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Recent advances in biodiesel production: challenges and solutions

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Abstract

Mono alkyl fatty acid ester or methyl ethyl esters (biodiesel) are the promising alternative for fossil fuel or petroleum derived diesel with similar properties and could reduce the carbon footprint and the green house gas emissions. Biodiesel can be produced from renewable and sustainable feedstocks like plant derived oils, and it is biodegradable and non-toxic to the ecosystem. The process for the biodiesel production is either through tradition chemical catalysts (Acid or Alkali Transesterification) or enzyme mediated transesterification, but as enzymes are natural catalysts with environmentally friendly working conditions, the process with enzymes are proposed to overcome the drawbacks of chemical synthesis. At present 95% of the biodiesel production is contributed by edible oils worldwide whereas recycled oils and animal fats contribute 10% and 6% respectively. Although every process has its own limitations, the enzyme efficiency, resistance to alcohols, and recovery rate are the crucial factors to be addressed. Without any benefit of doubt, production of biodiesel using renewable feedstocks and enzymes as the catalysts could be recommended for the commercial purpose, but further research on improving the efficiency could be an advantage.

Keywords: Biodiesel; Catalyst; Lipase; Greenhouse gas; Eco-friendly; Immobilized enzymes; Nanocatalyst

1. Introduction

Energy has been recognized as a key source for maintaining economic growth of any country and fossil fuels have been contributing as major source for fulfilment of this energy need, since its discovery (Bateni et al., 2017). But environmental issues caused by combustion of these fuels, increasing prices and depleting natural energy sources like petroleum reserves encouraged researchers to find the alternative, cost-effective, sustainable, renewable and efficient energy sources. As per report of Energy Information Administration - EIA (2016), there will be an increase in energy consumption between the years 2000 and 2030 by 71% worldwide and it is expected that increase in emission of carbon dioxide will also rise by up to approximately 35% (Jamil et al., 2018). Till date several renewable energy sources have been explored successfully to limit the fossil fuel usage in order to reduce the negative effects imposed by emission of greenhouse gas released by fossil fuels consumption. According to reported data approximately 28% energy is consumed by only the transportation sector worldwide and in that biofuels contribution is about 3% (Dewangan et al., 2018).

Due to energy security awareness biodiesel is popularized on the global fuel market. It is a petroleum diesel substitute and a mixture of free fatty acid alkyl esters and attracted significant attention because of its minimal toxicity and high biodegradability (Krawczyk, 1996). Mainly biomass oils are used for its generation (Huang et al., 2010). It is called biodiesel, since it originates from biological products and matches in performance with petrodiesel. It was in year 1893, when the Rudolf Diesel, German engineer foresaw that vegetable oils (pure form) could be

utilized as a fuel for machines and equipments used in agriculture (Singh and Singh, 2010). In 1900 Rudolf demonstrated first diesel engine utilizing peanut oil as the fuel at the world exhibition in Paris. Then in 1977 first industrial process for production of biodiesel was developed by Expedito Parente (Brazilian scientist) and in 1989 first biodiesel plant using rapeseed as a feedstock was functioned in Austria (Dewangan et al., 2018).

Biodiesel is regarded as clean energy source as it can provide protection to the environment by lowering the amount of direct and indirect green house gases like CO_2, CO, SO_2 and HC (Huang et al., 2012). Thus, utilizing biodiesel can help in maintaining ecological balance in comparison with the use of fossil fuel. For biodiesel production mainly four methodologies have been reported including direct use/blending of oils, pyrolysis (thermal cracking), micro-emulsion and transesterification (Ma and Hanna, 1999). Among them base-catalyzed alcoholysis (transesterification) is regarded as best economical technology, due to its low pressure and temperature conditions and maximum product yield (Fukuda et al., 2001). Currently either by transesterification (alcoholysis) of oils with alcohols in the presence/absence of catalyst or by the fatty acids esterification, biodiesel is produced (Zaher and Soliman, 2015).

On the basis of fats and oils origin some steps of pretreatment is also found important before processing. Green diesel is the other name given to the diesel fuel of renewable nature. Pure biodiesel i.e. 100% FAME (fatty acid methyl ester) is called B100 whereas biodiesel blends have lower concentrations often blended with petroleum diesel in different ratios like B2 (2% concentration), B5 (concentration of biodiesel up to 5%) and B20 (6%-20% biodiesel) (Hoekman et al., 2012). Transesterification reaction gives two final products- biodiesel and glycerol (1, 2, 3-propanetriol) and by sedimentation both can be separated as they form two different phases of liquid due to variation in their densities. Because of the triglycerides conversion into esters

biodiesel has molecular weight of one third of triglyceride and esters contain oxygen of 10-11% by weight that cause better combustion as compared to hydrocarbon- based diesel fuels. Also this clear amber-yellow colored liquid has high cetane number which is associated with its better ignition and retaining the quality of biodiesel even after blended with mineral diesel (Singh and Singh, 2010). Biodiesel has reduced vapor pressure that lowers its spontaneous flammable rate, which is because of the residual surfactant molecules that prevent steams saturation over the liquid. It has a typical smell, lower volatility and is hygroscopic in nature. It can easily dissolve several organic polymers like seals and rubber hoses.

According to economic point of view, biodiesel production is found to be feedstock-sensitive. Depending upon used feedstocks for production and several production technologies biodiesel is categorized into four generations. Using edible feedstocks like rapeseed, palm oil, corn etc gives first generation biodiesel, whereas non-edible feedstocks like rubber seed, neem, jatropha etc and waste oil can be used for biodiesel production of second generation. Feedstocks that do not compete with crops for land include microalgae is used for third generation biodiesel and fourth generation consists genetically-modified algae, electro-fuels as well as photo biological solar fuels and this generation is classified as a new field of research that needs intensive studies in future for more exploration (Syafiuddin et al., 2020).

One of the major advantages of this alternate fuel is its energy content that is same as conventional fuels which make it suitable in use either alone or blended with conventional diesel fuel and there is no requirement of modifications in existing diesel engines. Besides numerous advantages of this clean-burning fuel there are certain technical challenges that need attention in order to make profitable biofuel that includes modifications in present technologies for high quality production of biodiesel, development of cheaper and better catalysts as well as low cost

photo bioreactors, utilization of non fossil- based solvents etc. Moreover the biodiesel cost is the major hurdle in the commercialization of fuel (Yusuf et al., 2011). In developed countries biodiesel cost is about 1.5-3 times higher as compared to the cost of fossil diesel. Production of biodiesel is set to increase in forthcoming years as it offers several promising benefits associated with energy security, agriculture sector expansion, economics and limiting pollutant emission. This article gives a critical overview of different aspects of substrate, recent advancements, limitations as well as challenges involved in biodiesel production.

2. Substrate for biodiesel

Feedstock plays an important role in biodiesel production and type of raw material used for generating fuel differs across countries on the basis of their geographical locations as well as agricultural practices. According to the published data, till date more than 350 crops have been reported for production of biodiesel (Atabani et al., 2012). Information of some of the substrates used for biodiesel production is summarized in Table 1. There are certain aspects that needs close observation before selecting any raw material to be used as substrate first is its fatty acids profile like biodiesel having immense amount of esters obtained from monounsaturated fatty acids (MUFA) shows better performance as fuel and second is fats and oils processing. Although numerous sources of lipids have been investigated for biodiesel production such as vegetable and animal fat-based oils, but single cell oils (microbial oils) produced by several oleaginous microorganisms including bacteria, fungi, yeast and microalgae also proved potential feedstocks (Vicente et al. 2009). Microbial oils exhibit many advantages such as less dependency on climate and season, require less labor, and can be easily scale up as compared to animal fats and vegetable oils. However, there is still lot of scope to explore these oleaginous microorganisms for oil production. It is predicted that for biodiesel production, oils from microbial origin might

become major oil feedstocks in next several years. Feedstocks are divided into many groups discussed below.

2.1. Edible and non-edible crops

Edible sources include rapeseed, peanut, coconut, palm, canola, mustard, safflower, sunflower etc. Although it has been extensively reported for production process but these crops need large portions of land (Verma et al., 2016). Also biodiesel from edible oil has a higher tendency to cause ecological imbalances. As per reported data in many countries like Malaysia and Indonesia forests were cut down for plantation purposes and caused deforestation.

