Production of biodiesel from macaw palm (Acrocomia aculeata) oil with high acidity employing hydroesterification process

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Abstract:

The hydroesterification process has been investigated as an alternative to alkaline transesterification. This process consists of two associated steps, the hydrolysis step in which the triacylglycerol molecules are hydrolyzed to the respective acids and then the esterification step in which the fatty acids obtained in the first step are esterified with the desired alcohol. In this context, this work shows the results related to the enzymatic hydrolysis step of the macaw pulp oil using the enzyme extract from castor bean seed and subsequent esterification using HPW/Nb_2O_5 catalyst. The results showed that the enzymatic hydrolysis using enzymatic extract presented conversions of 45.81% in the hydrolysis of macaw pulp oil resulting in a hydrolyzate with 83% of free fatty acids (FFA) in 4h. The tests were performed using reaction conditions of 35°C, pH 4.5 and shaking of 1000rpm. The esterification step employing HPW/Nb_2O_5 showed conversions of 97% under the reaction conditions of 250°C, stirring of 700rpm, hydrolyzed ethanol ratio of 1:40 and 15% of the catalyst.

Keywords: Biodiesel, hydroesterifacion, hydrolysis, esterification, macaw oil

1. Introduction

Energy is currently considered the main vector of socioeconomic development.

The growing use of energy sources and the concern for environmental issues has led to new research into fuels derived from renewable and clean energy sources, such as biodiesel, which enables increased energy capacity in conjunction with sustainable economic development (Ramos, 2003).

Biodiesel is an alternative fuel consisting of alkyl esters, non-fossil, renewable, non-toxic and with excellent lubricating properties, which can totally or partially replace petroleum diesel in diesel cycle engines (Knothe, 2010, Santacesaria *et al*., 2012). This biofuel can be produced from the processing of oils and fats by transesterification reactions or the esterification of fatty materials and, despite the rapid growth of its use in the market, it still presents high production costs related to both the industrial process and the cost of the raw material. In addition, this process requires oils of low acidity, which prevents the use of a variety of low-quality oils.

Hydroesterification (hydrolysis followed by esterification) has been studied as a technological alternative to produce biodiesel because it has the possibility of working with raw materials of high acidity and humidity (Aranda, 2008). The advantage of hydroesterification over transesterification is that the free fatty acid is a reactant and, therefore, is not a limitation in terms of raw material specification (Di Serio *et al*., 2008). This allows the use of raw materials of high acidities, such as macaw pulp oil.

Some researches have been carried out and show that the hydroesterification process can be used successfully to obtain biodiesel, among them the work done by Díaz *et al*. (2013) in which niobium oxide was used in both the hydrolyzing and the esterifying phases. Another important study used biocatalysis in both phases, obtaining a conversion of up to 98% in biodiesel with a total reaction time of only 6h (Taludker *et al*., 2014). Other works varied the hydrolysis and esterification processes and the raw materials used, all with a minimum of 90% yield (Soares *et al*., 2013; Cavalcanti-Oliveira *et al*., 2011; Chen *et al*., 2010).

The great advantage of the process under study is that it enables the production of biodiesel from high acid oils with a relatively inexpensive process, because it uses, in the hydrolysis step, a catalyst from a natural and very available source, the seeds of Castor bean. A possible disadvantage may be the cost of the catalyst used in the esterification phase, however, this can be overcome by its regeneration and the reduction of the cost due to the scale-up of the process.

The objective of this work was to study the production of biodiesel through the hydroesterification process using macaw pulp oil. In the first step of the process, the oil of macaw pulp was hydrolyzed using low-cost enzymatic extract obtained from castor bean seeds. In the second step, the esterification reaction, HPW/Nb_2O_5 was used as a heterogeneous catalyst to produce ethyl esters from the concentrate of fatty acids obtained in the first stage.

Compared to other studies reported here, it has the advantage of using a low-cost catalyst and showing positive results for an oil whose species is very abundant throughout South America, is rarely used for this purpose and does not compete with the chain of food production.

