overcome technological barriers. This, in turn, pushed researchers to come up with another alternative called the third-generation biofuel production. The renewed promise is held in microalgae biomass as an alternative feedstock. This review deals with the important aspects of biofuel production from algae as a renewable resource. The production processes and their merits and demerits of algae capacity in producing biofuel are also discussed.

Keywords: Algae, Biofuel, Energy, Biomass, Benefits, Limitations

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#### Introduction

The fast growth of the world population and economic growth are the two factors that led to increases in universal energy consumption.<sup>1-3</sup> In line with this issue, there is a strong incentive in the world to reduce carbon emission and to also find alternative energy sources.<sup>4</sup> According to the US Energy Information Administration (EIA),<sup>5</sup> world biofuel production will increase from approximately 1.3 million barrels per day in 2010 to approximately 3.0 million barrels per day in 2040.6 In order to meet the global energy consumption, Nonrenewable resources (also called fossil fuel) such as carbon, petroleum and natural gas are mainly used as an energy source. Fossil fuel, mainly used in the sector of transportation and electricity sector to satisfy the huge energy need for industrialization, economic growth, and rising population. In fact, nonrenewable resources are limited resources and which pose an adverse impact on the environment. As a result, researchers look at options for sustainable energy sources called renewable resources.7-9

Renewable energy sources like liquid biofuels from freshwater microalgal biomass of bioethanol and biodiesel from microalgae feedstock have become an important issue related to the generation of alternative energy.<sup>10</sup> Microalgae is one of the best alternatives,11 also reserves the carbohydrate in the form of starch and cellulose in addition to lipids, which can be utilized for bioethanol production. Scenedesmus sp. is the eminent microalgae which has the capacity to accrue both lipid and starch, which are the valued sources for the production of bioethanol and biodiesel. The type of carbohydrates in microalgae biomass is mainly divided into starch and cellulose and no lignin. So, they can easily hydrolyze to monosaccharide compared to other lignocellulosic materials.<sup>12</sup> Some components like starch and cellulose presented in microalgae are cannot easily fermentable to produce bioethanol. Hence, the e pre-treatment processes which includes chemical and enzymatic hydrolysis are mandatory.<sup>13,14</sup> The cost of pre-treatment significantly affects the total cost of biomass conversion processes up to 30%.<sup>15</sup> In line with this, the pretreatment process greatly contributes in converting biomass to fermentable sugar.

Bioenergy produced by the trans-esterification process from renewable resources such as vegetable oil, animal fat and biomass decreases harmful emission combustion engine which is generated during energy produced from fossil fuel.<sup>16</sup> The common biodiesel feedstock's includes soybean oil, sunflower oil, peanut oil, corn oil, rice bran

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oil, palm oil, coconut oil, olive oil, rapeseed oil, jatropha, karanja, cottonseed oil, calophyllum, rubber seed oil, desert date, jojoba, neem oil, moringa and croton.<sup>17</sup> Based on their sources, biodiesel is grouped into three generations. First-generation biodiesel, which is produced from edible oil, second-generation biodiesel, which is produced from non-edible oil and third-generation biodiesel, is produced from microalgal biomass. First-generation biodiesel has effects on the environment and climate change as well as food. This led to discovering the potential of the second generation and third generation biodiesel.<sup>18</sup> The third generation biodiesel is a sustainable alternative fuel and has also many benefits to the world's energy infrastructure requirement.

The energy produced from fossil fuel is potentially substituted by bioenergy, which is produced from biological sources or renewable resources. Renewable energy that helps the production of bioenergy from biological sources where biomass is directly used and converted into liquid or gas form.<sup>19</sup> Bioenergy has less impact on climate change, is ecofriendly to the environment and sustainable resources. Biofuel is generated by many processes such as trans-esterification, fermentation, gasification and etc.20 The first-generation biofuel has reduced greenhouse gas emission, although has led to a shortage of food.<sup>21</sup> Second-generation biofuel is produced from non-food crops, which can overcome the intensifying food price concern, but became undesirable due to unsustainability and low yield of product.<sup>22</sup> As a result, the third-generation biofuel was developed in order to solve the problem that occurred during the first and second-generation biofuel. Therefore, the production of biofuel from algae is highly favorable unlike that of terrestrial plants. Additionally, it does not lead to an increase in the price of food cost and also has a lower impact on biodiversity and the environment.<sup>19,23</sup> This review focuses on the possible processes to produce bioethanol from microalgae. Also, the overall merits and demerits of bioethanol production from microalgae are discussed in this review as well.

