REVIEW: ENZYMATIC TRANESTERIFICATION OF WASTE COOKING OIL

M. V. Bagal[1*]; Mishal Shaju [2], Abhimanyu Yadav [3], Shubham Chavan [4],

[2,3,4]Undergraduate student, Department of Chemical Engineering, Bharati Vidyapeeth College of Engineering, Navi Mumbai, Maharashtra, India.

[1,5,6] Professor, Department of Chemical Engineering, Bharati Vidyapeeth College of Engineering, Navi Mumbai, Maharashtra, India.

Abstract

Conventional fuels are known for polluting air by emissions of sulfur dioxides, carbondioxides, particulate matter and other gases. Biodiesel is a renewable, biodegradable and non-toxic alternative fuel to 'fossil' diesel. Biodiesel manufacturers are focusing their attention on using low-cost feedstock such as waste cooking oil to ensure economic viability in biodiesel production. Waste cooking oil(WCO) is far less expensive than refined vegetable oils and therefore has become a promising alternative feedstock to produce biodiesel. WCO contain large amount of free fatty acid (FFA) and water. Enzymatic transesterification has drawn researcher's attention in last ten years due to the downstream processing problem such as huge amount of wastewater generation and difficulty in glycerol recovery posed by chemical transesterification in turn results in increase in the overall biodiesel production cost In contrast, enzyme catalysis proceeds without the generation of by-products, easy recovery of product, mild reaction condition, insensitive to high FFA oil and catalyst can be reused.

The present review focusses on the enzyme catalyzed biodiesel production which is eco friendly and promising alternative to the chemical process. High cost of enzyme is main disadvantage of enzymatic transesterification. So to overcome this drawback immobilization and pretreatment of enzyme is necessary. Along with that various lipase as biocatalyst and their operating conditions are discussed. With proper research focus and development, waste cooking oil can indeed become the next ideal feedstock for biodiesel production using enzyme as a catalyst.

Keywords: Biodiesel, waste cooking oil, enzymatic, transesterification.

1.Introduction

From many past years, the world has witnessed many changes in the energy field where many industries around the world created new goal based on reducing the environmental impact and achieving competitive cost of biofuel compared with fossil fuel. Biofuel has recently attracted huge attention in different countries all over the world because of its renewability, better gas emissions and its biodegradability. It is observed that biodiesel could replace approximately 10% of diesel fuel consumption within Europe and 5% of Southeast Asia's total fuel demand.[1] Recently, the petroleum prices have been setting record high in the history due to heavy dependence on petroleum as a major source of fuel for transportation and electricity generation. Both energy and environmental deterioration are serious crisis, which could possibly be reduced by adopting

alternative energy sources such as biofuels generation from renewable sources as well as the adoption of sustainable and environmental friendly methods for the generation of biodiesel.

Biodiesel extracted in more amount through vegetable oil. It have been reported that the biodiesel production in several ways: a) the effect of operating parameters b) the effect of the type of catalyst such as enzyme catalyst, heterogeneous catalyst and acid catalyst.Main obstacles in extraction of biodiesel is the raw material costs and limited availability of vegetable oil feedstock .The high cost of vegetable oil,which could be up to 75% of the total manufacturing cost has led to the production costs of biodiesel becoming approximately 1.5 times higher than that of diesel. Nevertheless, the price of waste cooking oils (WCO) is 2–3 times less than unused vegetable oils. Alternatively, the total manufacturing cost of biodiesel can be significantly reduced. Also, a similarity in the quality of biodiesel derived from WCO and from vegetable oils could be achieved at an optimum operating condition. Increasing food consumption has increased the production of a large amount of waste cooking oils, so it can be reused to produced valuable biofuel which is beneficial in environment and economic point of view. Enzyme transesterification has drawn attention due to the downstream process problem caused by chemical transesterification like acid or alkali catalyzed process. Enzyme catalysed production has proven to have high potential to be an eco-friendly process and a promising alternative to the chemical process.[1][2]

2.Waste cooking oil

Waste cooking oil is the used vegetable oil obtained from cooking food. Repeated frying for preparation of food makes the edible vegetable oil no longer viable for its intended use due to high free fatty acid (FFA) content. Waste oil has many disposal problems like water and soil pollution, human health concern and disturbance to the aquatic ecosystem, so rather than released it into environment and harming the environment ,it can be used as available feedstock to extract biodiesel. Furthermore, Animal fats with high acid value and fat-containing floating sludge released in water systems without proper pre-treatment are subject to environmental pollution due to their high pollutant potential.WCO collected can be use for various application such as soap formation and as additives for lubricants.

