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Immobilized *Candida antarctica* Lipase Catalyzed Transesterification of *Croton megalocarpus* Seed Oil for Biodiesel Production

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Abstract

Methanolic transesterification of *Croton megalocarpus* seed oil to produce biodiesel was investigated using Immobilized *Candida antarctica* lipase as a catalyst. The reactions were optimized by varying the temperature, amount of methanol and the weight of lipase. The transesterification process yielded 98.71 % biodiesel conversion at optimal conditions of 30 % enzyme (m/m), 50 ^{0}C reaction temperature and oil to alcohol molar ratio of 1:4. Biodiesel from this process had a remarkably high acid value. All the other fuel properties measured were within the range stipulated in the American Society for Testing and Materials (ASTM) and International Standards Organization (ISO) standards.

Keywords: Biodiesel, *Croton megalocarpus*, Transesterification, lipase

1. Introduction

The predicted shortage of fossil fuel coupled with the increase in fuel prices has encouraged the research for other substitutes such as biodiesel. Biodiesel can be defined as the alkyl ester of fatty acids, made by the transesterification of oils or fats, from plants or animals, with short chain alcohols such as methanol and ethanol (Pinto *et al*., 2005). The primary advantage of biodiesel is that it is renewable, non-toxic and biodegradable. The most commonly used catalysts for biodiesel production are sodium hydroxide, potassium hydroxide and sodium methoxide. The disadvantage of these base catalysts is that if biodiesel is prepared with a feedstock high in free fatty acids (FFA), the base reacts with the FFA forming soap. This process leads to loss of the catalyst and the soap will also inhibit later steps during processing such as water washing and glycerol separation. Enzymatic catalysis has an advantage in that even with very high FFA content in the oil a single-step process for biodiesel is feasible. The benefits of an enzyme-catalyzed process (Nielsen *et al*., 2008) can be summarized as follows: Compatibility with variations in the quality of the raw material; fewer process steps; higher quality of glycerol; improved phase separation (no emulsification from soaps) and reduced energy consumption and wastewater volumes. A range of lipases including those from *Candida antarctica* (Kose *et al.,* 2004), *Candida rugosa* (CRL) (Lee *et al*., 2006), *Thermomyces lanuginosa* (TLL) (Du *et al*., 2006) and other esterases have been tested for enzymatic biodiesel synthesis.

In this study the methanolysis of *Croton megalocarpus* seed oil with commercially available immobilized *candida antarctica* lipase as a catalyst was investigated and the optimum conditions were determined by varying the temperature, oil to alcohol ratio and enzyme weight. Most of the literature on enzymatic alcoholysis available report a biodiesel product characterized by its alkyl ester content but do not discuss if the obtained product is within the given specifications (Nielsen *et al*., 2008). This study therefore also aimed at measuring some of the properties of the biodiesel obtained from enzymatic alcoholysis to check whether it meets the standards.

2. Methods

2.1 Materials and Apparatus

Croton megalocarpus seeds were obtained from Machakos, Kenya and their identity confirmed at the herbarium in botany department, Jomo Kenyatta University of Agriculture and Technology. A screw press (Ruian grain and oil machinery factory Zhejiang China) was then used to crush and expel oil from the seeds. The oil was first filtered slowly using filter bags then refiltered again using a vacuum pump to remove any particles. Other reagents used in the experiments were of analytical grade. Enzymatic alcoholysis reactions were catalyzed with immobilized *Candida antarctica* lipase commercially called Novozym 435 which was a gift from Novo Nordisk Industry A/S (Bagsvaerd, Denmark).

2.2 Analytical methods

The acid value and FFA content was calculated using a simple titration procedure (Van Gerpen *et al*., 2004). Kinematic viscosity was measured using a capillary viscometer (No. 38, Kusano Scientific Instrument) immersed in a water bath. Specific gravity was measured using a pycnometer. More fuel properties were measured at Kenya Bureau of Standards (KEBS) Nairobi, using ISO and ASTM test methods.

