

Development of biofuels as an alternative source to petroleum

Abstract

Biofuel is a type of renewable energy created from living materials, as opposed to fossil fuels such as petroleum, coal, and natural gas, which are formed through slow natural processes. Biofuels can be liquid, gaseous and solid. Biofuel is commonly advocated as a cost effective and environmentally benign alternative to petroleum and other fossil fuels. Liquid biofuels are particularly appealing due to the extensive infrastructure already in place to support their use, particularly in transportation. The most common liquid biofuel is ethanol, which is produced by fermenting starch or sugars. Biodiesel is the second most prevalent liquid biofuel, and it's manufactured mostly from oily plants (such palm or soybean oil) and to a lesser amount from other oily sources. Biodiesel is used in diesel engines and is usually blended with petroleum diesel fuel in varying quantities. Some algae species have up to 40% lipids by weight, which can be used to make biodiesel or synthetic petroleum. This review described the four different types of biofuels, their advantages and disadvantages. In addition, to their economic and environmental considerations.

Keywords: biofuels, biomass, biodiesel, ethanol, feedstock, sustainability, biofuel generations.

Introduction

The combustion of fossil fuels leads to the emission of greenhouse gases (GHGs) that contributes to global warming and is associated with negative impacts to the ecosystem [1] (Menne et al., 2018). Depleting fossil fuel reserves and developing request for energy has necessitated the search for alternative energy resources from living materials such as microorganisms, plants and animals [2] (Keasling et al., 2021). Biofuel has developed as an elective source of energy to diminish the outflows of greenhouse gasses within the air and combat worldwide warming [3] (US-EPA, 2020). Biofuels are classified into first, second, third and fourth generations. Each of the biofuel generations points to meet the worldwide energy request whereas minimizing environmental impacts. Biofuels are an alternative to fossil fuels, which are liquid or gaseous fuels that are derived from biomass sources. Biofuels are produced from the fermentation of biological feedstocks, containing fermentable sugars, lipids, or carbohydrates. This is done by converting the biomass of the feedstocks into different forms of energy such as heat, electricity, biogas, and liquid fuels [4] (Gomez et al., 2008). Biofuels can be utilized alone or in combination with other fossil fuels. The first generation biofuels are bioethanol and biodiesel produced from starch and sugars and from seed oils respectively. The direct use of vegetable oils can prove harmful for the diesel engines due to their high viscosity, high density and various other problems that are related to them. So there is a need of converting these sources into biodiesel so that it can be used as a substitution for petroleum based diesel by a process known as bio-trans-esterification [5,6] (Kulkarni et al., 2006; Meher et al., 2006). The second generation biofuels used cellulosic raw materials that should grow on land that cannot be used to grow food. However, the third and fourth biofuel generations are

defined as types of biofuels that produced from algal biomass and specially designed engineered microorganisms and plants respectively [7] (Masjuki et al., 2013) This review article deals with the different generations types of biofuels and the conversion of non-edible and vegetable oils to biodiesel by bio-transesterification process as well as the conversion of sugars to bioethanol by optimization of the medium and substrates required for ethanol production or by the genetic modification of yeast cells.

Types of biofuels

The solution to avoid the disadvantages of fossil fuels is to turn to clean energy technologies such as biofuels. Biofuels exist in a variety of types and are used to suit a variety of energy demands. The following are the four common generation types of biofuels (Table 1).