#### **Algal Biomass as Potential Resources**

Algae are included under the kingdom Protista, which is a photosynthetic aquatic organism. They are grouped into two categories, microalgae and macroalgae, which are in unicellular mode and multicellular mode, respectively. Both have chlorophyll as a major photosynthetic pigment in order to fix atmospheric carbon dioxide through the photosynthesis process. Like a terrestrial plant, macroalgae have a true stem, leaves, and root, whereas microalgae do not have a true stem, leaves, and root. Marine algae are found on the sea, whereas fresh algae are found in ponds, lakes, rivers, and reservoirs. Surrounding temperature, amount of sunlight and nutrient, highly affect the growth of algae.<sup>24</sup>

Algal biomass is used to produce useful compounds, which include proteins, steroids, vitamins, carbohydrates, polysaccharides, and fatty acids that have valuable applications for the human being.<sup>25</sup> Additionally, algae have the ability to substitute synthetic compounds as a result of having the property such as anti-aging, anti-obesity and anti-oxidant.<sup>26</sup> Unlike a terrestrial plant, algae have a higher growth rate

and attain complete life cycle in short period of time. Due to this fact, there is a high bioenergy production from algae. Microalgae contain high lipid and carbohydrate content used for the production of biofuel.<sup>27</sup> Carbon dioxide, light, and water are required for the growth of algae and the generation of renewable energy. The schematic illustration of the possible biofuel production from algae is depicted in Figure 1.

### **Biofuel Production Sources**

Biofuel productions are categorized into different generations based on the type of raw materials used in the process (Figure 2). Mainly, there are two types of biofuel production ranges. Primary biofuel production has many drawbacks as compared to secondary biofuel production. The main reasons behind searching for the third generation are food security and energy efficiency.<sup>30</sup>

#### Microalgae

The algae, which is observed under a microscope is called microalgae. It requires sunlight, water, and nutrients such as carbon dioxide, nitrogen, and phosphorus for its growth. Based on species variety the composition of microalgae is different. Algae consist of chemical compounds such as lipid, carbohydrates, protein, and nucleic acid. The lipid content of algae is directly related to the yield of total biomass. Carbohydrate content also helps to produce biofuel such as bioethanol and biobutanol.<sup>31</sup> Because of low lignin content, the carbohydrate produced from algae is highly fermentable.<sup>32</sup>

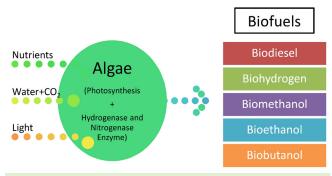
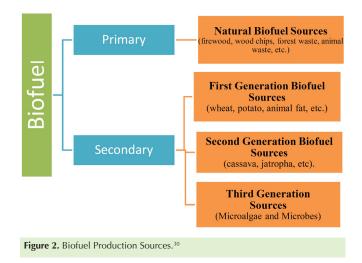


Figure 1. Possible Biofuel Production Mechanism From Microalgae.<sup>28,29</sup>



Many forms such as starch, paramylon, cellulose, and laminarin are stored carbohydrate in algae as energy reservoirs. Sulfonated polysaccharides and rigid, cellulosereinforced are mainly found in red algae and green algae, respectively.<sup>33</sup> Lipids present two forms in microalgae, such as polar or structural lipid and non-polar or neutral lipids. Polar lipids help to make the structure of cells and serve as food supplements, whereas non-polar lipid functions as energy reservoirs, mainly triacylglycerides (TAG). Neutral lipid is highly exploited for the production of biodiesel. The microalgae that contain high lipid content which are used for the production of biodiesel include species of the genera *Botryococcus, Chlorella, Nannochloropsis, Scenedesmus, Neochloris, Phaeodactylum,* and *Dunaliella* are listed in Table 1.

