Lipase Catalyzed Preparation of Biodiesel from a Lollipop Effluent Stream

Jorika Theart, Sanjib Kumar Karmee, Sanette Marx

***Abstract*—**Biodiesel fuel is produced from waste oil in order to make the production process more economically feasible. In this paper, the production of biodiesel from a lollipop effluent stream, using enzymatic transesterification methods, was investigated. The production of biodiesel through enzymatic methods offers several advantages over chemical catalyzed methods that is currently applied. By the screening of lipases, it was found that immobilized *Candida antarctica-B* lipase gave the highest conversion of oil to FAME. A 96 % biodiesel conversion was obtained at a temperature of 40°C and a molar ratio of 1:4 oil to methanol. In order to prevent the deactivation of the lipase a two-step addition of methanol was conducted over a time period of 12 hours.

***Index Terms*—**Confectionary effluent, Lipid,Immobilized lipase, Transesterification, Biodiesel

I. Introduction

The majority of the world's energy is supplied through natural gases, petrochemical sources and coal [1]. The transport industry relies heavily on fossil fuel resources particularly petroleum-based fuels such as diesel, gasoline, liquefied petroleum gas and compressed natural gas [2]. Petroleum based fuels play an extremely important role in the growth of the industrial and agricultural sectors of a country. Due to the diminishing petroleum reserves and environmental consequences of exhaust gasses from petroleum-fueled engines, the need for alternative fuel resources has become more urgent [3]. Bio-fuels are one of the leading forms of alternative petroleum-based fuels [4, 5]. Biodiesel contains no aromatics, is non-toxic, biodegradable and contains no sulphur [6].

It has been found that the biodiesel industry plays a significant role in the domestic economy. Due to the increasing consumption there has been a substantial increase in biodiesel production. This growth will require the construction of new production plants and the expansion of old ones, thus creating multiple employment opportunities [7, 8].

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The lipid feedstock accounts for 70% of the biodiesel production cost. By using waste oils as an alternative feedstock the production costs will be lowered significantly [9].

The development of many African countries is constrained by their inability to provide adequate energy services [10]. Access to energy is the key factor to improve the quality of life and industrial development in many of these countries [11].

Research has shown that biofuels can be produced from edible- and non- edible oils. Around the world several billions of liters of waste oil are produced by the food industry every year [12, 13]. The world's oil reservoirs are slowly depleting and a suitable long term strategy is required to produce fuel from zero value feedstocks. Waste from food industries can be considered as a zero-value feedstocks that contains high concentrations of sugars and lipids [14]. There are several small lollipop factories all around South-Africa that produce large quantities of effluent that is discarded in dumping sites. Lipid from this waste can be used for biodiesel production. Currently large quantities of biodiesel are produced from edible oils. A global imbalance in the food supply and demand can be caused by large scale production of bio-diesel from edible oils [15, 16]. Edible oils are more expensive than waste oils.

The transesterification process produces biodiesel and glycerol as a by-product [17]. This reaction is a stepwise process, with monoglycerides and diglycerides as intermediate products [18] (fig 1).

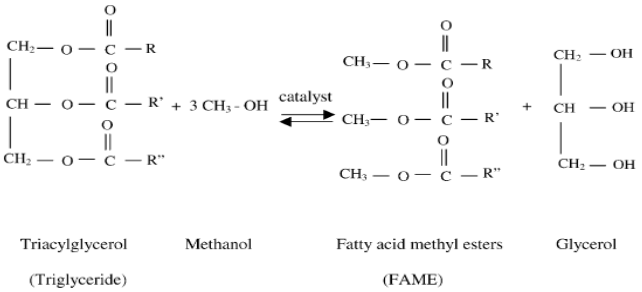

Fig. 1 Schematic representation of the transesterification reaction (taken from:[19])

The use of a catalyst (acid, base or lipase) accelerates the conversion.The use of biocatalyst is popular due to its environmentally friendly properties [20]. The most desired characteristics of the lipase are: (a) alcohol and temperature resistance; (b) ability to utilize all of the tri, di and monoglycerides as well as the free fatty acids; (c) low product inhibition and (d) high yield and activity in non-aqueous media [21]. For the production of biodiesel most lipases are of fungal or bacterial origin. Lipase catalyzed reactions are more efficient, consume less energy and are highly selective [13,9]. Therefore in this project lipases were used to convert lipid

waste from a lollipop factory to biodiesel with a high FAME(fatty acid methyl esters) content of 96 %.

