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BIOLOGICAL SCIENCES

Lipase production by *Aspergillus brasiliensis* in solid-state cultivation of malt bagasse in different bioreactors configurations

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Abstract: We evaluated the conditions to produce lipase in solid-state cultivation using a recently isolated strain of *Aspergillus brasiliensis* 157f in bioreactors of different configurations: static flat-bed, plugged-flow bed with forced water-saturated aeration, and pilot-scale rotating drum bioreactor, using malt bagasse as substrate. Lipase production was optimized applying experimental design analysis, which showed optima parameters defined as pH 7.7, addition of 11.3 % of soybean oil to the medium, and culture temperature of 32.7 °C, in static flat-bed. The highest enzyme activity (9.8 $U.g^{-1}$ substrate) was obtained in the plugged-flow bed with forced water-saturated aeration. The fermented culture medium was lyophilized to create a solid enzymatic preparation (SEP), which was used to test the possibility of using this cheap biocatalyst in bioreactors to mediate esterification and transesterification reactions. SEP presented lipase activities of 7.35 $U.g^{-1}$ substrate, indicating the possibility of further enhancing aspects of the use of such biocatalyst.

Key words: *Aspergillus brasiliensis*, brewery's spent malt, lipase production, Solid-state cultivation, transesterification reaction.

INTRODUCTION

Lipases (triacylglycerol hydrolases, EC 3.1.1.3), are enzymes that catalyze the hydrolysis of triglycerides, producing free fatty acids and glycerol. They also can catalyze alcoholysis, esterification, and transesterification of fatty acids (Treichel et al. 2010). Based on their versatility, selectivity, moderate reaction conditions, and susceptibility to immobilization, lipases are extremely interesting from a commercial point of view. Currently, these enzymes are used in various industrial processes such as the production of foods, pharmaceuticals, cosmetics, detergents, and biofuels, among other uses (Treichel et al. 2010, Choudhury & Bhunia 2015, Gupta et al. 2015).

The world market for enzymes in 2014 reached 4.2 billion dollars, projected to grow at rate of 7 % per year between 2015 and 2020, and the lipase market is expected to reach 590.5 million dollars in 2020 (Chauhan et al. 2016). During the last decade, lipases have been studied as a biocatalyst for the production of biodiesel, for its importance as biofuel. Some advantages of using lipases for biodiesel production are based on the use of acid oils without productivity reduction. The enzymatic route also generates pure glycerol as by-product and biodiesel of better quality compared to chemical synthesis, and the possibility of using ethanol, milder reaction conditions, and no need to neutralize the final product (De Castro et al. 2012).

Currently, lipases are produced from microbial sources because of high productivities achieved in bioprocesses (Hasan et al. 2006), either in submerged or solid-state cultivations. Solid-state cultivation (SSC) has some interesting characteristics such as the possibility of using agro-industrial waste or residues and the process is conducted in the absence of free water and buffering effect, thus reducing the costs of enzyme production and recovery (Edwinoliver et al. 2010). These advantages are fully exploited when SSC is conducted using fungi, based on the high capacity of these microorganisms to colonize the substrate and produce lytic enzymes (Thomas et al. 2013, Ashok et al. 2017). The majority of fungal lipases are usually excreted to the medium, which eases the unit operations of extraction and reducing production costs (Silva et al. 2005). *Aspergillus* is among the most important fungal lipase producers, with great potential as source of lipase, ability to degrade plant polysaccharides and interesting properties for industrial application (Pokorny et al. 1994, Contesini et al. 2010). This genus is found worldwide and has several known lipase producers species being generally recognized as safe (GRAS), acknowledged by the Food and Drug Administration (FDA) in USA (Contesini et al. 2010). Furthermore, the product of a SSC can be directly used as a solid enzymatic preparation (SEP), which is a technique in which the cultivated solid medium can be directly used for catalytic purposes, being reported to be simple and easy to perform, showing good results, having a low cost of production, and maintaining enzymatic activity over reuses (Rosa et al. 2006, Salum et al. 2010).

Malt bagasse (MB), also known as spent brewery's malt, is the largest by-product of the brewing industry, representing around 85 % of its waste generation (Mussatto et al. 2006). Approximately 20 kg of spent malt is generated for each hectoliter of beer produced (Mussatto et al. 2006, Thiago et al. 2014). Currently, is estimated an annual production of two billion hectoliters of beer worldwide, with China being the largest producer, followed by USA and Brazil (Kirin 2017), representing a potential of forty million tons of spent malt available that could be used in biorefinery processes every year, at low or no cost (Aliyu & Bala 2013).

Considering these aspects, the objective of this study was to evaluate optimal conditions for lipase production using a new strain of *Aspergillus brasiliensis* 157f, isolated by our group, growing under solid-state cultivation using spent malt bagasse. A central composite design analysis was performed to obtain optimal conditions of cultivation in bench scale bioreactors (static flat-bed). The optimal conditions were used for cultivation test in bioreactors with forced aeration (plugged-flow bed with forced water-saturated aeration), and in a pilot-scale rotating drum bioreactor using intermittent agitation. Finally, a solid enzymatic preparation was tested for its lipase activity as a preliminary study of possible applications of this cheap enzymatic preparation in reaction requiring the action of lipases.

MATERIALS AND METHODS

Microorganism, spent malt bagasse, and chemicals

Aspergillus brasiliensis 157f was isolated by our group from soil samples in Brazil and was used for the solid-state cultivations because it demonstrated the ability to produce lipase (Da Silva Menezes et al. 2017). This strain was isolated, genetically identified, and physiologically characterized for the production of several enzymes in a previous work, and certified lyophilized stocks are kept in our laboratory (Bioteclab, UFRGS, Porto Alegre, Brazil) (Da Silva

Menezes et al. 2017). For maintenance, the fungus was grown on Petri dishes containing potato dextrose (34 $g.L^{-1}$) and agar (15 $g.L^{-1}$) medium for 7 days at 37 °C for spore collection. The substrate used was spent malt bagasse (kindly provided by Ralf Beer Brewery Company, Alvorada, Brazil) collected immediately after the mashing process. Spent malt was stocked at -20 °C until utilization, without any further processing. The substrate was characterized for its content of lipids, reducing sugars, ashes, humidity, total proteins, cellulose, hemicelluloses, and lignin. Soybean oil, which was added to the substrate for cultivations as inducer of lipase production, was the commercial refined cooking oil bought on a local market. All chemicals used in this research were of analytical grade and purchased from Sigma-Aldrich (São Paulo, Brazil) unless otherwise informed.