The lipids extracted after microalgal farming can be utilized for the manufacture of biofuels and in certain cases, the use of wastewater and flue gases for the farming of lipid-rich microalgae importantly increases the economics of the process and offer cost modest biofuels.<sup>19,45</sup> The degree of transformation of the feedstock is signified as a percentage called the biodiesel conversion rate. The acid value of the product regulates the value of biodiesel, whereby the best quality biodiesel is specified by the lower acid value.<sup>19</sup> The transesterification of microalgal lipids to biomethane using anaerobic digestion process are summarized in Table 2.

#### Macroalgae

Seaweed mostly found in marine water are called macroalgae which require a nutrient supply for their growth. Macroalgae also found in freshwater, which are highly filamentous in nature, are used for the production of biofuel.<sup>51</sup> Macroalgae are

Table 1. Lipid Content of Selected Microalgae used for Biofuel Production

Microalgae Species	Lipid Content (%)	Reference
Botryococcus braunii	65-70	34
Botryococcus spp.	28.6	35
Chlorella vulgaris	55.9	36
Dunaliella tertiolecta	70.6-71.4	37
Isochrysis zhangjiangensis	53	38
Monoraphidium spp.	51.72	39
Nannochloropsis spp.	59.9	40
Neochloris oleabundans	56	41
Porphyridium cruentum	8	42
Scenedesmus spp.	53	43
Tetraselmis subcordiformis	29.77	44

 $\ensuremath{\text{Table 2.}}$  The Yield of Biomethane from Algae as Feedstock Through Anaerobic Digestion

Algae Species	Biomethane Yield	Reference
Chlorella vulgaris	240 mL/g volatile suspended solid	46
Microcystis sp.	140.48 mL/g volatile solid	47
Phaeodactylum tricornutum	0.36 L CH₄/g volatile solid	48
Scenedesmus sp AMDD	410 mL CH <sub>4</sub> /g total volatile solid	49
Pavlova cf. sp	0.51 L CH₄/g volatile solid	50

categorized into the three following groups: red (Rhodophyta), brown (Phaeophyceae) and green (Chlorophyta). Macroalgae composition differs based on species variety. Carbohydrate content is the major composition of macroalgae. In red algae, galactose-based polyglucans such as agar and carrageenan are majorly observed. A type of algae that contains alginate and a large amount of laminarin is called brown algae, whereas green algae are predominantly starch and cellulose.<sup>52</sup> Biofuel is produced from macroalgae carbohydrate, which is also used as a component for pharmaceuticals. Whereas, low lipid content in macroalgae, that yields lower production of biofuel irrespective to the carbohydrate contents. Due to high carbohydrate and low lipid (fatty acids) contents, macroalgae are not favorable for biodiesel production as compared to microalgae. Additionally, macroalgae contain high ash over microalgae.53

# The Production Processes and Extraction of Bioethanol from Microalgae

Biofuel production from microalgae involves many processes from raw material selection up to purification of the yield. Also, there are so many methods involved in bioethanol production from microalgae.<sup>54,55</sup> Figure 3 shows the methods used during the different periods of time for the production of bioethanol.

There are different ways to extract oil/lipids from microalgae including the mechanical/physical and chemical process of oil extraction under conventional methods. The mechanical/physical process are grouped in many ways, such as oil expeller, microwave-assisted extraction, and ultrasonic-assisted extraction process. In the same fashion, the chemical extraction method is classified as accelerated solvent extraction, supercritical fluid extraction, and soxhlet extraction process.<sup>56</sup> The merits and demerits of the mechanical and chemical methods of oil extraction are illustrated in Figure 4.

## **Biodiesel Production from Microalgae**

The transesterification process is a well-known method used

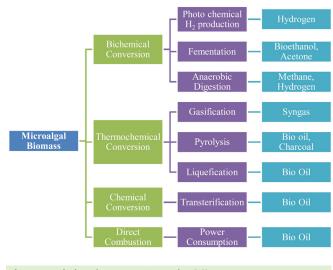
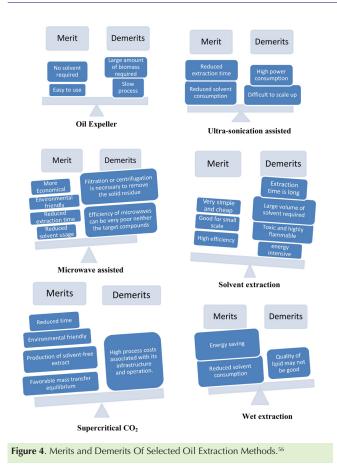