2.3 Enzymatic transesterification procedure

In this study a modified method of Shimada *et al* (2002) was used. The experiments were done in a solvent free system using a three-step batch methanolysis procedure. The reactions were conducted in conical flasks covered with aluminium foil and agitated in a temperature controlled water bath shaker set at 140 oscillations/minute. 50 g of the vegetable oil together with the appropriate amount of catalyst in a conical flask was first placed in the water bath to attain the required temperature. The amount of methanol to be used for the reactions was divided into three portions and added using a three step procedure. The first addition was done at the start of the experiment, the second after 10 hrs and the last addition was done after a total of 24 hrs. The reaction was continued for another 32 hrs to get a reaction time of 56 hrs in total. After completion of the reaction the contents of the flask were washed with chilled acetone and *Candida antarctica* B-lipase recovered by filtration. The acetone was then evaporated using a rotary vacuum evaporator and the biodiesel placed in a separating funnel for separation of glycerine. The experiments were done in triplicate and the average biodiesel yields obtained. The viscosity, acid value, percentage (%) yield and the specific gravity of the biodiesel obtained were determined.

3. Results and discussion

The crude *Croton megalocarpus* seed oil used for the experiments had an acid value of 7.5 mg KOH/g, FFA content of 3.7 % and viscosity of 28.30 mm^2/s. Enzymatic transesterification reactions were optimized by varying the oil to methanol ratio, reaction temperature and the weight of enzyme used. To investigate the effect of enzyme weight, reactions were performed with 4 % weight of catalyst (reaction 1) based on the weight of the reaction mixture at the start of the reaction (sum weight of methanol and vegetable oil at the start of reaction) and 10 % catalyst based on the weight of vegetable oil while the ratio of oil to methanol was kept at 1:3 (reaction 2). To investigate the effect of increasing temperature and enzyme weight, the reaction was repeated at a temperature of 50 ^{0}C with 30 % enzyme weight based on the weight of vegetable oil and oil to alcohol ratio of 1:3 (reaction 3). To investigate the effect of oil to methanol ratio the conditions chosen were 30 % enzyme weight based on the weight of vegetable oil, 1:4 oil to alcohol ratio and a temperature of 50 ^{0}C (reaction 4). Enzyme reuse was investigated by transferring used lipase recovered from reaction 4 to the first step procedure using fresh vegetable oil (reaction 5). The results are shown in Table 1.

3.1 Effect of enzyme weight on transesterification of Croton megalocarpus seed oil

Enzymatic catalysed transesterification carried out at 30 ^{0}C and using *Candida antarctica* B-lipase (CALB) 4 %

weight of the total reaction mixture gave a product that showed an acid value of 6.39 mg KOH/g which was lower than that of the crude vegetable oil, 7.5 mg KOH/g. This was consistent with observations made by Shimada *et al* (2002) while transesterifying waste edible oil using the same enzyme and enzyme weight equivalent to 4 % of the total reaction mixture. As expected the viscosity value of the product was much lower than that of the vegetable oil. Increasing the enzyme weight from 4 % weight of catalyst based on the weight of the reaction mixture at the start of the reaction to 10 % catalyst based on the weight of vegetable oil and retaining the other conditions, the resulting transesterified product gave a slightly lower viscosity value 6.61 mm^2/s than the viscosity 7.14 mm^2/s of 4 % wt. However, the acid value was found to increase by over 100 % for 10 % wt enzyme and over 200 % for 30 % wt enzyme. Esterification seems to have occurred together with hydrolysis in the reactions. In the reactions with 10 % wt enzyme and 30 % wt enzyme, glycerine separation was not visible and the viscosity was high suggesting that the biodiesel mixtures probably contained high amounts of triglycerides, monoglycerides and diglycerides in addition to methylesters. This was again consistent with observations made by Kose *et al* (2004) while studying transesterification of cotton seed oil using *Candida antarctica* B-lipase (CALB) enzyme as catalyst. Thus it was observed that the acid value of biodiesel increased with increase of enzyme quantity.

3.2 Effect of increasing enzyme weight and temperature on transesterification of Croton megalocarpus seed oil

Increasing the temperature and enzyme weight to 50 ^{0}C and 30 % of the weight of oil while leaving oil to methanol ratio at 1:3 lead to an increase in the acid value to 21.74 mg KOH/g. However, the viscosity was lowered to 6.23 mm^2/s indicating an increase in the ester content of the biodiesel obtained.

