

Production waste cooking oil as feedstock and



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Production of Biodiesel by Enzymatic Transesterification: using Waste Cooking Oil as feedstock and *Candida Antarctica* Lipase B as Biocatalyst.

CHAPTER 1 INTRODUCTION The high cost of bio-diesel, compared to petroleum-based diesel, is a major barrier to its commercialization. It has been reported that 60-90% of bio-diesel cost arises from the cost of the feedstock oil (C. C. Lai et al. , 2005). Studies showed the potential of waste-cooking oil (WCO) as a material for biodiesel production (Sulaiman Al-Zuhair, 2008).

Therefore, the use of WCO should greatly reduce the cost of bio-diesel. In addition to the choice of lipase employed, factors which make the transesterification process feasible and ready for commercialization are: enzyme modification, the selection of feedstock and alcohol, use of common solvents, pretreatment of the lipase , alcohol to oil molar ratio, water activity/content and reaction temperature. Optimization of these parameters is necessary in order to reduce the cost of biodiesel production.

Use of no/low cost waste materials such as the WCO will have double environmental benefits by reducing the environmental pollution potential of the wastes and producing an environmentally friendly fuel. In addition, production of bio-diesel from WCO is considered an important step in reducing and recycling waste. A fresh vegetable oil and its waste differ significantly in water and free fatty acids (FFAs) contents, which are around 2000 ppm and 10-15%, respectively (C. C. Lai et al. , 2005; Y. Zhang et al. , 2003). Because of this the traditional alkaline-catalyzed biodiesel production is unsuitable (Zhang et al. 2003). The use of the enzyme lipase as a biocatalyst for the transesterification reaction step in biodiesel production

has been extensively investigated. Lipase is produced by all living organisms and can be used intracellularly or extracellularly. In order to design an economically and environmentally sustainable biodiesel production process, a proper understanding of the factors affecting the process and their relative importance of enzyme-catalyzed biodiesel production is necessary. A general equation for transesterification (where group R is a fatty acid, R' is the length of the acyl acceptor and R'' is the rest of the triglyceride molecule) is as follows: Methanol is the most popular alcohol used in the transesterification process because of its relatively cheaper price compared to other alcohols. When methanol is used in the process, the reaction is known as methanolysis as shown in the following equation: Lipases from microorganisms (bacterial and fungal) are the most used as biocatalysts in biotechnological applications and organic chemistry. Fungal – source lipases have been found to produce high yields of lipases compare to the animal and plants.

Because their bulk production is easier, commercialization of microbial lipases and their involvement in enzymatic biodiesel production are more common than animal and plant ones (Hasan et al. , 2006; Akoh et al. , 2007; Antczak et al. , 2009). The lipase to be employed as the biocatalyst is *Candida Antarctica* lipase B (Novozyme CABL L), one of the most common fungal lipase used for the production of biodiesel (Vasudevan and Briggs, 2008). Lipases are capable of converting all the triglycerides derive from the feed stocks into their respective fatty acids methyl esters (FAMES).

They act on the ester bonds of carboxylic acids allowing them to carry out their primary reaction of hydrolyzing fats (Joseph et al. , 2008). Enzyme

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immobilization is an important approach that could be used as a tool to improve and optimize operation stability, activity and selectivity which allows the enzyme to study under harsher environmental condition and also provides their separation from the reaction mixture without filtration in case of packed bed reactor (Fernandez-Lafuente et al. , 1998; Bhushan et al. , 2009; Gao et al. 2006) and, hence, could lead to more favorable economical benefits. The cost of lipase makes up 90% of the total cost of enzymatic biodiesel production. A significant portion of that is associated with the use of expensive carrier or support materials (Dizge et al. , 2009a). Search for cheaper support materials has been ongoing in order to reduce the overall cost of enzymatic biodiesel production (Robles et al. , 2009). Thus it is important to immobilize lipase, to be able to recover and reuse it repeatedly (D. S. Clark, 1994; D.

