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Current strategies and prospects of biodiesel production: A review

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ABSTRACT

High fossil fuel prices, energy security concerns, and environmental issues—particularly climate change have motivated countries across the world to explore alternative sources of energy, such as biodiesel. It is a renewable substitute fuel for petroleum diesel or petrodiesel fuel made from vegetable or animal fats; it can be used in any mixture with petrodiesel fuel, as it has very similar characteristics, but with lower exhaust emissions. Biodiesel fuel has better properties than petrodiesel; it is renewable, biodegradable, non-toxic, and essentially free of sulfur and aromatics. Biodiesel seems to be a realistic fuel for future; it has become more attractive recently because of its environmental benefits. Primarily, biodiesel is produced from food crops and mostly oil seeds which are limited in their ability to achieve targets for replacement of fossil diesel, climate change and economic mitigation and their competition with the food has lead to the focus on microalgae which offer a better and reliable source for biodiesel production. This paper reviews the various aspects, methods and technologies associated with the production of biodiesel from available feedstocks such as vegetable oils, animal fats and microalgae with a comprehensive comparison among them.

Key words: Biodiesel, microalgae.

INTRODUCTION

Petroleum-based fuels are limited reserves concentrated in certain regions of the world. These sources are on the verge of reaching their peak production. The fossil fuel resources are shortening day by day. [1] The scarcity of conventional fossil fuels, growing emissions of combustion-generated pollutants, and their increasing costs has made biomass sources more attractive. [2] Biodiesel has recieved increasing attention worldwide as a blending component or a direct replacement for diesel fuel in vehicle engines. Biodiesel, as an alternative fuel for internal combustion engines, is defined as a mixture of monoalkyl esters of long chain fatty acids (FAME) derived from a renewable lipid feedstock, such as vegetable oil or animal fat. Biodiesel typically comprises alkyl fatty acid (chain length C_{14}–C_{22}) esters of short-chain alcohols, primarily, methanol or ethanol. Biodiesel is the best candidate for diesel fuels in diesel engines. The higher heating values (HHVs) of biodiesels are relatively high. The HHVs of biodiesels (39–41 MJ/kg) are slightly lower than those of gasoline (46 MJ/kg), petrodiesel (43 MJ/kg), or petroleum (42 MJ/kg), but higher than coal (32–37 MJ/kg) [3]. For the production of first generation biodiesel, edible vegetable oils such as soybean, rapeseed, and palm oils are used as the main feedstock. However, the use of edible oils as energy source has raised a lot of objections from public and non-government organizations. Thus, second generation biodiesel derived from non-edible oils such as *Jatropha curcas L.* appeared as an attractive alternative feedstock for the biodiesel industry. In fact, the use of jatropha oil in existing biodiesel plant does not require major modification on the equipments and process flow, mainly because the oil has similar properties to edible oils. Another advantage of using jatropha oil is that jatropha trees can grow easily on non-arable or wasteland. However, jatropha oil does contain higher concentration of free fatty acid (FFA) that may require an additional pre-treatment step. Nevertheless, regular irrigation, heavy fertilization and good management practices are required to ensure high oil yield. Due to these weaknesses, the search for a more sustainable biodiesel feedstock continues and now focuses on microalgae [4]. Microalgae are recognized as one of the oldest living microorganisms on earth [5]. They grow at an exceptional fast rate: 100 times faster than terrestrial plants and they can double their biomass in less than one day [6]. Apart from that, some microalgae strains are able to accumulate large quantity of lipid inside their cells, in which the lipid can be converted to biodiesel [7]. According to a recent study reported in the literature, a realistic value of microalgae biomass production lies between 15 and 25 tonne/ha/year. With an assumption of 30% lipid content in microalgae cells (without optimizing the growth condition), this is equivalent to a lipid production of 4.5–7.5 tonne/ha/ year [8]. This amount is higher compared to the production of oil from soybean (0.4 tonne/ha/year), rapeseed (0.68 tonne/ha/year), oil palm (3.62 tonne/ha/year) and jatropha (4.14 tonne/ha/year) [4] [7]. In other words, culturing microalgae for biodiesel production requires the least land area and holds an important key feature for effective land utilization. Apart from that, microalgae is also a superior feedstock for bioethanol production. Besides their high lipid content, some microalgae also contain carbohydrates (generally not cellulose) that can be used as carbon source or substrate for fermentation [9]. In addition, microalgae are also capable to fix CO_2 from the atmosphere, flue gases or soluble carbonate into their cells during growth while simultaneously capturing solar energy with efficiency 10–50 times greater than terrestrial plants [10] [11]. Based on these evidences, microalgae have successfully positioned itself as one of the most promising biodiesel feedstock. Biodiesel derived from microalgae are among third generation biofuels that totally open up a new dimension in the renewable energy industry. However it should be noted here that microalgae is not the only feedstock that can be used for the production of third generation biofuels. Other third generation biofuels are, for example biodiesel produced from yeast and fungus [12] and bioethanol produced via direct cellulose fermentation (consolidated bioprocessing) [13].

In recent years, the potential and prospect of biodiesel production from first and second generation feedstocks have been extensively reviewed. However there is a need for comparison between the biodiesel produced from first, second generation feedstocks and microalgae as they are foreseen to be the fuel of the future. In fact, microalgae biofuels have been placed globally as one of the leading research fields which can bring enormous benefits to human beings and the environment. This review aims to provide an insight about the processes and methods involved to obtain biodiesel and to give a clear guideline to researchers propelling the biodiesel industry to become economically more viable.

