A Short Review on Enzyme Catalyzed Transesterification
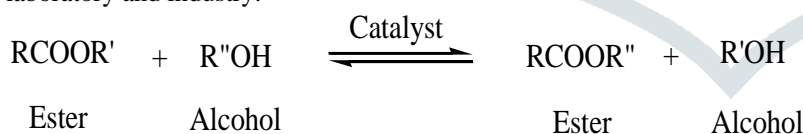
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Abstract : Transesterification is a chemical process that is used to convert esters into other esters and to produce biodiesel. It has become increasingly important to find alternative, clean, and renewable energy sources due to concerns about environmental degradation and the limited nature of fossil fuels. Biodiesel, also known as fatty acid methyl ester (FAME), is a biodegradable and renewable energy source that is produced through transesterification. This process involves mixing triglycerides from animal fats and vegetable oils with alcohol in the presence of a catalyst, which can be homogeneous or heterogeneous. Originally, transesterification was used to separate glycerin from soap. The resulting monoalkyl esters make up biodiesel. Biodiesel can be made from raw materials such as vegetable oils, animal fats, and waste products, which often have high water and free fatty acid (FFA) content. This can affect the performance of traditional homogeneous base catalysts. There have been several catalysts developed for transesterification, but research is ongoing to find more efficient options. The aim of this review is to discuss the role of enzyme catalysts in transesterification. Even though currently enzyme-catalyzed reaction is not the first choice for biodiesel production industry, it has a big potential to become one. An important task is to design a good enzymatic reaction with low operational cost but with an optimum amount of biodiesel yield. High yield of biodiesel can be obtained by controlling the reaction conditions, manipulating different factors affecting the reaction.

IndexTerms - **Transesterification, Biodiesel, enzyme, free fatty acid**

INTRODUCTION

Transesterification is the general term used to describe the important class of organic reactions where an ester is transformed into another through the interchange of the alkoxy moiety.[1,2] Transesterification is one of the classic organic reactions that have enjoyed numerous applications in academic laboratories as well as in industries.[3,4] It, also known as alcoholysis, is the displacement of alcohol from an ester by another alcohol in a process similar to hydrolysis, except that an alcohol is employed instead of water (Scheme 1). Since the reaction is an equilibrium process, the reaction occurs by essentially mixing the reactants. However, it gets accelerated in presence of a catalyst. It is a reversible reaction and alcohol has to be used in excess to achieve high yield of ester. Transesterification of esters with alcohols plays important role in the production of organic esters, especially for some important products and intermediates in the laboratory and industry.[5]

$$\text{RCOOR'} + \text{R''OH} \underset{}{\overset{\text{Catalyst}}{\rightleftharpoons}} \text{RCOOR''} + \text{R'OH}$$

Ester   Alcohol   Ester   Alcohol

Scheme 1: General equation of transesterification

Transesterification is a crucial step in several industrial processes such as (i) intramolecular transesterifications leading to lactones and macrocycles, (ii) biodiesel from vegetable oils, and (iii) co-synthesis of dimethyl carbonate and ethylene glycol (in polyester manufacturing) (iv) polyethylene terephthalate (PET) from dimethyl terephthalate (DMT) and ethylene glycol (in polyester manufacturing).[1,6,7] With increasing public concern over environmental degradation and future fossil fuel resources, it is increasingly necessary to develop alternative clean and renewable energy sources. The necessity to find alternatives to petroleum products and energy sources with low cost and with a reduced environmental impact is a reality that has been considered for several years. Fatty acid alkyl ester (FAME), which is called biodiesel fuel, is a well-known biodegradable and renewable energy source produced by transesterification.[8] This process has widest application in the production of biodiesel. Biodiesel is an oxygenated fuel that is produced by transesterification of triglycerides derived from animal fats and vegetable oils with alcohol in the presence of a homogeneous or heterogeneous catalyst. It has been used since mid-1800s.This method was originally used to distill out glycerin to make soap. The bye-products of this process are monoalkyl esters which are the constituents of biodiesel.[9]

Biodiesel fuel or fatty acid methyl ester (FAME) from vegetable oil, which primarily contains triglycerides and free fatty acids, is considered to be the best substitute for diesel fuel. It can be used in diesel engines and is used in neat (100% biodiesel) or can be blended with petroleum diesel.[10] It is an attractive and useful alternative to petroleum diesel fuel due to the following advantages[10]:

(i)   It is a renewable resource,
(ii)   It is biodegradable,
(iii)   It has a lower combustion emission profile (especially SOx),

(iv)   Reduced greenhouse gas emissions because of the closed CO_2 cycle,

(v)   It exhibits low toxicity,

(vi)   It can be used without engine modifications.