The properties of WCO can varies according to the frying conditions, such as temperature and cooking time. The chemical and physical properties of WCO are different from those of u oil since some changes due to chemical reactions - such as hydrolysis, oxidation, polymerization, and material transfer between food and vegetable oil occur during the frying process .It is observed that a vegetable oil subjected to thermal stress such as during frying can completely vary its chemical and physical original characteristics. The cooking process causes the vegetable oil, Triglyceride to split to form, Diglycerides, Monoglycerides, and free fatty acids (FFAs). Hydrolysis of triglycerides causes due to amount of heat and water in the frying which results in increases FFAs in WCO. Also, because of oxidation and polymerization reactions, there is an increase in the viscosity and the saponification number of the WCO when compared with the original oil .Saponification reaction occur during transesterification reaction due to presence of water in WCO sample, also high FFA content and high saponification number give rise to soap formation. Hydrolysis and saponification reactions

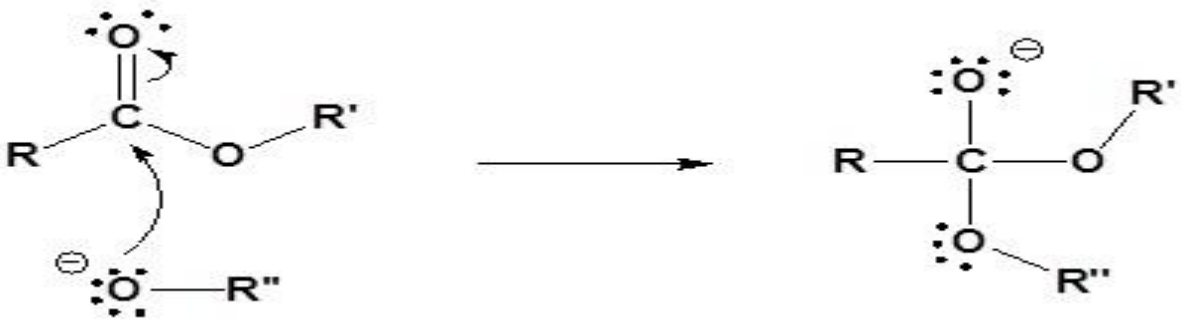
cause low biodiesel production and use of more quantity of catalyst. The knowledge about the physical–chemical properties of the many biodiesel sources available is a key issue to decide on investments for the development of crop production, processing, quality control, and engine adequacy. The properties of second used cooking oil is same as that of first time used cooking oil,only difference is second used cooking oil have high FFA content as compared to first used cooking oil which causes high cost to synthesis biodiesel and less product yield.[3]

3.Transesterification

In organic chemistry, transesterification is the process which involve an exchanging the organic group R″ of an ester with the organic group R′ of an alcohol. For biodiesel production mostly low molecular weight alcohol are preferred e.g. Methanol or ethanol. These reactions are generally catalyzed by the addition of an acid or base catalyst. The reaction can also be take place with the help of enzymes (biocatalysts) particularly lipases.

Methanol is a basic alcohol used in more amount as compared to other alcohol in transesterification of triglycerides to produce biodiesel fuel at a present of catalyst and it is known as methyl alcohol and wood alcohol and abbreviated as methanol. Methanol is light, volatile and colourless. Methanol is consider as a safe fuel. However, it is in fact flammable and burns with an invisible flame and it is biodegradable quickly when compared with petroleum fuels. The alcohols use in the transesterification reaction also act as surfactants because they are soluble in glycerol (polar phase) and in biodiesel (non-polar phase). The carbon chain of the alcohol molecule is responsible for its solubility in biodiesel, whereas the hydroxyl group exhibits affinity for the glycerol. When the interfacial tension between two liquids is reduced sufficiently less value due to presence of a surfactant ,the emulsification of their liquids occurs.[4][5]

Electrophilic can be attained by donating a proton by strong acid to the carbonyl group while base catalyse the reaction by removing a proton from the alcohol and nucleophilic occurs.



4. Enzyme transesterification –

Biodiesel can be obtained from waste cooking oil by various catalytic processes as we seen earlier among all of them enzyme transesterification has drawn attention due to the downstream processing problem caused by chemical transesterification. For the production of biodiesel fuel, an alkali -catalysis process has been

established that gives high conversion levels of oil to methyl esters, and at present this is the method that is generally employed in actual biodiesel production. However it has several drawbacks, including the difficulty of recycling of glycerol and the need for either removal of the catalyst or wastewater treatment. In particular ,several steps such as the evaporation of methanol, removal of saponified products, neutralization, concentration etc., are needed to recover glycerol as a by-product. To overcome this disadvantages which may causes limitations to availability of biodiesel fuel, enzymatic process using both extra- and intra- cellular lipase has recently been developed.

