INTERESTERIFICATION OF CRUDE PALM OIL BY *THERMOMYCES LANUGINOSA*: A MINI REVIEW

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ABSTRACT: This paper reviews the interesterification of crude palm oil by *Thermomyces Lanuginosa*. The advantages of supercritical and enzymatic interesterification and its disadvantages were also reviewed. Interesterification also has several advantages such as producing the highest biodiesel production yield. The reaction for the synthesis of biodiesel is based on the use of methyl acetate instead of the more commonly used alcohol. However, this method also has its disadvantages such as operations at very high pressures (20–40 MPa), high temperatures (350–400°C), and higher production costs. The performance of Lipozyme TL-IM for transesterification reaction was greatly improved by the ultrasound-assisted approach with a higher yield of biodiesel and a significant reduction of time of reaction from 24 to 4 h.

KEYWORDS: *Interesterification; Immobilized Lipase; Biodiesel; Ultrasonic; TLIM*

1. INTRODUCTION

This may be accomplished using a catalyst and an interesterification reaction between vegetable oil and methyl acetate as an alkyl group supply. Instead of glycerol, biodiesel, and a by-product in the form of triacetin are produced when methyl acetate replaces methanol Because of its mutually soluble nature, triacetin has a higher economic value than glycerol and may be used in biodiesel [1]. Other than that, there is no need for a by-product separation procedure because the interesterification process produces biodiesel with triacetin as a by-product, which may be utilized as a fuel additive. In addition, as compared to other methods, this interesterification process has a faster reaction time.

Triglycerides are transformed into methyl esters and triacetin during interesterification with methyl acetate. Interesterification is made up of three reversible processes in a row. Each stage releases a molecule of fatty acid methyl ester as the triglycerides are transformed into monoacetindiglycerides, diacetinmonoglycerides, and triacetin. Unlike transesterification, interesterification involves one ester exchanging its alcohol with another ester group. Because alcohol was not used as a reactive, the catalyst was only partially soluble in the reaction mixture [2].

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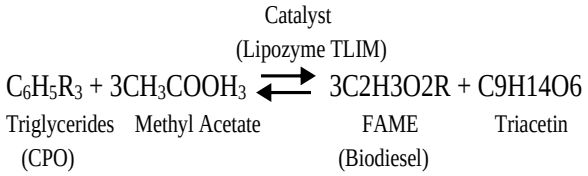

Fig. 1: Interesterification of Triglycerides with Methyl Acetate

The lipase of TLIM was used as the enzyme catalyst in this research. Lipase of TLIM was used because it has the shortest generation time and is able to produce a high yield of conversion of substrate into the product, great versatility to environmental conditions, simplicity in genetic manipulation, and cultivation conditions [4]. Lipase of TLIM is also renewable which is good for nature.

1.1 Enzymatic Interesterification

Some physical qualities of fats and oils, such as flexibility and consistency, have been modified through interesterification ("Modification of Fats and Oils," 1998). Temperature, catalyst ratio, particle size, and agitation strength all affect interesterification, which is an ester-ester exchange process [7]. To get around the glycerol surplus and the separation procedure, another method of producing biodiesel without glycerol is adopted. This may be accomplished using a catalyst and an interesterification reaction (non-alcoholic method) between vegetable oil and methyl acetate as an alkyl group supply [1]. Instead of glycerol, biodiesel and a by-product in the form of triacetin will be produced if methyl acetate is used to substitute methanol [8]. Transesterification catalyzed by lipase occurs in two steps: hydrolysis of the ester link and esterification with the second substrate [9].

Interesterification also has several advantages which are visualized in Table 1. Interesterification also has been mostly studied in the presence of enzymes or under supercritical conditions [10]–[12]. This process receives more attention as a high-efficiency, environmentally-friendly lipid modification method, lipase's high reaction efficiency, flexible reaction, and fewer by-products [13]. Table 1 summarizes the disadvantages of supercritical and enzymatic interesterification. The disadvantages include high temperature (350–400 °C) and pressure (20–40 MPa) operation, and higher heating and cooling costs which indirectly increase the production costs.

Table 1 also highlights that there is a need to create long-term process intensification technology for biodiesel synthesis from crude palm oil sources based on interesterification to lower processing costs [5], and interesterification has several benefits, however, there have been few articles published on this enzyme-based reaction pathway while using ethyl acetate as acyl acceptor [14].

