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Review Article

The Renewable Energy: Environmentally Friendly Algae Biofuel

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Abstract: Due to the limited availability of fossil fuels and the continuous increase in world energy demand, it will lead to an energy crisis in the future. The use of these energetic resources is responsible for the accumulation of greenhouse gases in the atmosphere that is associated with several adverse effects on the environment. Therefore, it is worth to search for different energy supplies that are renewable and environmentally friendly energy fuel. The alternative fuel is biodiesel, and its primary sources are oil seeds, used cooking oil, algae. Among which microalgae are photosynthetic microorganisms that can achieve high oil contents. This oil is suitable for producing biodiesel; thus, microalgae are considered a promising sustainable energetic resource that can reduce the dependence of fossil fuel. Biodiesel production from microalgae includes several steps, such as cell cultivation and harvesting, oil extraction, and biodiesel synthesis. Biodiesel is usually used by blending with Petro diesel, but it can also use in pure form. Biodiesel is a sustainable fuel, as it can be produced throughout the year and can run an engine with certain modifications. It can satisfy the needs and can also meet the demands of the future generation to come.

Keywords: Biodiesel, Extraction Method, Green Diesel, Microalgal Biomass, Transesterification.

1. Introduction

Since the occurrence of the 1973 energy crisis, countries in the world, especially developed countries, began to direct their attention to developing non-fossil energy sources from agriculture and forestry or other biological materials or often called bioenergy [1]. Petrol or premium fossil oil then given an alternative, namely bioethanol, while diesel or diesel oil with biodiesel [2]. Bioethanol made from starchy or sugar-containing substances such as molasses, coconut juice, sorghum stem juice, cassava starch, sago starch, and the like [3], [4]. Biodiesel made from vegetable oil from palm oil (CPO), coconut oil, and other plants that produce vegetable oil. The biofuel is known as Generation One Biofuel or provided by Generation One Biofuel Technology [5].

Recognizing the increasing demand for biofuels and at the same time will increase the demand for land and crop yields, the experts argue that Generation One Biofuels will compete and threaten the fulfillment of world food needs [6]. Along with the development of science and technology and the rapid development of world research, alternative techniques have developed to produce biofuels from non-food materials, which utilize agricultural and forestry solid biomass and solid waste that has known to contain lignocellulose. If lignin is separated, cellulose in this biomass can be processed into bioethanol and biodiesel. Biofuel, as a result of this technological process, is known as Second Generation Biofuel, which is by utilizing lignocellulose in solid biomass of forestry agriculture and its waste to produce bioethanol and biodiesel [7].

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The depletion of fossil fuels and the effect of exhaust gas emission on global climate change has stimulated the search for a sustainable source of energy that is carbon neutral or renewable [8], [9]. As an alternative energy resource, much attention has paid to biodiesel production from vegetable oil crops and animal fats. The oil extracted from vegetable have 10-20 times viscosity than petroleum fuel. Therefore, using vegetable oils directly as fuel can cause engine problems like injector fouling and particle agglomeration. Their effect can be reduced or eliminated through transesterification of vegetable oil to alkyl esters. This process decreases the viscosity of vegetable oil but maintains its properties like diesel fuel. Furthermore, producing biodiesel from vegetable crops is time-consuming and requires a significant area of arable land that would compete with the one used for food crops, leading to starvation of society.

To avoid the competition between energy and food production, attention now focused on evaluating the potential of microalgae as an oil source for biodiesel production [10]. Microalgae are photosynthetic microorganisms that present several advantages such as: (1) higher oil contents; (2) higher growth and biomass production rates; (3) shorter maturity rates; and (4) requires far less land. Despite the referred advantages, the production of biodiesel from microalgae is not economically viable. Technological improvement should be performed to reduce costs, including (1) improvement of photosynthetic efficiency, (2) reduction of water and CO_2 losses in microalgae cultures, (3) improvement of energy balances; (4) use of flue gases as a CO_2 source.