Non-edible includes pongamia oil, castor bean, jojoba, rubber seed, tobacco (*Nicotiana tabacum*), seed oil, okra (*Hibiscus esculentus*) seed oil, wild safflower (*Carthamus oxyacantha* Bieb) seed oil, sugar apple seed oil (*Annona squamosa*) etc. Non-edible oils are excellent alternative for decreasing edible oil dependency. As these contain toxic components that makes it unfit for human consumption, thus it reduces competition for feed and food, also wasteland can be used for growing these crops that are unsuitable for food crops (Janaun and Ellis, 2010). Moreover, they require less farmland but these sources may not be enough to meet the growing fuel demands.

2.2. Used cooking oils and waste animal fats

Cooking oils have high free fatty acids (FFAs). Used cooking oils are divided as per FFAs content 1. Brown grease (>15% FFA and water) 2. Yellow grease (< 15%FFA) and can be utilized as raw material after purification (Pikula et al., 2020). Fats, oils and grease are collectively known as FOG includes white, brown and yellow grease, fish oil, animal tallow (Ribeiro et al. 2011; Hotti and Hebbal 2014). Most of the animal fats have high quantity of

saturated fatty acids that cause hurdles in production process. The main limiting factor that hinders fat and oil wastes industrial application is the insufficient centralized system. Also, using animal fats as a feedstock for biodiesel production arises biosafety issue for the reason that fat may be extracted from infected animal.

2.3. Municipal sewage sludge (MSS)

MSS is collected from the wastewater treatment plants and contains various inorganic and organic compounds. Sewage sludge is mainly a mixture of industrial and domestic wastes and comprises of sugars, proteins, lipids, phenols and detergents. It also includes hazardous pollutants (inorganic and organic). Sewage sludge needs to be undergoing pretreatment processes prior to lipid extraction. Municipal wastewater sludge is undoubtedly one of the promising lipid feedstock but the primary constraint for scaling up is the need to remove high water content prior to lipid extraction (Olkiewicz et al., 2016). Also, sludge collection, lipid separation, bioreactor design, soap formation in transesterification are some of the challenges being faced in using sludge as raw material. Moreover, expensive drying methods used for sludge are not efficient for recovery of lipid and hence affects the biodiesel production process (Demirbas, 2017)

2.4. Oleaginous-microorganisms

Not all microbial biomass derived lipids are ideal for biodiesel production. Just lipids having FAE (fatty acid ester) linkages and free fatty acids are capable of generating FAMEs that can be utilized as biodiesel, if they meet the published standard specifications for biodiesel (Gujjala et al., 2019). Wide variety of microorganisms reported in literature as producers of oil includes *Rhodosporidium toruloides, Lipomyces starkeyi, Mortierella isabellina, Cryptococcus albidus, Yarrowia lipolytica, Trichosporon fermentans, Rhodotorula glutinis* etc (Chi et al., 2011). Arous et al reported that cheap raw materials could also be used in case of oil production by yeast, like

in their study they found *Wickerhamomyces anomalus* strain EC28 (oleaginous yeast) accumulates good quantity of lipid when grown on several agro-industrial wastewaters (confectionary industries wastewaters, olive mill wastewater and deproteinized cheese whey) and predicted properties of fuel as per FAME composition was found in the specified range defined by international biodiesel standard specifications (Arous et al., 2017). However there are certain reports on engineering of *E. coli* (*Escherichia coli)* for biodiesel production but still that requires detailed investigation so that engineered strains could be used on industrial scale (Steen et al., 2010; Nawabi et al., 2011).

There are many publications on microalgae describing its potency as feedstock for biodiesel production. Several researchers have investigated *Chlorella* sp. as it is easily available. Kookkhunthod and his co- workers had successfully extracted lipid for biodiesel feedstock from freshwater microalgae *Chlorella* sp. KKU-S2 grown in batch mode utilizing sugarcane juice hydrolysate (Kookkhunthod et al., 2016). In another study *Scenedesmus obliquus* has been reported as potential algae for biodiesel production (Mandal and Mallick, 2009). Despite various advantageous of micro-algae like they eliminate competition for land, can be grown in environments that's not suitable for crops for example on non-arable lands, in salt, brackish or fresh water and short life-cycle, it is not used widely because of high production cost (Getachew et al., 2020). However, there are certain challenges that need to be overcome in order to increase the large scale biodiesel production from microbial oils at reasonable cost. Some of them are efficient cell wall breaking methods and innovative oil extraction technique, screening and modification of oleaginous microbes, novel microporous catalysts as well as regeneration method (Ma et al., 2018).

2.5. Insect oil

Recently insect oils are also gaining interest of scientific community. As per literature insects of Lepidoptera order like *Phasus triangularis* and *Galleria mellonella* contain more than 60% fats (Pinzi et al., 2014). Percentage of fat content differs between orders and species. However in recently published article triglycerides obtained from black soldier fly (*Hermetial illucens*) larvae has been used for biodiesel production (Liu et al., 2019). Nutrition and performance of this saprophytic insect majorly depends upon the substrate used such as oil and a protein rich raw material favors the better accumulation of grease as well as protein (Nguyen et al. 2017; Wang et al., 2017). Detailed studies are still required in order to analyze the performance of biodiesel obtained from insect biomass.

3. Current scenario for biodiesel production

Due to rapidly growing population, energy consumption is also increasing that ultimately leads to concern about declining oil supplies. Biodiesel, also regarded as green technology is gaining world's attention. In 2016 U.S. and Brazil had been recorded as the highest producers of biodiesel. It was actually after the execution of Energy Policy Act of 2005 that gave tax incentives for some kinds of energy and in U.S biodiesel production began to rise. Transesterification reaction is applied for production of biodiesel and mostly base catalyst is used. Now a day's biocatalysts are hot topic as it has potential to surpass chemical catalysts in the future for the production of biodiesel. Lipases have been found very important enzyme for methyl esters (biodiesel) production by catalyzing esterification as well as transesterification reaction (Marchetti et al., 2007).

Generation of biodiesel by lipase catalyzed transesterification mainly occur in two steps - ester bond hydrolysis and esterification with II substrate. Although enzyme (lipase) has been

recovered from different sources that can catalyze reaction but mostly fungal and bacterial lipases are utilized in biodiesel production. Some reported microbial strains for lipase production are *Pseudomonas cepacia*, *Pseudomonas fluorescens*, *Aspergillus niger*, *Candida rugosa*, *Candida antarctica*, *Thermomyces lanuginosus*, *Mucor miehei*, *Chromobacterium viscosum*, *Rhizopus oryzae* etc. (Vasudevan and Briggs, 2008). Production cost of biodiesel is majorly influenced by the type of raw-materials used. Wide range of articles have been published on the several variety of substrates such as sunflower oil, olive oil, rice bran oil etc that are used for fuel production. Presently, more than 95% of the biodiesel production is contributed by edible oils worldwide whereas recycled oils and animal fats contribute 10% and 6% respectively (Naylor and Higgins, 2017). In India mainly *Pongamia pinnata* (Karanja) and *Jatropha curcas* (Jatropha) are used as raw materials (Sharma and Singh, 2009). Globally about 27% production of biodiesel is from soybean oil and one of the important industrial applications of this oil is its use in biodiesel blends and about 31% and 20 % biodiesel is produced from palm oil and rapeseed respectively (Changma et al., 2020). There are also oleaginous species (micro-organisms containing 20-25% lipids) that have been explored for oil production (Lopes da Silva et al., 2014).

According to report of United Nations if crops like sugarcane, palm oil, soya and maize continued to be used for fuel generation there could be irreversible and harmful consequences. So, examining biodiesel production from other non edible sources or waste oils is a better option. Already there are various studies reported on the animal fats and waste oils usage for biodiesel production. As per researchers opinion even oils from plant sources will not be sufficient in future to meet the global demand of biodiesel and therefore have investigated micro algae as a raw material and this algae-based biodiesel is currently a newly emerging field (Mata et al.,

2010; Ahmad et al., 2011). One of the biggest challenge in getting maximum oil from algae is that only stressed algae (mainly due to restrictions in nutrient) gives higher oil yield but that nutrient unavailability also effects growth pattern, so balancing proper growth for getting higher amount of oil in nutrient deficient medium is not a easy task. Schematic diagram for biodiesel production is depicted in Fig.1.