II. Materials

The lollipop effluent was obtained from a local confectionery factory. Methanol, diethyl ether and chloroform-d of analytical grade were purchased from Associated Chemical Enterprises (ARC), Johannesburg, South Africa. *Candida rugose, Pseudomonas fuorencens, Porcine pancreas* and *Candida antarctica-B* lipases were purchased from Sigma Aldrich, Kempton Park, South Africa.

III. Methods

A. Screening of lipases

Four different lipases were used during the screening process: i.e.,*Candida rugose, Pseudonas fuorencens, Porcine pancreas* and *Candida antarctica-B.* For the screening process a molar ratio of 1: 4 (oil to methanol) was used.

The mixture consisted of methanol (189 µl) , oil (1 g) and lipase (0.1 g ,10 wt%). The temperature was kept constant at 40 ℃ for a reaction time of 6 hours. After the mixture was removed from the oil bath, 2 ml diethyl ether was added to the mixture. The lipase was separated from the product using a centrifugation.

The product was then placed in a 50 ml round bottomed flask and dried at 70℃ for 30 minutes under vacuum. The product was then transferred to a NMR tube and 50 µl chloroform-d solvent was added. The sample was analyzed by ^{1}H-NMR.

B. Molar ratio optimization of lipase catalyzed reaction

Candida antarctica-B lipase was used to determine the best molar ratio. The mixture contained methanol, oil (1 g) and lipase (0.1 g, 10 wt%). The amount of methanol in the mixture was varied. Molar ratios of 1: 1 (47 µl), 1:4 (189 µl), 1:5 (240 µl), 1:6 (285 µl), 1:8 (379 µl) and 1:10 (480 µl) were used in order to determine the optimal molar ratio.

The temperature was kept constant at 40 ℃ for a reaction time of 6 hours. The work up and analyzes of the reactions were done according to the previous protocol.

C. Temperature optimization of lipase catalyzed reaction

Candida antarctica-B lipase was used to determine the best reaction temperature. Amoral ratio of 1:4 oil to methanol was used. The mixture contained methanol (149 µl), oil (1g) and lipase (0.1 g ,10 wt%). The temperatures (30 ° C, 50 ° C and 60 ° C) were varied in order to determind the reactiontemperature with the highest conversion of oil to FAME. Each experiment had a reaction time of 6 hours. The sample was analyzed by ^{1}H-NMR.

D. Two-step methanol addition process for biodiesel production

For the optimization of the reaction time, *Candida antarctica-B* lipase was used. The mixture consisted of methanol (149 µl), oil (1 g) and lipase (0.1 g ,10 wt%).

At 40°C and 1:4 molar ratio of oil to methanol the reaction was carried out for 6 hours. Then methanol (149) was added to the reaction mixture. The reaction was continued for another 6 hours. The sample was analyzed by ^{1}H-NMR.

IV. Results and Discussion

A. Screening of lipases for the transesterification reaction.

Lipases from different sources have been investigated for their transesterification activity. For the screening process *Candida rugosa, Pseudomonas fluorencens, Candida antrarctica-B* and *Procine pancreas* lipases were used. The reaction for each of the lipases were carried out at a molar ratio of 1:4 oil to methanol, 10 wt% of lipase, 6 hours reaction time and a reaction temperature of 40 °C.The conversion of oil to FAME for each lipase and how they compare can be seen in Fig 2.

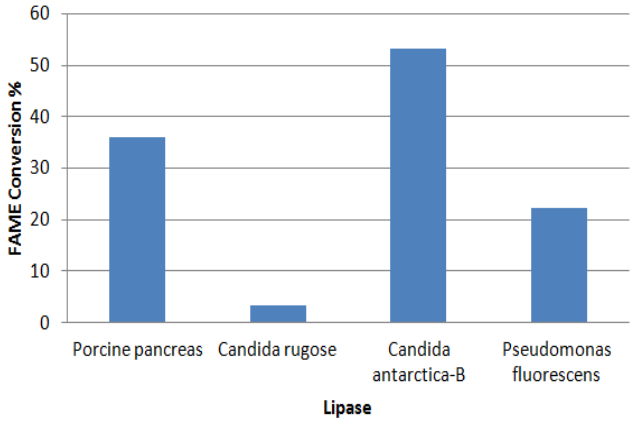

Fig. 2 Conversion of oil to FAME obtained for each lipase screened

As seen from Fig 2 lipases from different sources have different have different activity for biodiesel synthesis. *Candida rugosa, Pseudomonas fluorencens, Candida antrarctica-B* and *Procine pancreas* gave 3.27, 35.95, 53.25 and 22.15 % conversion respectively.