Figure 3. Biofuel Production From Microalgae<sup>54,55</sup>



to change crude oil into methyl ester. With the addition of a catalyst, the trans-esterification process has some consecutive reactions between vegetable oil and alcohol. Triglycerides are changed into monoglycerides during the trans-esterification process. The detail of the biodiesel production process from microalgae has been described by Haik et al.<sup>57</sup> Initially preheated crude oil with 60°C was added to the mixture of methanol (20% of total crude oil) and sodium hydroxide (3.50 g) for one liter of oil. The mixture was kept for 8 hours in order to settle on the trans-esterification reaction reactor and the glycerin was removed. The produced biodiesel was washed and filtered with appropriate techniques.<sup>58</sup> The production process of biodiesel flowchart is illustrated in Figure 5. It is necessary to check the properties of produced biodiesel before it is used in the engine. The essential fuel features of the produced biodiesel are described in Table 3.

# Advantages and Disadvantages of Microalgae Based Biofuel

Biofuel produced from algae, is a third-generation biofuel. This energy is considered as the best fuel that can reduce the use and need of non-renewable energy sources and can also minimize the number of greenhouse gases that are responsible for global warming. It is also believed that biomass production from microalgae has a great contribution in bringing nonpolluting energy. There are also some drawbacks that should be considered in producing bioethanol from algae. The summary of selected advantages and disadvantages are illustrated in Figure 6.

Microalgae have expressively rapid growth than terrestrial

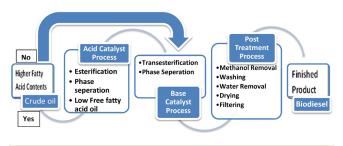


Figure 5. The Flowchart for the Biodiesel Production Process<sup>56,58</sup>

Table 3. The Properties of Biodiesel Produced from Microalgae

Fuel Property (Unit)	Microalgae Biodiesel	Reference
Cetane number	46.5	56,59
Acid value (mg KOH/g)	0.14	
Cloud point (°C)	16.1	
Density @15°C (kg/L)	0.912	
Flash point (closed cup) (°C)	95.0	
Higher heating value (MJ/kg)	39.86	
Kinematic Viscosity @40°C (mm²/s)	5.06	
Lower heating value (MJ/kg)	37.42	
Lubricity @°60 (mm)	0.136	
Sulphur content (mg/kg)	7.5	

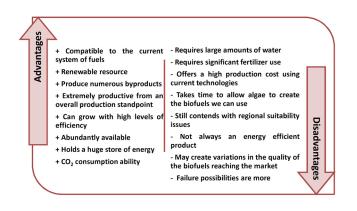


Figure 6. Selected Advantages and Disadvantages of Algae-Based Biofuel  $\ensuremath{\mathsf{Production}}$ 

crops and it is reported that the per unit area yield of oil from algae is projected to be from 20 000 to 80 000 per acre in 2017, which is 7-31 times larger than the next best crop, palm oil. Researchers consider that an acre of algae can yield about 5000 gallons of biodiesel, where an acre of corn can only yield 500 gallons of ethanol.<sup>60</sup> A study conducted in 2009 has reported that algae growth supplied fuel at a rate that would yield 1200 gal/acre/year, if constant for a year.<sup>61</sup> In addition, the algae do not require potable water or arid land for its growth unlike other oil crops and will not occupy the resources used for growing food.<sup>53</sup> However, the space and water needed to grow these algae are significant in order to grow them on a large scale. Moreover, the algae segregate the  $CO_2$  from the atmosphere and remove the nitrogen from wastewater, but the level of  $CO_2$  in the atmosphere may not be high enough to

spur exponential algal growth. So, there is a need for intensive ways to provide a mass level of nutrients, such as sulfur, iron and nitrogen and  $CO_2$  for the prolonged and high yield of algae in a controlled environment.<sup>60,62</sup>

### Conclusions

The conventional method of diesel production from nonrenewable resources increases emissions of gases and has an impact on the environment. Moreover, the production of diesel from edible crops has also led to food scarcity. In order to tackle those problems associated with first and secondgeneration biofuels, using third-generation feedstock for biofuel production is highly important due to its capacity for producing high-quality biofuel, outstanding productivity, and reduced environmental impact. Nowadays, the production of bioenergy from microalgae has become of great importance all over the world. The extraction process of biofuel from microalgae oil is similar to that of any other oilseed. The lipid content of microalgae produces more biofuel as compared to that of the carbohydrate content in microalgae. Finally, further investigation of microalgae biodiesel is required in order to create new techniques that could decrease the costs of biofuel production.

