Quality and composition of lipids used in biodiesel production and methods of transesterification: A review

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ABSTRACT

This study presents a brief overview of the composition and properties of various oils used in biodiesel production and the alkali, acidic and enzymatic transesterification reactions used in production. Nowadays, vegetable and microalgal oils are mostly used in biodiesel production. Recently, however, animal fats, processed oils, industrial oils and yeast and bacterial oils have also gained importance. Although all fats and oils are roughly similar, their saturated fatty acids (SFA), polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA), free fatty acids (FFA), cholesterol or glycerol contents may be different and affect the production and efficiency of the biodiesel. Triglycerides are the most significant lipids in biodiesel production. Triglycerides in vegetable oils dominantly contain C18:1ω9 and C18:2ω6 fatty acids. Whereas, triglycerides in animal fats mostly include C16:0 and C18:1ω9 fatty acids. Microalgae are rich in long-chain fatty acids such as C20 and C22. Also, fatty acids such as C14:0, C16:1ω7, C18:0, C20:0, C22:0, C24:0, C22:1ω9, C18:3ω6, C20:4ω6 and C20:5ω6 are present in oils and fats. However, their proportions change from feedstock to feedstock. Rendered animal-originated fats include larger quantities of FFA than waste vegetable oils and, as known, excessive amounts of FFA generate soap in reactions with the alkaline catalyst, reducing biodiesel efficiency. For this reason, generally vegetable and microalgal oils containing a large number of triglycerides and less FFA are preferred for biodiesel production. Bacteria can synthesize branched fatty acids. It has been reported that biodiesel produced from branched fatty acids has advantages according to biodiesel produced from other lipids. Consequently, the use of correct and reliable lipids in biodiesel production is very important for the quality of fuel.

Keywords: Fatty acids, lipid feedstocks, biodiesel, transesterification.

Biyoüzümden üretimde kullanılan yağların kalitesi, kompozisyonu ile transesterifikasyon yöntemleri: Bir derleme

ÖZ

Bu çalışma, biyoüzümden üretimde kullanılan çeşitli yağların kompozisyonu ve özellikleri ile üretimde kullanılan alkali, asidik ve enzimatik transesterifikasyon reaksiyonlarına kısa bir bakış sunmaktadır. Günümüzde, bitkisel ve mikroöl yağları çoğunlukla biyoüzümdede kullanılmaktadır. Ancak son zamanlarda hayvansal yağlar, işlenmiş yağlar, endüstriyel yağlar ve maya ve bakteri yağları da önem kazanmıştır. Bu yüzden suyu ve katı yağlar kabaca birbirine benzer olmasına rağmen, onların doymus yağ asiti (DYA), çoklu doymamış yağ asiti (ÇDYA), tekli doymamış yağ asiti (TDYA), serbest yağ asiti (SYA), kolesterol ve gliserol içerikleri farklı olabilir ve bu da biyoüzümden üretim ve verimini etkiler. Trigliseritler, biyoüzümden üretimde en önemli lipitlerdir. Bitkisel yağlardaki trigliseritler, bazıları C18:1ω9 ve C18:2ω6 yağ asitlerini içerirler. Oysa hayvansal yağlardaki trigliseritler çoğunlukla C16:0 ve C18:1ω9 yağ asitlerini ihtiva ederler. Mikroöl yağlar C20 ve C22 gibi uzun zincirli yağ asitleri bakımından zengindirler. Ayrıca, C14:0, C16:1ω7, C18:0, C20:0, C22:0, C24:0, C22:1ω9, C18:3ω6, C20:4ω6 ve C20:5ω6 gibi yağ asitleri, katı ve suvi yağlarının çoğununda bulunurlar. Fakat oranları hammaddeden hammaddeye değişmektedir. İşlenmiş yağlar kaynaklı yağlar, atk bitkisel yağlardan daha büyük miktarlarda FFA içerir ve bilendiği üzere, aşırı mikarda FFA, alkali katalizörle reaksiyonlarda sabot üreterek biyoüzümden verimini azaltır. Bu nedenle, genel olarak çok sayıda trigliserit ve daha az FFA içeren bitkisel ve mikroöl yağları biyoüzümden üretim için tercih edilir. Bakteriler ise dallanmış yağ asitlerini sentezleyebilirler. Dallanmış yağ asitlerinden üretilen biyoüzümden daha diğer yağlar ile kullanılan biyoüzümlerle göre avantajlarına sahip olduğu rapor edilmiştir. Sonuç olarak, biyoüzümden üretimde doğru ve güvenilir lipidlerin kullanılması yakının kalitesi için çok önemlidir.

Anahtar Kelimeler: Yağasitleri, lipit ham maddeleri, biyoüzüm, transesterifikasyon.
1. INTRODUCTION

Lipids can be classified into three groups, simple lipid (neutral lipids), complex lipid (phospholipids, lipoproteins, glycolipids), and primary lipid (fatty acids and sterols). In detail, lipids contain a variety of structural types, including fatty acids, triglycerides, diglycerides, monoglycerides, lipoprotein, glycolipids, terpenoids, terpenes, steroids, sphingolipids, phospholipids, eicosanoids, and waxes. They are often known by high solubility in nonpolar solvents such as chloroform, hexane and diethyl ether.

Physically, fats are solid and oils are liquid at room temperature. Lipids from fats and oils include triglycerides synthesized from three long-chain fatty acids and a glycerol molecule. Because of the high viscosity of the lipids, they cannot be directly used as fuel in a standard diesel engine. For this reason, triglycerides are converted into fatty acids methyl esters (FAME) by transesterification reaction to reduce viscosity. Hence, three molecules of FAME and one molecule of glycerol are picked up from one molecule of triglycerides. Glycerine is taken away as by-product, and FAME is used as biodiesel.

Most of the lipids are comprised of fatty acids containing long saturated or unsaturated aliphatic hydrocarbon chains and a terminal carboxyl group. Fatty acids generally have unbranched chains with even number of carbon atoms, usually 14 to 28 carbon-containing and they are mostly found in triglycerides and phospholipids. Saturated carbon chains containing fatty acids are called fats (mostly found in animals) having higher melting points than the unsaturated ones like vegetable oils. If a single fatty acid is not bonded to other molecules, it is called free fatty acid (FFA). The major fatty acids of the feedstocks are stearic (C18:0), palmitic (C16:0), oleic (C18:1ω9) and linoleic (C18:2ω6) acids with a low degree of unsaturation. It has been reported that density and viscosity of biodiesel can be predicted by the fatty acid profile of feedstock. If the main fatty acids of the feedstock have a long chain of carbon and low degree of unsaturation, the viscosity of the biodiesel will be high, and vice versa. Consequently, the increasing number of double bonds and reducing chain length make density higher.