A possible explanation for the variation in results may be due to the different lipase loading or different support. The process to immobilize the *Candida rugose* lipasedecreasesits activity [21]. This explains the low conversion of oil to FAME during the transesterification reaction with *Candida rugose* lipase.

Lipases shows specificity towards the chain length and type of the fatty acids [22].*Candida rugosa* as well as *Procine pancreas* lipases has a preference to short chain fatty acids. It is assumed that the cconfectionary lipid consists out of long chain fatty acids, thus resulting is a low FAME conversion when conducting the transesterification reaction with *Candida rugose* and*Procine pancreas.*

Candida antrarctica-B lipasehas a strong preference towards elaidic acid (trans fat found in hydrogenated oils), whereas*Candida rugosa* prefers oleic acid (fatty acid that occurs naturally in oils) [23].

Pseudomonas fluorencens is more sensitive to inactivation due to the alcohol present in the reaction mixture. *Candida antrarctica-B* lipase is a non-specific lipase that could act on all ester bonds and thus resulting in a high oil to FAME conversion

This concurs with previous studies done to produce biodiesel from cotton seed oil, castor oil, canola oil, sunflower oil and soybean oil using *Candida antrarctica-B* lipases[24]. From Fig 2 it can be seen that the highest conversion was obtained using *Candida antrarctica-B* and thus, all further experiments were done using this lipase.

When determining the economic potential of the enzymatic reaction the productivity calculations of the amount of ester produced per amount of lipase used is essential. As seen in Equation 1does the productivity depend on the FAME yield, number of times the lipase has been reused (N) and the lipase concentration. The productivity is measures in the kg of product produced per kg lipase that has been used [21].

$$Productivity = \frac{Yield\ (\%) * N * 100\%}{Enzyme\ concentration\ (wt\%)} \qquad (1)$$

The productivity for the transesterification reaction with *Candida antrarctica-B*lipase was found to be 533 kg product/ kg lipase. The productivity of NaOH is approximately 10 kg product per kg catalyst [21], thus making lipase catalyst 53 more productive than chemical catalysts.

B. Molar ratio optimization of lipase catalyzed reaction

For the production of biodiesel, short chain alcohols are widely preferred [9]. For the *Candida antrarctica-B* lipase catalysed reaction the molar ratio of oil to methanol was varied in order to determine the optimum molar ratio. The results are shown in Fig 3.

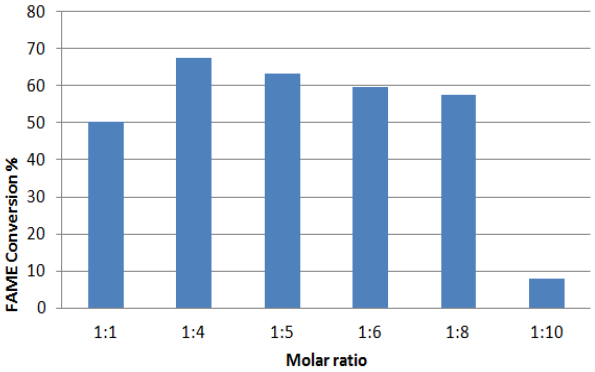

Fig. 3 Optimalization of molar ratio of oil to methanol

A maximum FAME conversion of 67.40 % was found at a molar ratio of 1: 4 oil to methanol. The FAME conversion increased by 17% when the molar ratio was increased from 1:1 to 1:4.

