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EFFECTS OF VARIABLES LIPASES PRODUCTION PROCESS BY *Aspergillus niger* C BY SUBMERGED FERMENTATION

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ABSTRACT

Lipases are hydrolytic enzymes and have been highlighted as biocatalysts with potential application. Most industrial microbial lipases derived from fungi are versatile in their properties and substrate specificity. This study aimed to evaluate the effects of variables lipases production process by Aspergillus niger C by submerged fermentation. The production assays were performed in shake flasks for 72 hours at 150 rpm and 32 °C. A fractional factorial design 2^{5-1} was carried out to evaluate the effect of the following independent variables: sucrose (10.0; 15.0; 20.0 gL^{-1}), ammonium sulfate (2.0; 4.0, 6.0 g L^{-1}), soybean oil (2.0; 4.0; 6.0 g L^{-1}), yeast extract (0.0; 1.0; 2.0 g L^{-1}) concentration and pH (6.0; 7.0; 8.0). The best average activity (13.12 U ml^{-1}) was obtained when using 15.0 g L^{-1} sucrose, 4.0 g L^{-1} ammonium sulfate, 4.0 g L^{-1} soybean oil and 1.0g L^{-1} yeast extract and pH 7.0.

Key-words: Lipases, Submerged fermentation, *Aspergillus niger*.

INTRODUCTION

Lipases (glycerol ester hydrolases EC 3.1.1.3) were first identified in the pancreas by J. Eberle in 1834 and by C.I. Bernard in 1856, together with amylases and proteases, constituting the three major known digestive enzymes and the most important group of biocatalyst for biotechnological applications. The number of available industrial lipases has increased considerably since the 1980s in response to an increasing demand for these biocatalysts (BORNSCHEUER U.T. et al., 2002). Lipase biosynthesis occurs in animals, plants and microorganisms. Many industrial lipases have been produced, purified and cloned from bacteria and fungi that include *Aspergillus* species. They are widely used in industrial applications, such as in dairy and food manufacture, leather and detergent industry. Recently they faced novel biotechnological applications such as production of cosmetics, enantiopure pharmaceuticals, agro-chemicals, flavor compounds and synthesis of biopolymers and biodiesel (HASAN F. et al., 2006). Despite the presence of a large number of already discovered and characterized lipolytic enzymes, the search for new lipase producers and the knowledge of production parameters remains important, which requires the availability of lipolytic enzymes displaying unique and specifically desired properties. The *Aspergillus niger* C was the strain tested for lipase production, by submerged fermentation (SmF) through the evaluation of the effects of some variables of process, using statistical design.

MATERIALS AND METHODS

Microorganism

The strain of *Aspergillus niger* C was provided by the culture collection of Embrapa Food Agroindustry, Rio de Janeiro, Brazil.

Microorganisms, maintenance and activation

The strain was maintained and activated in basic agar slant as previously described by Couri and Farias (1995) with modifications, with 2.0% (w/v) of olive oil as carbon source. The slants of *A. niger* were incubated for 7 days at 32^{o}C.

Inoculum preparation

Spores from 7-days-old agar slant culture were used to inoculate corn cob medium. After 5 to 7 days incubated at 32°C, a spore suspension was prepared with addition of 20 mL of 0.3% (v/v) Tween 80 per flask of corn cob medium, filtered in sterilized gauze (COURI & FARIAS, 1995). A spore suspension of about 10^6 conidia ml^{-1} was used as a source of inoculum. The number of conidia mL^{-1} in the suspension was count by the Neubauer chamber.

Experimental designs

In order to identify medium components that have a significant effect on lipase production. a fractional factorial design 2^{5-1} was carried out. A set of 19 experiments, including three replicates at the central point, was constructed for five medium components namely, pH, sucrose, soybean oil as inductor, yeast extract and ammonium sulfate concentration. Each variable was tested in two levels and the center point (Table 1). The dependent variable was the enzymatic activity (U mL^{-1}). "STATISTICA" (version 7.0) software was used for graphical analyses of the data obtained.

