Contents lists available at ScienceDirect

Food and Bioproducts Processing

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

Galacto-oligosaccharide production with immobilized β-galactosidase in a packed-bed reactor vs. free β-galactosidase in a batch reactor

Anja Warmerdam[a], *Eric Benjamins*[b,c], *Tom F. de Leeuw*[a], *Ton A. Broekhuis*[b], *Remko M. Boom*[a], *Anja E.M. Janssen*[a,*,1]

[a] *Food Process Engineering Group, Wageningen University, Bomenweg 2, 6703 HD Wageningen, The Netherlands*
[b] *Faculty of Mathematics and Natural Sciences, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands*
[c] *FrieslandCampina, P.O. 1551, 3800 BN Amersfoort, The Netherlands*

ABSTRACT

We report here that the usage of immobilized enzyme in a continuous packed bed reactor (PBR) can be a good alternative for GOS production instead of the traditional use of free enzyme in a batch reactor. The carbohydrate composition of the product of the PBR with immobilized enzyme was comparable to that of the batch reactor with free enzyme. The stability of the immobilized enzyme at a lactose concentration of 38% (w/v) and at 50 °C was very high: the half-life time of the immobilized enzyme was approximately 90 days. The enzymatic productivity of GOS production using immobilized enzyme in a PBR can be more than six times higher than that of GOS production with free enzyme in a batch reactor. Besides, when aiming for an equal volumetric productivity to the batch process in designing a PBR, the volume of the PBR can be much smaller than that of the batch reactor, depending on the enzyme dosage and the run time of a single batch.

Keywords: Enzyme immobilization; Packed bed reactor; Batch reactor; β-galactosidase; Biolacta N5; Eupergit C 250L

1. Introduction

The advantages of immobilized enzyme instead of free enzymes have been shown by existing large-scale processes such as the production of high fructose syrup (HFS) and penicillins such as 6-APA (Brodelius, 1978; Linko et al., 1983). Due to the reuse of the immobilized enzymes, the cost of enzyme and carrier can be reduced to a few cents per kg finished product (Katchalski-Katzir and Kraemer, 2000).

Usually, enzymes are immobilized onto insoluble polymeric beads, simplifying the recovery of the enzyme. Besides the ease of recovery, the immobilization often provides an increase in their stability (Boller et al., 2002; Campello et al., 2012; Cao, 2005; Chen et al., 2009; Hernaiz and Crout, 2000; Liu et al., 2012; Nakkharat and Haltrich, 2007; Nguyen et al., 2005; Zhou and Chen, 2001). The enhanced stability often comes at the expense of the enzyme activity (Sheldon, 2007). Nevertheless, the ability to reuse the enzyme often compensates for the loss in activity.

The current industrial process for the production of galacto-oligosaccharides (GOS) uses free β-galactosidase (FDA, 2007, 2009, 2010) in a batch reactor. GOS are produced on tonnage scale (Crittenden and Playne, 1996; Gänzle, 2012; Mahoney, 1998) and are applied in infant nutrition because of their prebiotic benefits for health and similarity to human milk oligosaccharides (HMO) (Macfarlane et al., 2008; Mahoney, 1998; Playne and Crittenden, 2009).

GOS are synthesized from lactose via enzymatic synthesis with β-galactosidases (Barreteau et al., 2006; Mahoney, 1998; Playne and Crittenden, 2009; Prenosil et al., 1987). Besides

* *Corresponding author*. Tel.: +31 317 48 22 31.
E-mail address: anja.janssen@wur.nl (A.E.M. Janssen).
Received 15 April 2013; Received in revised form 27 August 2013; Accepted 29 August 2013
Available online 7 September 2013
[1] P.O. Box 8129, 6700 EV Wageningen, The Netherlands.
0960-3085/$ – see front matter
http://dx.doi.org/10.1016/j.fbp.2013.08.014

forming GOS through transgalactosylation, β-galactosidases simultaneously hydrolyze lactose into galactose and glucose. Many factors such as the initial lactose concentration, the temperature, and the enzyme source, affect the ratio between hydrolysis and transgalactosylation (Boon et al., 2000).

Since in the GOS production process the enzyme is often still active after the reaction, it may be attractive to reuse the enzyme, which can be realized by immobilization of the enzyme. The aim of this study is therefore to investigate the feasibility of GOS production using immobilized beta-galactosidase in a continuous flow packed bed reactor (PBR) and to compare the performance of such system with the traditional batch system with free enzyme, in terms of enzymatic and volumetric productivity.

A β-galactosidase from *Bacillus circulans*, covalently immobilized on Eupergit C 250L was selected as our model immobilized enzyme. The β-galactosidase of *Bacillus circulans* is well known for its high GOS synthesizing activity. GOS synthesis using this enzyme is well described in the literature (Rodriguez-Colinas et al., 2012; Torres and Batista-Viera, 2012; Warmerdam et al., 2013). The immobilization carrier Eupergit C250L is a well-defined carrier consisting of porous acrylic microbeads. Covalent attachment was preferred in order to prevent leakage of enzyme during running time.

In order to evaluate the robustness of this system, the activity and stability of the immobilized β-galactosidase were studied. Besides, the final product composition is also compared to the product composition obtained with the free enzyme. Finally, the productivities of the packed-bed reactor with immobilized enzyme and the batch system with free enzyme are compared.

