Arabinose biopurification by *Ogataea zsoltii* yeast

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Summary

Arabinose is a five carbon sugar which exists in nature as a component of hemicellulose and pectin polymers, naturally occurs in L-configuration. It is used as diet controlling food additive, important intermediate for anti-virus drug synthesis and as base material in the production of vitamin B_2, among other applications. Commercial production of L-arabinose begins with the acid hydrolysis of gum arabic followed by expensive purification steps. Nowadays biopurification have become an attractive approach for purifying high-value sugars from lignocellulosic hydrolysates.

Ogataea zsoltii yeast was investigated in terms of its ability to perform arabinose biopurification on semidefined medium and on the supernatant of acidic hydrolysis of corn fibre. Arabinose biopurification was performed in shake flasks and bench-top bioreactor under aerobic conditions. Based on our results, this strain can be used for arabinose biopurification on both medium, resulting in near 90 % purity for arabinose.

Introduction

Arabinose is a five carbon sugar which exists in nature as a component of hemicellulose and pectin polymers [1]. The main sources of arabinose are arabinoxylans (AX) which consist of homopolymeric backbone chain of 1,4-linked-β-D-xylopyranose that is decorated with α-L-arabinofuranose units [4]. Naturally arabinose occurs in L-configuration, this is a so-called non-caloric sugar [5]. L-arabinose is used as a diet controlling food additive because it can repress the increase of blood sugar after consuming sucrose. It also inhibits the sucrase activity of intestinal mucosa [1]. Applications of L-arabinose for analytical purposes and bacteriological diagnostics were also reported [4]. It can be used as a starting material of non-ionic surfactants, an important intermediate for anti-virus drug synthesis and a precursor of the drug intermediate (R)-3,4-dihydroxybutryric acid, carnitine, agrichemicals and several antibiotics [4]. It can be utilized as a base material in the production of vitamin B_2 and it is a raw material of L-ribose production which is utilized for synthetic oligonucleotides [4,5]. Commercial production of L-arabinose begins with the acid hydrolysis of gum arabic followed by purification steps such as neutralization reaction and ion-exchange chromatography. These purification steps cause the high cost of pure L-arabinose [1]. Enzymatic hydrolysis is another way of producing L-arabinose from the materials mentioned. α-L-arabinofuranosidases hydrolyze non-reducing L-arabinose residues from L-arabinose-containing polysaccharides and oligosaccharides. Arabinoxylan α-L-arabinofuranohydrolases effectively release L-arabinose from arabinoxylans, however the high L-arabinose content of arabinoxylans could inhibit the activity of these enzymes because of steric hindrance among L-arabinose residues [6]. The complete depolymerisation of xylan is achieved by several enzymes which act on the xylan backbone, the side chains and decorating units (for example the synergistic action of endo-xylanases, xylosidases, arabinofuranosidases, ferulic acid esterases and uronosidases), producing fermentable oligosaccharides and monosaccharides [7]. The advantages of enzymatic hydrolysis compared with acid hydrolysis are the mild reaction conditions, the high substrate specificity and no waste of saccharide due to side reactions [2]. Nowadays biopurification have become an attractive approach for producing high-value sugars from lignocellulosic hydrolysates. Arabinose biopurification means the biologically

depletion of other sugars, such as xylose, glucose and galactose from an arabinose containing solution, thus enriching L-arabinose in this liquor and facilitating its crystallization by simple method [1].

The aim of our study is to investigate *Ogataea zsoltii* NCAIM Y.01540 strain in terms of its ability in biopurification of arabinose using semidefined medium and acidic hydrolysate of corn fibre. Biopurification experiments were carried out in Erlenmeyer flasks and bench-top bioreactor.

Materials and methods

Microorganism

Ogataea zsoltii NCAIM Y.01540 strain was obtained from the culture collection of National Collection of Agricultural and Industrial Microorganisms (Budapest, Hungary).

Yeast cultivation

Medium used for inoculum preparation contained 10 g/L yeast extract, 15 g/L KH_2PO_4, 1 g/L $MgSO_4*7H_2O$, 3 g/L $(NH_4)_2HPO_4$ and 30 g/L xylose. The xylose and the other components were sterilised separately at 120 °C for 15 minute in autoclave. Cells were cultivated in 750 mL Erlenmeyer flasks containing 150 mL inoculum and they were shaked at 220 rpm for 72 h at 30°C.

Acid treatment of corn fibre

The acidic hydrolysis was carried out in 1000 mL Duran flasks containing 700 g suspension (10 % corn fibre, 1,1 % sulphuric acid), it was resting in water bath at 90°C for 51 min (plus 15 min warm-up period). The flasks were cooled with cold water and the suspension was separated with nylon filter and Büchner funnel under vacuum. The supernatant was treated for 1 h at 120 °C in autoclave and stored at -10 °C. The hydrolysate is rich in glucose and arabinose. The hydrolysate (pH=1) was adjusted to pH 7,5 with calcium hydroxide and then with 10 % HCl to achieve pH 6. It was used in biopurification experiments after sterilization [3].

