



Status and prospects of supercritical alcohol transesterification for biodiesel production

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The growth in the global fuel consumption is expected to continue unabated. At the same time, nations around the globe are trying to reduce greenhouse gas emissions resulting from the transportation sector. These factors have led researchers to look for alternative sources of fuels. Biodiesel is one such alternative fuel that can complement or displace petroleum diesel with a potentially lower carbon footprint fuel, depending upon the feedstock and the production process. Biodiesel refers to the monoalkyl esters derived from a wide range of raw materials, like vegetable oils, animal fats, and algae lipids. Conventionally, biodiesel is produced by transesterification with the help of an acid, base or enzyme catalyst. Certain drawbacks, like slow reaction times, soap formation and intense pre- and post-processing, are associated with conventional transesterification, ultimately leading to increased capital and production costs. Supercritical transesterification is a relatively new technique promising to provide advantages, such as faster reaction times, catalyst-free operation, and higher purity of final product, over the conventional transesterification method. The most common feedstocks are virgin and used edible oils from crops like soybean, rapeseed, and African palm. There is an increasing interest in algae to avoid the utilization of food resources for energy production. Using algae as a lipid source, a more sustainable biodiesel production process could be developed to achieve large-scale production capabilities on a long-term basis without adverse effects on the food chain. This paper presents a critical review of supercritical transesterification and its prospects for biodiesel production. © 2017 Wiley Periodicals, Inc.

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INTRODUCTION

World consumption of fossil fuels is estimated to increase by about 38% over current demand reaching 119 million barrels per day by 2040 and with the transportation and industrial sectors

accounting for 92% of the global demand.¹ Most of the growth will result from developing countries, like China, India, and Middle Eastern nations. Comparatively, the usage is rather flat in developed countries like the United States, Europe, and Japan. Figure 1 shows these projections for the coming years in comparison with the numbers in 2010.

With increasing fuel demand in the near future, it is essential to secure feedstocks and develop conversion technologies that can provide the world with fuel security, particularly in the transportation sector. One such fuel is biodiesel, an alternative to diesel that can be synthesized from renewable sources, like vegetable oils, animal fats, used cooking oils, and

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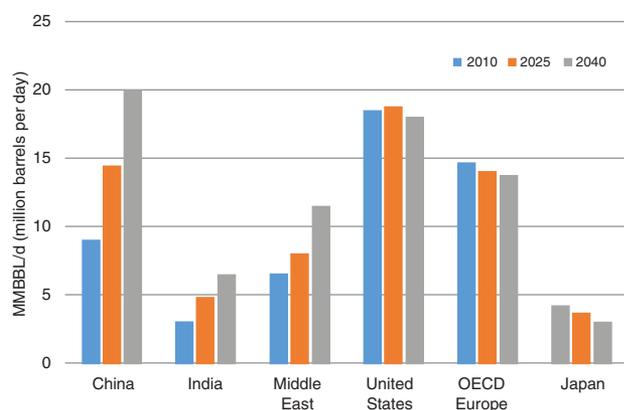


FIGURE 1 | Petroleum liquid fuel (non-renewable sources) consumption in selected countries (created with data from Ref 1).

algal lipids.² However, it is essential to take into account the conversion efficiencies of alternative fuels. Biodiesel derived from certain feedstocks can deliver more energy per unit than the energy it takes to produce it from the raw material.³ A study concluded that biodiesel produces about 3.2 units of energy per unit of fossil fuel energy consumed on a life cycle basis.⁴ Producing biodiesel from renewable sources, like vegetable oils and algal lipids, will reduce fossil energy consumption, thus enhancing energy security and environmental quality. Compared to biodiesel's 0.31 units of fossil fuel input required for each unit of energy output, petroleum diesel production requires about 1.2 units of fossil fuel energy for each unit of diesel.⁴ Hence, biodiesel appears to be significantly more sustainable. In other life cycle assessment studies it was concluded that biodiesel produced from palm oil is capable of achieving energy yield ratio (ratio of output energy to input energy) of 3.53. This indicates a strong net positive energy for biodiesel and a potential to become a major renewable source of energy in the near future.⁵

BIODIESEL PRODUCTION METHODS

Biodiesel is a mixture of mono-alkyl esters, which can be obtained from a wide range of feedstocks: vegetable oils (corn, soybean, jatropha, rapeseed, sunflower, peanut, and canola), algal lipids, animal fat, and used cooking oils. Although vegetable oils can be used directly as fuel by blending them with conventional diesel in a particular ratio, such use is not advisable since it may damage the engine in the long run, as vegetable oils have higher viscosities, cause acid contamination and free fatty acid formation, and lead to carbon deposition on the piston and

the head of the engine. Other problems like cold-weather ignition, plugging of filters, and engine knocking are also observed with direct use of vegetable oils. For these reasons, processing to biodiesel is needed to render the oils suitable for use as fuels.⁶ Biodiesel can be synthesized by a number of processes, as outlined next.

Pyrolysis

Pyrolysis or thermal cracking involves the use of high temperatures at atmospheric pressure to break the long chains of fatty acids in oils and deoxygenate them to form useful hydrocarbon-based fuel. The process often requires the presence of an activated catalyst. Pyrolysis is complex and endothermic in nature. Organic matter is heated in a non-reactive atmosphere, which results in simultaneous and successive reactions. The thermal decomposition of organic components begins at 350–500°C and continues as the temperature rises to 700–800°C in the absence of oxygen. At such high temperatures, the long chains of carbon, hydrogen, and oxygen break down into small molecules in the form of gases and vapor that can be condensed. Based on the operating conditions, pyrolysis can be classified into three types: conventional (slow), fast, and flash pyrolysis. In the conventional pyrolysis the major drawback is the long residence time.⁷ The process temperature is 300–700°C and the gas residence times are long (typically 7–8 min), as compared to other pyrolysis methods, affecting the yield and quality of the fuel produced. The process is also characterized by low heat transfer rates, required a significant energy input.⁸ Fast pyrolysis involves rapid heating of the feedstock in the absence of oxygen or air at higher temperatures of 600–1000°C and at much shorter residence times (1–10 seconds). Flash pyrolysis is instantaneous with gas residence times smaller than 1 second at temperatures ranging from 450°C to 1000°C and biodiesel yields reaching 75%.⁹ Although pyrolysis processes are quite fast, they have drawbacks, such as poor thermal stability, presence of solid particles in the generated fuel oil and increase in viscosity over time.

