EVALUATING THE OPERATING CONDITIONS OF ENZYMATIC HYDROLYSIS OF DEGUMMED SOYBEAN OIL

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ABSTRACT

There has been an increasing number of studies on enzymatic hydrolysis over the last few years, given that unrefined vegetable oils are capable of being used with the aim of producing fatty acids and glycerol, in addition to its potential application as a new biodiesel production route, where hydrolysis would function as an intermediate reaction in the process. Thus, the present work aimed to evaluate important parameters in the hydrolysis process of degummed soybean oil via the enzymatic route. Evaluated parameters were temperature, agitation, conversion profile, selected enzyme and enzyme loading and operational stability. Results revealed that the enzymatic catalysis of *Pseudomonas fluorescens* (PFL) alone was better than that mixed with porcine pancreas lipase (PPL). Optimal temperature was 35 °C, stirring at 700 rpm and the time taken for maximum conversion (˜90%) was 24 hours. Under these conditions, the catalyst was reused for 6 consecutive cycles and average productivity of 5.95 mmol of fatty acids per hour was reached. Results were rather promising, considering that they provided productivity comparable to that obtained with immobilized enzymes.

Keywords: Enzymatic hydrolysis. Degummed soybean oil. Biodiesel. Lipase.

1 INTRODUCTION

Industrial biodiesel production is carried out by homogeneous transesterification using an alkaline catalyst from vegetable oils with methanol. However, this route is disadvantageous due to the need to use refined vegetable oil with low acidity (< 5%) as raw material and the formation of a by-product (glycerol) containing several impurities, therefore restricting its use and having low commercial value[1].

Biodiesel production through hydroesterification consists of two steps: initially, an enzymatic hydrolysis of all glycerides (mono-, di- and triglycerides) contained in the oil is carried out, and fatty acids and glycerol are formed therefrom. Fatty acids are then reacted with alcohol to produce biodiesel. This route allows using oils having lower degree of purity, in addition to generating glycerol with a high degree of purity and, consequently, having greater commercial value[2].

The possibility of using an oil with a lower degree of purity offers a great advantage to the process since, according to the Energy Research Office (EPE in Brazilian Portuguese), 80% of the total costs of biodiesel production are associated with raw material purchase[3], thus justifying the choice of using degummed soybean oil in the hydrolysis stage of the present work.

2 MATERIAL & METHODS

Temperature assessment: Degummed oil hydrolysis was carried out at 30, 35 and 40 °C using LPF Pseudomonas fluorescens lipase) at enzyme load of 50 U/g of oil in an oil:water ratio of 1:0.5 (m/m), stirring at 450 rpm for 1 h of reaction. Afterwards, 10 ml of ethanol was added to stop conversion and three drops of 1% (w/v) phenolphthalein were used for titration with 0.02 M NaOH. The blank was prepared under the same conditions without adding the enzyme. All experiments were performed in duplicates using a jacketed glass reactor.

Conversion was calculated according to eq. (1):

$$Conversion(\%) = \frac{V_{NaOH} \cdot C_{NaOH} \cdot MM_{KOH} \cdot 1000}{IS \cdot M_{Oil}} x100 \quad (1)$$

Where V_{NaOH} is the volume (mL) of base used to titrate the sample minus that utilized for titrating the control; C_{NaOH} is the base concentration in mol/L, MM_{KOH} is the molecular mass of KOH (g/mol), IS is the saponification index of degummed soybean oil (mg_{KOH}/g of oil) and M_{Oil} is the mass of degummed soybean oil (g) used in the test.

Agitation evaluation: Degummed oil hydrolysis was carried out at 450, 750 and 1000 rpm using PFL at enzyme load of 50 U/g of oil in an oil:water ratio of 1:0.5 (m/m) at 35°C. The procedure for calculating conversion was the same as that described above.

Conversion profile was calculated as a function of time. Selected conditions used were: oil:water ratio of 1:0.5 (m/m) at 750 rpm using PFL and enzyme load of 50 U/g of oil at 35°C. Aliquots of 1 mL were removed for titration at 0; 0.5; 1; 3; 6; 12; 24 and 30 hours.

Evaluation of enzyme and enzyme load: The use of PFL alone (100 U/g of oil) and a mixture of enzymes, PFL (50 U/g of oil) and Porcine pancreas lipase (PPL, 50 U/g of oil) were evaluated along a period of 30 hours. Selected conditions as well as the time taken for removing samples were described above.

Operational stability: The catalyst was reused for six consecutive 24h-cycles. The light phase that remained at the end of a batch was removed and the heavy phase, which was the one containing the enzyme, water and glycerol, was kept into the reactor. Then, a new load of oil was prepared and a new batch was started. Selected conditions were: oil:water ratio of 1:0.5 (m/m) at 750 rpm using PFL and enzyme load of 100 U/g of oil at 35°C.

3 RESULTS & DISCUSSION

The results from the evaluation of temperature and agitation are shown in Figure 1. Conversion values were very close at 35°C and 40°C, therefore 35°C was adoted for further assays. However, regarding agitation, no difference was observed in the evaluated values. As for subsequent studies, there was an increase in reactor volume, an average agitation value (750 rpm) was adopted in order to ensure the generation of a homogenous solution.

