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Short communication

A study of cell disruption of *Candida mogii* by glass bead mill for the recovery of xylose reductase

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ABSTRACT

The release of xylose reductase (XR) from *Candida mogii* by cell disruption in a glass beads mill was studied using an experimental design. Statistical analysis of the results indicated that XR volumetric activity increases by using lower glass beads diameter and cell concentration, and by increasing the number of agitation pulses. Based on results attained in experimental design, assays were carried out aiming at the maximization of XR release. Under optimized conditions (300 μm glass beads, 45 g/l of cell concentration and 50 pulses), the XR volumetric activity reach 0.683 U/ml. Disruption with glass beads showed to be the most efficient method for XR release when compared to sonication process. The highest specific activity (0.175 U/mg of protein) was found in extracts obtained by suspension freezing and thawing, which suggests that this method can be used as a selective process of cell disruption for XR release.

1. Introduction

Various biomolecules of industrial interest are concentrated intracellularly. In such cases, disruption of the cellular wall is a mandatory step prior to product recovery and purification [1–3]. Disruption may be achieved by mechanical, chemical or enzymatic methods, although in large-scale processing the mechanical method is generally employed [4]. Xylose reductase (XR) is the enzyme responsible for the first step of xylose metabolism in yeasts, catalyzing the reduction of xylose into xylitol [5]. Xylitol is a poliol of economical interest that can be used as a dietetic and anticariogenic sweetener. Currently, it is produced by a chemical process, although several biotechnological studies [6,7] are being carried out in the last years to develop alternative technologies for its production (microbiological and enzymatic). The feasibility of xylitol attainment by enzymatic process requires primarily the optimization of the conditions for obtaining and recovering of the enzyme XR that will be used as the catalyst. The purification and characterization of intracellular XR have been investigated [8,9], and among the different techniques used for cell disruption aiming to release XR, mechanical methods, such as agitation with glass beads, sonication and high-pressure homogenization, have been more frequently utilized [8,10,11]. Suitable conditions for disruption of *Candida mogii* cells employing a glass beads mill were pursued in this work, aiming at XR release. The influences of three variables (diameter of glass beads, concentration of cell suspension and number of pulses employed for agitation) were evaluated using an experimental design. Based on the results, a wider range of number of pulses was studied using fixed beads diameter and cell concentration. Results of disruption were evaluated by comparing processes employing glass beads and ultrasound in conditions previously established in our laboratory.

2. Materials and methods

2.1. Microorganism cultivation

C. mogii NRRL Y-17032 was obtained from the Northern Regional Research Laboratory (Peoria, IL) and was kept at 4 °C in glucose agar slants. The cultivation medium consisted in rice straw hemicellulosic hydrolysate diluted with distilled water up to an initial xylose concentration of 50 g/l [12]. The cells were cultivated for 48 h either in a 7-l fermentor (Bioflo III-New Brunswick Scientific Co.) containing 5-l of medium (temperature of 30 °C, aeration rate of 0.4 vvm and agitation of 400 rpm) or in a rotary shaker using Erlenmeyer flasks and a flask volume-to-medium volume ratio of 5:1 (200 rpm and 30 °C). In both cases, the initial cell concentration was 1 g of dry cells/l.

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2.2. Preparation of cell suspension

Cells were harvested by centrifugation at 1100 × *g*, washed with 0.1 M potassium phosphate buffer (pH 7.2), centrifuged, resuspended with the same volume of the buffer and stored at −18 °C. Before disruption, they were thawed and diluted to concentrations established for each assay.

2.3. Cell disruption

Cell disruption in a glass beads mill was performed in a 60-ml jacketed mill, filled with 30 ml of glass beads (300, 500 and 700 μm diameter) and 20 ml of cell suspension containing 45 g of dry cells/l. The beads were agitated by four slitted disks mounted on a drive shaft in the centric position. The rotation was produced by a 100-W electrical motor and the cell disruption by 10 s agitation pulses with 20 s intervals. Cooling was made by circulating ethylene glycol solution at −5 °C through the jacket. In the sonication assays, a Sonics equipment (60 Hz frequency, 1 s pulses 1 s intervals) was used for disrupting 3 ml of suspension containing 15 g of dry cells/l during 35 min. For freezing and thawing method, 20 ml of suspension containing 45 g of dry cells/l was frozen at −18 °C for 3 days and thawed at 4 °C. After all the treatments, the suspensions obtained were then centrifuged 6000 × *g* for 10 min, at 4 °C and the supernatants (cell extract) were analyzed.