Microemulsions

Microemulsions are clear, isotropic, and thermodynamically stable dispersions of immiscible liquids in the presence of surfactants.¹⁰ The microemulsion droplets have diameters ranging from 100 to 1000 Å. A microemulsion could consist of a vegetable oil, alcohol, and a dispersant acting as a

co-solvent or a vegetable oil, alcohol and a surfactant. Microemulsions of methanol with vegetable oils perform similar to diesel fuels. Since microemulsions contain alcohol, the overall heating value is lower than that of diesel fuel, but the alcohols have high heat of vaporization and they cool off the combustion chamber much faster. Although some of the shortcomings of using direct vegetable oils as fuel are eliminated when microemulsions are used, issues with carbon deposition on exhaust valves and injector nozzles still persist.¹¹ Nevertheless, formation of microemulsions could be a promising method for obtaining low viscosity fuel blends with straight vegetable oils.¹²

Transesterification

Transesterification, by far the most popular biodiesel production technology, is a catalytic reaction that replaces the alkoxy moiety of the ester with another alkoxy moiety from an alcohol.¹³ When fatty acids are subjected to transesterification, they produce fatty acid alkyl esters and glycerol, with monoglycerides and diglycerides being formed as intermediates. Figure 2 shows the three-step transesterification reaction with the formation of intermediates.

The overall reaction is pseudo-first order, whereas the intermediate reactions are second order. An excess amount of alcohol is needed to shift the reaction equilibrium towards esters.¹⁴ As the reaction reaches equilibrium, a catalyst is needed to accelerate the process. The reaction can be catalyzed by bases,¹⁵ acids¹⁶ or enzymes.¹⁷

Base-catalyzed Transesterification

Biodiesel is most commonly produced using homogeneous base catalysts, like sodium hydroxide (NaOH) or potassium hydroxide (KOH).¹⁸ These alkalis provide certain advantages: ability to catalyze the reaction at low temperature and pressure, high conversion rates, low cost, and wide availability.¹⁹ However, the presence of free fatty acids (FAA) in the feedstock hampers the performance of base catalysts. FAA have a long carbon chain that is disconnected from the glycerol backbone. In the presence of FAA the alkali catalyst will typically react with it to form soap (esterification), thus reducing biodiesel yield.²⁰ As a result, the use of alkalis is limited to vegetable oils and other feedstocks with FAA content of no more than 0.5% by weight or acid value of less than 1 mg KOH/g.¹⁸ The presence of water also affects the transesterification reaction yield. At high temperatures, water is known to hydrolyse triglycerides to diglycerides, resulting in the formation of

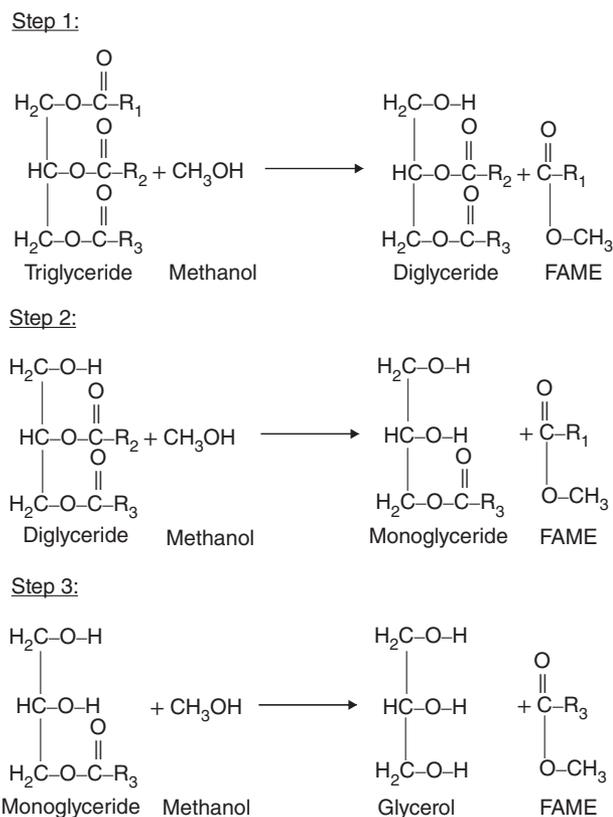


FIGURE 2 | Three-step transesterification reaction showing the intermediate reaction products.

FAA. These FAA will react with base catalysts to form soap. Further, excessive soap formation may cause the reaction mixture to gel at ambient temperatures, making it difficult to recover the individual components.²⁰

Using homogeneous base catalysts also creates issues with separation of the catalyst from the products, leading to an increase in the cost of separation.²⁰ Solid base catalysts, also known as heterogeneous base catalysts, have also been used for biodiesel production. In recent years, alkaline earth metal catalysts, like calcium oxide (CaO), have attracted attention due to their high basicity and low cost.²¹ CaO, prepared at 900°C for 1.5 h under helium gas flow, has proven to provide high yields of fatty acid methyl esters (FAME).²² Although these solid catalysts are easy to separate from the liquid products, some researchers have found that they may dissolve to some extent in the reaction products. For example, CaO could react with glycerol to form calcium diglycerate, which is soluble in the reaction products.²²

Acid-catalyzed Transesterification

Acid catalysts could provide some solutions to the problems associated with using base catalysts, as they

are not significantly affected by the presence of FFA. Acid catalysts can simultaneously catalyze both esterification and transesterification.¹⁹ So far, sulphuric acid has been the most widely used acid catalyst. Some other acids, like HCl, BF₃, H₃PO₄, and organic sulfonic acids, have also been used by researchers. Although acid-catalyzed transesterification gives high yields, the reactions are slow and need higher temperatures and longer reaction times than base catalysts.²³ Moreover, the presence of water in the oil may lead to the formation of carboxylic acids, reducing the yield of biodiesel. Therefore, it is essential that the reactants be free of moisture to avoid the formation of such undesirable by-products.²³ Current biodiesel research is focused on exploring heterogeneous acid catalysts, which are easily separated from the products.²⁰ Catalysts like zirconium oxide (ZrO₂), tin oxide (SnO₂), titanium oxide (TiO₂), and zeolites have been used for biodiesel production.²⁰ The use of non-metallic solid catalysts for biodiesel production has also been reported in the literature. Konwar et al. used sulfonated activated carbon based catalysts for FAME production.²⁴