Lipase used in biotechnology are normally of microbial origin and produced by fermentation processes. s. Whilst some are employed as free powders the majority are used as immobilized preparations. Huge amount of wastewater generation and difficulty in glycerol recovery are some of the problems that eventually increases the overall production cost of biodiesel and being not environmental benign. In contrast, enzyme catalysis is occurs without the generation of by products ,easy re cover product , mild reaction condition, insensitive to high FFA oil and catalyst can be reuse. Thus, enzyme catalysed production has prove to have high potential to be an eco-friendly process and a promising alternative to chemical process. The enzymatic production of biodiesel can be performed using organic solvents (usually hexane, heptanes, or petroleum ether) or simply using the mixture of substrates (lipids and alcohol) depending on the size of the chain of alcohol. If methanol or ethanol is used, a solvent can facilitate the oil solubility in alcohol and also decrease the viscosity of the reactional mixture, but there will be an additional cost for its removal (distillation or extraction) after the reaction. Mucormiehei (Lipozyme IM 60), Pseudonyms cepacia (PS 30), C Antarctica (Novozyme 435) , Bacillus subtilis. are some examples of biocatalystLipase used in biotechnology are normally of microbial origin and produced by fermentation processes. s. Whilst some are employed as free powders the majority are used as immobilized preparations. Huge amount of wastewater generation and difficulty in glycerol recovery are some of the problems that eventually increases the overall production cost of biodiesel and being not environmental benign. In contrast, enzyme catalysis is occurs without the generation of by products ,easy re cover product , mild reaction condition, insensitive to high FFA oil and catalyst can be reuse. Thus, enzyme catalysed production has prove to have high potential to be an eco-friendly process and a promising alternative to chemical process. The enzymatic production of biodiesel can be done using organic solvents depending on the size of the chain of alcohol. If methanol or ethanol is used, a solvent can provide the oil solubility in alcohol and also reduces the viscosity of the reactional mixture, but there will be an extra cost for its removal (distillation or extraction) after the reaction. Mucormiehei (Lipozyme IM 60), Pseudonyms cepacia (PS 30), C Antarctica (Novozyme 435) , Bacillus subtilis. are some examples of biocatalyst.

4.1 Lipase as biocatalyst in biodiesel synthesis–

Lipases are enzymes that catalyze the hydrolysis of carboxylic ester link in the triacylglycerol molecule to form free fatty acids, di- and monoglycerides and glycerol. Although their natural function is to catalyze hydrolysis of ester links, they can also catalyze the esterification, the creation of this link between alcohol

hydroxyl groups and carboxyl groups of carboxylic acids. So they can catalyze hydrolysis, alcoholysis, esterification and transesterification they have a wide spectrum of biotechnological applications. Also they are highly specific as chemo-, regio- and enantio selective catalysts. From lipases of plant, animal and microbial origins, most commonly used are microbial lipases. They have many advantages over lipases from animals and plants. Using microorganisms it is possible to achieve a higher yield of enzymes, and to genetically manipulate the producing strain in obtaining a low-cost lipase with desired properties for the conversion of natural fats and oils into biodiesel. In addition, the enzymatic yield is not depended on potential seasonal variations and it is possible to achieve rapid growth of microorganism in low-cost culture media.[6][8]The benefits of used of lipases as biocatalyst are mild reaction conditions and easy recovery of glycerol without purification or chemical waste production and production of very high purity product. [9]

Source	Catalyst/Enzyme	Operating conditions	Important results	References
Waste cooking (10 mmol)	Geotrichum sp. Lipase (50 gm)	Temperature-40°**C** Reaction time-6hr Solvent- t-butyl	Yield -85%	[9]
Salad and waste oil (2 gm)	Candida sp lipase 99-125 (Immbolised lipase about 20 wt% oil)	Temperature-40°**C** Reaction time-30 hr Solvent- n-hexane	96%	[8]
Olive,corn and waste oil	Pseudomonas cepacia lipase (50 gm)	Temperature-40°**C** Reaction time-12 hr Solvent- n-hexane	99.8	[15]
Waste oil (0.5 gm)	Candida antartica B	Temperature-40°**C** Reaction time-12hr	100%	[4]
Waste oil	Penicilliumexapansum (168U immbolised)	Temperature-35°**C** Reaction time-24 hr Solvent- t-amyl	92.80%	[10]

		alcohol		
Waste oil (2.75 mmol)	Thermocyceslanuginosa	Temperature-40°**C** Reaction time-10 hr Solvent- n-hexane	70-100%	[15]
Waste cooking oil	Candida sp. 99-125 (2252.5 U immobilised)	Temperature-45°**C** Reaction time-10 hr Solvent- n-hexane	91.80%	[5]
Waste cooking oil (68.85 gm)	Bacillus subtilis	Temperature-40°**C** Reaction time-72 hr	>90%	[11]

