Integrated Process of Arabinose Biopurification and Xylitol Fermentation Based on the Diverse Action of *Candida boidinii*

C. Fehér,[*] Z. Gazsó, B. Gál, A. Kontra, Z. Barta, and K. Réczey
Budapest University of Technology and Economics,
Faculty of Chemical Technology and Biotechnology,
Department of Applied Biotechnology and Food Science,
Hungary, H-1111 Szent Gellért tér 4

doi: 10.15255/CABEQ.2014.2145

Original scientific paper
Received: November 4, 2014
Accepted: December 1, 2015

Hemicellulosic hydrolysates of agro-residues are promising raw materials for xylitol and arabinose production through biotechnological methods.

Two-step acidic fractionation of corn fibre was developed to produce a glucose- and arabinose-rich hydrolysate and a xylose-rich hydrolysate. An integrated process of arabinose biopurification on the glucose- and arabinose-rich hydrolysate and xylitol fermentation on the xylose-rich hydrolysate using *Candida boidinii* NCAIM Y.01308 was introduced, in which cell mass produced in arabinose biopurification was used as inoculum in the xylitol fermentation. Aerobic biopurification resulted in an arabinose solution containing 9.2 g L^{-1} of arabinose with a purity of 90 %, based on total sugars. Xylitol fermentation under microaerobic conditions resulted in a xylitol yield of 53 % of theoretical and a xylitol concentration of 10.4 g L^{-1} in three days.

Hence, an integrated biorefinery process of hemicellulosic hydrolysates was developed based on the diverse action of *C. boidinii* to purify arabinose and produce xylitol.

Key words:
biorefinery, hemicellulosic hydrolysate, corn fibre, arabinose, xylitol, *Candida boidinii*

Introduction

Agricultural and agro-industrial residues (agro-residues) containing high amounts of carbohydrates, such as cellulose and hemicellulose, are produced in large amounts annually worldwide.[1,2] Agro-residues are attractive raw materials of biorefinery processes producing value-added products due to their relatively low commercial value, renewability and abundant availability.[3] Biorefining of agro-residues for biofuel and bioproducts does not compete with food production and is considered to be advantageous from the environmental point of view, as it contributes to waste management, and its products can replace fossil-derived ones.[4,5]

One of the most important steps in lignocellulose-based biorefining technologies is the fractionation of the lignocellulosic biomass into its core constituent (hemicellulose, cellulose and lignin). Several studies proved the applicability of dilute acid-catalysed hydrolysis to selectively solubilise the hemicellulose fraction, therefore it is widely used as part of lignocellulose fractionation.[2,3] Hemicellulosic hydrolysates containing high amounts of xylose have great potential as a raw material for microbial xylitol production[1], while hemicellulosic hydrolysates with considerable amounts of arabinose might be suitable for arabinose production.[6]

L-Arabinose can be used as a non-caloric sweetener in the food industry; moreover, it strongly inhibits intestinal sucrase uncompetitively and consequently inhibits the absorption of sucrose in the small intestine, which makes it a promising sugar substitute in treatment of diabetes.[6,7] L-Arabinose can be utilized for bacterial diagnostics,[8] as a starting material in the synthesis of non-ionic surfactants[9] and as intermediate for antiviral drug synthesis.[10] Commercial production of L-arabinose is a complex process consisting of acid hydrolysis of gum arabic, followed by multiple procedures of purification.[6] The relatively high cost of gum arabic and the expensive purification steps result in the high cost of pure arabinose,[11] which has evoked an increasing research effort to develop new cost-effective methods of arabinose production from agro-residues rich in hemicellulose or pectin, for example from sugar beet pulp,[11] corn hull,[12] xylose-mother liquor,[6] wheat bran[13] and corn fibre.[14] Biopurification of hemicellulosic hydrolysate is an interesting and inexpensive strategy to produce a pure arabinose solution through the depletion of other sugars (e.g. glucose, xylose, galactose) using

[*]Corresponding author: csaba_feher@mail.bme.hu, +36-1-463-2843

the adequate microorganisms.[15] The drawbacks of the biopurification process are wasting of the potential of other sugars by converting them into value-added products and the formation of a considerable amount of cell mass as by-product.

Xylitol has a potential use in the pharmaceutical, odontological and food industries as an alternative sweetener.[16] Besides its anticariogenic properties, xylitol can be used for diabetes treatments, since its metabolism is partially independent of insulin.[17] On an industrial scale, xylitol is produced through the chemical reduction of xylose derived from hemicellulosic hydrolysate of birchwood or other xylose-rich materials.[1] The purification and separation steps required to remove other by-products from xylose and xylitol makes the process relatively expensive.[1] As an alternative method, microbial production of xylitol is becoming more attractive, since the downstream processing is expected to be cheaper and, unlike the chemical route, mild reaction conditions are required.[16] Many studies have been conducted to produce xylitol from the hemicellulose portion of agro-residues, like rice straw,[18] corn cob,[19] brewer's spent grain,[20] sugarcane bagasse,[21] corn stover,[22] barley bran[23] and corn fibre[24] using microbial processes. Xylitol can be produced by some bacteria and filamentous fungi, but the best producers are yeasts, especially species of genus *Candida,* such as *C. guilliermondii*, *C. pelliculosa, C. parapsilosis*, *C. tropicalis* and *C. boidinii*.[25] Microbial production of xylitol from hemicellulose hydrolysate is influenced by several factors including the strain, the fermentation conditions (pH, dissolved oxygen concentration, initial cell concentration, temperature) employed in the process and the composition of the fermentation medium (initial xylose concentration, concentrations of other sugars and the ratios of these sugars, concentrations of inhibitor compounds and nutrients).[16,26] The use of high initial cell concentration results in increased volumetric productivity and reduces the toxic effect of inhibitors,[26] however, a considerable amount of carbon source is required to obtain an inoculum of high cell density.

In this study, arabinose biopurification and xylitol fermentation were investigated on a semidefined medium and real hemicellulosic hydrolysate derived from corn fibre, and an integrated process was developed, in which the cell mass obtained as by-product in the biopurification step was utilized to inoculate the xylitol fermentation step. This process resulted in more effective carbon utilization, as the cell propagation for the xylitol fermentation did not require an additional carbon source or it did not consume xylose convertible into xylitol in the xylitol fermentation.

Materials and methods

Corn fibre

Corn fibre was kindly donated by Hungrana Starch and Isosugar Manufacturing and Trading Co. Ltd. (Szabadegyháza, Hungary). It was dried and stored at room temperature.

Yeast strains and inoculum preparation

Candida boidinii NCAIM Y.01308, *Candida parapsilosis* NCAIM Y.01011, *Candida guilliermondii (Pichia guilliermondii)* NCAIM Y.01050, *Hansenula anomala* (*Pichia anomala)* Y.01499 were purchased from the National Collection of Agricultural and Industrial Microorganisms (Budapest, Hungary). Yeast strains were stored and maintained on agar slants containing 1 % (w/w) glucose, 1 % (w/w) peptone, 0.3 % (w/w) yeast extract and 2 % (w/w) agar at room temperature and at 4 °C, respectively. The medium used for inoculum preparation (pH = 6) contained 10 g L^{-1} yeast extract, 15 g L^{-1} KH_2PO_4, 1 g L^{-1} $MgSO_4 \cdot 7H_2O$, 3 g L^{-1} $(NH_4)_2HPO_4$ and 30 g L^{-1} xylose. The solutions containing xylose and the other components were sterilized separately at 120 °C for 15 minutes in an autoclave. The cells were cultivated in 750-mL cotton-plugged Erlenmeyer flasks containing 150 mL of inoculum medium at 220 rpm rotation speed in a rotary shaker at 30 °C for 72 h, subsequently recovered by centrifugation (1000 × g, 5 min), washed with sterile distilled water, and the adequate amount of cell mass was directly resuspended in the xylitol fermentation and biopurification media.

