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Xylitol from wood: study of some operational strategies

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Raw or alkali-extracted *Eucalyptus globulus* wood samples were treated with aqueous sulphuric acid in order to obtain xylose solutions. After neutralization and nutrient addition, the hydrolysates were used as culture media for the production of xylitol by the yeast *Debaryomyces hansenii* NRRL Y-7426. Several alternatives were considered for producing xylitol from wood. Either the original wood or the hydrolysates were subjected to selected treatment stages (NaOH extraction, concentration by evaporation or treatment with sulphite–bisulphite solutions). The results obtained are discussed in terms of volumetric productivity and product yield, and compared with data presented in the literature. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

There is an established commercial demand for bulk sugar substitutes that are suitable for diabetics and are non-cariogenic. In this field, one of the most promising sweeteners is xylitol, a pentitol that is found naturally in small quantities in many fruits and vegetables and which also acts as an intermediate metabolite in mammalian carbohydrate metabolism.

In the field of human nutrition, xylitol shows interesting properties, including: (1) it is less cariogenic than some other sweeteners (Emodi, 1978; Mäkinen, 1979), and shifts the equilibrium of the demineralization–remineralization towards the latter (Campillo, 1993); (2) cariogenic oral microorganisms are unable to utilize xylitol (Emodi, 1978; Mäkinen, 1979), but xylitol is metabolized by humans through normal pre-existing pathways (Ylikahri, 1979); (3) xylitol has been recommended for parenteral nutrition (Ylikahri, 1979); (4) it is claimed to have a better anticatabolic action than glucose in postoperative and post-traumatic states, since the first steps of xylitol metabolism are not insulin-dependent, avoiding insulin resistance; (5) it is suitable for diabetics, because its slow absorption avoids rapid changes in blood concentration (Ylikahri, 1979).

The technological characteristics of xylitol as a food are similar to those traditionally expected for bulk sweeteners. For example, its caloric value is similar to those of other sugar alcohols, and its relative sweetness is about the same as sucrose, being 2–2.5 times higher than sorbitol or mannitol (Emodi, 1978). It is chemically and thermally stable, and does not undergo Maillard reactions and caramelization because of the absence of aldo and keto groups. Xylitol solutions are less viscous than those of sucrose. Another remarkable characteristic is its endothermic dissolution, with a heat of solution more than eight times greater than that of sucrose and almost twice that of sorbitol (Hyvönen *et al.*, 1982). After long-term storage, products containing xylitol showed colour and taste properties superior to products containing other sweeteners (Hyvönen & Törmä, 1983). Its food applications include confectionery products (chewing gum, hard candy, toffee, compressed tablets, coatings and chocolate), ice cream, yoghourt, jams and marmalades, bakery products and drinks (Hyvönen & Slotte, 1983; Pepper & Olinger, 1988).

Extraction of xylitol from its natural sources is impractical and uneconomical because of its relatively small concentration. The basic principle of current commercial xylitol production is the recovery of xylose from xylan (a polysaccharide present in the hemicellulose fraction of hardwoods, sugarcane bagasse, straw or corn cobs) and its subsequent reduction by hydrogenation (Hyvönen *et al.*, 1982). However, extensive purification and separation steps are required to remove by-products.

The biotechnological way to produce xylitol, based on xylitol being an intermediate in the metabolism of D-xylose by yeasts and bacteria, is an interesting alternative to conventional chemical processes. Microbiological processes for bioconversion of xylose into xylitol by yeasts, bacteria, fungi or mixed cultures have recently been reviewed by Nigam & Singh (1995).

Yeasts are the most studied microorganisms for xylitol production. Among them, *Debaryomyces hansenii* has shown ability for producing xylitol with good yield and productivity from both commercial D-xylose (Girio *et al.*, 1990; Cultor, 1990; Roseiro *et al.*, 1991) and *Eucalyptus globulus* wood hydrolysates (Parajó *et al.*, 1995). Observed productivities were in the range of those reported in the literature for other yeasts such as *Candida* sp. (Chen & Gong, 1985), *Candida guilliermondii* (Barbosa *et al.*, 1988; Meyrial *et al.*, 1991), *Candida boidinii* (Vongsuvanlert & Tani, 1989), *Candida tropicalis* (Horitsu *et al.*, 1992; da Silva & Afschar, 1994) and *Candida parapsilosis* (Furlan *et al.*, 1991).