First generation: The first generation biofuels includes bioethanol and biodiesel produced from starch, sugars and seed oils, in addition to biogas which produced by anaerobic bacterial fermentation of organic materials [8] (Zhu et. al., 2008). Biogas is known to be a low carbon fuel source providing rural communities the best ways to meet their energy demand. In this respect, the direct use of vegetable oils can prove harmful for the diesel engines due to their high viscosity, high density and various other problems that are related to them. So there is a need of converting these sources into biodiesel so that it can be used as a substitution for petroleum based diesel by a process known as trans-esterification. Biodiesel is the second most prevalent liquid biofuel, and it's manufactured mostly from oily plants (such palm or soybean oil) and to a lesser amount from other oily sources [9] (Chew and Bhatia, 2008). The first generation differs from the second one biofuels in that their contents produced from plant crops origin and represent the bulk of biofuels currently in use. The main drawback of 1st generation biofuels is that they come from biomass that is also a food source [10] (Naik et al. 2010). Ethanol is generally produced on a large scale by the fermentation process of the six-carbon atoms sugars, such as glucose using the genetically modified yeast strain *Saccharomyces cerevisiae* [11,12] (Zhou et al., 2018; McAloon et al., 2000). Other different feedstocks such as sugarcane, corn, barley, sugarbeets and potato wastes are also used. Biodiesel is another biofuel produced on an industrial scale. Its production differs in ethanol one in that after extracting the oils there is a need to methanol to convert the long chain fatty acids to glycerol by a transesterification process.

Second generation: This type of biofuels is made from different organic feedstock (biomasses), lignocellulosic biomass or woody crops, agricultural forest residues, municipal solid wastes or waste plant materials. The raw material used to create second generation biofuels should grow on land that cannot be used to grow food successfully [13, 14] (Kalnes et al., 2007; Stevens and Verhe, 2004). The process of this biomass is complex and also depends on various technologies [15, 16] (Yadav et al. 2020; Lee and Lavoie 2013). Biomass for second-generation biofuels incorporate plants that are particularly developed either for bioenergy generation (bioenergy crops) on minimal lands or inedible parts of convention crops and forest trees that should be efficiently processed for bioenergy by improving the current technologies. Second-generation biofuels follow the conversion process of lignocellulosic biomass to biofuel in two different pathways, namely, thermochemical and biochemical pathways [17, 18, 10] (Zargar et al., 2017;

Bond-Watts et al, 2011; Naik et al. 2010). In the thermochemical pathway, biomass is heated at varying degrees of temperature with a minimum amount of an oxidizing agent leading to its conversion to three fractions, one solid called biochar, one liquid called bio oil or pyrolytic oil and the third a gas called syngas. In this pathway methanol can be produced from hydrogen and carbon monoxide under the action of a reducing catalyst [19-22] (Lavoie et al., 2012; Lavoie et al., 2011; Clarke and Preto, 2011; Zhang et al., 2007). In the biochemical pathway, cellulose is first isolated from the lignocellulosic biomass followed by an enzymatic fermentation process for the saccharification of cellulose [23-25] (Jin et al., 2010; Lavoie et al., 2010; Clark, 2007). Hemicellulose, the carbohydrate-based polymer composed of C5 and C6 nonosaccharide sugars, can be fermented by genetically modified yeast strains rather than classical one. In case of the presence of acetic and formic acid in the fermented biomass, additional operation is needed for detoxification [26] (Matsushika et al., 2009). Shabtai and his coworkers reported the possibility to convert lignin into added value compounds (such as phenol, guaiacol and catechol) and transportation jet fuels [27] (Shabtai et al., 1998). *Jatropha* is generally accepted as second generation feedstock as most of the species produce toxic seeds and are not used for food [28] (Pradhan et al., 2009).

Third generation: The third generation is defined as type of biofuels that would be produced from algal biomass, which has a very high quality and growth yield as compared with classical lignocellulosic biomass [29] (Brennana and Owendea, 2010). Algae produce oil that is easy to refine into diesel fuel, but algae stability is poorer than other biofuels as extremely unsaturated oils are volatile at high temperatures. Lipids extracted from algae such as *Chlorella* can be transferred to biodiesel through the transesterification process or can be processed by hydrogenolysis to produce kerosene [30] (Tran et al., 2010). Algae production to harvest oil for biofuels has not yet been carried out on a commercial scale, but feasibility studies have been carried out to arrive at the above yield estimate.