The use of ultrasound can result in a significant degree of process intensification due to the cavitation phenomena. At moderate reaction conditions in terms of temperature and pressure, cavitation effects can promote mass transfer, resulting in a quicker reaction rate, greater product yield, and perhaps a lower acyl acceptor to oil molar ratio and catalyst

loading [14]. At the microscale, cavitation causes severe turbulence and liquid circulation, which aids in the reduction of mass transfer resistances in heterogeneous systems [14].

Table 1: Disadvantages of Supercritical and Enzymatic Interesterification and Advantages of Interesterification

Advantages of interesterification	**Disadvantages of supercritical and enzymatic interesterification method.**
The highest biodiesel production yield of 63.3% was achieved by using ethyl acetate to oil molar ratio of 6:1 with 8% of the immobilized lipase Novozym 435 [14].	Operations at very high pressures (20–40 MPa) [15]
The interesterification reaction for the synthesis of biodiesel is based on the use of methyl acetate instead of the more commonly used alcohol [16].	Requirements for high temperatures (350–400°C) [15]
A maximum biodiesel yield of 92.34% was obtained by using 1.5 g of the enzyme, a 1:12 molar ratio of oil to methyl acetate, and a temperature of 35 °C in 60 h of reaction time [14].	Difficulties producing at larger scales due to the necessity for meticulous control of reaction conditions and the reaction's intrinsic slowness.[5]
	High oil, and methyl acetate ratios, which are typically set at 1:42 [15],
	The supercritical method entails higher costs for the evaporation of unreacted methyl acetate [5]
	Higher production costs [17]
	Resulting in much higher heating and cooling costs [15]

1.2 Immobilized Lipase

The utilization of lipases as catalysts for fatty acid conversions has received a lot of attention in recent years [18]. Lipases are mostly used to change the fatty acid contents of triglycerides by interesterification. Lipases are hydrolytic enzymes that can be used in various industrial applications for alcoholysis, acidolysis, aminolysis, and hydrolysis reactions. Biodiesel production is one of the applications that use lipase. Lipase-catalyzed biodiesel production was reported first by [19]. Microorganisms are favored for industrial enzyme synthesis because they have the quickest generation time [4]. Other benefits of microbes include high yields of substrate conversion to product, significant environmental adaptability, and ease of genetic modification and culture [4]. *Aspergillus niger*, *Candida antarctica*, *Candida rugosa*, *Chromobacterium viscosum*, *Mucor miehei*, *Pseudomonas cepacia*, *Pseudomonas fluorescens*, *Photobacterium lipolytic*, *Rhizopus oryzae*, *Streptomyces* sp., and *Thermomyces lanuginose* are the most often utilized bacterial and fungal lipase [4]. *Streptomyces* sp. was recently researched as a powerful lipase-generating microbe for biodiesel synthesis and proved to be useful in this sector [20]. Immobilized *Thermomyces lanuginosa* on silica gel (Lipozyme TL IM). Under the right circumstances, the application of sn-1,3-specific lipases can result in a biodiesel yield of more than 90% [21].

Enzyme immobilization was initially described in 1971 at the Enzyme Engineering Conference and has since gained widespread interest [4]. Immobilized lipases provide several advantages throughout the biodiesel manufacturing process, including quicker recovery and reuse, increased tolerance, improved pH and heat stability, and so on [22]. By altering the structure of the protein, the immobilized lipase may increase the activity and stability of the lipase when compared to free enzymes [22]. Chemical, physical, and gene expression strategies have all been developed in the last decade to produce more cost-effective, active, selective, and stable lipases [4]. The term "immobilization" refers to the process of connecting an enzyme to an insoluble solid support material [4]. By immobilization, more operational and temperature-stable lipases can be obtained, and lipases can be reused in the reactions. In addition, the reusability of lipases will be a possible solution to the high cost of the enzymes and make them suitable for applications on an industrial scale [4]. Comparative investigations indicated that the following immobilization onto different supports, the same lipase molecule might have substantially varied catalytic activity [4].

