



# Ultrasound assisted enzyme catalyzed hydrolysis of waste cooking oil under solvent free condition



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## ABSTRACT

The present work demonstrates the hydrolysis of waste cooking oil (WCO) under solvent free condition using commercial available immobilized lipase (Novozyme 435) under the influence of ultrasound irradiation. The process parameters were optimized using a sequence of experimental protocol to evaluate the effects of temperature, molar ratios of substrates, enzyme loading, duty cycle and ultrasound intensity. It has been observed that ultrasound-assisted lipase-catalyzed hydrolysis of WCO would be a promising alternative for conventional methods. A maximum conversion of 75.19% was obtained at mild operating parameters: molar ratio of oil to water (buffer pH 7) 3:1, catalyst loading of 1.25% (w/w), lower ultrasound power 100 W (ultrasound intensity –  $7356.68 \text{ W m}^{-2}$ ), duty cycle 50% and temperature ( $50^\circ\text{C}$ ) in a relatively short reaction time (2 h). The activation energy and thermodynamic study shows that the hydrolysis reaction is more feasible when ultrasound is combined with mechanical agitation as compared with the ultrasound alone and simple conventional stirring technique. Application of ultrasound considerably reduced the reaction time as compared to conventional reaction. The successive use of the catalyst for repetitive cycles under the optimum experimental conditions resulted in a loss of enzymatic activity and also minimized the product conversion.

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## 1. Introduction

With the increasing demand of the bio-based products and renewable resources there is necessity for the utilization of available raw materials. As vegetable oils are widely available in nature, both edible and non-edible forms, some part of edible oil get converted into waste after its use in cooking process. The disposal of such waste is the most imperative problem in the recent years as it has been banned in many countries for use as an animal feed as well as to dump it in the ground. The waste minimization and its utilization are the most vital problem for many governmental agencies. In the recent years, many ways have been explored for the utilization of the WCO such as its use in the production of surfactant, bio lubricants, soap production, synthetic detergents, greases, cosmetics and in the biodiesel industry. Among these applications production of biodiesel from waste cooking oil is the mostly focused area [1]. Biodiesel can be prepared from WCO by using two methods. First by direct transesterification of WCO with methanol or ethanol using acid, base or enzyme as catalyst and the second way is to first convert WCO to its corresponding fatty acids

by hydrolysis and then esterify to its fatty acid esters. The second method has advantages over first as it produces high purity biodiesel [2].

High temperature and high pressure processes are generally used for the industrial production of fatty acids and glycerol from vegetable oil, which require huge energy and there are chances of the formation of undesirable polymerized products. For example, at higher temperature unsaturated fatty acids can polymerize, and also forms anhydrides which on further decomposition gives ketones and hydrocarbons [3]. Recently, many researchers have published work on the minimization of such harsh conditions in industry by adopting newer techniques like microwave irradiation [4], ultrasonication [5], supercritical fluids [6], ionic liquids [7] as medium and enzyme as a biocatalyst. Enzyme catalyzed reactions is the growing research area as it has very wide industrial applications in the field of foods, pharmaceuticals, flavors, polymer, dyes, water treatment, textiles, cosmetics, fine and bulk chemicals, etc. due to its mild operating conditions, bioavailability, selectivity towards substrate and requirement of no special apparatus. Apart from its wide applications and environmentally operating conditions, it has certain limitations such as slow reaction rates and significantly higher cost of the enzymes. To overcome these limitations of the enzymatic reactions, we have used ultrasound assisted enzymatic reaction as a greener approach. In the recent

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years, combination of enzymatic catalysis assisted with ultrasonication is the most explored area as it gives the high quality of product due to the selective nature of enzymes, which ultimately produces less or no side products, low production cost due to reduction of harsh conditions, good reusability of catalyst, minimization of actual reaction time compared with conventional stirring processes and most importantly follows the protection of environmental laws as these process are ecofriendly [8–12].

Application of ultrasound produces cavitation phenomenon which mainly consists of formation, growth and violent implosion of microbubbles formed by the sound waves. Cavitation phenomenon acts through three main mechanism, in combination or alone. The first one is thermal effect due to generation of very high temperature during cavitation, second one is free radical generation by sonolysis and the third one is due to the mechanical (shear) forces formed due to the shock waves produced due to cavitation [13]. Considering the hydrolysis of oil there are several papers available using enzyme as a catalyst [14–22] but only few are reported using ultrasound assisted enzyme catalyzed hydrolysis [5,23–25]. Though there are many papers published on hydrolysis of WCO but limited data is available on ultrasound assisted enzyme catalyzed hydrolysis of WCO [26]. Present study deals with the application of low frequency ultrasound (22 kHz) with lipase Novozyme 435 as a catalyst for the hydrolysis of WCO using ultrasonic bath sonicator under solvent free conditions. The various process parameters were examined to get the maximum conversion of WCO.