Fourth generation: This type of biofuels is derived from specially designed engineered microorganisms, plants or biomasses that will have higher energy yields or lower boundaries to cellulosic breakdown or are able to be grown on non-agricultural land or bodies of water [31] (Correa et al., 2019). The fourth generation biofuel is also focuses on the genetic modification of the microalgae [32] (Hu et al., 2008). It is aimed to produce microalgae which can capture large amounts of CO₂, increase the biofuel productivity and the adaptability of microalgae in wastewater using advanced technologies [33, 34] (Hays and Ducat, 2015; Chisti, 2008). The genetically modified microalgae are considered as carbon negative because the amount of CO₂ released is lower than the amount of CO₂ take-up [35, 36] (Abdullah et al., 2019; Zhu et al., 2017). This type of biofuels is delivered by photosynthetic microorganisms to create photo-biological sun powered fills, by combining photovoltaics and microbial fuel generation, or by manufactured cell production lines or manufactured organelles particularly custom fitted for generation of wanted high-value chemicals and biofuels [37] (Wijffels et al., 2013). In the fourth generation biomass crops are seen as efficient 'carbon capturing' machines that take CO₂ out of the atmosphere and lock it in their branches, trunks and leaves. After that, the carbon-rich biomass is converted into fuel and gases by means of second generation techniques. The producing fuels and gases are not only renewable; they are also effectively carbon-negative.

The system not only captures and stores CO₂ from the atmosphere, it also reduces carbon dioxide emission by replacing fossil fuels.

Table1: Types of biofuels generations: Their biomasses sources and products

Biofuels 'generation	Source	Product
First generation	Starch, Sugars, Seed oils	Bioethanol, Biodiesel, Biogas
Second generation	Lignocellulosic Biomass or woody crops, Jatropha, Waste plant materials	Bio oil, or pyolytic oil, Syngas, Biochar
Third generation	Classical lignocellulosic biomass	Biodiesel, Unsaturated oils
Fourth generation	Specially designed engineered microorganisms and plants, Genetically modified microalgae	Carbon-negative fuels, Biohydrogen, Biomethane

Transesterification

The process of transesterification involves the reaction between a triglyceride and an alcohol molecule to give alkyl esters and glycerol as reaction products. This process proceeds under the influence of catalyst which helps to speed up the reaction. Transesterification is based on various parameters and by controlling those parameters, a high yield of methyl esters can be obtained [38, 39] (Dizge and Keskinler, 2008; Du et al., 2008). Those parameters include amount of catalyst, alcohol to oil ratio, reaction temperature and reaction time. Two approaches for transesterification of vegetable oils for the generation of biodiesel have been proposed. The first approach is the enzymatic one, in which lipase-catalyzed transesterification is carried out in non-aqueous environment. The second approach is a chemical one in which extracted oil is treated with methanol or ethanol in the presence of strong acid or base [40] (Fukuda et al., 2001).

Advantages and disadvantages of Biofuels

Biofuels costs have been declining and are likely to be far cheaper than petrol and other fossil fuels. Whereas oil is a finite resource and comes from unique materials, biofuels can be produced from a broad variety of ingredients, including crop waste, manure, and other by-products. This makes the recycling process an effective step. Moreover, it takes a very long time for fossil fuels to be produced, but biofuels are much more readily renewable as new crops are grown and waste materials are obtained [41] (Rodionova et al., 2017). The generation of biofuels can be domestic, which lowers the nation's reliance on foreign resources. The countries will then protect the integrity of their energy supply and ensuring they are protected from external impacts by reducing reliance on international sources [42] (Gehlhar et al., 2010). Biofuel production would also raise demand for sufficient biofuel crops, providing an economic boost to the agriculture industry. Biofuels have lower emissions, when they are burnt, they

