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# Utilisation of Waste Cooking Oil as Biodiesel through Bioprocess Technology

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**Abstract:** *Rising adverse effect towards environment due to consumption of conventional fuels, the requirement of environment friendly and alternative source of energy has gained considerable importance in the last few years. Biodiesel is proved to be the best alternative fuel due to its biodegradable nature, low toxicity, non-sulfur emission and environment friendly nature. The present research paper uses waste cooking oil (WCO) as the cheap source of biodiesel and transesterification reaction was followed between WCO and methanol in the presence of enzyme catalyst, Novozyme 40013 (Candida antarctica) maintaining definite reaction parameters like alcohol to oil molar ratio, reaction temperature, mixing intensity and biocatalyst concentration. The optimum reaction conditions were investigated and the final product was analysed. Studies show that WCO may be considered a good cheap source for the production of alternative fuel.*

**Keywords:** *Waste cooking oil, Biodiesel, Candida antarctica, Biocatalyst, Bioprocess*

## I. INTRODUCTION

The need for environmental friendly alternative energy sources is supposed to be one of the urgent essential demands in the present environmental tribulations and degradations. Energy crisis along with environmental issues developed the requirement for the new biofuels.

Biodiesel is a clean, biodegradable alternative energy fuel to fossil fuel. A green approach for biodiesel production through enzymatic method has gained a lot of attention due to the certain drawbacks of chemical methods. Alternative energy sources like biodiesel is prepared from different sources through different processes using chemical or biocatalyst. One of the important cheap raw materials for biodiesel production is WCO which is supposed to be the best resource regarding its availability and reuses. WCO has a potential to be used as an alternative fuel, biodiesel and diesel engine [1]. Any source of fatty acids can be used to produce biodiesel, thus, any animal or plant lipid can be used as substrate for the production of biodiesel. Biodiesel can be made through transesterification process of vegetable oil or animal fat reacts with alcohol and with presence of catalyst. This initiative is cheaper and environmentally friendly.

The WCO is generated from the fried food of different food shops, restaurants, hotels etc. which needs large amounts of oil at temperatures greater than 180°C. Due to the generation of high temperatures, the chemical and physical composition, as well as in its organoleptic properties of oil have been changed which affect both the food and oil quality. Also, repeated frying makes the edible vegetable oil no longer suitable for consumption due to high free fatty acid (FFA) and other toxic components [2]. Disposal of WCO to the environment creates a lot of problems like water and soil pollution which affects bad impact on human health and aquatic system [3,4]. So WCO can be used as a cost effective feedstock for biodiesel production as readily available raw materials. Several studies [5-10] on biodiesel synthesis from used cooking oil have been carried out.

Solikhah et al. [11] synthesized biodiesel from used cooking oil with the trans-esterification process. Wang et al. [12] synthesized biodiesel using a two-stage catalyst process, namely the esterification process with ferrous sulfate catalyst and potassium hydroxide base catalyst. The biodiesel processing process that uses two stages, namely esterification and transesterification requires double consumption of methanol.

The addition of catalyst can increase conversion percentage of biodiesel produced [13-14]. Chuah et al. [15] studied the kinetics of waste cooking oil into biodiesel via hydrodynamic cavitation and showed that the process was time saving and energy efficient compared to mechanical stirring. Present author also extracted functional foods from bad oil like deodorizer distillate which can be utilized in different food formulations [16]. Very few studies have been done regarding the utilization of enzyme as catalyst for the production of biodiesel from WCO. In the present research investigation, WCO is successfully utilized for the production of biodiesel using bioprocess technology observing the optimum reaction conditions.

## II. MATERIALS AND METHODS

WCO was obtained from different markets of Madhyamgram, Kolkata, West Bengal. The enzymes used in the present study was Novozyme 40013, an immobilized non specific lipase from *Candida antarctica* with ester synthesis activity of 10000 propyl laurate unit/g and it was a kind gift of Novozyme South Asia Pvt. Ltd. Bangalore, India. The chemicals monoglycerides and diglycerides were purchased from Scientific and Laboratory Instrument Co., Kolkata. Except otherwise specified all other chemicals were A.R. Grade.