Solid-state cultivation

Solid-state cultivation (SSC) for the experimental design was performed using 125 mL Erlenmeyer flasks containing 10 g of substrate (malt bagasse and oil), in which the percentages of each substrate were determined by experimental design (see below). Flasks containing substrate were autoclaved at 121 °C for 15 min, then, corresponding autoclaved buffer and autoclaved soybean oil were added. Buffers were added to provide maximum amount of water to be absorbed by the malt bagasse but avoiding any free water to accumulate, which was found to be 30 % (mass fraction to malt bagasse). Inoculum was prepared by pouring 10 mL of Tween 80 (0.01 % mass fraction) to a plate previously colonized by the fungus (as prepared in 1.1.), and smoothly scraping for spore collection. The resulted spore suspension was centrifuged (4,500 *g*, 20 °C, 5 min), and resuspended in sterile distilled water. Spores were counted in Neubauer chamber and inoculum was made by addition of $1{\cdot}10^6$ spores per gram of malt bagasse.

Experimental design and method validation

Central composite design (CCD) was employed as experimental design to evaluate the percentage of soybean oil, the cultivation temperature, and the pH of buffer used at the start of cultivation. Two different buffers were used: phosphate buffer 0.2 M for pH 6.0 and higher, and acetate buffer 0.2 M for pH lower than 6.0. Extracellular lipase activity was measured at different times of cultivation (0, 24, 48, 72, and 96 h), and the time of sampling with highest activity was used as response in the CCD analysis. Independent variables and their level are shown in Table I. This experimental design consisted of 16 treatments, with 8 factorial points, 6 axial points, and 2 central points. Experimental design and analysis were evaluated using STATISTICA software 10.0 (Statisoft, USA).

Lipase essay

For lipase extraction, 5 g of cultivated substrate was added to 25 mL of phosphate buffer (pH 7, 0.1 M), incubated at 37 ºC, 180 rpm, 30 min and filtered through qualitative filter paper (80 g), resulting in a crude enzymatic extract. Lipase assay was performed according to the literature (Burkert et al. 2004), with some modifications as follows. 2.5 mL of olive oil emulsion (25 % volume fraction of olive oil dispersed in a solution of Arabic gum 70 $g.L^{-1}$) was added to 1 mL of acetate buffer (pH 5, 0.1 M) and 0.5 mL of culture extract was added and incubated at 37 ºC, 180 rpm, for 30 min. Reaction was stopped by the addition of 7.5 mL of acetone-ethanol-water mixture (1:1:1 volume fraction), and free fatty acids were titrated with NaOH 0.02 M. One unit of lipase activity ($U.g^{-1}$) was considered to be the amount of enzyme that liberates 1 µmol

Table I. Variables analyzed in the CCD showing the levels and the real values for the experimental design to optimize lipase production by *A. brasiliensis* 157f cultivated in malt bagasse.

Treatment	Variables		
	pH	Soybean oil %(%)	Temperature (°C)
1 (A)*	-1 (5)	-1 (5)	-1 (25)
2 (B)	+1 (7)	-1 (5)	-1 (25)
3 (C)	-1 (5)	+1 (15)	-1 (25)
4 (D)	+1 (7)	+1 (15)	-1 (25)
5 (E)	-1 (5)	-1 (5)	+1 (35)
6 (F)	+1 (7)	-1 (5)	+1 (35)
7 (G)	-1 (5)	+1 (15)	+1 (35)
8 (H)	+1 (7)	+1 (15)	+1 (35)
9 (I)	-1.68 (4.3)	0 (10)	0 (30)
10 (J)	+1.68 (7.6) (7.68)	0 (10)	0 (30)
11 (K)	0 (6)	-1.68 (1.6)	0 (30)
12 (L)	0 (6)	+1.68 (18.4)	0 (30)
13 (M)	0 (6)	0 (10)	-1.68 (21.6)
14 (N)	0 (6)	0 (10)	+1.68 (38.4)
15 (O)	0 (6)	0 (10)	0 (30)
16 (P)	0 (6)	0 (10)	0 (30)

***Letters correspond to treatment number in text and statistical analysis.**

of fatty acid per minute per gram of cultivated substrate (Burkert et al. 2004).

Lipase production test in aerated bioreactor

Reactions for the experimental design and validation were performed using 125 mL flasks, which are usually known as static flat-bed or tray reactors (Mitchell et al. 2006). To have a more comprehensive understanding of culture conditions upon lipase production, the determined optimal conditions in the static flat-bed were used in different types of reactors. This experiment was performed in bioreactors, in which the bed is static, but air is forcefully blown through the cultivation. For this, cylindrical glass flasks were used, with 28 cm high, 5.5 cm of diameter and sintered glass on the bottom. In this case, water-saturated air passed forcefully through the malt bagasse bed, at an air flow rate of 2 $L.min^{-1}$, after passing through an air filter and distilled water to keep water saturation in aeration. For this experiment, 70 g of sterilized malt bagasse was used, keeping the same proportions of buffer and soybean oil defined by the CCD.

Lipase production test in pilot scale rotating drum bioreactor

To evaluate how this lipase production could behave under pilot scale conditions, a cultivation test was carried out in an rotating drum bioreactor, using 3 kg of non-autoclaved malt bagasse, keeping oil percentages and pH of buffer as obtained in the optimal conditions in

the CCD analysis. The rotating drum bioreactor was equipped with circulating air above the bed to remove excess of metabolic gases, and agitation conditions were established as 30 min of 1 rpm agitation every 24 h of cultivation, before sample collection.