1. Properties of biodiesel

Biodiesel may be defined as a domestic, renewable fuel for diesel engines derived from natural oils that meets the specifications of ASTM D 6751. In technical terms (ASTM D 6751) biodiesel is a diesel engine fuel comprised of monoalkyl esters of long-chain fatty acids derived from vegetable oils or animal fats or any other feedstock designated B100 and meeting the requirements of ASTM D 6751. Biodiesel, possesses a number of promising characteristics, including reduction of exhaust emissions [14]. It’s a mixture of methyl esters of long-chain fatty acids like lauric, palmitic, stearic, oleic, etc. The chemistry of conversion into biodiesel is essentially the same. Oil or fat reacts with methanol or ethanol in the presence of a sodium hydroxide or potassium hydroxide catalyst to form biodiesel, (m)ethyl esters, and glycerol. Properties of biodiesel are given in Table 1. Biodiesel is a clear amber-yellow liquid with a viscosity similar to that of petroleum diesel. Biodiesel is non-flammable and, in contrast to petrodiesel, is non-explosive, with a flash point of 423 K for biodiesel as compared to 337 K for petrodiesel. The cetane number is another important factor which determines the combustion quality of diesel fuel during compression ignition. The higher the cetane nimber the more easily the fuel will combust in a diesel engine. Biodiesel produced from vegetable oil has a cetane number between 46-52, animal based between 56-60 whereas the ASTM standard number is 47 minimum. Unlike petrodiesel, biodiesel is biodegradable and non-toxic, and it significantly reduces toxic and other emissions when burned as a fuel. Table 2 shows the fuel ASTM standards of biodiesel and petroleum diesel fuels. Important operating disadvantages of biodiesel in comparison with petrodiesel are cold start problems, lower energy content, higher copper strip corrosion, and fuel pumping difficulty from higher viscosity. Currently, biodiesel is more expensive to produce than petrodiesel, which appears to be the primary factor in preventing its more widespread use. Biodiesel is the first and only alternative fuel to have a complete evaluation of emission results. Biodiesel is derived from vegetative feedstock oils and hence is a renewable fuel. A renewable fuel such as biodiesel, along with lesser exhaust emissions is the need of the present scenario worldwide [15]. It is referred to as B100 or ‘‘neat” fuel when its 100% pure. Biodiesel blends are referred to as BXX. The XX indicates the amount of biodiesel in the blend (i.e., a B80 blend is 80% biodiesel and 20% petrodiesel).

Table. 1. Properties of biodiesel

Common name	Biodiesel (bio-diesel)
Common chemical name	Biodiesel (bio-diesel)
Chemical formula range	Fatty acid (m)ethyl ester
Chemical formula range	C14–C24 methyl esters or C 15-25 H 28-48 O2
Kinematic viscosity range (mm^2/s, at 313 K)	3.3–5.2
Density range (kg/m^3, at 288 K)	860–894
Boiling point range (K)	>475
Flash point range (K)	420–450
Distillation range (K)	470–600
Vapor pressure (mm Hg, at 295 K)	470–600
Solubility in water	Insoluble
Physical appearance	Light to dark yellow, clear liquid
Odor	Light musty/soapy odor
Biodegradability	More biodegradable than petroleum diesel
Reactivity	Stable, but avoid strong oxidizing agents

Table. 2. ASTM standards of biodiesel and petroleum diesel

Property	Test method	ASTM D975 (petrodiesel)	ASTM D6751 (biodiesel, B100)
Flash point	D 93	325 K min	403 K
Water and sediment	D 2709	0.05 max %vol	0.05 max %vol
Kinematic viscosity (at 313 K)	D 445	1.3 – 4.1 mm^2/s	1.9 – 6.0 mm^2/s
Sulphated ash	D 874	-	-
Ash	D 482	0.01 max %wt	-
Sulfur	D 5453	0.05 max %wt	-
Cetane number	D 613	40 min	47 min
Aromaticity	D 1319	35 max %vol	-
Carbon residue	D 4530	-	0.05 max %mass

Source: http://www.astm.org/Standards/D6751.htm

2. Biodiesel from vegetable oils and animal fats

Vegetable oils were thought to have the potential to replace a fraction of the petroleum distillates and petroleum-based petrochemicals in the near future. However, their direct use in compression engines was restricted due to high viscosity which resulted in poor fuel atomization, incomplete combustion and carbon deposition on the injector and the valve seats causing serious engine fouling [16, 17]. Although Rudolph Diesel tested vegetable oil as the fuel for his engine way back around 100 years ago [18], vegetable oils have become more attractive recently because of their environmental benefits and the fact that they are made from renewable resources.

3.1. Sources

Various oils have been in use in different countries as raw materials for biodiesel production owing to its availability. Soybean oil is commonly used in United States and rapeseed oil is used in many European countries for biodiesel production, whereas, coconut oil and palm oils are used in Malaysia and Indonesia for biodiesel production [11-14]. In India and southeast Asia, the Jatropha tree (*Jatropha cursas*) [15], Karanja (*Pongamia pinnata*) [12, 16, 17] and Mahua (*M. indica*) [11] is used as a significant fuel source. Commonly accepted biodiesel raw materials include the oils from soy, canola, corn, rapeseed, and palm. New plant oils that are under consideration include mustard seed, peanut, sunflower, and cotton seed. The most commonly considered animal fats include those derived from poultry, beef, and pork [18]. Rapeseed has been grown in Canada since 1936. Hundreds of years ago, Asians and Europeans used rapeseed oil in lamps. Cottonseed oil is used almost entirely as a food material. Sesame, olive, and peanut oils can be used to add flavor to a dish. Walnut oil is high-quality edible oil refined by purely physical means from quality walnuts.

They have become more attractive recently because of their environmental benefits and the fact that they are made from renewable resources. Vegetable oils are a renewable and potentially inexhaustible source of energy, with energy content close to that of diesel fuel. Global vegetable oil production increased from 56 million tons in 1990 to 88 million tons in 2000, following a below-normal increase. The source of this gain was distributed among the various oils. Global consumption rose 56–86 million tons, leaving world stocks comparatively tight [10]. A variety of biolipids can be used to produce biodiesel as shown in table 3. These are (a) virgin vegetable oil feedstock; rapeseed and soybean oils are most commonly used, though other crops such as mustard, palm oil, sunflower and hemp (b) waste vegetable oil; (c) animal fats including tallow, lard, and yellow grease; and (d) non-edible oils such as jatropha, neem oil, castor oil, and tall oil [6].