contain considerably less carbon output and fewer contaminants, making them a better solution to preserving atmospheric health and reducing air pollution [43] (Jeswani et al., 2020). In contrast to fossil fuels, which have resources in just a few nations, any country may start producing biofuels without interfering with other nations' energy sources. If a country can produce its own biofuel, it can easily determine its own product pricing without being constrained by global or regional constraints. Biofuels may be utilized to help eliminate the monopoly produced by fossil fuels because they are equivalent replacements [44] (Arshad et al., 2018). Biogas, for example, may be utilized similarly to natural gas. As a result, when natural gas prices rise, people will have the option of converting to biogas [45] (Eriksson, 2014). When the price of fossil fuels rises, motorists can switch to ethanol or butanol, which are superior alternatives. Among the many beneficial features of biofuels, there are still many drawbacks of these sources of fuels. Biofuels have a lower energy output than conventional fuels and thus need more to be used to generate the same amount of energy. The use of cropland to grow fuel crops could have an impact on food prices and could contribute to food shortages [43] (Jeswani et al., 2020). Bio crops can increase the cost of production by increasing land use and demand for water for crop irrigation. In addition, significant amounts of water are also required for the proper irrigation of biofuel crops as well as for the production of fuel that could strain local and regional water supplies. Some of the disadvantages of biofuels are mostly associated with low-diversity biofuel sources such as corn, sugarcane, soybeans, oil palms which are traditional agricultural crops. (Table 2).

Table 2: Advantages and disadvantages of biofuels

	Advantage		Disadvantage
1	Biofuels are a renewable source of energy.	1	Biofuels have a lower energy output than that of petroleum based fuels.
2	Biofuels can be produced from a broad variety of ingredients, including crop wastes and other by-products.	2	Biofuels release large quantities of carbon upon burning.
3	Biofuels are cheaper fuel than other fossil fuels.	3	Great concern about the valuable crop-land used for biofuel production.
4	Biofuels are environmentally friendly and their burning causes less pollution.	4	There is a shortages of food for human consumption if crops are grown for the production of biofuels.
5	The generation of biofuels decrease the nation' reliance on foreign resources.	5	Wasting a large amount of water for the proper maintenance of biofuel crops.
6	Biofuels production can greatly help in the reduction of greenhouse gas emissions.	6	Biofuels need to be used in large quantities to attain the same energy levels of petroleum based fuels.
7	Biofuels can be produced from various cheaper sources such as non-edible oils,	7	Biofuels are mostly associated with

	used vegetable oils, and remaining wastes from crops.		low-diversity biofuel sources.
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Environmental and economic considerations

The energy required to create biofuels must be considered when assessing their economic and financial benefits. The method of growing corn to produce ethanol consumes fossil fuels in farming equipment, in fertilizer manufacturing, in corn transportation, and in ethanol refining. In this respect, corn ethanol gives a generally minor energy gain; this energy from sugarcane is greater, and from cellulosic ethanol or algal biodiesel may be significantly more prominent [46] (Brännlund et al., 2008). Land use is also an important consideration in assessing the benefits of biofuels. The use of common feed-stocks like corn and soybeans as a main component of first-generation biofuels ignited a controversy about "food versus fuel". Biofuel production can modify the economics of food price and availability by diverting agricultural land and feed-stock from the human food chain [47] (Janda et al., 2013). Besides, vitality crops created for biofuel may compete for characteristic environments around the planet. One interesting advantage of biofuels is that, when combined with an upcoming technology known as carbon capture and capacity, the method of making and consuming biofuels may be capable of permanently eliminating CO₂, the greenhouse gas, from the environment [48-50] (Prasad et al., 2020; Gheewala et al., 2013; Zhang et al., 2013). Biofuel crops would remove carbon dioxide from the air as they grew, and energy facilities would capture the carbon dioxide given off as biofuels are burned to generate power. Carbon dioxide captured within the environment might be put away in long-term storehouses such as profound sea silt, geologic arrangements underneath the earth's surface, or indeed solids such as carbonates [51, 52] (Bertrand et al., 2016; Tilman et al., 2006).