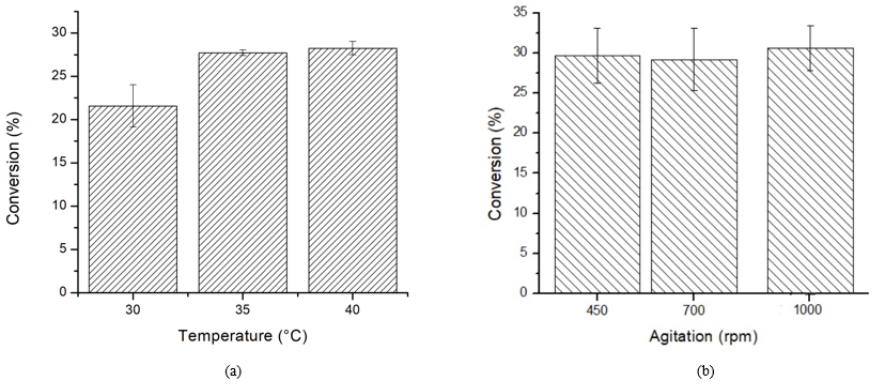

Figure 1 Results from the conversion of enzymatic hydrolysis reactions at (a) different temperatures and (b) different agitations using PFL and enzyme loading of 50 U/g of oil in a oil:water ratio of 1:0.5 (m/m) , T =35°C for 1 h of reaction.

The results found for the conversion profile as a function of time using only PFL at two different enzyme loads and a mixture of PFL and PPL are shown in Figure 2. PFL is a non-specific enzyme and allowed hydrolizying 94.19% ± 4.79 of the oil within 24 hours using 50 U/g of oil. However, when there was an increase in the amount of PFL load (100 U/g of oil), there was no increase in the hydrolysis yield , and the two curves were very close.

Therefore, in order to increase the hydrolysis yield of the oil, two enzymes were used in the same reaction. It was observed that the mixture of enzymes brought no advantage to the process, leading to the same hydrolysis yield within 30 hours of reaction, thus not justifying its use.

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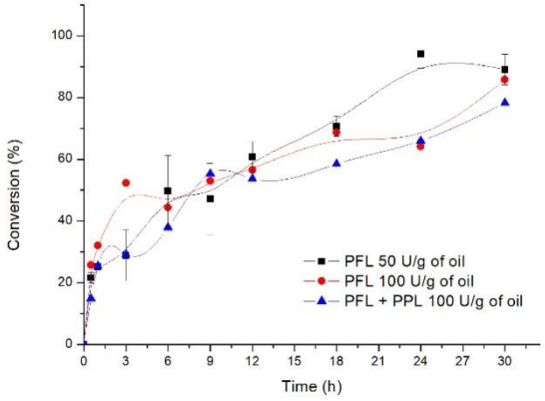

Figure 2 Conversion profile over time using PFL and PFL + PPL and different enzyme loads in an oil:water ratio 1:0.5 (m/m) at 35°C and 700 rpm.

Although it was previously demonstrated that enzyme load has not significantly altered reaction conversion, it was opted to use 100 U/g of oil to prevent conversion from being hampered by possible losses of enzyme the moment the light phase was removed.

Figure 3 shows the achieved results. In total, six batches were carried out, and average productivity between batches of 5.95 mmol/h was reached, but there was a slight loss of activity in the last three batches.

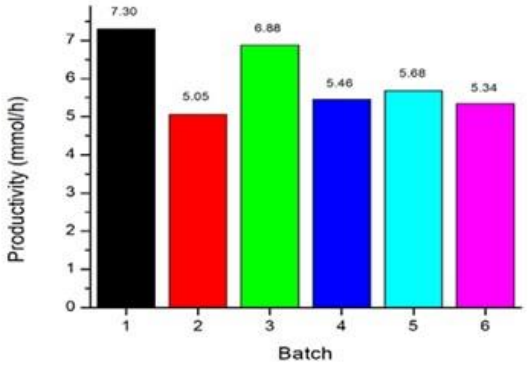

Figure 3 Productivity in mmol/h of fatty acids for each batch. Tests were carried out at 35°C, 700 rpm and 24h.

4 CONCLUSION

These results allowed obtaining more suitable parameters for the hydrolysis reaction of degummed soybean oil with a soluble enzyme. Parameters were: agitation at 700 rpm, 35°C, PFL (100 U/g of oil), oil ratio of 1:0.5 (m/m) and reaction time of 24 hours. Furthermore, in operational stability tests, it was possible to reuse the soluble enzyme for 6 consecutive cycles without great loss of activity, thus demonstrating that it is possible to use (and reuse) a soluble enzyme in the hydrolysis stage.

Thus, the present work showed that the use of the enzymatic hydrolysis reaction in the first stage of biodiesel production by hydroesterification was very advantageous, as it allowed the use of a cheaper raw material (degummed soybean oil), in addition to the use of soluble enzymes having lower market value when compared to immobilized enzymes and a by-product with high added value (glycerol) was generated, therefore contributing to the economic viability of the process.

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