Two-step acidic fractionation of corn fibre

The fractionation process of corn fibre includes two sequential hydrolyses catalysed by sulphuric acid (Fig. 1). The first acidic hydrolysis was carried out in 1000-mL closed glass-flasks containing 800 g of corn fibre suspensions at 90 °C for 51 minutes (plus 15 minutes warm-up period) without agitation in water bath. The corn fibre suspensions contained 10 % (w/w) dry matter and 1.1 % (w/w) sulphuric acid. Subsequently, the flasks were cooled in cold-water (10 °C) for 1 minute and the solid fractions were separated by vacuum filtration through a 150 µm-pore size nylon filter (Rimóczi és Társa Ltd., Hungary). The supernatants were collected and stored at –10 °C (first hydrolysate). The solid fractions were washed with distilled water until neutral pH, collected, then dried and stored at 40 °C (first solid residue). The first hydolysate was treated at 120 °C for 60 minutes to decompose the oligosaccharides. The solution obtained is referred to as glucose- and arabinose-rich hydrolysate. The first solid residue was used in the second acidic hydrolysis

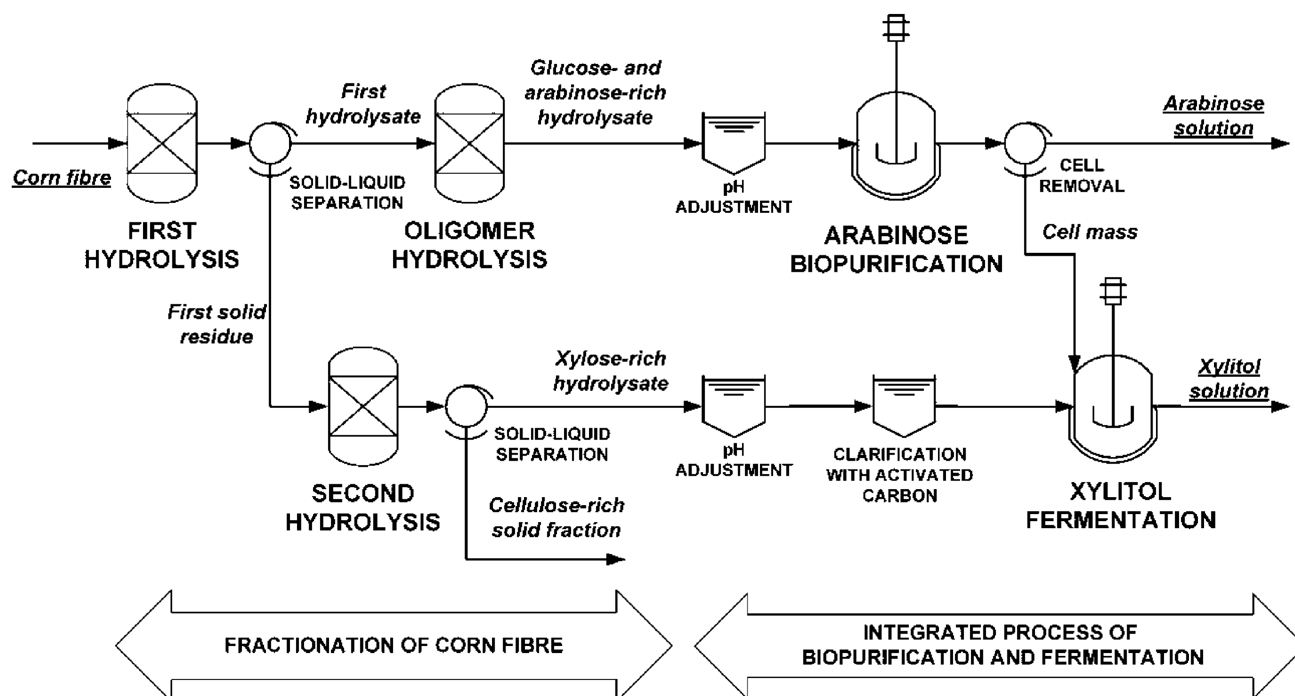

Fig. 1 – *Process scheme of fractionation of corn fibre and integration of arabinose biopurification and xylitol fermentation. Process steps and material streams are indicated in capital letters and italics, respectively.*

step, which was performed in 500-mL glass-flasks containing 300 g suspensions at 120 °C for 30 minutes in an autoclave. The suspensions of the first solid residue contained 10 % (w/w) dry matter and 1.1 % (w/w) sulphuric acid. After the flasks were removed from the autoclave, they were cooled in coldwater (10 °C) for 1 minute, and the solid fractions were separated by vacuum filtration through a nylon filter (150 µm). The supernatants were collected and stored at –10°C, and called as xylose-rich hydrolysate. The solid fractions were washed with distilled water until neutral pH, collected, then dried and stored at 40 °C (cellulose-rich solid fraction).

Biopurification, medium preparation

Biopurifications were carried out at 30 °C in a rotary shaker (220 rpm) for 96 hours in 100-mL cotton-plugged Erlenmeyer flasks containing 20 mL of the semidefined medium or glucose- and arabinose-rich hydrolysate of corn fibre, and monitored through daily sampling. The semidefined biopurification medium (pH = 6) contained 10 g L^{-1} yeast extract, 15 g L^{-1} KH_2PO_4, 1 g L^{-1} $MgSO_4 \cdot 7H_2O$, 3 g L^{-1} $(NH_4)_2HPO_4$, 15 g L^{-1} arabinose, 7.5 g L^{-1} xylose and 7.5 g L^{-1} galactose. The solution of the sugars and that of the other components were sterilized separately at 120 °C for 15 minutes in an autoclave. The glucose- and arabinose-rich hydrolysate was sterilized using the same conditions. Before sterilization, the pH of the glucose- and arabinose-rich hydrolysate (pH = 1) was adjusted to 6 by the addition of calcium hydroxide (powder). The precipitated gypsum was removed by filtration with a folding filter.

Xylitol fermentation, medium preparation

Xylitol fermentations were performed on a semidefined medium or xylose-rich hydrolysate of corn fibre at 30 °C in a rotary shaker for 96 hours, and monitored through daily sampling. The semidefined fermentation medium (pH = 6) contained 10 g L^{-1} yeast extract, 15 g L^{-1} KH_2PO_4, 1 g L^{-1} $MgSO_4 \cdot 7H_2O$, 3 g L^{-1} $(NH_4)_2HPO_4$, and 30 g L^{-1} or 70 g L^{-1} xylose. The solution of the xylose and that of the other components were sterilized separately at 120 °C for 15 minutes in an autoclave. The xylose-rich hydrolysate was sterilized using the same conditions. Before sterilization, pH adjustment and clarification of the xylose-rich hydrolysate were performed. The pH of xylose-rich hydrolysate (pH = 1) was adjusted to 6 by adding calcium hydroxide (powder). The precipitated gypsum was removed by filtration with a folding filter. After pH adjustment, the xylose-rich hydrolysate was clarified by activated carbon (0.05 g/100 g hydrolysate) at room temperature for 30 minutes with continuous agitation, subsequently, the activated carbon was removed by filtration with a folding filter. Activated carbon (Norit DX ULTRA 8005.3) was kindly donated by Cabot Norit Activated Carbon (Amersfoort, The Netherlands). Fermentations were carried out in 100-mL Erlenmeyer flasks closed with cotton plugs. In order to simulate different aeration conditions, three levels of medium volume (35, 50 and

65 mL) were used at two levels of rotation speed (125 and 220 rpm). Xylitol yields were considered to evaluate the experiments. Xylitol yield was calculated from the highest xylitol concentration obtained during the fermentation and expressed as percentage of theoretical. Theoretical xylitol yield was calculated from the initial xylose concentration by assuming a complete (stoichiometric) conversion. Xylitol volumetric productivities were also calculated at the time of the maximum xylitol concentration.