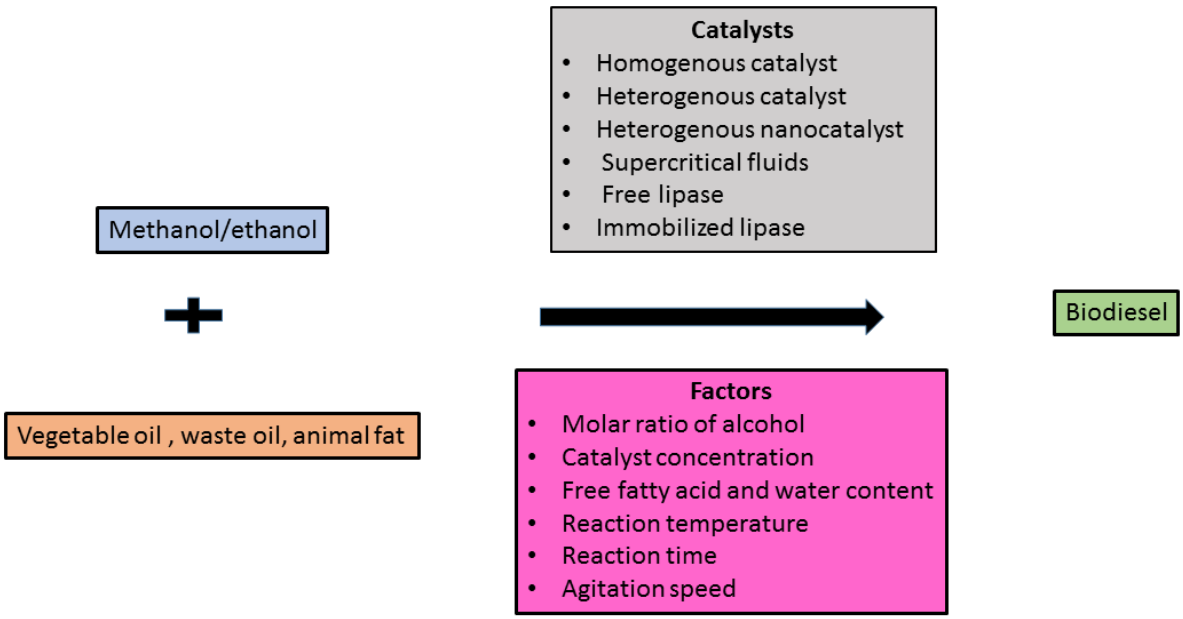

Fig.1. Schematic diagram for biodiesel production

4. Factors affecting on biodiesel production

Among all the substrates there are extensive studies done by researchers on vegetable oils to be used as diesel fuel. However, higher viscosity in vegetable oils hinders their direct application as fuel in diesel engines. As mentioned in introduction section several known methods include micro emulsification, pyrolysis, blending and transesterification that can be employed for viscosity reduction. It has been observed that pyrolysis method gives higher amount of biogasoline as compared to biodiesel fuel. And presently in order to boost the fuel property of

oils by lowering the viscosity transesterification methods are generally applied. There are several factors mentioned below that strongly influence the transesterification reaction for biodiesel production.

4.1. Molar ratio of alcohol

The ester yield is greatly affected by the alcohol to triglyceride molar ratio. It is one of the critical factors as it affects conversion that ultimately decides production cost and biodiesel yield. Usually in transesterification process for 1 mol of triglycerides to 3 mol of fatty acid esters and 1glycerol mol the reaction requires 3 mol of alcohol. Increased alcohol to oil ratio also increases purity and yield of biodiesel. But it is up to certain concentration of alcohol that gives maximum fuel yield. Moreover the molar ratio is related to the type of utilized catalyst.

According to reported data most of the alkali-catalyzed reaction requires approximately 6:1 molar ratio of methanol: oil for biodiesel production (yield-> 98w/w %) and this ratio is enough for breaking fatty acid-glycerol linkages (Musa, 2016). In case the ratio is increased beyond that range, there will be no rise in yield but that variation in ratio could obstruct separation process of glycerol as well as final reaction. This 6:1 ratio had been used and reported for transesterification of beef tallow with methanol (Zhang, 1994). It is noted that failure in by-product glycerol separation means it will remain in the solution and shifts equilibrium back to the left and hence lower the quantity of alkyl esters. Also ethanol can be used in place of methanol only under optimized conditions and some reports suggest molar ratio for ethanol should be 9:1. And using ethanol obtained from sugarcane or soybean will lead biofuel production completely from renewable sources. This ratio was fixed after conducting several experiments and was found in case of ethanol molar ratio less than 6:1 leads to incomplete reaction and ratio of 15:1 caused problems in by product separation. For the oil samples having high FFA (high free fatty acid)

content alkali catalyst fail to carry forward the reaction and in that case acid catalyst can be used for catalyzing the reaction but alcohol used will be of higher concentration than alkali catalyst. For example in case of acid catalyzed reaction reported by Leung and Guo by utilizing waste cooking oil alcohol ratio was 15:1 (Leung and Guo, 2006). And for microalgal oil scientists revealed 56:1 M ratio (Miao and Wu, 2006).

4.2. Catalyst concentration

In transesterification reactions different kinds of catalyst (alkali, acid, or enzyme) are known to be used and for attaining desirable yield each catalyst's concentrations i.e. their optimum value should be determined carefully by titration. It has been reported that alkali catalyzes reactions at a faster rate than acid ones (Basha and Raja Gopal, 2012). In case of using higher amount of catalyst than the optimum range that results in increased soap formation that ultimately lowers the final yield of methyl esters. Alkali catalysts include sodium hydroxide (NaOH), sodium hydride, sodium amide, sodium methoxide, potassium amide, potassium hydroxide (KOH), potassium hydride, potassium methoxide and acid catalyst could be phosphoric acid, organic sulfonic acid, sulfuric acid or hydrochloric acid. And among all these for biodiesel production most commonly NaOH and KOH are used as catalyst.

4.3. Free fatty acid (FFA) and water content

Reaction is affected to a higher extent due to FFA and water content. For alkali catalyzed alcoholysis, oil samples used should have FFA value less than one and all materials should be dehydrated (Demirbas, 2009). In case of higher content of FFA (greater than 1%) there will be need of more base catalyst for neutralizing FFA. Water also hinders the reaction by frothing and soap formation that results in increase in viscosity. This soap consumes the available catalyst and reduces its efficiency whereas foams and gels make the glycerol separation difficult. FFA

content and water effect have been investigated on alcoholysis of beef tallow (BT) with methanol (CH_3OH). And the study showed that for best conversion, FFA content and water of BT should be maintained below 0.5% w/w and 0.06% w/w respectively (Ma et al., 1998).

4.4. Reaction temperature

Temperature also greatly influences the esters yield and reaction rate. High temperatures lead reduction in viscosity of oils that result in high reaction rate as well as reduction in the reaction time. However it has been also found that if the temperature is increased beyond the desirable range that lowers the biodiesel yield because of the saponification of triglycerides accelerated by high temperature. For preventing alcohol evaporation generally temperature should be maintained below the boiling point of alcohol. Range of optimum temperatures is between 50°C and 60°C and depends on the kind of oils or fats to be processed. In some cases like utilizing supercritical methanol temperature of 350°C was found optimum for reaction (Kusdiana and Saka, 2001).

4.5. Reaction time

It is also an important parameter in biodiesel production. As with reaction time there is increase in conversion rate and it ultimately affect the reaction outcome. Proper time duration is critical for the reaction. If enough time is not given to the reaction some part of oil may remain un reacted and ultimately decrease ester yield and in case reaction duration exceeds than the normal time, it affect the final end product and also lead soap formation. There are different durations reported for particular oil such using soybean oil within less than 90 min conversion of ester was achieved (Freedman et al., 1986). Different reaction times used for different substrates are shown in Table 1.

4.6. Agitation/ Mixing

For the reaction agitation is mandatory and its speed has an important role for end product formation. In case of higher stirring speed there is soap formation and lower stirring cause less product formation. So, agitation speed also needs to be chosen very carefully.