Lignocellulosic materials are inexpensive sources of sugar polymers that can be used as raw material for bioconversion into fuels, chemicals or food ingredients. A selective hydrolysis of the hemicellulose fraction of wood can be achieved with a mild acid hydrolysis (prehydrolysis), leading to xylose solutions useful for making culture media that can be used to produce xylitol. In the present study, several alternatives were explored for producing xylitol. Untreated or alkali-extracted *Eucalyptus globulus* wood samples were subjected to acid hydrolysis, and the products (directly or after processing) were used for xylitol production with *Debaryomyces hansenii*. Concentration of hydrolysates by evaporation allowed significant improvements in productivity and product yield in relation to other production schemes evaluated.

MATERIALS AND METHODS

Raw material

Eucalyptus globulus wood chips were obtained from a local pulp mill. These chips were milled to pass through a 1 mm screen, homogenized in a single lot, air-dried, stored and used for experimentation.

Acid hydrolysis of wood (prehydrolysis)

Wood samples were autoclaved at 130 °C with 3% sulphuric acid solutions for 1 h using a liquid/wood ratio of 8 g g^{-1}. These operational conditions were chosen on the basis of a study reported by Parajó *et al.*, 1994. The composition of hydrolysates was established using the high-performance liquid chromatography (HPLC) method reported elsewhere (Parajó *et al.*, 1994). The hydrolysates contained 18 g xylose $litre^{-1}$, 5.2 g acetic acid $litre^{-1}$, 3.6 g glucose $litre^{-1}$, 0.6 g arabinose $litre^{-1}$ and less than 0.5 g furfural $litre^{-1}$.

Alkaline treatment of wood

Milled wood samples were treated at 100 °C in a batch reactor with 1% NaOH solution for 1 h using a liquid/wood ratio of 8 g g^{-1}. The solid residue was submitted to prehydrolysis under the same conditions specified above for raw wood.

Neutralization and concentration of hydrolysates

Hydrolysates of raw or alkali-treated wood were neutralized with $CaCO_3$, and the $CaSO_4$ formed was separated by centrifugation. The neutralized hydrolysates were concentrated under vacuum in a rotary evaporator at 50 °C until the volume was reduced to either three-quarters or one-half that of the original.

Sulphitation of hydrolysates

Sodium sulphite (0.5%) and sodium bisulphite (0.5%) were added to concentrated hydrolysates, and the solutions obtained were supplemented with nutrients, autoclaved and used as culture media.

Microorganism

The bioconversion of xylose into xylitol was performed using the yeast strain *Debaryomyces hansenii* NRRL Y-7426 (obtained from the Northern Regional Research Laboratory, USDA, Peoria, IL, USA). This yeast was adapted to hydrolysates by sequential fermentation batches with cell reutilization to overcome (at least in part) the effect of inhibitors present in the culture medium. The lyophilized broth from NRRL was grown in a fermentation medium containing 10 g commercial xylose $litre^{-1}$, 5 g peptone $litre^{-1}$ and 3 g yeast extract $litre^{-1}$. The microorganism was maintained in agar slant tubes containing a medium formulated with the same components and concentrations as above plus 20 g agar $litre^{-1}$.

Fermentation media

After neutralization with $CaCO_3$, the hydrolysates were supplemented with nutrients (3.5 g $litre^{-1}$ of yeast extract and 3.5 g $litre^{-1}$ peptone), autoclaved at 121 °C and used as fermentation media for xylitol production.

Fermentation

Fermentations were carried out at 30 °C in a Braun Biostat B fermentor using a working volume of 500 ml. After each fermentation trial, the cells were decanted, and the supernatant was discarded. Fresh fermentation medium was then added to the cell suspension in order to start a new experiment.

Analytical methods

Samples from the fermentation media were centrifuged, filtered through 0.45 μm membranes and analysed by the same HPLC method used to study the composition of hydrolysates.

