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Response Surface Methodology to Evaluation the Recovery of Amylases by Hollow Fiber Membrane

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ABSTRACT

*This work aimed to study the pH and the transmembrane pressure effects during the recovery of α and β amylases enzymes from corn malt (*Zea mays*) by hollow fiber membrane. The optimal condition was obtained for a statistical model, established by response surface methodology (RSM). The response surface analysis showed that the best operation condition for amylolitics enzymes recovery by hollow fiber membrane was 0.05 bar and pH 5.00, while the enzymes were purified about of 26 times.*

Key words: Hollow fiber membrane, corn malt, α and β amylase, RSM

INTRODUCTION

Of late, the use of enzymes in feeding, pharmaceutics, textile and others industries is increased for being economically viable. They are also used in the manufacture of alcoholic drinks, detergent, biosensors, and diagnostic kits and in the management of environment polluents (Jesus, 2002). α and β - amylases are commercial enzymes used its many applications, mainly in the starch hydrolyses (Fogarty and Kelly, 1979; Wiseman, 1987). They are obtained commonly from barley malt or microorganisms.

α-amylase (EC 3.3.1.1; α - 1,4 glucan, 4 - glucanhydroxilase) is extracellulas enzyme that hydrolyses of the α - 1,4 bonds, of the amylose, amylopectin, glycogen and dextrin molecules, but can not hydrolyses a-1,6 bonds. It has molecular weight about 50 kDa, with isoelectric point 5.4, very good enzymatic activity about pH 4.7 and 75 °C (properties enzymes depends from source) (Reguly, 1996; Wiseman, 1987).

β-amylase (EC 3.2.1.1, α-1,4, glucanmalthydrolase) is a extracellular enzyme that hydrolyses of amylopectin and glycogen, breaking each second α - 1,4 bond. It has molecular weight about 50 kDa, with isoelectric point 5.4, very good enzymatic activity about pH 4.5-6.5 and 55-57 °C, it is inactive after temperatures above of 60 °C (Reguly, 1996; Wiseman, 1987).

In biotechnology, separation and purification of biomolecules from large-scale fermentation represents the major manufacturing cost, therefore

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competitive advantage in commercialization will depend not only on biomolecule production, but also on innovation and optimization of downstream process. Downstream process more recent were appeared in this last time with the biotechnology advances, principally, with the several genes sequencing of divers organisms, including to the full human genome, the three principals separation process are: aqueous two-phase systems extraction, membrane separation and fluidized bed sorption (Asenjo, 1990).

Membranes separation process is an existing alternative among separation processes that it has been more used in recent years. A membrane is defined as a barrier to separate two phases and total restricting or presented various chemical species partial transporting into phase. The membrane controls the species transporting relative rate and, as all separation, furnishes one exit current that is poor in certain components and another, rich in these components (Cheryan, 1986; Noble and Way, 1993).

Cross flow filtration occurs when to solution or suspension flow runs parallel to the membrane and perpendicular to the permeate flow. The processes that involve cross flow filtration are used to recovery, to concentrate or to purify solutions. One of the principal membrane modules utilized in cross flow filtration is the flat membranes of plates and blocks (Santos, 1996).

Processes with membranes become viable in an industrial scale with the adequate choice of membranes to the processing of each determined product (Basseti, 2002).

Response surface methodology (RSM) is a tool very used in process optimization, due to be easily application and fast response. The analysis of variance (ANOVA) is employed for the determination of significant variables. ANOVA consists of classifying and cross-classifying statistical results and was tested by the means of a specified classification difference, which was carried out by Fisher's statistical test (*F*-test). The *F*-value is defined as the ratio of the mean square of regression (MRR) to the error (MRe) (*F*=MRR/MRe), representing the significance of each controlled variable on the tested model. The regression equations were also submitted to the *F*-test to determine the coefficient R^2 (Barros Neto et al., 2001; Higuti et al., 2004).

This work aimed to study the effect of the pH and of the transmembrane pressure during the recovery of the α and β amylases enzymes from corn malt (*Zea mays*) by hollow fiber membrane the optimal conditions obtaining it was for a statistical model, established by response surface methodology (RSM).

MATERIALS AND METHODS

Maize malt obtaining

The maize seeds were selected, their weight was measured, washed, the seeds were carried to water absorption until 40- 45 % (w/w) and germinated in laboratory. The germination time was between 4 – 5 days. The maize malt was dried at 55 °C (Biazus et al., 2005; Malavasi et al., 2004; Santana, 2003).

Enzyme activity

Amylase was assayed according to Milles laboratory method Nirmala and Muralikrishna (2003), Reguly (1996). 20 mL of 2 % soluble starch at pH 4.8 (0.1 M acetate buffer) were hydrolyzed by 500µL of enzyme sample in 10 min at 37 °C. One unit of enzyme activity was defined as µmol of glucose released for min under the assay conditions. The specific activity was calculated as activity for mg protein.