5. Technologies for biodiesel production

5.1. Chemical methods for biodiesel production:

Biodiesel is produced by the transesterification of triglycerides in the presence of methanol. Traditional methods for biodiesel production are by esterification and transesterification. In the traditional esterification process, methanol is added along with a homogenous acid catalyst like sulphuric acid to convert the free fatty acids (FFA) into esters. In the traditional transesterification process, homogenous base catalyst like sodium methylate/ potassium methylate in the presence of methanol convert the free fatty acids to biodiesel and glycerol. Transesterification reactions are dependent on the type and concentration of catalyst used, molar ratio of alcohol, reaction temperature and time and the percent of conversion of triglycerides in the transesterification reaction to biodiesel.

The transesterification process the facilitated mainly by catalysts.

5.1. 1. Catalyst in biodiesel production

A catalyst is a substance (chemical or biological) that helps in increasing the rate of chemical reaction without being consumed in the process (Talha and Sulaiman, 2016). In the biodiesel production process, there are 6 main types of catalysts namely: a) homogenous catalyst b) heterogenous catalyst c) heterogenous nanocatalyst d) supercritical fluids e) free lipases f)

immobilised lipases (Thangaraj et al., 2014). The advantages and disadvantages of these catalysts are depicted in Table 2.

5.1.1.1. Homogenous catalyst

Homogenous catalysts function in the same phase as that of the reactants whether they are in the liquid or gaseous state. Ideally, homogenous catalysts are dissolved in a solvent along with the substrates, which could be an acid or base. Acid or base catalysts are used for the transesterification processes for biodiesel production.

5.1.1.1.1. Homogenous acid catalyst

As an acid catalyst, sulphuric acid aids in the transesterification of the triglyceride. Acid catalysts have better tolerance level in processing waste oils for biodiesel production than alkali. In two-step transesterification processes, acid is preferred as a catalyst first followed by alkali for better results, especially when using organic substrates (Thangaraj et al., 2014, Bharathiraja et al., 2014). Although the reaction rate is slower while using acid catalysts, they yield better yield of alkyl esters. The commonly used homogenous acid catalysts are sulphuric acid, hydrochloric acid, phosphoric acid, Boron Triflouride and sulfonic acids. Among them, sulphuric acid and sulfonic acids gave 99% of transesterification in comparison to other acid catalyst (Marchetti et al., 2007). According to Freedman et al. (1986) 99% of soybean oil was converted to biodiesel using 1 mol% H_2SO_4 (molar ratio of 30:1) at 65˚C for 50 hrs. Triglyceride production was more when excess acid was added for catalysis. Some of the advantages for acid based catalysts for biodiesel production are a) direct biodiesel production using low lipid feed stocks that have FFA level >6% (Meher et al., 2006), b) their ability to carry out esterification and transesterification

processes simultaneously and c) they are insensitive to free fatty acids and water and d) better yield of alkyl esters.

Homogenous acid catalyzed reactions are not preferred for commercial applications due to their slower rate of reaction; higher temperature conditions, high molar ratio of alcohol over oil and their corrosive effluents released which requires neutralization (Lam et al., 2010). When acid is added as a catalyst, it causes the protonates the carbonyl group of the ester causing carbocation (carbon atom that has a positive charge and three bonds), forming a tetrahedral intermediate, which removes glycerol producing a new ester with the generation of the added catalyst (Noureddini and Zhu, 1997, Bharathiraja et al., 2014). Sagiroglu et al., 2011 used 1.85 wt% sulphuric acid to produce a biodiesel yield of 95.2% at 100˚C with sunflower oil. Cao et al., 2013 used the microalgae *Chlorella pyrenoidosa* with 0.5 wt% of H_2SO_4 as a catalyst produced biodiesel with 92.5% yield.

5.1.1.1.2. Homogenous base catalyst

The most common base catalysts are NaOH, KOH and alkali metal oxides like Sodium methoxide (CH_3ONa) and potassium methoxide (CH_3OK) are preferred to be more active than other alkali hydroxides. When Sodium methoxide is used as a catalyst in transesterification, it produces water due to the interaction between NaOH and methanol. The water and fatty acids produce soaps, which interfere with the yield of biodiesel and product quality. Normally biodiesel is prepared from high quality vegetable oil using alkaline catalyst (Canakci and Van Gerpen, 1999).

In recent years, technology has been developed to obtain biodiesel from waste oil derived from cooking, frying, vegetable wastes etc. However, processing of these kinds of wastes can produce

significant amounts of FFA and water using alkaline catalyst. This could result in the formation of soap and interfere with the biodiesel production and their quality. The homogenous base catalyst are advantageous as they are able to undergo transesterification reaction at low atmospheric pressure and temperature, conversion rate is faster in a short period of time and is cost effective (Lam et al., 2010).The rate of alkali-based catalysts are 4000 times faster than acid based catalysts (Fukuda et al., 2001). For homogenous base catalysed reactions, it is ideal that the FFA content oil feed is between 0.5% to less than 2%. Loreto et al. (2005) observed that for cooking oil with more than 6% FFA, base catalysed transesterification is not possible. According to Silitonga et al. (2014) the highest biodiesel production was using *Calophyllum inophyllum* producing a maximum yield of 98.53% using KOH (1% wt) as catalyst with a methanol to oil ratio of 9:1. Silva et al. (2011) produced 95% of Biodiesel yield using 1.3% NaOH with soybean oil, and ethanol to oil ratio of 9:1.

5.1.1.2. Heterogenous catalyst

Heterogenous catalysts occur in a different state than their reactants. Most of the heterogenous catalysts are solid in nature, where their reactants are either in liquid or gaseous forms. Heterogenous base catalysts have been suggested over homogenous catalyst for biodiesel production as homogenous catalysts formed soap (undergo saponification), toxic effluent release and difficulty in regenerating the catalyst and causes corrosion in the reactor.

5.1.1.2.1. Heterogenous base catalysts

Some of the solid base catalyst involved in the biodiesel includes zeolites, hydrotalcites and alkaline metal oxides like calcium oxide. Calcination of calcium hydroxide, calcium carbonate results in the formation of CaO. Due to their low soluble nature in methanol, high catalytic

activity and high basic strength and easy availability as a raw material from limestone, bone, seashells etc. calcium oxide has been used as a heterogeneous catalyst in the transesterification (Zabeti et al., 2009; Widayat et al., 2017). In some cases it was observed that CaO was leached out during the transesterification reaction. Granados et al. (2007) observed that in the process of transesterification of sunflower and methanol, CaO was found solubilize with the reactants. When calcium oxide reacts with the reactants sunflower oil and methanol, it required extra purification step to remove the soluble fraction with ion exchange resin (Kouzu et al., 2009). When activated CaO was used as a heterogeneous catalyst, in transesterification of sunflower oil, they resulted in carbonation of air and hydration, thus affecting the active sites of CaO (Granados et al., 2007). However, their active sites can be revived after heating at 700˚C for the removal of carbonate group from the surface.

Magnesium oxide is used as a heterogenous base catalyst for the transesterification reaction of soybean oil and higher temperature of 180˚C (Di Serio et al., 2006). At a lower reaction temperature of 100˚C, the catalytic activity was very low producing less than 20% fatty acid methyl esters (FAME). No/ less catalytic activity was observed when the transesterification temperature was 60 ˚C (Gryglewicz S. 1999; Cantrell et al., 2005). Among the group II alkali oxides, Magnesium oxide showed the weakest catalytic activity in comparison to CaO and SrO (Kouzu et al., 2008). Mg-Al oxide is another heterogenous base catalyst that was prepared by the calcination of hydrotalcites ($(Mg_6Al_2(OH)16CO_34H_2O$) at a higher temperature (Xie et al., 2006). Leaching out of Mg and Al to the reactants occurred resulting in further step of purification. Di Serio et al., 2006 observed that 90% of FAME was obtained after transesterification reaction.

Nakatani et al. (2009) obtained 96.55% biodiesel yield using 25% w of oyster shell (containing CaO) as catalyst at 65˚C using methanol in the ratio of 6:1 for a period of 5 hrs. Similarly, eggshells have been used as catalyst on vegetable oil according to Wei et al. (2009) and Viriya-empikul et al. (2012).Viriya-empikul et al. (2010) used palmolein oil to undergo transesterification at 60˚C for 2 hrs with a methanol to oil ratio of 15:1 using 10% eggshell catalyst to produce 90% biodiesel yield.