Table. 3. Different oil sources for biodiesel production

Group	Source of oil
Inedible oils	Babassu tree, copaiba, honge, jatropha or ratanjyote, jojoba, karanja or honge, mahua, milk bush, nagchampa, neem, petroleum nut, rubber seed tree, silk cotton tree, and tall, castor.
Major oils	Coconut (copra), corn (maize), cottonseed, canola (a variety of rapeseed), olive, peanut (groundnut), safflower, sesame, soybean, and sunflower
Nut oils	Almond, cashew, hazelnut, macadamia, pecan, pistachio and walnut
Other edible oils	Amaranth, apricot, argan, artichoke, avocado, babassu, bay laurel, beech nut, ben, Borneo tallow nut, carob pod (algaroba), cohune, coriander seed, false flax, grape seed, hemp, kapok seed, lallemantia, lemon seed, macauba fruit (*Acrocomia sclerocarpa*), meadowfoam seed, mustard, okra seed (hibiscus seed), perilla seed, pequi, (*Caryocar brasiliensis* seed), pine nut, poppy seed, prune kernel, quinoa, ramtil (*Guizotia abyssinica* seed or Niger pea), rice bran,tallow, tea (camellia), thistle (*Silybum marianum* seed), and wheat germ

3.2 Potential Technologies for Biodiesel Production

3.2.1 Direct use of vegetable oils in Diesel engines

Beginning in 1980, there was considerable discussion regarding use of vegetable oil as a fuel. Bartholomew (1981) addressed the concept of using food for fuel, indicating that petroleum should be the alternative fuel rather than vegetable oil and alcohol being the alternatives and some form of renewable energy must begin to take the place of the nonrenewable resources. The most advanced work with sunflower oil occurred in South Africa because of the oil embargo. Caterpillar Brazil, in 1980, used pre-combustion chamber engines with a mixture of 10% vegetable oil to maintain total power without any alterations or adjustments to the engine. At that point, it was not practical to substitute 100% vegetable oil for diesel fuel, but a blend of 20% vegetable oil and 80% diesel fuel was successful. Some short-term experiments used up to a 50/50 ratio. The first International Conference on Plant and Vegetable Oils as fuels was held in Fargo, North Dakota in August 1982. The primary concerns discussed were the cost of the fuel, the effects of vegetable oil fuels on engine performance and durability and fuel preparation, specifications and additives. Oil production, oilseed processing and extraction also were considered in this meeting (ASAE, 1982) [19]. The main advantages of use of vegetable oils as diesel fuel are ready availability, renewability, lower sulfur and aromatic content, and biodegradability [20]. The main disadvantages of vegetable oils as diesel fuel are higher viscosity, lower volatility, and the reactivity of unsaturated hydrocarbon chains. The problems met in long-term engine tests, according to results obtained by earlier researchers, may be classified as follows: choking on injectors, more carbon deposits, oil ring sticking, and thickening and gelling of the engine lubricant oil [3-5]. Thus, they require modifications of the engines [21]. Vegetable oils could only replace a very small fraction of transport fuel.

3.2.2 Dilution of vegetable oils

Dilution of vegetable oils with solvents lowers the viscosity, thus reduces engine performance problems such as injector choking and more carbon deposits. The viscosity of oil can be lowered by blending with pure ethanol/diesel. A study was conducted by blending twenty-five parts of sunflower oil and 75 parts of diesel. The viscosity of blended fuel was 4.88 cSt at 313 K, while the maximum specified ASTM value is 4.0 cSt at 313 K [22].

3.2.3 Microemulsion of oils

To overcome the high viscosity of the vegetable oils, microemulsions with solvents such as methanol, ethanol and 1-butanol are used. A microemulsion is defned as a colloidal equilibrium dispersion of optically isotropic fuid microstructures with dimensions generally in the 1-150 nm range formed spontaneously from two normally immiscible liquids and one or more ionic or non-ionic amphiphiles [23]. They can improve spray characteristics by explosive vaporization of the low boiling constituents in the micelles [24]. Short term performances of both ionic and non-ionic microemulsions of aqueous ethanol in soybean oil were nearly as good as that of No. 2 diesel, in spite of the lower cetane number and energy content [25]. The 2-octanol was found an effective amphiphile in the micellar solubilization of methanol in triolein and soybean oil [19].

3.2.4 Pyrolysis and catalytic cracking

Pyrolysis is the conversion of one substance into another by means of heat or by heat with the aid of a catalyst [26]. Pyrolysis and catalytic cracking of oils and fats result in production of alkanes, alkenes, alkadienes, cycloalkanes, alkylbenzenes, carboxylic acids, aromatics and small amounts of gaseous products [27,28]. It involves heating in the absence of air or oxygen and cleavage of chemical bonds to yield small molecules. The pyrolyzed material can be vegetable oils, animal fats, natural fatty acids and methyl esters of fatty acids. Soybean oil was thermally decomposed and distilled in air and nitrogen sparged with a standard ASTM distillation apparatus [29]. The main components were alkanes and alkenes, which accounted for approximately 60% of the total weight. Carboxylic acids accounted for another 9.6–16.1%. Catalytic cracking of vegetable oils to produce biofuels has been studied [30]. Copra oil and palm oil were cracked over a standard petroleum catalyst SiO_2/Al_2O_3 at 723 K to produce gases, liquids and solids with lower molecular weights. The condensed organic phase was fractionated to produce bio-gasoline and biodiesel fuels. The liquid fuel produced from pyrolysis has similar chemical components to conventional petroleum diesel fuel [31].

3.2.5 Transesterification of oils and fats

Transesterification is a chemical reaction between triglyceride and alcohol in the presence of catalyst. It consists of a sequence of three consecutive reversible reactions where triglycerides converted to diglycerides and then diglycerides are converted to monoglycerides followed by the conversion of monoglycerides to glycerol. In each step an ester is produced and thus three ester molecules are produced from one molecule of triglycerides. The transesterification reaction requires a catalyst such as sodium hydroxide to split the oil molecules and an alcohol (methanol or ethanol) to combine with the separated esters. A catalyst is usually used to improve the reaction rate and yield. Because the reaction is reversible, excess alcohol is used to shift the equilibrium to the product side. Acid catalyst and alkali catalyst are used depending upon the nature of the oil used for biodiesel production. Another catalyst in study is lipase. Lipase has advantage over acid and alkali catalyst but its cost is a limiting factor for its use at large scale production of biodiesel. Choice of acid and alkali catalyst depends on the free fatty acids (FFA) content in the raw oil.