2. Materials and methods

2.1. Materials

Lactose monohydrate (Lactochem) and Biolacta N5, a β-galactosidase from *Bacillus circulans*, (Amano Enzyme Inc., Nagoya, Japan) were gifts from FrieslandCampina (Beilen, The Netherlands). The enzyme preparation was previously found to have a protein content of 19 ± 3% (Warmerdam et al., 2013). In all calculations, the total enzyme concentration is assumed to be equal to the total protein concentration, because the actual enzyme concentration is not known.

Eupergit C 250L was a kind gift from Evonik (Darmstadt, Germany). D(+)-Galactose, D(+)-glucose, maltotriose, maltotetraose, maltopentaose, maltohexaose, maltoheptaose, sulfuric acid, sodium hydroxide, *o*-nitrophenyl β-D-galactopyranoside (oNPG), *o*-nitrophenol (oNP), and ethanolamine were purchased from Sigma–Aldrich (Steinheim, Germany).

McIlvaine's buffer was prepared by adding together 0.1 M citric acid and 0.2 M disodium hydrogen phosphate in the correct ratio to reach a pH of 6.0. Sodium carbonate, citric acid monohydrate, disodium hydrogen phosphate, dipotassium hydrogen phosphate, potassium dihydrogen phosphate, and sodium chloride were purchased from Merck (Darmstadt, Germany).

2.2. Methods

2.2.1. Immobilization of Biolacta N5

Eupergit C 250L is used as a carrier for immobilization of Biolacta N5. Eupergit C 250L is well-known carrier consisting of porous acrylic beads with oxirane functionality (Boller et al., 2002; Sheldon, 2007). Covalent immobilization proceeds through reaction of thiol and amino groups of the enzyme with the epoxide groups of the carrier.

Eupergit C 250L beads were washed with excess MilliQ water and dried over a glass filter by vacuum suction. The last washing step was performed with 0.2 M potassium phosphate buffer of pH 7.5. The resulting carrier, after drying over a glass filter with vacuum suction, was defined as wet Eupergit and had a mass of approximately four times its initial dry mass. This is in accordance to the work of Bortone et al. (2012). The increase in mass is caused by the uptake of water by the carrier beads. An enzyme solution of 20 g Biolacta N5 L^{-1} was prepared by dissolving Biolacta N5 in 1 M potassium phosphate buffer of pH 8.5. The carrier beads were added to the enzyme solution in a ratio of 1.0 g Biolacta N5 per 10 g wet Eupergit and incubated at room temperature in an IKA HS 250 horizontal shaker under gentle shaking for 24 h. After washing with MilliQ water and 0.5 M NaCl, the beads were incubated in 20% (v/v) ethanolamine in 0.1 M potassium phosphate buffer of pH 7.5 to block the remaining epoxide groups and provide a more hydrophilic surface of the carrier beads. After rinsing with excess MilliQ water again, the immobilized enzyme was stored in McIlvaine's buffer of pH 6.0 in the refrigerator. The amount of immobilized enzyme is defined as wet Eupergit with immobilized enzyme.

2.2.2. Enzyme activity measurements

The oNPG converting activity of the free enzyme, the supernatant after immobilization, the washing liquid, and the immobilized enzyme were determined. These measurements were performed as previously described (Warmerdam et al., 2013). An Eppendorf tube with 979 μL of 0.20% (w/w) oNPG-in-McIlvaine's buffer of pH 6.0 was preheated in an Eppendorf Thermomixer at 40 °C and 600 rpm for 10 min. Subsequently, 21 μL of sample was added and these mixtures were incubated for another 10 min at 40 °C and 600 rpm. A volume of 1.0 mL of 10% (w/w) Na_2CO_3 solution was added to stop the reaction and, afterwards, the absorbance of oNP was measured at 420 nm. The oNP concentration was determined using the law of Lambert–Beer of which the extinction coefficient was determined to be 4576 M^{-1} cm^{-1}. The oNP formation was found to be linear during the first 10 min of the reaction. This initial rate of oNP formation was expressed in mmol min^{-1} g protein^{-1}. Measurements were performed in duplicate and the average enzyme activity was used. A blank measurement was performed by using 21 μL McIlvaine's buffer instead of 21 μL sample.

The assay was slightly adapted to measure the enzyme activity of the immobilized enzyme, although the oNPG concentration in the assay remained at 0.20% (w/w). A weighted amount of approximately 5 mg of wet Eupergit with immobilized enzyme was suspended in 1.21 mL McIlvaine's buffer and added to 790 μL preheated 0.50% (w/w) oNPG-in-buffer. After an incubation time of 10 min at 40 °C and 900 rpm, the oNPG-immobilized enzyme solution was transferred to a tube containing 2.0 mL Na_2CO_3 solution. Before measuring the absorbance, the solution was filtered with a 0.2 μm Minisart filter.

2.2.3. Experimental setup PBR

Lactose conversion in a PBR with immobilized Biolacta N5 was followed in time at various ingoing lactose concentrations. Lactose was dissolved in McIlvaine's buffer of pH 6.0 at 60 °C.