5.1.1.2.2. Heterogenous acid catalyst

Although homogenous acid catalysts are effective, they can result in the contamination with reactants requiring further purification steps for product recovery and increasing the cost (Lotero et al., 2005). Heterogenous acid catalyst are not sensitive to the free fatty acids, they can carry out esterification and transesterification processes simultaneously (Kulkarni and Dalai, 2006), terminate washing steps for biodiesel (Jitputti et al., 2006) and help in the easy purification steps for the removal of catalyst. Solid based acid catalysts are preferred over liquid acid catalysts.

5.1.1.2.3. Heterogenous nanocatalysts

Heterogenous catalyst limits their usage in industrial applications as they reduce the reaction rates and produce undesirable reactions leading to the saponification of glycerides and methyl esters (Veljkovic et al., 2009). Nanocatalyst is preferred due to the high specific surface area, greater catalytic efficiency, prevents saponification and has excellent rigidity (Wen et al., 2010). The feedstocks for the nanocatalyst have high catalytic efficiency, decrease pollution, cost effective and non-toxic in nature. Some of the common heterogenous nanocatalysts used in biofuel production are zinc oxide nanocatalyst, calcium oxide nanocatalyst, magnesium oxide

nanocatalyst, zirconium oxide nanocatalyst, titanium dioxide nanocatalyst, nanohydrotalcites and nanozeolites.

Madhuvilakku and his team (2013) loaded 200mg TiO_2-ZnO nanocatalyst, for 5 hrs at 60˚C with 6:1 methanol to oil ratio to obtain 92.2% biodiesel yield with palm oil. Baskar et al. (2017) used Mn-ZnO nanocatalyst (8%), methanol to oil ratio of 7:1, at 50˚C for 5 hrs to get a biodiesel yield of 97% with Mahua oil. A hetero polyacid based nanocatalyst (5 to 29 nm size, derived by co-precipitation and calcination) was used for the transesterification of madhuca oil at 55˚C for 5 hrs to get a biodiesel yield of 95%. Bai et al. (2009) utilized 3% calcium oxide microspheres (15 to 100 nm) with methanol to oil ratio of 9:1 for 3 hrs at 65˚C to produce a biodiesel yield of 98.72% by the transesterification of soybean oil. Hu et al. (2011) synthesized nanomagnetic catalyst KF/CaO-Fe_3O_4 (50 nm) using methanol to oil ratio of 12:1 at 65˚C for 3 hrs producing an yield of 95% using stillingia oil for biodiesel production. Tang et al. (2012) obtained a biodiesel yield of 98.71% using rapeseed oil with nanomagnetic calcium prepared by calcium aluminate and iron (lll) oxide at the ratio of 5:1 (Ca: Fe) at 600˚C for 6 hrs.

Pandit and Fulekar (2017) used chicken eggshell waste as a raw material for the development of calcium oxide nanocatalyst. Using Response surface methodology, biodiesel was produced from algal biomass produced and yield of 86.41%. Vahid et al. (2016) used 3% magnesium oxide doped magnesium aluminate ($MgO/MgAl_2O_4$) nanocatalyst for the transesterification of sunflower oil, at 110˚C for 3 hrs, methanol to sunflower ratio of 12:1 leading to 95% diesel yield. Magnesium oxide coupled lanthanum oxide ($MgO-La_2O_3$) for the transesterification of sunflower oil using methanol to oil ratio of 18:1 at 338 K for 5hrs to produce 97.7% biodiesel yield (Feyzi et al 2017).

Dehghani et al., (2019) used 5% w/w of Mg/CeM biofunctional nanocatalyst for the transesterification of waste cooking oil with methanol to oil ratio of 9:1, at 70˚C for 6 hrs leading to 88.7% biodiesel yield and the nanocatalyst could be reused for 7 cycles. ZrO_2 loaded with $C_4H_4O_6HK$ was used for the transesterification of soybean oil using methanol. The nanocatalyst had particle size of 10-40nm and could be reused for 5 successive cycles for transesterification. 98.03% of biodiesel was produced by the following optimized conditions with 6% w/w catalyst incubated at 60˚C for 2 hrs at a methanol to oil ratio of 16:1 (Qiu et al., 2011).

Nanohydrotalcites are heterogenous nanocatalysts synthesized by the co-precipitation method using magnesium and aluminium hydroxides as a 2 D arrangement of hydroxides as double layer. 1% of nanohydrotalcite of Mg/Al, at a temperature of 303K for 1.5 hrs with a methanol to oil ratio of 95.2% biodiesel yield from transesterification of jatropa oil (Deng et al., 2011).

Nanozeolites have strong acid sites, ability to disperse in aqueous and organic solvents and play an important role in the transesterification process. Saeedi et al. (2016) synthesized KNa/ZIF8 complex and used this complex for the transesterification of soybean oil with methanol to oil ratio 10:1 for 3.5 hrs. Heterogenous nanocatalysts are advantageous for biodiesel production due to their reusability, generation of minimal waste and purification ease.

5.1.2. Supercritical fluids for biodiesel production

Supercritical fluids (SCF) have been used for the transesterification of vegetable oil to form biodiesel in the absence of a catalyst (Manojkumar et al., 2021). Some of the recent supercritical fluids are listed in Table 3. Supercritical fluids have several advantages over conventional catalytic processes, as their downstream processing is faster, environmentally friendly and faster in reaction (Sarangi et al., 2018; Reddy et al., 2018). SCF forms a single phase due to the

supercritical methanol rather than the two-phase mixtures (methanol and oil) which is difficult to separate. Saka and Kusdiana (2001) prepared biodiesel from the rapeseed oil using supercritical methanol. Addition of catalyst like calcium oxide to the supercritical fluids can also enhance the efficiency of transesterification (Demirbas, 2002; Deslandes et al., 1998).

Addition of water along with supercritical methanol for biodiesel production has been explored. This water added supercritical method is economical is it allows easy separation of by-products like glycerol due to their greater solubility in water (Kusdiana and Saka, 2004). Yin et al., 2008, produced 98% of methyl esters by the addition of co-solvents hexane, carbon dioxide and potassium hydroxide with soybean oil. The optimum conditions were 160˚C, 20 min with methanol to oil ratio of 24:1, with 0.1% KOH.

Green solvents are added as an alternative to reduce the alcohol to oil in biodiesel production by non-catalytic methods. Carbon dioxide and hydrocarbons are added to enhance the biodiesel yields by replacing conventional supercritical alcohols (Tan et al. 2010b; Muppaneni et al. 2012). The usage of carbon dioxide results in high biodiesel yields requiring low separation costs by reducing the pressure. The products formed and the catalysts can be easily separated, as they cannot dissolve in CO_2. Other solvents like those that dimethyl carbonate is used for the biodiesel production at an atmospheric pressure of 20 MPa and temperature of 350˚C (Ilham and Saka, 2009).

Supercritical methyl acetate is used as a solvent for biodiesel production (Goembira and Saka 2013; Campanelli et al., 2010). Campanelli et al. (2010) studied the biodiesel synthesis using edible, non-edible, and waste oils. Complete oil conversion was observed at 50 min at 345˚C for 20MPa, with methyl acetate to oil ratio of 42:1. Supercritical Tert-butyl methyl ether was added

for the biodiesel production from canola oil (Farobie et al., 2014). This method overcame the problem of saponification, low yield and produced a high value product namely Glycerol Tert Butyl Ether (GTBE); a product with high cetane number with the ability to blend with diesel.

Although methanol and ethanol are utilized as supercritical fluids in transesterification, they are found to be corrosive, low energy content and hygroscopic. These alcohols are replaced by 1-propanol, a high carbon alcohol which helps in the major conversion of oil to biodiesel. Farobie et al. (2016) observed that supercritical propanol (SCP) could convert oil to obtain a biodiesel yield of 93.8 mol% at 350°C and 20 MPa for 30 min.

5.2. Enzymatic method of biodiesel

Due to the high temperature conditions, incubation time, methanol to oil ratio of the operating condition of homogenous and heterogenous catalysts, greener approaches are considered for the biodiesel production.

