Application of immobilized Lipura® Flex lipase in the interesterification reaction of waste oils to obtain biodiesel

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ABSTRACT

Fossil fuels are responsible for high emission of pollutants, which motivates the search for alternative fuels. Among these, biodiesel stands out as a biofuel obtained from renewable sources, like vegetable oils and animal fats. Enzymatic catalysis for biodiesel production using low-cost oil, has attracted much attention recently. Enzymatic interesterification is a process that can uses waste oils as substrate. The use of this feedstock is of great interest, since its disposal is often incorrectly made, which causes damage to the environment. In this context, the objective of this work is the synthesis of biodiesel by enzymatic interesterification of waste oil and methyl acetate, with immobilized lipase Lipura® Flex. For evaluation of the conversion of the methyl esters a CCD was performed. Independent variables were temperature (40-60°C), molar ratio of oil: methyl acetate (1:5-1:15) and enzyme concentration (7-13% w/w). The highest ester concentration was obtained with molar ratio 1:15, 13% of enzyme and 60°C with conversion of 52.2%. The results showed that using waste oils in enzymatic interesterification are viable alternative for biodiesel production because it allows cost and environmental impact reduction.

Keywords: Lipase. Lipura® Flex. Biodiesel. Interesterification. Methyl ester.

1 INTRODUCTION

Biocatalysis for biodiesel synthesis using low-cost, high-acid and lower-grade waste oils have attracted much attention due mainly to economic issues and sustainable benefits. Production costs is also reduced, since the raw material can represent more than 75% of the overall biodiesel synthesis costs, still avoids the incorrect disposal of waste oils, which has a major impact on the environment[1]. Among the benefits of enzymatic catalysts are very tolerant to high acid and water contents present in waste oils, and the fact avoiding the typical soap formation due to alkaline catalysis.

Lipases, among the enzymes available, are those that catalyze the hydrolysis of triglyceride ester bonds. Due to their great structural and functional versatility, lipases are of great importance for industrial applications in various sectors, such as food, pharmaceuticals, detergents, textiles, biodiesel production and cosmetics[2].

The lipases used to obtain biodiesel are more expensive than conventional acid and basic catalysts. However, the emphasis on the use of lipases is due to their high catalytic efficiency, lower waste generation in processes and low environmental impact. It can also be said that when immobilized, the immobilization method can improve their stability and facilitate their reuse in the bioprocess[3].

Due high cost of enzymes, reusability is a highly desirable characteristic. Chemical bonding or physical entrapment of enzymes in supports facilitates separation from the reaction medium, favoring reuse[4]. In biotechnological synthesis, the use of immobilized to replace soluble enzymes facilitates the recovery of the reaction's bioproducts[4].

Biodiesel is a renewable fuel produced from vegetable oils or animal fats, which burns more cleanly than fossil fuels. Currently, the conventional method for obtaining industrial biodiesel is via chemical transesterification, using chemical catalysts[5]. In transesterification, triacylglycerols present in oils and fats react with a short-chain alcohol in the presence of catalysts, generating esters and glycerin[6]. An alternative, less explored method for obtaining biofuels is interesterification, which consists of the reaction between two esters, thus resulting in the formation of biodiesel and triacetin[7]. In interesterification, one mole of triacylglycerol reacts with three moles of a short-chain ester, leading to the formation of three moles of fatty acid esters and one mole of triacetin. The interesterification reaction has the advantage of not generating glycerin as a by-product of the reaction, eliminating the need for additional purification steps and generating triacetin, a good biolubricant[7].

In this context, this study sought to verify the best conditions for the interesterification reaction of waste oil (OR) and methyl acetate, using central composite design (CCD). A little-explored lipase (Lipura® Flex) was used as a biocatalyst.

2 MATERIAL & METHODS

Methyl acetate (99.8%, Neon) and waste oil were used as substrate. The waste oil was obtained from local restaurants in the city of Araraquara (São Paulo, Brazil). The biocatalyst was Lipura® Flex kindly provided from Novozymes S/A (Araucária, PR, Brazil).

The reactions were carried out in a stirred tank reactor operating in batch mode (BSTR). The experimental apparatus was a jacketed glass reactor (8.5 cm height and 5.5 cm diameter) coupled with a reflux condenser connected to thermostatic bath (Marconi, model MA 184/6). The temperature of reactor was controlled by thermostatic bath (Solab, model SL-152) and the agitation by magnetic stirrer (Fisatom, model 752). A 2^3 central composite design (CCD) (Table 1) was used to determine the experimental conditions needed to achieve maximum methyl ester conversion in a BSTR. Independent variables studied were temperature (40-60°C), molar ratio of oil: methyl acetate (1/5-1/15) and enzyme concentration (7-13% w/w). After 24 h of reaction, the samples were centrifuged 32300 xg 25 °C/20 min (Hitachi®, model CR22N) and analyzed by gas chromatography (Shimadzu, model 2010) according EN 14103. The effects of variables on the responses of the 2^3 factorial design were assessed at $p < 0.05$ using Protimiza Experimental Design (http://experimentaldesign.protimiza.com.br/).

3 RESULTS & DISCUSSION

A 2^3 central composite design (CCD) (table 1) was used to determine the experimental conditions needed to achieve maximum methyl ester conversion in a BSTR. CCD was carried out with a total of 11 experiments (Table 1). The independent variables studied were temperature (°C), oil:methyl acetate molar ratio and enzyme concentration (% m/m), and the response variable was conversion to methyl esters (% m/m).