Acid-catalyzed transesterification methods

Sulfuric acid, hydrochloric acid, and sulfonic acid are usually preferred as acid catalysts. The catalyst is dissolved into methanol by vigorous stirring in a small reactor. The oil is transferred into the biodiesel reactor and then the catalyst/alcohol mixture is pumped into the oil. The transesterification process is catalyzed by Bronsted acids, preferably by sulfonic and sulfuric acids. These catalysts give very high yields in alkyl esters, but the reactions are slow [32].

Alkali catalytic transesterification methods

In the alkali catalytic methanol transesterification method, the catalyst (KOH or NaOH) is dissolved into methanol by vigorous stirring in a small reactor. The oil is transferred into a biodiesel reactor and then the catalyst/alcohol mixture is pumped into the oil. The final mixture is stirred vigorously for 2 h at 340 K in ambient pressure. A successful transesterification reaction produces two liquid phases: ester and crude glycerol. The alkali-catalyzed transesterification of vegetable oils proceeds faster than the acid-catalyzed reaction. Alkaline metal alkoxides (as CH3ONa for methanolysis) are the most active catalysts since they give very high yields (>98%) in short reaction times (30 min) even if they are applied at low molar concentrations (0.5 mol%). However, they require the absence of water, which makes them inappropriate for typical industrial processes [33].

Enzyme-catalyzed transesterification

Biodiesel can be obtained from enzyme or biocatalytic transesterification methods [34, 35]. In recent work three different lipases (*Chromobacterium viscosum*, *Candida rugosa*, and Porcine pancreas) were screened for a transesterification reaction of jatropha oil in a solvent-free system to produce biodiesel; only lipase from C. viscosum was found to give appreciable yield [36]. Immobilization of lipase (*C. viscosum*) on Celite-545 enhanced the biodiesel yield to 71% from the 62% yield obtained by using free tuned enzyme preparation with a process time of 8 h at 113 K. Immobilized C. viscosum lipase can be used for ethanolysis of oil. It was seen that immobilization of lipases and optimization of transesterification conditions resulted in adequate yield of biodiesel in the case of the enzyme-based process [36].

3.2.6 Other types of transesterifications

Transesterification of oils and fats was done using branched-chain alcohols, such as isopropyl or 2-butyl (1:66) to reduce the crystallization temperature of biodiesel [37]. The crystal temperatures of isopropyl and 2-butyl esters of soybean oil were 7-11 and 12-14°C lower than that of soybean oil methyl esters, respectively. The crystallization onset temperatures (T_{CO}) of isopropyl esters of lard and tallow were similar to that of methyl esters of soybean oil. Comparison between the conventional and in-situ processes and found the acid catalyzed in-situ process for sunfower seed oil was better [38]. Ethyl, propyl and butyl esters of soybean fatty acids were obtained directly, in high yields, by in-situ transesterification of soybean oil [39]. It was also suggested that the utilization of a higher shear mixing device for making esters from animal fat. Glycerolysis was investigated using a high shearing mixing device. The separated glycerol reacted with triglycerides to produce mono- and diglycerides, which are valuable chemical intermediates for detergents and emulsifiers. This process was thought to lower the production cost of biodiesel fuel [40].

3.2.7 Non-catalytic transesterification methods

There are two non-catalyzed transesterification processes. These are the BIOX process and the supercritical alcohol (methanol) process. Biodiesel production with BIOX cosolvent process. The BIOX (cosolvent) process is a new Canadian process developed originally by Professor David Boocock of the University of Toronto that has attracted considerable attention. Dr. Boocock has transformed the production process through the selection of inert cosolvents that generate an oil-rich one-phase system. This reaction is over 99% complete in seconds at ambient temperatures, compared to previous processes that required several hours. BIOX is a technology development company that is a joint venture of the University of Toronto Innovations Foundation and Madison Ventures Ltd. BIOX's patented production process converts first the free fatty acids (by way of acid esterification) up to 10% FFA content and then the triglycerides (by way of transesterification), through the addition of a co-solvent, in a two-step, single phase, continuous process at atmospheric pressures and near-ambient temperatures. The co-solvent is then recycled and reused continuously in the process. The unique feature of the BIOX process is that it uses inert reclaimable cosolvents in a single-pass reaction taking only seconds at ambient temperature and pressure. The developers are aiming to produce biodiesel that is cost competitive with petrodiesel. The BIOX process handles not only grain-based feedstocks but also waste cooking greases and animal fats [41]. The BIOX process uses a cosolvent, tetrahydrofuran, to solubilize the methanol. Cosolvent options are designed to overcome slow reaction times caused by the extremely low solubility of the alcohol in the triglyceride phase. The result is a fast reaction, on the order of 5–10 min, and no catalyst residues in either the ester or the glycerol phase.

3.2.8 Supercritical alcohol transesterification

In the conventional transesterification of animal fats and vegetable oils for biodiesel production, free fatty acids and water always produce negative effects since the presence of free fatty acids and water causes soap formation, consumes the catalyst, and reduces catalyst effectiveness, all of which results in a low conversion [42]. The transesterification reaction may be carried out using either basic or acidic catalysts, but these processes require relatively time-consuming and complicated separation of the product and the catalyst, which results in high production costs and energy consumption. To overcome these problems, Kusdiana and Saka [43] and Demirbas [44] have proposed that biodiesel fuels may be prepared from vegetable oil via noncatalytic transesterification with supercritical methanol (SCM). A novel process of biodiesel fuel production has been developed by a non-catalytic supercritical methanol method. Supercritical methanol is believed to solve the problems associated with the two-phase nature of normal methanol/oil mixtures by forming a single phase as a result of the lower value of the dielectric constant of methanol in the supercritical state. As a result, the reaction was found to be complete in a very short time [43]. Compared with the catalytic processes under barometric pressure, the supercritical methanol process is non-catalytic, involves a much simpler purification of products, has a lower reaction time, is more environmentally friendly, and requires lower energy use. However, the reaction requires temperatures of 525–675 K and pressures of 35–60 MPa [43]. The supercritical methanol process is non-catalytic, involves simpler purification, has a lower reaction time, and is less energy intensive. Therefore, the supercritical methanol method would be more effective and efficient than the common commercial process [45].

