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β-Glucosidases as biocatalysts for the enzymatic synthesis of oligosaccharides

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ABSTRACT

β-Glucosidases under certain reaction conditions are capable of reversing the hydrolysis reaction for the synthesis of oligosaccharides of industrial interest. A comparative study of the conversion of glucose in oligosaccharides was performed using β-glucosidases from fungal and plant sources. Using β-glucosidases from Aspergillus niger, Aspergillus awamori, and Prunus dulcis, total conversions were 29.2%, 32.3%, and 22.9%, respectively, when a initial glucose concentration of ~70% (w/v) was evaluated. Disaccharide gentiobiose was the principal product and its concentrations were 103.3, 79.6, and 69.7 g/L using A. niger, A. awamori, and P. dulcis, respectively. The yield of gentiobiose synthesis using A. niger was 16.5%, which is one of the highest yields reported for the synthesis of this disaccharide. A preparative chromatography method was developed to allow an efficient separation of disaccharides from residual glucose.

Keywords: Reverse hydrolysis, transglycosylation, β-glucosidase, gentiobiose, cellobiose.

INTRODUCTION

Oligosaccharides are important compounds that have different industrial applications. For example, they can be used as prebiotic, sweetening, and anti-hygroscopic agents in the food industry, as well as in the formulation of cosmetics, pharmaceuticals, and agricultural products (Patel and Goyal, 2011). The chemical synthesis of oligosaccharides is challenging due to the necessity of several protecting groups to control the stereoselectivity of glycosylation reactions, which increases with the size of the oligosaccharide (Nilsson, 1988).

β-glucosidases, which are commonly reported as essential enzymes for the complete hydrolysis of cellulose to glucose, under certain reaction conditions of low water activity and high substrate concentration 60–90% (w/v) are capable to promote the synthesis of oligosaccharides by transglycosylation or reverse hydrolysis mechanisms (Monsan and Paul, 1995). Previous studies reported the preferential formation of disaccharides such as gentiobiose (β-1,6), cellobiose (β-1,4), sophorose (β-1,2) and laminaribiose (β-1,3) (Ravert and Legoy, 1993).

All β-glucosidases that have been described so far fall in glycoside hydrolase (GH) families GH1, GH3, GH5, GH9, GH30 and GH116, with the major numbers of characterized enzymes classified in family 1, followed by family 3. Most plants β-glucosidases fall in GH1 family, while most fungal beta-glucosidases are found in GH3 (Bathia et al., 2002). All characterized GH1 β-glucosidases present a (β/α)8-barrel structure, while GH3 known structures display a variety of domains (Cairns and Esen, 2010). Therefore, this difference in

structure between GH1 and GH3 can also be reflected in their properties and selectivity for the synthesis of oligosaccharides. The present work is a comparative study of glucose conversion into oligosaccharides, using two different fonts of β-glucosidases from fungi (*Aspergillus niger*, *Aspergillus awamori*) and one from plant source (*Prunus dulcis* - sweet almond) aiming to compare the enzyme properties and selectivity.

MATERIALS AND METHODS

β-Glucosidases from *Aspergillus niger* (Novozyme 188, Novozymes, Denmark), *Aspergillus awamori* (culture supernatant, provided by the Bioethanol Laboratory, from UFRJ) and *Prunus dulcis* (Sweet almond, Toyobo, Japan), were compared for the synthesis of oligosaccharides via reverse hydrolysis reaction. Glucose syrups of 66, 64, or 70% (w/v), solubilized in 0.05 M sodium acetate buffer (pH 4.0), were used as substrates in the reactions catalyzed by *A. niger*, *A. awamori*, and *P. dulcis*, respectively. The reactions were carried out with 7 IU/mL β-glucosidase activity at 65°C under stirring and reflux for 120 h. Another experiment was performed using 90% (w/v) glucose as initial concentration for reactions catalyzed by β-glucosidases from *A. niger* and *A. awamori*, using the same experimental conditions mentioned above. Glucose conversion into di- and oligosaccharides were analyzed by high-performance liquid chromatography (HPLC).

A preparative chromatographic method was developed and applied for the separation of residual glucose from the synthesized oligosaccharides. A 1:1 mixture of carbon/celite was used as stationary phase and a gradient of EtOH/H_2O as the mobile phase. It was applied to the column 10 mL of the reaction medium containing glucose, cellobiose and gentiobiose, the concentrations were 545.6, 9.15 and 140.9 g/L, respectively. The monitoring of the fractions was performed by Thin-layer chromatography and HPLC.