Compositional analysis

Composition of the carbohydrate fractions of corn fibre and the solid residues (first solid residue and cellulose-rich solid fraction) derived from fractionation of corn fibre were determined using the method of the National Renewable Energy Laboratory with minor modifications.[27] Half a gram of dry matter was mixed with 2.5 mL of 72 % (w/w) sulphuric acid and the mixture was kept at room temperature for 2 hours. Then, 75 mL of distilled water were added and the suspension was treated at 120 °C in the autoclave for 1 hour in 100-mL glass-flasks. The supernatant was analysed using high-performance liquid chromatography (HPLC) to determine its monosaccharide content. The starch content of the corn fibre was determined using thermostable α-amylase. Ground, air-dried corn fibre was suspended in a sodium acetate buffer (pH = 4.8, 100 mmol L^{-1}) at 3 % (w/w) dry matter content, and then treated by thermostable α-amylase (20 mmol min^{-1} kg^{-1} dry matter) in 1-L closed glass-flasks at 90 °C for 3 hours with continuous agitation (250 rpm) in a water bath. The thermostable α-amylase enzyme preparation with a volumetric enzyme activity of 4 mmol min^{-1} mL^{-1}) (defined on starch substrate at 25 °C) was donated by Hungrana Starch and Isosugar Manufacturing and Trading Co. Ltd. The supernatant was separated by vacuum filtration through a nylon filter (150 µm), mixed with 8 % (w/w) sulphuric acid at a volume ratio of 1:1, and treated at 120 °C in an autoclave for 15 minutes to decompose the oligosaccharides. Then it was analysed for glucose by HPLC. The cellulose content was calculated as the difference of the total glucan and starch content. The compositional analysis was carried out in triplicate.

Analytical methods

Concentrations of glucose, xylose, arabinose, xylitol, ethanol, methanol and acetic acid were determined by HPLC using BioRad (Hercules, CA, USA) Aminex HPX-87H (300 × 7.8 mm) column equipped with Micro-Guard Cation H^+ Refill Cartridge (30 × 4.6 mm) pre-column at 65 °C, and a refractive index detector. The eluent was 5 mmol L^{-1} sulphuric acid at a flow rate of 0.5 mL min^{-1}. Galactose was determined by using Phenomenex (Torrance, CA, USA) Rezex RPM-Monosaccharide Pb^{2+} (300 × 7.8 mm) column equipped with Carbo-Pb Security Guard Cartridge (4 × 3.0 mm) pre-column at 80 °C, and a refractive index detector. The eluent was ultra-pure (milli-Q) filtered water at a flow rate of 0.5 mL min^{-1}. The sample volume was 40 µL.

Cell concentration in the inoculum and fermentation samples was calculated from the optical density of the sample using a calibration curve based on the relationship of optical density and cell dry weight. Cell dry weight was determined gravimetrically after separation of a certain volume of inoculation broth (48 hours) by centrifuge (1000 × g, 5 minutes), washing it with distilled water, and drying the cells at 105 °C. Optical density was determined by spectrophotometer (Ultrospec III, Pharmacia LKB, Uppsala, Sweden) at a wavelength of 600 nm.

Total phenolics content was estimated using Folin-Ciocalteu reagent according to the method described by Guo *et al.*[28]

Gas-liquid mass transfer coefficients (K_La) of oxygen from the headspace of the flask to the media of xylitol fermentation (semidefined medium and xylose-rich hydrolysate) were determined by using a non-fermentative gassing-out method.[29] A 100-mL Erlenmeyer flask was equipped with an optical oxygen sensor (VisiFerm DO 120, HAMILTON Bonaduz AG, Switzerland) to measure dissolved oxygen concentration (C). After gassing out of the fermentation media with nitrogen, the increase in the dissolved oxygen concentration due to the shaking of the flask was measured until constant level of the dissolved oxygen concentration (C^*). The measurements were performed at 30 °C by using different levels of medium volume (35, 50 and 65 mL) and rotation speed (125 and 220 rpm). The values of the expression $-\ln(1-(C/C^*))$ were plotted as a function of time. The slope of the fitted linear curve gave the value of K_La (h^{-1}).[29] The maximum oxygen transfer rate (OTR) was calculated by multiplying K_La (h^{-1}) and C^* (mmol L^{-1}).

Results and discussion

Arabinose biopurification on semidefined medium

The capability of the investigated yeast strains for arabinose biopurification was tested on a semidefined medium containing sugars typically present in hemicellulosic hydrolysates, such as xylose, arabinose and galactose. The aim of the biopurification is the selective depletion of other sugars

beside the arabinose by using the adequate microorganism, which results in a pure arabinose solution. The absence of other sugars enables the effective crystallization of pure arabinose.[15] During aerobic biopurification, the undesired sugars are utilized mainly to produce cell mass, which occurs as a by-product of the process. Biopurification offers an inexpensive method to purify arabinose from hemicellulosic hydrolysates, however due to the loss of the other valuable sugars, this method might be reasonable only in the case of utilization of arabinose-rich hemicellulosic hydrolysates. Hence, the concentration of arabinose in the semidefined medium used in this study was two times higher than the concentrations of xylose and galactose. Four xylitol-fermenting yeast strains, namely *C. boidinii*, *C. guilliermondii*, *C. parapsilosis* and *H. anomala* were cultured in shake flasks under aerobic conditions to investigate their sugar consumption from the mixed sugar solution.

C. guilliermondii, *C. parapsilosis* and *H. anomala* utilized xylose, galactose and arabinose simultaneously to produce cell mass. *C. parapsilosis* consumed all of the sugars in two days, *C. guilliermondii* and *H. anomala* depleted xylose and galactose in two days, and arabinose in three days (data not shown). Hence, these strains are found to be inappropriate for arabinose biopurification from hemicellulosic hydrolysate. *C. boidinii* metabolized almost all of the xylose in one day (Fig. 2). The galactose concentration continuously decreased, 85 % of the initial was consumed in two days and 7 % of the initial (0.4 g L^{-1}) remained after four days (Fig. 2). *C. boidinii* did not consume arabinose, even if the other carbon sources were present in negligible quantities (Fig. 2). The cell concentration increased from 0.5 g L^{-1} to 5.5 g L^{-1} during the biopurification (Fig. 2), which corresponded to a cell yield of 0.38 g dry cell mass/g sugar consumed. The purity of the arabinose solution was defined as the ratio of arabinose content to total sugar content, in percentage. After four days, the biopurified medium contained 13 g L^{-1} arabinose and 0.4 g L^{-1} galactose, which resulted in a purity of 97 %. As *C. boidinii* was found to be appropriate for arabinose biopurification on semidefined media, it was selected for further investigations.