Enzyme-catalyzed Transesterification

Enzyme catalysts are an attractive option, since they can prevent the formation of soap and operate in the presence of water and under ambient conditions. Enzymes like lipases have been found to be effective in catalyzing transesterification reactions, when immobilized on a suitable support. By immobilizing, the enzymes can be reused repeatedly without the need to separate them from the reaction products.⁶ The reaction temperatures are lower, about 50°C, as compared to the reactions using bases or acids. On the downside, the commercial application of enzymes is limited due to their higher costs, slower reaction times, and deactivation issues.²⁰

Supercritical Transesterification

The existence of supercritical fluids was first understood back in 1822, when Baron Cagniard de la Tour reported the disappearance of the gas–liquid phase boundary when materials were exposed to certain temperature and pressure conditions.²⁵ This temperature and pressure are referred to as the critical temperature (T_c) and critical pressure (P_c) and represent the co-ordinates of a substance's critical point on the phase diagram. Beyond the critical point the distinction between the gas and liquid phases disappears. Figure 3 shows the phase diagram for a pure substance. The region above and to the right of the critical point is thus known as the supercritical region. It is in this region that the fluid exhibits both

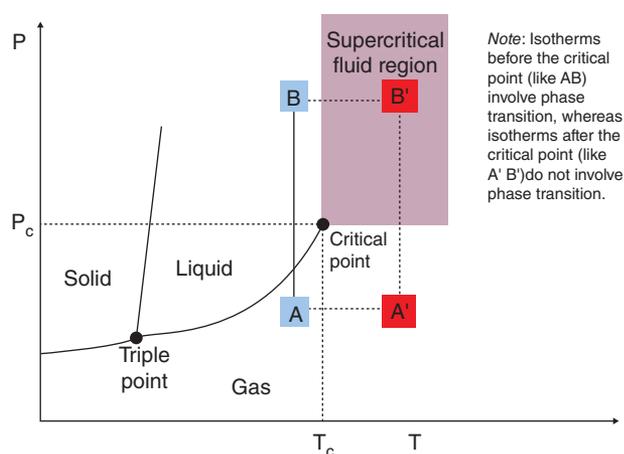


FIGURE 3 | Generic phase diagram of a pure substance showing the supercritical state (created with information from Ref 26).

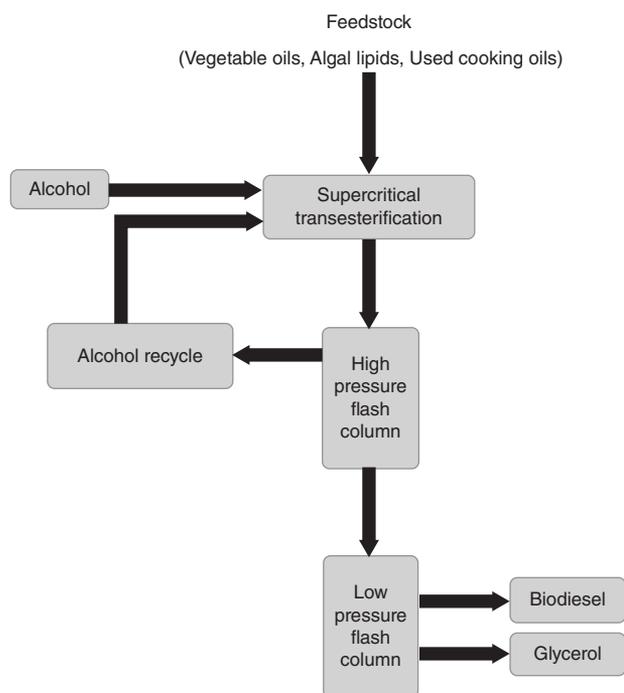
gas-like and liquid-like properties and exists as a non-condensable dense fluid whose density ranges from 20% to 50% of that in its liquid state and whose viscosity is close to that in its gaseous state. Table 1 shows a comparison between typical values of physical properties of gases, liquids, and supercritical fluids.

As mentioned earlier, catalyzed transesterification comes with certain drawbacks, like long reaction times and higher costs associated with catalyst poisoning, catalyst separation and regeneration, saponification, and biodiesel washing. One of the means to address the problems of catalyzed transesterification is by eliminating the need for catalysts with the use of supercritical operating conditions.²⁸ Supercritical transesterification simplifies the biodiesel production, as the process requires no catalyst, thereby reducing the complexity and the costs associated with separating or recovering the catalyst. Additionally, there is no wastewater generated and no soap formation. The reaction times are much shorter than the traditional alkali-catalyzed reaction (minutes vs. hours), yet the yields can be higher. The process is robust and, unlike conventional transesterification, is not affected by the presence of water or free fatty acids in the reactants. This allows the use of a significantly wider range of feedstocks, including lower quality and hence lower cost feedstocks, such as used oils and animal fats, without any need for pre-treatment.²⁹ Figure 4 depicts the flow diagram of biodiesel production from various feedstocks via the supercritical transesterification route.

The supercritical production of biodiesel was pioneered by Saka and Kusdiana in 2001. In the supercritical state, fluids undergo drastic changes in

TABLE 1 | Comparison of Typical Values of Transport Properties of Gases, Liquids, and Supercritical Fluids (adapted from Ref 27)

State	Defining Condition	Property		
		Density (kg/m ³)	Diffusivity (m ² /s)	Viscosity (kg/ms)
Gas	1 atm, 25°C	0.6–2	$1-4 \times 10^{-5}$	$1-3 \times 10^{-5}$
Liquid	1 atm, 25°C	500–1600	$0.2-2 \times 10^{-9}$	$0.2-3 \times 10^{-3}$
SC fluid	T_c, P_c	200–500	$0.5-4 \times 10^{-7}$	$1-3 \times 10^{-5}$
SC fluid	$T_c, 4P_c$	400–900	$0.1-1 \times 10^{-7}$	$3-9 \times 10^{-5}$

