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# Ultrasound assisted intensification of biodiesel production using enzymatic interesterification

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ABSTRACT

Ultrasound assisted intensification of synthesis of biodiesel from waste cooking oil using methyl acetate and immobilized lipase obtained from *Thermomyces lanuginosus* (Lipozyme TLIM) as a catalyst has been investigated in the present work. The reaction has also been investigated using the conventional approach based on stirring so as to establish the beneficial effects obtained due to the use of ultrasound. Effect of operating conditions such as reactant molar ratio (oil and methyl acetate), temperature and enzyme loading on the yield of biodiesel has been investigated. Optimum conditions for the conventional approach (without ultrasound) were established as reactant molar ratio of 1:12 (oil:methyl acetate), enzyme loading of 6% (w/v), temperature of 40 °C and reaction time of 24 h and under these conditions, 90.1% biodiesel yield was obtained. The optimum conditions for the ultrasound assisted approach were oil to methyl acetate molar ratio of 1:9, enzyme loading of 3% (w/v), and reaction time of 3 h and the biodiesel yield obtained under these conditions was 96.1%. Use of ultrasound resulted in significant reduction in the reaction time with higher yields and lower requirement of the enzyme loading. The obtained results have clearly established that ultrasound assisted interesterification was a fast and efficient approach for biodiesel production giving significant benefits, which can help in reducing the costs of production. Reusability studies for the enzyme were also performed but it was observed that reuse of the catalyst under the optimum experimental condition resulted in reduced enzyme activity and biodiesel yield.



## 1. Introduction

Biodiesel offers a substitute to the petroleum based fuels offering greener processing and reduced emissions. Biodiesel (monoalkyl fatty acid methyl esters), which is generally produced by the transesterification reaction of triglycerides with methanol, has become significantly important in recent years due to the diminishing petroleum reserves, strong dependence of the national economies on the fuel prices and the ecological concerns of released gases from petroleum-based fuel [1]. Even though biodiesel has been generally produced using the chemical synthesis approach, there are numerous problems associated with this approach such as recovery of glycerol, removal of inorganic salts and significant processing costs especially with the separation of excess methanol being used in the synthesis [2,3].

Enzyme catalyzed approach for production of biodiesel partially addresses these issues and offers an environmentally more attractive alternative to the chemical synthesis routes [4–6]. The enzyme catalyzed biodiesel production under moderate environments allows the easy removal of the biocatalyst and glycerol recovery by centrifugation resulting in achieving biodiesel production with high purity in simple steps [7]. Typically short-chain alcohols such as methanol are used as the acyl acceptor for biodiesel production, however, usage of methanol in excess leads to deactivation of enzyme, and glycerol which is a major by-product of the reaction, also has inhibitory effects on the enzymatic activity apart from offering low commercial value. These associated issues with the enzymatic route have offered considerable restrictions for the effective application at industrial scale [8]. Replacing the alcohol as alkyl acceptor by alkyl acetate helps to solve these problems and the reaction is described as interesterification.

The present work deals with production of biodiesel using the interesterification reaction based on methyl acetate as an acyl acceptor instead of methanol and the by-product formed in the reaction is triacetin instead of glycerol (Scheme 1). Triacetin has higher commercial value as compared to glycerol due to the use as a gelatinizing agent as well as additives in tobacco, pharmaceutical and cosmetic industries [9]. Moreover, triacetin has no adverse effect on the activity of lipase enzyme [10]. Though

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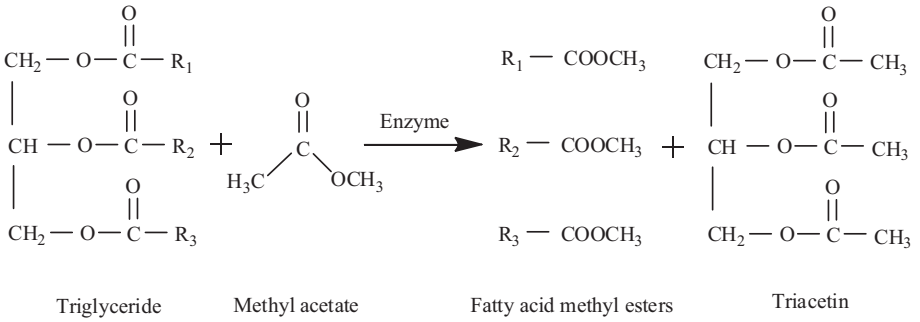


**Scheme 1.** Production of fatty acid methyl esters by enzymatic interesterification of triglyceride with methyl acetate.

interesterification offers significant advantages, very few papers dealing with this reaction route involving enzymes have been reported. Kim et al. [11] used ethyl acetate as acyl acceptor and highest biodiesel production yield of 63.3% was achieved by using an ethyl acetate to oil molar ratio of 6:1 with 8% of the immobilized lipase Novozym 435. Surendhiran and Vijay [12] studied the interesterification reaction for synthesis of biodiesel based on the use of methyl acetate instead of more commonly used alcohol. Maximum biodiesel yield of 92.34% was obtained by using 1.5 g of enzyme, 1:12 molar ratio of oil to methyl acetate, temperature of 35 °C in 60 h of reaction time.

