OPTIMIZATION OF FREE FATTY ACIDS PRODUCTION BY ENZYMATIC HYDROLYSIS OF WASTE FRYING OIL WITH LIPASE BONDED TO *Rhizopus oryzae* MYCELIUM

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ABSTRACT

A liter of improperly waste frying oil can contaminate up to 25 thousand liters of water. In light of environmental problems thereof, seeking alternatives for reusing it becomes crucial. The filamentous fungus *Rhizopus oryzae* (*R. oryzae*), known for its ability to produce several enzymes, including lipases, has been explored as biocatalyst in the hydrolysis of oils and fats to produce free fatty acids for quite some time. Thus, the present work aimed to use lipase bonded to the mycelium of *R. oryzae* for the hydrolysis of waste frying oil to produce free fatty acids. A 2^2 factorial design was carried out on enzymatic hydrolysis reactions, and independent variables were the influence of the amount of biomass produced and gum arabic concentration. Tests were conducted in 250 mL jacketed reactors for 240 min. Results showed that, at gum arabic concentration of 2% and biomass activity of 324 U/g of mycelium (2%), conversion into free fatty acids of 208.30 mM was reached, which corresponded to approximately 81% hydrolysis after 240 min of reaction.

Keywords:1. Lipase 2. Hydrolysis 3. Residual oil 4. Factorial Design 5. Free Fatty Acids

1 INTRODUCTION

An incorrect disposal of various types of waste causes environmental problems such as pollution and contamination of soil, water bodies, among others. Vegetable oil used in the kitchen, after its use in frying, is commonly discarded improperly, in addition to being one of the greatest water polluters, compromising aquatic ecosystems and reducing biodiversity. Therefore, its reuse in a hydrolysis reaction to produce free fatty acids (FFA) is a green alternative.

Fatty acids are typically used as raw materials in the production of products such as lubricating greases, plasticizers, emulsifiers and as ingredients in the manufacture of soaps and detergents, in addition to being able to produce biodiesel through an alternative route known as hydroesterification. However, large-scale production often involves extreme conditions such as high pressure and temperature, which can result in unwanted reactions such as oxidation and polymerization. Enzymatic hydrolysis, catalyzed by lipases, offers a gentler alternative, given the fact that it can operate at moderate temperatures and pressures, thus minimizing the formation of undesirable byproducts[1,2].

In the production of biodiesel, the use of waste frying oil as raw material in alternative routes to basic chemical catalysis is an advantageous approach aimed at preserving the environment and reducing production costs. In hydroesterification, an oil hydrolysis reaction initially occurs, followed by an esterification reaction of FFA obtained in the previous reaction. By adding the use of lipases as biocatalysts in the process, it is possible to obtain biodiesel with no generation of toxic by-products and pollutants[2].

In view of such a great potential, research focused on this area has reported an increased application of enzymatic methods aimed at obtaining FFA concentrates from residual frying oil[3]. In this context, the objective of the present work was to produce FFA concentrates from the hydrolysis of waste frying oil catalyzed by lipase linked to the *R.oryzae mycelium*.

2 MATERIAL & METHODS

The fungus *R. oryzae* previously identified as a potential source of lipase linked to the mycelium[3] was used. As a substrate, waste frying oil from residential homes in the city of Alfenas, Minas Gerais, was used. All other reagents used were of analytical grade obtained from Synth (São Paulo, SP).

An enzymatic hydrolysis of waste frying oil was carried out in a stirred tank reactor: Reactions to obtain FFA were carried out in batch mode by waste frying oil hydrolysis using a central composite rotatable design (DCCR) and two independent variables: the amounts of enzyme used in the reaction (% m/v) and gum arabic used (% m/v), and real and coded values for the independent variables are found in Table 1. Samples were collected at reaction times of 60, 120, 180 and 240 min. Conversion was calculated by determining FFA concentration formed during the hydrolysis reaction[4]. Data were analyzed at 5% significance using Protimiza.

3 RESULTS & DISCUSSION

The proposed 2^2 factorial design, together with results obtained in terms of percentage of hydrolysis achieved after 60, 120, 180 and 240 min of reaction is shown in Table 1.

Table 1 2^2 CCRD factorial design with real and coded values of independent variables used to determine the percentage of hydrolysis.

Trial	Independent Variables		Responses			
	Enzyme (% m/v)	Gum Arabic (% m/v)	Hydrolysis (% m/v)			
			60 min	120 min	180 min	240 min
1	-1 (1.29)	-1 (1.29)	14.41	27.09	28.87	32.66
2	+1 (2.71)	-1 (1.29)	3.58	15.96	23.71	36.50
3	-1 (1.29)	+1 (2.71)	46.91	35.21	50.53	48.99
4	+1 (2.71)	+1 (2.71)	4.44	26.20	84.60	35.69
5	-1.41 (1.00)	0 (2.00)	9.37	24.19	39.63	44.82
6	+1.41 (3.00)	0 (2.00)	38.08	51.71	51.29	71.95
7	0 (2.00)	-1.41 (1.00)	21.05	30.10	58.46	61.15
8	0 (2.00)	+1.41 (3.00)	9.50	13.60	29.44	37.20
9	0 (2.00)	0 (2.00)	28.32	68.78	73.55	82.31
10	0 (2.00)	0 (2.00)	23.51	65.00	79.59	81.90
11	0 (2.00)	0 (2.00)	21.99	62.55	78.80	78.80