Table 1. Oil Content in Some Micro Algae Organisms

No.	Micro Algae	Oil content (% dry weight)
1	*Botryococcus braunii*	25 – 75
2	*Chlorella sp.*	28 – 32
3	*Crypthecodinium cohnii*	20
4	*Cylindrotheca sp.*	16 – 37
5	*Nitzschia sp.*	45 – 47
6	*Phaeodactylum tricornutum*	20 – 30
7	*Schizochytrium sp.*	50 – 77
8	*Tetraselmis Suecia*	15 – 23

Algae are aquatic environmental organisms that use light and carbon dioxide to produce biomass. Algae grouped into two sections based on size, namely macroalgae and microalgae. The most efficient use of algae is the use of oil to produce biofuels. Some types of algae even produce hydrogen gas if it is grown under certain conditions. Biomass from algae can also be burned, like wood, to produce heat and electricity.

Algal biomass consists of three main components, including carbohydrates, proteins, and lipids (natural oils) [11]. Microalgae grow very fast compared to terrestrial plants. Algae generally grow two times larger than the previous size every 24 hours. During the peak of the growth phase, some microalgae can double every 3.5 hours. The oil content of microalgae is usually between 20% and 50% (dry weight), then some strains can reach as high as 80%. This production is much higher compared to oil produced by various terrestrial plants, which only contain a maximum of about 5% dry weight of oil from microalgae [12].

The cost of producing algal oil depends on many factors, such as the yield of biomass from a cultural system, the oil content, the scale of the production system, and the cost of oil recovery from algal biomass. At present, algal oil production is still far more expensive than diesel fuel. However, this problem expected to be overcome or minimized by technological development. Given the enormous potential of microalgae as the most efficient primary producer. Biofuels from algae are ideal candidates for biofuels that can eventually replace petroleum-based fuels because of several advantages, such as high oil content, high production, less land. At present, the production of biofuel algae is still too expensive to be commercialized because of the static costs associated with extracting oil and biodiesel.

2. Different Generation of Biofuel

Biofuel is energy sources made from recently grown biomass (plant or animals' matter). Biofuels are a renewable resource because they continually replenished.

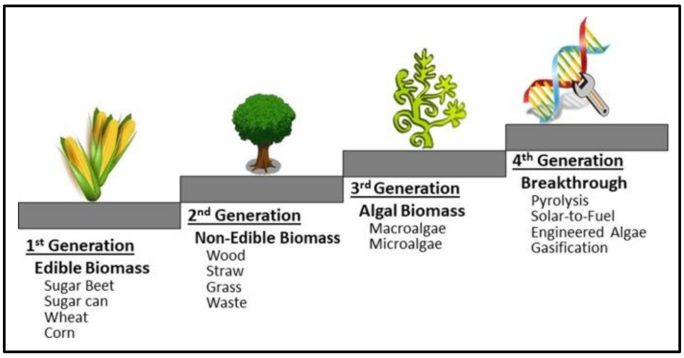

Figure 1. Evolution of Biofuel Generations [13].

2.1. First Generation Biofuels

First Generation Biofuels are produced directly from food crops by abstracting the oils for use in biodiesel or producing bioethanol through fermentation. Crops such as wheat and sugar are the most widely used feedstock for ethanol. However, first-generation biofuels have specific problems like the CO_2 gas emission from produce biofuel is higher than being consumed in the production of biofuel. Secondly, it has an issue of 'Fuel vs. Food.' As the majority of biofuels are produced directly from food crops, the rise in demand for biofuels has lead to an increase in the volume of crops diverted away from the global food market [14].

