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Research article

# Scaling-up batch conditions for efficient sucrose hydrolysis catalyzed by an immobilized recombinant *Pichia pastoris* cells in a stirrer tank reactor

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ABSTRACT

*Background:* Invert sugar is used greatly in food and pharmaceutical industries. This paper describes scaling-up batch conditions for sucrose inversion catalyzed by the recombinant *Pichia pastoris* BfrA4X whole cells expressing *Thermotoga maritima* invertase entrapped in calcium alginate beads. For the first time, we describe the application of a kinetic model to predict the fractional conversion expected during sucrose hydrolysis reaction in both, a model and a prototype bioreactor with 0.5- and 5-L working volume, respectively.
*Results:* Different scaled-up criteria used to operate the 0.5-L bioreactor were analyzed to explore the invert sugar large scale production. After model inversion studies, a 5-L scaled-up reaction system was performed in a 7-L stirred reactor. Both scaled-up criteria, immobilized biocatalyst dosage and stirring speed, were analyzed in each type of bioreactors and the collected data were used to ensure an efficient scale-up of this biocatalyst.
*Conclusions:* To date, there is not enough information to describe the large-scale production of invert sugar using different scaled-up criteria such as dose of immobilized biocatalyst and stirring speed effect on mass transfer. The present study results constitute a valuable tool to successfully carry out this type of high-scale operation for industrial purposes.



## 1. Introduction

An ideal enzymatic biocatalyst for industrial invert sugar manufactu ring should optimally operate in a highly concentrated sucrose solution (60%, w/v) to minimize microbial contamination and to avoid an initial dilution and final concentration steps. Concentrated sucrose solutions however, can reduce the diffusion of substrate into immobilized systems [1]; thus, the biocatalyst needs to be operated at pasteurization temperatures (60–70°C) and high agitation speed to reduce mass transfer problems.

Stirred tanks reactors are often the system of choice when a bioreactor is designed, and a common approach to enhance mass transfer in these systems is by increasing the impeller speed. However, impeller speed increment also increases the power-volume ratio, which is not economically feasible for large reactors because of high power cost [2]. Therefore, the impeller speed should be chosen such that it is adequate to make all the solid surface areas available for mass transfer [3]. If a satisfactory solid suspension is obtained in a small tank, and this fact becomes evident by visual observations, together with particle velocities or mass transfer rates then the safe scale-up rule is to keep geometrical similarity. In agitated reactors, this type of scale-up criterion is often derived from studies on the minimum rotational speed for complete solid suspension. Scale-up criterion involves selecting mixing variables to achieve the desired performance in both model and pilot scales [4].

Invert sugar has been extensively researched because of its importance in food and pharmaceutical industries [1,5,6,7,8,9,10,11]. Most of the research studies have focused to find the adequate support and immobilization methods to increase invertase thermostability and reuse. Despite the studies on scale-up and/or mass transfer with calcium alginate-immobilized cell-based systems for different processes [12,13,14,15], very few contributions have reported

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stirrer influence in mass transfer, scale-up criteria, and kinetics studies in immobilized cell systems for sucrose hydrolysis.

Thus, the present research focuses on a large-scale reaction system for sucrose hydrolysis catalyzed by the recombinant *Pichia pastoris* BfrA4X whole cells expressing *Thermotoga maritima* invertase entrapped in calcium alginate beads. For the first time, a useful tool for scaling up the sucrose inversion reaction with the PpABfrA biocatalyst is described, together with a new application of a kinetic model [10] to predict the fractional conversion expected during the sucrose hydrolysis reaction in both model and prototype reactors.

## 2. Material and methods

### 2.1. Strain, culture conditions, and cell inactivation

*P. pastoris* GS115 strain BfrA4X was obtained from the culture collection of the Center for Genetic Engineering and Biotechnology, Havana, Cuba [16].

Fed-batch fermentation was performed in a 7.5-L fermenter (INFORS) as described by Martínez et al. [10]. The culture broth was centrifuged; 150 g of the pellet was resuspended in 300 mL deionized water, and the yeast cells were heat-inactivated at 70°C for 30 min. The heat-killed yeast cells were then pelleted by centrifugation and used for whole cell immobilization or as a free cell source. A total of 150 g (wet weight) of the heat-killed cells was resuspended in distilled water (500 mL) to achieve final biomass concentrations of 300 g $L^{-1}$.

### 2.2. Preparation of calcium alginate beads

Sodium alginate solutions were prepared by the stepwise addition of 10 g of alginate powder to 150 g of wet biomass suspended in 500 mL of deionized water and stirred thoroughly to ensure a homogenous distribution of the cells in the alginate solution; the volume of the mixture was adjusted to 500 mL with deionized water before extrusion in the $CaCl_2$ solution. The alginate/cell suspension was added drop-wise through a silicone tube (using a peristaltic pump at a flow rate of 20 mL $min^{-1}$ and a fine needle) to 5 L $CaCl_2$ (0.55% w/v) solution. The $CaCl_2$ solution was stirred at constant speed (100 rpm) using an impeller-type marine propeller to avoid droplet aggregation. Gelation time was restricted to 1 h, after which the $CaCl_2$ solution was discarded. Subsequently, the beads were washed three times and stored in 1.46 M sucrose solution at 4°C. The resulting biocatalyst was named PpABfrA.