#### Authors' Contributions

DG, KIM, and GG contributed equally to the writing of the article. KAM designed the figure and paper structure, and contributed to the editing and revising of the article.

#### **Conflict of Interest Disclosures**

The authors declare they have no conflicts of interest.

#### References

- Kim I, Seo YH, Kim GY, Han JI. Co-production of bioethanol and biodiesel from corn stover pretreated with nitric acid. Fuel. 2015;143:285-289. doi:10.1016/j.fuel.2014.11.031.
- Pejin JD, Mojović LV, Pejin DJ, et al. Bioethanol production from triticale by simultaneous saccharification and fermentation with magnesium or calcium ions addition. Fuel. 2015;142:58-64. doi:10.1016/j.fuel.2014.10.077.
- 3. Imamoglu E, Sukan FV. The effects of single and combined cellulosic agrowaste substrates on bioethanol production. Fuel. 2014;134:477-484. doi:10.1016/j.fuel.2014.05.087.
- 4. Vassilev SV, Vassileva CG. Composition, properties and challenges of algae biomass for biofuel application: an overview. Fuel. 2016;181:1-33. doi:10.1016/j.fuel.2016.04.106.
- Ullah K, Ahmad M, Sofia, et al. Assessing the potential of algal biomass opportunities for bioenergy industry: a review. Fuel. 2015;143:414-423. doi:10.1016/j.fuel.2014.10.064.
- U.S. Energy Information Administration (EIA). 2017. Annual Energy Outlook report. https://www.eia.gov/pressroom/presentations/ sieminski\_01052017.pdf.
- Bastos RG. Biofules form microalgae: bioethanol. In: Jacob-Lopes E, Zepka LQ, Queiroz MI, eds. Energy from Microalgae. Cham: Springer International Publishing AG; 2018. doi:10.1007/978-3-319-69093-3.
- Mahmudul HM, Hagos FY, Mamat R, Adam AA, Ishak WFW, Alenezi R. Production, characterization and performance of biodiesel as an alternative fuel in diesel engines–a review. Renew Sustain Energy Rev. 2017;72:497-509. doi:10.1016/j. rser.2017.01.001.
- Srithar K, Arun Balasubramanian K, Pavendan V, Ashok Kumar B. Experimental investigations on mixing of two biodiesels blended with diesel as alternative fuel for diesel engines. J. King Saud Univ

Eng Sci. 2017;29(1):50-56. doi:10.1016/j.jksues.2014.04.008.