Solid enzymatic preparation

To obtain the Solid Enzymatic Preparation (SEP), malt bagasse was cultivated for 96 h under optimized conditions in cylindrical flasks with aeration through the bed, according to procedures described in the solid-state cultivation section. The cultivated malt bagasse was placed in a vacuum freeze dryer LGJ-12 lyophilizer for 24 h and stocked at -20 °C before use. The SEP obtained was tested according to section describing the lipase assay, using 0.5 g of SEP, to preliminarily evaluate possible practical uses of this product.

Analytical procedures

Lipid content was measured by Soxhlet direct extraction using petroleum ether as solvent in an automatic extractor SOXTEC Avanti 2055. Reducing sugars were measured by dinitrosalicylic acid method (DNS) and the results were determined in spectrophotometer (540 nm) (Miller et al. 1959). Ashes were determined according to literature (Silva & Queiroz 2002). Moisture content of malt bagasse was measured using a moisture analyzer (Ohaus MB32). Total protein content was measured using the Kjeldahl method, with conversion factor of 6.25 (Horwitz 1975). Chemical characterization of spent malt (cellulose, hemicellulose, and lignin) was performed according to literature, using H_2SO_4 72 % for hydrolysis and sugar content was measured at HPLC with column HPX-87H at 45 °C, with 5 mM H_2SO_4 solution as mobile phase (Gouveia et al. 2009). During cultivation, lipase activity, soluble protein content, and pH were analyzed. Bradford method was used to measure soluble proteins, where Bradford reagent was added to the enzyme extract, and after 30 min incubation (20 °C), the content was read in a spectrophotometer (595 nm) (Zaia et al. 1998). For pH measurements, 5 g of total cultivated substrate was resuspended in 50 mL of distilled water, and allowed to rest for 15 min, under casual manual agitation. After 15 min, pH was obtained using a digital pH meter.

RESULTS AND DISCUSSION

Spent malt bagasse characterization

Results for the characterization of the spent malt bagasse used in this work are shown in Table II. Moisture values were similar to those found by Cordeiro et al. (2012) with 75.5 % of water content in the biomass (Cordeiro et al. 2012). Moisture is an important factor in SSC, with fungi needing low moisture content, usually around 40 to 60 %, thus, the malt bagasse was already appropriate for cultivation (Singhania et al. 2009). These water content values would, however, increase transportation costs and increase the risk of bacterial contamination (Cordeiro et al. 2012) and therefore should be addressed in the industrial scale.

Lipid and ash contents (Table II) were similar to values found in the literature (Mello & Mali 2014). For lipase production, lipid content in malt bagasse may not be sufficient to induce enzyme activity, making necessary oil supplementation. In the production of lipase by *Candida rugosa* in SSC using rice bran as substrate, the most significant variables affecting enzyme production were found to be oil content, urea, and maltose (Venkata Rao et al. 1993). Although studies showed that *Aspergillus* could produce extracellular lipase without lipid addition in substrate, adding oil as inducing agent can significantly improve lipase

Table II. Physico-chemical characterization of the spent malt bagasse used in this work.

Composition (% components in dry basis)	
Moisture	76.56 ± 0.94*
Lipids	4.00 ± 0.39
Proteins	15.06 ± 2.27
Ashes	3.64 ± 0.09
Cellulose	24.32 ± 0.08
Hemicellulose	22.27 ± 0.05
Soluble Lignin	3.79 ± 0.01
Insoluble Lignin	18.87 ± 3.30

***Standard deviations for composition.**

production. However, increasing the amount of lipid addition in cultivation does not necessarily correlate to higher lipase yields in *Aspergillus* cultivation (Ohnishi 1994, Pokorny 1994).

Hemicellulose and lignin composition of the malt bagasse (Table II) were similar to values found by Mello et al. (2014) (23.41 % of hemicellulose and 26.13 % of lignin), and by Mussato et al. (2006) (28 % of hemicellulose and 28 % of lignin) (Mussatto et al. 2006, Mello & Mali 2014). Cellulose, however, was higher than values reported by Mello et al. (2014) and Steinmacher et al. (2012), (12.29 % and 11.35 %, respectively), which can be explained by malt bagasse composition varying based on barley varieties used, harvest time, conditions in malting of barley, and other conditions particular to each brewery (Santos et al. 2003, Steinmacher et al. 2012). Some filamentous fungi, such as *Aspergillus*, are good producers of cellulases, liberating sugars used in their metabolism (Liu et al. 2011), thus the use of biomass with high cellulose content could be advantageous to reduce costs in nutritional supplementation in SSC using this microorganism.

CCD results

The experimental conditions and lipase activity values for 96 h (samples showing the highest activities in all experiments) of cultivation are shown in Table III. The response surface of variables pH, temperature, and percentage of oil are shown in Figure 1.

The highest activity was 9.1 $U.g^{-1}$, using phosphate buffer pH 7.0, 5 % of soybean oil and cultivation at 35 °C, whereas the lowest activity of 1.9 $U.g^{-1}$ was obtained using acetate buffer at pH 4.7, 10 % of oil, and cultivation at 30 °C. Figure 2 presents the kinetics of lipase activity evolution during the CCD trails. Though we found higher lipase activity at 96 h of cultivation, some authors found longer times of cultivation time (120 h) for highest lipase activity in cultures of *Aspergillus* (Mahadik et al. 2002, Colla et al. 2015), but this difference is explained by the use of different cultivation conditions.

Analysis of the experimental data in Table III yielded the following second order equation (Eq. 1), relating the response variable (Y: lipase activity) to dependent factors (X_1: pH, X_2: % of soybean oil, and X_3: temperature). In Table IV are presented optimal parameter values predicted by CCD analysis, and maximum predicted

Table III. CCD experimental conditions and lipase activity for 96 h cultivation showing standard deviation. Experiments were run using the static flat-bed bioreactor configuration.