Biodiesel consists of a mixture of fatty acid formed chain length between C14-C22alkyl esters, obtained from a sustainable lipid feedstock, such as animal fat, vegetable or algae oil. Just in case of using ethanol or methanol as reactants, the mixture of fatty acid ethyl esters (FAEE) or FAME is formed, respectively. By any means, methanol is generally used in transesterification reactions by its low-priced and availability. Sometimes butanol and isopropanol might also be used in transesterification reactions instead of conventional alcohol. Water content is the main factor for primary alcohol. Water is not good for biodiesel production because it can give rise to poor yields and converts FFA and triglycerides to soap. FAME is the most important part of biodiesel and can be used in conventional diesel engines with no significant modifications. The advantages of biodiesel over diesel fuel are its non-toxic feature, higher combustion performance, biodegradability, renewability, higher cetane number, portability, easy availability, lower aromatic and sulfur content, safer handling and better emission profile. Compared to petroleum-based fuels, biodiesel is less toxic and has a low environmental impact with its emissions and does not include sulfur, therefore SOx is not emitted. On the other hand, proper combustion can be achieved because of the high flash point of biodiesel and producing lower emissions of hydrocarbons, CO, CO2, and NOx.

High-quality vegetable oils such as soybean, sunflower, rapeseed (canola), corn, and safflower are generally used as biodiesel raw materials. These raw materials have a high-cost, which is currently responsible for 85% of biodiesel production expenses. Recently, a variety of investigations are released on biodiesel production from low-priced feedstocks. Therefore, there are a lot of explorations on new generation oily seeds, rendered and frying oils, different animal fats, algae oils, and microbial lipids. The most efficient and low-priced feedstock has not been advanced exactly. More than 95% of total biodiesel production is edible oil feedstock. For example, sunflower oils (13%) and rapeseed oils (84%) are major feedstock in biodiesel synthesis followed by palm oil (1%). The other is produced from groundnut, corn, coconut, peanut, soybean, and canola (2%). Some of the non-edible oil sources utilized for biodiesel are rubber (Ficus elastica), Jatropha (Jatropha curcas), karanja (Milletia pinnata), polanga (Calophyllum inophyllum), tobacco (Nicotiana tabacum), caster (Ricinus communis) and mahua. Animal-originated products such as poultry fat, tallow, and lard have also been explored for their capacities as raw materials of biodiesel. The restaurants and food processing oils such as rendered cooking oil and other waste oil have been evaluated as feedstock. Animal fats derived biodiesel has the superiority of a higher cetane number, the most significant indicator of diesel combustion property as compared to vegetable originated biodiesel. Nonetheless, the biodiesel from animal fats has higher cold filter plugging point due to the high content of saturated fatty acids, and it is less stable to oxidation owing to the lack of natural antioxidants which is mostly found in the biodiesel from vegetable oil. Thereby, biodiesel from animal fat may not be appropriate to use it 100% pure in vehicles during cold weather; however, it can be utilized as 100% pure in the boiler systems for heat generation.

This review paper describes the lipid composition of all kinds of feedstocks mentioned up to now such as vegetable oil, animal fat, algal oil, waste, and industrial oil and microbial lipids to be used in transeste-
rhification reactions for producing biodiesel, as well as comparing the advantages, disadvantages and positive and negative aspects of biodiesel feedstocks. Thence, the purpose of this work is to present an overview of the different type of raw materials adopted for the production of biodiesel, and additionally it describes alkaline, acidic and enzymatic transesterification methods.

2. TYPES OF FEEDSTOCKS AND THEIR QUALITIES

2.1. Composition of vegetable oils as a feedstock for biodiesel

More than 95 % of biodiesel primarily has been obtained from edible vegetable oils, biodiesel first generation, which is substantially obtainable from the agricultural industry. Most countries are searching for different types of vegetable oils as a supplement for their diesel fuels depending on their climate conditions and soil types. The purpose is to grow plants that are cheaper and have higher oil content. Latterly biodiesel is predominantly produced from rapeseed in Canada, soybean in the USA, sunflower in Europe, palm in Southeast Asia (mainly in Malaysia and Indonesia), and safflower in India and rarely in the Middle East. Furthermore, the rapeseed and the sunflower are produced in the north of Europe and the Mediterranean countries, respectively. Sunflower oil has good properties in biodiesel production. However, to obtain economical biodiesel, the high cost of sunflower oil is a serious problem. For this reason, a large scale of biodiesel production from edible oils has nowadays been of great concern because they compete with food materials. Let's not miss it; the malnourished human proportion is approximately 60% across the world. The largest producers of biodiesel were the European Union, the United States, Brazil, and Indonesia, which used edible oil for about 8.6 million tones of biodiesel production. The approximate development in using edible oil for biodiesel production was 6.6 million tons from 2004 to 2007, which would ascribe to 34% of the increase in global consumption to biodiesel. Alternative vegetable oil from Cynara cardunculus (Cardoon), Brassica carinata (Ethiopian mustard), Camelina sativa, Crambe abyssinica, Pogianus, Jatropha curcas are recently been the raw material for biodiesel. Additionally, some of the non-edible oils containing plants, e.g. Jatropha, pongamia and karanj play a noteworthy role in resources. Both these plants may be grown on a massive scale on agricultural, degraded or wastelands.