- Rahman MM, Rasul MG, Hassan NMS, Azad AK, Uddin MN. Effect of small proportion of butanol additive on the performance, emission, and combustion of Australian native first- and secondgeneration biodiesel in a diesel engine. Environ Sci Pollut Res Int. 2017;24(28):22402-22413. doi:10.1007/s11356-017-9920-6.
- Kim HM, Lee YG, Patel DH, Lee KH, Lee DS, Bae HJ. Characteristics of bifunctional acidic endoglucanase (Cel5B) from Gloeophyllum trabeum. J Ind Microbiol Biotechnol. 2012;39(7):1081-1089. doi:10.1007/s10295-012-1110-4.
- Ho SH, Huang SW, Chen CY, Hasunuma T, Kondo A, Chang JS. Bioethanol production using carbohydrate-rich microalgae biomass as feedstock. Bioresour Technol. 2013;135:191-198. doi:10.1016/j.biortech.2012.10.015.
- Gírio FM, Fonseca C, Carvalheiro F, Duarte LC, Marques S, Bogel-Łukasik R. Hemicelluloses for fuel ethanol: a review. Bioresour Technol. 2010;101(13):4775-4800. doi:10.1016/j. biortech.2010.01.088.
- 14. Hahn-Hägerdal B, Karhumaa K, Fonseca C, Spencer-Martins I, Gorwa-Grauslund MF. Towards industrial pentose-fermenting yeast strains. Appl Microbiol Biotechnol. 2007;74(5):937-953. doi:10.1007/s00253-006-0827-2.
- 15. Talebnia F, Karakashev D, Angelidaki I. Production of bioethanol from wheat straw: an overview on pretreatment, hydrolysis and fermentation. Bioresour Technol. 2010;101(13):4744-4753. doi:10.1016/j.biortech.2009.11.080.
- Mofijur M, Masjuki HH, Kalam MA, et al. Properties and use of Moringa oleifera biodiesel and diesel fuel blends in a multicylinder diesel engine. Energy Convers Manag. 2014;82:169-176. doi:10.1016/j.enconman.2014.02.073.
- Imdadul HK, Zulkifli NW, Masjuki HH, et al. Experimental assessment of non-edible candlenut biodiesel and its blend characteristics as diesel engine fuel. Environ Sci Pollut Res Int. 2017;24(3):2350-2363. doi:10.1007/s11356-016-7847-y.
- Eisentraut A. Sustainable Production of Second-Generation Biofuels: Potential and Perspectives in Major Economies and Developing Countries. Paris: OECD Publishing; 2010. doi:10.1787/5kmh3njpt6r0-en.
- Chia SR, Ong HC, Chew KW, et al. Sustainable approaches for algae utilisation in bioenergy production. Renew Energy. 2018;129(Pt B):838-852. doi:10.1016/j.renene.2017.04.001.
- Kwon EE, Jeon YJ, Yi H. New candidate for biofuel feedstock beyond terrestrial biomass for thermo-chemical process (pyrolysis/ gasification) enhanced by carbon dioxide (CO2). Bioresour Technol. 2012;123:673-677. doi:10.1016/j.biortech.2012.07.035.
- Raghavi S, Sindhu R, Binod P, Gnansounou E, Pandey A. Development of a novel sequential pretreatment strategy for the production of bioethanol from sugarcane trash. Bioresour Technol. 2016;199:202-210. doi:10.1016/j.biortech.2015.08.062.
- 22. Ho DP, Ngo HH, Guo W. A mini review on renewable sources for biofuel. Bioresour Technol. 2014;169:742-749. doi:10.1016/j. biortech.2014.07.022.
- Quinn JC, Hanif A, Sharvelle S, Bradley TH. Microalgae to biofuels: life cycle impacts of methane production of anaerobically digested lipid extracted algae. Bioresour Technol. 2014;171:37-43. doi:10.1016/j.biortech.2014.08.037.
- Zhang W, Zhao Y, Cui B, Wang H, Liu T. Evaluation of filamentous green algae as feedstocks for biofuel production. Bioresour Technol. 2016;220:407-413. doi:10.1016/j.biortech.2016.08.106.
- Wang HD, Chen CC, Huynh P, Chang JS. Exploring the potential of using algae in cosmetics. Bioresour Technol. 2015;184:355-362. doi:10.1016/j.biortech.2014.12.001.
- Varshney P, Mikulic P, Vonshak A, Beardall J, Wangikar PP. Extremophilic micro-algae and their potential contribution in biotechnology. Bioresour Technol. 2015;184:363-372. doi:10.1016/j.biortech.2014.11.040.
- 27. Yoo C, Jun SY, Lee JY, Ahn CY, Oh HM. Selection of microalgae for lipid production under high levels carbon dioxide.

Bioresour Technol. 2010;101 Suppl 1:S71-74. doi:10.1016/j. biortech.2009.03.030.