Run	Variables			Lipase Activity ($U.g^{-1}$)
	pH	% oil	Temperature	96 h
1	5.00	5.00	25.00	4.11 ± 0.2*
2	7.00	5.00	25.00	6.46 ± 0.9
3	5.00	15.00	25.00	4.00 ± 0.5
4	7.00	15.00	25.00	7.22 ± 1.2
5	5.00	5.00	35.00	3.95 ± 0.5
6	7.00	5.00	35.00	9.11 ± 1.0
7	5.00	15.00	35.00	4.31 ± 0.3
8	7.00	15.00	35.00	8.06 ± 0.3
9	4.68	10.00	30.00	1.91 ± 0.2
10	7.68	10.00	30.00	7.93 ± 0.7
11	6.00	1.60	30.00	5.53 ± 0.6
12	6.00	18.40	30.00	6.31 ± 0.3
13	6.00	10.00	21.60	6.75 ± 0.2
14	6.00	10.00	38.40	5.48 ± 0.1
15	6.00	10.00	30.00	6.80 ± 0.5
16	6.00	10.00	30.00	6.42 ± 0.3

***Standard deviations for lipase activity.**

value of lipase activity in optimal conditions of cultivation was 8.2 $U.g^{-1}$. Optimum pH and temperature obtained in this study for fungal lipase activity are similar to those already reported in literature, being around 30-40 °C and pH 6.0 to 8.0 (Colla et al. 2015, Cyndy et al. 2015).

$$Y = 7.32 + 3.80\,X_1 - 1.95\,X_1^2 - 2.16\,X_2 + 1.12\,X_2^2 + 0.14\,X_3 + 0.96\,X_3^2 - 1.73\,X_1X_2 - 0.68X_1X_3\,xz + 0.51X_2X_3 \quad (1)$$

Other studies also found pH value to be a contributing factor on lipase activity. For example, in the SSC of *Rhodotorula glutini* and *C. rugosa* in sugarcane bagasse and nutrient solution, highest lipase activity was achieved at pH range 7.0 to 8.0 (Ferrarezi et al. 2014). The highest lipase activity in SSC of *C. rugosa* in groundnut oil cake was obtained at pH 6.0 (Rekha et al. 2012).

As it is observed, highest lipase activity result was obtained at pH 7.0, using phosphate buffer. In Figure 3, it is possible to follow the variation of pH during the CCD experiment. It can be seen that those treatments with initial pH 6.00 or higher showed a decrease in pH at 48 h, stabilizing at a pH range of 3.0 to 4.0, coinciding with spore formation, indicating *Aspergillus* stress. In treatments with initial pH below 5.5, the formation of spores was not observed.

In this work, the variables oil percentage and temperature of cultivation were not found to be statistically significant, contrasting with other studies assessing lipase production by fungi in SSC (Treichel et al. 2010, Rekha et al. 2012). Lipid content in the cultivation could be just an inducer, which corroborates with observed results in this work, in which higher percentages of oil were not paralleled with

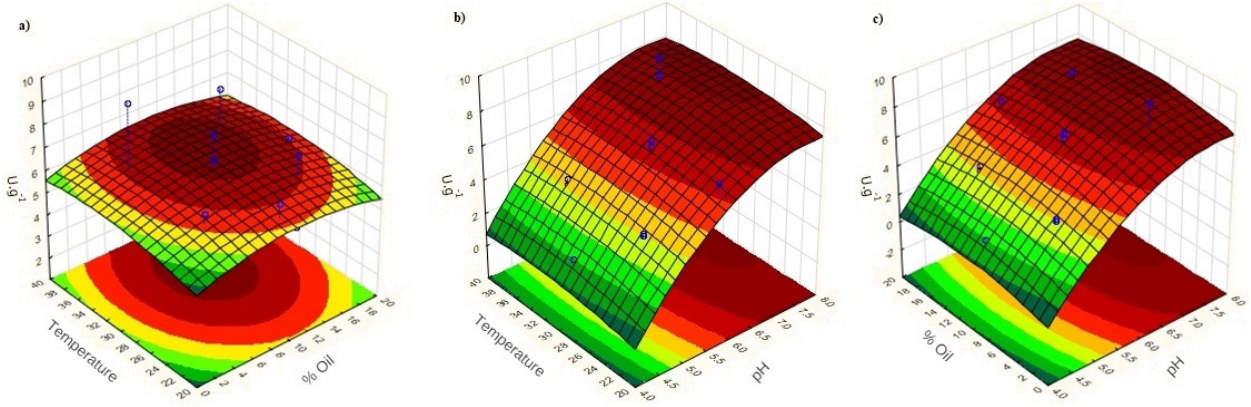

Figure 1. Response surfaces from CCD analysis of 96 h cultivation, of *Aspergillus brasiliensis* in malt bagasse, where (a) is influence of temperature and % of oil, (b) is influence of temperature and pH, and (c) is influence of % of oil and pH in lipase activity. Experiments were run using the static flat-bed bioreactor configuration.

increased lipase production (Mahadik et al. 2002). In the present work, unlike most, no mineral salts were added to the medium in the SSC because we intended to produce lipase under the least expensive conditions. It has been demonstrated that some nutrients in culture medium might have significant influence in lipase production such as soybean meal and K_2HPO_4, as observed in cultures of *Candida* sp. in submerged fermentation (He & Tan 2006). Many reports on the use of agro-industrial residues for SSC used supplementations, such as NH_4NO_3, yeast extract, peptone, NH_4Cl, and $NaNO_3$, suggesting that one important factor is the C:N ratio (Kempka et al. 2008, Mahanta et al. 2008, Treichel et al. 2010).

ANOVA results, in Table V, shows that the linear effect of pH was significant for lipase activity (p<0.05). The model prediction could explain 90.20 % of the lipase activity (R^2 of 0.90), suggesting a good correlation between experimental results and theoretical values predicted by the model.