The majority of vegetable oils mostly have shown similar fatty acids profiles, usually dominated with monounsaturated or polyunsaturated fatty acids. On the other hand, they can show similarities in total but also contain differences in detail. Knowing the fatty acid content of a plant helps to achieve a high quality and efficient biodiesel production. Rapeseed oil is distinguished by low level (7%) of saturated fatty acids and considerable amounts of monounsaturated fatty acids and polyunsaturated fatty acids, including 61% C18:1ω9, 21% C18:2ω6 and 11% C18:3ω3, plant sterols (0.53-0.97%). In some other studies, it is stated that rapeseed has contained 60% of C18:1ω9 and soybeans have contained 50-60% of C18:2ω6 (Table 1). The saturated fatty acid contents of rapeseed, safflower, sunflower, corn, olive, and soybean oil is determined at low levels; however, the ratio of monounsaturated fatty acid in canola and the ratio of polyunsaturated fatty acid in safflower, sunflower, corn, olive, and soybean are detected at a high level. On the other hand, coconut has contained more than 90% saturated fatty acids. Soybean contains a significant amount of triglycerides, primer neutral lipids. There is a high content of unsaturated fatty acids in soybean oil, as approximately all the triglyceride molecules contain at least two unsaturated fatty acids. Palm oil has equivalent fatty acid compositions in which the level of saturated fatty acid is nearly equal to unsaturated fatty acids. Palmitic acids (44-45%) and C18:1ω9 (39-40%) are major fatty acids along with C18:2ω6 (10-11%). Total ratio of the safflower seed oil ranges between 30 and 45% based on the dry weight and its oil is used by both food industry and food producers. Safflower oil which contains high C18:2ω6 ratio is mostly used in human nutrition, however, recent market demand has drastically shifted from the traditional high C18:2ω6 to high C18:1ω9. High C18:1ω9 safflower oil is lower in the saturated fatty acids and higher in the monounsaturated fatty acids compared to olive oil. High C18:1ω9 safflower oil has promising solutions for the environment as a pollutant-reducing diesel fuel due to reducing smoke and particulate emissions. In Europe, canola oil is seen as a major source of oil, as well as contributing nearly 85% of the oil for biodiesel production, additionally, sunflower, soybean, and palm oils are the other three important vegetable oils for biodiesel. Some other resources for vegetable oil extraction to be used in biodiesel production are coconut kernel, castor berry, cotton seed, palm pulp, palm kernel oil, peanut grain, and babassu kernel. In a study on some non-traditional seed oils, fatty acid profiles of seed oils of 75 plant species having 30% or more fixed oil in their seed/kernel were analyzed. FAMEs of the oils from 26 species including Calophyllum inophyllum, Jatropha curcas, Pongamia pinnata, and Azadirachta indica showed these plants as most proper species for biodiesel production and they meet the correct definition of biodiesel standards of USA, Germany and European Standard Organization. The FAMEs of the other 11 species meet the specification of USA biodiesel standard. It is concluded that perceived plants have a great potential for biodiesel. The carbon number of the diesel fuel is roughly nearly; it is similar to vegetable oil with 14-18 carbons.
The major dissimilarity between animal fat and vegetable oils their fatty acid content. Vegetable oils have a high content of unsaturated fatty acids, mostly C18:1ω9 and C18:2ω6, whereas animal fat has a higher proportion of saturated fatty acids. Although there are lots of economical oil and fats for example waste oils and unused animal fats, their major problem is the high amount of FFA. Their conversions to biodiesel are quite difficult by transesterification reactions. For biodiesel largely the materials including triglycerides are preferred. Vegetable oil contains more triglycerides and thus preferentially has been preferred for biodiesel. Molecular weights of triglycerides are between 800 and 900 g mol⁻¹ and for this reason, they are nearly four times larger than typical diesel (C_{12}H_{25}S) fuel. Because of higher molecular weights, vegetable oils have low volatility and due to their unsaturation, they are naturally more reactive than diesel fuels. For these reasons, they are much more susceptible to oxidation and thermal polymerization reactions. Thus, the triglycerides are preferred for formation of FAME by transesterification.

A lot of vegetable oils are differentiated by their different fatty acid compositions. The types of fatty acids in triglycerides are important for biodiesel quality. In triglycerides of vegetable oil, the dominant fatty acids are C16:0, C18:1ω9 and C18:2ω6. Additionally, the fatty acids C14:0, C16:1ω7, C18:0, C20:0, C22:0, C24:0, C22:1ω9 and C18:3ω6 are generally present in vegetable oils and their proportions may vary from plant to plant.

| Fatty acids | C12:0 | C14:0 | C16:0 | C16:1 | C18:0 | C18:1 | C18:2 | C18:3 | C20:4 | > C20 |
|-------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| **Vegetable oils** |       |       |       |       |       |       |       |       |       |       |
| Soybean     | -     | 0.1   | 6-10.2| -     | 2-5   | 20-30 | 50-60 | -     | -     | -     |
| Rapeseed    | 0.2   | 0.1   | 3.9   | 0.2   | 1.7   | 60    | 18.8  | 9.5   | -     | 4.0   |
| Corn        | -     | 1-2   | 8-12  | 0.1   | 2-5   | 19-49 | 34-62 | 0.7   | -     | 2.0   |
| Olive       | -     | -     | 9-10  | -     | 2-3   | 73-84 | 10-12 | Traces| -     | -     |
| Cotton      | -     | -     | 20-25 | -     | 1-2   | 23-35 | 40-50 | Traces| -     | -     |
| **Animal fats** |       |       |       |       |       |       |       |       |       |       |
| Lard        | 0.1   | 1-2   | 23.6-30| 2.8 | 12-18 | 40-50 | 7-13  | 0-1   | 1.7   | 1.3   |
| Tallow      | 0.1   | 3-6   | 23.3-32| 4.4 | 19-25 | 37-43 | 2-3   | 0.6-0.9 | 0.2  | 1.8   |
| Fish        | 0.2   | 6.1   | 14.3  | 10.0  | 3.0   | 15.1  | 1.4   | 0.7   | 0.7   | 56.5  |
| Butter      | -     | 7-10  | 24-26 | 2.3   | 10-13 | 1-2.5 | 2.5   | -     | -     | -     |
| Chicken     | 0.1   | 1-1.3 | 17-20.7| 5.4 | 6-12  | 42.7  | 20.7  | 0.7-1.3 | 0.1  | 1.6   |

The inedible tallow, fats, edible tallow, lard, yellow and brown grease, all these raw materials are mainly from animals such as beef, pork, chicken fat, and poultry, as these are most used on a large scale in the food industry and for biodiesel production. Mixtures of vegetable oils and animal fats can be used as yellow greases. It is also known to be lower-quality grades of the cow or sheep fat (tallow); however, it usually comes from vegetable oil and termed waste oil, cooking oil or used vegetable oil. The tallow is made of beef fat, the lard is pork fat and the chicken fat is from poultry. Brown grease comes from restaurant fats and sewage plants. The brown grease is gelatinized at room temperature. Yellow, brown grease and tallow can be used for biodiesel, in spite of the high costs of processing and low yield of biodiesel. Each year, over 1.4 billion gallons of used cooking oil and animal fat are provided by the United States. Indeed, nearly 74% of the inedible tallow and grease are fabricated as animal feed and also the remainder is used to make lubricants, soaps, biodiesel and other products. Due to their high content of saturated fatty acids, animal fats have high viscosity and are in solid form at room temperature. Incomplete combustion and poor atomization of fuel are based on the high viscosity of the fuel. Subsequently, particulate in the exhaust gas and high emissions of pollutants increases.

Animal fats are easily existing due to good managing product control and handling procedures of meat industries. Animal fats are mostly used as animal feeds; however, this application recently decreases owing to the possible diseases. For this reason, the using of animal fats for biodiesel is a good alternative to evaluate the wastes. Another problem is that biodiesel fromanimal fat and cooking oil is less resistant to cold weather than biodiesel from vegetable oils. Therefore, additive productshave been developed particularly for biodiesel.
production. The biggest problem of animal fats for biodiesel is that they contain a large quantity of FFA that is very difficult to be converted to biodiesel by alkaline transesterification because of formation of fatty acids salts (soap). Soaps can prevent the dissociation of the biodiesel from glycerine fraction and cause poor quality. In research about alkaline transesterification of beef tallow, it has been stated that FFA content needs to be kept below 0.5% (1 mg KOH g⁻¹); this means that the fats and vegetable oils must be refined to remove FFA. FFA content influences the yield of biodiesel and causes a delay in the process of biodiesel production. Immiscibility of methanol and animal fats is another problem in biodiesel production. Stirring the solution does not thoroughly form an emulsion, resulting in low production of FAME. For this reason, the solution should contain catalysts because it helps to the conversion of triglycerides to biodiesel.