Transesterification is more advantageous than the esterification reaction of carboxylic acid and alcohols. For instance, some carboxylic acids are sparingly soluble in organic solvents. Common synthetic routes to esters include condensation reactions of carboxylic acids with alcohols or acylating alcohols with highly reactive acylating reagents such as acyl halides and acid anhydrides.[11] These reactions can be made spontaneous by using Dean-Stark apparatus or high temperature conditions.[3] On the other hand esters are soluble in most organic solvents. Transesterification is useful when the parent carboxylic acids are labile and difficult to isolate.[7] It is more advantageous than esterification due to handling ease and high stability of esters as well as their high solubility in most organic solvents.[7,12]

Transesterification is an equilibrium reaction and it is difficult to attain high conversions. The following methods have been used to force the reaction toward the product side: (i) use of excess amounts of either of the reactants (ii) use of an enol ester as a reactant, accompanied by the formation of the corresponding aldehyde or ketone and (iii) removal of the resulting lower alcohol by molecular sieves or continuous distillation. The last approach is the most ideal method, and several catalytic transesterifications at high temperature using esters of lower alcohols were developed using this approach.[11]

1. Biodiesel synthesis by transesterification

Transesterification of vegetable oil is a sequence of three consecutive and reversible reactions, in which di and monoglycerides are formed as intermediates leading to the product fatty acid alkyl ester. The stoichiometric reaction requires 1 mol of a triglyceride and 3 mol of the alcohol. However, an excess of alcohol is used to increase the yields of the alkyl esters.[1]
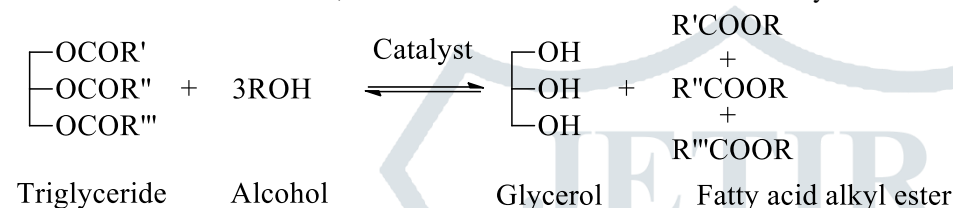
Scheme 2: General equation for transesterification of a triglyceride

Generally, alcohols used in the transesterification are methanol, ethanol, propanol, butanol and amyl alcohol. Methanol and ethanol are utilized most frequently, especially methanol because of its low cost and its physical and chemical advantages. This process has been widely used to reduce the high viscosity of triglycerides,[13] thereby enhancing the physical properties of renewable fuels to improve engine performance.[14] The first step is the conversion of triglycerides to diglycerides, followed by the conversion of diglycerides to monoglycerides, and of monoglycerides to glycerol, yielding one alkyl ester molecule per mole of glyceride at each step.

Scheme 3: The transesterification reactions of vegetable oil with alcohol to esters and glycerol

2. Catalysts used in transesterification

Transesterification reactions are catalyzed by acids, bases and biocatalysts. Classification of the catalysts are given in the flow chart.
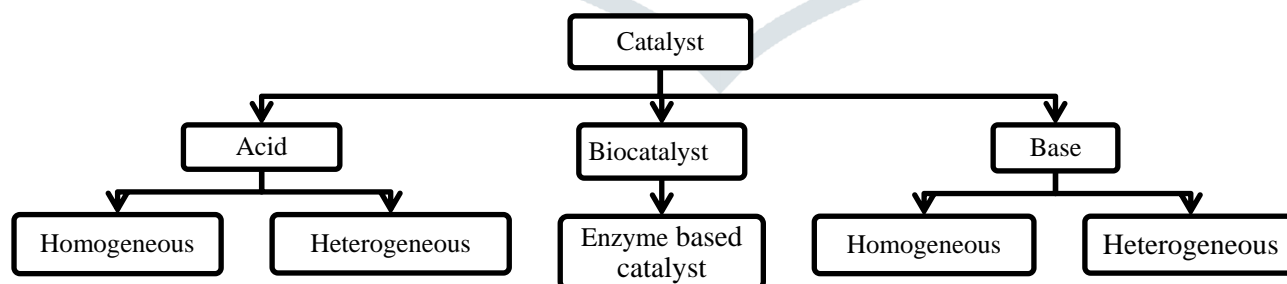
All the three types of catalysts have been attempted and reported. Acid catalyzed reactions are slower than the base catalyzed reaction.[15] According to an acid catalyzed mechanism for esterification, carboxylic acid can be readily formed by hydrolysis of the carbocation intermediate formed upon protonation of the ester. This suggests that acid catalyzed transesterification should be carried out in the absence of the water to avoid the competitive formation of carboxylic acids and concomitant reduction in the yields of alkyl ester.[16] The most common solid base catalysts are more effective than acid catalysts and enzyme catalyst. The solid base catalyst system eliminates corrosion problems and unwanted waste product formation. Heterogeneous catalysts are always advantageous over homogeneous ones. Homogeneous catalysts act in the same liquid phase as the reaction mixture, whereas heterogeneous catalysts act in a different phase from the reaction mixture, usually as a solid. Heterogeneous catalysts can be easily separated from the reaction mixture and thus they can be recovered and reused. Due to their long lifespan, they are economic too. In general, the heterogeneous catalyzed transesterification processes have less number of unit operations, with simple product separation and purification steps and no neutralization process is required.[17] It decreases the cost of separation of the catalyst. The effectiveness of the heterogeneous catalytic