First-generation biofuels produced from food sources, where the source is natural to process. These foods contain sugar, starch, or vegetable oil—examples such as cassava, corn, and sweet potatoes. The manufacturing process tends to be easy because the extraction of biofuels from these three compounds does not require a complicated process. However, in its application, this biofuel experienced a lot of controversies. The first thing is the dilemma between food and energy because, in addition to our many brothers having a food crisis, the use of energy food also feared to be able to disrupt future food supplies [15]. This generation of biofuels also requires large tracts of land for planting the necessary resources, so in the end, we may have to cut down forests to clear the area.

2.2. Second Generation Biofuels

Second Generation Biofuels has developed to overcome the limitations of first-generation biofuels. They produced from non-food crops such as wood, organic waste, food crop waste, and specific biomass biofuel. These are being more cost-competitive concerning existing fossil fuels. The second generation of biofuels uses lignocellulosic materials such as wood and agricultural wastes. Although the process of making this generation of biofuels is more complicated than the first generation, this second-generation biofuel based on non-food, so there is no 'energy versus food' problem in this generation. The analogy is our first generation is like making noodles with flour raw material. This second-generation, to make noodles with flour that we must make from sweet potatoes first [12]. This generation can certainly reduce waste and can continue along with human food needs.

2.3. Third Generation Biofuels

Third Generation Biofuels based on improvements in the production of biomass. It takes advantage of energy crops such as algae as its energy source. The algae are cultured to act as low-cost, high-energy, and entirely renewable feedstock. Algae can also be grown using land and water unsuitable for food production, therefore reducing the strain on already depleted water sources. Third generation biofuels are algae-based biofuels. Algae are unicellular (single-celled) or multicellular (multicellular) plants that have very high growth rates that live in marine or freshwater [12]. The number of species of algae is estimated to be above 50,000.

Due to its more straightforward structure and faster-growing speed, microalgae are currently being applied more for biofuel production than macroalgae. Microalgae can be cultivated in open ponds or with a machine called bioreactor incubation. Under optimum conditions, microalgae can divide several times a day. When compared to plants such as jatropha or palm oil, Algae can produce at least 15 times more oil products per hectare. Based on comparative data with petroleum, it turns out that the potential for microalgae is still more significant. In 1 hectare of the oil field, it can only be extracted by 0.83 barrels of oil per day, until it finished and no longer producing. While in the same area, microalgae cultivation can produce 2 barrels of oil per day [16]. In addition to the potential growth speed of the Algae, Algae has amazing content. Microalga has very high oil content, which can reach 40-85% of dry weight [17], even compared to palm oil, which has been the main icon of biodiesel much higher in oil content. The content of palm oil is only 20%. Algae are more productive than other plants because they continue to make fuel regardless of the weather [18]. Unlike the first and second generation who stumble on the production of raw materials and both are equally facing limited land. Algae can grow in many countries, without the need for fertile soil and abundant freshwater. Production and cultivation of algae for the distant future will not face the problem of land limitations because algae can be cultivated in any waters, including the ocean, or even a pool of wastewater.

2.4. Fourth Generation Biofuels

Fourth Generation Biofuels aimed at not only producing sustainable energy but also a way of capturing and storing CO_2. This process differs from second and third-generation biofuel is such that at all stages of production, the CO_2 captured using a process such as oxy-fuel combustion. The fourth-generation biofuel is almost the same as the third generation, which does not use arable land. It is just the difference; fourth generation biofuels do not involve the destruction of biomass. These include electro fuel and photobiological solar fuel. The advantage of this generation of biofuel is that raw materials are not destroyed in the production process, so they can be used many times. This biofuel is still under development.

