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Functional cello-oligosaccharides production from the corncob residues of xylo-oligosaccharides manufacture

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ABSTRACT

An integrated process has been developed, consisting of the "adsorption–separation" of cellulase enzymes to selectively remove β-glucosidase, and multi-stage enzymatic hydrolysis of corncob residues from xylo-oligosaccharides manufacture with the β-glucosidase deficient cellulase, aiming to obtain a high yield of cello-oligosaccharides production. After the "adsorption–separation" process, 79.50% of the endo-glucanase was retained in substrate, whereas 90.67% of β-glucosidase was removed with the separated liquid fraction, utilizing the different adsorbability of these enzymes to the substrate. A three-stage enzymatic hydrolysis of corncob residues with the β-glucosidase deficient cellulase was proposed in which the first, the second and the third stage were conducted for 6, 6 h and 12 h, respectively. Analysis indicated that the removal of hydrolysis products (glucose and cello-oligosaccharides) at each stage improved cello-oligosaccharides productivity and enzymatic hydrolysis yield. The cello-oligosaccharides yield and enzymatic hydrolysis yield in three-stage enzymatic hydrolysis were significantly improved to 51.78% and 75.56%, respectively, which were 36.00% and 25.10% higher than single-stage hydrolysis with original cellulase enzymes.

1. Introduction

Industrial process residues are widely considered as an important source for the production of various value-added products like biofuels, animal feeds, chemicals, enzymes, etc. [1]. Corncob residues from xylo-oligosaccharides manufacture are now considered as an excellent substrate for cello-oligosaccharides production, because of the abundance, high cellulose content (70–80%) and loose structure after extraction of xylan for xylo-oligosaccharides production. Due to lack of effective utilization, corncob residues after extraction of xylan are abandoned in the open field, causing serious environmental pollution. Utilization of these materials not only solves the proper disposal of the wastes, but also provides an attractive opportunity for more sustainable development of agricultural resources.

Cello-oligosaccharides are defined as saccharides consisting of 2 to 6 glucose by β-1,4-linkages, which contain mainly cellobiose [2,3]. Cello-oligosaccharides are important functional oligosaccharides [4], and are significant in the feed and food field [2,5,6]. However, there is still limited information available for the large-scale production of cello-oligosaccharides. In general, there are two main strategies of producing cello-oligosaccharides, namely acid-based and enzyme-based hydrolysis of the insoluble cellulose. According to Carvalho et al. [7], acid hydrolysis is not recommended, for the production of undesirable products such as furfural and hydroxymethylfurfural, the large amount of monosaccharides released [8]. However, enzymatic hydrolysis is considered more attractive due to the relatively mild reaction condition (less by-products) and the easier controlled cleavage breaking (less monomers). However, during the enzymatic hydrolysis of insoluble cellulose, cello-oligosaccharides that are produced in an intermediate step are hydrolysed quickly to glucose by β-glucosidase [9]. To overcome this problem, it is necessary to retain endo-glucanases on the substrate, which works randomly in the middle of the cellulose chains to release short cello-oligosaccharides. On the other hand, the removal of β-glucosidase from the cellulase mixture is also considered to have influence on cello-oligosaccharides production [10]. And it is well known that β-glucosidase, unlike the canonical cellulase enzymes (exoglucanases and endoglucanases), exhibits very low binding affinity to cellulosic substrates due to the lack of cellulose-binding domain. Therefore, it is hypothesized that β-glucosidase could be selectively removed from the liquid phase after other cellulase enzymes adsorbed to the insoluble cellulosic substrates. Comparing to the traditional enzyme separation strategies, such as membrane separation and ultrafiltration [11], separation of β-glucosidase from other cellulase

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components by their different adsorbability to substrate has been considered as a simple, inexpensive method [12]. However, β-glucosidase removal from cellulase enzymes by substrate using "adsorption–separation" strategy for cello-oligosaccharides production has not been reported.