5.2.1 Biodiesel production using free lipase

Lipases are enzymes that breakdown the ester bonds of triglycerides by reducing the number of steps in transesterification and require less energy (Marchetti et al., 2007). Lipase based transesterification process was first attempted by Mittlebach and Trathnigg (1990) using sunflower oil in the presence of different types of alcohols with temperatures ranging from 25-65°C. High quality of biodiesel is obtained using different feedstock requiring minimal steps in downstream processing of biodiesel (Fukuda et al., 2001). Enzymes are easily degraded at temperatures above 50°C, limiting their use at high temperatures (Sim et al., 2010). The optimal condition for methanol to oil ratio is between 3:1 to 4:1 and if the ratio is increased it decreased the catalytic efficiency (Kumari et al., 2009). Methanol removes the water molecules on the

surface of lipase resulting in the change of configuration and decrease in enzyme activity (Fjerbaek et al., 2009).

Lipases are isolated from bacteria (*Staphylococcus hyicus*, *Bacillus thermoleovorans*, *Pseudomonas putida, P. cepacia, Pseudomonas fluorescens*, *P. aeruginosa*), fungi and yeast (*Candida cylindracea, Candida rugose)* and are used in the transesterification of biodiesel (Borrelli and Trono, 2015; Thangaraj et al., 2014). Fungal lipases for transesterification of triglycerides predominantly belonged to *Rhizopus* species namely: *Rhizopus arrhizus, R. usamii, R stolonifer, R. fusiformis, R. circinans, R. thermosus, R. microsporus, R. japonicas, R. chinensis, R. delemar, R. oryzae, R. podiformis* and *R. boreas*. Other fungal species include *Aspergillus niger, Chromobacterium viscosum, Pencillum cyclopium, Humicola lanuginose, Fusarium hetrosporum, F. oxysporum, Mucor meihei* etc (Tan et al., 2010; Gog et al., 2012; Akoh et al., 2007; Zhang et al., 2012). The most commonly used commercial enzymes biodiesel production from different stock are Novozyme 435, Lipozyme RM-IM, Lipozyme TL and Lipase PS-C (Reddy et al., 2018).

Some of the disadvantages in using free lipases for biodiesel production compared to other catalyst are; a) high enzyme loading is required for the access of active sites for the transesterification reaction to occur, b) expensive c) the by-product of biodiesel transesterification inhibits the substrates from reaching the active sites by forming a hydrophilic layer around the lipase enzyme (Varma and Madras 2007; Taher et al. 2011; Xu et al., 2011). Microbial whole cells have been used directly for the transesterification as a cheaper alternative. However, it has been observed that the yield of biodiesel production was relatively low when compared to using enzymes directly (Li et al., 2007).

5.2.2 Biodiesel production with immobilized lipase

Biodiesel production is due to the transesterification process of oils/fats with the aid of chemical or biological catalysts. Enzymes like lipases are used as a greener approach for the conversion of fats to fatty acid alkyl ethers as chemical methods require high energy, difficulty of recovering the catalyst and glycerol after transesterification process and environmental pollution generated as a result of acid/alkali used in the chemical catalysis (Narwal and Gupta, 2013; Lara Pizzaro and Park, 2003). Lipases catalyse reactions at lower temperatures, high stability in non-aqueous media, very good catalytic activity and hence are used by researchers globally (Tan et al., 2010; Shimada et al., 2002; Lu et al., 2008). One of the bottlenecks of using lipase is their high cost, which can be reduced by the immobilizing the lipase enzyme to enhance their stability and reusability (Cao, 2005; Salis et al., 2008).

Different methods for immobilizing lipase enzymes are a) Adsorption b) covalent bond c) cross linking d) Entrapment. Fig 2 depicts the different strategies adopted for enzyme immobilization and Fig.3. depicts enzymatic transesterification of fatty acid esters to produce a mixture of fatty esters (biodiesel) and glycerol (by product). Table 4 explains the immobilization methods used to adhere enzymes for the conversion of oils to biodiesel.

5.2.2.1. Adsorption

Adsorption is the process of attaching the lipase enzyme of a carrier matrix with the help of weak forces like hydrophobic interactions, van der Waal's interaction and dispersive forces (Jegannathan et al., 2008). It is the most widely used method for lipase immobilization. The process of adsorption is relatively easy, operates under mild conditions without loss of catalytic activity, the carrier can be recovered for repeating the immobilization process and hence is cost

effective. The immobilized lipase for biodiesel production are obtained from, yeast species *Candida antartica* (Du et al., 2004; Wang et al., 2007), *Candida* sp 99-125 (Lu et al., 2010; Nie et al., 2006 ; Tan et al., 2006; Lv et al., 2008) bacteria namely *Pseudomonas cepacia* (Salis et al., 2005 ; Shah and Gupta, 2007), *Pseudomonas fluorescens* (Salis et al., 2008; Soumanou and Bornscheuer, 2003), *Chromobacterium viscosum* (Shah et al., 2004) fungal species namely *Rhizomucor meihei* and animal source like Porcine pancreatic lipase (Shah et al., 2004; Shieh et al., 2003; Yesiloglu, 2004).

Some of the carriers used in adsorption are diatomaceous earth, acrylic resin, textile membranes, celite, synthetic polymers like polypropylene, polystyrene macroporous resin, electrospunpolyacrylonitrile nanofibers, polymethacrylate (Li & Yan, 2010, Salis et al., 2009, Sakai et al., 2010). When vegetable oil/ waste cooking were used as the raw material, the biodiesel yield was about 80%. The commonly used commercial lipases belonged to *Candida* species. Different acyl receptors are used for the transesterification using immobilized lipase. Ethyl and methyl acetate acyl receptors were preferred more that methanol due to its inhibition on the lipase enzyme activity (Du et al., 2004; Kim et al., 2007).

Du et al. (2004) observed that when methyl acetate was used as an acyl acceptor (12:1 methyl acetate to oil ratio) with acryl resin as carrier for transesterification of soybean oil, 92% of biodiesel was produced using Novozym-435 (*Candida* lipase). Lu et al., (2008) used textile membrane as carrier using immobilized lipase from *Candida* species 99-125 using methanol as acyl acceptor providing a yield >87%. Iso et al. (2001) showed highest catalytic activity using *P. fluorescence* with porous kaolinite (Toyonite 200-M) as carrier with using triolein and safflower oil with methanol in comparison to other lipases from *Mucor javanicus*, *Candida rugosa* and

Rhizopus niveus. Zeng et al. (2009) improved the immobilization of *S. cerevisiae* lipase by adsorption on Mg-Al hydrotalcite that retained 95% enzyme activity at 50˚C.

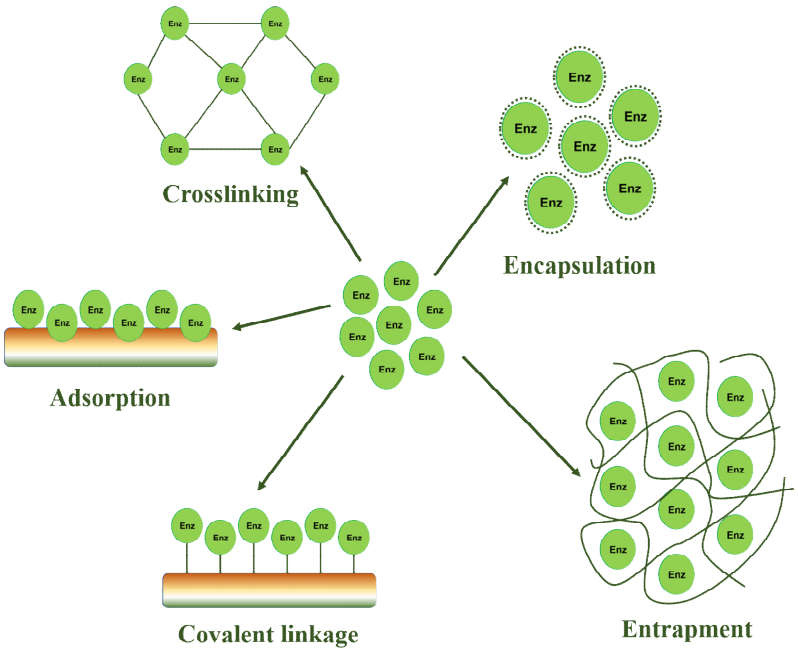

Fig.2. Schematic diagram for enzyme immobilization techniques

5.2.2.2. Lipid immobilization by entrapment/ encapsulation

Entrapment is the process of capturing the enzyme within the matrix of a polymer and encapsulation is the process of forming a membrane like structure around the enzyme (Cao, 2005). Entrapment involves capture of the enzyme within a matrix of a polymer, although enzyme encapsulation refers to the formation of a membrane-like physical barrier around the enzyme preparation (Cao, 2005). The matrix development occurs during immobilization. This process of enzyme entrapment/ encapsulation is relatively simple and the stability is better than adsorption method. Some of the carriers suggested for entrapment/encapsulation includes κ-carrageenan, silica gel, silica aerogel, celite supported sol gel etc (Stoytcheva et al., 2011).

