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A SIMPLE APPROACH FOR HYDROLYSIS OF HETEROGENEOUS SUBSTRATES USING LIPASE

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ABSTRACT

A simple approach for the hydrolysis of heterogeneous substrates using lipase is proposed. The hydrolysis of coconut oil was evaluated as a model substrate, and Novozym 435 (CALB) was used as biocatalysts. Reaction parameters were optimized, three variables were identified: temperature, molar ratio and the enzyme amount on the hydrolysis yield. The mixture of coconut oil, water and lipase were monitored each hour to verify the conversion in fatty acids. Results showed that, the optimum condition for the reaction at 48 °C, 10% of the enzyme relative to oil mass and 1:9 coconut oil: water molar ratio giving a conversion of 19.43%. These results are initial studies, which should be further investigated. The simple procedure might be a useful technology for reactions including full modification of heterogeneous substrates.

1. INTRODUCTION

Fatty acids are ingredients used in various products of industrial origin, for example surfactants, detergents and food (Goswami et al. 2013). Traditionally, the fatty acids can be produced by the hydrolysis of oils and fats via chemical route. In this work a simple route is proposed, through enzymatic hydrolysis, for the production of fatty acids. The use of the enzymes is advantageous because it requires less energy and temperature, and still presents greater selectivity (dos Santos et al. 2017). One class of enzymes that have natural substrate oils and fats are lipases. In this work, was used the Novozym 435 (CALB), an immobilized preparation of the lipase B from *Candida antarctica* (CALB) on the hydrophobic resin Lewatit VP OC 1600. Lipase B from *Candida antarctica* (CALB) has been selected, as model lipase, due to its spread use in processes involving enzymatic applications (dos Santos et al. 2017, Goswami et al. 2013). On the other hand, other lipases will be used in the continuation of this study. As model substrate, it was chosen coconut oil, abundant and one of the

cheapest vegetable oils, which has a heterogeneous composition of fatty acids. Central composite design and response surface methodology were used in order to optimize reaction parameters in the software Statistica 10.0 (Statsoft, USA).

2. METHODOLOGY

2.1. Enzymes and other materials

Lipase from *C. Antarctica* (Novozym 435) was donated by Novozymes (Novozymes, Spain). Extra-virgin coconut oil was purchased at a local market. The assumed composition of the acids was (as mass fraction): caproic (0.38%), caprylic (5.56%), capric (4.99%), lauric (45.78%), myristic (18.56%), palmitic (8.85%), stearic (3.39%), oleic (5.65%), linoleic (0.94%).

2.2. Hydrolysis of oil

Different molar ratios of water: oil were added to 2 mL flasks, added of varying concentrations of biocatalyst according to the experimental design. The mixtures of coconut oil, water, and lipase were stirred in an orbital shaker (150 rpm) for the specific time and temperature. For each point of the experimental design or time course reaction, samples were collected at the desired times to measure the hydrolysis degree. The progress of hydrolysis was monitored by determination of the free fatty acid released by titration of samples containing the maximum of supernatant volume possible to extract using 0.01 M NaOH using phenolphthalein as pH indicator and 5 mL of ne ethanol as quenching agent. In this study, emulsifying agents, such as arabic gum, were not used, as they may interfere the enzymatic activity and have a high commercial value. Here, the proposal is to present a simple methodology, with easy realization, and low commercial value.

2.3. Central composite design

A central composite design of 3 variables were designed using Statistica 10.0 (Starsoft, USA) in order to obtain the optimal conditions for the hydrolysis reaction. The variables and their coded and real values are presented in Table 1. Table 2 shows 17 treatments of the 3 variables each at 5 levels. The design was constructed of 8 factorial points, 6 axial points and 3 replications at the central point. In each case, the percentage of conversion for hydrolysis was determined after 4 h.

2.4. Statistical analysis

The analysis of results were also using software Statistica 10.0 (Statsoft, USA).

Table 1. Process variables and their levels used in CCD

		Coded Levels				
Variables	**Name**	**-1,68**	**-1**	**0**	**1**	**1,68**
X_1	Temperature (°C)	32	35	40	45	48
X_2	Biocatalyst content (% relative to the oil mass)	5,8	7,5	10	12,5	14,2
X_3	Substrate molar ratio (coconut oil: water)	1:1,4	1:3	1:9	1:12	1:16,6

3. RESULTS

3.1. Statistical analysis

Some results are not possible to be presented in here, because there is space limitation. On the other hand, in the present work, is being proposed the design of a lipase biocatalyst strategy for the simultaneous hydrolysis of a mixture of different substrates, an initial study. The results of every treatment from the CCD. The highest hydrolysis conversion was 19.43% (48 °C; 10% of enzyme relative to oil mass; 1:9 coconut oil: water molar ratio), graphically represented in Fig. 1.

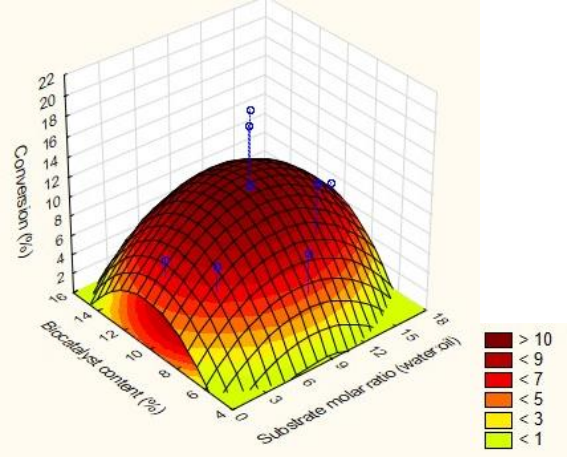

Figure 1. 3-D surface plot for conversion of hydrolysis of coconut oil. Interaction between biocatalyst loading versus substrate molar ratio at 48 °C.

In general, these results can be should be probably attributed to the protein diffusional limitations imposed by the considerably high amount of deposits of reaction product accumulated on the matrix surface, and possibly also inside the pores (Verdasco-Martín et al. 2016). In consequence, caused mass transfer restrictions to the protein molecules to go outside and that makes difficult to follow the protein immobilized on the support (Villalba et al. 2016). The tree variables presented positive effects, meaning that changing the variable level from −1 to 1 the response was increased. Temperature was the variable showing the highest effect, while the amount of biocatalyst was the lowest. Increasing temperature improves the enzymatic activity because of higher solubility of oil and its mobility on the porous support (Alves et al. 2014). The interactions between substrate molar ratio with the amount of biocatalyst, and with temperature strongly suggest that increasing the water content positively affects the hydrolysis rate. Water, which is a substrate of this reaction, is an important factor to keep the enzyme activity and stability (Alves et al. 2014).

4. CONCLUSION

This study objective was find the best conditions for Novozym 435 to catalyze a hydrolysis reaction. The best condition founded was 48 °C, 10% of enzyme relative to oil mass and 1:9 coconut oil: water molar ratio giving a conversion of 19.43%. The operational performance of Novozym 435 can be improbed. The next step for the research is improve the technique applied seeking for better results. In the continuation of this study, other lipases will be used as lipase from *Thermomyces lanuginosus* and lipase from *Rhizomucor miehei*, in order to show the versatility of the methodology.

5. REFERENCES

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