- 28. Converti A, Casazza AA, Ortiz EY, Perego P, Del Borghi M. Effect of temperature and nitrogen concentration on the growth and lipid content of Nannochloropsis oculata and Chlorella vulgaris for biodiesel production. Chem Eng Process. 2009;48(6):1146-1151. doi:10.1016/j.cep.2009.03.006.
- 29. Rodionova MV, Poudyal RS, Tiwari I, et al. Biofuel production: challenges and opportunities. Int J Hydrogen Energy. 2017;42(12):8450-8461. doi:10.1016/j.ijhydene.2016.11.125.
- Alam F, Date A, Rasjidin R, Mobin S, Moria H, Baqui A. Biofuel from algae-is it a viable alternative? Procedia Eng. 2012;49:221-227. doi:10.1016/j.proeng.2012.10.131.
- Chen CY, Zhao XQ, Yen HW, et al. Microalgae-based carbohydrates for biofuel production. Biochem Eng J. 2013;78:1-10. doi:10.1016/j.bej.2013.03.006.
- 32. Strickberger MW. Evolution. 3rd ed. Sudbury, USA: Jones & Bartlett Publishers; 2005.
- Huang H, Yuan X, Zeng G, et al. Thermochemical liquefaction characteristics of microalgae in sub-and supercritical ethanol. Fuel Processing Technology. 2011;92(1):147-153. doi:10.1016/j. fuproc.2010.09.018.
- 34. Cheng P, Ji B, Gao L, Zhang W, Wang J, Liu T. The growth, lipid and hydrocarbon production of Botryococcus braunii with attached cultivation. Bioresour Technol. 2013;138:95-100. doi:10.1016/j. biortech.2013.03.150.
- Anjos M, Fernandes BD, Vicente AA, Teixeira JA, Dragone G. Optimization of CO(2) bio-mitigation by Chlorella vulgaris. Bioresour Technol. 2013;139:149-154. doi:10.1016/j.biortech.2013.04.032.
- Feng Y, Li C, Zhang D. Lipid production of Chlorella vulgaris cultured in artificial wastewater medium. Bioresour Technol. 2011;102(1):101-105. doi:10.1016/j.biortech.2010.06.016.
- Takagi M, Watanabe K, Yamaberi K, Yoshida T. Limited feeding of potassium nitrate for intracellular lipid and triglyceride accumulation of Nannochloris sp. UTEX LB1999. Appl Microbiol Biotechnol. 2000;54(1):112-117. doi:10.1007/s002530000333.
- Feng D, Chen Z, Xue S, Zhang W. Increased lipid production of the marine oleaginous microalgae Isochrysis zhangjiangensis (Chrysophyta) by nitrogen supplement. Bioresour Technol. 2011;102(12):6710-6716. doi:10.1016/j.biortech.2011.04.006.
- 39. Zhao P, Yu X, Li J, Tang X, Huang Z. Enhancing lipid productivity by co-cultivation of Chlorella sp. U4341 and Monoraphidium sp. FXY-10. J Biosci Bioeng. 2014;118(1):72-77. doi:10.1016/j. jbiosc.2013.12.014.
- Jiang L, Luo S, Fan X, Yang Z, Guo R. Biomass and lipid production of marine microalgae using municipal wastewater and high concentration of CO(2). Appl Energy. 2011;88(10):3336-3341. doi:10.1016/j.apenergy.2011.03.043.
- Gouveia L, Marques AE, da Silva TL, Reis A. Neochloris oleabundans UTEX #1185: a suitable renewable lipid source for biofuel production. J Ind Microbiol Biotechnol. 2009;36(6):821-826. doi:10.1007/s10295-009-0559-2.
- 42. Biller P, Ross AB. Potential yields and properties of oil from the hydrothermal liquefaction of microalgae with different biochemical content. Bioresour Technol. 2011;102(1):215-225. doi:10.1016/j.biortech.2010.06.028.
- 43. Xin L, Hu HY, Ke G, Sun YX. Effects of different nitrogen and phosphorus concentrations on the growth, nutrient uptake, and lipid accumulation of a freshwater microalga Scenedesmus sp. Bioresour Technol. 2010;101(14):5494-5500. doi:10.1016/j. biortech.2010.02.016.
- Huang X, Huang Z, Wen W, Yan J. Effects of nitrogen supplementation of the culture medium on the growth, total lipid content and fatty acid profiles of three microalgae (Tetraselmis subcordiformis, Nannochloropsis oculata and Pavlova viridis). J Appl Phycol. 2013;25(1):129-137. doi:10.1007/s10811-012-9846-9.