conversion depends on the activity of the solid catalyst used. Homogeneous catalyzed transesterification downstream processing disadvantages have motivated intense research on heterogeneous catalyzed transesterification methods.

There are several techniques investigated for the transesterification - transesterification via radio frequency microwave, alcohol reflux temperature, alcohol supercritical temperature and ultrasonication.[17]

3. Enzyme catalyzed transesterification

Enzyme catalysis is another growing area of research in the field of catalytic transesterification. Enzymes are well-known as nature's catalyst, performing various biological processes about which we can just wonder and that are difficult to study in laboratory. However, in past few decades, numerous efforts have been made to mimic various biochemical reactions, such as hydrolysis, esterification, transesterification, amidation, oxidation-reduction reaction, methyl like group transfer reaction, in the laboratory using enzymes as a biocatalyst for synthesis of industrially important compounds.[18]

Due to their ready availability and the ease with which they can be handled, hydrolytic enzymes have been widely applied in organic synthesis. They do not require any coenzymes, are reasonably stable, and often tolerate organic solvents. Their potential for regioselective and especially for enantioselective synthesis makes them valuable tools. The common aspects of these studies consist in optimizing the reaction conditions (solvent, temperature, pH, type of microorganism which generates the enzyme, etc.) in order to establish suitable characteristics for an industrial application.

Ultrasound assisted enzyme catalyzed transesterification of waste cooking oil with dimethyl carbonate in solvent free system by immobilized enzyme (Novozym 435) was demonstrated by Gharat et al.[19] As compared with the conventional stirring method, where FAME conversion was 38.69% at 4 h, the Ultrasonic Irradiation without stirring significantly increases the conversion of enzymatic transesterification to 57.68% in the same reaction time. However, the reaction rate was further increased under the condition of ultrasonication coupled with stirring and resulted into higher conversion of 86.61% for the same reaction time.
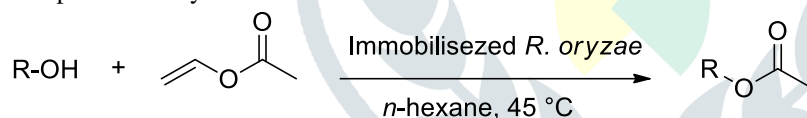
Lipase-catalyzed transesterification of vinyl esters having aromatic ring in acyl moiety with 2-phenyl-l-propanol have been studied and investigated by Masashi Kawasaki and his co-workers.[20]

The use of biocatalysts in transesterification provides an opportunity for carrying out reactions under milder conditions leading to better quality products suitable in fragrance and flavor industry. Yadav et al.[21] studied transesterification of vinyl acetate with n-octanol at 30 °C as a model reaction in presence of different lipases as catalysts such as Pseudomonas species lipase immobilized on diatomite, free Candida rugosa lipase, Novozym 435 (lipase B from Candida antarctica; immobilized on macro-porous polyacrylic resin beads) and Lipozyme IM 20 (Mucor miehei lipase immobilized on anionic resin). Novozym 435 was found to be the most active catalyst in heptane as a solvent. A conversion of 82% with 100% selectivity of n-octyl acetate was obtained at 30 °C in 90 min using equimolar quantities of the reactants with 0.833 g L^{-1} of Novozym 435. The order of transesterification reactivity of vinyl acetate with various alcohols in presence of Novozym 435 under identical conditions at 30 °C was found to be as follows:

n-octanol > n-decanol > benzyl alcohol > cinnamyl alcohol > 2-ethyl-1-hexanol > 2-phenyl ethyl alcohol > 1-phenyl ethyl alcohol.