3. Microalgae Description

Algae are thallophyte organisms, organisms that do not have real roots, stems, and leaves that have chlorophyll as the primary photosynthetic pigment and do not have sterile cells that protect their reproductive cells. Algae do not show one taxonomic group that is close together, but it is a group of organisms that have photosynthetic diversity that exchanges only a few characteristics [19]. There are two basic types of algae cells, namely prokaryotic and eukaryotic. Prokaryotic cells do not have membranous organelles (plastids, mitochondria, nuclei, Golgi bodies, and flagella). Eukaryotic cells surrounded by cell walls composed of polysaccharides, and inside have a plasma membrane that regulates the entry and exit of compounds in the protoplasm—the nucleus surrounded by a double cell membrane that has pores. The chloroplast has a membrane sac called thylakoids, which carry light reactions for photosynthesis. The chloroplast also wrapped in a double membrane. While the Golgi body is composed of many membrane sacs (membrane sacs) called the cysteine. Flagella are composed of axons from a doublet microtubule surrounded by two central microtubules, which are all encased in a cell plasma membrane.

Microalgae are marine organisms characterized by their high biomass productivity, photosynthesis efficiency, and exciting oil content, which makes them a promising feedstock for biodiesel production. Algae can reach a high level of oil content, like 75% of the dry basis for *Botryococcus braunii*. The primary constituent of most algal oil is unsaturated fatty acids, such as palmitic acid (C16:0), along with significant amounts of highly unsaturated species [20], [21].

There are different metabolic behaviors in microalgae including (1) Autotrophic, i.e., algae utilizing light as a sole energy source, they convert light energy to chemical energy using CO_2 through photosynthesis; (2) Mixotrophic, i.e., algae performing photosynthesis as the primary energy source, but they need both organic compounds and CO_2; (3) Heterotrophic, i.e., algae utilizing only organic compounds as energy and carbon source; and (4) Photoheterotrophic, i.e., algae utilizing light to use organic compounds as carbon source. Algae can change the metabolic pathway according to the changes in the environmental condition. Algae for biodiesel production should be selected to grow in photoautotrophic mode to reduce the cultivation cost and to utilize CO_2 as much as possible for CO_2 sequestration.

3.1. Microalgae Biomass Production Systems

One method used to capture sunlight and CO2 efficiently is to use a system: an open pond or a photobioreactor. Photobioreactors are carried out by using transparent pipes, using natural sunlight, and feeding with the graffiti method. Mixing with CO2 bubbles is another method used to maximize CO2 capture and reduce costs [22].

Cultivation of microalgae can be done in an open-culture system or in a highly controlled closed-culture system called Photobioreactors (PBRs). Open ponds are about 20-40 cm deep and are designed to copy the conditions of the algae’s natural environment. It has a paddlewheel that maintains circulation in the system to mix the algal cells and nutrients and baffles designed to maintain the flow in the system.

These nutrients can be supplied by using wastewater rather than clean water. Its benefits are: it is cheaper to build, clean, and easy to maintain as compare with PBRs. While the disadvantages of open ponds are a lot of water loss due to evaporation, which results in the system having an enormous demand for daily water supply to compensate for the loss. Environment temperature impacts on the growth rate of microalgae, the optimal annual temperature for high productivity is 18-23°C while below 20°C the productivity declines drastically [23]–[25].

Figure 2. Open Raceway Pond

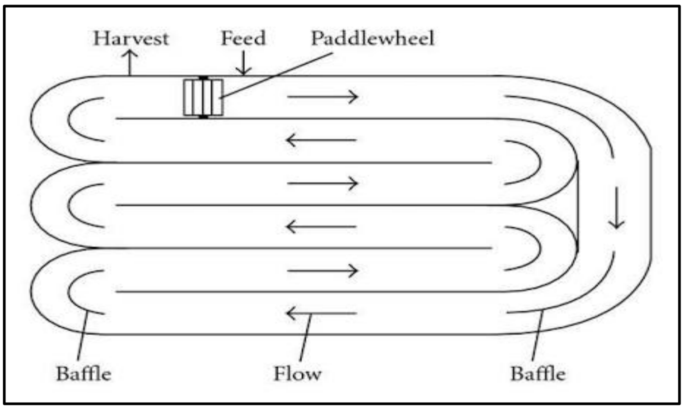

Figure 3. Open Raceway Structure

Even in the desert region, it can be challenging to maintain high productivity of algae due to low nighttime temperatures. These impacts photosynthesis rate because the pond must warm up again. To overcome these issues, some tried remedies are covering ponds, increasing the depth of the pond.