2. Material and Methods

a. Material

Castor bean seeds were acquired from BRSeeds Ltd (Araçatuba,SP, Brazil). Macaw palm oil with high free fatty acid content was supplied by Association of Small Farmers D'Antas (Minas Gerais, Brazil). Hydrated niobium oxide HY340 (amorphous) with high surface area (BET ~170 ^{2}/g) containing 80% Nb_2O_5 was supplied by *Companhia Brasileira e Metalurgia eMineração* - CBMM and calcinated at 300°C for 3h before use. Anhydrous ethanol (98.0%) were purchased from VETEC®Sigma-Aldrich. Anhydrous sodium sulfate, Acetone, and hexane (65.0%) were supplied by Cromoline.

b. Preparation of the enzyme extract from castor oil

The preparation of the enzyme extract from castor bean was carried out according to the methodology described by Avelar *et al*. (2013). Initially, the endosperm tissues were carefully removed and the shells of the seedling discarded. The endosperms (20g) were triturated in a mixer during 10 min by adding chilled acetone (5mL). The samples were mixed with chilled acetone (ratio 1:5w/v) under stirring at 150 rpm and 4ºC for 15 min (PIEROZAN *et al*. 2009). The suspension was filtered under vacuum via a Buchner funnel and washed with chilled acetone. The delipidated extract prepared was sieved to obtain average particles size of 75–90μm. The product was defined as crude enzymatic extract and used to catalyze the hydrolysis of the macaw pulp oil.

For the determination of the hydrolytic activity, an emulsion was prepared with 15g of olive oil, 45g of gum arabic solution 30g/L. 0.1 g of enzyme extract from castor oil, 5 ml of emulsion, 5 ml of phosphate buffer pH 7.0 (0.01 mol/L) were then added in 125 ml erlenmeyers. The vials were incubated at 37 ° C for 5 minutes in a shaker with the constant shaking of 150 rpm (AVELAR *et al*.,2013). After the incubation period, the reactions were stopped with the addition of 10 mL of a 1: 1 solution of ethanol and acetone. The fatty acids released during the reaction were titrated with 0.025 mol/ KOH standard solution, using phenolphthalein as indicator. Calculations were performed by Equation 1 and one unit of activity was defined as the amount of enzyme that releases 1 μmol of fatty acid per minute of reaction under the assay conditions (37ºC, 5 min). Activities were expressed in units of activity per gram of lipase. (U/g)

$$Activity = \frac{(V_A - V_B).M.1000}{t.m} \quad (1)$$

c. Hydrolysis reactions

The hydrolysis reactions of macaw oil were conducted in 100 mL jacketed cylindrical glass reactors containing 50 g of reaction media maintained at 35 ºC, pH 4.5, under mechanical stirring at 1000 rpm for 6 hours. In this stage, the raw material type (macaw almond oil and macaw pulp oil) was evaluated in different concentrations of oil in the reaction medium (25%, 35% and 50% w/w), as well as the influence of acidity of the oil in the enzymatic hydrolysis using the castor bean enzyme extract. The course of the hydrolysis was checked by the removal of aliquots of approximately 0.50g. Fatty acid concentrations were quantified by titration with 0.025 mol/L KOH standard solution and phenolphthalein indicator. The percentage of free fatty acids at the end of the reaction (FFAfinal) was calculated according to Equation 2. To determine the percentage of hydrolysis, Equation 3.

$$FFA_{final}(\%) = \frac{V \times 10^{-3} \times M \times MM}{m \times f} \times 100 \quad (2)$$

$$\%Hidrolysis = \%FFA_{final} - \%FFA_{start} \quad (3)$$

Where: V is the volume of KOH solution used in the titration; M is the molar concentration of the KOH solution (mol/L); MM is the mean molar mass of macaw oil fatty acids (g/mol); m is the mass of the withdrawn sample (g) and f is the fraction of oil in the substrate solution.