RESULTS AND DISCUSSION

- **Synthesis reactions:** In reactions conducted with 64-70% (w/v) initial glucose concentration, the disaccharides gentiobiose (β-1,6) and cellobiose (β-1,4) were the major products synthesized by all the β-glucosidases evaluated. The total conversions of glucose mediated by *A. niger*, *A. awamori*, and *P. dulcis* enzymes were 29.2%, 32.3% and 22.9%, respectively. The sum of gentiobiose and cellobiose corresponded to 67.4%, 57.8%, and 54.1% of the total products for *A. niger*, *A. awamori*, and *P. dulcis*, respectively. Although only glucose, gentiobiose, and cellobiose were quantified, HPLC analysis revealed the synthesis of other oligosaccharides, probably tri- and tetrasaccharides, indicating that glucose consumption in the reaction was not entirely directed toward disaccharides synthesis. Concentrations of 103.3, 79.6, and 69.7 g/L of gentiobiose were obtained using *A. niger*, *A. awamori and P; dulcis* (Figure 1), respectively. The yield of gentiobiose synthesis using *A. niger* was 16.5%, which is one of the highest yields reported for the synthesis of this disaccharide. Compared to gentiobiose, lower cellobiose concentrations of 20.4, 33.9, and 12.6 g/L were obtained for *A. niger, A. awamori and P. dulcis*, respectively. The results indicate that at the studied conditions the fungal enzyme were more effective in converting glucose into oligosaccharides and β-glucosidase from *A. awamori* showed higher selectivity to form the β-1,6 glycosidic bond. β-glucosidase from *P. dulcis* exhibited much faster

kinetics, showing synthetic activity in the first 12 h, while with fungal enzymes the disaccharides synthesis was detected only after 24 h reaction.

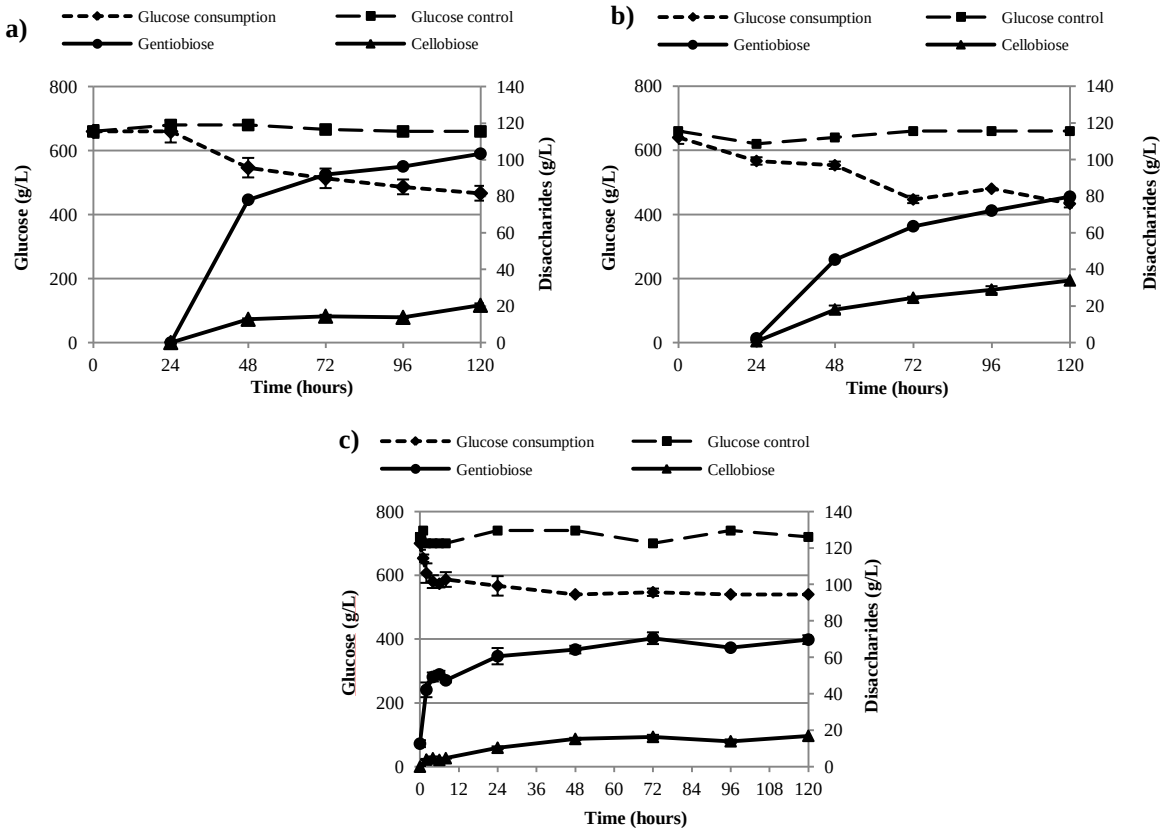

Figure 1. Synthesis of disaccharide using β-glucosidase from a) *A. niger* , b) *A. awamori*, and c) *P. dulcis*. Initial glucose concentrations were of 66, 64% and 70% (w/v), respectively, and reactions were conducted at pH 4.0, 65°C for 120 h under stirring.