Xylitol fermentation on semidefined medium

Xylitol fermentations were performed on a semidefined medium to determine the most favourable conditions for xylitol production by *C. boidinii*. One of the most important factors affecting microbial production of xylitol is the aeration.[16] In order to simulate different aeration conditions in shake flasks, the fermentations were performed at three levels of filling ratio (0.35, 0.5 and 0.65), each

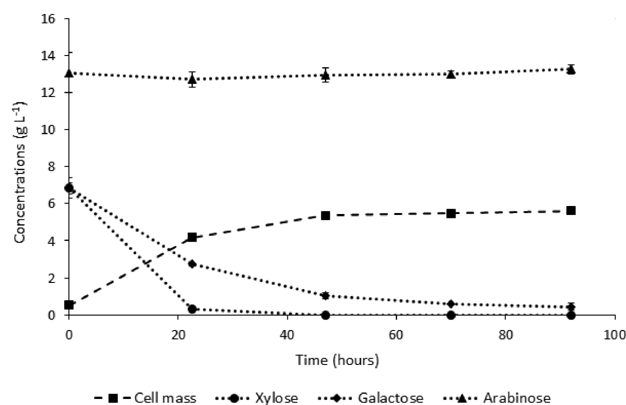

Fig. 2 – *Arabinose biopurification on semidefined medium using Candida boidinii NCAIM Y.01308. Standard deviations are calculated from duplicates.*

of them at two levels of rotation speed (125 and 220 rpm) (Table 1). The filling ratio is defined as the ratio of the medium volume to the flask volume. A higher filling ratio results in lower oxygen supply, while a higher rotation speed intensifies the dissolution of oxygen, during shake flask fermentations. In order to confirm that, OTR values of the different settings of filling ratio and rotation speed were determined (Table 1). The effects of initial cell concentration, initial xylose concentration, and methanol addition were also investigated. The different sets of the variables are shown in Table 1. Fermentations were carried out for four days. The investigation of different aeration conditions was performed with 1 g L^{-1} initial cell concentration and 30 g L^{-1} initial xylose concentration (Table 1). Xylitol fermentations under these initial conditions using *C. boidinii* followed the same trends. One example is demonstrated in Fig. 3a. Xylitol concentration keeps increasing as long as xylose is available in the fermentation broth. After xylose is depleted, *C. boidinii* starts to consume xylitol, hence xylitol concentration has a maximum value during the fermentation. Therefore, in the case of industrial implementation, it is crucial to terminate the process at the highest xylitol concentration, for which continuous monitoring of xylitol and xylose concentrations is necessary. Small amounts of ethanol is always produced simultaneously with xylitol, however, after xylose depletion the ethanol is also consumed. The cell mass continuously increased, which indicated that xylitol and ethanol were used to form cell mass after xylose depletion. Similar behaviour was observed by Walther *et al.* using *C. tropicalis*.[30]

At the rotation speed of 220 rpm, the OTR value decreased from 6.6 mmol L^{-1} h^{-1} to 5.2 mmol L^{-1} h^{-1} by increasing the filling ratio from 0.35 to 0.65 (Table 1). At the filling ratios of 0.35, 0.5 and 0.65 xylitol yields of 22 %, 25 % and 28 % were obtained

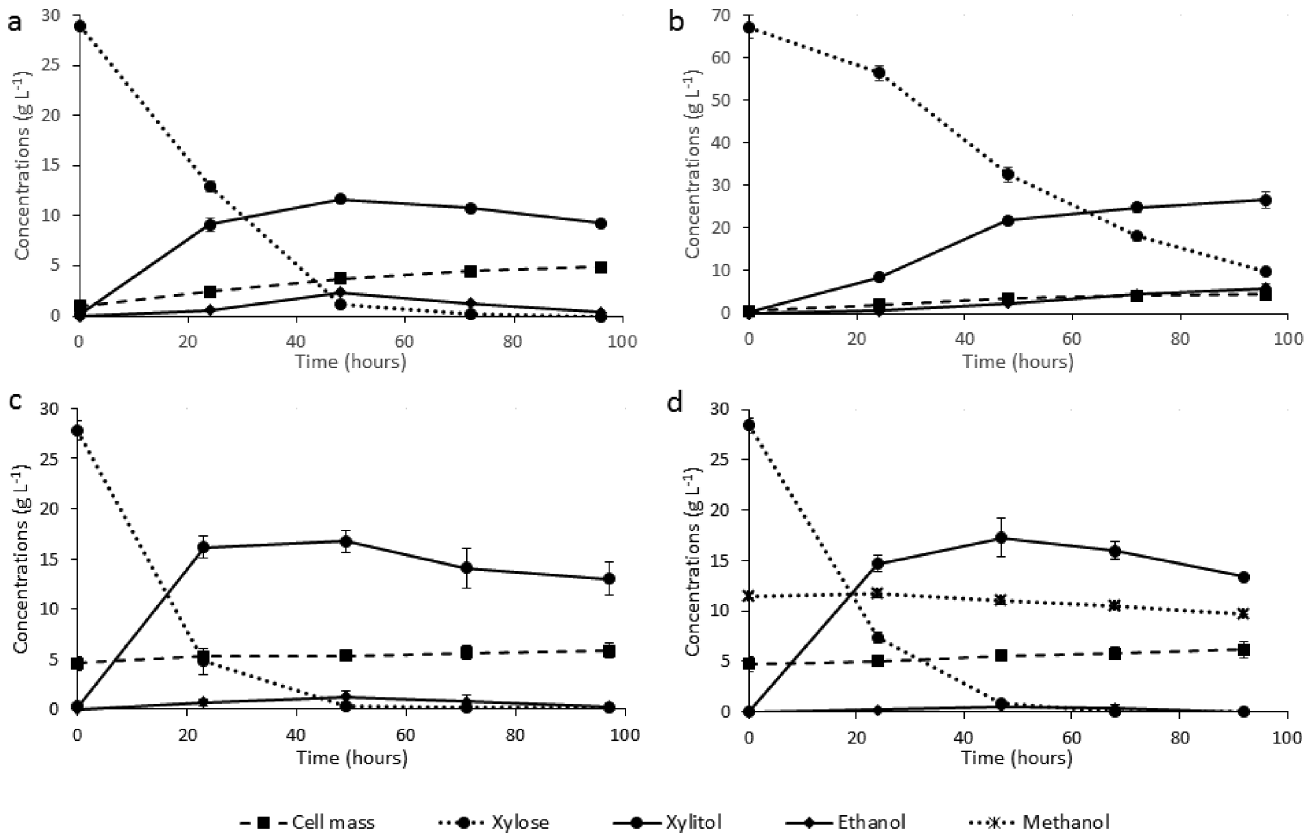

Fig. 3 – *Fermentation profiles of xylitol production on semidefined medium using Candida boidinii NCAIM Y.01308 under different conditions. Fermentations were carried out under microaerobic conditions (0.5 filling ratio, 125 rpm) using (a) 1 g L^{-1} initial cell concentration and 30 g L^{-1} initial xylose (base case), (b) 1 g L^{-1} initial cell concentration and 70 g L^{-1} initial xylose, (c) 5 g L^{-1} initial cell concentration and 30 g L^{-1} initial xylose and (d) 5 g L^{-1} initial cell concentration, 30 g L^{-1} initial xylose and 12 g L^{-1} methanol. Standard deviations are calculated from triplicates.*

Table 1 – *Fermentation conditions, maximal xylitol yields achieved on semidefined media and times required to achieve the maximal yield. Standard deviations are calculated from triplicates and indicated in parenthesis.*