3.2.9 Catalytic supercritical methanol transesterification

Catalytic supercritical methanol transesterification is carried out in an autoclave in the presence of 1–5% NaOH, CaO, and MgO as catalyst at 520 K. In the catalytic supercritical methanol transesterification method, the yield of conversion rises to 60–90% for the first minute [46]. Transesterification reaction of the crude oil of rapeseed with supercritical/subcritical methanol in the presence of a relatively low amount (1%) of NaOH was successfully carried out, where soap formation did not occur [47].

Of the several methods available for producing biodiesel, transesterification of natural oils and fats is currently the method of choice. The purpose of the process is to lower the viscosity of the oil or fat. Although blending of oils and other solvents and microemulsions of vegetable oils lowers the viscosity, engine performance problems, such as carbon deposit and lubricating oil contamination, still exist. Pyrolysis produces more biogasoline than biodiesel fuel.

Transesterification is basically a sequential reaction. Triglycerides are first reduced to diglycerides. The diglycerides are subsequently reduced to monoglycerides. The monoglycerides are finally reduced to fatty acid esters. The order of the reaction changes with the reaction conditions. The main factors affecting transesterification are molar ratio of glycerides to alcohol, catalysts, reaction temperature and time and the contents of free fatty acids and water in oils and fats. The commonly accepted molar ratio of alcohol to glycerides is 6:1. Base catalysts are more effective than acid catalysts and enzymes. The recommended amount of base used to use is between 0.1 and 1% w/w of oils and fats. Higher reaction temperatures speed up the reaction and shorten the reaction time. The reaction is slow at the beginning for a short time and proceeds quickly and then slows down again. Base catalyzed transesterifications are basically finished within one hour. The oils or fats used in transesterification should be substantially anhydrous (60.06% w/w) and free of fatty acids (>0.5% w/w).

3. Microalgae based biodiesel

Microalgae are prokaryotic or eukaryotic photosynthetic microorganisms that can grow rapidly and live in harsh conditions due to their unicellular or simple multicellular structure. Examples of prokaryotic microorganisms are Cyanobacteria (Cyanophyceae) and eukaryotic microalgae are for example green algae (Chlorophyta) and diatoms (Bacillariophyta) [48, 49]. Microalgae are present in all existing earth ecosystems, not just aquatic but also terrestrial, representing a big variety of species living in a wide range of environmental conditions. It is estimated that more than 50,000 species exist, but only a limited number, of around 30,000, have been studied and analyzed [50]. Many research reports and articles described many advantages of using microalgae for biodiesel production in comparison with other available feedstocks [48, 49, 51]. From a practical point of view, they are easy to cultivate, can grow with little or even no attention, using water unsuitable for human consumption and easy to obtain nutrients. Microalgae reproduce themselves using photosynthesis to convert sun energy into chemical energy, completing an entire growth cycle every few days [51]. Moreover they can grow almost anywhere, requiring sunlight and some simple nutrients, although the growth rates can be accelerated by the addition of specific nutrients and sufficient aeration [52-54]. Different microalgae species can be adapted to live in a variety of environmental conditions. Thus, it is possible to find species best suited to local environments or specific growth characteristics, which is not possible to do with other current biodiesel feedstocks (e.g. soybean, rapeseed, sunflower and palm oil). They have much higher growth rates and productivity when compared to conventional forestry, agricultural crops, and other aquatic plants, requiring much less land area than other biodiesel feedstocks of agricultural origin, up to 49 or 132 times less when compared to rapeseed or soybean crops, for a 30% (w/w) of oil content in algae biomass [7]. Therefore, the competition for arable soil with other crops, in particular for human consumption, is greatly reduced. Table 4 shows that although the oil contents are similar between seed plants and microalgae there are significant variations in the overall biomass productivity and resulting oil yield and biodiesel productivity with a clear advantage for microalgae. [55]. Fig.1 shows the overall process of biodiesel production from microalgae.

Table .4. Comparison of microalgae with other biodiesel feedstocks [55]

Plant source	Seed oil content (%oil by wt in biomass)	Oil yield (L oil/ha year)	Land use (m^2 year/kg biodiesel)	Biodiesel productivity (kg biodiesel/ha year)
Corn/Maize (*Zea mays L.*)	44	172	66	152
Hemp (*Cannabis sativa L.*)	33	363	31	321
Soybean (*Glycine max L.*)	18	636	18	562
Jatropha (*Jatropha curcas L.*)	28	741	15	656
Canola/Rapeseed (*Brassica napus L.*)	41	974	12	862
Sunflower (*Helianthus annuus L.*)	40	1070	11	946
Castor (*Ricinus communis*)	48	1307	9	1156
Palm oil (*Elaies guineensis*)	36	5366	2	4747
Microalgae (low oil content)	30	58,700	0.2	51,927
Microalgae (medium oil content)	50	97,800	0.1	86,515
Microalgae (high oil content)	70	136,900	0.1	121,104

