Contents lists available at ScienceDirect

Food and Bioproducts Processing

journal homepage: www.elsevier.com/locate/fbp

Fructooligosacharides production in aqueous medium with inulinase from *Aspergillus niger* and *Kluyveromyces marxianus* NRRL Y-7571 immobilized and treated in pressurized CO_2

Marceli Fernandes Silva[a], *Simone Maria Golunski*[a], *Diane Rigo*[a], *Vinícius Mossi*[a], *Marco Di Luccio*[b], *Marcio A. Mazutti*[c], *Débora Oliveira*[b], *J. Vladimir Oliveira*[b], *Marcus Vinícius Tres*[a], *Helen Treichel*[d,*]

[a] *Department of Food Engineering, URI – Campus de Erechim, Avenida Sete de Setembro, 1621, Erechim, RS 99700-000, Brazil*
[b] *Department of Chemistry and Food Engineering, Universidade Federal de Santa Catarina, UFSC, Caixa Postal 476, Florianópolis, SC 88040-900, Brazil*
[c] *Department of Chemical Engineering, Federal University of Santa Maria, Avenida Roraima, 1000, Santa Maria, RS 97105-900, Brazil*
[d] *Federal University of Fronteira Sul – UFFS, Avenida Dom João Hoffman, 313, Erechim, RS 99700-000, Brazil*

ABSTRACT

This work investigated the influence of compressed CO_2 treatment on the enzymatic activity of immobilized inulinases, and the production of fructooligosacharides in aqueous medium using these enzymes. The effects of system pressure, exposure time and depressurization rate on the enzymatic activity were evaluated through central composite designs (CCD) 2^3. Inulinase from *Kluyveromyces marxianus* NRRL Y-7571 presented an increase of 104% in the residual activity using CO_2 at 275 bar submitted to 6 h treatment, at a depressurization rate of 10 kg m^{-3} min^{-1}. For *Aspergillus niger* commercial inulinase, a decrease in enzyme activity was observed (residual activity of 39%) using CO_2 treatment at 75 bar for 6 h exposure at the highest depressurization rate (200 kg m^{-3} min^{-1}). Enzymatic activities changed significantly depending on the enzyme source and the experimental treatment conditions investigated. The values of FOS obtained using inulinases from *A. niger* were 30.64% of GF2; 13.90% of GF3 and 2.88% of GF4 in the medium containing inulin as substrate. Results demonstrate that the use of compressed CO_2 might be of technological importance as a preceding, preparation step, to improve enzyme activity, hence helping the development of new biotransformation processes.

© 2013 The Institution of Chemical Engineers. Published by Elsevier B.V. All rights reserved.

Keywords: Inulinase; Compressed CO_2; Residual activity; Pretreatment; Fructooligosacharides

1. Introduction

Inulinases are 2,1-β-D fructan furohydrolases (EC 3.2.1.7) that convert inulin to fructose. This enzyme can be applied to the production of high fructose syrups, which are extensively used in the food and beverage industry (Ettalibi and Baratti, 2001). The inulinases can also be applied in the production of fructooligosacharides. These macromolecules have drawn much attention due to their application as functional ingredients (Silva-Santisteban and Maugeri, 2005).

Nowadays, the high cost involved in enzyme production has been evidenced as one of the major obstacles to the commercialization of enzyme-catalyzed processes. However, recent advances in enzyme technology, such as the use of solvent-tolerant and/or immobilized inulinases, which make possible the re-utilization of the catalyst, have been

* Corresponding author. Tel.: +55 5481126456.
E-mail address: helentreichel@gmail.com (H. Treichel).
Received 23 January 2013; Received in revised form 27 March 2013; Accepted 29 April 2013
0960-3085/$ – see front matter © 2013 The Institution of Chemical Engineers. Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.fbp.2013.04.009

made to develop cost-effective systems (Risso et al., 2010, 2012).

Over the past recent years, many investigations regarding the utilization of alternative solvents for biocatalysis have been presented in the literature (Zhao et al., 2006; Knez and Habulin, 2002). Considerable efforts have been reported in the literature toward green chemistry reactions, with emphasis on enzymatic reactions carried out in ionic liquids (Lou et al., 2006; Kim et al., 2001; Lau et al., 2000; Erbeldinger et al., 2006) and in sub- and supercritical fluids (Kumar et al., 2004; Jessop and Leitner, 1999; Oliveira and Oliveira, 2000). The use of compressed fluids as solvents (liquefied gases) for chemical reactions may be a promising route to completely eliminate solvent traces from reaction products. In addition, manufacturing processes in compressed and near-critical fluids can be advantageous in terms of energy consumption, easier product recovery, adjustable solvation ability, and reduction of side reactions.

Undoubtedly, supercritical carbon dioxide has been elected as a green, environmentally friendly, solvent due to its special characteristics, such as low toxicity, working temperature compatible with the optimal temperature for enzymes and favorable transport properties that can accelerate mass-transfer-limited enzymatic reactions (Kumar et al., 2004; Jessop and Leitner, 1999; Kao et al., 1997; Chen and Yang, 2000; Habulin and Knez, 2001).