**FIGURE 4** | Process block diagram for supercritical transesterification.

their thermo-physical properties, like dissociation constant, viscosity, specific gravity, and polarity. The dissociation constant, which is a measure of a compound's tendency to dissociate into simpler ions, greatly increases under supercritical conditions enabling the reaction to produce biodiesel without the need for a catalyst.³⁰ When methanol was reacted with canola oil at supercritical conditions (350°C and 45–65 MPa) in a small 5 cm³ reactor, a 95% conversion to biodiesel was achieved in 4 min.³¹ This demonstrates the potential of the supercritical transesterification technology. The formation of high quality biodiesel is the result of certain phenomena that occur under supercritical conditions. Hydrogen bonds in the triglycerides are weakened and, as the temperature increases, the dielectric constant decreases allowing the alcohol to form a single homogenous mixture with the triglyceride.³² This

homogeneity greatly promotes the extent and speed of the transesterification reaction.³³

On the downside, supercritical transesterification requires an environment of high temperature and pressure. The reacting alcohol needs to be at supercritical conditions, which for methanol are 81 bar and 240°C. Such conditions are harsh thus shortening the useful life of equipment, so more costly materials of construction are used for process equipment, which results in higher capital cost. Although reaction times are short, caution is required not to run the reaction for longer times as this may cause thermal degradation of the esters, leading to a lower yield of esters.¹⁴ Supercritical transesterification requires a high molar ratio of alcohol to feedstock to drive the equilibrium towards the formation of esters. A high molar ratio requires more energy input for heating the reactants and recycling the excess alcohols. Moreover, the presence of higher volumes of alcohol in the biodiesel product could impede the biodiesel-glycerol phase separation.

Although the absence of pre-treatment, soap removal, and catalyst removal steps significantly reduces the biodiesel production costs, the energy needed to meet the pressure and temperature requirements for this process adds to the operating costs.³⁴ Simulation models were employed in a conceptual design to analyze different scenarios and evaluate productivity, raw material requirements, environmental impacts, and economic advantages of different processes for biodiesel production.³⁵ It was concluded that although supercritical transesterification technology has the most technical advantages, it has the highest capital investment when compared to other technologies and the highest cost of biodiesel produced. On the other hand, the significantly shorter reaction time of supercritical transesterification allows continuous mode of operation and leads to enhanced productivity, when compared to conventional catalyzed processes. Higher productivity will reduce operating costs and offset the higher capital cost, helping the technology become more cost-effective. Furthermore, heat integration steps can be used to

significantly reduce the energy demands of the process and further improve its economic feasibility.³⁶

A major advantage of the supercritical process is its ability to handle a wide range of feedstocks. Many waste streams like used cooking oils, waste animal fats, and greases, which are available at negligible costs, can be efficiently converted to biodiesel with supercritical transesterification, whereas conventional transesterification requires costly pretreatment steps. A few companies have already established commercial scale supercritical biodiesel production facilities with capacities ranging from 1 to 5 million gallons per year. This demonstrates the scalability as well as the deployment readiness of the technology.

FEEDSTOCKS FOR SUPERCRITICAL BIODIESEL PRODUCTION

The production cost of biodiesel depends heavily on the cost of feedstock and the cost of other reactants, purification processes, and storage. Out of these, by far the most important factor is the cost of feedstock, which accounts for 88% of the operating cost.³⁷ Thus, abundant and low-cost feedstocks are needed to reduce the cost of biodiesel and make its use more widespread. Such advantageous feedstocks, in turn, need conversion technologies, such as supercritical transesterification, which can handle lower quality (and hence lower cost) feedstocks without compromising the quality of the produced biodiesel.³⁷

Vegetable Oils

Decades ago, vegetable oils were used to directly power conventional diesel engines. Eventually, vegetable oils came into use as raw materials to synthesize biodiesel. They consist of about 98% triglycerides, along with small amounts of mono- and diglycerides.³⁸ The triglyceride (or triacylglycerol) molecule consists of three esters of the fatty acid chain attached to a glycerol group. This being the standard structure of vegetable oils, the only difference between various vegetable oils is the type of fatty acid attached to the triglyceride molecule.³⁹ Vegetable oils used for biodiesel production depend on the locale of production and its climate. Soybean oil is dominant in the United States, canola (rapeseed) oil in Canada and the European countries, and palm oil in Malaysia, Indonesia, and Latin America.³⁹

Although plentiful, vegetable oils represent food staples and their use for biodiesel production raises serious sustainability questions. Moreover, vegetable oils are not the most economical raw material for biodiesel production. Instead, if used cooking oils

and non-edible oils (such as jatropha, camelina, and carinata) were used for biodiesel production, their lower cost could reduce the overall production cost of biodiesel.⁴⁰ The potential of used cooking oil is considerable, as 100 million gallons per day are generated in the United States alone at an average of 4 kg of used oil per capita. If this used oil is collected and utilized, the economic viability of biodiesel production would be significantly enhanced.⁴¹ One critical consideration, when selecting used cooking oil as feedstock, is the change in properties of the oil itself, as it undergoes thermolytic, oxidative, and hydrolytic reactions during frying.^{42,43} The combined effects of these reactions lead to the formation of many undesirable compounds. Repetitive heating results in an increased polar content that negatively affects the quality of the produced biodiesel.⁴⁴

Significant work has been done on the supercritical synthesis of biodiesel from vegetable oils, both virgin and used, as summarized in Table 2. Rapeseed oil was reacted with supercritical methanol to produce biodiesel with a 95% conversion.³¹ In another study, cottonseed, hazelnut kernel, poppyseed, rapeseed, safflower-seed, and sunflower-seed oils were transesterified for biodiesel production.⁴⁵ The samples were subjected to supercritical methanolysis in a 100-mL stainless steel autoclave reactor and the yield of methyl esters was found to be closely dependent on the reaction temperature and pressure. Other researchers used refined sunflower oil to synthesize biodiesel with supercritical methanol and were able to achieve 96% conversion of FAME.⁴⁶ Another study reported up to 99% yields in 30 min for supercritical methanolysis of used cooking oil in a 100-mL reactor.⁴⁸

Soybean oil subjected to continuous transesterification with supercritical methanol reached biodiesel yields of over 96%.²⁹ The initial reaction temperature was found to play a crucial role in the ester yield with 100–170°C generating the best yields. When supercritical methanolysis of jatropha oil was conducted under various temperatures and pressures, a 100% methyl ester yield was achieved with supercritical methanol in just 4 min of reaction time.⁴⁷ Continuous production of biodiesel from vegetable oils was investigated using supercritical ethanol and supercritical methanol with carbon dioxide as a co-solvent.⁴⁹ In that study, sunflower-based oil was subjected to supercritical ethanolysis and supercritical methanolysis in a fixed bed titanium reactor. The performance of supercritical methanol was superior to that of supercritical ethanol with the yields being 90% and 80%, respectively, for the two alcohols in 4 min of reaction time.