Cheng Li et al.[22] synthesized valuable methionol-derived esters from the readily available natural material butter oil as the fatty acid source through Lipozyme TL IM-mediated transesterification. The long fatty acid methionyl esters with high odour thresholds can provide long-lasting flavouring in food products.

Hydroxypropyl methyl cellulose (HPMC) and polyvinyl alcohol (PVA) film immobilized Rhizopus oryzae lipase was effectively applied by Dhake et al.[18] to various alcohols for synthesis of industrially important acetates providing good to excellent yields of desired products. Catalytic activity of immobilized lipase is found to be 4-fold greater than that of free lipase for transesterification reaction. The immobilized biocatalyst was effectively recycled for four consecutive cycles and exhibited remarkable stability for a period 90 days.

R = Alkyl, aryl

Scheme 4: Immobilized *Rhizopus oryzae* lipase catalyzed transesterification

Hsu et al.[23] studied the optimization of alkyl ester production from grease using a phyllosilicate sol–gel immobilized lipase. According to the studies, it was concluded that the immobilized lipase was active from 40 to 70 °C. Ester contents of 60–97% were highest when using a ratio of reactants of 2 mmol grease to 8 mmol alcohol and the biocatalyst was 10% (w/w) in the presence of a molecular sieve.

Watanabe et al.[24] studied the enzymatic transesterification of waste edible oil in a fixed-bed bioreactor. Three-step methanoloysis and one step methanoloysis of waste oil were conducted using Candida antarctica lipase. 90% conversion was achieved in both the cases. The immobilized biocatalyst could be used for 100 d in the two reaction systems without significant decrease in its activity.

Shimada et al.[25] found that Candida antarctica lipase can be deactivated by shaking it in a mixture containing more than 1.5 M eq. of methanol to oil. Above this concentration, methanol is partially present as small droplets in the oil phase. These droplets are believed to cause enzyme deactivation. Therefore, methanol was added stepwise; after the addition of the third methanol equivalent, conversion to methyl esters was almost complete. The enzyme could be reused 50 times without loss of activity and its catalytic activity is not affected by the occurrence of free fatty acids.

Lipase from Pseudomonas cepacia was used as a catalyst for converting the oil from Madhuca indica into ethyl esters by Kumari et al.[26] The lipase immobilized on accurel gave 96% conversion in 6 h. The best results were obtained using modified biocatalyst formulations, which are called cross-linked enzyme aggregates (CLEAs) and protein-coated microcrystals (PCMCs). While free enzyme powder after process optimization gave 98% conversion in 6 h using 50 mg of lipase, CLEAs gave 92% conversion in 2.5 h (using an equivalent of 50 mg of enzyme) and PCMCs gave 99% conversion in 2.5 h using the same amount of enzyme.

Nelson et al.[27] also screened the potential of Lipases for their ability to transesterify triglycerides with short-chain alcohols to alkyl esters. The lipase from Mucor miehei was most efficient for converting triglycerides to their alkyl esters with primary alcohols, whereas the lipase from Candida antarctica was most efficient for transesterifying triglycerides with secondary alcohols to give branched alkyl esters. However, the efficiency of the P. cepacia (PS 30) enzyme in catalyzing the transesterification reaction was low,

even when solvent was introduced into the reaction mixture. Biodiesel yield obtained was merely 13.9–28.8% for primary alcohols (methanol, ethanol and isobutanol) and 44.1% for secondary alcohol (isopropanol). The reaction conditions reported are; reaction temperature at 45 °C, stirring speed of 200 rpm, 5 h reaction time, 0.34 molar of triglyceride in hexane, methanol to oil molar ratio of 3:1 and 12.5–25% enzyme by weight of tallow.[27,30] Screening reactions for transesterification of tallow with secondary alcohols showed a completely different trend, in which the lipases from *C. antarctica* and *P. cepacia* gave higher ester conversions than *Lipozyme* IM 60.

Chen et al.[28] investigated the enzymatic conversion of waste cooking oil using immobilized *R. oryzae lipase*. A three-step batch transesterification reactor was used and a stepwise process was introduced in the reactor to reduce the poisoning of enzyme by methanol. The optimum reaction condition was reported at 40 °C, methanol to oil molar ratio of 4, immobilized lipase to oils weight ratio of 30%, pressure of 1 atm and reaction time of 30 h. Biodiesel yield in the range of 88–90% can be obtained under these conditions.