Experimental validation

Using the optimized conditions obtained in statistical model (Table IV), a validating experiment was carried out in triplicate, with results shown in Figure 4. In 96 h, cultivation reached a maximum of 8.19 ± 0.34 $U.g^{-1}$ of lipase activity, which is very similar to value predicted by the statistical model (8.17 $U.g^{-1}$). Figure 4 also shows pH profile in cultivation, with similar behavior observed for treatments with initial pH of 6.0 or higher, showing a decrease of pH in 48 h with further stabilization in range of pH 3.0 to 4.0.

Lipase production test in bioreactors with fixed bed and forced aeration

Lipase production test in the plugged-flow bed with forced water-saturated aeration, had similar behavior as observed in the validation experiment using the static flat-bed conformation (Figure 5). Lipase activity at 96 h cultivation (9.83 $U.g^{-1}$) was higher than found for the validation (8.19 $U.g^{-1}$). Solid-state bioreactors with forced aeration trough fixed bed have better heat transfer rates and capacity to remove metabolic heat and CO_2 from substrate (Mitchell et al. 2000). For instance, in SSC of *P. decumbens* in wheat straw and wheat bran to produce cellulases, highest enzyme productions were observed by using forced aeration in fixed bed configurations (Mo et al. 2004).

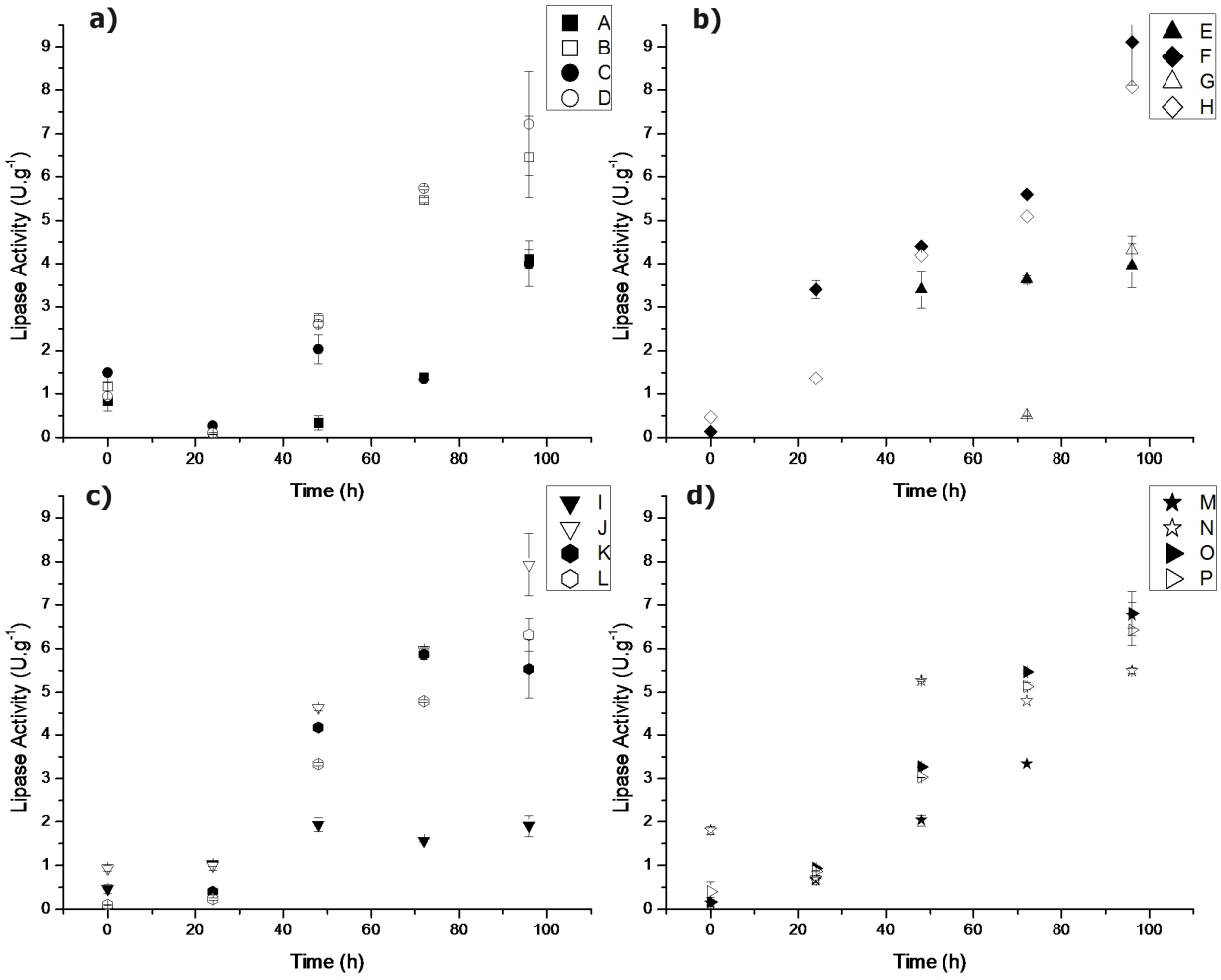

Figure 2. Kinetics of lipase activity during the CCD experiment for *Apergillus brasiliensis* 157f cultivation in the static flat-bed bioreactor configuration. Panels (a): treatments A (■), B (□), C (●), and D (○); (b): treatments E (▲), F (◆), G (△), and H (◇); (c): treatments I (▼), J (▽), K (⬢), and L (⬡); and (d): treatments M (★), N (☆), O (▶), and P (▷).

Table IV. Optimal conditions and solution predicted by the CCD analysis.