Total protein content

It was determined according to the dye binding method of Bradford (1976) with bovine serum albumin as protein standard.

Membrane module

The experiments were conducted in a module of membrane of hollow fiber type (MMHF-01), that is shown in Figure 1. The polyssulfone membranes (AMICON – H1MP01-43 model) used had an area of 0.03 m^2 and a pore size of 0.1 µm. Under the pH and pressure used in assay (to see Table 1), 2% corn malt was used with feed, the flow was circulated tangentially to the membrane surface, until full-process.

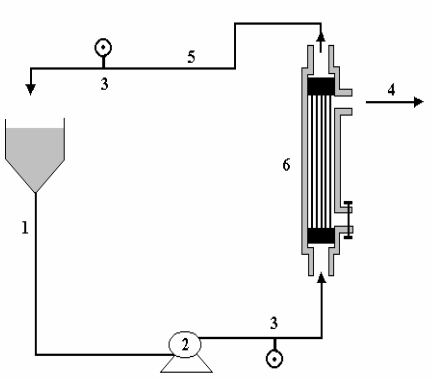

Figure 1 - Experimental MMHF-01 Microfiltration Unit.

Where: 1 – feed flow, 2 – pump, 3 – manometer, 4 – permeate, 5 – concentrate, 6 – hollow fiber membrane.

Experimental design

The influence of transmembrane pressure (***P***) and of pH on purification factor (***PF***) of α and β amylases enzymes from corn malt (*Zea mays*) were studied by 2^2 experimental planning with hexagon design (showed in Table 1), and response surface methodology (RSM) was used for optimization of recovering process. The least square method was used to estimate the model parameters, and the model fitting was made by analysis of variance methodology (ANOVA), all showed in Barros Neto *et al.* (1995 and 2001). The following variables codifications were used:

$$x_1 = pH - 6 \qquad (1)$$

$$x_2 = \frac{P - 0.10}{0.05} \qquad (2)$$

RESULTS AND DISCUSSION

Experimental results obtained in this work are shown in Table 1, which describes the assays carried out to optimize the recovery of α and β amylases enzymes.

Table 2 is contained the results of the statistical analysis of model validity by the analysis of variance methodology (ANOVA) to obtain the optimal empirical model for describing the system behavior studied Barros Neto et al., (1995 and 2001).

Table 1 - Planning matrix of the experiments with the optimal model data.

Assays	x_1	x_2	*pH*	*P* (bar)	$PF_{exp.}$	PF_{pred}
1	-1	-1	5.00	0.05	26.2488	25.9385
2	1	-1	7.00	0.05	4.3314	4.6417
3	-1	1	5.00	0.15	1.9514	1.6411
4	1	1	7.00	0.15	3.7225	4.0327
5	0	0	6.00	0.10	3.5204	4.9195
6	0	0	6.00	0.10	5.2900	4.9195
7	0	0	6.00	0.10	5.9480	4.9195
8	-1.41	0	4.59	0.10	13.8103	14.2491
9	1.41	0	7.41	0.10	1.3200	0.8811

Table 2 - Variance analysis (ANOVA) of the fitting model.

Source	Square sum	Free degree	Mean square	F_{calc}	F_{tab}
Regression	503.629	5	100.726		
Residual	3.923	3	1.308	77.033	9.01
Fitting fault	0.770	1	0.770		
Error	3.152	2	1.576	0.489	18.51
Total	507.551	8			
% explainig variance =			98.763		
% maximum explaning variance =			99.677		
Determination coefficient (R^2) =			0.9876		

Results of the F test, whole the first test (F_{calc}/F_{tab}) indicated that the model was statistical significant and the predict data were approaching the experimental data. The second test (F_{tab}/F_{calc}) indicated that the data were fitting and they were statically representing the response surface. For the ***F*** tests are statistical considerate the rate much be equal or more that 4 (Barros Neto *et al*. 1995). According to Barros Neto *et al*. (1995) bout F test must be > 4 for the model is statistical significant and predictive. Results showed that the model was statically significant and that the data get to describe well the response surface.

Equation 3 gives purification factor (***PF***) as a function of factors x_1 (***pH***) and x_2 (***P***, transmembrane pressure) as a square empirical model obtained by quadratic regression. This empirical model shows responses on individual and multiple effects of the factors Barros Neto et al., (1995 and 2001).

$$PF = 176.0011 - 32.5471pH - 1060.2499P + 118.4425(pH)\cdot(P) + ... \\ ... + 1.3308pH^2 + 1125.3168P^2 \quad (3)$$

In Figs. 2 and 3 the response surface and contour lines generated by data plotting of the optimal curve (Equation 2) for purification factor (PF) as a function of the factors are shown.