TABLE 2 | Summary of Reported Biodiesel Production under Supercritical Conditions from Vegetable Oils

Author & Year	Research Focus	Pressure (psi)	Temperature (°C)	Reaction Time (min)	Ratio v/wt. (or wt./v)	Yield %
Saka and Kusdiana (2001) ³¹	Supercritical methanolysis of rapeseed oil	6500–9400	342	4	42:1 (molar)	95
Demirbas (2002) ⁴⁵	Transesterification in supercritical methanol of six different vegetable oils	Up to 14500	Up to 576	15	1:3 to 1:9	No Report
Madras et al. (2004) ⁴⁶	Synthesis of biodiesel in supercritical fluids from refined sunflower oil	2900	200 to 400	30	40:1	80 to 100
He et al. (2007) ²⁹	Continuous production of biodiesel from soybean oil via supercritical methanolysis	1450 to 5800	240 to 400	12.5 to 50	6:1 to 80:1	96
Hawash et al. (2009) ⁴⁷	Biodiesel from Jatropha oil via supercritical methanol transesterification	850 to 1250 1218	240 to 340 320	4	43:1	100
Demirbas (2009) ⁴⁸	Biodiesel from waste cooking oil via supercritical methanol transesterification	No report	246–266 266	30	1:6–1:41	99
Santana et al. (2012) ⁴⁹	Continuous production of biodiesel from vegetable oil using supercritical ethanol and CO ₂ mixtures	2100 to 2900, 2900	150 to 200 200	2 to 10 4	1:20 to 1:45 1:25	80 (SCE) 90 (SCM)
Micic et al. (2014) ⁵⁰	Transesterification of rapeseed oil in supercritical fluids (ethanol, methanol, 1-propanol)	1150 to 1750	250 to 350	15, 20, 25	42:1	93 (SCM) 91.9 (SCE) 91.1 (SCP)

Text in bold indicates optimal conditions as reported by the authors.

Animal Fats

Animal fats refer to the greases primarily collected from animal meat-processing facilities.⁴⁰ They are inexpensive and abundantly available and hence they constitute another promising source for biodiesel production. Animal fats have similar chemical structures to vegetable oils, but with a different distribution of fatty acids. Although they are considered a good feedstock for biodiesel production, they have not been studied as extensively as vegetable oils.⁵¹ Biodiesel can be obtained by conventional transesterification from different types of animal fat, like pork lard, beef tallow, chicken fat, brown grease, and yellow grease.⁵² Studies have reported the conversion of animal fats to biodiesel by using supercritical methanol. A FAME yield of 89.91% was obtained in 15 min by supercritical methanolysis of waste lard obtained from restaurants at 335°C, 20 MPa and 45:1 molar ratio of methanol to lard.⁵³

The significant presence of FFA in animal fats poses a challenge to conventional transesterification as it increases processing costs and decreases ester yield. In contrast, supercritical transesterification can readily use such feedstocks. Kusdiana and Saka have reported the conversion of free fatty acids to methyl esters by using supercritical methanol. About 95% conversion was reported for methyl esters

derived by reaction of FFA's and methanol at 350°C.⁵⁴

Biodiesel obtained from animal fats has a higher cetane number, but is more vulnerable to oxidation, since animal fats lack the presence of the natural antioxidants of vegetable oils.⁵⁵ Research suggests that methyl esters obtained from animal fats, like tallow, have lower flash point and lower heating value compared to petroleum diesel fuel. Furthermore, they also have a lower pour point, which may make problematic their use in cold weather conditions.⁵⁶

Microalgae, a Sustainable Feedstock

Up until now the main raw materials for biodiesel production have been edible vegetable oils, as they are readily available on a large scale. As a result, 95% of the world biodiesel production is based on vegetable oils.⁵⁷ Countries like China, India, and the European Union are major importers of oils and fats to fulfill their local consumption. However, since vegetable oils are food staples, a shortage in the availability of edible oils, like soybean, palm, and rapeseed, would push food prices higher. Continued demand for edible oil conversion to biodiesel raises concerns over the fact that biodiesel production will compete with food supply over the long run.⁵⁸

Microalgae, on the other hand, do not compete for land needed to grow food crops. Moreover, the growth rate of microalgae is much higher than oil-producing crops (days instead of months). Many species are rich in lipids, which can be readily converted to biodiesel.⁵⁹ Moreover, unlike food crops, microalgae could be a sustainable source of biodiesel as they can be cultivated in brackish or salt-water, instead of potable water, on non-arable land.⁶⁰ Thus, they could provide a promising sustainable alternative to the use of edible crops and eliminate the food vs. fuel competition.⁶¹

Moreover, from a supply chain standpoint, first generation biofuels produced from food crops and oil seeds are limited in their ability to provide the huge fuel quantities needed to replace petroleum fuels in a sustainable way.⁶² Consequently, interest has developed in exploring new frontiers for raw materials, like microalgae. Microalgae are capable of producing higher product yields per unit of land as compared to other feedstocks because of their fast growth rate. Certain species with higher oil content

are capable of oil production yields as high as 25 times that of conventional oil crops, like palm and soybean.⁶¹ The land area required for cultivation of microalgae can be up to 49–132 times less than for agricultural crops, like soybean or rapeseed, based on a 30% w/w oil content in algal biomass.⁶² Lipid content varies from 10% to over 80% of the dry weight of microalgae depending on the particular strain.⁶³ A significant portion of these lipids can be extracted and serve as feedstock for biodiesel. This valuable portion consists of triacylglycerols (TAG), which can be converted to biodiesel via transesterification.⁶³