	Observed - Minimum	Optimal Predicted	Observed - Maximum
pH	4.31	7.71	7.68
% Oil	1.59	11.34	18.40
Temperature	21.59	32.74	38.40

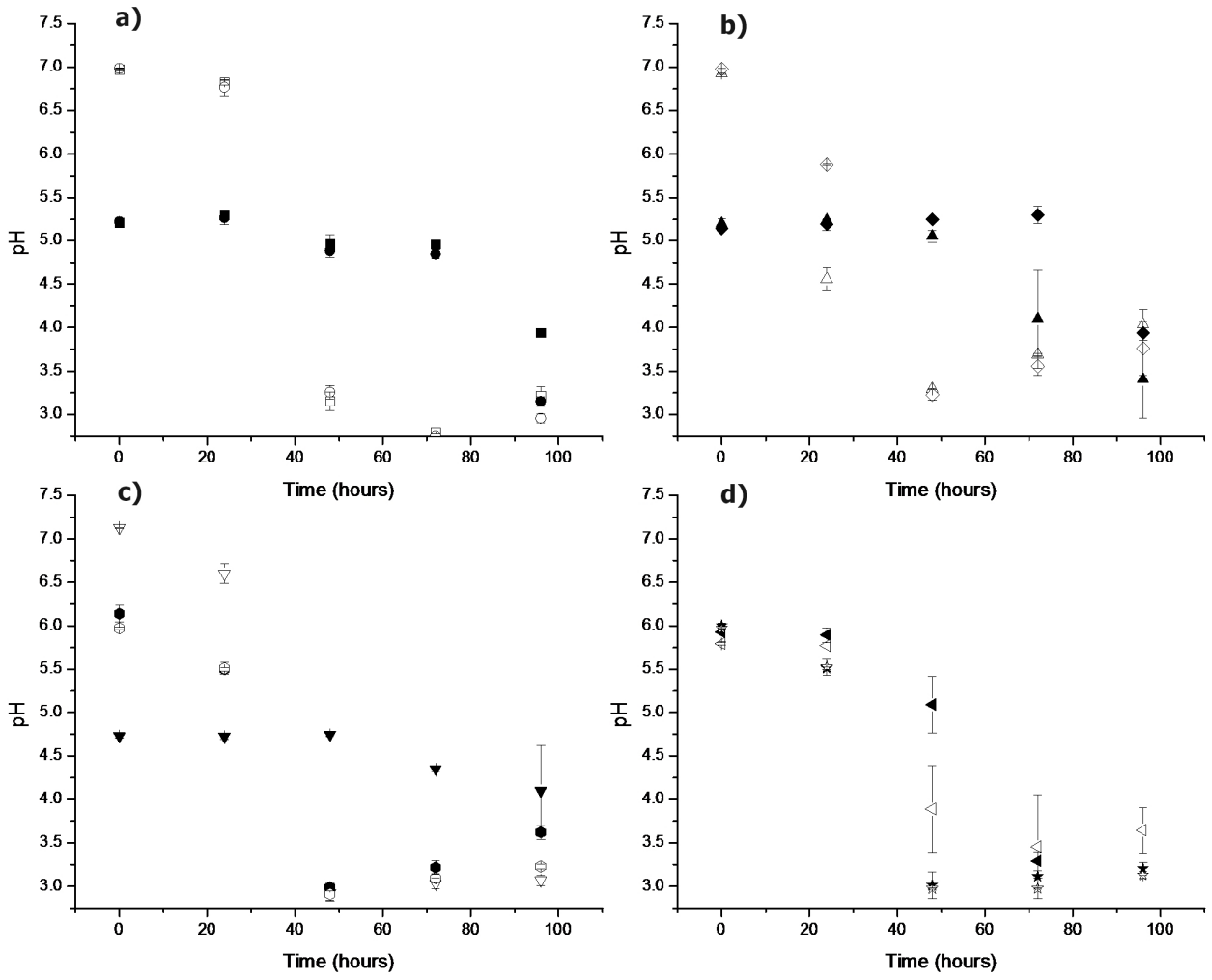

Figure 3. Kinetics of pH variation during the CCD experiment for *Apergillus brasiliensis* 157f cultivation in the static flat-bed bioreactor configuration. Panels (a): treatments A (■), B (□), C (●), and D (○); (b): treatments E (▲), F (△), G (◆), and H (◇); (c): treatments I (▼), J (▽), K (⬢), and L (⬡); and (d): treatments M (◀), N (◁), O (★), and P (☆).

Table V. ANOVA results from the CCD analysis of *Aspergillus brasiliensis* bench scale SSC in malt bagasse.

	SS	df	MS	F	*p*
(1) pH (L)	44.37315	1	44.37315	77.63922	0.000010*
pH (Q)	2.55707	1	2.55707	4.47408	0.063531
(2) % Oil (L)	0.11692	1	0.11692	0.20457	0.661762
% Oil(Q)	0.27350	1	0.27350	0.47854	0.506536
(3) Temperature (L)	0.16788	1	0.16788	0.29374	0.600993
Temperature (Q)	0.09471	1	0.09471	0.16571	0.693462
Error	5.14377	9	0.57153		
Total SS	52.52346	15			
Determination coefficient (R^2)	0.902				