The increase of transmembrane pressure can to provoke a modification in the structure conformational of the enzymes, through of the passage by membrane pores, decreasing the specific enzymatic activity of amylolitics enzymes during the separation process, verified by the loss of activity (seen Figures 2 and 3). Behavior similar it was obtained in Lopes *et al*. (2004 and 2005), during the recovery of bromelain enzymes from pineapple by membranes.

Aforesaid behavior it was observed with the increase of the pH modifying the structure conformational of the enzymes due the alterations in the hydrogenionic concentration, decreasing the specific activity enzymatic of the amylolitics enzymes (Borzani *et al*., 2001).

Through analysis of these surfaces it can be observed that purification factor increased with reduction of the transmembrane pressure and of the pH. This shows that the best operational conditions for α and β amylase enzymes recovery by the hollow fiber membrane were a transmembrane pressure of 0.05 bar and a pH of 5.00.

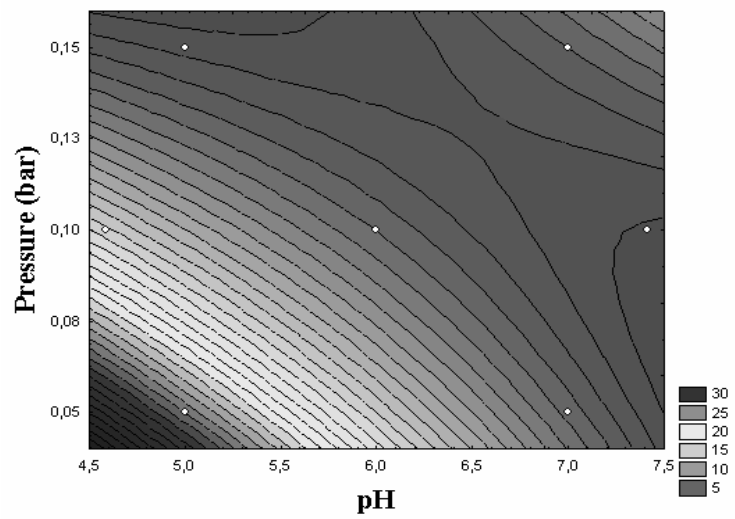

Figure 2 - 2D response surface for understanding the purification factor dependency with the pH and transmembrane pressure during the microfiltration of α e β amylases from corn malt.

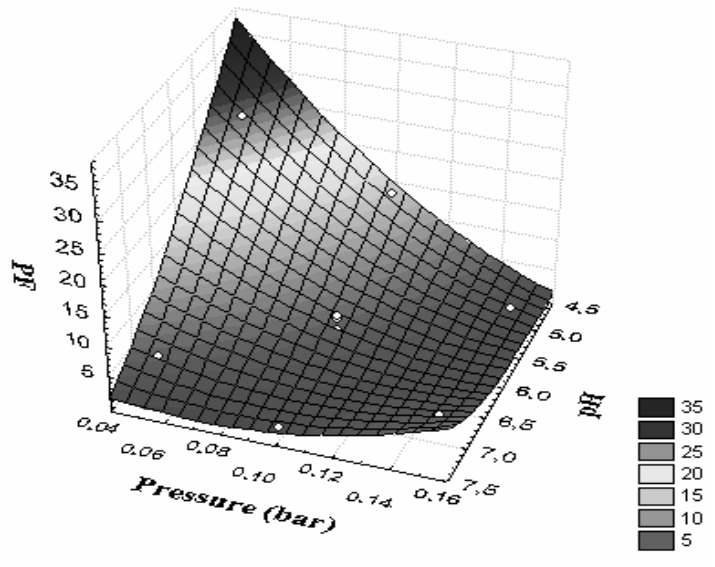

Figure 2 - 3D response surface for understanding the purification factor dependency with the pH and transmembrane pressure during the microfiltration of α e β amylases from corn malt.

CONCLUSIONS

Under the conditions adopted for this research, and based on the results obtained, the following conclusions can be reached:

- The variance analysis (ANOVA) showed that the best fitting model introduced a square dependency of the purification factor, with the pH and the transmembrane pressure.
- The response surface analysis showed that the best operation condition for amylolitics enzymes recovery by hollow fiber membrane was 0.05 bar and pH 5.00.

RESUMO

Este trabalho objetivou estudar o efeito do pH e da pressão trans-membrana durante a recuperação das enzimas α e β amilases do malte de milho (*Zea mays*) por membranas de fibras ocas, a obtenção das condições ótimas foi feita por um modelo estatístico, estabelecido pela metodologia de superfície de resposta (RSM). A análise da superfície de resposta mostrou que as melhores condições operacionais para a recuperação das enzimas amiloliticas por membranas de fibras ocas foi 0,05 bar e pH 5,00; onde as enzimas foram purificadas cerca de 26 vezes.

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