Tallow which is one of the most important animal fats has been used mostly in recent work on biodiesel. The fatty acids contents of tallow mainly comprise of C18:1ω9 (26-50%), C18:0 (6-40%), C16:0 (17-37%), C14:0 (1-8%), C18:2ω6 (0-5%) and other branched-chain acids. It has been also reported that C18:0 (19-25%) is the most abundant fatty acid, C18:1ω9 is found between 37-43% in tallow. On the other hand, in lard, C16:0 (23.6-30%), C18:0 (12-18%) and C18:2ω6 (40-50%) have been found to be predominant fatty acids (Table 1). In the tallow, the fatty acids are mostly saturated and monounsaturated fatty acids and only a minimum amount of essential fatty acids can be found. Additionally, tallow has a high cholesterol level and lacks in natural antioxidants. In another study, it is stated that the fatty acid composition of lard basically comprises C18:1ω9 (41.6%), C16:0 (23.9%) and some C18:2ω6 (13.2%), and it contains (67.3%) of C16:0 in the sn-2 position of triglycerides. Because of its composition, the lard can be melted easily physically. Besides, the lard contains high levels of cholesterol and does not contain natural antioxidants, so the level of oxidation must be preserved by adding natural or synthetic antioxidants for preventing oxidation of fatty acids. It is normal to have different results regarding animal fats. Because, in the analysis of fatty acids, animal fats from different regions have been selected. It is emphasized that fatty acids which are detected at high levels in the fat are dominant in many studies. On average, the lipid composition of the animal fat is between 38-43% for saturated fatty acids, 56-62% for unsaturated fatty acids, 47-50% for monounsaturated fatty acids and 6-10% for polyunsaturated fatty acids.

The content of fatty acids in milk fats, rendered animal fats, and fish oil are determined at different levels. The short-chain fatty acids from milk are larger than other fats, varying between C4:0 to C10:0 carbon-containing fatty acids. Animal fats have more saturated fatty acids and monounsaturated fatty acids whereas fish oils consist of more polyunsaturated fatty acids. The composition of fatty acids in animal fats is important for qualified biodiesel production. There are many reasons affecting the fatty acid ratio of animals. Genetic, feeding behavior, animal physiology, environmental and climatic factors are some of these factors, but the most important factor is nutrition. For instance, the fatty acids composition (particularly C18:2ω6) can be altered by increasing the grains in the animal diet, in poultry. Increases in peanut and corn in pig diet could produce softer lard.

However, when compared with biodiesel based on vegetable oil, animal fat originated biodiesel has some important advantages as follows: the animal biodiesel blend (B20) cetane number is higher than standard diesel fuel, and therefore, the engine runs very smoothly with animal fat-based biodiesel. It has higher calorific value and higher cetane number and low sulfur and aromatic contents. Net sulfur dioxide (SO₂) and carbon dioxide (CO₂) emissions are reduced by 100%, preventing in this way global warming, acid rain, and visible smoke and noxious odors. Without any major modification in machines, animal FAME as biodiesel blended with diesel fuel can be used in conventional diesel engines, generators, and boilers. Magnesium and nickel-based additives reduce the pour point, flash point and viscosity of biodiesel. Additionally, it is a clean liquid fuel and biodegradable in aqueous solution, 95% resolve within 28 days. A flashpoint of 150°C which compares very favorably to diesel oil whose value is 50°C. Several studies in the U.S. have shown that biodiesel reduces by 90% the risk of cancer. Positive health reduces the effects of carcinogenic aromatic polycyclic hydrocarbon (PAH), particularly following cancer-causing agents: benzo[a]fluoranthren 56% reduction, benzoypyrene 71% reduction and benzantrn 97% reduction. Hydrocarbons and soot emissions are reduced by 10-50% and 40-60%, respectively. The major disadvantages of animal fat originated biodiesel arethaving a higher cold filter plugging point owing to the greater content of SFAs and being less stable to oxidation on account of the absence of antioxidants.

2.3. Composition of algae lipids as a feedstock for biodiesel

In comparison to higher plants, microalgae have several advantages as higher photosynthetic efficiency, growth rate, the production rate of biochemicals and so on. In addition to the high content of lipids, microalgae biomass encompasses a significant amount of carbohydrates, proteins, and some other nutrients, therefore the residual biomass from biodiesel production processes can be used potentially as animal feed also.
Other constituents apart from lipids, e.g. pigments, proteins, carbohydrates in microalgae have commercial importance as nutraceuticals and pharmaceuticals. These constituents can be produced from microalgae in an integrated biorefinery approach along with biodiesel production.42 Some of the residual biomass may be used to produce methane by anaerobic digestion, for generating the necessary electrical power for running the microalgae biomass production facility. Overall, it is used as a very outstanding raw material in producing of biodiesel due to the high content of the oil. In comparison to other oil crops, microalgae have beneficial potentials in biodiesel production. When compared to land plants, First, much land is not needed for the cultivation of microalgae.43 Also, biodiesel from microalgae does not compromise the production of food and other products obtained from crops. Second, microalgae growmore quickly and algal species are rich in oils than conventional crops.44 Oil from microalgae does not burn entirely and form deposits in the fuel injector of diesel engines due to low volatility and high viscosity (about 10-20 times higher than diesel fuel). However, the transesterification of microalgae oils will greatly reduce the original viscosity and increase the fluidity.

Unlike other oil crops, microalgae grow pretty quickly and most of them are fairly rich in oil. They can multiply twice their biomass within 24 h. In some microalgae, lipid quantity can exceed 80% by weight of dry biomass.45,46 The proportion of algae lipid generally ranges from 20 to 80% varying from species to species. Medium composition, temperature, illumination intensity, the ratio of light/dark cycle and aeration rate can affect the composition and fatty acid profile of lipids extracted from particular algal species.47 Some certain microalgae can accumulate the substantial amounts of lipids up to 50% of dry cell weight,43 which is 40 times more oil per acre than other plants used for biodiesel.48 There are numerous microalgal species including high lipid on the basis of dry weight, for instance Dunaliella tertiolecta (37-40%), Chlorella protothecoides (38-51%), Chlorella ellipsoidea (84%), Botryococcus braunii (68%-74%), Nitzschia (49-51%), Nannochloropsis sp. (39-42%), Schizochytrium sp (50-77%) and Neochlorisoleoabundans (35-54%) (Table 2).43,49 Microalgae producing lipid with higher triglycerides more opt for the biodiesel production43 because triglycerides are the main lipid class for biodiesel. The synthesis of triglycerides in microalgae may provide of the formation of acetyl coenzyme A in the cytoplasm, the denaturation, and elongation of the carbon chain of fatty acids and the biosynthesis of triglycerides.43 Above all, the development of new algal species by genetic recombination should be considered that for higher triglyceride synthesis, fast reproduction ability, and more lipid accumulation capacity.