Jegannathan et al., 2010, suggested the used of κ-carrageenan as a carrier matrix due to the low cost, easy biodegradation and low toxicity. *Burkholderia* lipase encapsulated in κ-carrageenan retained about 82% of the lipase activity after 5 cycles, leading to 100% methyl ester conversion of palm oil transesterification. Sol gel method is another method involved in the entrapment/encapsulation. This process involves a chemical catalyst (NaOH, HCl, NaF etc.) along with enzyme solution and a matrix precursor. The hydrolytic reaction as well as condensation results in the formation of amorphous silica matrix which surrounds around the enzyme. This method has been employed in encapsulation of lipase from *R. meihei* (Macario et al., 2009). This encapsulated form decreased the rate of leaching and improved the stability of the enzyme. Noureddini et al. (2005) entrapped lipase PS using sol gel matrix using tetramethoxysilane and isobutyltrimethoxysilane. The entrapped lipase showed better transesterification of soybean oil than using free lipase.

Enzymes immobilized in sol gels increase their enzyme activity (Aucoin et al., 2004). However, enzymes immobilized on sol gels have limitations in internal mass transfer and hence require a double immobilization process involving immobilization of sol gel in an inert material. The internal mass transfer can be improved with the help of inert material. Celite ® R632 is a supporting material for improving the application of sol gels in enzyme immobilization. It has lower adsorption capacity, and does not entrap lipase in its pores and can be used for biodiesel production (Meunier and Legge, 2013).

5.2.2.3. Cross linking

Crosslinking is the method involved in the formation of 3D network between the enzyme, the carrier and coupling reagent. In the crosslinking method, the immobilized enzymes are stable due to the interaction between the enzyme and the carrier, but the enzyme activity was observed to be

low due to their intense cross-linking conditions. This method has disadvantages due to the absence of amino groups of the support causing low enzyme activity. For better results, cross linking can be combined with adsorption method for better immobilization. Chitosan can be added as the source for amino groups, enhances the enzyme immobilization on anion exchange macroporus resin, and gave a biodiesel yield of 50.79% (Alamsyah et al., 2017). Addition of glutaraldehyde as a cross linking agent could improve the enzyme stability in transesterification reactions. Whole cell of *Rhizopus oryzae* IFO 4697 was used as a biocatalyst in the transesterification process of renewable oils using methanol and crosslinking with glutaraldehyde to improve the stability of immobilized cells for better stability and thereby used several times (Sun et al., 2009).

5.2.2.4. Covalent bond formation

Enzymes immobilized to a solid support by covalent bonding are more stable unlike other bonds and form a stable complex. The main functional groups of the enzymes involved in covalent linkage formation are amino, carboxyl, phenolic, indole, sulfhydryl, thiol, imidazole, hydroxyl, imidazole etc. When the enzyme binds on the surface of the solid support, it involves the activation of the surface linker molecules like glutaraldehyde followed by covalent bonding of the enzyme to the activated support. Covalent bonding makes the enzyme and support stronger, prevents enzyme leakage from the surface area of the matrix (Trevan, 1988). Lipases from *P. fluorescens* and *Thermomyces lanuginosus* were immobilized onto Toyopearl AF-amino-650 resin by covalent bond. Mendes et al., (2011) immobilized *T. lanuginosus* lipase on glyoxyl-resin derivatives and it was 30 times more stable than free lipase. This immobilized enzyme was successfully used for the transesterification of palm oil with methanol to produce biodiesel yield ranging from 93.5 to 100% (Mendes et al., 2011).

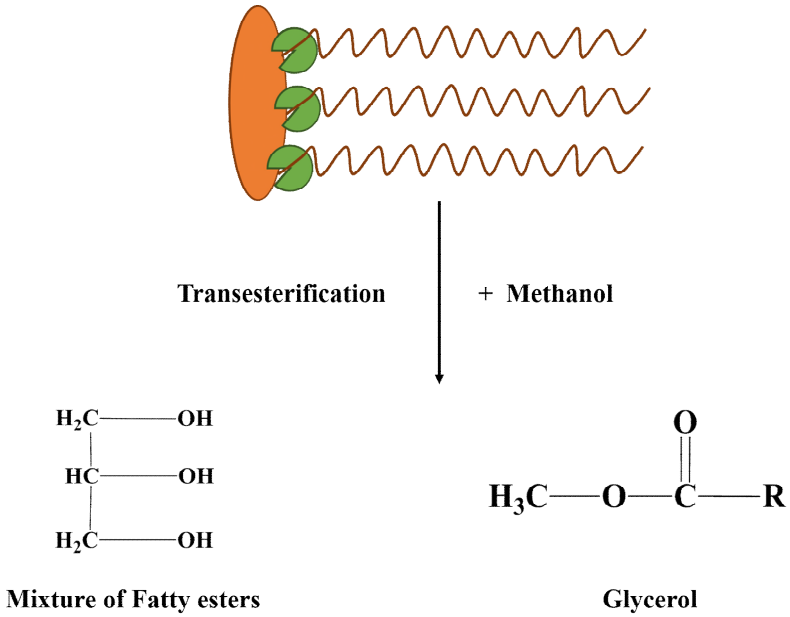

Fig.3. Enzymatic transesterification of fatty acid esters to produce a mixture of fatty esters (biodiesel) and glycerol (by product)

7. Challenges and limitations of pilot scale biodiesel production

In 2020, the prices of natural gas, gasoline, diesel and biodiesel were US$ 2.18, 2.18, 2.4, 3.33 per gallon respectively (Alternative Fuels Data Center: Fuel Prices (energy.gov)). In USA, the biodiesel is sold at US$ 1.0 with subsidy which is the only country where biodiesel was found economical (Agarwal et al., 2017), and in India with a production capacity of 11 million to 280 million liters in six different plants the average cost is Rs. 52.7 per liter. In a commercial point of view, substrate availability, substrate cost, cost of the catalyst, suitable bioreactor or reactor design (Capital investment), and production cost interms of energy and chemicals (Operating cost). As oil or fattyacids is the major feedstock for production of biodiesel it tends to cost 80 – 85% of the biodiesel production cost.

In a comprehensive review of Christopher et al., 2014, described the cost of the catalyst per kilogram of the biodiesel produced is around 0.14 and 0.006US$ for enzyme and alkali mediated

transesterification respectively. In terms of processing cost, a base catalyzed process using Jatropha feedstock is about US$ 0.15/L, and cooking oil as the feedstock with acid and base catalysis sums up to 1.2 and 0.23 US$/L respectively. So the situation of biodiesel is the production cost is comparatively higher than the petro-diesel, than the selling price, which has become a question of viability in commercial scale, unless the government bodies provides subsidy. When Jatropha feedstock was replaced by used or waste cooking oil, the substrate cost is reduced to 55% which makes the process commercially viable, but the limitation is procurement of the feedstock. With the increased availability of feedstock or improvement in the supply chain management, along with increase in crude oil prices could favor the economics of biodiesel market (Agarwal et al., 2017; Christopher et al., 2014).

The major challenges to be addressed in enzyme mediated processes are (i) lowering the cost of the enzyme by improving the efficiency, (ii) ways to recycle the catalysts (immobilized or entrapped), and (iii) improve the resistance of the enzyme to short or medium chain alcohols.