Table 3 summarizes the work done to date on biodiesel production from microalgae. Levine et al.⁶⁵ reported yields ranging from 66.4% to 100% for *in situ* hydrolysis of wet algae by supercritical ethanol. Patil et al. have a significant contribution towards algal transesterification by supercritical ethanol as well as methanol. They have reported yields ranging from 32% for microwave-mediated supercritical ethanol transesterification,⁶⁸ to as high as 90% for

TABLE 3 | Summary of Reported Biodiesel Production from Algal Lipids

Author (Year)	Method	Pressure (psi)	Temperature (°C)	Reaction Time (min)	Ratio v/wt. (or wt./v)	Yield %
Demirbas (2008) ⁶⁴	Soxhlet Extraction of algae lipids followed by SC methanolysis	2030	350	4	42:1 (molar)	No report
Levine et al. (2010) ⁶⁵	<i>In situ</i> lipid hydrolysis of wet algae	No Report	225, 250 , 300	10, 20, 30, 45	45–50	66.4
	SC ethanolysis			60, 120	6.6–7.5	94–100
Patil et al. (2011) ⁶⁶	Direct conversion of wet algae with SC Methanol	1200	240–260, 255	10–30	1:4–1:12	90
				25	1:9	
Patil et al. (2012) ⁶⁷	Direct transesterification with SC methanol & Microwave-assisted reaction	1200	255	25	1:9	84.15
		14.5	60–64	4–5	1:12	80.13
Patil et al. (2013) ⁶⁸	Direct conversion of wet algal biomass by microwave mediated SC ethanolysis	942–1160	245–285, 260	10–30, 25	1:6–1:15, 1:9	30.9
Patil et al. (2013) ⁶⁹	Microwave mediated non-catalytic transesterification by SC ethanol	942–1160, 1160	245–285, 265	10, 15, 20 , 25	1:6–1:15, 1:12	32
Levine et al. (2013) ⁷⁰	Direct supercritical transesterification of algal biomass	1450–2500	275	180	20:1	89
Reddy et al. (2014) ⁷¹	Direct conversion of wet algae with supercritical ethanol	1200–1350	245–270, 265	2–30	1:6–1:15	67
				20	1:9	
Jazzar et al. (2015) ⁷²	Direct conversion of wet algal biomass by SC methanol	1232, 1450, 3045 , 6526	245, 255 , 265, 275	10, 20, 35, 50	6:1, 8:1, 10:1 , 12:1	46–48
Liu et al. (2015) ⁷³	Conventional catalytic method using H ₂ SO ₄	14.50	65	1440	30:1	98.7
	Supercritical methanolysis	2175, 2900 , 4351	350, 385 , 400	0.5–10, 4	6:1, 9:1 , 12:1	95.5

Text in bold indicates optimal conditions as reported by the authors.

direct conversion of wet algae by supercritical methanol. Liu et al. have reported yield of 98.7% with conventional catalyzed transesterification using H_2SO_4 and 95.5% with supercritical methanol.

It should be noted that microbial oils derived from yeasts, bacteria, and fungi are an additional resource for biodiesel production. Oleaginous microorganisms producing significant amounts of lipids include the bacteria *Rhodococcus opacus* and *Arthrobacter* species, the fungi *Mortierella isabellina*, and *Mortierella ramanniana*, and numerous yeasts, like *Cryptococcus albidus*, *Cryptococcus curvatus*, *Lipomyces lipofera*, *Lipomyces starkeyi*, *Rhodospiridium toruloides*, *Rhodotorula glutinis*, *Trichosporon pullulan*, and *Yarrowia lipolytica*.^{74,75} Such microbes constitute a promising feedstock for biodiesel since, like algae, they are renewable, have rapid growth rates, and do not require arable land. However, they are not photosynthetic, so conventional or cellulosic sugars are needed to cultivate them for biodiesel production.

MICROALGAE PREPARATION FOR BIODIESEL SYNTHESIS

Although microalgae are a promising candidate for producing biodiesel, they require a series of preparation and processing steps, as outlined in Figure 5. First, the selected algae strain needs to be cultivated in large-scale open pond systems (or sometimes in more expensive closed bioreactors) and then harvested and dewatered.⁷⁶ Separation methods like centrifugation, flocculation, filtration, gravity sedimentation, floatation, and electrophoresis are used to concentrate the algae from as low as 0.1–0.2 g/L (dry basis) to 10–450 g/L.^{77,78} In fact, dewatering consumes almost 60% of the total energy spent on producing algal biodiesel.⁷⁹ The dewatering

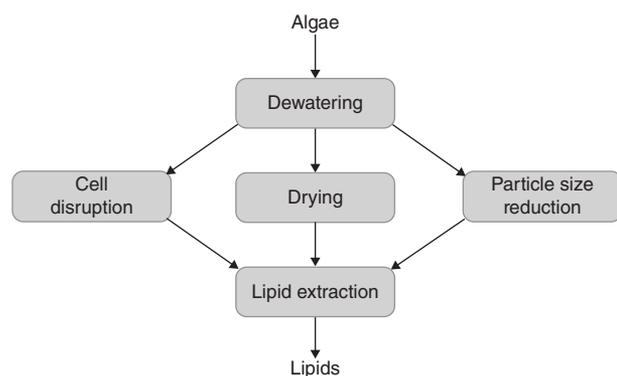


FIGURE 5 | Flowchart of algae processing before algal lipid conversion to biodiesel.

method is chosen based on strain characteristics, such as rate of water removal, solid content of recovered microalgae-water slurry, and efficiency of dewatering.⁷⁸ Since flocculation can be applied to a broad spectrum of algae species, it is considered to be superior to other techniques, like centrifugation or filtration.⁸⁰

Subsequently, algae undergo cell disruption to release the intracellular lipids to the surrounding media and improve the efficiency of the subsequent lipid extraction step.⁷⁷ The cell walls of algae are robust and thick, trapping the lipids inside the cell.⁷² Mechanical cell disruption is often used to release the lipids from the cells,⁸¹ followed by organic solvent extraction or supercritical fluid extraction to concentrate the lipids⁸² and then convert them to biodiesel via transesterification. Since lipids exhibit high solubility in organic solvents, like benzene, hexane, cyclohexane, acetone, and chloroform, such solvents are often used for lipid extraction.⁸³ The efficiency of the solvent extraction is high, if the solvent polarity matches that of the target compound. For example, hexane, a non-polar solvent, is commonly used for extraction of non-polar lipids from algae.⁸¹ Polar modifiers can be used to enhance the extraction of polar compounds.⁸⁴ Often, solvent extraction is carried out at higher temperatures and pressures to change the polarity of the solvent, thereby making it more selective towards algal lipids. This is termed accelerated solvent extraction (ASE) and results in better efficiency of extraction and lower use of solvent compared to conventional extraction.⁸⁵ Another technique applied to lipid extraction is supercritical fluid extraction. CO_2 is the most commonly used supercritical fluid, since its critical temperature and pressure are relatively low, it is abundant and non-toxic, and it can extract non-polar as well as moderately polar compounds.