According to Ravert *et al*. (1993) increasing the initial concentration of substrate reduces the water activity and may enable higher yield in product conversion via the reverse hydrolysis reaction. Therefore, experiments were performed using an initial concentration of 90% (w/v) glucose. The total glucose conversions corresponded to 36.0% and 33.6% for *A. niger* and *A. awamori*, respectively (Figure 2a and 2b). Reactions with *P.dulcis* were not conducted. Gentiobiose was produced in concentrations of 79.64 and 54.16 g/L, while cellobiose production was 2.79 and 30.44 g/L for *A. niger* and *A. awamori*, respectively.

Although the total conversion of glucose was increased or kept stable when glucose concentration was increased from ~70% to 90% (w/v), the selectivity for the formation of the disaccharides gentiobiose and cellobiose decreased, as the sum of those disaccharides corresponded to 26.6% and 29% of the total products of reactions conducted with *A. niger* and *A. awamori* β-glucosidases, respectively. It is probably that this condition has favored the formation of higher oligosaccharides, however further experiments will be needed to better characterize the products formed.

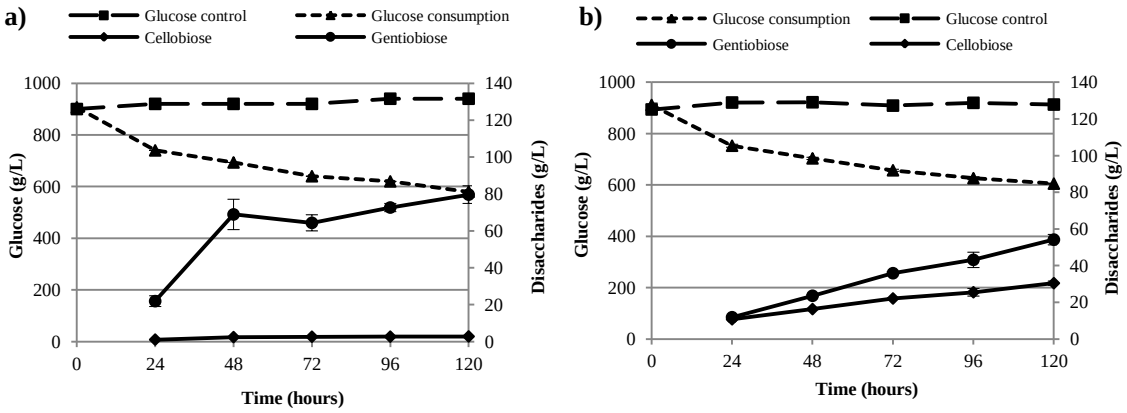

Figure 2. Synthesis of disaccharides using β-glucosidases from a) *A. niger* and b) *A. awamori*. Initial glucose concentrations were of 90% (w/v), at pH 4.0 and 65°C for 120 hours of reaction with stirring.

- Separation of disaccharides from residual glucose: The results obtained with thin-layer chromatography (TLC) plates revealed that glucose was eluted in the first fractions (100% H_2O, EtOH/H_2O 1 to 5%), followed by disaccharides (EtOH/H_2O, 5 to 20%). Subsequently, the fractions were mixed according to the TLC profile, and were concentrated on a rotary evaporator and analyzed by HPLC. Efficient separation of disaccharides was observed from residual glucose by preparative chromatography column with the following recovery rates: 93.3%, 137.7% and 62.5% for glucose, cellobiose and gentiobiose, respectively.

CONCLUSIONS

Gentiobiose and cellobiose were synthesized using β-glucosidases from *A. awamori*, *A. niger* and *P. dulcis*, being gentiobiose the major product produced when initial glucose concentration of ~70% (w/v) was used. We observed a yield 16.5% of gentiobiose using β-glucosidase from *A. niger*, which is to our knowledge the highest yield reported for the enzymatic synthesis of this disaccharide. Further experiments will be needed to evaluate the best conditions to increase the selectivity of the enzyme towards one product. The enzymatic synthesis of these disaccharides from glucose may become a simple and inexpensive method in comparison to the current chemical process that are used.

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