Cowan, 1996). Immobilization of lipase is the attachment of the enzyme onto a solid support or the confinement of the enzyme in a region of space (Jegannathan et al. , 2008). When proper strategy for the lipase immobilization technology is employed , it provides a number of important benefits including: (a)enzyme reuse, (b) easy of separation of product from enzyme and (c) the potential to run continuous processes via packed-bed reactors (Peilow and Misbah, 2001). Methods of immobilization include chemical and physical means.

Among these, the physical immobilization by way of entrapment is the most widely-used method, in which enzymes are entrapped within the sol-gel matrix prepared by hydrolysis and polycondensation of precursors (Ko Woon Lee, et al. 2010). Tetramethylorthosilicate (TMOS) is a widely used precursor <https://assignbuster.com/production-waste-cooking-oil-as-feedstock-and/>

for sol-gel immobilization of the enzyme. However, CALB is unstable and shows low catalytic efficiency in the reaction media contains high concentration of methanol and the lipase is also inhibited by the by-product of glycerol.

To overcome this, an amphiphilic matrix is developed to immobilize the lipase ((Ko Woon Lee, et al. 2010). The use of solvent in the transesterification process is also considered. Solvents are used to protect the enzyme from denaturation by alcohol by increasing alcohol solubility (Kumari et al. , 2009). The solvent can also increase the solubility of glycerol which is beneficial since the byproduct can coat the enzyme and inhibit its performance (Royon et al. , 2007).

The use of a common solvent for the reactants and products not only reduces enzyme inhibition but also ensures a homogeneous reaction mixture, reduces the reaction mixture viscosity and stabilizes the immobilized enzyme (Ranganathan et al. , 2008; Fjerbaek et al. , 2009). This is beneficial because homogeneous reaction mixture decreases problems associated with a multiple phase reaction mixture and a reduced viscosity reduces mass transfer problems around the enzyme (Fjerbaek et al. , 2009). The use of solvents significantly increases the reaction rate in comparison to solvent free systems (Vasudevan and Briggs, 2008).

Some study also showed that methanolysis conversion using *Candida antarctica* was increased when tert-butanol was added to the system (Royon et al. , 2007). This serve as the basis for the choice of tert-butyl to be the solvent use in the system, in order to reduce the inhibition cause of the use

of a lower chain alcohol, in this case, the methanol. **OBJECTIVES** This study aims to produce economical source of feedstocks such as waste-cooking oil for the production of biodiesel and the use of enzyme *Candida Antarctica* Lipase B, to catalyze to transterification reaction.

To be able to determine the yield biodiesel through Gas Chromatographic Analysis (Chrompack CP 9001, Holland). **SIGNIFICANCE OF THE STUDY** Oil is one of the most commonly reported types of water pollution, causing nearly a quarter of all pollution incidents. Careless disposal of oil into drainage systems, onto land or to watercourses is an offense. It can harm river birds, fish and other wildlife. Because of the way oil spreads, even a small quantity can cause a lot of harm.

It is estimated that UK caterers produce between 50 – 90 million litres of waste cooking oil each year. If this is not disposed of correctly the effects of oil pollution on the environment could be quite devastating. According to the Environmental Protection Agency (EPA), estimates that over 200 million gallons of used oil ends up in the trash, and poured into the water each year. This study aims to promote conventional and economic source for the production of biodiesel by using home waste material such as waste cooking oil.

Thus, resolving high cost of biodiesel production making it commercially producible and reduce devastation of environment due to high consumption of crude oils from fossil sources. This study will be a significant endeavour in promoting the social needs and to resolve the high prices of the gasoline which is the major economical crisis face in the present society. The

advantages of using lipases in biodiesel production are: (a) ability to work in very different media which include biphasic systems, monophasic system (in the presence of hydrophilic or hydrophobic (Am.

J. Biochem. & Biotech. , 6 (2): 54-76, 2010), (b) they are robust and versatile enzymes that can be produce in bulk because of their extracellular nature in most producing system, (c) many lipases show considerable activity to catalyze transesterification with long or branched chain alcohols, which can hardly be converted to fatty acid esters in the presence of conventional alkaline catalysts, (d) products and byproduct separation in downstream process are xtremely easier, (e) the immobilization of lipases on a carrier has facilitated the repeated use of enzymes after removal from the reaction mixture and when the lipase is in a packed bed reactor, no separation is necessary after transesterification and (f) higher thermostability and short-chain alcohol-tolerant capabilities of lipase make it very convenient for use in biodiesel production (Bacovsky et al. , 2007; Kato et al. , 2007; Robles et al. , 2009). SCOPE AND LIMITATION Like any method for enzymatic biodiesel production, the cost of the lipase to be used is one of great consideration .