### 2.3. Invertase activity assay

Samples were withdrawn at regular intervals from the stirrer tank reactor to measure the reducing sugars. As described by Miller [17], an equimolar mixture of glucose and fructose was used as standard. Sucrose hydrolysis was determined by measuring the release of reducing sugars using 3′5-dinitrosalicylic acid (DNS).

### 2.4. Batch process in a 0.5-L model scale reactor

The immobilized cell biocatalyst was tested in a constant-volume batch reactor with a 0.7-L total reactor volume and 0.5-L working volume. The tank was 0.09 m in diameter and the marine impeller was 0.03 m diameter, resulting in an impeller to tank diameter ratio of 0.33. The impeller was placed 0.027 m from the bottom of the tank, resulting in a height to impeller diameter ratio of 0.9. The tank was fabricated of glass to enable the observation of flow patterns in the tank while conducting experiments. Calcium alginate beads of immobilized cells at a concentration of 100 and 200 g $L^{-1}$ were incubated 3 h at a sucrose concentration of 1.75 M at 60°C. To determine the agitation speed needed for satisfactory bead suspension in the model reactor, the speed of 0, 50, 100, and 200 rpm was assayed. To evaluate the sucrose hydrolysis profile, 100 g $L^{-1}$of the biocatalyst was incubated for 12 h at a sucrose concentration of 1.75 M at 60°C at a constant stirring of 100 rpm. Samples were withdrawn at regular intervals to measure the reducing sugars as described above.

### 2.5. Batch process in a 5-L prototype reactor

The immobilized cell biocatalyst was tested in a constant-volume batch reactor with a jacketed heat transfer fluid and with 7-L total reactor volume and 5-L working volume. The tank was 0.15 m in diameter and the marine impeller was 0.05 m diameter, resulting in an impeller to tank diameter ratio of 0.33. The impeller was placed 0.045 m from the bottom of the tank, resulting in a height to impeller diameter ratio of 0.9. To evaluate the sucrose hydrolysis profile, 100 g $L^{-1}$ of the biocatalyst was incubated for 12 h at a sucrose concentration of 1.75 M at 60°C and a constant stirring of 60 rpm. Samples were withdrawn at regular intervals to measure the reducing sugars as described above.

### 2.6. Kinetic equation evaluation for sucrose hydrolysis

The experimental fractional conversions ($X_A$) in the model and pilot-scale reactors were compared with the theoretical $X_A$ expected according to the kinetic equation $-\ln(1 - X_A) = kt$ previously reported by Martínez et al. [10]. From the equation, the theoretical $X_A$ expected at different time intervals was calculated as $X_A = 1 - e^{-kt}$ where $t$ is the time (h); $S_{A0}$ is the initial sucrose concentration (mol $L^{-1}$), $W$ is the biocatalyst weight ($gL^{-1}$), and k is the reaction kinetic coefficient: $k = (-0.0432\ g^{-1}\ h^{-1}\ M^{-1} S_{Ao} + 0.1054) W\ (h^{-1})$ according to Martínez et al. [10].

### 2.7. Statistical analysis

The statistical package for social sciences (SPSS) 15.0 was used for the data analyses. The data are presented as means ± S.D. The level of significance used in this study was $P < 0.05$.

## 3. Results and discussion

### 3.1. Effect of agitation and biocatalyst loading on sucrose hydrolysis in a model bioreactor

The effect of agitation and biocatalyst loading on mixtures and sucrose hydrolysis was determined in a 0.5-L reactor through a bifactorial design for the agitation factor, with four speed levels of 0, 50, 100, and 200 rpm and two load levels of 100 and 200 g $L^{-1}$of the biocatalyst. Operational conditions of 60°C, sucrose concentration of 1.75 M, and reaction time of 3 h remained constant.

The percentages of sucrose hydrolysis under the assayed conditions described above are shown in Table 1. The biocatalyst loading affects the percentage of sucrose hydrolysis, regardless of agitation speed. With the increase in the biocatalyst load, a significantly increased percentage of sucrose hydrolysis was achieved ($F = 13.0$; $p = 0.005$).

**Table 1**
Influence of agitation and biocatalyst loading on the percentage of sucrose hydrolysis in the model bioreactor.

| Biocatalyst loading (g $L^{-1}$) | Sucrose hydrolysis (%) | | | |
|---|---|---|---|---|
| | 0 rpm | 50 rpm | 100 rpm | 200 rpm |
| 200 | 14.9 ± 1.4[a] | 24.7 ± 1.2[a] | 51.4 ± 17.7[b] | 45.2 ± 16.4[b] |
| 100 | 8.0 ± 2.1[a] | 18.7 ± 0.9[a] | 36.7 ± 5.5[b] | 32.0 ± 4.6[b] |

Tabulated data are the means of triplicate measurements ± standard deviation. Different letters imply significant differences between sucrose hydrolysis results for the same biocatalyst loading (Tukey DHS test: $F = 28.4$; $p \leq 0.000$).