***Significant at $p<0.05$; (L) linear effect; (Q) quadratic effect.**

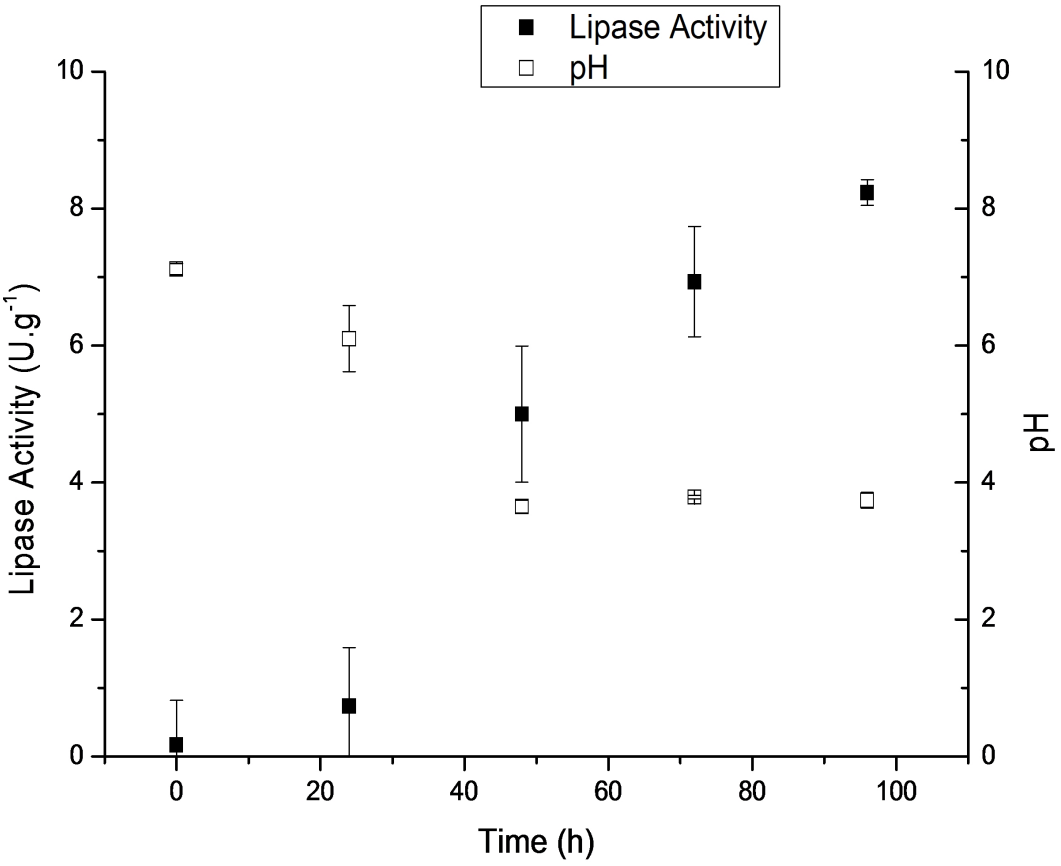

Figure 4. Profiles for lipase activity (■) and pH (□), during statistical validation of SSC of *Aspergillus brasiliensis* 157f in malt bagasse.

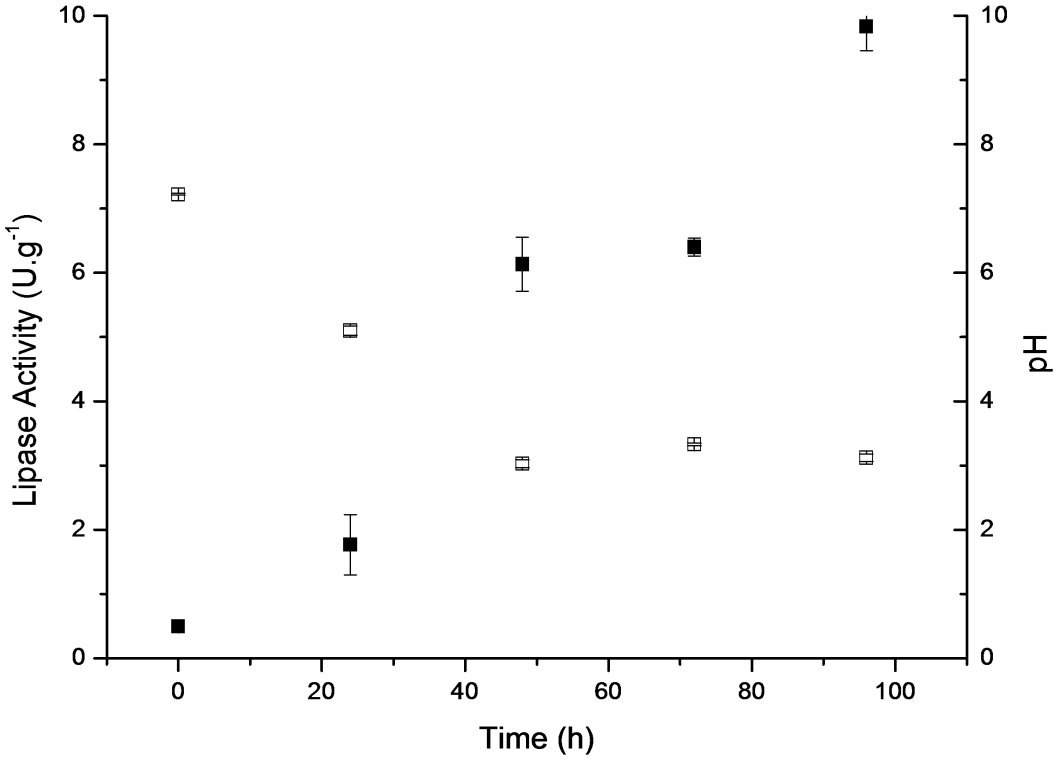

Figure 5. Lipase activity (■) and pH (□) profiles of SSC of *Aspergillus brasiliensis* 157f in plugged-flow bed with forced water-saturated aeration bioreactors (malt bagasse).

Lipase production test in a pilot-scale rotating drum bioreactor with intermittent agitation

Lipase activity and pH results for rotating drum bioreactor with intermittent agitation (pilot-scale test) are shown in Figure 6. Lipase activity at 96 h (6.37 ± 0.02 $U.g^{-1}$) was lower than results found for the other types of bioreactors tested. The pH profile had different behavior as observed in validation and cylindrical bioreactor with forced aeration. Lowest pH in this cultivation was 4.9. Mechanical mixing of the substrate was performed every 24 h for 30 min for homogenization. This agitation might have disrupted certain structures of the filamentous fungus, but could also facilitate cultivation in cases of strong bed packing problems (Mitchell et al. 2000, 2006).