Initially 500 mL of WCO was filtered and taken in an Erlenmeyer flask and heated up to 80°C to drive off moisture by continuous stirring for about 1 h. After that, alcohol was added to it for transesterification reaction through stepwise manner in an appropriate proportion using solvent hexane fitted with a water condenser and stirred by a magnetic stirrer at a specified temperature for 6 hours. To the reaction mixture, immobilized enzyme Novozyme 40013 was added in definite proportion (w/w). Stepwise addition of methanol was allowed to minimize the deactivation of enzyme.

During the reaction, continuous sampling and analysis were done by withdrawing the sample in to a capped vial and removing enzyme through centrifugation.

The progress of reaction or production of biodiesel was monitored by thin layer chromatographic (TLC) method and the typical yield of each reaction product was determined separately by column chromatography. TLC was done by spotting the lipid mixture on a silica-gel G plate (0.2 mm thick) using hexane-diethyl ether-acetic acid (90:10:1) as a developing solvent. The lipid spots were identified by iodine absorption with triacylglycerol (TG), diacylglycerol (DG), monoacylglycerol (MG) and BD as standard. The composition of WCO esters was determined by column chromatography using silicic acid as an adsorbent and 160 mL of hexane-diethyl ether: 99:1 as eluting solvent. After completion of reaction, the enzyme was washed with hexane, dried and reused for the next experiment. Biodiesel characterization was done according to the American Standard Testing Method (ASTM). Values are reported as mean  $\pm$  s.d., where n=3 (n=no of observations).

## III.RESULTS AND DISCUSSIONS

The analytical characteristics of WCO was shown in Table 1. It was observed from Table 1 that the density and acid value of WCO are 0.92 g/mL and  $6.21 \pm 0.16$  mg KOH/g WCO respectively. Due to the heating and cooling processes several times, FFA content increases which is shown through acid value. The permissible limit for the acidity index is 1.5 mg KOH / g oil for biodiesel production [13].

Carlini et al. [14] established that solids content of waste oil for biodiesel production should be lower than 0.5 %. The solids content of the WCO in the present study is 1.16%. So filtration is done before processing. It has also been established [9] that the humidity percentage of WCO should be lower than 0.5% for the production of biodiesel, for which it is necessary to dehydrate the WCO. Before enzymatic transesterification, WCO was thoroughly bleached to remove peroxides.

TABLE I  
CHARACTERISTICS OF WCO

Sl.no	Waste cooking oil		
	Characteristics	Value	Test methods
1	Density (gm/mL)	0.92	ASTMD 1298
2	Acid value mg KOH/g WCO	$6.21 \pm 0.16$	ISO 660-2009
3	Saponification value	$175 \pm 0.46$	ASTM D558
4	Humidity	$1.34 \pm 0.03$	Gravimetry
5	Iodine value	$93.79 \pm 0.57$	ISO 3961-2009
6	Sediments content	$1.16 \pm 0.02$	Gravimetry
7	Dynamic Viscosity	$4.44 \pm 0.02$	ASTM-D445

### A. Optimization of alcohol to WCO molar ratio

For identifying optimum molar ratio, different ratios of alcohol were treated with definite amount of WCO. In the present study, transesterification reaction was carried out at 60°C using 6% (wt/wt) immobilized enzyme for 10 hours using different molar ratios as shown in Fig. 1. It has been observed from the figure that optimum biodiesel conversion was achieved with a 5:1 molar ratio of methanol to WCO. Increasing concentration of methanol did not enhance the conversion as evidenced from the figure. This may be due to the fact that with 5:1 molar ratio, all the active sites of enzymes are fully occupied and these did not allow any further increment of methanol for higher conversion of biodiesel.