At the end of the reactions, 50 mL of hexane: acetone solution (1: 1) was added to the reaction medium. The reaction medium was then centrifuged at 5000rpm for 20min. The aqueous phase was discarded and the organic phase was concentrated in a rotary evaporator at 70°C for about 30 min.

d. Catalyst preparation

The catalyst (HPW/Nb_2O_5) was prepared by the incipient-wetness impregnation method. For impregnation, the phosphotungstic acid (HPW) was dissolved in 70% w/w alcoholic solution at room temperature, transferred to a melting pot containing the support (Nb_2O_5) and mixed with the aid of a spatula. The solid was then oven dried at 100° C for 30 min and then calcined at 300°C for 1 h. This step was repeated two more times to obtain an impregnation of 30% of the acid support. At the end of the third impregnation, the catalytic solid was dried at 100°C for 1h, followed by calcination at 300°C for 4h. The catalyst was thermally activated at 110°C for 2 h before it was employed in the esterification reactions.

e. Esterification reactions

The esterification reactions were performed in a Parr Series 5000 stainless steel reactor equipped with electric heating, temperature control, and pressure indication. The reaction time was 2h at 700rpm agitation, the reaction volume was 50mL. The reaction conditions studied were temperature (150°C, 200°C, and 250°C) and hydrolyzate/ethanol molar ratio (1:20, 1:40 and 1:60); the catalyst concentration was 15%.

After the esterification reaction, the reaction medium was centrifuged for 15min at 1570rpm, after centrifugation the supernatant was washed with a 100ml aliquot of warm water and was taken to the separation funnel for 24h. After this period, the procedure was repeated for another 3 times. The reaction mixture was then roto-evaporated at 70°C to remove the residual ethanol.

The esterification reactions were quantified by titration employing KOH (0.1 mol/L) and the conversion to ethyl esters was calculated by Equation 4.

$$\%EEC = \left(1 - \frac{IA_{final}}{IA_{hidrolizado}}\right) \times 100 \qquad (4)$$

Where: EEC is the conversion of ethyl esters; IAfinal is the acid value of the reaction medium after purification, IA hydrolyzate is the acid value of the hydrolyzate

3. Results and Discussion

a. Influence of the lipid raw material on enzymatic hydrolysis:

To study the influence of the type of lipid raw material in the enzymatic hydrolysis using the enzymatic extract from the castor bean, the oils from the almond and from the pulp of macaw were used. The reactions were conducted without an emulsifying agent, using oil to water mass concentration of 35% and 288 units of activity. According to the results shown in Table 1, it was verified that there was no difference in the percentage of hydrolysis in the two lipid sources, both raw materials reached hydrolysis conversions around 44%. However, as the percentage of free fatty acids (final FFA%) accumulated in the macaw pulp oil was 70%, this oil was chosen for the other tests.

b. *Influence of the different concentrations of pulp oil in the reaction medium*

To evaluate the influence of the oil mass concentration on the hydrolysis reaction, the mass concentrations of 25, 35 and 50% (w/w) were chosen. As can be seen in Figure 1a, with 25% of oil in the reaction medium, a conversion of 57.1 ± 0.8% was achieved, with 35% of the oil the conversion was 44.5 ± 0.9% and with 50% of the oil, the conversion was only 20.0 ± 0.8%. From these results, it can be concluded that the mass concentration of the oil had a significant effect on the hydrolysis, and with the increase of this concentration, there was a decrease in the conversion of fatty acids. Similar results were reported in the literature by Avelar *et al*. (2013) that evaluated the influence of the canola oil mass concentration on the hydrolysis. The reaction conditions were: pH 4.5 (acetate buffer), stirring at 1000 rpm and temperature of 25°C. The mass ratios ranged from 22.1 to 50% (oil: water). The results reported by the authors demonstrated that an increase in the mass concentration up to 30% led to increases in the conversion of free fatty acids. However, with mass concentrations above 30%, there was a decrease in conversion, since high concentrations of oil lead to the aggregation of droplets to form larger droplets, which worsens lipase contact with the substrate. Based on the results obtained, the mass ratio chosen to proceed with the work was 25% of macaw pulp oil.