Lipase production

The medium for lipase production optimization is: yeast extract (0, 1, 2 g L^{-1}); sucrose (10, 15, 20 g L^{-1}), soybean oil (1, 4, 6 g L^{-1}), $(NH_4)_2SO_4$ (2, 4, 6 g L^{-1}), KH_2PO_4, (2 g L^{-1}), $MgSO_4.7H_2O$ (0.5 g L^{-1}), KCl, (0.5 g L^{-1}), $ZnSO_4$, (5 mg L^{-1}), $FeSO_4.7H_2O$ (23 mg L^{-1}), $CuSO_4$. $5H_2O$ (6 mg L^{-1}), $MgSO_4$. $5H_2O$ (20 µg L^{-1}), pH (6, 7, 8) in 1 L of distilled water. The experiments were carried out in 250 mL Erlenmeyer flasks containing 50 mL of production media kept in the temperature controlled in a rotary shaker for 72h at 150 rpm and 32^{0}C.

Enzyme assay

Determination of lipase activity in the crude enzyme extract was done using the titrimetric method (DAMASO, M.C.T. et al., 2008). One unit (U) of lipase activity was defined as the amount of enzyme which produces 1 µmol of fatty acids per minute under assay conditions.

RESULTS AND DISCUSSION

The first step in the optimization strategy was to identify medium components that have a significant effect in the production of lipases by submerged fermentation. For this, it was used a fractional factorial design 2^{5-1}, where 5 independent variables were analyzed: sucrose, soybean oil, yeast extract, ammonium sulfate and pH. Table 1 presents the results of the experimental design performed to achieve the lipase optimization.

Table 1 - Matrix 2^{5-1} fractional factorial design for the production of lipase with their coded and real levels.

Assays	Sucrose ($g\ L^{-1}$)	$(NH_4)_2SO_4$ ($g\ L^{-1}$)	Soybean oil ($g\ L^{-1}$)	Yeast extract ($g\ L^{-1}$)	pH	Enzymatic activity ($U\ mL^{-1}$)	Reducing sugar ($g\ L^{-1}$)	Dry weight ($g\ mL^{-1}$)
1	-1 (10)	-1(2)	-1(2)	-1(0)	1(8)	**1.24**	0,173	0,0046
2	1(20)	-1(2)	-1(2)	-1(0)	-1(6)	**0.97**	0,153	0,0096
3	-1(10)	1(6)	-1(2)	-1(0)	-1(6)	**0.00**	0,115	0,0053
4	1(20)	1(6)	-1(2)	-1(0)	1(8)	**1.60**	0,283	0,0100
5	-1(10)	-1(2)	1(6)	-1(0)	-1(6)	**2.72**	0,272	0,0050
6	1(20)	-1(2)	1(6)	-1(0)	1(8)	**2.67**	0,275	0,0095
7	-1(10)	1(6)	1(6)	-1(0)	1(8)	**0.88**	0,000	0,0076
8	1(20)	1(6)	1(6)	-1(0)	-1(6)	**3.76**	0,000	0,0098
9	-1(10)	-1(2)	-1(2)	1(2)	-1(6)	**1.51**	0,000	0,0066
10	1(20)	-1(2)	-1(2)	1(2)	1(8)	**0.22**	0,203	0,0207
11	-1(10)	1(6)	-1(2)	1(2)	1(8)	**0.44**	0,218	0,0202
12	1(20)	1(6)	-1(2)	1(2)	-1(6)	**0.39**	0,248	0,0206
13	-1(10)	-1(2)	1(6)	1(2)	1(8)	**0.30**	0,228	0,0121
14	1(20)	-1(2)	1(6)	1(2)	-1(6)	**5.43**	0,230	0,0236
15	-1(10)	1(6)	1(6)	1(2)	-1(6)	**3.53**	0,270	0,0164
16	1(20)	1(6)	1(6)	1(2)	1(8)	**1.51**	0,258	0,0259
17*	0 (15)	0(4)	0(4)	0(1)	0(7)	**13.01**	0,462	0,0179
18*	0 (15)	0(4)	0(4)	0(1)	0(7)	**12.48**	0,419	0,0175
19*	0 (15)	0(4)	0(4)	0(1)	0(7)	**13.87**	0,482	0,0169