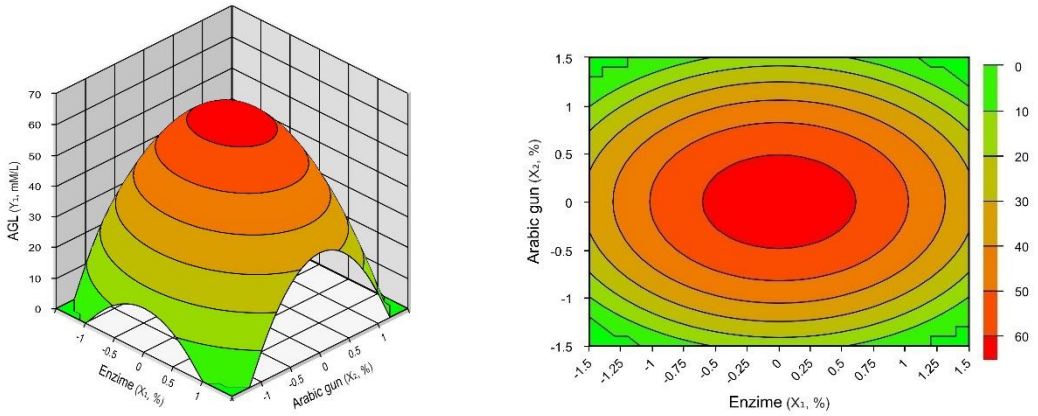
Optimal hydrolysis values (82.31 and 81.90%) were obtained in trials 9 and 10 of the CCRD factorial design after 240 min of reaction. At 120 min, the highest percentage hydrolysis was 68.78%, however the statistical analysis has showed that among the times analyzed, only hydrolysis after 120 min had statistically significance, given that 5% level was reached, thus being the time selected for the statistical analysis. Statistically significant terms at 120 min time were the quadratic terms of independent variables. Therefore, non-significant terms were removed from the model and reported as residuals, and regression coefficients were recalculated and used to generate Eq. (1).

$$Y = 65{,}44 - 14{,}69\ x_1^2 - 22{,}74\ x_2^2 \qquad \text{Eq (1)}$$

The ANOVA, generated only for statistically significant coefficients, is shown in Table 2. F_{calc} (17.7) was greater than F_{tab} (4.46) and the coefficient of determination was good (0.82). It was found that the pure error was low (19.7), indicating that trials are reproducible and experiments are in fact under control, therefore the highest percentage of residues observed was due to the lack of fit by the model to the experimental data. Thus, the construction of the response surface (Fig. 1a) and the contour curve (Fig. 1b) was carried out for the studied variables through ANOVA.

Table 2 2^2 CCRD ANOVA using statistically significant regression coefficients at 5% significance level

Source of Variance	Sum of squares	Degrees of freedom	Mean square	F_{calc}	P-value
Regression	3316.9	2	1658.5	17.7	0.00116
Residue	750.0	8	93.7		
Lack of fit	130.3		121.7		
Pure error	19.7		9.9		
Total	4066.9	10			
$R^2 = 0.82$; $F_{Tab\ 2;8;0.05} = 4.46$					



(a) (b)

Figure 1(a) Response surface (b) Contour curve for the 2^2 CCRD concerning the hydrolysis reaction using waste frying oil after 120 min of reaction.

For both studied variables, the highest percentage of hydrolysis occurred in the region around center points. Thus, to validate the CCRD, the center points condition was selected, in which the percentage of enzyme was 2 (% m/v) and that of gum arabic was 2 (% m/v), but the time of reaction was extended to 240 min, aiming at an increase in conversion, since for the trial lasting the longest (240 min), a higher percentage of conversion had been achieved (see Table 1). Trials were carried out in triplicate, and hydrolysis percentage was 80.67 ± 1.65 (% m/v), i.e. FFA conversion of 208.30 mM.

4 CONCLUSION

According to these results, lipase bonded to *R. oryzae* mycelium showed excellent results in the hydrolysis of waste frying oil. This strategy is technically attractive for the production of FFA from waste oils, as they are costless raw materials whose incorrect disposal can be avoided, given that it leads to environmental pollution. Furthermore, it can be applied in the production of biodiesel in one of the stages of an alternative route instead of using basic chemical catalysis in order to contribute to a process with no generation of toxic or unwanted waste and at lower production costs, thus suggested as future efforts to replace diesel from petroleum with biodiesel.

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ACKNOWLEDGEMENTS

The authors are grateful to Fundação de Amparo à Pesquisa do Estado de Minas Gerais - FAPEMIG (APQ-03161-22) for its financial support and Programa Institucional de Iniciação Científica e Tecnológica da Fundação de Amparo à Pesquisa do Estado de Minas Gerais - PIBICT/FAPEMIG for the scientific initiation scholarship granted (EDITAL n° 017/2021) and UNIFAL-MG.