3.3 Effect of oil to methanol ratio on transesterification of Croton megalocarpus seed oil

Changing the oil to methanol ratio from 1:3 to 1:4 and carrying out the process at 50 ^{0}C gave biodiesel with a viscosity of 4.7 mm^2/s which indicated a further increase of the methylester content of the biodiesel obtained. This is in agreement with Kose *et al* (2004) who in an experiment using cotton seed oil and the same enzyme, obtained optimum transesterification conditions of 1:4 oil to alcohol ratio, 30 % enzyme based on the weight of oil, and a temperature of 50 ^{0}C. This seemed to be the optimum conditions for the lipase. A clear and colorless glycerine layer was also observed.

3.4 Effect of using reused enzyme on transesterification of Croton megalocarpus seed oil

When used enzyme was reactivated using chilled acetone and applied under optimum conditions, the biodiesel obtained had a viscosity value of 4.17 mm^2/s and acid value of 13.69 mg KOH/g. The enzyme seems to have been conditioned from the previous experiment. A clear and colorless glycerine layer was also observed in this experiment.

3.5 Measurement of fuel properties

A batch experiment to determine more fuel properties was setup and the tests were carried out at Kenya Bureau of Standards (KEBS) Nairobi, using ISO and ASTM test methods. The results are shown in Table 2. The results show that the process of transesterification greatly reduces the viscosities of the vegetable oil which leads to improved fuel properties of the *Croton megalocarpus* seed oil. The flash point for the fuel measured was above 150 ^{0}C indicating that no residual alcohol was left after processing and hence it is safe to use as there is no risk of explosion. It can be seen from the results that the fuel is not corrosive to copper indicating very little effect on corrosion of the engine. All the other properties measured which include: kinematic viscosity, density and Astm colour meet the particular ASTM or ISO standard.

5. Conclusion

Enzymatic transesterification was successful considering the viscosity (4.74 mm^2/s) of the biodiesel product obtained at the optimum conditions of 30 % enzyme amount, temperature 50 ^{0}C and oil to alcohol ratio 1:4. Enzymatic

process also gave high yields. A clean and pure glycerine layer was also observed. The only drawback of the enzymatic transesterification at optimum conditions is the high acid value (13.86 mg KOH/g) of the biodiesel obtained which was not within the ASTM standard of a maximum of 0.8 mg KOH/g. However, the excess free fatty acids in the biodiesel can be removed as soaps by use of caustic stripping.

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Table 1. Enzymatic transesterification of *Croton megalocarpus* seed oil

Reaction	Enzyme amount	Temperature (^{0}C)	Oil to methanol ratio	Yield (%)	Viscosity (mm^2/s) (40 ^{0}C)	Specific Gravity (40 ^{0}C)	Acid Value (mgKOH/g)
1	4 wt. %	30 ^{0}C	1:3	97.64±1.63	7.14±0.13	0.90±0.01	6.39± 0.49
2	10 wt. %	30 ^{0}C	1:3	98.47±0.97	6.61 ±0.26	0.88±0.00	14.81±0.67
3	30 wt. %	50 ^{0}C	1:3	98.87±0.82	6.23 ±0.21	0.89± 0.00	21.74± 0.56
4	30 wt. %	50 ^{0}C	1:4	98.98±0.38	4.74 ±0.49	0.89± 0.00	13.86± 0.42
5	30 wt. %	50 ^{0}C	1:4	98.71±1.07	4.17 ±0.07	0.88 ± 0.00	13.69± 0.39

(1:4 oil to methanol ratio = 7.29 g methanol, 1:3 oil to methanol ratio = 5.46 g methanol)

Table 2. Fuel properties measurement

Property	Method	Apparatus	limits	CMEE*
Kinematic viscosity at 40 ^{0}C (mm^2/s)	ISO 3104	Automatic viscometer (HMV 472 HERZOG)	3.5-5.0	4.32
Astm colour	ASTMD 1500	Tintometer (Lovibond PFX880)	Max 3.5	1.9
Copper strip corrosion (3 h at 50 ^{0}C rating, Max)	ISO 2160	Air oven (Memmert)	Class 1	No tarnish
Density @15 ^{0}C (kg/m^3)	ISO 12185	Density meter (DMA4500)	860-900	891
Flash point ^{0}C, Min	ASTMD 93	Pensky Martens closed cup tester	130 Min	>150

*Biodiesel from enzymatic process using *Croton megalocarpus* seed oil, 7.29 g methanol (1:4 oil to alcohol ratio), 15 g enzyme (30 % enzyme based on the weight of oil) and 50 ^{0}C temperature.

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