2.4. Determination of enzymatic activity

The activities of XR were determined through the oxidation of the coenzyme (NADPH) by the change of absorbance at 340 nm at 38 °C. Assays were run in a 1-ml cuvette containing 50 μl 1 M phosphate buffer, pH 6.5; 100 μl 0.1 M mercaptoethanol; 100 μl 1.2 mM NADPH; 100 μl 0.5 M D-xylose and 650 μl diluted sample. The reaction was started up by the addition of D-xylose. Total proteins concentration was determined by a colorimetric method using bovine serum albumin as standard [13]. One unit of XR activity (U) was defined as the amount of enzyme catalyzing the oxidation of 1 μmol of NADPH per minute.

2.5. Experimental design

Assays were carried out according to a 2^3 factorial design with five repetitions at the central point (Table 1). The variables diameter of glass beads (300, 500 and 700 μm), number of pulses (24, 42 and 60) and cell concentration in dry weight basis (36, 63 and 90 g/l) were represented by coded levels (+1, 0 and −1, respectively).

Table 2
Effect estimates (EE), standard errors (S.E.) and *t*-test results for XR release, according to a 2^3 full-factorial design

Variables and interactions	XR activity		
	EE	S.E.	*t*-Value
A = ∅ Beads	−0.040	±0.017	−2.236[a]
B = No. Pulses	0.035	±0.017	2.044[a]
C = Cell concentration	0.010	±0.017	0.584
A × *B*	−0.055	±0.017	−3.213[b]
A × *C*	0.050	±0.017	2.921[b]
B × *C*	0.025	±0.017	1.460
Curvature	−0.066	±0.027	−2.391[a]

[a] Significant at 90% confidence level.
[b] Significant at 95% confidence level.

3. Results and discussion

In the present work, an experimental design was proposed to evaluate the effect of the operational variables on the disruption of *C. mogii* for xylose reductase (XR) release. Cells cultivated in bench fermentor were disrupted by varying the number of agitation pulses from 0 to 60, glass beads diameter from 300 to 700 μm and concentration of cell suspension from 36 to 90 g/l. The results expressed as volumetric activity of XR are presented in Table 1. As it can be seen, the highest XR activity (0.15 U/ml) was obtained in assays employing the smallest diameter of glass beads (300 μm) and the highest number of pulses (60), independently of the concentration of the cell suspension used. Table 1 also shows that the xylose reductase release was strongly influenced by the diameter of glass beads since its increase from 300 μm (assay 3) to 700 μm (assay 4) resulted in an decrease of 650% (from 0.15 to 0.02 U/ml) in the XR activity. To identify the variables that have the greatest influence on the XR release, an analysis of the estimated effects of the variables studied was thus performed (Table 2). Such analysis showed that only the interactions between glass beads diameter and number of pulses (*A* × *B*) and between glass beads diameter and cell concentration (*A* × *C*) were significant at 95% confidence level. The main effect of glass beads diameter was also significant, but at 90% confidence level.

The negative effect of the interaction *A* × *B* indicates that the highest XR activity can be achieved by increasing the values of one variable and decreasing the values of the other. Considering the significant negative main effect of glass beads diameter (*A*), this variable was set at the lowest level whereas the number of pulses (*B*) was at the highest level. In the same way, the positive effect of the interaction (*A* × *C*) indicates that the simultaneous reduction in the values of both variables led to an increase in XR activity, since the main effect of the beads diameter was negative. In fact, the

Table 1
2^3 factorial design of the study of *C. mogii* cell disruption for XR release

Assay	Coded levels			Actual values			Activity (U/ml)
	∅ Beads (*A*)	Pulses (*B*)	Cell conc. (*C*)	∅ Beads (μm)	Pulses	Cell conc. (g/l)	
1	−1	−1	−1	300	24	36	0.10
2	+1	−1	−1	700	24	36	0.05
3	−1	+1	−1	300	60	36	0.15
4	+1	+1	−1	700	60	36	0.02
5	−1	−1	+1	300	24	90	0.02
6	+1	−1	+1	700	24	90	0.10
7	−1	+1	+1	300	60	90	0.15
8	+1	+1	+1	700	60	90	0.09
9	0	0	0	500	42	63	0.09
10	0	0	0	500	42	63	0.06
11	0	0	0	500	42	63	0.03
12	0	0	0	500	42	63	0.05
13	0	0	0	500	42	63	0.03