Arabinose biopurification

Biopurifications were carried out in 2,5 L Applikon Biotechnology Autoclavable Bioreactor containing the hydrolysate of corn fibre and in 100 mL Erlenmeyer flasks containing 20 mL semidefined medium (10 g/L yeast extract, 15 g/L KH_2PO_4, 1 g/L $MgSO_4\times7H_2O$, 3 g/L $(NH_4)_2HPO_4$, 10 g/L arabinose, 10 g/L glucose, 5 g/L xylose and 5 g/L galactose) or hydolysate of corn fibre. The conditions in bioreactor were the following: 1,2 L hydrolysate, pH=6, 37 °C, 1 g/L centrifuged yeast cells, aeration rate of 1 or 0,8 vvm and 400 rpm rotation speed. The conditions for shake flasks experiments were: pH=6, 37 °C, 1 g/L centrifuged yeast cells, 220 rpm rotation speed.

Cell concentration

The cell concentration in the inoculum and fermentation samples was calculated from the optical density of the samples, that is measured at a wavelength of 600 nm by spectrophotometer (Ultrospec III, Pharmacia LKB, Uppsala, Sweden).

Sugar concentration

Concentration of glucose, xylose, arabinose and xylitol was determined by high-performance liquid chromatography (HPLC) using BioRad (Hercules, CA, USA) Aminex HPX-87H (300 × 7.8 mm) column at 65°C. The eluent was 5 mmol/L sulphuric acid at a flow rate of 0.5 mL/min. Determination of galactose was performed using Phenomenex (Torrance, CA, USA) Rezex RPM-Monosaccharide Pb+2 (300 × 7.8 mm) column at 80°C. The eluent was ultra-pure (milli-Q) water at a flow rate of 0.5 mL/min [3].

Results and discussion

Arabinose biopurification on semidefined medium

Arabinose biopurification was carried out on semidefined medium. *Ogataea zsoltii* metabolised glucose and xylose within 24 hours, galactose was also consumed in 30 hours. As the amount of other carbohydrates was getting to run out, the yeast started to utilize arabinose (24 hours). Based on Figure 1, biopurification on semidefined medium should be terminated at 24 hours. At this time the purity of the medium was 93 % for arabinose regarding total sugars.

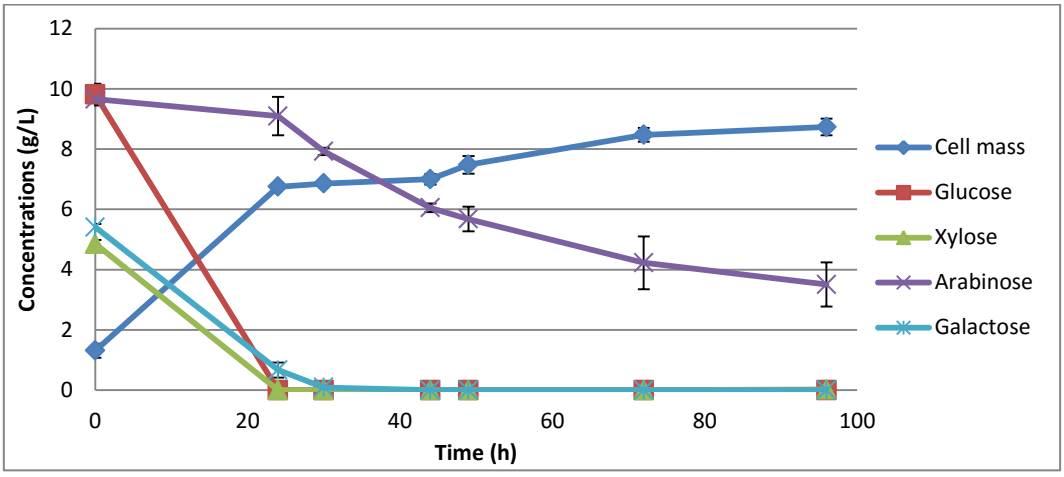

Figure 1: Arabinose biopurification on semidefined medium in Erlenmeyer flasks using *Ogataea zsoltii* strain

Arabinose biopurification on acidic hydrolysate of corn fibre

This experiment was intended to investigate the utilization of corn fibre hydrolysate for arabinose production. *Ogataea zsoltii* utilized glucose in 24 hours. The amount of xylose (and galactose) was decreased in 44 hours, while the arabinose concentration remained constant. Arabinose was not used for cell growth by the yeast. At the end of the fermentation, the biopurificated medium contained 1,1 g/L xylose (and galactose) and 8,5 g/L arabinose, which resulted in the purity of 89 % for arabinose (Figure 2).