Conclusion

Serious shortage of fossil fuels is projected as inevitable in near future coupled with a drastic environmental implication. Consequently, the need for an alternative renewable clean energy such as wind, solar, tidal and fusion energy is vital. Biofuels are a best alternative to petroleum based fuels since their best combustion profile and environment friendly nature. Besides, the feedstocks that are required to synthesize biofuels can be gotten effectively. Biofuels have an advantage over petroleum fuels since biofuels can be produced from waste products such as utilized vegetable oils as well as less costly sources including non-edible oils such as jatropha and neem oils, etc. Biofuels can too be delivered utilizing green growth and parasites as crude materials. The renewable nature of biofuels makes them way better than ordinary petrol and diesel and consequently biofuels can be utilized in place of fossil fuels which are not renewable. In spite of the advantages of biofuels over petrol and diesel, there are many disadvantages related to them but the overall effects of biofuels are advantageous over them. Many countries including Brazil, India, Indonesia, etc. are pioneer in this field of manufacturing biofuels. Use of biofuels gives advantageous to engines since their lubricating property is enhanced using biofuels. Biofuels mainly include bioethanol and biodiesel which are produced by the fermentation and transesterification processes respectively. Different techniques such as ultrasounds, microwave and irradiations can be used to increase and improve the productivity

of biofuels. These two processes can increase the yield of these two products by modifying the production processes. Molecular and genetic engineering strategies in the raw materials can moreover be utilized to improve the biofuel yield. In general, biofuels can give a valuable way to reduce the dependency on non-renewable fossil fuels as well as can prove beneficial to the environment around us. The future of biofuels may not rely solely on one generation, but may be a combination of the four generations to cope with increased worldwide request as a result of depletion in the world's oil resources.

REFERENCES

- [1] Menne MJ, Williams CN, Gleason BE, Rennie JJ, Lawrimore JH. The Global Historical Climatology Network Monthly Temperature Dataset, Version 4. *Journal of Climate* 2008; 31:9835-9854. <https://doi.org/10.1175/JCLI-D-18-0094.1>
- [2] Keasling J, Garcia Martin H, Lee TS, Mukhopadhyay A, Singer SW, Sundstrom E. Microbial production of advanced biofuels. *Nat Rev Microbiol.* 2021;19(11):701-715. <https://doi:10.1038/s41579-021-00577-w>. Epub 2021 Jun 25. PMID: 34172951.
- [3] US Environmental Protection Agency. Sources of greenhouse gas emissions. EPA, 2020; <https://www.epa.gov/ghgemissions/sources-greenhouse-gas-emissions>.
- [4] Gomez LD, Clare GS, McQueen-Mason J. Sustainable liquid biofuels from biomass: the writing's on the walls. *New Phytol* 2008;178: 473–85. <https://doi.org/10.1111/j.1469-8137.2008.02422.x>
- [5] Kulkarni M, Gopinath R, Meher LC, Dalai AK. Solid acid catalyzed biodiesel production by simultaneous esterification and transesterification. *Green Chem* 2006; 8:1056–1062. <https://doi:10.1039/B605713F>
- [6] Meher LC, Vidyasagar D, Naik SN. Technical aspects of biodiesel production by transesterification—a review. *Renewable Sustain Energy Rev* 2006;10:248–68. <http://dx.doi.org/10.1016/j.rser.2004.09.002>
- [7] Masjuki Hj, Hassan, Md. Abul Kalam. An overview of biofuel as a renewable energy source: development and challenges / *Procedia Engineering* 2013; 56:39–53. <https://doi:10.1016/j.proeng.2013.03.087>
- [8] Zhu LY, Zong MH, Wu H. Efficient lipid production with *Trichosporon fermentans* and its use for biodiesel preparation. *Bioresour Technol.* 2008; 99(16):7881-7885. <https://doi:10.1016/j.biortech.2008.02.033>. Epub 2008 Apr 3. PMID: 18394882.
- [9] Chew TL, Bhatia S. Catalytic processes towards the production of biofuels in a palm oil and oil palm biomass-based biorefinery. *Bioresour. Technol.* 2008; 99: 7911–22. <https://doi:10.1016/j.biortech.2008.03.009>
- [10] Naik SN, Goud VV, Rout PK, Dalai AK. Production of first and second generation biofuels: a comprehensive review. *Renewable Sustainable Energy Reviews* 2010; 14:578–597. <https://doi.org/10.1016/j.rser.2009.10.003>
- [11] Zhou Y, Li G, Dong J, Xing X, Dai J, Zhang C. MiYA, an efficient machine-learning workflow in conjunction with the YeastFab assembly strategy for combinatorial optimization of