Ying et al.[29] was the first group of researchers that used *Bacillus subtilis* for transesterification of waste cooking oil to biodiesel. *B. subtilis* was initially encapsulated within the net of hydrophobic carrier with magnetic particles (Fe_3O_4), and then the secreted lipase can be conjugated with carboxyl at the magnetic polymicrosphere surface. This magnetic cell biocatalyst (MCB) was claimed to have better dispersion during transesterification and can easily be separated from the reaction mixture by subjecting to an external magnetic field. From the study, it was found that biodiesel yield could reach up to 90% at reaction temperature 40 °C, pH 6.5, loading of 3.0% MCB, adding methanol in two stepwise and 72 h reaction time. Furthermore, MCB can be easily regenerated without losing its enzymatic activity.

J.Sebastian et al.[31] studied the enzyme catalysed transesterification of rubber oil. Unrefined rubber seed oil contains high levels of free fatty acids and moisture, and hence the efficiency of conventional chemical catalysts are very less for transesterification. In this case, enzyme catalyzed triggered transesterification is well suited for biodiesel production from rubber seed oil as the enzymes are insensitive to the free fatty acids. It was found that Thermomysis Lanugonosus Lipase was suitable for the transesterification of rubber seed oil with a biodiesel conversion of 92.83% at a molar ratio of 4% and 5% (w/v) enzyme concentration in solvent free reaction medium.

J. Bauwelinck et al.[32] studied enzymatic transesterification of methyl acrylate and with several industrially interesting alcohol substrates varied from linear molecules, such as 1-octanol, to bulky and sterically hindered molecules, such as 2-hexyl-1-decanol. Highly specific alkyl acrylates were produced by this method, but the reaction kinetics is greatly influenced by the structure of the alcohol as well as of the methyl ester substrate. Reaction Conditions were optimized for the reaction of methyl acrylate and octanol to octyl acrylate using Novozym 435 as enzyme catalyst.

H. Wan et al.[33] studied the efficacy of *Candida antarctica* lipase B (CALB). The enzyme was immobilized in a commercial resin "NKA" by a hydrophobic interaction, and the immobilized enzyme was PEGylated by a series reaction. The authors reported that the catalytic efficiency of NKA-CALB-PEGylated (80.1%) in the transesterification reaction system is slightly higher than those of NKA-CALB (71.7%) and Novozyme435 (69.3%), and significantly higher than that of Sol gel-CALB (60%) published in their previous literature. Additionally, NKA-CALB-PEGylated can be reused and retained an initial activity of 92.4% after repeated use six times.

Jambulingam et al.[34,35] studied synthesis of biodiesel using immobilized lipase functionalized magnetic nanocatalyst from the lipid of oleaginous fungal. Different microbes were isolated from the oil-contaminated soil and tannery effluent and investigated for high lipid content. The fungal strain SF2 was identified as *Aspergillus niger* and was found to have the highest lipid content among all of the isolates, with a reported 44.02% of lipid being produced. The lipase enzyme was immobilized onto Fe_3O_4 magnetic nanoparticles, which were synthesized through the co-precipitation method. TEM analysis showed that the particle size was 20±5 nm. The stability of both free and immobilized enzymes was tested under various conditions, and it was found that the immobilized enzymes displayed better stability, being able to be stored for 60 days and reused up to 5 cycles without significant loss of activity. Using the lipase immobilized on the magnetic nanoparticle, biodiesel was produced, and the immobilized catalyst was found to be reusable for 5 cycles. The properties of the biodiesel were within the limits specified by the ASTM standard.

4 Conclusion

From the survey of literature, we have come to know about different types of enzyme catalysts for transesterification. Most of the catalysts reported till now are efficient and some of them are reusable. Immobilized lipases possess high methanol tolerance with good catalytic efficiency and good protection against harsh conditions. They can maintain their efficiency even after their several uses. Highly efficient enzyme catalysed transesterification reactions can be obtained by controlling and manipulating the reaction conditions. Enzymatic reaction still needs to be modified for biodiesel synthesis compared to chemical catalysts, and this is due to the higher cost of enzyme catalysts, lower reaction rate, and enzyme inhibition. Immobilized lipases possess high methanol tolerance, high catalytic efficiency, and good protection against harsh conditions and can keep their efficiency even after their many uses. Highly efficient enzyme catalysed transesterification reactions are obtained by controlling and manipulating the reaction conditions.

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