

Stability evaluation of an immobilized enzyme system for inulin hydrolysis

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Received 8 August 2005; received in revised form 16 January 2006; accepted 16 January 2006

Abstract

The stability of free and Amberlite-immobilized inulinase, aiming at inulin hydrolysis was evaluated. The apparent activation energy of the biotransformation decreased when the immobilized biocatalyst was used, suggesting diffusional limitations, despite a decrease in the optimal temperature for catalytic activity for the immobilized biocatalyst. Thermal deactivation, of both forms of the biocatalyst, was evaluated by the linear inverted model. Inulinase immobilization consistently enhanced half-life of the enzyme, which increased up to 6-fold, as compared to the free form. Mean enzymatic activity was computed for both forms of the biocatalyst, and evidenced a decrease of optimal temperature with increased incubation periods. The deactivation energies estimated by an Arrhenius plot, evidenced a decrease of roughly 20% when free inulinase was used. The immobilized biocatalyst was effectively reused in successive batch runs for the hydrolysis of a 5% inulin solution.

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Keywords: Enzyme immobilization; Inulin hydrolysis; Inulinase; Thermal stability

1. Introduction

Inulin is a polysaccharide consisting of linear β -2,1 linked polyfructose units (Kaur & Gupta, 2002), terminated by a glucose residue through a sucrose-type linkage at the reducing end (Yun et al., 2000), that can be found in Jerusalem artichoke, dahlia tubers, chicory roots, garlic, asparagus root and salsify (Kaur & Gupta, 2002). The use of this polyfructan as raw material for the production of high fructose syrups (Peters & Kerkhofs, 1983; Vandamme & Derycke, 1983; Wenling, Huiying, & Shiyuan, 1999; Zittan, 1981) or inulooligosaccharides (Cho, Sinha, Park, & Yun, 2001a, 2001b; Yun, Song, Choi, Choi, & Song, 1999; Yun et al., 2000) has been established. The

total or partial hydrolysis of inulin, leading to syrups with high fructose content, usually designated ultra-high fructose syrups (UHFS) or to functional sweeteners, respectively, is achieved by the action of exoinulinase (EC 3.2.1.80) (Kulminkaya et al., 2003) or the synergistic action of exoinulinase and endoinulinase (EC 3.2.1.7) (Nakamura, Ogata, Shitara, Nakamura, & Ohta, 1995), if the goal is UHFS; or by the action of endoinulinase, if short chain fructans, namely oligofructose, are aimed at, as a result of partial enzymatic inulin hydrolysis (Kaur & Gupta, 2002; Yun, Kim, Yoon, & Song, 1997a).

Inulinases are fructofuranosyl hydrolases produced by a wide array of microorganisms, comprehending bacteria, fungi and yeast. Among these, the most common sources for inulinases are *Aspergillus* spp. and *Kluyveromyces* spp. (Pendey et al., 1999), alongside with *Pseudomonas* spp. (Kim, Choi, Song, & Yun, 1997; Yun, Kim, Kim, & Song, 1997b), *Xanthomonas* spp. (Park, Bae, You, Kim, & Yun,

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1999), *Penicillium* spp. (Onodera & Shiomi, 1988), *Chrysosporium* spp. (Xiao, Tanida, & Takao, 1989) and *Bacillus* spp. (Zherebtsov, Shelamova, & Abramova, 2002). More recently, the production of recombinant inulinases has also been reported (Park, Jeong, Kim, Yang, & Chae, 2001; Yun et al., 1999; Zhang, Zhao, Zhu, Ohta, & Wang, 2004). Both the products, from total or partial hydrolysis, are commercially relevant.