Lipases catalyze triglycerides, including oils and fats with significant levels of free fatty acids, under moderate circumstances and with a range of substrates. Positional-specific lipase, fatty acid-specific lipases, and lipases specific for certain classes of acyl glycerides are the three types of lipases classified by specificity or selectivity [2]. Lipases conduct their catalytic activity under mild conditions and with a variety of substrates triglycerides, including oils and fats with high values of free fatty acids [2]. The optimum temperature determined for various lipases ranges from 30 to 55 °C [23], a compromise between the lipase's operational stability and the transesterification reaction rate. However, due to the expensive cost of separation, purification, and immobilization on a carrier, as well as the limited stability of lipase in methanol, the enzymatic generation of biodiesel has yet to be commercialized [24], [25]. We have focused whole-cell biocatalysts for biodiesel synthesis on microorganisms that have lipase intracellularly or in their cell wall to address this difficulty [16].

1.3 Entrapment Immobilized lipase

The capture of enzymes within a polymeric network or microcapsules of polymers that allows the substrate and products to pass through but retains the enzyme is referred to as entrapment immobilization. Lipase proteins are not attached to the polymeric matrix or capsule after entrapment, but their diffusion is restricted. Entrapment-mobilized lipases are more stable than physically adsorbed lipases. Entrapment immobilization is less difficult to perform than covalent bonding, and lipase activity is preserved. When entrapped lipases are used to produce biodiesel, the conversion rate is relatively low. Furthermore, the entrapped lipases have low stability [26].

Entrapment is the method of cell immobilization that has received the most attention. It works by enclosing cells within a rigid network. The entrapment itself is tight enough to prevent cell release while still allowing substrate and product diffusion. It is one of the least disruptive methods of cell immobilization because it is less likely to cause direct changes in the cell's functions. It is widely used in biotransformation and fermentation processes that generate antibiotics, organic acids, enzymes, and alcohol [26].

1.4 Lipozyme TLIM

Lipozyme TLIM is a good catalyst under anhydrous conditions. This result suggests that biodiesel production using the Lipozyme TLIM enzyme might be carried out under no water

content [27]. Lipozyme TL IM from *Thermomyces Lanuginosa* has been used for the acidolysis of corn oil with CA in n-hexane; under optimized conditions, 21.5% mol of CA was incorporated [28].

Caballero et al., 2014, reported that there is no MAG was observed in Lipozyme TL IM. The explanation for this result is related to both the reaction time (24 h), which allowed the conversion of MAG to DAG and TAG, and the lipase mechanism. Thus, if the interesterification mechanism is sequential, the second nucleophilic substitution (sn-1or sn-3positions) would occur just after the first one has been substituted with CA, corresponding to an ordered mechanism, which is supported by Itabaiana and co-workers.

Mohd Hassim et al., 2018, reported that cocoa butter equivalent having 85% 1,3-distearoyl-2-oleoyl glycerol (SOS) can be produced through enzymatic interesterification by acidolysis reaction. The oil blend for the reaction's substrate includes a combination of two or more oils from various oils and fats sources such as palm oil, palm stearin, palm kernel oil, and palm kernel olein. The sn-1,3 regiospecific *Thermomyces lanuginose* lipase (Lipozyme TLIM) was used as the catalyst. The ratio for the substrate oil and acyl donor (stearic acid) was between 1:2 to 1:6 with water content below 0.02% and a reaction time of between 40°C-50°C. The cocoa butter equivalent has solid fat content (SFC) of between 40%-45% SFC at 20°C and 0%-0.5% at 35°C.

Subhedar et al., 2015, reported that the performance of Lipozyme TL-IM for transesterification reaction was greatly improved by the ultrasound-assisted approach as compared to the conventional enzymatic transesterification. The ultrasonic-assisted reaction resulted in a higher yield of biodiesel in significantly less time compared to the conventional method. The time of reaction was reduced from 24 to 4 h due to the use of ultrasonic irradiation. Under the optimum conditions (oil to methanol molar ratio of 1:3, 3% Lipozyme TL IM, 40 °C, 120W power at 40 kHz frequency), a 96% yield of biodiesel could be achieved.

2.0 Conclusion

The use of biocatalysts such as Lipozyme TLIM shows that biodiesel can be produced effectively through the process of interesterification. The performance of Lipozyme TL-IM for transesterification reaction was greatly improved by the ultrasound-assisted approach. Overall, the significant decrease in the reaction time and enzyme loading with enhanced effectiveness are the most attractive features of ultrasound-assisted enzymatic transesterification reactions.

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