Solid enzymatic preparation preliminary study

A possible strategy for reducing costs associated with the enzymatic route in biodiesel production is the use of a fermented solid, rich in lipase, produced by SSC, for the catalysis of reactions of esterification and transesterification (Salum et al. 2010). In this case, not only the substrate would have low cost, using an agro-industrial waste, but also the costs of purification and immobilization of lipases would be reduced. To have a preliminarily evaluation of the possible use of the solid enzymatic preparation obtained after 96 h of SSC of *A. brasiliensis* 157f under the optimized parameters, we prepared a SEP of this

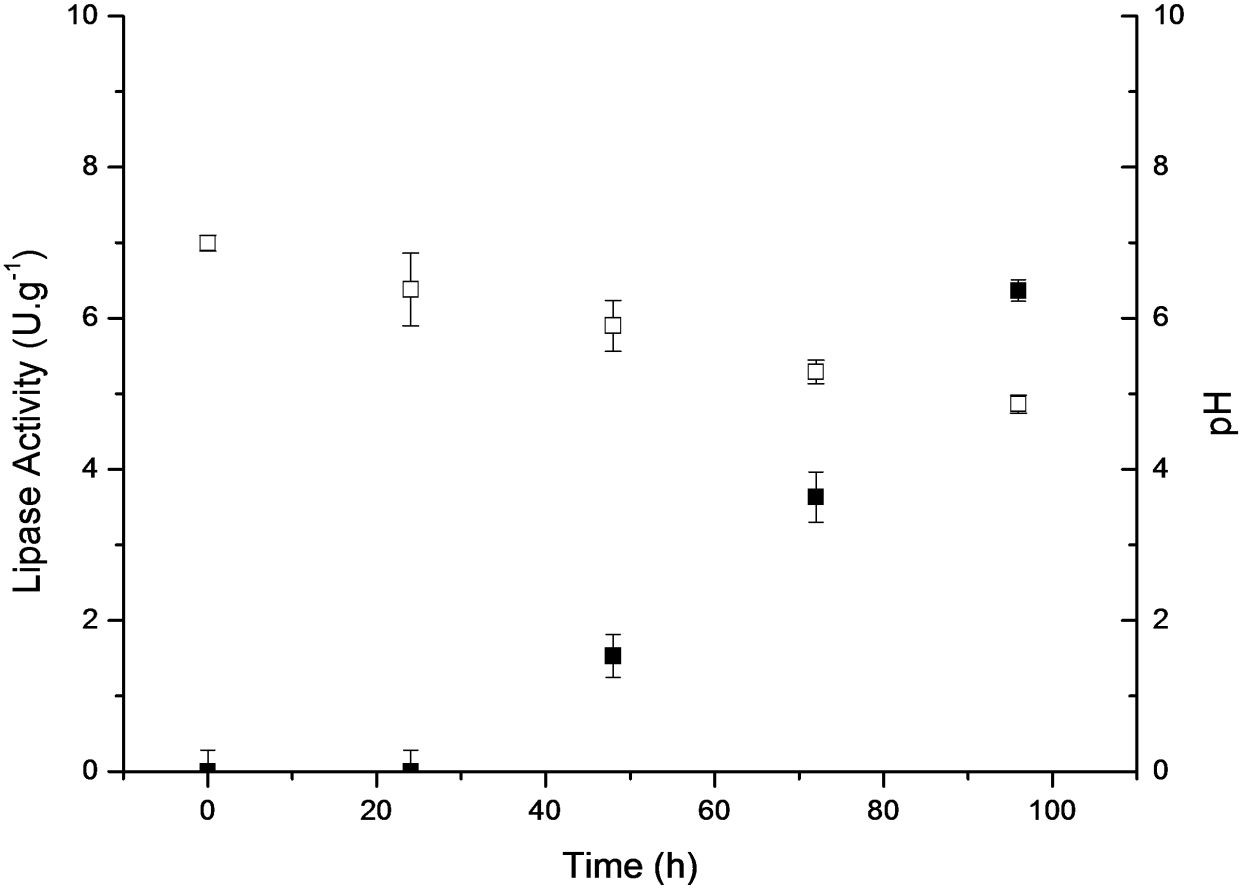

Figure 6. Lipase activity (■) and pH (□) profiles of SSC in the pilot-scale test in a rotating drum bioreactor of *Aspergillus brasiliensis* in malt bagasse.

material. Lipase activity measured in the SEP, after lyophilization, was 7.35 ± 0.13 $U.g^{1}$. In this study, a preliminary test was used to evaluate final lipase activity in SEP and further studies are necessary to assess possible applications of this biocatalyst. This technique is interesting to be exploited because it avoids the need for expensive processes such as lipase purification and immobilization, having, for example, the potential to reduce costs in lipase-catalyzed biodiesel production (Salum et al. 2010). This technique has been reported in literature for waste treatment and biodiesel production, as "Fermented Solid with Lipase Activity", presenting a promising bio-product for industries (Rosa et al. 2006, Zago et al. 2014). In effluent treatment tests with oil and grease, Rosa et al. (2006) were able to use SEP with lipolytic activity of 32.3 $U.g^{-1}$ produced in SSC of *Penicillium restrictum* using residues of the babassu oil industry as substrate, managing to make the effluent be under environmental legal disposal conditions. Such works represent an important contribution as an aid in biological treatment of effluents with high oil contents (Rosa et al. 2006). In a similar work, producing lipase-rich SEP with *R. microspores*, Zago et al. (2014) found high transesterification conversions of 68 % in 72 h of reaction time.

CONCLUSIONS

In this work, lipase production in solid-state cultivation in tray-type bioreactors (static flat-bed) was optimized using CCD analysis and validated. Under the same conditions, plugged-flow bed with forced water-saturated aeration bioreactors showed better results of lipase activity. These results demonstrated that forced aeration bioreactors could present better performances in industrial processes. Pilot-scale test in a rotating drum bioreactor showed lower lipase activity than observed for the other equipment, possibly because of fungi disruption. The solid enzymatic preparation showed lipase activity compatible with the previous cultivations and the lipolytic activity evaluated in the SEP and its low production costs justifies new researches for its possible application in industrial processes.

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