Currently the commercial production of high fructose syrups, which are used as low caloric sweeteners, given the enhanced sweetness of fructose over sucrose, roughly a 1.3-fold increase in a solids weight basis (<http://www.lsbu.ac.uk/biology/enztech/maltose.html>, accessed 11 April 2005), relies multi-enzymatic starch hydrolysis, combined with glucose isomerization, and a separation step in a chromatographic column, if a fructose content above 42% is envisaged, to yield high fructose corn syrups, (HFCS) (Huisman & Gray, 2002; Toumi & Engell, 2004). The consumption of HFCS has been rising steadily, as evidenced by the production in the USA, which increased from 2.2 million ton in 1980 to 9.4 million tons in 1999 (Coulston & Johnson, 2002). Lower complexity, higher fructose yield and concomitant cost reduction are easily foreseen for a production process based on inulin hydrolysis. On the other hand, the functional food world market which includes functional sweeteners, such as inulooligosaccharides (or oligo-fructose or fructo-oligosaccharide) (Cho et al., 2001a, Cho, Sinha, Park, & Yun, 2001b; Park et al., 2001), is quite huge and the particular segment embracing these sweeteners is still growing (Holzapfel & Schillinger, 2002). The broad use of oligo-fructose as a food ingredient is related to its acknowledged health benefits, namely its positive interaction with intestinal flora (Alles, Scholtens, & Bindels, 2004; Van Loo et al., 1999), and its role as low caloric sugar replacement in chocolate, ice cream or chewing gum (Park et al., 2001). Furthermore, a single given inulinase based system can be used for either fructose or oligo-fructose production, depending on the selection of the appropriate operational conditions (Nakamura et al., 1995).

The application of enzymes in practical processes often requires biocatalyst immobilization, which enhances thermal stability, allows repeated or continuous use of the biocatalyst and, furthermore eases downstream processing (Schmid et al., 2001; Van Beilen & Li, 2002). Such an approach has been used for inulin hydrolysis to high fructose syrups, using either enzymes (Ettalibi & Baratti, 1992; Gupta, Kaur, Kaur, & Singh, 1992; Kim, Byun, & Uhm, 1982; Nakamura et al., 1995; Peters & Kerkhooft, 1983; Wenling et al., 1999) or whole cells with inulinase activity (Barranco-Flórido, García-Garibay, Gómez-Ruiz, & Azola, 2001). A hydrolytic system, based in on inexpensive immobilization method, may prove quite useful, particularly if large scale application is foreseen (Katchalski-Katzir & Kraemer, 2000; Schmid et al., 2001). A thorough characterization of a given bioconversion system is necessary in order to evaluate its feasibility. In a previous paper

the use of a simple, low-cost methodology (Obón, Castellar, Iborra, & Manjón, 2000) for inulinase immobilization and concomitant application on inulin hydrolysis to fructose was ascertained, and kinetic characterization was performed (Rocha, Catana, Ferreira, Cabral, & Fernandes, 2006). The present work seeks to provide a deeper insight of this bioconversion system based in the immobilization of a commercial inulinase preparation onto Amberlite. In order to do so, the thermal stability of the immobilized enzyme preparation was evaluated and matched to the free form of the biocatalyst. Biocatalyst reuse, in successive batch runs with an initial inulin concentration of 5.0% (w/v), was performed to assess the operational stability of the biotransformation system.

2. Materials and methods

2.1. Biocatalyst

Fructozyme L, a commercial preparation of inulinases from *Aspergillus niger*, was provided by Novozymes.

2.2. Enzyme immobilization

Fructozyme L was immobilized onto Amberlite IRC 50 (Rohm and Haas), as described previously (Rocha et al., 2006).

2.3. Effect of temperature in hydrolytic activity

The biotransformations were started by adding 10 µl of a 10-fold diluted solution of Fructozyme L in 100 mM acetate buffer, pH 5.5, or a given amount of the immobilized biocatalyst, so that similar enzyme concentrations were present, to a 5 g l⁻¹ solution of inulin, also in 100 mM acetate buffer, pH 5.5. Bioconversion runs were performed in a temperature range of 30–70 °C, and with a stirring speed of 600 rpm, in order to prevent external mass transfer resistances, as determined in preliminary trials. All trials were performed in duplicate, at least, in a magnetically stirred jacketed glass vessel, of 25 ml volume. Samples (50 µl) were taken periodically, in 1 min gaps up to 5 min, or up to 25 min in 5 min gaps, when the free or immobilized biocatalyst were evaluated, respectively, and immediately assayed for quantification of reducing sugars.

2.4. Thermal stability

A given amount of free or immobilized biocatalyst was incubated in 100 mM acetate buffer, pH 5.5. Briefly, a 5-fold diluted commercial enzyme preparation was incubated at different temperatures. Periodically, 10 µl samples were taken periodically and added to 3 ml of a 5 g l⁻¹ solution of inulin in 100 mM acetate buffer, pH 5.5, to quantify residual catalytic activity. For the immobilized biocatalyst, 6 g of Amberlite containing immobilized inulinase were incubated in the buffer solution. Periodically 5 ml samples

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