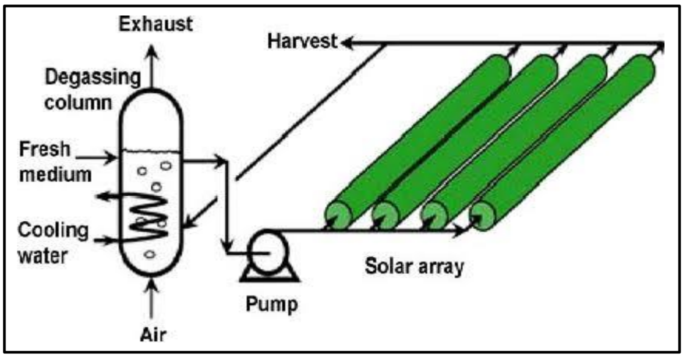

Figure 4. Photobioreactor Structure

Figure 5. Photobioreactor

Photobioreactor tubes are made of glass or acrylic, allowing daylight to enter the system for photosynthesis to occur. Nutrients and water are injected into a feeding vessel and are circulated into the system by a pump. PBRs are a more controlled system and can be optimized according to the biological and physiological characteristics of the cultivated algal species, allowing the cultivation of algal species that cannot be grown in open ponds. PBRs offer better control over culture conditions and growth parameters (pH, temperature, mixing, CO_2, and O_2) to prevent evaporation, reduce CO_2 losses, allow to attain higher microalgae densities. Despite its advantage, PBRs has its limitations like overheating, bio-fouling oxygen accumulation, difficulty in scaling up, the high cost of building and operating of algal biomass cultivation cell, cell damage by shear stress and deterioration of material used for photo-stage.

4. Manufacturing of Biodiesel

Biodiesel production from microalgal, emphasize two process concepts: (1) Indirect route, in which after a facultative cell wall disruption method, microalgal oil is recovered in an appropriate solvent and then converted into biodiesel through transesterification and (2) Direct route, in which biodiesel is produced directly from the harvested biomass. High biodiesel yields obtained when both routes are preceded by a cell wall disruption method, in indirect route, it is possible to apply three different types of solvent to recover microalgal oil [26], [27].

The most promising and cost-effective alternative for lipid recovery is n-hexane. The available information proposes that the direct route is the most efficient. The downstream process towards biodiesel production includes (1) Harvesting, (2) Drying; (3) Cell disruption and oil extraction; and (4) Transesterification.