## 2. Materials and methods

### 2.1. Materials

The WCO was procured from a local restaurant in Mumbai with an acid value of 2.4 mg of KOH g<sup>-1</sup> and saponification value 182 mg of KOH g<sup>-1</sup>. Novozyme 435 (lipase B from *Candida antarctica*; immobilized on macro-porous poly acrylic resin beads, bead size 0.3–0.9 mm, bulk density 0.430 g/cm<sup>3</sup>) was received as a gift sample from Zytex India Pvt. Ltd., Mumbai. All other chemicals such as ethanol 99%, acetone, potassium hydroxide, and phenolphthalein were purchased from Himedia Laboratories Pvt. Ltd., Mumbai.

### 2.2. Hydrolysis of waste cooking oil

Hydrolysis of WCO was carried out using an ultrasonic bath reactor with internal dimensions 300 × 150 × 150 having five transducers placed at the bottom along with length of the bath. The reactions were carried out in a 50 mL flat bottom baffled glass batch reactor having 4.5 cm i.d. The whole reactor assembly was immersed in ultrasonic water bath having the provision to control the temperature at the accuracy of ±2 °C. The bath was connected to the ultrasonic frequency controller having dual mode of frequencies i.e. 22 and 40 kHz. The maximum input power of bath operation was 200 W. Mechanical agitation was provided using overhead motor stirrer having three blade glass turbine impellers. In order to obtain the maximum cavitation intensity, the position of reactor in the ultrasonic bath was selected based on earlier report [27].

In 50 mL glass reactor, 12.0 g WCO and 4.0 g buffer solution of pH 7 was mixed properly to form uniform oil/water emulsion and 0.150 g of Novozyme 435 was added to start the reaction. Addition of enzymes was considered as the starting point of the reaction and periodic samples were taken and centrifuged at 8000 rpm to separate two phases. The progress of the reaction was carried out by analyzing the oil phase for acid value using

standard titrimetric method. All the reactions were performed at various temperature ranging from 40–60 °C, at fixed frequency (22 kHz), varying input irradiated power between 50–150 W, enzyme loading 1–1.5% and duty cycle 30–70%.

## 3. Analytical techniques

### 3.1. Determination of acid value and degree of hydrolysis

The progress of the reaction was estimated by the determination of acid value (AV) using the official method approved by American Oil Chemist Society (Ca 5a-40). 0.1–0.2 g of sample was taken in 100 mL conical flask and 20 mL of ethyl alcohol was added to dissolve the sample, the flask was well shaken in hot water to make dissolution easier. After dissolution the samples were titrated with 0.003 N Potassium hydroxide (KOH) using phenolphthalein indicator. The appearance of pink color indicates the end point of the titration.

The acid value of the sample was determine using following formula,

$$\text{Acid value} = \frac{56.1 \times N \times V}{W} \quad (1)$$

where  $N$  = Normality of alkali required to neutralized the acid,  $V$  = Volume of alkali required to neutralized the acid,  $W$  = Weight of sample taken for analysis in g.

The degree of hydrolysis (%) was determined by using following formula [3],

$$\text{Degree of hydrolysis (\%)} = \frac{AV_2 - AV_1}{SV - AV_1} \times 100 \quad (2)$$

where  $AV_1$  and  $AV_2$  are the acid value of the sample at initial and at time  $t$ ,  $SV$  is the saponification value.

### 3.2. Statistical analysis

Statistical analysis was useful in the data validation, summarization and its interpretation of obtained data. The analysis was carried out using single factor ANOVA with Microsoft excel 2013. All experiments were carried out at least in triplicate and the obtained data is reported as mean ± SD. The result was considered statistically significant for  $P$  value less than 0.05.

### 3.3. Determination of kinetic and thermodynamic parameters

To determine the kinetic parameters, pseudo 1st order kinetics is used in the current work. By considering these kinetic constants the activation energy of the reaction was determined using Arrhenius plot. Eq. (3) represents Arrhenius equation,

$$k = A \exp\left(\frac{-E_a}{RT}\right) \quad (3)$$

where  $k$  is kinetic constant,  $A$  is frequency factor,  $E_a$  is activation energy of the reaction,  $R$  is the universal gas constant and  $T$  is the absolute temperature. The Arrhenius plot  $\ln k$  vs.  $1/T$  gives the value of slope as  $-E_a/R$  and  $\ln A$  as the  $y$  intercept. Thermodynamic parameters of the reactions were determined by using Eyring transition state theory and some basic thermodynamic Eqs. (4)–(6) [28].

$$\ln \frac{k}{T} = \frac{-\Delta H}{R} \frac{1}{T} + \ln \frac{k_B}{h} + \frac{\Delta S}{R} \quad (4)$$

$$\Delta H = E - RT \quad (5)$$

$$\Delta G = \Delta H - T\Delta S \quad (6)$$

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