Xylanase Recovery Using Continuous Extraction with Reversed Micelles—A Statistical Approach

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Xylanase recovery from *Penicillium janthinellum* with a reversed micellar system consisting of a cationic surfactant using a continuous process was evaluated. A statistical approach applied to the results showed the highest xylanase recovery (43.5%), which was indicated by the model and was attained at an ionic strength of 10 mS/cm and a volumetric flow at 0.5 ml/min.

Introduction

Reverse micellar systems have been studied as a technique for extraction and purification of proteins (Pessoa, Jr. and Vitolo, 1997; Rodrigues *et al*., 1999). However, few examples of this type of extraction can be found in literature, namely, the extraction of α-amylase in an aqueous solution by TOMAC (tri-octyl methyl ammonium chloride) reversed the micellar phase using two mixer-settlers units (Dekker *et al*., 1989), the extraction of pure recombinant cutinase by AOT (sodium di-2-ethylhexyl sulfosuccinate) reversed micelles with a perforated rotating disc contactor (Carneiro-da-Cunha *et al*., 1994, 1996) and the recovery of intracellular proteins from *Candida utilis* by reversed micellar extraction in a spray column (Han *et al*., 1994). The present work describes the continuous extraction of extracellular xylanase from *P. janthinellum* to a reversed micellar phase of the cationic surfactant BDBAC [*n*-benzyl-*n*-dodecyl-*n*-bis(2-hidroxyethy)-ammonium chloride)] and the influence of the following factors: ionic strength, pulsation frequency and the volumetric flow rate.

1. Materials and Methods

1.1 Microorganism and growth conditions

Penicillium janthinellum isolated from a decaying wood (Milagres *et al*., 1993) was cultivated at 30°C for 5 days in the medium containing 2% (w/v) glucose, a 0.25% (w/v) yeast extract, a 2% (v/v) concentrated salt solution based on Vogel's medium and 2% (w/v) agar. The medium was sterilized at 112°C for 15 min. Spores were suspended in water and the suspension was filtered through a gauze placed on Erlenmeyer flasks to give a final spore concentration of 10^5 ml^{-1} for inoculation.

1.2 Preparation of sugar cane bagasse acid hydrolysate

Hydrolysate for cultivation came from 800 g of dry milled bagasse was mixed with 8 litres H_2SO_4 (0.25% v/v) and autoclaved for 45 min at 121°C. The liquid fraction was separated by filtration and the pH adjusted to 5.5 with NaOH.

1.3 Liquid-liquid extraction

The liquid-liquid extraction was performed using a pulsed column, as shown in **Fig. 1**. The column was made from a Perspex tube of 2.54 cm i.d. and 19 cm high. Three caps separated by 4 cm were mounted on a central shaft. The reverse micellar systems consisted of an organic phase of 0.2 M BDBAC-reversed-micelles in isooctane/hexanol (92.5:7.5 v/v). The volumetric flow of organic and aqueous phases were kept constant by using a multi-channel peristaltic pump. Both aqueous phases were assayed to determine enzyme activity.

1.4 Determination of conductivity

Conductivity were determined using a conduvimeter (Analyser model 650). The results were expressed in mS/cm.

1.5 Determination of enzyme activities

Xylanase activities were determined according to Bailey *et al*. (1992). The release of reducing sugars was determined using the 3,5-dinitrosalicylic acid method with xylose as a standard reference (Miller, 1959).

1.6 Factorial design methodology

Xylanase recovery was studied with a 2^3 full factorial central composite design with six star points and four replicates at the center point, 2 and eighth cube points to fit a second-order polynomial model, which

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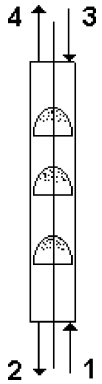

Fig. 1 Experimental column with 100 ml volume and 19 cm high and 2.54 cm i.d. Where: 1, inlet organic phase (0.2 M BDBAC-reversed-micelles in isooctane/hexanol 92.5:7.5 v/v); 2, outlet aqueous solution; 3, inlet aqueous phase that contains the studied enzyme; 4, outlet micellar solution

indicated that 18 experiments were required for this procedure. Ionic strength, frequency pulsed and volumetric flow were selected as experimental factors.

2. Results and Discussion

Liquid-liquid extraction of xylanase by reversed micelles was evaluated under different experimental conditions using an experimental design. A 2^3 full factorial design in two levels with 4 centre points was employed. The yield extraction values obtained from the 12 assays were 34.2%. However, under the best extraction conditions (ionic strength = 10.0 mS/cm, pulsation frequency = 1:3 and volumetric flow = 1 ml/min) the variables were significant.

As a function of these results, a 2^3 full factorial design was performed and all the factors were studied (ionic strength, pulsation frequency and volumetric flow). A statistical analysis indicates a significant curvature in the range of the experimental conditions. As a function of the first-order model, a new model was defined, now of the second-order, using non linear regression. Eighteen experiments were carried out (2^3 full factorial design + 4 centre points and 6 centre faces). **Table 1** presents the matrix employed in this design. The percentage of xylanase recovery under the best conditions (43.5%) (ionic strength = 10.0 mS/cm, pulsation frequency = 1:2 and volumetric flow = 0.5 ml/min).

Statistical analysis of the results showed significant effects only for the main variables *A* and *C*, and no evidence of any interactions involving these variables were obtained. Analysis of variance was performed and the regression coefficient of the model (R^2 = 0.73) calculated. The mathematical model which represents the continuous extraction process can be expressed by Eq. (1), where *Y* = xylanase recovery [%]: *A* = ionic strength (0) and *C* = volumetric flow (–1)

Table 1 Xylanse recovery [%] using the 2^3-full factorial design + 4 centre points and 6 centre faces under different treatments*

Assay	Factors			Xylanse recovery
	A	*B*	*C*	[%]
1	–1	–1	–1	28.2
2	1	–1	–1	12.9
3	–1	1	–1	32.7
4	1	1	–1	35.1
5	–1	–1	1	16.8
6	1	–1	1	2.7
7	–1	1	1	20.8
8	1	1	1	6.3
9	–1	0	0	9.1
10	1	0	0	3.3
11	0	–1	0	7.3
12	0	1	0	5.0
13	0	0	–1	43.5
14	0	0	1	10.5
15	0	0	0	9.4
16	0	0	0	11.8
17	0	0	0	16.8
18	0	0	0	12.6

*A = ionic strength [mS/cm] (–1 = 7.5; 0 = 10.0; 1 = 12.5); B = pulsation frequency [1/min] (–1 = 1:1; 0 = 1:2; 1 = 1:3); C = volumetric flow [ml/min] (–1 = 0.5; 0 = 1.0; 1 = 1.5); residence time [min] (–1 = 2.4; 0 = 3.0; 1 = 3.6)
Average values: 2 repetitions

$$Y = 9.413 - 4.73A - 9.53C + 11.538C^2 \quad (1)$$

Using the best conditions shown in Table 1, which was given by a statistical approach, we obtained 30.5% xylanase recovery (A = 0; 10.0 mS/cm and C = –1; 0.5 ml/min). Comparing the recovery for the best statistical conditions, given by Eq. (1), and the obtained experimental results, we have an maximum error above 30%.

Conclusion

Continuous xylanase extraction, using BDBAC-reversed micelles, is best at a low ionic strength of the medium and a low flow rate. The highest enzyme recovery was roughly 43.5%, with an enrichment factor of 1.3. These optimized conditions were: the ionic strength 10.0 mS/cm and the volumetric flow rate 0.5 ml/min.

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