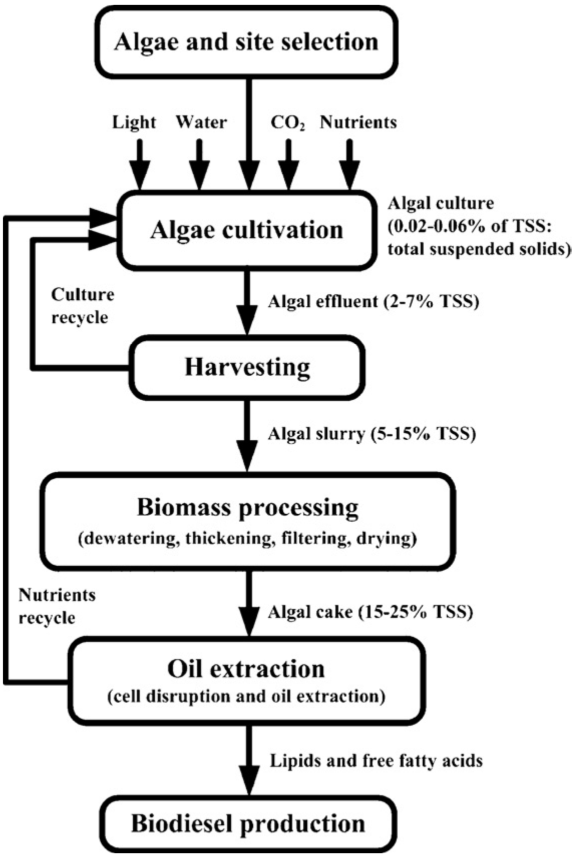

Fig:1. Biodiesel production from microalgae

4.1. Nutrient Sources and culture methods

Culturing of microalgae at industrial scale for biofuels production requires substantial amount of nutrients, typically nitrogen (usually in the form of nitrate) and phosphorus (usually in the form of ortho-phosphate). These nutrients are normally from chemical or inorganic fertilizers that are used to achieve promising growth rate of microalgae and to obtain bulk quantity of biomass. The use of chemical fertilizer has the advantage of reducing contamination in culturing medium and thus promotes water reutilization to re-culture microalgae. Recent studies have estimated that, the culturing of microalgae consumes more chemical fertilizers than the oil bearing crops. N-fertlizer contributed nearly 80-85% of overall chemical fertilizers composition required to culture microalgae [57-60]. In such a scenario recycling and reusing the excess nutrients in the culture medium should be encouraged to improve the life cycle energy balance of microalgae biodiesel. Due to the severe impact of chemical fertilizers towards the overall energy balance in microalgae cultivation, there is an urgent need to search for alternative and low cost nutrient sources to ensure long-term sustainability. In this case, using wastewater to culture microalgae appears as an attractive and economical alternative. Normally, secondary and tertiary wastewaters contain significant amount of nitrate and ortho-phosphate which are not removed during primary treatment. If these nutrients are to be removed, an additional 60 to 80% of energy will be consumed in the wastewater treatment plant [61]. Instead, these nutrients can be used to culture microalgae and at the same time, microalgae play an important role as reagent to purify the wastewater. In contrast, wastewater is not a suitable nutrient source for terrestrial crops as the highly concentrated nutrients can easily leached out from soil and cause serious eutrophication of surface waters [62]. For example, even trace amount of nitrate in drinking water can be hazardous to human health and nitrite is exceptionally toxic to aquatic species [62]. Hence, culturing of microalgae in wastewater does not only offers an inexpensive alternative to conventional way of wastewater treatment, but also substantially reduces the need of chemical fertilizers and their associated life cycle burden [61]. However , the real potential of using the waste water to culture microalgae is still uncertain since it contains several contaminants such as bacteria and virus which can devastate the colonies of microalgae.

Microalgae may assume many types of metabolisms (e.g. autotrophic, heterotrophic, mixotrophic, photoheterotrophic) and are capable of a metabolic shift as a response to changes in the environmental conditions. For example some organisms can grow [63]:

✓Photoautotrophically, i.e. using light as a sole energy source that is converted to chemical energy through photosynthetic reactions.

✓Heterotrophically, i.e. utilizing only organic compounds as carbon and energy source.

✓Mixotrophically, i.e. performing photosynthesis as the main energy source, though both organic compounds and CO_2 are essential. Amphitrophy, subtype of mixotrophy, means that organisms are able to live either autotrophically or heterotrophically, depending on the concentration of organic compounds and light intensity available.

✓Photoheterotrophycally, also known as photoorganitrophy, photoassimilation, photometabolism, describes the metabolism in which light is required to use organic compounds as carbon source. The photoheterotrophic and mixotrophic metabolisms are not well distinguished, in particular they can be defined according to a difference of the energy source required to perform growth and specific metabolite production.

4.2. Open versus closed-culture systems

Apart from nutrients supplement, microalgae cultivation system also plays an important role to determine the successfulness of the industry. An effective culture system should consist of the following criteria: (1) effective illumination area, (2) optimal gas–liquid transfer, (3) easy to operate, (4) low contamination level, (5) low capital and production cost and (6) minimal land area requirement [64].

Microalgae cultivation can be done in open-culture systems such as lakes or ponds and in highly controlled closed-culture systems called photo-bioreactors (PBRs).

Open-culture systems are normally less expensive to build and operate, more durable than large closed reactors and with a large production capacity when compared with closed systems. However According to Richmond [65] ponds use more energy to homogenize nutrients and the water level cannot be kept much lower than 15 cm (or 150 L/m^2) for the microalgae to receive enough solar energy to grow. Generally ponds are more susceptive to weather conditions, not allowing control of water temperature, evaporation and lighting. Also, they may produce large quantities of microalgae, but occupy more extensive land area and are more susceptible to contaminations from other microalgae or bacteria. Moreover, since atmosphere only contains 0.03–0.06% CO_2 it is expected that mass transfer limitation could slow down the cell growth of microalgae.

PBRs are flexible systems that can be optimized according to the biological and physiological characteristics of the algal species being cultivated, allowing one to cultivate algal species that cannot be grown in open ponds. On a PBR, direct exchange of gases and contaminants (e.g. microorganisms, dust) between the cultivated cells and atmosphere are limited or not allowed by the reactor’s walls. Also, a great proportion of light does not impinge directly on the culture surface but has to cross the transparent reactor walls. In terms of productivity PBRs surpass ponds by eight times higher and cell concentration about sixteen times higher.