c. Influence of acidity on enzymatic hydrolysis

To evaluate the influence of the acidity of the pulp oil on the hydrolysis, macaw pulp oils with different degrees of acidity were used. In Figure 1b it is observed that the acidity influenced negatively the conversion of fatty acids. It is possible to realize that as the acidity of the macaw pulp oil increased the conversion to fatty acids decreased. Table 2 presents the initial free fatty acid percentage, the conversion, and the percentage of free fatty acid at the end of the hydrolyzate, it is possible to notice that the increase of the acidity causes, in addition to the conversion drop, a decrease in the percentage of free fat acid in the end. Bressani *et al*. (2015) reached 100% conversion after 110 min of reaction, but the oil of macaw pulp used had an acidity of 9.3mgKOH / g. These results suggest that acidity is a limiting agent of the hydrolysis step using the enzyme extract from the castor bean.

d. Esterification reaction

Effect of temperature on the esterification reaction

Analyzing Figure 2a it is possible to notice that the temperature influenced the esterification reaction and the maximum ethyl ester conversion (EEC) was 97% and it was obtained when the reaction temperature reached 250ºC. The effect of temperature on ester esterification is reported by many researchers as important mainly when it regards to heterogeneous catalysis. High temperatures can accelerate the reaction rate and decrease the mass transfer limitation between the reagent and the catalyst.

Tabel 1. Percent hydrolysis of pulp and macaw almond oils

Raw-material	$\%FFA_{start}$	$\%FFA_{final}$	% Hydrolysis
Macaw almond oil	16	59,0±0,7	43,5±0,8
Pulp macaw oil	25	70,0±0,8	44,5±0,9

Tabel 2. Percent hydrolysis and percentage of free fatty acids in the final hydrolyzate ($\%FFA_{final}$)

Acid Value (mgKOH/g)	$\%FFA_{start}$	%Hydrolysis	$\%FFA_{final}$
63	25	56	83
72	36	40	76
98	47	25	72
118	56	11	67

In addition, they reduce the possibility that the water molecules formed during the reaction remain on the surface of the catalyst and possibly bind to the active sites, these water molecules are difficult to remove even when the catalyst is washed and regenerated (LIU *et al.*, 2014)

Almarales *et al*., (2012) state that high temperatures result in high ester conversion rates. The conversion of esters tends to increase rapidly at the beginning of the reaction and then, it becomes slow until a plateau is formed indicating that the reaction has reached maximum conversion. These authors studied the influence of temperature, molar ratio and catalyst (Nb_2O_5, Nb_2O_5/Al_2O_3) on esterification of the hydrolyzate of the oil obtained from *Nannochloropsis oculata*. The results showed that at temperatures of 200°C and 1:3 molar ratio (methanol), the reactions reached 92.24 and 87.43% conversion using 20% of Nb_2O_5/Al_2O_3 and Nb_2O_5 respectively.

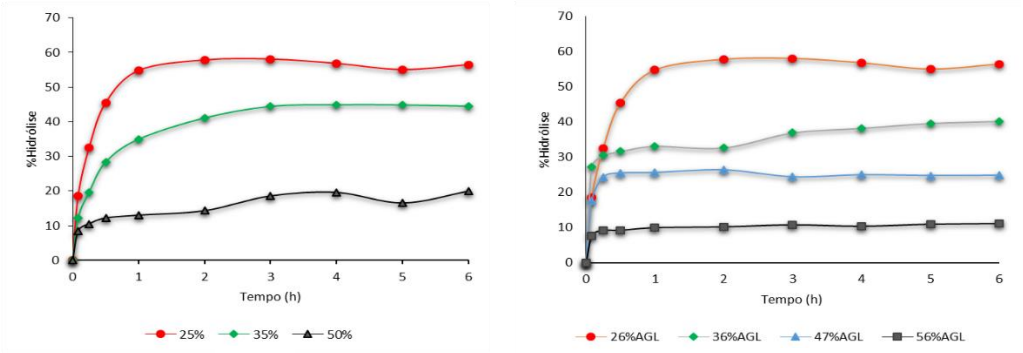
Effect of molar ratio on the esterification reaction