SUPERCritical TRANSESTERIFICATION OF MICROALGAL LIPIDS

In recent years, biodiesel synthesis using supercritical transesterification has gained interest, particularly from vegetable oils like jatropha,⁴⁷ sunflower,⁴⁹ rapeseed,⁸⁶ and soybean,⁸⁷ as outlined earlier. In contrast, only a few studies have been published on biodiesel synthesis using supercritical transesterification of algae lipids.^{65–72,88} These studies employ one of two options for synthesizing biodiesel: (1) dewatering of algae followed by lipid extraction using organic solvents or supercritical CO_2 as the primary

steps before supercritical transesterification of lipids or (2) direct supercritical transesterification of wet algae (whole cells) to synthesize biodiesel.

Researchers have used the Soxhlet technique to extract lipids from the microalga *Chlorella protothecoides* with hexane as the solvent.⁶⁴ The lipids were then subjected to supercritical transesterification in a 100-mL cylindrical reactor and biodiesel was successfully synthesized at the reaction conditions reported by Kusdiana and Saka in 2001. In another study *in situ* lipid extraction was followed by supercritical ethanolysis to produce biodiesel.⁶⁵ In those experiments the lipid-rich alga *Chlorella vulgaris* (80% lipid content) was disrupted in subcritical water to produce a nutrient-rich aqueous phase, along with a solid phase retaining the lipids. The solids were then subjected to supercritical transesterification with ethanol and a yield of 66.4% was reported.

As stated earlier, the dewatering step in the extraction-transesterification procedure makes the process energy-intensive and consequently costly. This has been a major limiting factor in commercializing algal biodiesel.⁷² In response, direct supercritical transesterification of wet algae (whole cells) has emerged as a promising alternative that renders the dewatering step unnecessary. A few studies have been reported to demonstrate the capabilities of direct conversion of wet algae to biodiesel by supercritical methanolysis, where the water in wet algae is believed to serve as an active co-solvent along with supercritical alcohol to convert the algal lipids into fatty acid methyl esters by not only increasing the rate of conversion, but also increasing lipid solubility and acidity.⁶⁶ Others have also demonstrated the capabilities of direct supercritical transesterification and studied the effects of process parameters on the yield of biodiesel.⁷² Key parameters are the molar ratio of alcohol to algae, reaction time and temperature, and reaction pressure. Good understanding of the effects of these parameters is crucial as they can help identify ways to render direct supercritical transesterification of algae commercially viable for such a more sustainable feedstock.

Key Process Factors in Supercritical Transesterification of Algae

From research findings to date it is clear that the yield of the supercritical transesterification process depends heavily on three key factors: ratio of alcohol to algae, reaction temperature and pressure, and residence (reaction) time.^{66,71–73}

Ratio of Alcohol to Algae

In studies of supercritical transesterification, alcohol has been used in an excess amount of what is required stoichiometrically. Presence of excess alcohol is key, since it helps break the glycerine-fatty acid links and drives the reaction equilibrium towards the formation of fatty acid esters.⁷² Some studies indicate that excess alcohol may actually help to reduce the required critical temperature of the alcohol-triglyceride mixture, which subsequently allows for the reaction to take place in a homogenous supercritical phase.^{28,87,89} In one of the studies, a single step approach was employed for extraction of lipids and supercritical transesterification using methanol.⁷² Here, methanol acted as a solvent, catalyst precursor, and reactant at the same time. It was observed that, under a set of reaction conditions, the FAME yield reached 43.1%, for a methanol to dry algae ratio (v/wt.) of 10:1 and the yield decreased as the methanol to dry algae ratio (v/wt.) increased to 12:1.⁷² The lowest yield of 31.9% was obtained at a ratio of 6:1.

In addition to methanol, ethanol has also been used under supercritical conditions with wet algae to produce biodiesel. The maximum yield of fatty acid ethyl esters (FAEE) was observed at an ethanol to algae volume by weight ratio of 9:1. Increasing the ratio of alcohol to algae resulted in a significant decrease in the yield.⁷¹ A higher ratio reduces the critical temperature of the alcohol-oil mixture and this appears to lead to decomposition of biodiesel and hence to a lower yield of the supercritical transesterification reaction.⁹⁰ A similar trend was reported by other researchers, as the reaction yield was found to decrease when the ethanol to algae ratio (v/wt.) exceeded 9:1.⁶⁹ Moreover, it was observed that the separation of final products became more energy intensive due to the presence of excess alcohol. Figures 6 and 7 show the relationship between the alcohol to algae ratio and the yield of the transesterification reaction, as reported.

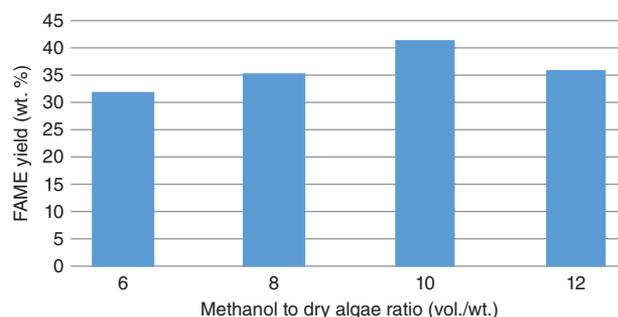


FIGURE 6 | Effect of the methanol-to-dry-algae ratio on the yield of fatty acid methyl esters (FAME) (created with data from Ref 72).