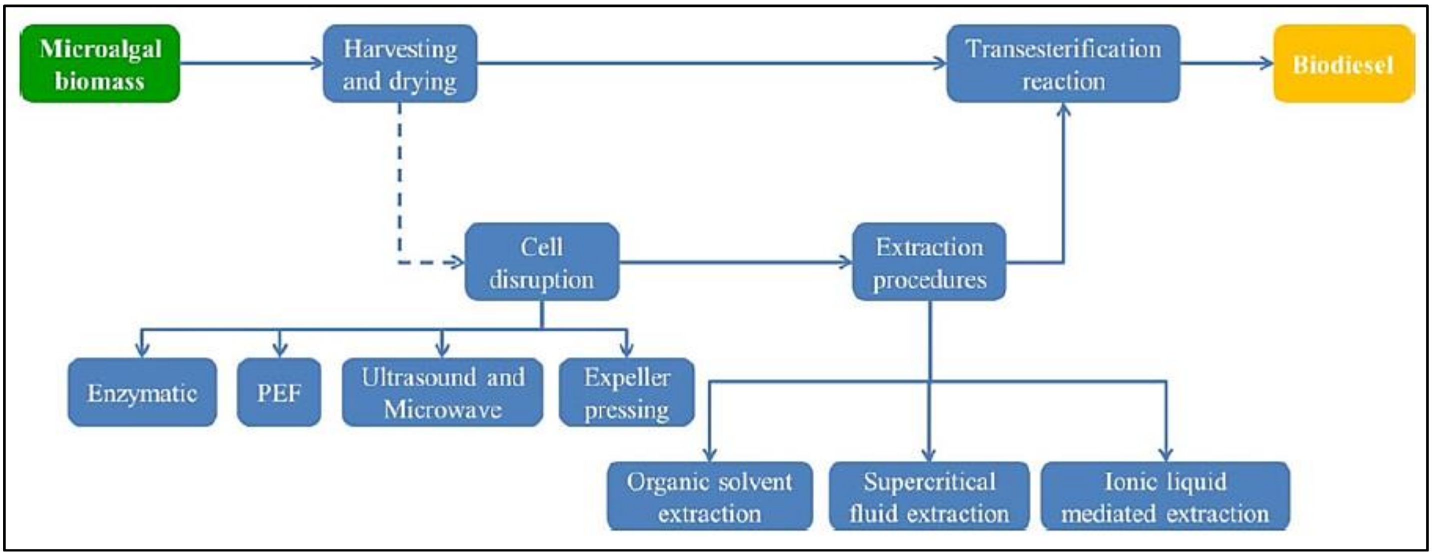

Figure 6. Steps of Biodiesel Production

The most common methods to recover oil from microalgae are adding organic solvents, supercritical fluids, and ionic liquids. The oil is not extracted from dewatered biomass, as it results in a decrease in efficiency because microalgal cell walls block the access of solvents to the cytosol, where most lipids accumulate [28].

4.1. Cell Disruption Methods

The cell disruption method aims to burst the cell wall to allow a free path to release intracellular components (like lipid) into an adequate solvent. Some methods are:

- Enzymatic Disruption
  Enzymes can be applied in oil extraction from microalgae, as they can mediate the hydrolysis of cell walls, enabling the release of their content. Cellulose is the most applied enzyme .
- Pulsed Electric Field (PEF)
  Pulsed Electric Field (PEF) technology has its potential as an alternative for oil extraction from microalgae. This technique uses pulses of a strong electric field to cells, which induces non-thermal permeabilization of the membrane. This lead to the complete disruption of the cell into fragments. It requires less time and energy than other applied methods [29]. This method used where the less organic solvent (usually presenting high toxicity) rather than the conventional organic solvent extraction method.
- Ultrasound and Microwave
  In the ultrasonic-assisted method, microalgal oil recovered by cavitation. This technique can improve efficiency by reducing extraction time and increasing oil recovery yield [26], [30]. Example: The experiment *Crypthecodinium cohnii* microalgae showed that cell disruption using ultrasounds increased oil extraction yield from 4.8% when applying Soxhlet extraction with n-hexane to 25.9%.
- Expeller Pressing
  When microalgae put under high pressure, they start to crush, releasing their contents [31]. After pressing microalgae, the oil recovered by using a solvent system containing ethanol and n-hexane in a 1:1 (v/v) ratio. Example: Increase in oil yield in Scenedesmus dimorphous found from 6.3 to 21.2% (wt.).

5. Oil Extraction Methods

Extraction of microalgal oil can be performed directly to harvested biomass or in addition to a cell wall disruption method. It is essential to choose an appropriate solvent, aiming to improve yield and to reduce process costs.