Rotation speed (rpm)	Filling ratio	OTR (mmol L^{-1} h^{-1})	Initial cell concentration (g L^{-1})	Initial xylose concentration (g L^{-1})	Co-substrate	Xylitol yield (% of theoretical)	Time (days)
220	0.35	6.6	1	30	–	22 (1)	2
220	0.5	6.1	1	30	–	25 (0)	2
220	0.65	5.2	1	30	–	28 (0)	2
125	0.35	4.2	1	30	–	36 (1)	1
125	0.5	2.8	1	30	–	44 (4)	2
125	0.65	1.9	1	30	–	27 (1)	2
125	0.5	2.8	1	70	–	40 (1)	4
125	0.5	2.8	5	30	–	58 (5)	1
125	0.5	2.8	5	30	12 g L^{-1} MeOH	60 (5)	2

in two days (Table 1), which resulted in volumetric productivities of 0.14 g L^{-1} h^{-1}, 0.16 g L^{-1} h^{-1} and 0.18 g L^{-1} h^{-1}, respectively. In the case of 125 rpm rotation speed, the highest xylitol yield, 44 %, was achieved at the filling ratio of 0.5, where an OTR value of 2.8 mmol L^{-1} h^{-1} was measured (Table 1). The maximum xylitol concentration was observed in two days, which resulted in 0.28 g L^{-1} h^{-1} volumetric productivity. At 0.35 and 0.65 filling ratios, 4.2 mmol L^{-1} h^{-1} and 1.9 mmol L^{-1} h^{-1} OTR was observed, respectively (Table 1). At 0.35 filling ratio, a xylitol yield of 36 % was obtained in one day (Ta-

ble 1), resulting in a volumetric productivity of 0.46 g L^{-1} h^{-1}. At the filling ratio of 0.65, the xylitol yield was 27 %, which was obtained in two days (Table 1). The volumetric productivity was 0.17 g L^{-1} h^{-1}. In terms of the xylitol yields achieved, the most favourable aeration condition in shake flask fermentation using *C. boidinii* was obtained at the filling ratio of 0.5 with 125 rpm rotation speed, hence such conditions were set for the subsequent experiments. These conditions of aeration can be considered as microaerobic conditions according to Walther *et al.*[30] The xylitol fermentation performed at 1 g L^{-1} initial cell concentration and 30 g L^{-1} initial xylose concentration under microaerobic conditions is referred to as the base case in the following sections.

The effect of high initial xylose concentration for the fermentative capacity of *C. boidinii* was investigated using 70 g L^{-1} initial xylose concentration (Fig. 3b). The high initial xylose concentration resulted in a xylitol yield of 40 % (Table 1), which is slightly lower compared to that obtained in the base case (44 %). The xylitol concentration continuously increased until the end of the fermentation (Fig. 3b), resulting in a volumetric productivity of 0.3 g L^{-1} h^{-1}. The continuous increase in xylitol concentration through four days was due to the presence of xylose during the entire fermentation. After four days, 10 g L^{-1} xylose remained in the medium, hence the cells did not consume any accumulated xylitol (Fig. 3b). The ethanol formation showed a similar trend to that of xylitol (Fig. 3b). The ethanol concentration increased until 6 g L^{-1} (Fig. 3b). The cell mass also continuously increased during the fermentation, which resulted in a final cell concentration of 4.5 g L^{-1} (Fig. 3b). Although it has been published in many studies that high initial xylose concentration increases the xylitol yield,[1,16] it was not observed in this study. The relatively high amount of ethanol accumulated during the fermentation could negatively affect the xylitol production.[30] Vandeska *et al.* investigated the effect of initial xylose concentration on xylitol production of *C. boidinii* NRRL Y-17213 by varying xylose concentration from 20 g L^{-1} to 200 g L^{-1}, and it was found that 150 g L^{-1} initial xylose concentration is the most favourable, as it results in a xylitol yield of 0.47 g g^{-1} xylose consumed.[31] In our study, similar xylitol yield (0.46 g g^{-1} xylose consumed) was obtained using 70 g L^{-1} initial xylose concentration and 1 g L^{-1} initial cell concentration. However, Vongsuvanlert and Tani reported that increasing xylose concentration up to 150 g L^{-1} resulted in lower xylitol production than that with 100 g L^{-1} of xylose, using *C. boidinii* no. 2201.[32] High initial xylose concentration negatively affects the xylitol production, which might be due to the osmotic stress on the cells of *C. boidinii*.[32]

The effect of high cell density on the fermentation process was investigated using 5 g L^{-1} initial cell concentration. High initial cell density resulted in significantly higher xylitol yield within shorter fermentation time compared to the base case, as 58 % xylitol yield was achieved in one day (Table 1). The volumetric productivity of the fermentation was 0.73 g L^{-1} h^{-1}. The cell concentration was almost constant throughout the fermentation, less than 1 g L^{-1} was formed (Fig. 3c), in contrast with the cases of low (1 g L^{-1}) initial cell density (Fig. 3a and 3b). Due to the reduced cell formation, more xylose was available in the cells for xylitol production, which probably contributed to the higher xylitol yield. The ethanol formation was also reduced, only 1 g L^{-1} was produced in two days (Fig. 3c), which is half of that obtained in the base case (Fig. 3a). Hence, high initial cell concentration was found to be favourable in terms of the xylitol yield, the xylitol productivity, and the ratio of xylitol to ethanol. The improvement of xylitol yield (g xylitol/g xylose consumed) by increasing the initial cell density of *C. boidinii* NRRL Y-17213 was reported by Vandeska *et al.*[31]

Methanol addition is considered to be favourable for polyols production using methylotrophic yeasts, since the oxidation of methanol results in the formation of NADH, which is needed for the reduction of sugars.[1] However, the addition of methanol as a co-substrate did not result in a significant increase in xylitol yield (Fig. 3d vs. 3c). A xylitol yield of 60 % was obtained when 12 g L^{-1} of methanol was added to the fermentation broth containing 30 g L^{-1} of initial xylose and 5 g L^{-1} of initial cell concentrations (Table 1). However, a longer time was needed (2 days) to reach the highest xylitol concentration compared to that without methanol addition (1 day) (Fig. 3d vs. 3c), which resulted in a volumetric productivity of 0.38 g L^{-1} h^{-1}. The methanol concentration decreased until 10 g L^{-1} in four days (Fig. 3d). The cell concentration slowly increased during the fermentation, and 6 g L^{-1} of final cell concentration was obtained (Fig. 3d). Only 0.5 g L^{-1} ethanol, half of that obtained without methanol addition, was produced simultaneously with xylitol, which indicates that methanol addition can repress the formation of ethanol (Fig. 3d). In contrast, Vongsuvanlert and Tani reported that xylitol production using *C. boidinii* no. 2201 had significantly increased by adding methanol, and the methanol addition resulted in increased ethanol formation.[32]

According to our investigation of xylitol production on semidefined media using *C. boidinii*, aeration and initial cell density have the greatest effects on xylitol yield. Xylitol production is favoured under microaerobic conditions (2.8 mmol L^{-1} h^{-1} OTR) using increased (5 g L^{-1}) initial cell concentration.

Fractionation of corn fibre

The fractionation process of corn fibre includes two sequential hydrolyses catalysed by sulphuric acid (Fig. 1). Through the fractionation steps, xylose and galactose were determined as one component, which is referred to as xylose (+galactose).

In the first step of the fractionation, corn fibre was treated under mild acidic conditions to solubilise the starch content and liberate most of the arabinose.[14] After the first hydrolysis, a considerable amount of oligosaccharides was obtained in the supernatant, thus an oligomer hydrolysis step was required to recover the sugars in monomeric form (Fig. 1). According to preliminary results, additional acid was not required to decompose oligomers of the first hydrolysate, when the oligomer hydrolysis step was carried out at 120 °C for 1 hour (data not shown). After the first and oligomer hydrolyses, the glucose, arabinose and xylose (+galactose) were recovered in the supernatant with yields of 103 %, 74 % and 32 % of theoretical based on the raw material composition (Table 2), respectively. In this case, the glucose yield was calculated from the starch content of corn fibre as the cellulose was assumed to be intact under mild conditions applied in the first hydrolysis. Glucose yield of 103 % could be explained by the presence of a small amount of glucose in the hemicellulose fraction, which can be easily hydrolysed under mild acidic treatments.[14] The glucose- and arabinose-rich hydrolysate contained 15 g L^{-1} glucose, 8.4 g L^{-1} xylose (+galactose), 10.5 g L^{-1} arabinose and 0.9 g L^{-1} acetic acid. It was utilized as the fermentation medium for biopurification after pH adjustment (pH = 6) and sterilization.