(*) = Central points / Real values between parenthesis

As indicated in Table 1 the highest lipase activity values (average 13.12 $U\ mL^{-1}$) and lowest sucrose consumption were found at the central point (15.0 $g\ L^{-1}$ sucrose, 4.0 $g\ L^{-1}$ ammonium sulfate, 4.0 $g\ L^{-1}$ of soybean oil, 1.0 $g\ L^{-1}$ yeast extract and pH 7.0), while experiments performed in the test 3 presented no activity. The higher biomasses were obtained with highest peptone concentration, except for the experiments 9 and 12, where the influence of the combination of lower concentrations of sucrose and ammonium sulfate was observed. Usually, the lipase production is improved by the inducer concentration and in this study, using olive oil, the highest lipase production was achieved with the medium concentration (4g/L).

Freire et al. (1997) used the *Penicillium restrictum* for lipase production by submerged fermentation for 80h, the best activity found in this study was 14 $U\ mL^{-1}$. In another study for lipase production by solid state fermentation in aerated columns using the same strain of *A. niger* C, the authors found the activity value around 254 $U\ g^{-1}$ corresponding to approximately 41 $U\ mL^{-1}$ (MUCURI, 2012).

The fractional factorial design 2^{5-1} used in this study indicated that the lipase production by submerged fermentation with *A. niger* C strain, under the conditions tested, was influenced by the concentration of inducer and the initial pH of the medium at 90% confidence level. The Pareto chart (Figure 1) shows that the production of the lipase by *A. niger* C can be improved by increasing the inducer concentration and pH decrease of the fermentation medium. The other independent variables did not show a significant effect.

Similar results were achieved by Vici et al. (2011), which had better lipase activity at pH of 3.0 to 6.0.

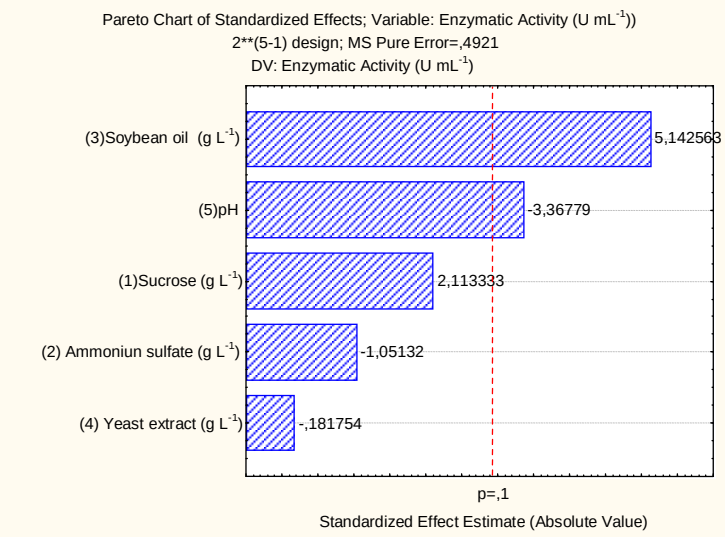

Figure 1 - Pareto Chart for lipase production by *A. niger* C

CONCLUSIONS

The Pareto chart shows that the production of the lipase bi *A. niger* C can be improved by increasing the inducer concentration and pH decrease of the fermentation medium. These results were essential to the second stage of the work which will be used a second experimental planning, CCRD 2^2 in order to improve the production process.

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