Apart from possible inactivation of enzyme, another key shortcoming in the commercialization of biodiesel production is the cost of operation especially dominated by the higher costs of the enzyme, raw materials and significantly longer reaction times. Even though, biodiesel has an enormous potential to substitute exhaustible fossil fuel, for all the current processing technologies, the cost of biodiesel is about 50–100% higher than the petroleum based diesel fuel [13]. The significant cost associated with the raw materials [14] can be reduced by using waste cooking oil (WCO) or other sustainable resources. The mass transfer limitations due to the heterogeneous nature of reactants can be reduced based on the use of intensification approaches such as using ultrasound. Use of interesterification approach instead of transesterification can also help in producing a more valuable co-product with the biodiesel. Considering these aspects, interesterification of waste cooking oil using enzymatic route and intensification using ultrasound has been investigated in the present work.

Application of ultrasound can give substantial degree of process intensification based on cavitation phenomenon. The cavitational effects can improve the mass transfer at mild reaction conditions in terms of temperature and pressure resulting into faster reaction rate, higher product yield [15,16] and possibly requirement of lower acyl acceptor to oil molar ratio and catalyst loading. Cavitation creates intense turbulence and liquid circulation at micro scale which help in reducing the mass transfer resistances in heterogeneous systems [17]. Although researchers have used ultrasound for the enhancement of enzymatic transesterification route for biodiesel synthesis [18–20], there is practically no information available for its application for the enzymatic interesterification route of biodiesel production. The main aim of the present work was to investigate the influence of ultrasound on the enzymatic interesterification of waste cooking oil using immobilized lipase. Furthermore, different reaction conditions including molar ratio, enzyme loading, reaction temperature and time which might influence the yield of FAMEs have been investigated so as to maximize the yield. Comparison of the ultrasound based approach with the conventional approach has been presented so as to establish the benefits of using ultrasound as a source of mixing.

## 2. Materials and methods

### 2.1. Materials

Waste cooking oil (WCO) was obtained from a local restaurant in Mumbai, India. The initial analysis of oil revealed that the main contents were unsaturated fatty acids (linoleic and oleic acids as a total of 91%) with very less quantum of saturated fatty acids (palmitic and stearic acid). The properties of WCO used in the work are shown in Table 1. Methyl acetate was acquired from S.D. Fine Chemicals Ltd., Mumbai. Acetonitrile and acetone (HPLC grade) used as HPLC solvents were obtained from J.T. Baker, Mumbai. The methyl oleate and methyl linoleate standards were procured from Sigma–Aldrich. Lipase enzyme, Lipozyme TL IM, immobilized on silica granules, was kindly provided as a gift sample by Brenntag India Pvt. Ltd., Mumbai, India.

### 2.2. Experimental Methodology

#### 2.2.1. Conventional approach of interesterification of waste cooking oil

A 100 mL glass reactor equipped with mechanical stirrer and baffles to avoid vortex formation was used for the conventional approach of interesterification. The temperature of the reaction was maintained constant using water bath. Reactor was also equipped with a condenser to achieve complete reflux conditions and recycle the methyl acetate vapors back to the reaction mixture. WCO and methyl acetate were first fed to the reactor and heating was started. After reaching the desired temperature, lipase enzyme was added into the reactor. 0.5 mL samples were withdrawn at specific intervals and analyzed using HPLC. It was observed that the pH of the reaction mixture did not change with time. The effect of operating parameters such as reactant molar ratio, temperature and enzyme loading has been investigated. The reproducibility of the data was tested by performing the experiments in duplicate and average values have been reported. The observed errors were within 2% of the reported average value.

**Table 1**
Composition and properties of waste cooking oil.

| Property | Value |
|---|---|
| Linoleic acid (%) | 73.4 |
| Oleic acid (%) | 18.3 |
| Palmitic acid (%) | 6.7 |
| Stearic acid (%) | 1.6 |
| Saponification value (mg KOH/g of oil) | 198 |
| Density (kg/m$^3$) | 930 |
| Acid value (mg KOH/g oil) | 4.3 |
| Viscosity (mm$^2$/s) | 54.3 |