According to stoichiometry a minimum of 1 mole of triglyceride and 3 moles of methanol is required [21]. As seen in Fig. 3 as when the molar ratio exceeded the optimal molar ratio a decreasing trend was experienced. At a molar ratio of 1:10 of oil to methanol a FAME conversion of 7.81 % was found. An excess of methanol in the mixture dilutes the oil and thus reduces the collision frequency of the oil and lipase catalyst [27].

C. Temperature optimization of lipase catalyzed reaction

Temperature has an influence on the reaction rate and the FAME conversion during lipase catalysed transesterification [21]. The optimum temperature is dependent on the oil to methanol molar ratio, type of organic solvent and lipase stability. The effect of the temperature on the immobilized *Candida antrarctica-B* lipasetransesterification reaction was studied within a temperature range of 30 – 60 °C at a molar ratio of 1:4 oil to methanol. The results are shown in Fig. 4.

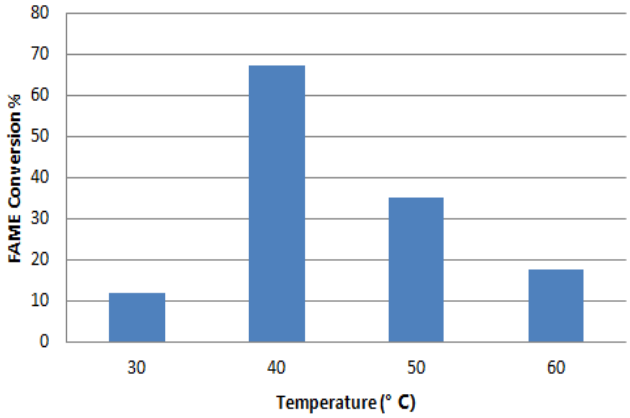

Fig. 4. Optimalization of the reaction temperature

At 30 °C a FAME conversion of 11.88 % was obtained. At this low temperature the lipid remained semi solid, resulting in a low FAME conversion.

The optimum temperature was founded at 40°C. At this temperature a biodiesel yield of 67.4 % was achieved. A lower optimum temperature reduces the energy requirement for the process, thus making it more economically feasible [25].

It was found that at temperatures above 50 °C the activity of the lipase decreases. At 60 °C a conversion of 17.57% was achieved. The decrease of FAME yield is due to the following three reasons: (a) the loss of the solvents (methanol) through evaporation, (b) degradation of substrates and products at high temperatures and (c) increased formation of by-products. At these high temperatures the separation of glycerol becomes more difficult and results in a lower FAME conversion.

D. Twostep methanol addition

The twostep methanol addition reaction took place in the presence of Candida *antrarctica-B* lipase. In order to achieve the highest FAME yield, a stepwise addition of methanol was required. For the total experimental time of 12 hours a FAME conversion of 96.7% was achieved. The FAME yield increased with 29 % with the addition of methanol during the second step.

By adding the methanol to the oil stepwisethe methanol concentration was kept low, therefore eliminating the risk of deactivating the lipase [28].If all of the methanol was added at the start of the 12-hour reaction time period the excess of methanol would block the reaction sites of the lipase and decrease the efficiency of the lipase and thus decrease the FAME yield. The productivity of the transesterification process with a twostep methanol addition, at optimal conditions, was calculated to be 967. Making this reaction 97 time more productive than chemical catalysed processes.

V. Conclusion

Lipid was obtained from a waste lollipop effluent stream and used as a potential feedstock for the production of biodiesel using biocatalyst. The transesterification reaction was conducted using four different types of lipases. It was found that *Candida antrarctica-B* lipase produces the highest FAME yield. For the optimization of the reaction conditions a molar ratio of 1:4 oil to methanol and a temperature of 40° C were found. In order to prevent the inactivation of the lipases, a twostep methanol addition was used to obtain a biodiesel yield

of 96.7 %. The results that were obtained proved that at low reaction time and temperature a high FAME yield can be achieved using waste oil.

This study has shown that an enzymatic approach to biodiesel production is more efficient due to the lipase specificity and selectivity and it consumes less energy due to the mild reaction conditions. Another advantage is that this approach can be considered as environmentally friendly due to the limited release of by-products or waste.

VI. Recommendations

It is recommended to test the reusability of the lipase in order to maximum amount of times the it can be reused. By testing the reusability, the economic feasibility if the process can be optimized.

A round bottom flask is advised to ensure that proper mixing takes place.

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