The limitations of using lipases in biodiesel production include (a) initial activity may be lost because of volume of the oil molecule (Marchetti et al. , 2008; Robles et al. , 2009), (b) the use of solvent does not guarantee the complete protection of enzyme from the inhibitory effect of low chain alcohol, methanol (c) Although lipase is not affected by the high content of FFAs in WCO, the high water content remains a problem (d) the lipase in the biodiesel production is limited on a specific feedstock to be used because of the regioselectivity of the enzyme lipase.

CHAPTER 2 REVIEW OF RELATED LITERATURE Biodiesel has shown its ability to meet the energy demand of the world in the transportation, agriculture, commercial and industrial sectors of the economy (Akoh et al. , 2007; Basha et al. , 2009; Shafiee and Topal, 2009; Robles et al. , 2009). The annual world consumption of diesel is approximately 934 million tons, of which Canada and the United States consume 2. 14 and 19. 06%, respectively (Marchetti et al. , 2008).

As a green renewable and potentially unlimited, biodiesel has recently come out as the superlative alternative fuel which can be used in compression ignition engines with minor or no modifications (Xu and Wu, 2003; Vasudevan and Briggs, 2008; Robles et al. , 2009; Leung et al. , 2010). The concept of biofuel is not new. Rudolph Diesel was the first to use a vegetable oil (peanut oil) in a diesel engine in 1911 (Akoh et al. , 2007; Antczak et al. , 2009). The use of biofuels in place of conventional fuels would slow the regression of global warming by reducing sulfur and carbon oxides and hydrocarbon emissions (Fjerbaek et al. , 2009). Because of economic benefits and more power output, biodiesel is often blended with diesel fuel in ratios of 2, 5 and 20% (Vasudevan and Briggs, 2008). The higher the ratio of biodiesel to diesel the lower the carbon dioxide emission (Fukuda et al. , 2001; Harding et al. , 2007). Using a mixture containing 20% biodiesel reduces carbon dioxide net emissions by 15. 66% (Fukuda et al. 2001) while using pure biodiesel makes the net emission of carbon dioxide zero (Vasudevan and Briggs, 2008). The simplest and most efficient route for biodiesel production in large quantities, against less ecofriendly, costly and eventual low yield methods is transesterification. One of the classic organic

reactions (transesterification) is the step wise reversible reactions of a triglyceride (fat/oil) with an alcohol to form esters and glycerol. Little excess of alcohol is used to shift the equilibrium towards the formation of esters.

Transesterification using an alcohol is a sequence of three reversible consecutive steps. In the first step, triglycerides are converted to diglycerides. In the second step, diglycerides are converted to monoglycerides. In the third step, monoglycerides are converted to glycerin molecules (Freedman et al. , 1984; Nouredini and Zhu, 1997; Marchetti et al. , 2008). Each conversion step yields one FFAE molecule, giving a total of three FFAEs per triglyceride molecule as described by the following equations (Murugesan et al. , 2009). 1. Conversion of triglycerides to diglycerides . Conversion of diglycerides to monoglycerides 3. Conversion of monoglycerides to glycerin molecules In order for the transesterification reaction to be applicable for biodiesel production, the process must be accelerated by the use of catalyst which may be alkaline, acids or enzymes (Bacovsky et al. , 2007; Murugesan et al. , 2009; et al. , 2010). The catalyst employed directly affects the purity of the feedstock required, the reaction rate and the extent of post reaction processing needed (McNeff et al. , 2008). To speed up the reaction, heat is also applied.

However, this process is very energy intensive and inefficient since FFAE yield below 350°C is very low and temperatures above 400°C degrade the ester bonds (Ranganathan et al. , 2008). Generally, the reaction mix is kept just above the boiling point of the alcohol (71-72°C) to speed up the reaction. The variables known to affect the reaction are: temperature, alcohol to oil molar ratio, catalyst concentration and mixing intensity

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(Marchetti et al. , 2007). Transesterification catalysts: The transesterification process is catalyzed by alkalis, acids or enzymes.