A large number of lipases from various sources have been utilized for biodiesel production as shown in Table . Candida antarctica B lipase is the most studied enzyme for biodiesel production among various reaction systems. It catalyzed acyl transfer reactions of various oils and acyl acceptor showing more stability in organic solvents and wide substrate specificity. However, the lipase from Pseudomonas has also many benefits considering enzyme stability in a water-containing system in the presence of methanol. It has been reported that the lipase from P. cepacia has higher methanol resistance than those from Rhizopusoryzae, P. roqueferoti, C. lipolytica and even C. antarctica, made it more attractive for use as a biocatalyst in methanolysis reaction processes. In addition, commercially available lipases (Lipozyme, Novozyme) appear to be more suitable catalysts for large scale application, since the use of free Pseudomonas or other lipases requires the additional immobilization step.

4.2 Lipase immobilization –

One of the main disadvantages for industrial application of lipases is the high cost of biocatalysts. Hence, immobilization of lipases, which allows their reusability, is a necessity to make them more attractive for industrial biodiesel processes. The immobilization is used to increase lipases properties such as thermo stability and activity in non-aqueous media, and to improve handling, recovery and recycling of biocatalyst. Recycling of immobilized enzymes reduces the cost of the production, so the most promising immobilization supports and methods could make the enzymatic biodiesel production competitive to chemical processes. Different immobilization techniques of lipases used as catalysts in synthesis of biodiesel are summarized in Table .

Adsorption is the most widely used method for lipase immobilization primarily because procedure is easy, conditions are mild and the method itself is cheap . This technique consist of no toxic chemicals, supports can be regenerated for repeated use, and there is no major activity loss. Various supports have been used such as: textile membrane, alumina, ceramics, sepharose, sepadex, cellulose, hydrotalcite, different type of zeolites, celite, silica gel, polyethylene, polypropylene and others. The nature of support strongly analysed catalytic properties of the lipase such as activity, selectivity, stability. Some study explored the influence of pore diameter of non polar and weakly polar resin on degree of immobilization and reported that the degree of immobilization increases with increasing pore diameter. Also, the higher lipase activity produced employing non polar resins. It has been observed that lipase activity is generally higher with hydrophobic support. [3]

Table 4.2. Immobilization methods with their specification

Immobilization method	Source of lipase	Content for procedure	Important result	References
Adsorption	Burkholderiacepacie	Carrier used- celit Acyl acceptor – Ethanol Oil-waste cooking oil,jatropha oil	98%	[14]
Adsorption	Candida sp 99-125	Carrier used- cotton membrane Acyl acceptor – methanol Oil-waste cooking oil	92%	[8]
Adsorption	Thermocyceslanuginosus	Carrier used- Hydrotalcite& zeolite Acyl acceptor – methanol Oil-waste cooking oil	92.80	[7]
Cross linked enzyme	Burkholderiacepacie	Carrier used- none Acyl acceptor – methanol Oil-mahul oil	92%	[12]
Entrapment	Burkholderiacepacie	Carrier used- phyllosilicate sol-gel matrix Acyl acceptor – Ethanol Oil-waste oil	96%	[13]
Covalent bond	Thermocyceslanuginosus	Carrier used- novel	63.80%	[2]

		microporouspolymeric matrix Acyl acceptor – methanol		

Lipases immobilized by entrapment are more useful and stable than physically adsorbed lipase. Entrapment of lipase includes trapping of the lipase within a matrix of a polymer Encapsulation is the imprisonment, of enzyme within a porous membrane forming a bilayer .Help of this process it prevents enzyme leaching making the resulting immobilized enzyme a highly reusable biocatalyst. Encapsulation is carried out by using natural polymers, like alginate and carrageenan, synthetic polymers (photo-cross linkable resins and polyurethane polymers), acrylic polymers, hydrogels, microemulsion based gels and those produced by sol–gel methods Covalent immobilization via the carboxy group of supports is often used in immobilization of lipase on the polymer derivates of acrylic acid. It must be noted that these active derivatives enable mild covalent coupling of lipase. Polymers consist of amino groups can be activated by the addition of the diazo groups. Enzymes are then easily connected through their α- or ε-amino groups and less by sulfhydryl, hydroxyl, imidazole or carboxy groups. The immobilized lipase proved to be very important, stable and active in transesterification reaction. It gives high reaction activity in the presence of excess alcohol under the optimal conditions. This method is an excellent step in decreasing the cost of biodiesel by reducing the cost of enzyme, making it competitive with the chemical process.[3][7][15]