RESULTS AND DISCUSSION

The bioconversion of xylose into xylitol has been assayed in both synthetic xylose solutions and hemicellulose

hydrolysates. In several studies using culture media derived from straw or sugarcane bagasse hydrolysates, xylitol productivities in the range 0.19–0.56 g litre^{-1} h^{-1} have been reported in fermentations with *Candida guillermondii* (Roberto *et al.*, 1991, 1994), with product yields of 0.36–0.69 g xylitol g^{-1} xylose. Less favourable fermentation media were obtained when hardwood hemicellulose hydrolysates were used for making culture media: in bioconversion experiments with *Pachysolen tannophilus*, the volumetric xylitol productivities were in the range 0.025–0.104 g litre^{-1} h^{-1} (Perego *et al.*, 1990).

In this work, several strategies for enhancing the bioconversion of *Eucalyptus* wood hydrolysates into xylitol are explored.

Results obtained with hydrolysates of raw wood

As a first step in the bioconversion of hydrolysates, the influence of the oxygenation rate on the xylitol production was analysed using hydrolysates made from milled wood samples. For this purpose, several fermentation trials were performed with agitation speeds of 100, 300, 500 or 700 rpm. In these experiments, air was flushed into the fermentor for 45 min after sampling (flow rate 2.5 litre min^{-1}), in order to provide the oxygen necessary for an efficient bioconversion. The results obtained are shown in Fig. 1. Xylose consumption (Fig. 1(a)) was similar in all the experiments: more than 50% of substrate was utilized by the yeast after 200 h of fermentation. Figure 1(b) shows that increased agitation rates (and thus increased concentrations of dissolved oxygen in the fermentation media) resulted in decreased xylitol concentrations. This behaviour can be explained on the basis of yeast metabolism: under conditions defined by high concentrations of dissolved oxygen, the xylose is preferentially utilized for biomass generation, whereas under semi-anaerobic conditions the production of xylitol is favoured (Barbosa *et al.*, 1988; Furlan *et al.*, 1991; Girio *et al.*, 1990, 1994). It can be seen from Fig. 1(b) that the operational conditions leading to the maximum xylitol concentration corresponded to the experiment performed with the least agitation speed (100 rpm).

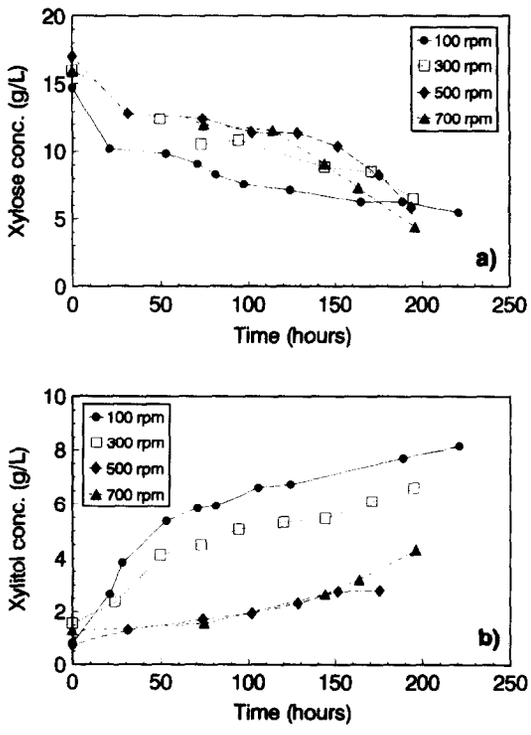

Fig. 1. Time course of substrate and product concentrations in fermentation runs carried out at the various agitation speeds assayed.

Figure 2 shows the dependence of the volumetric rate of product generation (Q_p) and substrate consumption (Q_s) as well as that of the product yield ($Y_{p/s}$) on the agitation speed. The results derived from the above experiments confirmed, quantitatively, the trends observed. The maximum $Y_{p/s}$ (0.79 g xylitol produced g^{-1} xylose consumed) and the maximum Q_p (0.033 g xylitol produced litre^{-1} h^{-1}) corresponded to the experiment performed with the least agitation speed, conditions under which the substrate was consumed slowly ($Q_s = 0.042$ g xylose consumed litre^{-1} h^{-1}). Higher agitation speeds resulted in faster xylose consumption, but the metabolism is directed towards biomass generation, leading to decreased values of Q_p and $Y_{p/s}$. The best $Y_{p/s}$ obtained in this work doubled the maximum value determined previously by Parajó *et al.* (1995) in experiments carried out in an orbital shaker, whereas Q_p was reduced by about 20% in comparison with the same case.