In order to conduct enzyme-catalyzed reactions at high pressures, the enzyme behavior in compressed fluids is of primary importance, as the loss of enzyme activity may lead to undesirable poor reaction rates and low yields of target products (Ceni et al., 2010; Brusamarello et al., 2010). In fact, enzyme stability and activity may depend on the nature of the enzyme, the characteristics of the compressed fluid, the water content of the enzyme/support and the process variables involved, which means that very distinct effects can be achieved depending on the characteristics of the system under investigation (Kamat et al., 1993; Oliveira et al., 2006a, 2006b; Fricks et al., 2006).

Based on these aspects, the main focus of this study was to investigate the enzymatic activity of two immobilized inulinases in compressed CO_2 using a commercial enzyme from *A. niger* and a home-made one from *Kluyveromyces marxianus* NRRL Y-7571 and the fructooligosacharides production in aqueous medium using these enzymes. The present report is part of a broader project and reflects our efforts to help developing new enzyme-catalyzed processes in alternative fluid media (Ceni et al., 2010; Brusamarello et al., 2010; Andrade et al., 2008; Dalla Rosa et al., 2008, 2009; Kuhn et al., 2010; Franken et al., 2010; Manera et al., 2011).

2. Materials and methods

2.1. Chemicals and enzymes

Carbon dioxide (minimum purity of 99.5%) was purchased from White Martins S.A. Commercial inulinase from *A. niger* was purchased from Sigma–Aldrich. Non-commercial inulinases were produced from *K. marxianus* NRRL Y-7571. The extract containing extracellular inulinase was obtained by solid-state fermentation using sugarcane bagasse as substrate. The medium composition was optimized in a previous work by our research group as following: 2 kg of sugarcane bagasse supplemented with pre-treated cane molasses 15 wt%, corn steep liquor (CSL) 30 wt%, and soybean bran 20 wt%. The moisture content was set up at 65 wt% and autoclaved at 121 °C for 20 min. The fermentation runs were started with the inoculation of an optimized volume corresponding to a cell mass of 14 g. All experiments were carried out for 24 h (Mazutti et al., 2010). After fermentation, the enzyme was extracted from the sugarcane bagasse by adding sodium acetate buffer 0.1 mol L^{-1} pH 4.8 in a solid/liquid ratio of 1:10, following incubation at 50 °C and 150 rpm for 30 min (Bender et al., 2008).

2.2. Inulinase immobilization

Inulinases from both microorganisms were immobilized according to the methodology described by Risso et al. (2010). Initially, a gel solution was prepared containing 16.5 g of distilled water and 0.75 g of sodium alginate, and maintained under mild heating. After complete dissolution of the alginate, 12.5 g of sucrose were added, followed by 5 mL of the solution containing the recovered inulinase, 3.5 mL of glutaraldehyde and 0.75 g of activated carbon.

For sphere formation, the gel solution was dropped into a 0.2 M calcium chloride solution in sodium acetate buffer (0.1 M and pH = 4.8) containing 3.5 wt% of glutaraldehyde, and stirred slowly at 10 °C. The immobilized inulinase was maintained at 4 °C for 24 h and then washed with sodium acetate buffer (0.1 M and pH = 4.8). To maintain the structure integrity, the immobilized spheres (around 0.005 m in diameter) were immersed in a 0.2 M calcium chloride solution in sodium acetate buffer (0.1 M and pH = 4.8).

2.3. High-pressure treatment of enzymes

The experiments involving the immobilized inulinases were performed in a laboratory-scale unit similar to that employed by Kuhn et al. (2010) which consists basically of a solvent (CO_2) reservoir, two thermostatic baths, a syringe pump (ISCO 260D), a stainless steel vessel (cell) with an internal volume of 3 mL, an absolute pressure transducer (Smar, LD301) equipped with a portable programmer (Smar, HT201) with a precision of ±0.37 bar, as schematically represented in Fig. 1. All lines of the experimental setup consisted of 1/16 in. OD tubing of stainless steel (HIP). Between the pump and solvent reservoir a check (one way) valve (HIP 15-41AF1-T 316SS) was positioned to avoid solvent back flow to the head of solvent cylinder. Two additional micrometric valves (HIP 15-11AF2 316SS) completed the experimental apparatus. One valve was located after the syringe pump, at the entrance of high-pressure cell, to allow solvent loading. The other valve was positioned just after the cell to perform solvent discharge. The high-pressure cell was submerged into the water bath and was supported by a simple device while the micrometric valves were located outside the bath.

The experimental procedure adopted for enzyme treatment in pressurized fluid consisted firstly in adjusting the thermostatic bath to 40 °C, the temperature established in the present work for all experimental runs. Then, the enzymatic preparation (0.7 g) in immobilized form was loaded into the cell. After this procedure, the system was submitted to pressurization under different exposure times, according to pre-established conditions following an experimental design, keeping a constant pressurization rate (10 kg m^{-3} min^{-1}). The system was finally depressurized at different pre-established rates, according to the experimental design, by a programmed

Download English Version:

https://daneshyari.com/en/article/19135

Download Persian Version:

https://daneshyari.com/article/19135

Daneshyari.com