The solid residue of the first step was utilized in the second acidic hydrolysis to produce the xylose-rich hydrolysate (Fig. 1). Glucose, xylose (+galactose) and arabinose were recovered in the supernatant with the yields of 10 %, 88 % and 63 % of theoretical based on the composition of the first solid residue, respectively. The lower yield of arabinose than that of xylose might be due to arabinose degradation. The low glucose recovery during the second hydrolysis resulted in a cellulose-rich solid fraction (Fig. 1) containing 50 % cellulose based on dry weight. The xylose-rich liquid fraction contained 3 g L^{-1} glucose, 28 g L^{-1} xylose (+galactose), 6.6 g L^{-1} arabinose, and 2.3 g L^{-1} acetic acid. The total phenolics content was 5.3 g L^{-1}. The xylose-rich hydrolysate was utilized for xylitol production after pH adjustment (pH = 6), treatment with activated carbon and sterilization.

Table 2 – *Carbohydrates and acetate content of corn fibre. Standard deviations are calculated from quintuplicate and indicated in parenthesis.*

Component	Percentage of dry weight
Starch	13 (0.6)
Cellulose	17 (0.8)
Xylan	19 (0.7)
Arabinan	12 (0.5)
Galactan	4 (0.1)
Acetate	3 (0.4)

Arabinose biopurification on glucose- and arabinose-rich hydrolysate of corn fibre

The glucose- and arabinose-rich hydrolysate derived from the fractionation process of corn fibre was utilized to produce a pure arabinose solution through biopurification using *C. boidinii*. Biopurification was carried out for three days. The biopurification medium contained 13.5 g L^{-1} glucose, 9.2 g L^{-1} arabinose, 5.8 g L^{-1} xylose and 2.3 g L^{-1} galactose after the inoculation, and the initial cell concentration was 0.5 g L^{-1} (Fig. 4). It also contained 0.8 g L^{-1} acetic acid. During the biopurification of glucose- and arabinose-rich hydrolysate of corn fibre, the consumptions of glucose, xylose and galactose occurred simultaneously, however with different consumption rates. The consumption rate of glucose was the highest, followed by that of xylose, while the galactose consumption was the slowest (Fig. 4). Glucose and xylose were totally depleted in one day and two days, respectively (Fig. 4). Galactose was not consumed completely, 32 % and 57 % of the initial amount were removed in one day and four days, respectively (Fig. 4). Acetic acid was consumed within one day (data not shown). Most of the cell mass (~4.5 g L^{-1}) was formed in one day; however cell concentration increased continuously until 6.5 g L^{-1} (Fig. 4). Beside the cell formation, a considerable amount of ethanol was produced (2 g L^{-1}) in one day, however it was consumed within the next day (Fig. 4). In the case of biopurification of glucose-free semidefined medium, ethanol formation was not observed, which might indicate that the ethanol was formed from glucose during biopurification of the corn fibre hydrolysate. To find the exact explanation of ethanol production under aerobic biopurification, further investigation of the metabolism of *C. boidinii* is required. The arabinose concentration was constant through the whole biopurification process (Fig. 4). After three days of biopurification, the medium contained 9.2 g L^{-1} arabinose and 1 g L^{-1} galactose (Fig. 4), i.e., the purity of arabinose was 90 % of total sugars, which exceeds the purity requirement for crys-

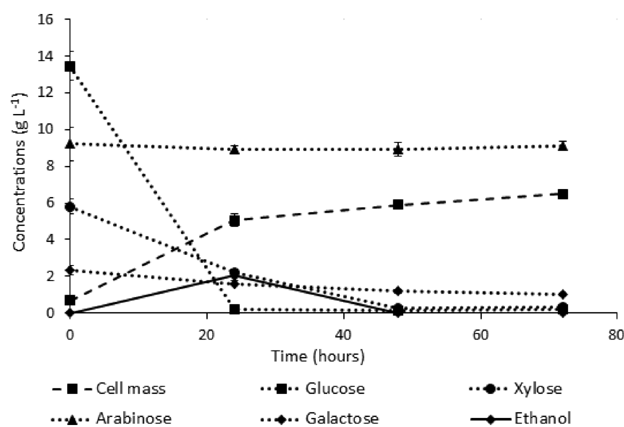

Fig. 4 – *Arabinose biopurification on glucose- and arabinose-rich hydrolysate of corn fibre using Candida boidinii NCAIM Y.01308. Standard deviations are calculated from triplicates.*

tallization of L-arabinose from a solution.[6] These results prove the applicability of *C. boidinii* to produce arabinose-rich solution from crude hemicellulosic hydrolysates through aerobic biopurification.

Yeast mediated biopurification of arabinose from hemicellulosic hydrolysate was published by Cheng *et al.* (2011) and Park *et al.* (2001). Cheng *et al.* performed arabinose biopurification on xylose mother liquor using *P. anomala*.[6] The purity of arabinose obtained under optimized conditions of biopurification was 86 % of total sugars, the optimal time of biopurification was 75 hours. Park *et al.* developed a method to produce arabinose from purified corn fibre arabinoxylan by enzymatic hydrolysis and arabinose biopurification.[15] *Williopsis saturnus* yeast strain was selected to perform the arabinose biopurification on enzymatic hydrolysate of corn fibre arabinoxylan. Almost all of the xylose was consumed in three days with no loss of arabinose however data of the concentrations of other components were not reported. Compared to these methods, arabinose biopurification from corn fibre hydrolysate using *C. boidinii* is a competitive strategy.

Xylitol fermentation on xylose-rich hydrolysate of corn fibre, integration of xylitol fermentation and arabinose biopurification

The xylose-rich hydrolysate derived from the fractionation of corn fibre was utilized to produce xylitol, however it contained a significant amount of phenolic compounds, which are strong inhibitors of microbial xylitol production.[26] In order to remove phenolic substances, the xylose-rich hydrolysate was treated with activated carbon before xylitol fermentation (Fig. 1). Activated carbon treatment is widely considered as an inexpensive and efficient method to reduce the content of inhibitory compounds in hemicellulosic hydrolysates.[26] After the activated carbon treatment, the total phenolic content reduced to 0.3 g L^{-1}. Xylose concentration during activated carbon treatment did not change. The initial composition of the xylitol fermentation medium was the following: 2.2 g L^{-1} glucose, 24.1 g L^{-1} xylose (+galactose) and 6.1 g L^{-1} arabinose. The acetic acid concentration (2.3 g L^{-1}) had not changed significantly through the treatments of the xylose-rich hydrolysate.

The fermentation was performed under microaerobic conditions (2.8 mmol L^{-1} h^{-1} OTR) using 5 g L^{-1} initial cell concentration. The *C. boidinii* cell mass used to inoculate the fermentation was harvested from the arabinose biopurification (Fig. 1), in contrast with the fermentations on semidefined medium, in which cell mass for inoculation was propagated on semidefined inoculum medium. This kind of integration of xylitol fermentation and arabinose biopurification (Fig. 1) enables the utilization of the by-product cell mass of biopurification and results in a more effective carbon utilization, as the cell propagation of xylitol fermentation does not require additional carbon source or it does not consume xylose convertible into xylitol in the fermentation step. Utilization of on-site by-products and integration of different production routes are crucial for developing a viable biorefinery process.

