Journal of Industrial and Engineering Chemistry xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Journal of Industrial and Engineering Chemistry

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

Application of *Bacillus pumilus* β-xylosidase reaction and simulated moving bed purification to efficient production of high-purity xylobiose from xylose

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ARTICLE INFO

Article history:
Received 21 November 2016
Received in revised form 14 December 2016
Accepted 17 December 2016
Available online xxx

Keywords:
Simulated moving bed
Xylobiose
Enzyme reaction
Separation
Purification

ABSTRACT

Xylobiose (X2) is recognized to possess great prebiotic function and to be highly favorable for application in food and prebiotic industries. In this study, we demonstrated that the cloned β-xylosidase of *Bacillus pumilus* IPO could be utilized to produce X2 via the reaction of xylose(X1) → xylobiose(X2). The use of such enzyme in the X1 → X2 reaction was found to give much higher X2 reaction yield and reaction efficiency, compared to those reported in the literature. Furthermore, we developed an efficient simulated moving bed (SMB) chromatographic process that could recover X2 from the reaction output with nearly 100% purity and 92% recovery on a continuous-separation mode. The developed SMB process could also recover the unreacted X1 almost completely, which leaves room for a further increase in the overall X2 reaction yield by reusing the recovered X1 from the SMB as the reactant of the upstream processing (i.e., *B. pumilus* IPO β-xylosidase X1 → X2 reaction). The results of this study will enable a highly economical and environmentally-friendly production of high-purity X2 from X1.

Introduction

Xylobiose (X2), which is an useful disaccharide that consists of two molecules of xylose (X1), has been recently highlighted as a high-value food supplement with outstanding prebiotic function [1–4]. In particular, X2 is recognized to possess the highest prebiotic activity in the proliferation of bifidobacteria, which plays an important role in the maintenance of the healthy intestinal microflora [1,5–8]. Furthermore, X2 is known to have the most favorable condition in terms of industrial applicability [5–8]. For these reasons, there has been significant interest in developing an economical method for the large-scale production of high-purity X2 (>99.9%) in food and prebiotic industries.

In regard to the production of X2, it is worth paying attention to the recent studies on the feasibility of converting X1 to X2 via the β-xylosidase reaction [9,10]. In these studies, the β-xylosidases from *Sporotrichum thermophile* and *Talaromyces thermophilus* were employed as the enzymes for the X1 → X2 reaction, and a buffer solution was used as the reaction solvent [9,10]. The highest X2 reaction yield was 5.65%, which was attained in the latest study [10]. The X2 reaction yield, however, was achieved by the addition of a third component (sorbitol) into the reaction solvent, which should be removed after the reaction and would thus be unfavorable to downstream processing. In addition, the reaction times (i.e., the times required for attaining the maximum X2 reaction yield) in the previous studies were all longer than 3 days, and the X1 loading concentrations were kept lower than 60% (w/v). More importantly, the recovery of X2 from the reaction output was not attempted in the previous studies.

To establish a highly economical X2 production process for industrial application, the efficiency of the aforementioned X1 → X2 reaction needs to be improved further in the following

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http://dx.doi.org/10.1016/j.jiec.2016.12.015
1226-086X/

directions. First, the X2 reaction yield should be increased, and the reaction time should be shortened. Secondly, the applicable X1 loading concentration should be as high as possible for the purpose of ensuring high throughput. Thirdly, the addition of a third component into the reaction system needs to be avoided. Fourthly, using water rather than a buffer solution as the reaction solvent needs to be considered because it can reduce the separation load and separation costs for downstream processing. In addition to the above-mentioned requirements on the upstream processing (i.e., X1 → X2 reaction), it is essential to fulfill the task of separating the reaction product (X2) and the unreactant (X1) with high purities and high recoveries for both X2 and X1.

The goal of this study is to accomplish the aforementioned work, i.e. to propose an enhanced X1 → X2 reaction process that can lead to higher X2 reaction yield and shorter reaction time without using a third component and a buffer solution, and further to develop a highly efficient X2–X1 separation process that is based on a favorable operation mode for the large-scale X2 production (i.e., continuous-separation mode). As a first step for this work, we explored another enzyme source of β-xylosidase that could make a substantial improvement in the efficiency of the X1 → X2 reaction process. It was found in this study that the β-xylosidase from *Bacillus pumilus* IPO could surpass the previously adopted β-xylosidase enzymes [9,10] to a large extent in every respect. To date, the use of *B. pumilus* IPO β-xylosidase for the X1 → X2 reaction has not been reported in the literature. Furthermore, a continuous-mode chromatographic separation process for recovering high-purity X2 from the reaction output was developed in this study on the basis of the process structure and the operation principle of a simulated moving bed (SMB) technology [11–14]. It was confirmed from the experimental results that the developed separation process in this study was successful in recovering X2 from the reaction output on a continuous-separation mode, through which the X2 product of nearly 100% purity was obtained with 92% recovery. The unreacted X1 molecules were also recovered from the developed process without any loss, which is meaningful because they could be reused in the upstream X1 → X2 reaction.