In place of microalgae, lipid-producing heterotrophic microorganisms grown omnatural resources of organic carbon such as sugar is considered to be used for biodiesel production; yet, heterotrophic production of lipid is not as effective as photosynthetic microalgae production.40,43 For why, the prolongable organic carbon sources for heterotrophic microorganisms are constructed by crop plant photosynthesis. The production of algae lipids is related to the fact that high amounts of lipid-rich microalgae biomass can be produced inexpensively compared to the other organisms.43 Commonly, both organic carbon sources such as glucose, acetate, etc. and inorganic carbon (CO2) can be used by microalgae for the production of lipids.29

Microalgae usually contain long-chain fatty acids like C20 and C22, and the ratio and type of fatty acids vary from species to species. Generally, short-chain fatty acids like C14 and C18 carbon-containing which are the main constituents of biodiesel are predominant fatty acids in Chlorella sp.; however, hydrocarbons and high amount of long-chain fatty acids can be seen in some particular microalgaespecies. Therefore, it is essential to distinguish convenient microalgae species as raw materials for biodiesel production. Microalgal lipids are contrary to most vegetable oils in being pretty rich in PUFA with four or more double bonds.32 For instance, docosahexaenoic acid (DHA, C22:6ω3) and eicosapentaenoic acid (EPA, C20:5ω3) can be found in algal oils. Fatty acids and FAME with four and more double bonds are vulnerable to oxidation during storage and for this reason, their suitability for biodiesel becomes decreasing. Some vegetable oils such as canola oil containing large proportions of C18:2ω6 and C18:3ω3 have also this problem. Even though these fatty acids have much higher oxidative steadiness compared with C22:6ω3 and C20:5ω3, the European Standard limits C18:2ω6 and C18:3ω3 methyl esters content in biodiesel for the vehicle to 12% (mol).43 The fatty acid content of some important algae has been emphasized in many studies. For instance, Nannochloropsis sp. Contains 28.83% C16:0, 32.93% C16:1ω7 and 21.16% C18:1ω9; Chlamydomonas reinhardtii contains 23.77% C16:0, 25.49% C18:3ω3; Scenedesmus sp. contains 18.42% C16:0 and 49.64% C18:1ω9 (Table 3). The high content of saturated and monounsaturated fatty acids in these algae indicates that they may be a good raw material for biodiesel.

One of the essential macro nutrients in the production of lipids is nitrogen. Complex nitrogen source can be better to simple nitrogen source in the heterotrophic culture of microalgae due to providing vitamins, amino acids, and essential growth factors. For the cultivation of microalgae, industrial nitrogen-rich wastewater can also be evaluated. Diluted monosodium glutamate waste is well treated as an inexpensive fermentation medium for Rhodotorula glutinis to synthesize lipids.44
starvation. Also, a study of microalgae has showed that Chlorella pyrenoidosa can be induced to accumulate 70% of its dry weight as lipids by nitrogen starvation. Nonetheless, nitrogen starvation cannot always finish in a boost in total lipid content in microalgae but can alter their lipid composition. Zhila and co-workers reported that the algae Botryococcus braunii deposited high content (28.4-38.4%) of C18:1ω9 under nitrogen limitation, however, the scope of total lipids and triglycerides did not change.

2.4. Composition of rendered oils and fats as a feedstock for biodiesel

The annual percentage of recycled vegetable oil obtained from potato processing plants, cookie factories, fast-food restaurants, industrial deep fryers is often more than expected. If all of this can be recycled, it can be protected partly the petroleum fuel in place. Waste cooking oil is mostly obtained from large quantities of oil used for fried food, since it needs full immersion of the food at temperatures above 180°C. The high temperature causes the changes in the chemical and physical composition of the oil and food, and in its organoleptic features which affect both the oil and food quality. Physically and chemically altered oils and fats directly affect the productivity and quality of the biodiesel. Increasing FFA and unsaturated fatty acid chains are undesirable molecules in oils and fats during biodiesel production.

Recently, the rendered animal fats have got attention as raw material for biodiesel industry, because they are easily obtainable and found in excess quantity with relatively low-priced in regions with excessive livestock. Most of the waste cooking oil collected from eating houses and food-service places contain animal fats. Biodiesel produced from animal-derived fats by a transesterification includes substantial quantities of long-chain saturated FAME, resulting in low temperature properties. Another hardship is that the waste of animal-derived fats comprises larger quantities of FFA than waste vegetable oil, and the FFA forms soap in the reactions with the alkaline catalyst reducing biodiesel efficiency. The soaps can stop the separation of the biodiesel from the glycerine fraction. An alternative arrangement is to use acid catalysts, which can esterify FFA. Black and yellow greases are present among the important industrial waste fats. Black greases are elucidated loosely as greases arising from sewage or other unusual oil sources. It has a low conversion factor to biodiesel on account of its high FFA content. Brown greases are largely described as a composition of greases and obtained from the slaughter industry. Yellow greases consist of the oils and greases coming from the fast-food industry.
compositions of the rendered oils mostly depend on raw material types.

Rendered waste oil contains decomposition products which degrade the quality of the oil and leads to a loss of efficiency in the transesterification reaction. Accordingly, this can cause unwanted by-products, damaging the final output. Therefore, it is significant to refine rendered waste oil for biodiesel production. Such purification has an effect of 67% to 87% in reaction yield after bleaching. The processes that can be applied to assess the adequacy of waste oils are de-acidification, filtration, neutralization, and bleaching. The processes of deodorization and degumming are not required since the oils have been treated previously before use. Although odors appear during degradation, its removal is not requisite for the biodiesel production.62

The rendered oils and fats must have a low acid amount, to prepare high yielding esters to use alkaline catalysis. The acid amount is an indication of the number of acidic functional groups in a sample and is evaluated in terms of the quantity of potassium hydroxide which is needed to neutralize the sample. In the alkali-catalyzed transesterification reaction, the FFA is converted into the ester by reaction with the catalyst to form soap. In acid-catalyzed processes, the reaction of FFA with alcohol forms ester, and water is produced which restricts the transesterification of triglycerides.64 The association between free FFA level and triglycerides transesterification in the course of acid-catalyzed biodiesel investigated in a study. The sample compounds were made ready by adding C16:0 to soybean oil to acquire FFA level of 5-33%. The transformation rate of soybean oil to methyl ester decreased from 90.54% to 58.77% as the FFA level rose from 5% to 33%.65