The oligosaccharides and monomers are formed which may cause an inhibition of the enzymes involved [13]. Especially, the produced cello-oligosaccharides, mainly cellobiose, have inhibition on exoglucanases and endoglucanases. It has been reported that cellobiose may inhibit the binding of cellulases by forming an enzyme–saccharides complex [14], and further have an impact on hydrolysis efficiency. Aim to alleviate the product inhibitions on enzymes, multi-stage enzymatic hydrolysis was carried out, in which enzymatic hydrolysis was separated into some stages, and the product formed was removed after each stage. And it has been reported that multi-stage enzymatic hydrolysis could effectively remove product inhibition and significantly enhance hydrolysis efficiency [15,16]. It is also hypothesized that β-glucosidase could be further removed during multi-stage enzymatic hydrolysis, for β-glucosidase has no cellulose-binding domain.

The objective of this study was to explore an effective approach for cello-oligosaccharides production from corncob residues of xylo-oligosaccharides manufacture. Firstly, the selective removal of β-glucosidase from other cellulase components in the commercial cellulase mixture was investigated, using an adsorption–separation strategy. Secondly, multi-stage enzymatic hydrolysis by the β-glucosidase deficient cellulase was assessed, aiming to alleviate the product inhibition at each stage. And to our knowledge, no reports to describe cello-oligosaccharides production from lignocellulosic materials, especially from the corncob residues of xylo-oligosaccharides manufacture.

2. Materials and methods

2.1. Substrates and enzyme preparations

The corncob residues from xylo-oligosaccharides manufacture were friendly provided by Jiangsu Comverse Bio Co., Ltd. The corncob residues were neutralized with HCl and washed by tap water with a total solid-to-liquid ratio of 1:20 (g:ml), and the operation was as follows: Mixture of 500 g dry corncob residues and 5 L tap water was adjusted to pH 4.5–5.0 using 6 M HCl at room temperature, followed by a filtration to separate the solid and liquid; Subsequently, the separated solid subjected to washing with another 5 L tap water, and was filtrated to collect the solid residues. The residues after filtration were stored in plastic bags at 4 °C for further utilization. The main composition of the washed corncob residues was as follows (dry weight basis): glucan, 73.21%; xylan, 13.58%; lignin, 4.20% and others, 7.21%. Cellulase enzymes preparation (C2730) was purchased from Sigma, USA. The filter paper activity (101.37 FPU), CMCase activity (endo-glucanase, 805.59 U), β-glucosidase activity (38.20 U) per gram enzyme were detected.

2.2. Optimization of adsorption of cellulases to corncob residues

The reaction parameters affecting cellulases adsorption to substrate, such as the adsorption temperature, pH and incubation time were evaluated individually, and the best condition for maximizing CMCase adsorption and minimizing β-glucosidase adsorption to corncob residues was established. All these adsorption tests were carried out in 250 ml Erlenmeyer flasks with 50 ml reaction mixture at cellulose consistency of 5% (2.5 g cellulose per 50 ml reaction mixture) with cellulase dosage of 15 FPU per gram cellulose and shaking at 150 rpm. After adsorption, the supernatant was collected at 3000 rpm, 4 °C for 10 min for determination of CMCase activity and β-glucosidase activity. All experiments were performed in duplicate and average results are given. Enzyme retention rate and ratio of CMCase to β-glucosidase in the solid residue were calculated as characteristics of adsorption.

$$\text{Enzyme retention rate (\%)} = \frac{\text{total enzyme loading (U)} - \text{enzyme in supernatant (U)}}{\text{total enzyme loading (U)}} \times 100 \quad (1)$$

$$\text{Ratio of CMCase to BG} = \frac{\text{CMCase activity in solid residue}}{\text{BG activity in solid residue}} \quad (2)$$

2.3. Enzymatic hydrolysis of corncob residues after "adsorption–separation" of cellulases

The cellulase adsorption to corncob residues was performed at cellulose consistency of 5% (2.5 g cellulose per 50 ml reaction mixture), pH 7.0 (Na_2HPO_4–citric acid buffer) at 10 °C for 30 min. After adsorption, the supernatant which contains most β-glucosidase was removed by centrifugation at 3000 rpm for 10 min. Fresh citrate buffer (50 mM, pH 4.8) with same volume of the removed supernatant was added to the solid residue to make the enzymatic hydrolysis volume consistent. Enzymatic hydrolysis experiments of the solid residue were then performed at 50 °C with shaking at 150 rpm for 24 h. As a control, enzymatic hydrolysis with the original cellulase was also performed. At the end of hydrolysis, samples were heated at 100 °C for 10 min to inactivate the enzymes. Supernatants were collected after centrifugation at 3000 rpm for 10 min. All experiments were performed in duplicate and average results are given.