The profiles of utilization of sugars and formation of products through xylitol fermentation of the integrated process are shown in Fig. 5. Glucose was present in the medium in a small concentration and it was depleted within one day (Fig. 5). The arabinose concentration was unaltered throughout the fermentation (Fig. 5). The cell concentration was almost constant, less than 0.5 g L^{-1} was produced in four days (Fig. 5). Xylitol concentration increased until the third day, when it was 10.4 g L^{-1} (Fig. 5), resulting in a volumetric productivity of 0.14 g L^{-1} h^{-1}. This concentration corresponds to a xylitol yield of 43 % of theoretical based on the initial concentration of xylose (+galactose). However, according to our previous results, 80 % of the component measured as xylose (+galactose) in the xylose-rich hydrolysate was xylose, thus the initial xylose concentration can be assumed to be 19.3 g L^{-1}. By using this assumption during the calculation of the concentration of theoretically achievable xylitol, a xylitol yield of 53 % was obtained. This is only 5 % less, than the xylitol yield of the case performed under the same conditions (microaerobic aeration, 5 g L^{-1} initial cell concentration) on semidefined media, however, on semidefined media the xylitol yield was close to the maximum in one day (Fig. 3c). During the fermentation of xylose-rich hydrolysate, a higher amount of ethanol (3 g L^{-1}) was produced in two days, than that obtained under the same conditions on semidefined medium (1 g L^{-1}) (Fig. 5 vs Fig. 3c). The increased ethanol formation

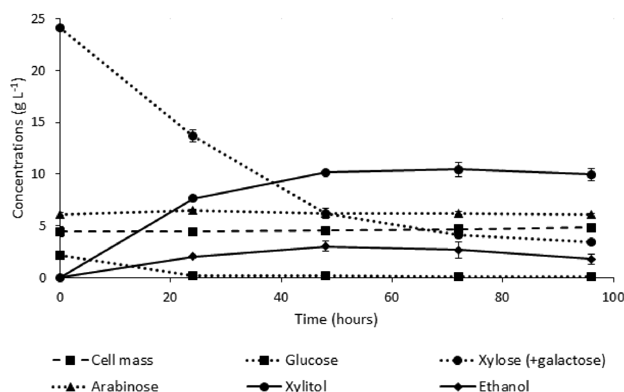

Fig. 5 – *Xylitol fermentation on xylose-rich hydrolysate of corn fibre in the integrated process using Candida boidinii NCAIM Y.01308. In the integrated process, the cell mass used for inoculation of xylitol fermentation is obtained in the arabinose biopurification step. Standard deviations are calculated from triplicates.*

might contribute to the reduced yield and slow accumulation of xylitol. After the third day of xylitol fermentation on xylose-rich hydrolysate of corn fibre, the fermentation broth contained 10.4 g L^{-1} xylitol, 6.1 g L^{-1} arabinose, 4.1 g L^{-1} xylose (+galactose) and 2.7 g L^{-1} ethanol (Fig. 5). As the xylitol concentration is approximately the same as the concentration of the residual sugars, further purification steps might be required to enable xylitol crystallization from the broth.

Leathers and Dien developed a two-stage, sequential fermentation process for xylitol and arabitol production from neutralized and deionized corn fibre hydrolysate using *P. guilliermondii*.[33] This strategy resulted in a xylitol yield of 0.27 g xylitol/g initial xylose within four days, which yield is around half of that achieved in our process. Rao *et al.* investigated xylitol production from corn fibre hydrolysate, which was neutralized, treated with activated charcoal and ion exchange resins.[24] *C. tropicalis* cells were adapted by sub-culturing in hydrolyaste containing medium for 20 cycles. This method resulted in a xylitol yield of 0.58 g/g xylose utilized, within two days. In our process, a xylitol yield of 0.54 g/g xylose utilized was obtained within three days by assuming that all of the initial xylose was utilized, however the deionization step was not included. Buhner and Agblevor investigated different detoxification methods to produce xylitol from concentrated corn fibre hydrolysate by using *C. tropicalis*.[34] The highest xylitol yield, 0.4 g/g xylose utilized, was obtained within four days in the case of the highest concentrations (three times the original hydrolysate) that had been partially neutralized by adding calcium hydroxide and treated with activated charcoal prior to the fermentation. Hence, xylitol production from corn fibre obtained in our integrated process is competitive with those methods found in the literature.

Conclusions

The two-step acidic fractionation of corn fibre was developed to produce a glucose- and arabinose-rich hydrolysate containing 15 g L^{-1} glucose, 10.5 g L^{-1} arabinose, 8.4 g L^{-1} xylose (+galactose), and a xylose-rich hydrolysate containing 28 g L^{-1} xylose (+galactose), 6.6 g L^{-1} arabinose, 3 g L^{-1} glucose. *C. boidinii* NCAIM Y.01308, a natural isolate yeast strain, was found to be suitable for producing an arabinose solution with a purity of 90 % from the glucose- and arabinose-rich hydrolysate through aerobic biopurification, as well as appropriate for xylitol production from the xylose-rich hydrolysate using high initial cell concentration (5 g L^{-1}) under microaerobic conditions (2.8 mmol L^{-1} h^{-1} OTR). In order to eliminate the separate cell propagation step prior to xylitol fermentation and to solve the utilization of cell mass by-product obtained in biopurification, an integrated biorefinery process based on the diverse action of *C. boidinii* was developed, in which the cell mass produced in biopurification was used to perform the xylitol fermentation. Xylitol fermentation resulted in a xylitol yield of 53 % of theoretical in three days. Hence, an integrated biorefinery process was developed to purify arabinose and produce xylitol from hemicellulosic hydrolysates by using *C. boidinii* yeast strain.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the Project OTKA PD-108389 of the Hungarian Research Fund and the Pro Progressio Foundation for their financial support. This work is connected to the scientific program of the "Development of quality-oriented and harmonized R+D+I strategy and functional model at BME" project. This project is supported by the New Hungary Development Plan (Project ID: TÁMOP-4.2.1/B-09/1/KMR-2010-0002).

References

1. *Nigam, P., Singh, D.*, Processes of fermentative production of Xylitol – A sugar substitute, Process Biochem. **30** (1995) 117.
doi: http://dx.doi.org/10.1016/0032-9592(95)95709-r
2. *Kumar, R., Singh, S., Singh, O. V.*, Bioconversion of lignocellulosic biomass: Biochemical and molecular perspectives, J. Ind. Microbiol. Biotechnol. **35** (2008) 377.
doi: http://dx.doi.org/10.1007/s10295-008-0327-8
3. *Mtui, G.*, Recent advances in pretreatment of lignocellulosic wastes and production of value added products, African J. Biotechnol. **8** (2009) 1398.
4. *Cherubini, F., Ulgiati, S.*, Crop residues as raw materials for biorefinery systems – An LCA case study, Appl. Energy **87** (2010) 47.
doi: http://dx.doi.org/10.1016/j.apenergy.2009.08.024