### 2.5. Composition of yeast and bacteria lipids as a feedstock for biodiesel

In recent years, very important and comprehensive researches have been done on biodiesel production capacities of microorganisms. Most of the microorganisms, particularly yeast and bacteria, are seen as a hopeful source for obtaining high-value lipids and supply considerable advantages over conventionally used vegetable or animal-based oils and fats. The yeasts can produce lipids in a very short time with a low cost. Additionally, bacteria have a less complicated genome and metabolism and can produce a wider variety of lipids than multicellular eukaryotes. On the other hand, they can generally be genetically altered more readily to obtain optimized strains that increase further the prolificacy of the entire process.66,67 The production of "third-generation biofuels" purposes to create efficient processes for the production of the lipids obtained from organic wastes of microorganisms. In some studies, it is expressed that special anaerobic microorganisms accumulate lipid of triglycerides during wastewater treatment.68 Oleaginous photosynthetic microorganisms efficiently convert carbon dioxide into lipids via photosynthesis. It is known that microalgae store triglycerides under nutritional and environmental stress, some cyanobacteria accumulate lipid in the form of diglycerides in significant quantities on their thylakoid membranes and may even be genetically engineered to provide overproduction of FFA.69

In a paper on yeast, with some applications, yeast could accumulate lipids up to 37% of its dry weight, which is normally around 5%. It has been found that biodiesel produced by yeast has a very similar structure.
compared to biodiesel generated with animal or plant-based raw materials in which C16 and C18 fatty acid chains are predominant. Furthermore, the study stated that FAME obtained from yeast grown under normal condition showed only four distinguished peaks of C16:0, C16:1ω7, C18:0 and C18:1ω9; whereas, the product of yeast cultivated in metabolic stress circumstances has given peaks of C16:0, C16:1ω7, C18:0, C18:1ω9, C18:2ω6, C20:0 and C22:0 of FAME along with several other peaks of structural lipids. In the experiment, the cells were forced to accumulate lipids by stress condition, so the concentration of the fatty acids had increased up to detectable quantities. With stress-based experiments and genetic modifications applied to the yeast, these microorganisms could allow them to accumulate higher lipid concentration. So that biodiesel can be obtained more cheaply and easily in the future.

Like yeast, bacteria also accumulate high amount of lipid in their bodies. For instance, the bacteria such as Arthrobacter, Dietzia, Gordonia, Nocardia, Rhodococcus, and Streptomyces have the ability of accumulation of triglycerides in significant amounts as intracellular storage deposits. However, Rhodococcus opacus, commonly known with impressive triglycerides content of up to about 80%. At the same time, it is seen as a very encouraging strain for industrial lipid production by rapid growth, the acceptance of high substrate tolerance and a large carbon source. Though Arthrobacter was announced several years ago to gather similar high quantity, this strain has not been employed in further studies. Gordonia or Rhodococcus strains express lipase suitable for the conversion of industrial waste materials for examplewastes from agro-industrial processes, molasses, dairy wastewater, etc. or lignocellulosic hydrolysates, whilst a Streptomyces can make use of cellulose to build up to 50% fatty acids. Unlike triglyceride synthesized by Rhodococcus, which are primarily composed of straight-chain of C16 and C18 fatty acid molecules, Streptomyces obtain the special capability to synthesize branched-chain fatty acids. As an alternative, ethanol can be used to get biodiesel from fatty acid ethyl esters (FAEE), which can exhibits superior physiochemical properties as per FAME, while a large majority of biodiesel is now produced with methanol derived from fossil sources to produce FAME. In a study, some initiatives were under taken to entirely synthesize FAEE de novo from irrelevant carbon sources in Escherichia coli. In a study, the over expression of a WS/DGAT gene was normally joined with the ethanol synthesis pathway from Zymomonas mobilis. Up to now, the highest FAEE amounts of 1.5 g l⁻¹ have been obtained only in glucose and small-scale cultivations. The major handicap of Escherichia coli is that it could not metabolize lignocellulose, but the first evidence of passage could be obtained by establishing an optimized strain using switchgrass hydrolysis. Another important property of bacteria is that they can synthesize branched fatty acids. Biodiesel produced from branched fatty acids has advantages over biodiesel produced from other feedstock. In a study, it is stated that the synthesis of fatty acid alkyl esters from branched short-chain alcohols and branched fatty acids (e.g., isoamyl alcohol or isobutanol); making use of the biosynthesis pathway for branched-chain amino acid synthesis is getting importance recently. There are advantages of biodiesel produced mainly from branched fatty acids found in microorganisms. For instances, branched fatty acid esters exhibit improved quality at low temperatures, where conventional biodiesel generally has relatively low efficiency. On the other hand, the synthesis of branched FAEE is still not achieved in significant quantities. Alternatively, bacteria incorporating inherently branched fatty acids into triglycerides such as Streptomyces may be favorable for biodiesel production with a mixture of branched and straight-chain fatty acid remnants.

3. COMMON TRANSESTERIFICATION REACTIONS FOR BIODIESEL

Vegetable oils or animal fats are not preferred to be used directly as fuel. They have high viscosity, and it is necessary to keep down their viscosity to use them in a common diesel engine. There are generally four methods used to reduce the viscosity; blending with petrodiesel, pyrolysis, micro-emulsification, and transesterification. The products produced by the transesterification reaction are generally known as biodiesel. Biodiesel is usually obtained by transesterification of fats and oils from lipid-containing raw materials such as soya, safflower, corn, palm, rapeseed, sunflower, jatropha, pongamia, cottonseed, tallow, lard, yellow and brown grease, cooking and waste oils. Clearly speaking, transesterification is done by monoglycerides, diglycerides and triglycerides from the almost all types of lipids of raw materials and can be affected by various factors including raw material composition, catalyst type, catalyst ratio, content of FFA, water concentration, the ratio of alcohol to triglycerides, alcohol type, pressure, temperature, mixing time, etc. Many studies have evaluated the variables that affect fatty acid alkyl esters known as biodiesel yields and their interactions.