Table . 5. Comparison of PBR and Open/ Raceway ponds [7]

Variable	PBR facility	Open/Raceway ponds
Annual Biomass production (kg)	100,000	100,000
Volumetric productivity (kg m^{-3} d^{-1})	1.535	0.117
Biomass concentration in broth (kg m^{-3})	4	0.14
Dilution rate (d^{-1})	0.384	0.25
Area needed (m^2)	5681	7828
Oil yield (m^3 ha^{-1})	136.9[a]	99.4[a]
	58.7[b]	42.6[b]
Annual CO_2 consumption	183,333	183,333

[a] *Based on 70% by wt oil in biomass.*

[b] *Based on 30% by wt oil in biomass.*

4.3. Harvesting of microalgae biomass

Algal harvesting consists of biomass recovery from the culture medium that may contribute to 20–30% of the total biomass production cost [83]. When microalgae culture has reached the stationary phase, the next step is to separate the microalgae from water and to recover their biomass for downstream processing. Nevertheless, microalgae harvesting process posed a challenging task since microalgae are small size microorganism (generally, 1–20 μm) and suspended in liquid. Currently, there are several methods to harvest microalgae: (1) bulk harvesting – to separate microalgae from suspension, such as natural gravity sedimentation, flocculation and floatation and (2) thickening – to concentrate the microalgae slurry after bulk harvesting, such as centrifugation and filtration [66]. Although centrifugation and filtration are good harvesting methods for microalgae, studies have shown that they are not energy feasible methods to harvest microalgae in commercial scale [67]. They involve high energy consumption, and high capital and maintenance cost resulting to unsustainable practice for long term operation.

On the other hand , flocculation offers a relatively low energy way to harvest microalgae. The microalgae cells always carry negative charge due to which they repel each other and remain suspended in the liquid for a longer time. By introducing coagulant that is positively charged into the culture medium, the negative charge surrounding the microalgae cells will be neutralized. At the same time, flocculant can be added to promote agglomeration by creating bridges between the neutralized cells to become dense flocs and settle due to natural gravity [68].

Another possible method to harvest microalgae is through immobilization biotechnology, in which microalgae are embedded in an entrapment matrix and continuously grown within the matrix. Once microalgae have grown to stationary phase and mixing is terminated, the immobilized microalgae beads will settle immediately at the bottom of the culture medium. Consequently, the relatively large size microalgae beads can be easily separated from water through simple filtration method (e.g. sieve) that does not require high amount of energy.

4.4 Drying of microalgae biomass

The microalgae biomass requires extensive drying as the presence of water will inhibit the downstream processes such as extraction of lipids and transesterification. Several methods have been employed to dry microalgae such as *Chlorella, Scenedesmus* and *Spirulina*, where the most common include spray-drying, drumdrying, freeze-drying and sun drying [65]. Because of the high water content of algal biomass sun-drying is not a very effective method for algal powder production and spray-drying is not economically feasible for low value products, such as biodiesel or protein. At the commercial scale solar drying is assumed to be the best method of drying wet microalgae paste after harvesting process. However this may not be possible in the temperate countries due to limited sunlight.

4.5 Microalgae lipid extraction

After harvesting and drying microalgae biomass, the subsequent step is lipid extraction. Effective lipid extraction is required particularly for microalgae with low lipid content as losing the lipid during extraction process may bring a significant impact towards the production cost of microalgae biofuels [69]. Several methods can be used depending on the microalgae wall and on the product nature to be obtained either based on mechanical action (e.g. cell homogenizers, bead mills, ultrasounds, autoclave, and spray drying) or non-mechanical action (e.g. freezing, organic solvents and osmotic shock and acid, base and enzyme reactions). Different from terrestrial energy crops, lipid extraction from microalgae biomass is relatively difficult due to the presence of thick cell wall that prevents the release of intra-lipid. Hence, mechanical press which is effective to extract oil from terrestrial energy crops is generally not applied to microalgae biomass. The following section depicts two lipid extraction methods: (1) solvent extraction-suitable for dry microalgae biomass and (2) supercritical fluid extraction-suitable for wet-paste microalgae biomass.

Solvent Extraction

Chemical solvent extraction is the most common method used to extract lipid from microalgae biomass. This is because chemical solvent has high selectivity and solubility towards lipid and therefore, even inter-lipid can be extracted out through diffusion across microalgae cell wall [69]. Nevertheless, the disadvantages of using chemical solvent are mostly related to their high toxicity towards human and surrounding environment. Chemical solvents such as n-hexane, methanol, ethanol and mixed methanol–chloroform (2:1 v/v) (Bligh and Dyer method) are effective to extract microalgae lipid, but the extraction efficiency is highly dependent on microalgae strains. Several enhanced strategies have been applied along with solvents to improve the overall extraction of lipids from the biomass. For extraction using chemical solvent, diffusion is always the rate limiting factor in the overall mechanism. However, this factor becomes more serious in microalgae as the cell wall further prohibits solvent from diffusing into the inner cell for lipid extraction. Therefore, cells disruption method can be introduced to enhance solvent diffusion efficiency and consequently, to improve microalgae lipid recovery rate. There are several techniques to disrupt microalgae cell wall, such as autoclave, bead-beater, ultrasonication and microwave as osmotic shock.

Supercritical fluid extraction

In the recent few years, research in extraction and reaction field has entered a new dynamic era with the introduction of supercritical fluid technology. The basic principal of this technology is achieving a certain phase (supercritical) that is beyond the critical point of a fluid, in which meniscus separating the liquid and vapor phases disappears, leaving only single homogeneous phase [70]. At supercritical phase, thermophysical properties such as density, viscosity, diffusivity and dielectric constant of a fluid will change drastically depending on temperature and pressure. Consequently, the changes of the thermophysical properties transform the fluid into a super-solvent and thus, improve extraction and reaction efficiency. Several supercritical fluids that are currently being explored are ethylene, CO_2, ethane, methanol, ethanol, benzene, toluene and water [71, 70] Among these, supercritical-CO_2 has received the most interest typically in extraction of pharmaceutical and health related products from microalgae [72-75]. In fact, supercritical-CO_2 offers several advantages in comparison with chemical solvent extraction: (1) non-toxic and provide non-oxidizing environment to avoid degradation of extracts, (2) low critical temperature (around 31 °C) which prevent thermal degradation of products, (3) high diffusivity and low surface tension which allow penetration of pores smaller than those accessible by chemical solvents and (4) easy separation of CO_2 at ambient temperature after extraction [72-75].