Table 1 2^3 central composite design matrix showing coded and actual values of independent variables and responses in terms of methyl esters conversion after 24 h of reaction

Run	Temperature (°C)	Oil/methyl acetate molar ratio	Enzyme loading (% w/w)	Methyl esters (% w/w)
1	-1(40)	-1(1/5)	-1(7)	36.9
2	1(60)	-1(1/5)	-1(7)	30.3
3	-1(40)	1(1/15)	-1(7)	35.8
4	1(60)	1(1/15)	-1(7)	33.5
5	-1(40)	-1(1/5)	1(13)	45.3
6	1(60)	-1(1/5)	1(13)	26.5
7	-1(40)	1(1/15)	1(13)	40.6
8	1(60)	1(1/15)	1(13)	52.2
9	0(50)	0(1/10)	0(10)	49.1
10	0(50)	0 (1/10)	0(10)	49.5
11	0(50)	0(1/10)	0(10)	47.4

Statistical analysis of the experimental results obtained in the CCD was carried out in order to evaluate the effect of the variables studied on the interesterification of waste oil (Y) and to build a predictive model of the process (Eq. 1).

$$Y = 40.65 - 2x_1 + 2.9x_2 + 3.5x_3 + 4.35x_1x_2 + 0.2x_1x_2 + 2.35x_2x_3 \quad (1)$$

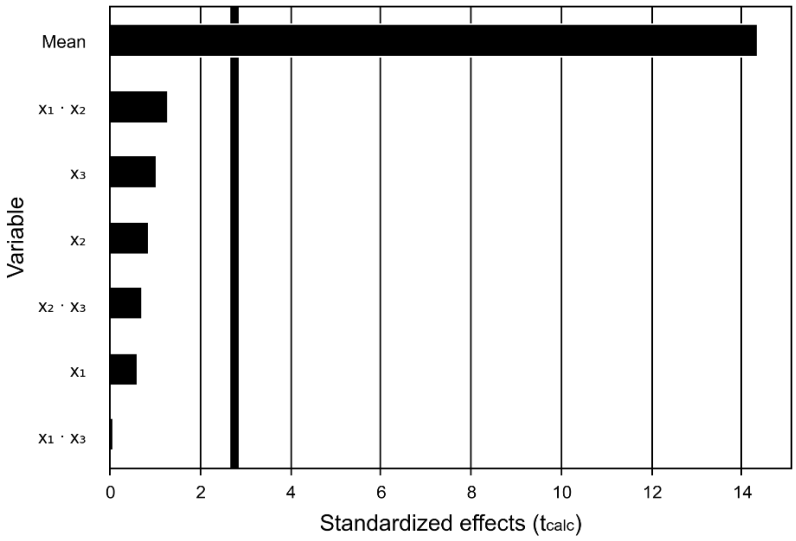

Figure 1 Pareto chart of the linear, quadratic, and interaction terms of temperature (X_1), molar ratio (X_2), enzyme loading (X_3) and their effects on the methyl esters convertion.

As can be seen in the Pareto chart (Figure 1) that in the range studied temperature (X_1), substrate molar ratio (X_2), and enzyme loading (X_3), did not exert significant ($p < 0.05$) effects on methyl ester conversion. According to ANOVA (Analysis of variance) the R^2 was 52,78, which means that the model explains 52.78% of the variability of the response variable. his information, combined with the fact that the F_{cal} found was 0.7 and the F_{tab} was 6.16 ($p < 0.05$), so $F_{cal} < F_{tab}$, indicates that the predictive model obtained (Eq. 1) was not considered valid, and therefore cannot be used to predict the bioprocess.

Despite not being able to obtain a mathematical model that describes the process, valuable information was obtained in this study. By analyzing the answers obtained in the CCD matrix (table 1), it is possible to observe that relevant methyl ester conversion results have been achieved. The run 8 showing the highest conversion to methyl esters, 52.2% w/w, with the conditions: temperature 60 °C, molar ratio of substrates oil: methyl acetate 1/15 and enzyme loading 13% w/w. In other words, the highest ester conversion was obtained at levels +1 of the independent variables studied. Thus, the excess of methyl acetate was beneficial in the reaction because it may have improved the fluidity of the medium, resulting in greater catalyst-substrate contact[9]. Similar results using high molar ratios of methyl acetate/oil, with an excess of methyl acetate were obtained[10,11,12]. Lipura Flex lipase was also able to produce significant amounts of methyl esters (30-52% w/w) at different temperatures (40-60 °C). The fact that the independent variables were not significant in the ranges studied may provide greater flexibility in the conditions for producing esters via interesterification.

This is a preliminary study of the interesterification process using waste oil and a new biocatalyst (Lipura® Flex). In order to better understand the reaction kinetics, it is still necessary to study the influence of time and substrates on the reaction.

4 CONCLUSION

Lipura® Flex lipase is a new enzyme with high potential for application in the interesterification reaction. Relevant conversions of methyl esters were obtained ~52% w/w. However, these are preliminary results; studies must still be carried out to better understand the reaction kinetics and achieve greater yields of esters.

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ACKNOWLEDGEMENTS

Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, nº 2020/09592-1), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq ,proc. no. 304399/2022-1 and 424931/2018-4), Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-Capes and FCFAr- UNESP.