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Continuous production of fructooligosaccharides and invert sugar by chitosan immobilized enzymes: Comparison between in fluidized and packed bed reactors

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ABSTRACT

In this work, β-fructofuranosidase and β-fructosyltransferase were covalently immobilized on chitosan spheres, using glutaraldehyde as a coupling agent, in order to produce invert sugar and fructooligosaccharides (FOS). Maxinvert L was used to make β-fructofuranosidase biocatalyst yielding 7000 HU/g. A partial purified β-fructosyltransferase from Viscozyme L was used to prepare the other biocatalyst yielding 2100 TU/g. The production of invert sugar and FOS was evaluated using different continuous enzymatic reactors: two packed bed reactors (PBR) and two fluidized bed reactors (FBR). The invert sugar production achieved a yield of 98% (grams of product per grams of initial sucrose) in the PBR and 94% in the FBR, whereas FOS production achieved a yield of 59% in the PBR and 54% in the FBR. It was also observed in both cases that varying the flow rate it is possible to modulate the FOS composition in terms of nystose and kestose concentrations. The operational stability of FOS produced in the PBR was evaluated for 40 days showing no reductions in yields.

1. Introduction

The commercial viability of industrial biotransformations is heavily dependent on the cost of the enzyme. Industrial applications of enzymes are often hampered by the lack of long-term operational stability, difficult recovery and reuse of the enzyme. These drawbacks can be overcome by immobilization of the enzyme [1,2].

Notwithstanding all these advantages compared to the free enzymes, immobilized enzymes usually have their activities reduced, producing unfavorable effects on their overall catalytic performances. This alteration can result from mass transfer limitations, which may be reduced by applying appropriate reactor designs and immobilization techniques [3,4]. Among immobilization techniques, the use of chitosan [(1 → 4)-2-amino-2-deoxy-β-D-glucan] as a support for covalent attachment is widely used for a multiplicity of purposes, mostly in industrial applications. This fact is due to several qualities of chitosan, such as the presence of reactive functional groups for direct reactions and for chemical modifications resulting in high affinity to proteins, hydrophilicity, good biocompatibility, non-toxicity, improved resistance to chemical degradation and ease of preparation in a variety of physical forms [3,5]. Recently, several works reported the development of packed bed or fluidized bed reactors filled with chitosan biocatalysts for industrial purposes [6–9].

Packed bed reactors (PBR) consist of an immobile stack of particles within a column, through which a reactant solution is pumped. These reactors are preferred over other reactor types because of simpler technology, high bed volume, ease of operation, and scaling up [10,11]. Fluidized bed reactors (FBR) consist of particles within a column, which are kept in movement (fluidization), by the liquid flow. The particles are retained by a hydrodynamic balance between gravity and drag forces promoted by the upflow substrate stream [12,13].

Both types of reactors enable continuous production without the need of a prior separation of the enzyme resulting in lower process costs [7,12]. In comparison to PBR, FBR are less susceptible to column clogging, formation of preferential flow paths and compression due to bed weigh [11,14]. FBR also present higher axial

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dispersions than PBR. On the other hand, PBR present lower shear stress on immobilized enzymes, generally leading to long-term enzyme stability [11].

Invertase (or β-fructofuranosidase, E.C. 3.2.1.26) is a suitable enzyme for carrying out studies concerning enzymatic processes due to its stability, no need for any cofactors and its commercial significance [15,16]. This enzyme is used for sucrose (β-D-fructofuranosyl(2 → 1)β-D-glucopyranoside) hydrolysis, resulting in an equimolar mixture of glucose (α-D-glucose) and fructose (β-D-fructose) known as invert sugar. This product is widely used in food and beverage industries due to its higher sweetness and lower susceptibility to crystallization [17]. However, the application of immobilized invertase in enzyme reactors for sucrose hydrolysis, on the industrial scale, is still in the development phase [18].

β-Fructosyltransferase (E.C.2.4.1.9) is another suitable enzyme for studying the reactor design in enzymatic processes. This enzyme is used for short chain fructooligosaccharides (FOS) synthesis from sucrose. FOS are prebiotic food ingredients [19], and short chain FOS present sweet taste and may be used as a sweetener by diabetics [20]. Although the enzymatic synthesis of FOS also requires sucrose as substrate, the concentration of sucrose required is higher than for sucrose hydrolysis. This solution is more viscous, and the use of a highly viscous liquid in PBR may lead to the fatal problem of column clogging [11], therefore this effect must be evaluated.

In this context, the aim of this work is to evaluate the best reactor design to be used for invert sugar and FOS production catalyzed by immobilized invertase and β-fructosyltransferase, respectively. Both enzymes have been recently immobilized in chitosan particles yielding stable biocatalysts [21,22]. Packed-bed and fluidized-bed reactors were compared for sucrose hydrolysis and FOS synthesis. Furthermore, it was evaluated the influence of operational conditions on the FOS composition.