5. *Doherty, W. O. S., Mousavioun, P., Fellows, C. M.*, Value-adding to cellulosic ethanol: Lignin polymers, Ind. Crops Prod. **33** (2011) 259.
doi: http://dx.doi.org/10.1016/j.indcrop.2010.10.022
6. *Cheng, H., Wang, H., Lv, J., Jiang, M., Lin, S., Deng, Z.*, A novel method to prepare L-arabinose from xylose mother liquor by yeast-mediated biopurification, Microb. Cell Fact. **10** (2011) 43.
doi: http://dx.doi.org/10.1186/1475-2859-10-43
7. *Loeza-Corte, J. M., Verde-Calvo, J. R., Cruz-Sosa, F., Vernon-Carter, E. J., Huerta-Ochoa, S.*, L-Arabinose Production by Hydrolysis of Mesquite Gum by a Crude Extract with α-L-arabinofuranosidase Activity from *Aspergillus niger*, Rev. Mex. Ing. Química **6** (2007) 259.
8. *Otto, L. A., Pickett, M. J.*, Rapid method for identification of gram-negative, nonfermentative bacilli, J. Clin. Microbiol. **3** (1976) 566.
9. *Bouquillon, S.*, D-Xylose and L-arabinose-based surfactants: Synthesis, reactivity and physico-chemical properties, Comptes Rendus Chim. **14** (2011) 716.
doi: http://dx.doi.org/10.1016/j.crci.2010.06.016
10. *Du, J., Choi, Y., Lee, K., Chun, B. K., Hong, J. H., Chu, C. K.*, A Practical Synthesis of L-FMAU from L-Arabinose, Nucleosides and Nucleotides **18** (1999) 187.
doi: http://dx.doi.org/10.1080/15257779908043066
11. *Wu, R., Zhang, H., Zeng, X.*, L-Arabinose and oligosaccharides production from sugar beet pulp by xylanase and acid hydrolysis, African J. Biotechnol. **10** (2011) 1907.
doi: http://dx.doi.org/10.5897/AJB11.1927
12. *Kurakake, M., Takao, J., Asano, O., Tanimoto, H., Komaki, T.*, Production of L-arabinose from corn hull arabinoxylan by *Arthrobacter aurescens* MK5 α-L-arabinofuranosidase, J. Food Sci. **76** (2011) C231.
doi: http://dx.doi.org/10.1111/j.1750-3841.2010.02028.x
13. *Aguedo, M., Vanderghem, C., Goffin, D., Richel, A., Paquot, M.*, Fast and high yield recovery of arabinose from destarched wheat bran, Ind. Crops Prod. **43** (2013) 318.
doi: http://dx.doi.org/10.1016/j.indcrop.2012.07.029
14. *Fehér, C., Gazsó, Z., Tatijarern, P., Molnár, M., Barta, Z., Réczey, K.*, Investigation of selective arabinose release from corn fibre by acid hydrolysis under mild conditions, J. Chem. Technol. Biotechnol. (2014) in press.
15. *Park, N. H., Yoshida, S., Takakashi, A., Kawabata, Y., Sun, H. J.*, A new method for the preparation of crystalline L-arabinose from arabinoxylan by enzymatic hydrolysis and selective fermentation with yeast, Biotechnol. Lett. **23** (2001) 411.
doi: http://dx.doi.org/10.1023/A:1005681032082
16. *Winkelhausen, E., Kuzmanova, S.*, Microbial conversion of D-Xylose to Xylitol, J. Ferment. Bioeng. **86** (1998) 1.
doi: http://dx.doi.org/10.1016/S0922-338X(98)80026-3
17. *Natah, S., Hussien, K.*, Metabolic response to lactitol and xylitol in healthy men, Am. J. Clin. Nutr. **65** (1997) 947.
18. *Mayerhoff, Z., Roberto, I., Silva, S.*, Xylitol production from rice straw hemicellulose hydrolysate using different yeast strains, Biotechnol. Lett. **19** (1997) 407.
doi: http://dx.doi.org/10.1023/A:1018375506584
19. *Tada, K., Kanno, T., Horiuchi, J.*, Enhanced production of bioxylitol from corn cobs by *Candida magnoliae*, Ind. Eng. Chem. Res. **51** (2012) 10008.
doi: http://dx.doi.org/10.1021/ie202800h
20. *Mussatto, S. I., Dragone, G., Roberto, I. C.*, Influence of the toxic compounds present in brewer's spent grain hemicellulosic hydrolysate on xylose-to-xylitol bioconversion by *Candida guilliermondii*, Process Biochem. **40** (2005) 3801.
doi: http://dx.doi.org/10.1016/j.procbio.2005.06.024
21. *Rodrigues, R., Sene, L., Matos, G.*, Enhanced xylitol production by precultivation of *Candida guilliermondii* cells in sugarcane bagasse hemicellulosic hydrolysate, Curr. Microbiol. **53** (2006) 53.
doi: http://dx.doi.org/10.1007/s00284-005-0242-4
22. *Maciel de Mancilha, I., Karim, M. N.*, Evaluation of ion exchange resins for removal of inhibitory compounds from corn stover hydrolyzate for xylitol fermentation, Biotechnol. Prog. **19** (2003) 1837.
doi: http://dx.doi.org/10.1021/bp034069x
23. *Cruz, J., Domínguez, J., Domínguez, H., Parajó, J.*, Xylitol production from barley bran hydrolysates by continuous fermentation with *Debaryomyces hansenii*, Biotechnol. Lett. **22** (2000) 1895.
doi: http://dx.doi.org/10.1023/A:1005693709338
24. *Rao, R. S., Jyothi, C. P., Prakasham, R. S., Sarma, P. N., Rao, L. V.*, Xylitol production from corn fiber and sugarcane bagasse hydrolysates by *Candida tropicalis*, Bioresour. Technol. **97** (2006) 1974.
doi: http://dx.doi.org/10.1016/j.biortech.2005.08.015
25. *Vandeska, E., Amartey, S., Kuzmanova, S., Jeffries, T.*, Fed-batch culture for xylitol production by *Candida boidinii*, Process Biochem. **31** (1996) 265.
doi: http://dx.doi.org/10.1016/0032-9592(95)00058-5
26. *Parajó, J., Dominguez, H., Domínguez, J.*, Biotechnological production of xylitol. Part 3: Operation in culture media made from lignocellulose hydrolysates, Bioresour. Technol. **66** (1998) 25.
doi: http://dx.doi.org/10.1016/S0960-8524(98)00037-6
27. *Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Nrel, D. C.*, Determination of Structural Carbohydrates and Lignin in Biomass, in: Laboratory Analytical Procedure NREL/TP-510-42618, National Renewable Energy Laboratory, Golden, CO., 2012, pp 1-15.
28. *Guo, X., Cavka, A., Jönsson, L. J., Hong, F.*, Comparison of methods for detoxification of spruce hydrolysate for bacterial cellulose production, Microb. Cell Fact. **12** (2013) 1.
doi: http://dx.doi.org/10.1186/1475-2859-12-93
29. *Roseiro, J. C., Peito, M. A., Gírio, F. M., Amaral-Collaço, M. T.,* The effects of the oxygen transfer coefficient and substrate concentration of the xylose fermentation by *Debaryomyces hansenii*, Arch. Microbiol. **156** (1991) 484.
30. *Walther, T., Hensirisak, P., Agblevor, F. A.*, The influence of aeration and hemicellulosic sugars on xylitol production by *Candida tropicalis*, Bioresour. Technol. **76** (2001) 213.
doi: http://dx.doi.org/10.1016/S0960-8524(00)00113-9
31. *Vandeska, E., Amartey, S., Kuzmanova, S., Jeffries, T.*, Effects of environmental conditions on production of xylitol by *Candida boidinii*, World J. Microbiol. Biotechnol. **11** (1995) 213.
doi: http://dx.doi.org/10.1007/BF00704652
32. *Vongsuvanlert, V., Tani, Y.*, Xylitol production by a methanol yeast, *Candida boidinii* (*Kloeckera* sp.) No. 2201, J. Ferment. Bioeng. **67** (1989) 35.
doi: http://dx.doi.org/10.1016/0922-338X(89)90083-4
33. *Leathers, T. D., Dien, B. S.*, Xylitol production from corn fibre hydrolysates by a two-stage fermentation process, Process Biochem. **35** (2000) 765.
doi: http://dx.doi.org/10.1016/S0032-9592(99)00137-5
34. *Buhner, J., Agblevor, F. A.*, Effect of detoxification of dilute-acid corn fiber hydrolysate on xylitol production, Appl. Biochem. Biotechnol. **119** (2004) 13.
doi: http://dx.doi.org/10.1385/ABAB:119:1:13