- Organic Solvent Extraction
  It is the most applied method to extract microalgal oil. The selection of organic solvent should make under the consideration of cost, toxicity, and recycling after use. The most applied organic solvents are n-hexane, cyclohexane, benzene, ethanol, acetone, and chloroform. Example: Mixture of chloroform and methanol (1:1, v/v) to extract oil from organisms Botryococcus specie and Chlorella Vulgaris, their yields were 7.9%, 4.9% respectively.
- Supercritical Fluid Extraction
  Supercritical fluids are compounds that behave both as liquid or gas when exposed to temperature and pressure above their critical values. The most used supercritical fluid for oil extraction is CO2 because it has a low critical temperature (31.1^{o}C) and pressure (72.9 atm). Example: Oil extraction of Nannochloropsis specie yield 23% by CO2 and 12% yield by using n-hexane (by organic solvent extraction method.
- Ionic Liquid-Mediated Extraction
  Ionic liquids (ILs) used as an alternative for volatile and toxic organic solvents because of their non-volatile character, thermal stability, and high solvation capacity. This (ILs) are salts of relatively large asymmetric organic cations coupled with smaller organic or inorganic anions. Example: Ionic liquid extraction from microalgae Dunaliella sp. and Chlorella sp. resulted in an oil yield of 8.6% and 38% by using a mixture of 1-ethyl-3-methylimidazolium methyl sulfate [Ethyl-mim] $MeSO_4$ and methanol.

6. Biodiesel Production

Biodiesel made through a chemical process called transesterification, where glycerin separated from vegetable oils. This process produces two products, namely methyl esters (biodiesel)/mono-alkyl esters and glycerin, which are by-products. The primary raw materials for making biodiesel include vegetable oils, animal fats, used fats/recycled fats. All these raw materials contain triglycerides, free fatty acids, and pollutants, which depend on the preliminary treatment of these raw materials, meanwhile, as a supporting raw material, namely alcohol. In this process, biodiesel needed for the catalyst for the esterification process and the catalyst needed because alcohol dissolves in oil. The vegetable oil content of free fatty acids is lower than animal fat, vegetable oil usually contains not only free fatty acids but also phospholipids, phospholipids can be removed in the degumming process and free fatty acids removed in the refining process. Vegetable oil used can be in the form of oil Biodiesel products depend on vegetable oil used as raw material after preliminary processing of the raw material [32].

Alcohol used as a reactant for vegetable oils is methanol, but can also use ethanol, isopropanol, or butyl, but it should also note the water content in the alcohol. If the water content is high, it will affect the yield of low-quality biodiesel because of the content of soap, free fatty acids, and trig. Besides that, biodiesel yield also influenced by the high operating temperature of the production process, the length of time of mixing, or the speed of mixing alcohol [33], [34].

Once the oil has extracted from algae, it becomes necessary to investigate transesterification, which is a process that can convert the oil into biodiesel that can now serve a purpose as fuel. Transesterification is the process in which the alcohol molecule and an ester molecule react in either presence of acid or base to form a new ester. To simplify the purification of fatty acid methyl ester (FAMEs) and to recover glycerol quickly, enzymatic transesterification has used. Then there is acid-catalyzed transesterification where the reaction is much slower, but instead, this reaction is more suitable for glycerides that have relatively high free fatty acid content and more water. Thus, transesterification is a nucleophilic substitution reaction that consists of three reversible reactions. Firstly, triglycerides converted into diglycerides, which further converted into monoglycerides [35]. Lastly, the monoglycerides converted into glycerol. These steps are shown below:

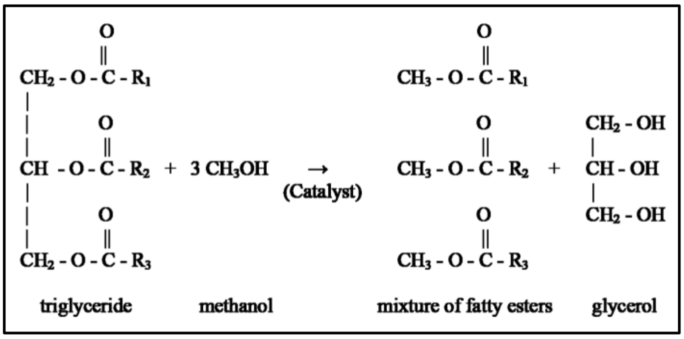

Figure 7. Reaction Equation of the Transesterification Process [36].