7. Conclusion and future prospects

With respect to properties like heat of combustion and octane number the biodiesel is comparable to petroleum diesel, but the particulate, CO_2, and sulphur emissions are significantly lower when biodiesel is burnt. The process of biodiesel production is a simple transesterification of triacyl glycerol or fatty oils mediated by alkali or acidic catalysts. With the limitations of using acid or alkali as the catalysts, environmentally friendly enzymes provided a less energy intense production process with limited by-products. Replacement of Jatropha or palm oil was the feedstock with waste cooking oil, the cost of the process can be considerably reduced and also the problem of discharge of waste cooking oil can be addressed. In the chemical transesterification process methanol is used as the initiator or alcohol, but methanol can be toxic

to whole cell or cell free enzymes, and even hard to recycle after the reaction, hence another suitable alcohol ethanol can be used. An effective and sustainable process development for the biodiesel production could be a key for major economic development. Hence focused research concerning the barrier of feedstock supply, innovation in process i.e., reactor configuration that can withstand harsh conditions, or development of cost-effective enzyme, would be required.

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Table 1: List of some reported feedstocks for biodiesel production and used alcohol: oil molar ratio, catalyst, temperature, duration for reactions and the percentage of final yield

S. No.	Feedstock	Alcohol: oil molar ratio	Catalyst	Temperature and duration	Yield (%)	Reference
1.	**Edible-oils**					
	Crude palm oil	Methanol: oil (40:1)	H_2SO_4 5% (vol/wt)	95°C for 9h	97	Crabbe et al. 2001
	Soybean oil	Methanol: oil (20:1)	Hydrotalcite 5.0%(w/wt)	64°C for 10h	94.8	Martins et al. 2013
	Rapeseed oil	Methanol: oil (7:1)	1.0% NaOH and KOH	75°C for 60min* & 65°C for 90min**	92.17* and 93.80**	Zhang and You 2015
2.	**Non-edible oils**					
	Jatropha oil	Methanol: oil (6:1)	1%NaOH	65°C for 1h	97.7	El Diwani et al. (2009)

	Rubber seed oil	Methanol: oil (4:1)	5.0% wt (limestone based catalyst obtained from cement clinker)	65°C for 4h	96.9%	Gimbun et al. 2013
	Castor oil	Ethanol: oil (10:1)	1.5% KOH	60°C for 10min	80.1	Hincapie et al. 2014
3.	**Waste oils**					
	Deodoriedpalm oil (DDPO)	Methanol: DDPO(60:1)	5% (derived from waste material-amazon flint kaolin	160°C for 4h	92.8	do Nascimento et al. 2011
	Waste cooking oil (Sunflower oil)	Methanol: oil (6:1)	NaOH (1%wt/wt)	60±1°C for1-3h	80	Thirumarimurugan et al. 2012
	Second used cooking oil	Methanol: Oil (6.18:1)	KOH (1%wt/wt)	66.5 °C for 1hour	92.76	Kawentar and Budiman 2013

	Waste animal fat	Ethanol: fat (7:1)	KOH (0.96wt %)	30 °C for 30min.	83.5	Cunha et al. 2013
	Waste frying oil	Methanol: Oil (15:1)	NaOH (0.5-1%wt/wt)	55°C for 1-1.5h)	94	Banerjee et al. 2014
	Waste cooking oil	Methanol: oil (15:1)	Heterogeneous catalyst 5% wt/wt (Derived from chicken bones)	65°C for 4 h	89.33	Farooq and Ramli 2015
4.	**Micro-algal oils**					
	Spirulina maxima	Methanol: oil (9:1)	KOH 0.75% (wt %)	65 °C for 20min	86.1***	Rahman et al. 2017
	Spirulina sp.	Methanol: Palm oil (10:1) & Palm oilᵠ: microalgae(5:1)	KOH (1% w/w palm oil)	60°C 2h	82.28 (Partial yield from palm oil-99.015 and partial from dry microal	Pradana et al. 2020)

				gae - 16.69)	
Chlorella vulgaris oil	Methanol: microalgae oil (6:1)	KF/KOH-Fe_3O_4, 1.5 (wt%) and CaO/KOH-Fe_3O_4, 1.5 (wt %)	25°C for 120 min	96.8$^{\Psi\Psi}$ & 92.2$^{\Psi\Psi\Psi}$	Farrokheh et al. 2020

* utilizing NaOH catalyst; ** utilizing KOH catalyst

*** Yield obtained was after first step of esterification that was conducted for lowering acid value optimum conditions were - (Methanol: oil (12:1) with 1% H_2SO_4 for 90min. at 60°C]

$^{\Psi}$ Palm oil used as co-solvent of methanol

$^{\Psi\Psi}$ using catalyst- KF/KOH-Fe_3O_4; $^{\Psi\Psi\Psi}$ using catalyst - CaO/KOH-Fe_3O_4

Table 2: Comparison of catalysts used in Biodiesel Production

Catalyst	Examples	Advantage	Disadvantage
Homogenous acid catalyst	H_2SO_4, HCl, sulphonic acid	Improved catalysis	Corrosion of reactor, slower reaction rate
Heterogenous acid catalyst	Bronsted type and Lewis type acid catalyst ; Keggin type heteropolyacids	Reusable and easy separation	Catalyst preparation is expensive, harsh reaction conditions
Homogenous base catalyst	NaOH, KOH, Sodium methylate	High rate of catalysis, noncorrosive, easily available	Saponification at high FFA, non-reusable
Heterogenous base catalyst	Alkaline metal caronates, alkaline earth metal carbonates and oxides	Reusable, simple purification of products, less waste generation	Costly, leaching of active sites
Heterogenous nanocatalyst	zinc oxide nanocatalyst, calcium oxide nanocatalyst, magnesium oxide nanocatalyst, zirconium oxide nanocatalyst, titanium dioxide nanocatalyst,	Catalyst recovery is easy, non-toxic and cost effective, reusable	Process of synthesizing heterogenous nanocatalyst is longer

	nanohydrotalcites and nanozeolites.		
Supercritical fluids	No catalysts used. Solvents like CO_2 and methanol are added to enhance biodiesel production	High reaction rate, not sensitive to water/FFA	They require high temperature
Free lipase	Lipase from bacteria, yeast and fungi	Biodiesel yield is higher	Low catalytic activity, difficulty recovery of glycerol and the catalyst
Immobilized lipase	Commercial lipases	easy recovery of glycerol, biodiesel yield is higher	Low catalytic activity

Table 3: Heterogenous nanocatalysts used in recent years for biodiesel production

Oil source	**Nanocatalyst**	**Biodiesel Yield**	**Reference**
Moringa oleifera oil	CuO-CaO	95.24	Niju et al., 2019
Soybean oil	CaO nanocatalyst	97.61	Bharti et al., 2019
Mahua oil	Manganese doped ZnO	97	Baskar et al., 2017
Simarouba oil	Ag-ZnO	84.5	Nagaraju et al., 2017
Canola oil	$ZnO/BiFeO_3$	95.43	Salimi and Hosseini, 2019
Non-edible oil	Cobalt doped ZnO	98.03	Borah et al., 2019
Moringa oleifera oil	MgO nanocatalyst	93.69	Esmaeili et al., 2019
Waste cooking oil	MgO-NaOH nanocatalyst	97	Rafati et al., 2019

Sunflower oil	MgO over cerium-doped MCM-41 nanocatalyst	94.3	Dehghani and Haghighi, 2019
Goat fat	MgO nanocatalyst	93.12	Rasouli and Esmaeili, 2019

Table 4: Immobilization methodology adopted for lipases in biodiesel production

Oil Source	Source of lipase	Immobilization method	Carrier	Acyl receptor	Reference
Soybean oil and waste cooking oil	*Candida antartica*	Adsorption	Acrylic resin	Methanol, propanol, methyl acetate	Du et al., 2004; Wang et al., 2007
Jatropa oil, vegetable oil	*Pseudomonas cepacia*	Adsorption	Diatomaceous earth, Celite	Ethanol, Butanol	Shah and Gupta., 2007; Salis et al., 2005
Lard, waste oil	*Candida* species	Adsorption	Textile membrane	Hydrophobic solvents	Lu et al., 2007; Tan et al., 2006
Babassu oil and Palm	*T. lanuginosus*	Covalent bond	Toyopearl-AF-amino-650M	Glycidol, glutaraldehyde	Mendes et al., 2011

oil			resin	and epichlorohydrin	
Soybean oil and triolein	*Pseudomonas cepacia*	Entrapment	Hydrophobic sol-gel support with and without Celite® R632	Methanol, ethanol	Meunier and Legge., 2010; Noureddini et al., 2005
Renewable oils	*Rhizopus oryzae* IFO 4697	Cross linking	Glutaraldehyde	Methanol	Sun et al., 2009