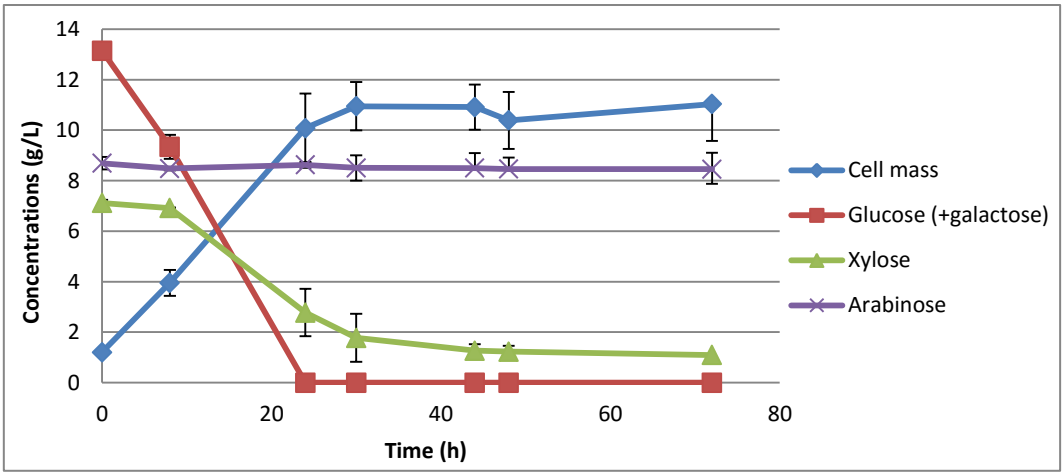

Figure 2: Arabinose biopurification on hydrolysate of corn fibre in Erlenmeyer flasks using *Ogataea zsoltii* strain

Arabinose biopurification in bench-top bioreactor

Arabinose biopurification was carried out on hydrolysate of corn fibre. *Ogataea zsoltii* metabolised glucose in 24 hours and xylose (and galactose) concentration started to decrease at the same time. The arabinose concentration was nearly constant during the fermentation. After three days, the medium contained 1,3 g/L xylose (and galactose) and 11,6 g/L arabinose, which resulted in the purity of 90 % for arabinose (Figure 3).

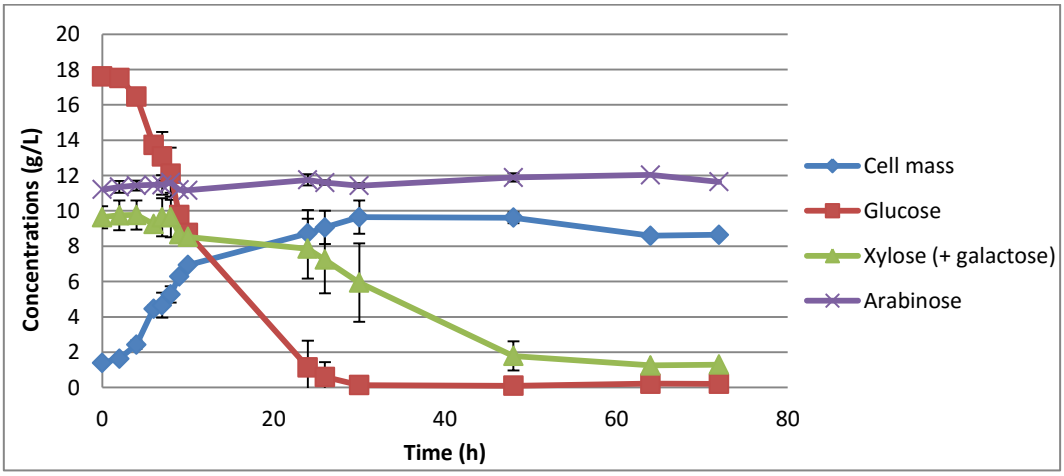

Figure 3: Arabinose biopurification implemented in bioreactor on first hydrolysate of corn fibre using *Ogataea zsoltii* strain

Conclusion

In this study, we described that *Ogataea zsoltii* NCAIM Y.01540, has the ability to perform the biopurification of arabinose on semidefined medium and corn fibre hydrolysate too. In every case the purity of the biopurificated medium was near 90 % for arabinose. Using hydrolysate of corn fibre, the quantity of arabinose remained constant for three days, while on semidefined medium arabinose concentration decreased when other carbon sources were depleted. Arabinose biopurification was fulfilled in 100 mL Erlenmeyer flasks as well as 2,5 L Applikon Biotechnology Autoclavable Bioreactor.

References

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