The transesterification is the reaction of fats or oils (mostly 15 and 23 carbon atoms containing glycerides) with alcohol of low molecular weight (CH₃OH or C₂H₅OH) by using an alkaline, acidic or enzymatic catalyst, to produce fatty acid alkyl esters and glycerine. Triglycerides are converted to diglycerides followed by monoglycerides and at last glycerol. One molecule of a fatty acid alkyl ester is produced at each step.
The transesterification process consists of three continuous steps: firstly, the conversion of triglycerides to diglycerides, secondly the conversion of diglycerides to monoglycerides and then lastly, the transformation of monoglycerides to fatty acid alkyl ester and glycerine. One fatty acid alkyl ester molecule is produced from each conversion of fats and oils by alcohol as shown in Figure 1. When methanol alcohol opts-in transesterification reaction, the process is called methanolysis and the product is known as FAME; however, when ethanol alcohol is used, the process is called ethanolysis and the product is known as FAEE. Methanolysis is the most common process for producing biodiesel. Generally, acid, alkaline and enzymatic catalysts are used to increase the rate of transesterification reaction. Using the right catalyst may affect the rate of reaction, purification process and quality of biodiesel. FFA can react with the alkaline catalyst to form water and soap, which leads to the loss of the alkaline catalysts during the reaction. For this reason, additional catalysts should be added to compensate for the catalyst loss to soap. If the FFA level is over 5%, the produced soap will prevent disconnection of the methyl esters and glycerol from each other, resulting in emulsion formation during the water washing. Thereby, it is essential to convert FFA to methyl esters firstly to decrease their contents, and then transesterification is carried out with an alkaline catalyst to convert the pretreated triglycerides with low FFA to methyl esters. On the contrary, the enzymes used in transesterification are well tolerated at FFA proportion of the raw material, but the enzymes increase the cost and may not ensure the required degree of reaction.

3.1. Alkali catalyzed transesterification

Alkali-catalyzed transesterification of triglycerides is nearly 4000 times faster than the acid-catalyzed reaction. Alkali-catalyzed transesterification requires a lower amount of catalyst to accomplish the reaction. Sodium hydroxide (NaOH) and potassium hydroxide (KOH) are regularly used as commercial catalysts at a percentage of about 1% by weight of oil or fat. Alkoxides like sodium methoxide (NaOCH₃) are even better catalysts. Use of lipase provides important benefits, however are not currently practicable on account of the relatively high price of the enzyme catalyst. On the other hand, alkaline catalysts sometimes have drawbacks. Especially, they are very sensitive to the lipid raw materials and need a pretreatment to enhance the transesterification purity, mainly by operating in batch mode by needing large reaction times to obtain a complete transformation of oil, and also has complex biodiesel purification stages after the reaction. Alkaline catalysts produce soap from FFA in oils and fats. For this reason, if the lipid contains a high amount of FFA, it is not suitable for biodiesel production by an alkaline catalyst. With the high amount of alkaline catalyst, alkaline will react with the fatty acids and form emulsions between water and soaps molecules. More soap will be produced which consume the catalyst and reduces the catalytic efficiency as the catalyst concentration increases. When the high concentration of KOH is used as catalyzer, crude biodiesel will be purified by washing with sodium chloride (NaCl) solution. Saponification will also bring other problems such as the formation of gels, increases in viscosity and difficulty in achieving
separation of glycerine. Elimination of these saponified catalysts is technically arduous and it requires extra expenditure.\textsuperscript{87} Alkaline catalyst transesterification is used extensively throughout the world for vegetable, algal and used oils with low FFA content despite all these drawbacks. Homogeneous alkaline catalysis is mostly used for biodiesel production because it reacts at low temperature and high conversion output can succeed in a short period.\textsuperscript{86} The basic reason for this is that it is kinetically much faster than heterogeneously catalyzed transesterification and is economically effective. Ethanol, methanol, or butanol is preferred in the reactions to obtain methyl, ethyl, or butyl esters, respectively. Generally, the ratio of alcohol and oil is 6:1, at a reaction temperature of about 60°C if methanol is used, or 70°C if ethanol is used. The amount of catalyst used in the mixture is in the range of 0.5-1.0% (w/w).\textsuperscript{84,85}

3.2. Acid-catalyzed transesterification

Direct use of animal fats and vegetable oil is generally considered to be objectionable because of low volatility and high kinematic viscosity and leads to operational problems in a diesel engine, for instance, the formation of deposits and injector coking due to poorer atomization in the combustion chamber.\textsuperscript{88} Different methods have been devised to achieve sufficient engine compatibility for high-quality diesel fuels produced from vegetable oils and animal fats, including derivatization (transesterification, hydrotreating, and ozonation), dilution, blending, pyrolysis, gasification, and micro-emulsification. Transesterification process takes away glycerol from the triglycerides and alters it with alcohol mostly methanol. The process reduces the viscosity, however preserves heating value and the cetane number.\textsuperscript{89} Transesterification reaction reduces the viscosity of the lipid to a range (usually 4-5 mm s\textsuperscript{-1}) adjacent to that of petrodiesel.\textsuperscript{88}

In the existence of water and FFA, saponification reactions occur during alkaline catalysts because FFA reacts with the alkaline catalyst to produce soap, thus reduce FAME yield and even inhibit the transesterification reaction. Therefore, it is more convenient to use an acid catalyst for transesterification of triglycerides and esterification of FFA. Strong acid catalysts are less susceptible to FFA and can perform in transesterification and esterification reactions simultaneously. Despite all these advantages, alkaline-catalyzed reactions are still preferred today, because acid-catalyzed reactions are much slower than alkaline catalyzed reactions. During the acid-catalyzed reactions, the FFA can react with alcohol to form biodiesel and more suitable for converting FFA with high biodiesel yield.\textsuperscript{1} High acidic animal fat (more than 2.5% w/w of FFA) requires pretreatment to minimize their FFA content. This is normally fulfilled by acid-catalyzed esterification, using methanol as a reagent and \( \text{H}_2\text{SO}_4 \) as catalyst.\textsuperscript{87}

The acid-catalyzed transesterification reaction is usually done by \( \text{HCl}, \text{BF}_3, \text{H}_3\text{PO}_4, \text{H}_2\text{SO}_4, \) and \( \text{R}-\text{SO}_3\text{OH}, \) especially \( \text{R}-\text{SO}_3\text{OH} \) and \( \text{H}_2\text{SO}_4 \) are mostly utilized acids. These acid catalysts give very high efficiency in alkyl esters as biodiesel; however, the reaction is quite slow, needing temperatures above 100°C and from 3-48 h to catch up complete conversion.\textsuperscript{90} Besides the type of catalyst used, the type of alcohol used also affects the rate and efficiency of the reaction. In a study, it was stated that soybean oil methanolation is in the existence of 1 mol \% of \( \text{H}_2\text{SO}_4 \) with an alcohol/oil molar ratio of 30:1 at 65°C, prolongs 50h to completed reactions (>99%); whereas, the butanolysis (at 117°C) took 3 h and ethanolysis (at 78°C) took 18 h with the equal quantities of catalyst and alcohol.\textsuperscript{91} In fact, a way of accelerating these slow-acid catalyzed reactions has been emphasized in some studies. By the use of an extensive amount of acid catalyst, transesterification reaction rates may be increased. In most of the papers, acidic catalyst percentage (\( \text{H}_2\text{SO}_4 \)) in the reaction mixture varies from 1 to 5 wt %.\textsuperscript{91} However, the use of acid in such a high concentration is not preferred because it will ascend the cost of biodiesel production protein.