4.6. Biodiesel production from microalgae

Lipid extracted from microalgae biomass is now ready to be converted to biodiesel which is nothing but a mixture of fatty acid alkyl esters obtained by transesterification (ester exchange reaction). These lipids are composed by 90–98% (weight) of triglycerides and small amounts of mono and diglycerides, free fatty acids (1–5%), and residual amounts of phospholipids, phosphatides, carotenes, tocopherols, sulphur compounds, and traces of water [9]. Transesterification is a multiple step reaction, including three reversible steps in series, where triglycerides are converted to diglycerides, then diglycerides are converted to monoglycerides, and monoglycerides are then converted to esters (biodiesel) and glycerol (by-product). The overall transesterification reaction is described in Fig. 3 where the radicals R_1, R_2, R_3 represent long chain hydrocarbons, known as fatty acids. For the transesterification reaction oil or fat and a short chain alcohol (usually methanol) are used as reagents in the presence of a catalyst (usually NaOH). Although the alcohol:oil theoretical molar ratio is 3:1, the molar ratio of 6:1 is generally used to complete the reaction accurately. The relationship between the feedstock mass input and biodiesel mass output is about 1:1, which means that theoretically, 1 kg of oil results in about 1 kg of biodiesel. A homogeneous or heterogeneous, acid or basic catalyst can be used to enhance the transesterification reaction rate, although for some processes using supercritical fluids (methanol or ethanol) it may not be necessary to use a catalyst [76].

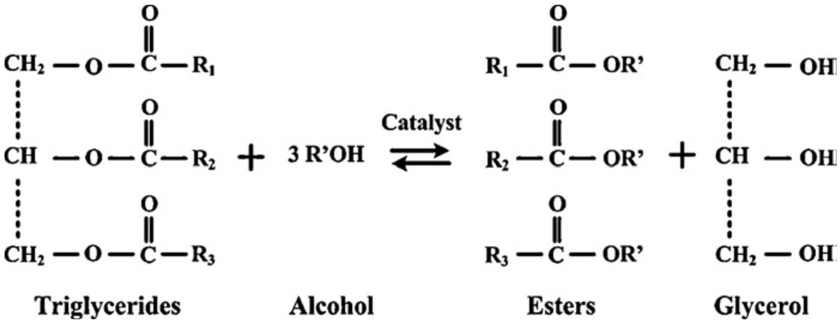

Fig:2. Transesterification of triglycerides (overall reaction)

Homogeneous base catalyst (e.g. KOH and NaOH) is usually used to accelerate the transesterification reaction. However, the presence of high free fatty acid (FFA) content in microalgae lipid (more than 0.5% w/w) prevented the use of homogeneous base catalyst for transesterification reaction [77-79]. This is because FFA will react with base catalyst to form soap leading to lower biodiesel yield and increase the difficulty to separate biodiesel from glycerol (co-product). Alternatively, acid catalyst (e.g. sulfuric acid, H_2SO_4) will be a better option as the catalyst is not sensitive towards FFA level in oil and thus, esterification (FFA is converted to alkyl ester) and transesterification can occur simultaneously.

Heterogeneous catalyst (base or acid) has also been explored extensively for transesterification reaction to produce biodiesel. Unlike homogeneous catalyst, heterogeneous catalyst can be recycled, regenerated and reused for subsequently transesterification reaction cycles, thus enhancing cost effectiveness in biodiesel production.

Furthermore, the catalyst can be easily separated out at the end of reaction through filtration and therefore, minimizing product contamination and number of water washing cycle (purification). To date, study on the application of heterogeneous catalyst in microalgae biodiesel production is still limited, mainly because it is a relatively new feedstock and not commercially available in the market. Common heterogeneous base catalyst, CaO supported with Al_2O_3 was recently tested for the very first time in transesterification of *Nannochloropsis oculata* microalgae lipid [80].

Conventional method to produce biodiesel mainly consists of two separate steps: extraction and followed by transesterification. In contrast, in-situ transesterification simplifies the process by allowing extraction and transesterification to occur in one single step, in which oil/lipid-bearing biomass is directly contacted with chemical solvent in the presence of catalyst. Chemical solvent plays two significant roles in this process: (1) as an solvent to extract oil/lipid out from biomass and (2) as a reactant in transesterification reaction. In-situ transesterification offers several advantages over conventional biodiesel production method such as minimize solvent separation step, reduce processing time and consequently, cut down the overall biodiesel production cost [81]. Furthermore, it is foreseen that in-situ transesterification can bring a greater advantage to produce biodiesel from microalgae biomass since mechanical extraction (e.g. extrusion and expeller) is ineffective to extract lipid from microalgae biomass. In a typical study of in-situ transesterification from dried *Chlorella* biomass, about 90% of biodiesel yield was attained at reaction temperature of 60 °C, methanol to lipid molar ratio of 315:1, H_2SO_4 concentration of 0.04 mol and reaction time of 4 h [77].

CONCLUSION

Biodiesel production is expected to offer new opportunities to diversify income and fuel supply sources, to promote employment in rural areas, to develop long term replacement of fossil fuels, and to reduce GHG emissions, boosting the decarbonisation of transportation fuels and increasing the security of energy supply. Current policies at regional and national levels and the expected cost and difficulties in obtaining fossil fuels will necessarily lead to an increase in biodiesel production and of other types of renewable energy. To become a more viable alternative fuel and to survive in the market, biodiesel must compete economically with diesel. The end cost of biodiesel mainly depends on the price of the feedstocks that accounts for 60–75% of the total cost of biodiesel fuel [82]. In order to not compete with edible vegetable oils, the low-cost and profitable biodiesel should be produced from low-cost feedstocks such as non-edible oils, used frying oils, animal fats, soap-stocks, and greases. However the available quantities of waste oils and animal fats are not enough to match the today demands for biodiesel. Thus transition to second generation biodiesel, such as microalgae, can contribute to a reduction in land requirements due to their presumed higher energy yields per hectare as well as to their non-requirement of agricultural land. For long-term sustainability and environmental benefits, all the processing stages of microalgae biodiesel should be simplified without involvement of extensive energy input. In addition, the processes should be easily adopted in the existing biodiesel industry and can be implemented immediately especially in third world countries. This is because culturing microalgae for biodiesel production is not only meant for profit making and benefiting the environment, but also to help people from the bottom billions in terms of food and energy security. Additionally, biodiesel needs to have lower environmental impacts and ensure the same level of performance of existing fuels.

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