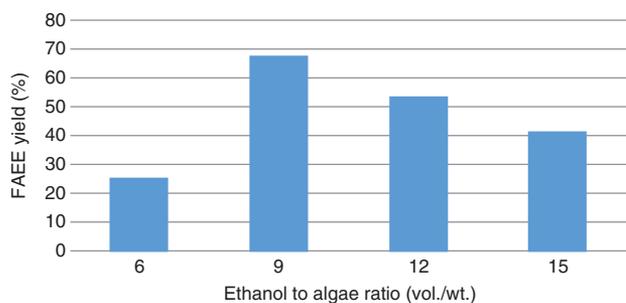


FIGURE 7 | Effect of the ethanol-to-algae ratio on the yield of FAEE (created with data from Ref 71).

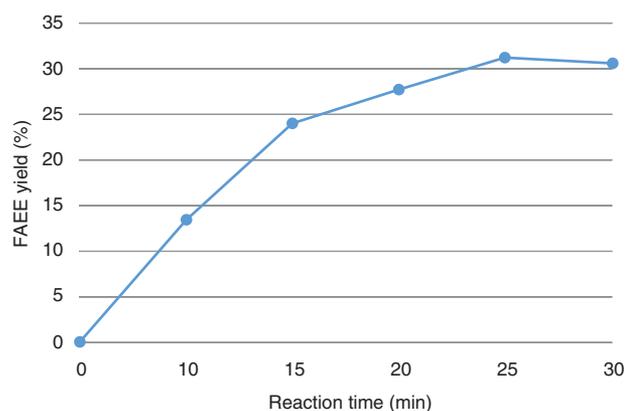


FIGURE 8 | Yield of ethyl esters from whole-cell wet algae as a function of reaction time (created with data from Ref 69).

Reaction Time and Temperature

Reaction time plays a crucial role in the supercritical transesterification process. Regardless of the type of alcohol used for transesterification (methanol or ethanol), reaction time affects the yield and rate of the reaction.⁵⁰ It has been observed that longer reaction times (20–25 min) allow the supercritical transesterification reaction to proceed to completion, resulting in higher yields, as shown in Figure 8. Still, these reaction times are an order of magnitude shorter than conventional transesterification, which requires at least 2–3 h to come to completion. However, it is likely that extending the reaction time further at a given temperature and alcohol to algae ratio may lead to lower yields due to biodiesel degradation reactions.⁹¹

Biodiesel yield decreases at temperatures higher than 300°C. This is primarily due to the unstable nature of C16 and C18 esters. These are stable below 300°C for optimum reaction times, but as the temperature rises to about 350°C, they start to decompose.⁹² In the case of FAEE, the yield may decrease due to the slow degradation of the poly-unsaturated fatty acids present in the algal lipids. This usually occurs when the double bond functional group

isomerizes from a cis-type carbon C=C into a trans-type carbon C=C.⁹²

Pressure

The properties of the supercritical fluid, like density, hydrogen bond intensity, and solubility, are affected by pressure.²⁹ An increase in pressure results in higher yields of biodiesel as the molar density of alcohol becomes higher, the solubility of alcohol in oil increases, and the unsaturated molecules undergo splitting, which subsequently improves conversion to biodiesel.⁵⁰ As the pressure increases, the biodiesel yield increases as well, until a point is reached beyond which the yield remains unchanged despite further increases in pressure. Interestingly, the impact of pressure on the yield of biodiesel varies depending on the molecular weight of alcohol used. When the pressure was increased from 8 MPa to 12 MPa, the yields of FAME, FAEE, and fatty acid propyl esters (FAPE) increased by 10.6%, 25.4% and 29.9%, respectively.⁵⁰

OTHER TECHNOLOGIES FOR BIODIESEL PRODUCTION

Apart from the conventional and supercritical technologies, other technologies like microwave-assisted biodiesel production and co-solvent transesterification have also been proposed. Patil et al. demonstrated the capabilities of microwave mediated supercritical transesterification.⁶⁸ Their process consisted of a single-step microwave-mediated transesterification with ethanol and dry algal biomass and produced about 32% yield of FAEE in 25 min. Reported advantages included improvement in efficiency of algal extraction and reduced extractive-transesterification time.

Another non-catalytic process is co-solvent transesterification, which utilizes a co-solvent, tetrahydrofuran, soluble in both vegetable oil and methanol.⁹³ In this process the triglycerides and the free fatty acids are converted in a two-stage continuous process. The co-solvent assists in overcoming the slow reaction rates due to the low solubilities of alcohol in oil. The co-solvent led to a clean phase separation between biodiesel and glycerol after the reaction and allowed single-step recovery of excess alcohol as well as of the co-solvent.

CONCLUSION

In recent years biodiesel has become an attractive bio-fuel to blend with or replace petroleum diesel. Although conventional transesterification is the main

production process practiced by the biodiesel industry, supercritical transesterification in the last few years has shown significant promise as a means of manufacturing biodiesel not only from vegetable oils, but also from promising renewable resources, like algae, and from low-value resources, like used cooking oil and animal fat. Supercritical transesterification is capable of synthesizing biodiesel without the use of a catalyst and in a much shorter time (by an order of magnitude) compared to conventional transesterification. Furthermore, the cumbersome post-transesterification separation steps are avoided. Although, the process needs higher temperatures and pressures, its ability to reach high yields within just a few minutes of reaction time, instead of hours, is indicative of its promising future.

One of the challenges in producing biodiesel on a large scale is the availability of abundant, inexpensive and sustainable raw materials. Until now, the major raw materials for biodiesel have been edible vegetable oils, like soybean, rapeseed and palm, all of which are food staples. As a result concerns have been raised worldwide about the use of edible oils

for biodiesel production. In response, increased attention has been directed towards microalgae, which have a high and tunable growth rate, require much less land area than oil crops, have a high lipid content, and are adaptable to growth conditions around the world.

Researchers have started demonstrating the ability of the supercritical process to convert algal lipids or even whole algae cells into biodiesel. Direct supercritical transesterification of whole algae cells may be the process of choice, as it appears to be more efficient than transesterification of algae-derived lipids by eliminating the need for energy-intensive cell mass drying and lipid extraction with organic solvents. Combining the merits of supercritical transesterification with the potential of algae, biodiesel production from algae could soon lead to more sustainable and cost-effective biofuels at large scale. Technology holds significant promise, although it still needs improvements and scale-up efforts to optimize its kinetics and thermodynamics and bring it to the commercial arena.

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