3.3. Enzyme catalyzed transesterification

In enzyme-catalyzed transesterification reactions, the lipase enzyme and alcohol are used to convert lipids to alkyl esters. Lipase enzymes are mostly obtained from bacteria and fungi because of their high enzyme yield and reproducibility in dense quantities. The microorganisms used to obtain the lipase enzyme are mostly: \textit{Burkholderia cepacia}, \textit{Candida antarctica} (Novo enzyme 435), \textit{Aspergillus niger}, \textit{Bacillus thermoleovorans}, \textit{Fusariumheterosporum}, \textit{F. oxysporum}, \textit{C. cylindracea}, \textit{C. rugosa}, \textit{Chromobacteriumviscosum}, \textit{Getrichum candidum}, \textit{Thermomyces lanuginose}, \textit{Mucormiehei}, \textit{Humicola lanuginose}, \textit{Pseudomonas cepacia}, \textit{P. roqueforti}, \textit{P. aeruginosa}, \textit{P. putida}, \textit{R. arrhizus}, \textit{P. fluorescens}, \textit{Rhizomucormiehei}, \textit{Oosporalactis}, \textit{Rhodotorularabra}, \textit{Saccharomyces cerevisiae} and \textit{Staphylococcus hyicus}, \textit{Penicilliumcyclopium}, \textit{Rhizopusoryzae}, \textit{R. japonicus} (NR400), \textit{R. stolonifer} (NRRL1478), \textit{R. chinensis} \textit{R. delemr}, \textit{R. fusiformis}, \textit{R. circinans}.\textsuperscript{92,93} Among these microorganisms, \textit{C. antarctica}, \textit{C. rugosa}, \textit{R. miehei}, \textit{R. chinensis}, \textit{P. cepacia}, \textit{P. fluorescens}, \textit{R. oryzae} and \textit{T. lanuginosa} have produced the most effective lipase enzymes.\textsuperscript{84} Lipases can be classified based on their extracellular and intracellular applications. Extracellular lipases are extracted and purified from organisms; while intracellular lipases are enzymes that are present within the organisms.\textsuperscript{95}
The conventional synthesizing method of biodiesel, which includes acid and alkaline catalyst, is accomplished at a high temperature closer to methanol or ethanol boiling point; therefore some unwanted by-products such as soap are created. To put away these drawbacks, it has been proposed that the enzymes can be used instead of chemical catalysts owing to their mild operative circumstances and high particularity. Besides, there is no need for feedstock purification because biocatalysts like lipase can esterify feedstock FFA along with the transesterification of triglycerides. Some studies have indicated essential data about the temperature tolerances of the enzymes from microorganisms. C. antarctica was fulfilled in a temperature between 25 and 55°C by methanolysis. The optimum temperature was detected at 40°C, the temperature above 40°C leads to a diminution in conversion. P. cepacia was performed in a temperature extended from 20 to 70°C and found the optimal temperature to be 50°C after 1 h which decreased to 40°C after 2 h by butanolysis. Methanolysis with R. chinesis enzymes had an optimal temperature of 30°C.

There are many advantages of using enzymes instead of acid and alkaline catalysts including absence of soap formation, producing a higher quality glycerol, ability to process large variation in raw material, having ability to esterify both FFA and triglycerides in one stage without the need for a washing step, works under milder conditions leading to less energy consumption, and high FFA consisting animal fat and waste oil can be catalyzed with completely to alkyl esters. On the other hand, enzymatic transesterification has many disadvantages, such as longer response time, requiring a higher catalyst amount to complete the reaction and high fabrication cost. Additionally, lipase becomes ineffective in 100 days of application, although it can be used repeatedly after immobilization of the lipase on the carrier.

4. CONCLUSIONS

To overcome the energy crisis in the world, the search for biologically lipid-rich materials for using in biodiesel productions effectively has attracted much interest. New modified microbial, animal, algal and vegetable raw materials are considered positively for biodiesel production in terms of short development cycles, high lipid content, and ease of replacement via biotechnological methods. Raw materials commercially exploited by countries constitute mostly edible lipids commonly supplied from soybean, rapeseed, coconut, corn, palm, sunflower, safflower, etc. It is thought that the use of such edible oil to produce biodiesel in the world is not correct because such oils have a big gap in supply and demand around the world. Increasing pressure to increase edible oil production also limits the biodiesel production. For this reason, many of today's examinations are related to algae and microbial lipids which are of great promise for biodiesel.

Generally, short-chain fatty acids like C14 and C18 carbon-containing raw materials are more suitable for biodiesel productions. Saturated fatty acids have got more advantages than unsaturated fatty acids because FAME with four and more double bonds is susceptible to oxidation during storage, and for this property, their acceptability for use in biodiesel becomes decreasing. Less FFA content in the oils or fats is more advantageous because it prevents the formation of soap. The importance of lipid content in a high proportion of acylglycerol provides a greater advantage, even if the raw material has high oil content.

Biodiesel has several important advantages. Most of which can be listed as follows: the emission profile of the biodiesel is much better than petroleum diesel, reducing emissions of unburned hydrocarbons, sulfates, carbon monoxide, nitrated polycyclic aromatic hydrocarbons, and particulate matter. It is non-toxic renewable and biodegradable fuel, besides it can be used as blended or pure with petrodiesel in engines without any modifications. Transportation and storage of the biodiesel are less dangerous than the petroleum diesel because the flash point temperature of biodiesel is nearly 170°C, whereas it is 60 to 80°C for petroleum diesel. The combustion process is better than petrodiesel and smoke is less. It can diminish unburned hydrocarbons emissions by 90%. Higher lubrication capacity prolongs engine life. The biodiesel embodies at least 11% oxygen and it causes better burning than petroleum diesel. On the other hand, it reduces the release of PAH (Polycyclic aromatic hydrocarbons) (cancer-causing compound). The CO2 released from biodiesel combustion is an approximate amount for photosynthesis of plants. In other words, the vegetable oil carbon percentage is nearly 77.8%, the average percentage of animal fats is 76.1%, whereas carbon percentage of fossil diesel is 86.7%.

To sum up, biodiesel production recently has attracted much attention due to its many advantages over traditional energy sources. At present, the high cost of oily raw materials is the main problem preventing commercial biodiesel production. For this reason, to develop new transesterification technologies and to obtain cheap raw materials that are rich in fat or oil content seem like the most important solution for an efficient biodiesel production.

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Conflict of interests

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