- heterologous metabolic pathways in *Saccharomyces cerevisiae*. *Metab. Eng.* 2018; 47: 294–302. <https://doi.org/10.1016/j.ymben.2018.03.020>
- [12] McAloon A, Taylor F, Yee W, Ibsen K, Wooley R. Determining the cost of producing ethanol from corn starch and lignocellulosic feedstocks. National Renewable Energy Laboratory, 2000; Golden, CO. <https://doi.org/10.2172/766198>
- [13] Kalnes T, Marker T, Shonnard DR. Green diesel: a second generation biofuel. *Int J Chem Reactor Eng.* 2007; 5: 748. <https://doi.org/10.2202/1542-6580.1554>
- [14] Stevens CV, Verhe R. Renewable bioresources scope and modification for nonfood application, 2004. England: John Wiley and Sons Ltd.; ISBN: 978-0-470-85447-1; 330 pages.
- [15] Yadav AN, Yadav N, Rastegari A, Gaur R. Biofuels production– sustainability and advances in microbial bioresources. Springer, Cham. 2020; ISBN: 978-3-030-53933-7; <https://doi.org/10.1007/978-3-030-53933-7>
- [16] Lee RA, Lavoie JM. From first- to third-generation biofuels: challenges of producing a commodity from a biomass of increasing complexity. *Animal Frontiers* 2013; 3:6–11. <https://doi.org/10.2527/af.2013-0010>
- [17] Zargar A, Bailey CB, Haushalter RW, Eiben CB, Katz L, Keasling JD. Leveraging microbial biosynthetic pathways for the generation of 'drop-in' biofuels. *Curr Opin Biotechnol.* 2017;45 :156-163. <https://doi.org/10.1016/j.copbio.2017.03.004>. Epub 2017 Apr 17. PMID: 28427010; PMCID: PMC6283405.
- [18] Bond-Watts BB, Bellerose RJ, Chang MCY. Enzyme mechanism as a kinetic control element for designing synthetic biofuel pathways. *Nat. Chem. Biol.* 2011; 7:222–227. <https://doi.org/10.1038/nchembio.537>
- [19] Lavoie JM, Marie-Rose S, Lynch D. Non-homogeneous residual feedstocks to biofuels and chemicals via the methanol route. *Biomass Convers. Biorefin.* 2012;3(1):39–44. <https://doi.org/10.1007/S13399-012-0050-6>
- [20] Lavoie JM, Beauchet R, Berberi V, Chornet M. Biorefining lignocellulosic biomass via the feedstock impregnation rapid and sequential steam treatment. In: *Biofuel's Engineering Process Technology*. M. Bernardes, ed. Intech publishing, Croatia, 2011; 685–714. <https://doi.org/10.5772/18186>
- [21] Clarke S, Preto F. Biomass densification for energy production. <http://www.omafra.gov.on.ca/english/engineer/facts/11-035.pdf>. Ontario Ministry of Agriculture and Food factsheet, 2011. https://doi.org/10.1007/978-3-319-74482-7_2
- [22] Zhang Q, Chang J, Wang T, Xu Y. Review of biomass pyrolysis oil properties and upgrading research. *Energy Convers. Manage.* 2007; 48:87–92. <https://doi.org/10.1016/j.enconman.2006.05.010>
- [23] Jin, Y, Jameel H, Chang HM, and Phillips R. Green liquor pretreatment of mixed hardwood for ethanol production in a repurposed kraft pulp mill. *J. Wood Chem. Technol.* 2010; 30:86–104. <https://doi.org/10.1080/02773810903578360>
- [24] Lavoie, J.-M., Capek-Menard E, Gauvin H, and Chornet E. Production of pulp from *Salix viminalis* energy crops using the FIRSST process. *Bioresour. Technol.* 2010; 101:4940–4946. <https://doi.org/10.1016/j.biortech.2009.09.021>
- [25] Clark JH. Green chemistry for the second generation biorefinery-sustainable chemical manufacturing based on biomass. *J Chem. Technol. Biotechnol.* 2007; 82:603–9. <https://doi.org/10.1002/jctb.1710>