2. Experimental

2.1. Materials

Invertase from *Saccharomyces cerevisiae* (Maxinvert L 10000) was kindly donated by DSM Food Specialties (The Netherlands). Commercial enzyme preparation from *Aspergillus aculeatus* with β-fructosyltransferase activity (Viscozyme L), produced by Novozymes, was kindly donated by LNF Latino Americana (Brazil). Chitosan from shrimp shells (>75% deacetylated) was purchased from Sigma–Aldrich (USA). Sucrose and glutaraldehyde 25% were purchased from Vetec Química Fina Ltda (Brazil). Enzymatic glucose (D-glucose) determination kit was purchased from In Vitro Diagnóstica Ltda (Brazil). All other chemicals were analytical or HPLC grade obtained from readily available commercial sources.

2.2. Enzymatic activities

Maxinvert L (invertase) hydrolytic activity was evaluated using a substrate solution of 100 g/L of sucrose in 50 mM sodium acetate buffer (pH 4.5) at 50 °C. Samples were taken at regular intervals and the reaction was stopped by the addition of 0.1 M sodium carbonate buffer, pH 10.0. Glucose released was measured with the enzymatic glucose determination kit. One hydrolytic unit of enzymatic activity (HU) was defined as the amount of enzyme that produces 1 μmol of glucose per minute at test conditions.

Viscozyme L (β-fructosyltransferase) transfructosylation activity was carried out at 50 °C using a solution of sucrose 600 g/L in 50 mM sodium acetate buffer pH 5.5. Fifty chitosan particles (17.1 mg) were incubated in 2 mL of sucrose solution. Samples were taken after 15 min and the concentration of saccharides (sucrose, glucose, fructose, kestose and nystose) were measured on HPLC. On unit of transfructosylation activity (TU) was defined as the amount of enzyme that produces 1 μmol of kestose or nystose from sucrose per minute [22].

All the activities of immobilized enzymes were carried out under agitation. Protein content of the enzyme solutions was determined by the Lowry assay.

2.3. Chitosan spheres synthesis

The chitosan particles were prepared adding dropwise a chitosan solution into an alkaline coagulation solution, previously described by Klein et al. [23]. The prepared particles had a spherical shape with a diameter of approximately 2 mm and a dry weight of 0.343 ± 0.008 mg per sphere.

Activation of chitosan particles was carried out incubating the chitosan particles with glutaraldehyde solution as it was previously described in Lorenzoni et al. [22].

2.4. Enzyme immobilization

Invertase immobilization was carried out incubating the particles with a solution of enzyme diluted in activity buffer (50 mM sodium acetate, pH 4.5) at room temperature under gentle shaking for 3 h, in an orbital shaker at 120 rpm with 25 mm of orbital diameter. The amount of enzyme applied to the support was 50 mg per gram of support, in a ratio of enzymatic solution to solid of 146 μL per milligram of support in Erlenmeyer flasks. The amount of support in an immobilization batch varied from 50 to 1400 chitosan spheres, i.e., 17.1–480 mg, with the same results.

β-Fructosyltransferase was partially purified and immobilized as previously described by Lorenzoni et al. [22]. The amount of enzyme applied to the support was 180 mg per gram of support.

After immobilization, both biocatalysts were washed with buffer NaCl (1.0 M) and ethylene glycol (30%, volume fraction) in order to eliminate non-covalently bonded enzymes. The immobilization yield (IY) and immobilization efficiency (IE) were calculated by Eqs. (1) and (2), respectively, previously described in Valerio et al. [21], and Sheldon and Van Pelt [1]:

$$\mathrm{IY} = \frac{\text{immobilized activity}}{\text{starting activity}} \quad (1)$$

$$\mathrm{IE} = \frac{\text{observed activity}}{\text{immobilized activity}} \quad (2)$$

Immobilized activity is the difference between the activity applied to the support and the activity measured in the washing fractions. Starting activity is the activity of the enzyme added to the support. Observed activity is the activity on the chitosan beads after immobilization.

2.5. Reactors setup

The reactors consisted of a water-jacketed glass column, each filled with 930 units of chitosan spheres (280 mg of dry weight). Both enzymatic preparations were evaluated in packed bed and fluidized bed reactors. The reactors were flow rate controlled with adjustable peristaltic pumps.

The packed bed column (height, 85 mm; inner diameter, 10 mm; volume, 6.67 mL) has an entrance at the bottom and an exit at the top, which were fitted with a sintered glass disk to retain the particles within the column. The fluidized bed column (height, 230 mm; inner diameter, 10 mm; volume, 18.0 mL) was equipped with one additional exit at the top and one additional entrance at the bottom for external liquid recirculation, also fitted with sintered glass disk. This design enables fluidization of the biocatalysts independently