The cello-oligosaccharide was expressed as the amount of glucose using acid hydrolysis, cello-oligosaccharides solution was hydrolyzed by 4% H_2SO_4 at 121 °C for 1 h and so as to convert cello-oligosaccharides to glucose. The glucose concentration before and after acid hydrolysis was measured using HPLC. Cello-oligosaccharides concentration was expressed as follows:

$$\text{Cello-oligosaccharides conc. (g L}^{-1}\text{)} = (\text{glucose conc. after hydrolysis} - \text{glucose conc. before hydrolysis}) \times 0.9 \quad (3)$$

2.4. Multi-stage hydrolysis of corncob residues for cello-oligosaccharides production

Multi-stage hydrolysis experiments were carried out to alleviate the product inhibition. The hydrolysis was conducted in 250 ml Erlenmeyer flasks at 50 °C using 50 mM citrate buffer (pH 4.8) with shaking at 150 rpm. At the end of each stage, the solid residue was separated from the liquid phase by centrifugation. The fresh buffer (pH 4.8) with the same volume of the removed supernatant was added to the solid residue to carry out the next stage hydrolysis. Multi-stage hydrolysis experiments included two-stage (strategy 1: 6 + 18 h; strategy 2: 10 + 14 h; strategy 3: 12 + 12 h; strategy 4: 14 + 10 h; strategy 5: 18 + 6 h) and three-stage (strategy 6: 6 + 6 + 12 h; strategy 7: 6 + 8 + 10 h; strategy 8: 8 + 8 + 8 h; strategy 9: 6 + 12 + 6 h) hydrolysis. The supernatants of hydrolysis experiments were analyzed for soluble sugars production. All experiments were performed in duplicate and average results were given.

$$\text{Enzymatic hydrolysis yield (\%)} = \frac{\text{cello-oligosaccharides (g)} + \text{glucose} \times 0.9\ \text{(g)}}{\text{total cellulose in substrate (g)}} \times 100 \quad (4)$$

$$\text{Cello-oligosaccharides yield (\%)} = \frac{\text{cello-oligosaccharides production (g)}}{\text{total cellulose in substrate (g)}} \times 100 \quad (5)$$

$$\text{Cello-oligosaccharides selectivity (\%)} = \frac{\text{cello-oligosaccharides (g)}}{\text{cello-oligosaccharides (g)} + \text{glucose} \times 0.9\ \text{(g)}} \times 100 \quad (6)$$

2.5. Analytical methods

Endo-glucanase activity (CMCase activity) was measured using an assay based on the Ghose methodology [17], using 2% CMC (Sigma, USA) solution as substrate. One unit of endo-glucanase activity corresponds to 1 μmol of glucose released per minute at pH 4.8 and 50 °C. The quantification of the reducing groups released was done according to the DNS method [18]. One unit of β-glucosidase is defined as the amount of enzyme that hydrolysing 1 μmol of 4-nitrophenyl-β-D-glucopyranoside (pNPG) per min. Glucose was determined using Agilent high performance liquid chromatography with a refractive index detector and Bio-rad Aminex HPX- 87H column (300 × 7.8 mm) at 55 °C, using 5 mmol H_2SO_4 as the eluent at a flow rate of 0.6 ml/min [19].

3. Results and discussion

3.1. Separation of β-glucosidase from cellulase mixture by "adsorption–separation" strategy

As mentioned before, the effective removal of β-glucosidases from cellulase components should be beneficial for the production of cello-oligosaccharides. Although the adsorption profile of

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