The esterification is a reversible reaction, and thus, high conversions are obtained if the reverse reaction rate is minimized. To solve this problem, two measures can be taken: (1) simultaneously removing the water from the product or (2) using the excess of one of the reactants (ethanol). In this system, it is not possible to remove the water because the reactor is closed. Thus, the second option was applied. The reaction conditions of this stage of the study were the temperature of 200ºC and 15% of the catalyst. Observing Figure 2b it can be concluded that an increase in the molar ratio caused a decrease in the conversion of ethyl esters, thus, the 1:40 molar ratio is suitable for esterification, since when the temperature was 250 ° C the maximum conversion of 97% was obtained.

Torpecêlo *et al*. (2010) evaluated the effect of the molar ratio on the esterification of palmitic acid with methanol using as catalyst tungtophosphoric acid immobilized in SBA-15. They used molar ratios 1: 6, 1:31, 1:63 and 1:95 in their studies. It was found that an increase in the molar ratio from 1:6 to 1:95 resulted in a conversion increase of 88 to 96%. However, by comparing the intermediate ratios, at 1:31 and 1:63 the conversions were 92 and 94% respectively, resulting in a slight increase in ester conversion.

4. Conclusion

According to the experiments carried out, it can be considered that the hydroesterification process (hydrolysis followed by esterification) using an enzymatic extract from castor bean seed as a catalyst in the hydrolysis step and chemical catalysis in the esterification step is an alternative to the conventional process of production of biodiesel.



(a) (b)

Figure 1. Enzymatic hydrolysis using enzyme extract from castor bean; (A) Influence of the mass concentrations of pulp oil in the reaction medium; (B) Influence of the acidity of the pulp oil in the reaction medium

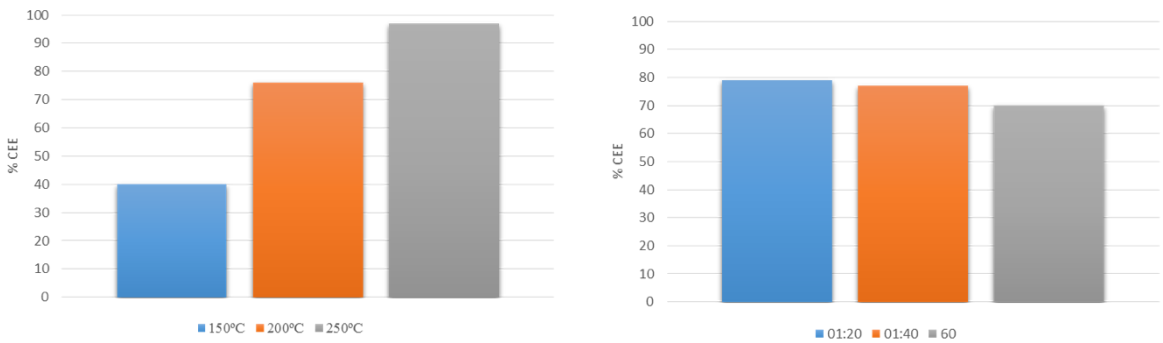


Figure 2. Conversion of ethyl esters from the esterification reaction employing HPW/Nb_2O_5; (A) Influence of temperature on the esterification reaction; (B) Influence of the molar ratio on the esterification reaction

The application of low-cost crude enzyme extract prepared from dormant castor seeds in the hydrolysis of vegetable oil is an economically attractive strategy for the production of fatty acid concentrates since it reached a conversion of 82%. For this purpose, the reaction conditions used were 35ºC in sodium acetate buffer pH 4.5, 25% oil-water mass concentration and 288U under mechanical stirring at 1000 rpm for 2 hours of reaction.

In the esterification step, conversions of 97% were achieved when using molar ratios of 1:40 and temperature of 250°C.

So, hydroesterification, as it was reported in this study, can be a promising solution to deal with high acid value oils, to produce biodiesel.

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