Materials and methods

Cloning and expression of the β-xylosidase gene from B. pumilus *IPO*

The *B. pumilus* IPO (*BP*I) β-xylosidase, which was adopted as the enzyme for the X1 → X2 reaction in this study, was obtained through cloning and expressing its gene in *Escherichia coli*. This was carried out by referring to the procedures reported previously [15–19]. First, the information on the *BP*I XynB DNA (2207 bp) was acquired from the NCBI (national center for biotechnology information). Using this information, the *BP*I β-xylosidase DNA was synthesized at BIONEER Corporation (Daejeon, Korea). The synthesized β-xylosidase DNA was ligated into pET21a, and then transformed into *E. coli* BL21(DE3). The recombinant *E. coli* was cultured in Luria-Bertani (LB) medium that was supplemented with 100 μg/mL of ampicillin (Sigma-Aldrich Co., USA). When the OD_{600} of the culture medium became 0.8, IPTG at 0.5 mM (BIONEER Corporation, Korea) was added and the culture was continued at 20 °C and 100 rpm for 16 h.

After the culture, the cells were harvested by centrifugation and suspended in disruption buffer (50 mM Tris–HCl, 300 mM NaCl, 10 mM imidazole, pH 8.0). The cell suspension was sonicated by using a VC-505P ultrasonic processor (Sonic & Materials Inc., USA), followed by being centrifuged at 8000 rpm (6576 g-force) for 20 min. The supernatant obtained by the centrifugation was filtered through a 0.45 μm Minisart® RC filter (Sartorius Co., Germany), and then loaded on a Ni-NTA agarose column (Qiagen Co., Germany) for purification purpose. After then, the washing buffer (50 mM Tris–HCl, 300 mM NaCl, 20 mM imidazol, pH 8.0) and the elution buffer (50 mM Tris–HCl, 300 mM NaCl, 250 mM imidazol, pH 8.0) were introduced sequentially into the Ni-NTA agarose column in order to remove impurities and recover β-xylosidase. The active β-xylosidase fractions that were collected from the Ni-NTA agarose column were dialyzed with 50 mM sodium phosphate buffer, pH 7, and subsequently concentrated by ultrafiltration on an Amicon®Ultra system (Merck Millipore Co., Germany).

Implementation of the X1 → X2 reaction using the cloned β-xylosidase of B. pumilus *IPO (BPI)*

Xylose, which was supplied from Samin Chemical Co. (Siheung, Korea), was dissolved in distilled water by boiling in order to prepare 500 mL of aqueous xylose solution that contained xylose at 90% (w/v). The prepared xylose solution was placed in a drying oven at 50 °C. When the temperature of the xylose solution was equilibrated at 50 °C, the reaction began by adding 50 U of the *BP*I β-xylosidase (0.1 U/mL) to the solution. The reaction mixture consisting of the xylose solution and the β-xylosidase was incubated at 50 °C for 24 h. This reaction was stopped by heating the reaction mixture at 99 °C for 15 min. Then, the reaction output was diluted with distilled water and centrifuged at 7000 rpm for 20 min to remove the denatured β-xylosidase. The supernatant solution from the centrifugation was placed in a drying oven at 45 °C for 2 h, and then filtered through a 0.22 μm Stericup-GP (Merck Millipore Co., Germany). The resulting solution was then loaded into the downstream SMB separation process for recovery of high-purity xylobiose (X2).

Resin pretreatment and pulse-injection experiment for estimating the intrinsic parameters of the solutes in the reaction output

A commercial ion-exchange resin Dowex-50WX4, which was reported to be effective in oligosaccharide separation in the literature [20,21], was adopted in this study as the solid phase of the considered SMB process for recovery of X2 from the *BP*I β-xylosidase reaction output. The Dowex-50WX4 resin was supplied in hydrogen form from the Sigma-Aldrich Co. (St. Louis, MO), and it was converted to the sodium form prior to use. This resin was packed into an omnifit chromatographic column, which was purchased from the Bio-Chem Fluidics Co. (Boonton, NJ). The diameter and length of this column are 3.5 cm and 21.7 cm respectively. The bed voidage and particle porosity of the packed column were 0.3 and 0.629 respectively, which were obtained from a series of tracer-molecule pulse tests. The average diameter of the resin particle is 55.5 μm.

Using the column packed with the aforementioned Dowex-50WX4 resin, the pulse-injection experiment for estimating the intrinsic parameters was carried out in the following manner. First, the packed column was installed in a ÄKTA™ fast protein liquid chromatography (FPLC) system (Amersham Biosciences Co., USA), which consisted of two pumps (Amersham Biosciences P-920) and a fraction collector (Amersham FPLC Frac-900). One of the two pumps delivered deionized distilled water (DDW) and the other pump the mixture solution coming from the upstream process of the *BP*I β-xylosidase X1 → X2 reaction. Prior to the experiment, DDW was pumped into the column for a sufficiently long period of time. The experiment was started by switching the pump flow from DDW to the mixture solution and simultaneously collecting the column effluent in the fraction collector at intervals of 0.6 mL. The amount of the mixture solution loaded into the column was set at 20 mL. Immediately after the completion of such loading, the pump flow was switched back to DDW. The flow rates of both

Please cite this article in press as: C. Park, et al., Application of *Bacillus pumilus* β-xylosidase reaction and simulated moving bed purification to efficient production of high-purity xylobiose from xylose, J. Ind. Eng. Chem. (2016), http://dx.doi.org/10.1016/j.jiec.2016.12.015