- [26] Matsushika A, Inoue H, Kodaki T, Sawayama S. Ethanol production from xylose in engineered *Saccharomyces cerevisiae* strains: Current state and perspectives. *Appl. Microbiol. Biotechnol.* 2009; 84:37–53. <https://doi:10.1007/s00253-009-2101-x>
- [27] Shabtai J, Zmierczak W, Chornet E. Process for conversion of lignin to reformulated hydrocarbon gasoline: The University of Utah Research Foundation, 1998. United States Patent #5959167 Sep 28, 1999; Appl No 9/136336; Assignee: The University of Utah Research Foundation (Salt Lake City, UT).
- [28] Pradhan RC, Naik SN, Bhatnagar N, Vijay VK. Moisture-dependent physical properties of jatropha fruit. *Ind. Crop. Prod.* 2009; 29:341–347. <https://doi:10.1016/j.indcrop.2008.07.002>
- [29] Brennana, L., and Owendea P. Biofuels from microalgae—A review of technologies for production, processing, and extractions of biofuels and coproducts. *Renewable Sustainable Energy Rev.* 2010; 14:557–577. <https://doi:10.1016/j.rser.2009.10.009>
- [30] Tran, N., J. Bartlett, G. Kannangara, A. Milev, H. Volk, and M. Wilson. Catalytic upgrading of bio-refinery oil from micro-algae. *Fuel* 2010; 89:265–274. <https://doi:10.1016/J.FUEL.2009.08.015>
- [31] Correa DF, Beyer HL, Fargione JE, Hill,JD, Possingham HP, Thomas-Hall SR, SchenkPM. Towards the implementation of sustainable biofuel production systems. *Renewable Sustainable Energy Rev.* 2019; 107: 250-263. <https://doi.org/10.1016/j.rser.2019.03.005>
- [32] Hu Q, Sommerfeld M, Jarvis E, Ghirardi M, Posewitz M, Seibert M, Darzins A. Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. *Plant J.* 2008; 54:621–39. <https://doi:10.1111/j.1365-313X.2008.03492.x>
- [33] Hays SG, Ducat DC. Engineering cyanobacteria as photosynthetic feedstock factories. *Photosynthesis Research* 215; 123:285–295. <https://doi:10.1007/s11120-014-9980-0>
- [34] Chisti Y. Biodiesel from microalgae beats bioethanol. *Trends Biotechnol.* 2008; 26(3):126–31. <https://doi:10.1016/j.tibtech.2007.12.002>
- [35] Abdullah B, Syed Muhammad SAF ad, Shokravi Z, et al. Fourth generation biofuel: a review on risks and mitigation strategies. *Renewable Sustainable Energy Rev.* 2019; 107:37–50. <https://doi.org/10.1016/j.rser.2019.02.018>
- [36] Zhu B, Chen G, Cao X, Wei D. Molecular characterization of CO₂ sequestration and assimilation in microalgae and its biotechnological applications. *Bioresour Technol.* 2017; 244:1207-1215. <https://doi.org/10.1016/j.biortech.2017.05.199>
- [37] Wijffels RH, Kruse O, Hellingwerf KJ. Potential of industrial biotechnology with cyanobacteria and eukaryotic microalgae. *Current Opinion in Biotechnology* 2013; 24:405–413. <https://doi:10.1016/j.copbio.2013.04.004>
- [38] Dizge N, Keskinler B. Enzymatic Production of Biodiesel from Canola Oil using Immobilized lipase. *Biomass Bioenergy* 2008; 32:1274-1278. <https://doi.org/10.1016/j.biombioe.2008.03.005>
- [39] Du W, Li W, Sun T, Chen X, Liu D. Perspectives for biotechnological production of biodiesel and impacts. *Applied Microbiology and Biotechnology* 2008; 79:331-337. <https://doi:10.1007/s00253-008-1448-8>
- [40] Fukuda, H, Kondo A and Noda H. Biodiesel fuel production by transesterification of oils. *Journal of Biosci. Bioeng.* 2001; 92: 405-416, <https://doi:10.1263/jbb.92.405>