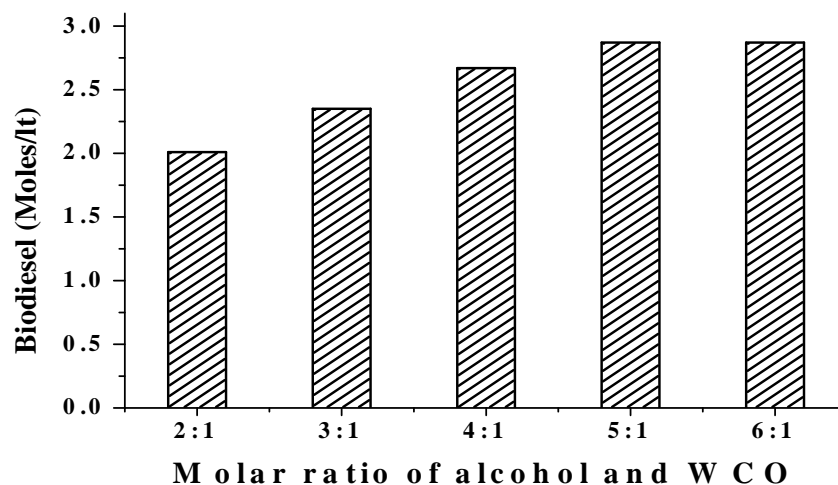


Fig. 1 Effect of Molar ratio of alcohol to WCO for BD production

### B. Optimization of Reaction Temperature

Energy of activation of any reaction depends on temperature and hence temperature plays a significant role for optimization of reaction. For identifying optimum reaction temperature, different temperatures e.g. 40, 50, 60 and 70°C were applied using 6% (wt/wt) immobilized enzyme for 10 hours with 5:1 molar ration of alcohol and WCO. It has been observed from Fig. 2 that maximum conversion of biodiesel has been achieved at 60°C beyond which the production did not enhance rather it has been decreased. This may be due to the enzyme deactivation after a certain temperature. Higher temperature also volatilize methanol which hampers the proper ratio of methanol: JCO.

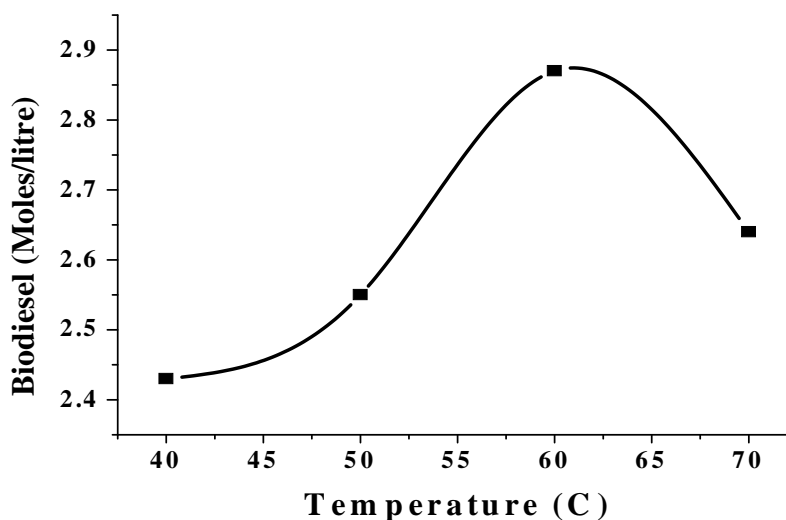


Fig. 2 Effect of temperature for BD production



### C. Optimization of Mixing Intensity

Proper mixing of reactants is essential for optimum conversion of product within a specific time span. Mixing not only transfer the reactants within the reaction vessel from surface to bulk and vice versa but also helps the reactants to reach the active sites of enzyme. The effect of speed of agitation was investigated in the present study in the range of 300 to 700 rpm at 60°C using 6% (wt/wt) immobilized enzyme for 10 hours with 5:1 molar ration of alcohol and WCO. It was found from Fig 3 that maximum conversion has been achieved at mixing intensity of 600 rpm. Beyond this mixing intensity, conversion has not been improved. This may be due to the fact that higher stirring intensity hampers the contact between reactants and active sites of enzymes which ultimately decreases the conversion efficiency.

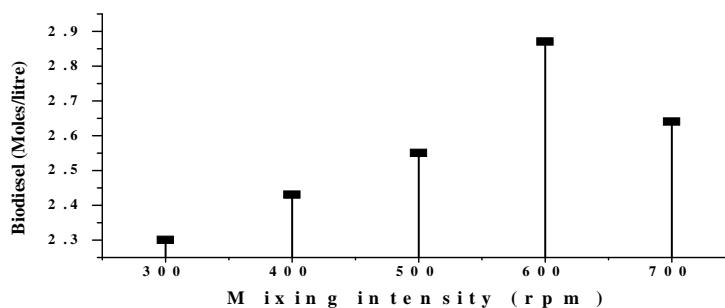


Fig. 3 Effect of mixing intensity for BD production

### D. Optimization of Biocatalyst Concentration

Biocatalyst optimization not only saves time for production but also reduces cost of production by recycling it. In the present study, effect of biocatalyst concentration was studied (Fig. 4) and maximum biodiesel was obtained using 6% (w/w) concentration of enzyme at 60°C for 10 hours with 5:1 molar ration of alcohol and WCO maintaining 600 rpm mixing intensity. By increasing catalyst concentration further, the conversion efficiency for biodiesel production did not enhance. One of the reasons may be higher amount of enzyme contributes higher amount of active sites but due to possible aggregation of enzymes, all active sites would not be effective.