4.3 Pre-treatment of Lipase–

Enzyme transesterification mostly not preferred due to high cost because it cannot be reused. So in order to reduce cost we introduce lipase immobilization method. But before immobilisation enzyme should be pre treated to avoid impurities related to that.

Pre-treatments of lipase by various methods have a goal of improving enzyme activity, enzyme stability and, especially important for biodiesel synthesis, to improve methanol tolerance. Literature review explains that when immobilized enzyme is successfully pretreated enzyme activity is increased and the ability of the enzyme to resist deactivation by methanol is higher, thereby enabling multiple reuses of enzymes for industrial production. Generally, pre-treatment reagents can be classified as: 1) substrates or their analogues; 2) organic solvents; 3) salts; 4) enzyme lyoprotectants such as crown ethers. Treatments with different reagents have, more or less, the same goal, keeping the conformation of lipases in its active form, by making the conformational change of active site from closed to open form. The most commonly used method is the usage of substrates or their analogues as pre-treatment reagents. This treatment enhanced the enzyme activity and methanol tolerance in three step methanol addition and even in one step methanol addition.[11] However, there were no enhancements for both, the initial rates and the equilibrium biodiesel yields with lipase pretreated with short chain alcohols: n-propyl alcohol, n-butanol, isopropyl alcohol, t-butanol, isobutyl alcohol. The most promising results were achieved when the lipase was treated with 1mM solution of CaCl2 and MgCl2., since lipase activity, methanol tolerance and operational stability were drastically improved. It is

assumed that salts could incorporate with the protein to form a more stable molecule, which could resist conformational change induced by high methanol concentration. Lipases from different origins show to have distinct properties and one activation methods might not be versatile for other lipases. The immobilized lipase from C. antarctica was pretreated, immersed, in alcohols: isopropanol, 2-butanol, t-butanol. The activity of the commercial immobilized enzyme, Novozyme 435, increased about ten times in comparison to the enzyme not subjected to any pre-treatment and the methyl ester yield was about 7 to 10 times higher. It achieved 97% biodiesel yield in methanolysis of plant oil after 3.5 hours with preincubated Novozym 435. Methanolysis proceed much faster when Novozym 435 was preincubated in methyl oleate for 0.5h and subsequently in soybean oil for 12h. This procedure make process economically feasible[3][12]

5.Conclusion –

In recent years, biodiesel has become more attractive as an alternative fuel for diesel engines because of its environmental benefits and the fact that it is made from renewable resources. Used oils can also be utilized for making biodiesel fuel, thus helping to reduce the cost of wastewater treatment in sewerage systems and generally assisting in the recycling of resources. Biodiesel fuel production now exceeds 100,000 tonnes per year in several countries, including Belgium, France, Germany, Italy, and the United States. In Japan, small quantities of biodiesel fuel are now being produced from used oils in many localities.

For the production of biodiesel fuel, an alkali-catalysis process has been established that gives high conversion levels of oils to methyl esters, and at present this is the method that is generally employed in actual biodiesel production. However, it has several drawbacks, including the difficulty of recycling glycerol and the need for either removal of the catalyst or wastewater treatment. In particular, several steps such as the evaporation of methanol, removal of saponified products, neutralization, concentration, etc., are needed to recover glycerol as a by-product. To overcome these drawbacks, which may limit the availability of biodiesel fuel, enzymatic processes using both extracellular and intracellular lipases have recently been developed. Incomparative tabular column for biodiesel fuel production by the alkali- and a lipase-catalysis processes are presented. The latter process is much easy therefore recovery of unreacted methanol and wastewater treatment are unnecessary. In addition, only a simple concentration is required to recover glycerol.

It has been shown that enzymatic transesterification can be carried out successfully with variety of lipases with higher yields using a large variety of oil, fats and acyl acceptors. Higher FFA and water content of substrate can be catalyzed with complete conversion to alkyl esters with significantly condensed amount of wastewater. The stability of losses has been further increased by pre-treatment of loaded, selection of adequate immobilization procedure or use of whole cell biocatalyst, leading to prolonged activity of biocatalyst.

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