- [41] Rodionova MV, Poudyal RS, Tiwari I, Voloshin RA, Zharmukhamedov SK, Nam HG, Zayadan BK, Bruce BD, Hou HJM, Allakhverdiev SI Biofuel production: Challenges and opportunities. *International Journal of Hydrogen Energy* 2017; 42(12):8450-8461; [https://doi: 10.1016/j.ijhydene.2016.11.125](https://doi.org/10.1016/j.ijhydene.2016.11.125)
- [42] Gehlhar MJ, Somwaru A, Winston A. Effects of Increased Biofuels on the U.S. Economy in 2022 (October 1, 2010). USDA-ERS Economic Research Report No. 102. <http://dx.doi.org/10.2139/ssrn.1711353>
- [43] Jeswani HK, Chilvers A, Azapagic A. Environmental sustainability of biofuels: a review. *Proceedings. Mathematical, physical, and engineering sciences* 2020; 476(2243), 20200351. <https://doi.org/10.1098/rspa.2020.0351>
- [44] Arshad M, Zia MA, Shah FA, Ahmad M. An Overview of Biofuel. In: Arshad, M. (eds). *Perspectives on Water Usage for Biofuels Production*, 2018. Springer, Cham. https://doi.org/10.1007/978-3-319-66408-8_1
- [45] Eriksson O. Biogas and natural gas. In book: *Government 11 Publisher: Pan European Networks Government*, 2014;11,www.paneuropeannetworks.com;[https://doi: 10.13140/2.1.5190.1128](https://doi.org/10.13140/2.1.5190.1128)
- [46] Brännlund R, Kriström B, Lundgren T, Marklund P. *International Review of Environmental and Resource Economics* 2008; 2(736); [https://doi: 10.1561/101.00000017](https://doi.org/10.1561/101.00000017)
- [47] Janda K, Kristoufek L, Zilberman D. Biofuels: Policies and impacts; *Agricultural Economics (AGRICECON)* 2013; 58(8):372-386; [https://doi: 10.17221/124/2011-AGRICECON](https://doi.org/10.17221/124/2011-AGRICECON).
- [48] Prasad S, Yadav AN, Singh A. Impact of Climate Change on Sustainable Biofuel Production. In: Yadav, A.N., Rastegari, A.A., Yadav, N., Gaur, R. (eds) *Biofuels Production Sustainability and Advances in Microbial Bioresources. Biofuel and Biorefinery Technologies*, 2020; vol 11. Springer, Cham. https://doi.org/10.1007/978-3-030-53933-7_5
- [49] Gheewala SH, Damen B, Shi X. Biofuels: Economic, environmental and social benefits and costs for developing countries in Asia. *WIREs Clim Change* 2013; 4(6): 497–511. Available from:<https://www.researchgate.net/publication/250309394>; <https://doi.org/10.1002/wcc.241>
- [50] Zhang W, Yu E, Rozelle S, Yang J, Msangi S. The impact of biofuel growth on agriculture: Why is the range of estimates so wide? *Food Policy* 2013; 38:227-239. <https://doi.org/10.1016/j.foodpol.2012.12.002>
- [51] Bertrand, E., Pradel, M., Dussap, CG. Economic and Environmental Aspects of Biofuels. In: Soccol, C., Brar, S., Faulds, C., Ramos, L. (eds), 2016. *Green Fuels Technology. Green Energy and Technology*. Springer, Cham. https://doi.org/10.1007/978-3-319-30205-8_22
- [52] Tilman D, Hill J, Lehman C. Carbon-Negative Biofuels from Low-Input High-Diversity Grassland Biomass. *Science* 2006; 314(5805), 1598-1600. [https://doi:10.1126/science.1133306](https://doi.org/10.1126/science.1133306)

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