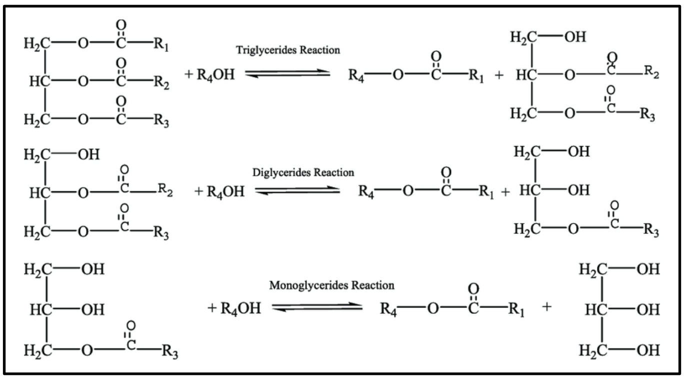

Figure 8. Steps of Biodiesel Production Separate Individual Reaction of Transesterification

This generally done with ethanol and sodium ethanol ate (It can produce by reacting ethanol with sodium). It is used as a catalyst. The reaction is between an ester of one alcohol and secondary alcohol. The result will be an ester of the second alcohol and alcohol from the first ester. Chemically, this means taking a triglyceride molecule (or a fatty acid molecule), neutralize the fatty acids and remove the glycerin to create an alcohol ester. This is done by merely mixing methanol with sodium or potassium hydroxide to make sodium or potassium methoxide. This liquid then mixed with the triglycerides. The mixture settles after some time, as glycerin in the bottom while the methyl esters (or biodiesel) left on top. Thus, with sodium ethanoate as a catalyst, ethanol reacts with triglyceride giving the final products of biodiesel, sodium ethanoate, and glycerol. Ether and saltwater are added to the mixture to separate it into two phases. After some time, the mixture will separate into two layers, the bottom layer containing a mixture of ether and biodiesel. This layer then separated from the ether via vaporizing under a high vacuum.

A catalyst is also needed to increase the solubility during the reaction. Generally, the catalyst used is a strong base, NaOH, or KOH or sodium methoxide. The catalyst to be chosen depends on the vegetable oil used, if it is used crude oil with a free fatty acid content of less than 2%, besides soap and glycerin are formed. The catalyst is generally very hygroscopic and reacts to form a chemical solution that will be destroyed by an alcohol reactant. If the catalyst absorbs much water, the catalyst is not working well, so that biodiesel products are not right. After the reaction is complete, the addition of strong mineral acids must neutralize the catalyst. After biodiesel is washed the neutralization process can also be done by adding washing water, HCl can also be used for the neutralization process of fundamental catalysts, if used phosphoric acid will produce phosphate fertilizer (K_3PO_4).

7. Conclusion

Algae species can use as raw material for biodiesel production. The process mainly involves two steps, i.e., oil extraction and transesterification. For oil extraction, organic solvent extraction is the most efficient method. The main drawback of applying organic solvents for oil extraction is related to the harmfulness of these compounds. However, the amount of organic solvent required can be reduced by the previous application of a cell wall disruption method. These procedures are also responsible for an increase in oil extraction efficiencies.

Further, Enzymatic and Pulsed Electric Field extraction seems to be profitable because enzymes show high selectivity towards cell walls, and Pulsed Electric Field has reduced energetic cost compared to the other disruption methods. Another possibility to avoid the use of organic solvents is to use ILs as solvents. These compounds are safer for the environment; however, their cost is also high. An alternative for these expensive methods is the application of n-hexane, as it is less harmful and has a higher selectivity for neutral oil fractions than other organic solvents. While in transesterification, the variables affecting the process should be taken into consideration and to be maintained to attain the maximum yield of biodiesel production. Thus, in the coming era of depleting fossil fuel, biodiesel will be the future of all fuel, and microalgae can be the best source of biodiesel production.

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