However, the use of alkali catalysts is 100% in commercial sector. The most common alkaline catalysts are sodium hydroxide (NaOH) METHODOLOGY * LIPASE CABL (Novozyme CABL L) can be purchased from Novozyme (Denmark). All other chemicals can be purchased from Sigma- Aldrich (St. Louis, MO, USA). Grown in the laboratory, Candida appears as large, round, white or cream (albicans is from Latin meaning ' whitish') colonies with a yeasty odor on agar plates at room temperature. IMMOBILIZATION OF LIPASE Sol - gel immobilization in an amphiphilic matrix was shown in figure below; mL of CABL (8. 2 mg/ml) is to be placed in a 50-ml Falcon tube with 1-mL of 0. 2 M phosphate buffer (pH 7). As a catalyst, 50 microliter of 1M sodium fluoride is to be added and the mixture is to be shaken with a vortex mixer. Then, TMOS (2 mM) and the following hydrophobic alkylsilanes (8 mM) is added; methyltrimethoxysilanes (MTMS), ethyltrimethoxysilane (ETMS), propyltrimethoxysilanes (PTMS), and iso-butyltrimethoxysilane (iso-BTMS). Gelation is usually observed within a few minutes while a reaction vessel is gently shaken.

Following complete polymerization for 12 hours in a closed Falcon tube, the gel was dried for 24 hours in an open Falcon tube. The gel is to washed with 10 mL of distilled water, 10 mL of 99. 8% iso-propanol, and 10 mL of 95% n-hexane respectively. The immobilized CALB is to be filtered using filter paper, dried at 30 for oC for 36 hours and then ground with mortar and pestle. The particles were sorted using 500 and 300 micrometer sieves and stored at 4 oC until use. ENZYME SOLUTION Immobilized P. cepacia lipase <https://assignbuster.com/production-waste-cooking-oil-as-feedstock-and/>

solution is prepared by adding 0.1 g of lipase to 1 ml of distilled water and soaked in water for 30 minutes, prior to being used. This step is found

experimentally essential to activate the enzymes. WASTE-COOKING OIL

PREPARATION In order to ensure consistency, waste cooking oil is simulated from the commercially available palm oil by heating 1 L of palm oil on a hot plate (Stuart, U. K.), set at its maximum heating power for two hours. The oil is then allowed to cool to room temperature and then 5 ml of water (around 5000 ppm) is to be added. The sample is shelved for two weeks before being used.

Fresh WCO samples were prepared every two weeks. Bio-Diesel Production in tert-butyl Solvent System Using *C. Antarctica* Lipase The experiment will be conducted in a specially designed 150 ml capacity jacketed reactor cell. The cell will be kept on a magnetic stirrer (Velp Scientifica, Italy) to facilitate the agitation of the mixture. Water from a temperature controlled water bath (Grant Instruments, UK) circulated through the jacket and will be set to maintain the temperature of the reaction media constant at 45 °C.

The temperature used was that presented in the literature to be the optimum (M. M. Soumanou, et al, 2003; H. Fukuda, et al, 2001) and an agitation speed was chosen to provide suitable mixing without affecting the activity of the enzyme. In this part, the working volume at the beginning of each experiment was 50 ml, consisting of 5 g of WCO, different volumes of methanol, in the range of 0.4 to 0.8 ml (correspond to 0.57 to 1.14 molar equivalents of ester bonds in the triglyceride chain), and tert-butyl solution to make up the total volume.

The cell is to be covered tightly throughout the progress of the experiments to prevent evaporation. After thermal equilibrium is ensured, 1 ml of enzyme solution containing 0.4% g of *C. Antarctica* lipase per g oil is added to initiate the reaction. At suitable intervals, 1.5 ml samples are withdrawn into a capped vial, immediately immersed in boiling water for at least 5 minutes to denature the enzyme and stop the reaction, and then send for analysis. The amounts of FAMES in the samples are to be determine by using Gas Chromatograph (Chrompack CP 9001, Holland).