- 45. Harwood JL, Guschina IA. The versatility of algae and their lipid metabolism. Biochimie. 2009;91(6):679-684. doi:10.1016/j. biochi.2008.11.004.
- Ras M, Lardon L, Bruno S, Bernet N, Steyer JP. Experimental study on a coupled process of production and anaerobic digestion of Chlorella vulgaris. Bioresour Technol. 2011;102(1):200-206. doi:10.1016/j.biortech.2010.06.146.
- Zeng S, Yuan X, Shi X, Qiu Y. Effect of inoculum/substrate ratio on methane yield and orthophosphate release from anaerobic digestion of Microcystis spp. J Hazard Mater. 2010;178(1-3):89-93. doi:10.1016/j.jhazmat.2010.01.047.
- Zamalloa C, Boon N, Verstraete W. Anaerobic digestibility of Scenedesmus obliquus and Phaeodactylum tricornutum under mesophilic and thermophilic conditions. Appl Energy. 2012;92:733-738. doi:10.1016/j.apenergy.2011.08.017.
- Frigon JC, Matteau-Lebrun F, Hamani Abdou R, McGinn PJ, O'Leary SJB, Guiot SR. Screening microalgae strains for their productivity in methane following anaerobic digestion. Appl Energy. 2013;108:100-107. doi:10.1016/j.apenergy.2013.02.051.
- Bohutskyi P, Betenbaugh MJ, Bouwer EJ. The effects of alternative pretreatment strategies on anaerobic digestion and methane production from different algal strains. Bioresour Technol. 2014;155:366-372. doi:10.1016/j.biortech.2013.12.095.
- 51. Zhao P, Yu X, Li J, Tang X, Huang Z. Enhancing lipid productivity by co-cultivation of Chlorella sp. U4341 and Monoraphidium sp. FXY-10. J Biosci Bioeng. 2014;118(1):72-77. doi:10.1016/j. jbiosc.2013.12.014.
- Takagi M, Karseno, Yoshida T. Effect of salt concentration on intracellular accumulation of lipids and triacylglyceride in marine microalgae Dunaliella cells. J Biosci Bioeng. 2006;101(3):223-226. doi:10.1263/jbb.101.223.
- Milledge JJ, Heaven S. A review of the harvesting of micro-algae for biofuel production. Rev Environ Sci Biotechnol. 2013;12(2):165-178. doi:10.1007/s11157-012-9301-z.
- Dragone G, Fernandes B, Vicente AA, Teixeira JA. Third generation biofuels from microalgae. In: Méndez-Vilas A, ed. Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology. Badajoz, Spain: Formatex; 2010:1355-1366.
- Wang B, Li Y, Wu N, Lan CQ. CO(2) bio-mitigation using microalgae. Appl Microbiol Biotechnol. 2008;79(5):707-718. doi:10.1007/s00253-008-1518-y.
- Mofijur M, Rasul MG, Hassan NMS, Nabi MN. Recent development in the production of third generation biodiesel from microalgae. Energy Procedia. 2019;156:53-58. doi:10.1016/j. egypro.2018.11.088.
- Haik Y, Selim MYE, Abdulrehman T. Combustion of algae oil methyl ester in an indirect injection diesel engine. Energy. 2011;36(3):1827-1835. doi:10.1016/j.energy.2010.11.017.
- Galadima A, Muraza O. Biodiesel production from algae by using heterogeneous catalysts: a critical review. Energy. 2014;78:72-83. doi:10.1016/j.energy.2014.06.018.
- Islam MA, Rahman MM, Heimann K, et al. Combustion analysis of microalgae methyl ester in a common rail direct injection diesel engine. Fuel. 2015;143:351-360. doi:10.1016/j.fuel.2014.11.063.
- Frank B. The Challenges of Producing Biofuel from Algae. Stanford University; 2017. http://large.stanford.edu/courses/2017/ph240/ buncom2/.
- Woertz I, Feffer A, Lundquist T, Nelson Y. Algae grown on dairy and municipal wastewater for simultaneous nutrient removal and lipid production for biofuel feedstock. J Environ Eng. 2009;135(11):1115-1122. doi:10.1061/(ASCE)EE.1943-7870.0000129.
- 62. Hannon M, Gimpel J, Tran M, Rasala B, Mayfield S. Biofuels from algae: challenges and potential. Biofuels. 2010;1(5):763-784. doi:10.4155/bfs.10.44.