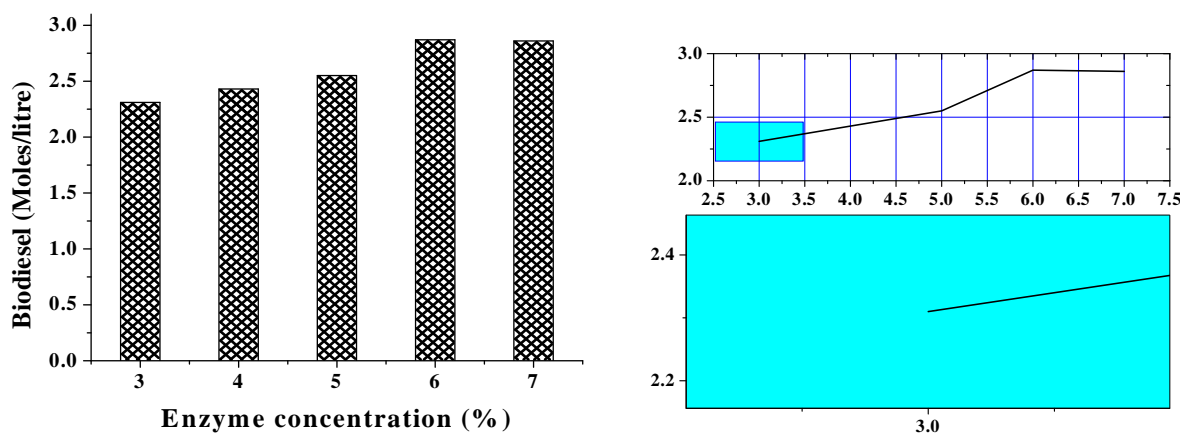


Fig. 4 Effect of enzyme concentration for BD production

### E. Analysis of TG, DG, MG and BD production

The optimum parameters identified for biodiesel production from WCO were 5:1 molar ratio of methanol to WCO by using 6% (wt/wt) immobilized enzyme for 10 hours at a temperature of 60°C. During the reaction process, TG, DG, MG and biodiesel were identified as shown in Fig. 5. Initially amount of TG, DG and MG were 67.47, 24.73 and 7.8% respectively along with some free fatty acids. During the transesterification reaction, TG, DG and MG were decreasing while amount of BD was increasing as shown in the Fig. 5. So the parameters optimized in the process were correctly identified and applied. Finally, 95.35% conversion has been achieved for successful production of biodiesel.

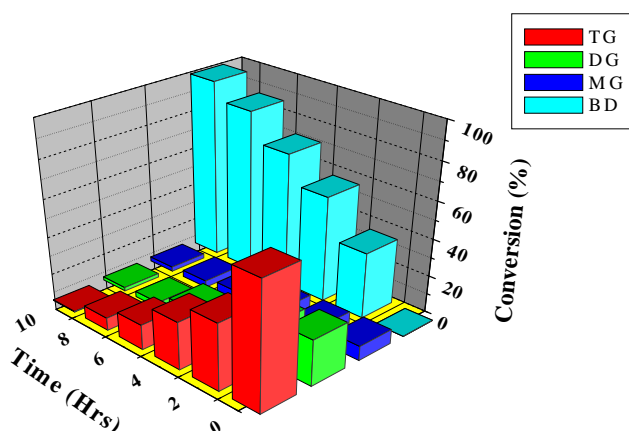


Fig. 5 Analysis of TG, DG, MG and BD during the reaction

#### IV. CONCLUSIONS

Production of biodiesel through transesterification reaction between waste cooking oil and methanol using enzyme as catalyst is a novel technology for the alternative solution of fossil fuel. Present bioprocess technology identifies suitable reaction parameters which can be applied in the recycling of biocatalyst and recycling technology helps to reduce the process cost. This technology can be applied to other cheap raw materials for the production of alternative fuel in the near future for the researchers.

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