In order to improve the above results, three different strategies were analysed: alkaline extraction of the raw material, concentration of hydrolysates by evaporation and sulphitation of hydrolysates. The objectives of these treatments and the results obtained are described in the following sections.

Alkaline treatment of wood

An alkaline extraction of wood was performed before the prehydrolysis step in order to achieve two main objectives: to remove the extractives from the hydrolysates and to break the acetyl linkages of xylan and lignin in order to decrease the concentration of acetic

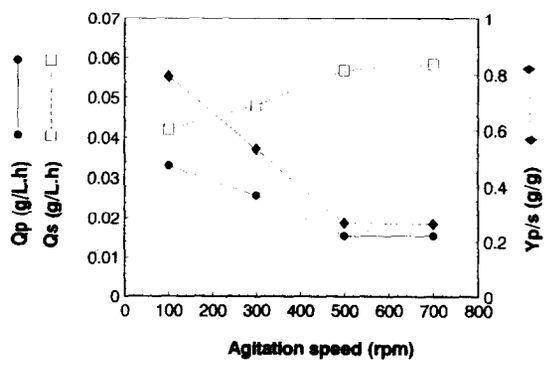

Fig. 2. Dependence on the agitation speed of the substrate and product volumetric productivities (Q_s and Q_p) and product yield ($Y_{p/s}$) under the selected operational conditions.

acid in the reaction media. Both objectives were effectively reached by treatment stages performed under the conditions assayed. As an example, Fig. 3 shows two chromatograms corresponding to prehydrolysates of untreated and alkali-extracted wood, in which it can be seen that the acetic acid concentration was negligible in the second case in comparison with the 4.21 g acetic acid litre^{-1} in the first. However, these favourable analytical data did not result in improvements of the bioconversion process. The experimental results (70% xylose conversion after 215 h, $Q_p = 0.015$ g litre^{-1} h^{-1}, $Y_{p/s} = 0.67$), suggested that the alkaline processing of wood resulted in hydrolysates containing increased concentrations of lignin-derived inhibitors (such as acid-soluble lignin) which hindered the bioconversion process.

Concentration of hydrolysates by evaporation

Acid hydrolysates of untreated wood were subjected to vacuum evaporation in order to obtain culture media with increased xylose concentration. Two different degrees of concentration were assayed, corresponding to [final volume]/[initial volume] ratios of 3/4 and 1/2, respectively.

Figure 4 shows the time course of fermentation trials carried out with hydrolysates concentrated in the proportions cited above. In the experiment performed with a concentration ratio of 3/4, Fig. 4(b) shows a remarkable increase in xylitol concentration (up to 10 g litre^{-1}), with a Q_p of 0.053 g litre^{-1} h^{-1} (about 1.6 times the optimum value determined in the above sections). The product yield $Y_{p/s}$ decreased to 0.63, suggesting a higher biomass production, which was also confirmed by the comparatively high substrate consumption (about twice the values obtained in the previous section). The decrease observed in $Y_{p/s}$ suggests that agitation speeds lower than 100 rpm could result in a more efficient utilization of this substrate for xylitol production. In the experiment performed with hydrolysates concentrated by 1/2 (Fig. 4(a)), the utilization of xylose was hindered in comparison with the previous experiment, a fact that could be related to the increased concentration of inhibitors in this fermentation medium and/or with the requirements for higher oxygenation for an effective bioconversion. For example, it is well known that, under conditions of oxygen limitation, the acetic acid concentration in culture media negatively affects the productivity, the specific growth rate, the product yield and the substrate utilization when its concentration exceeds a given level (Van Zyl *et al.*, 1991; Perego *et al.*, 1990).

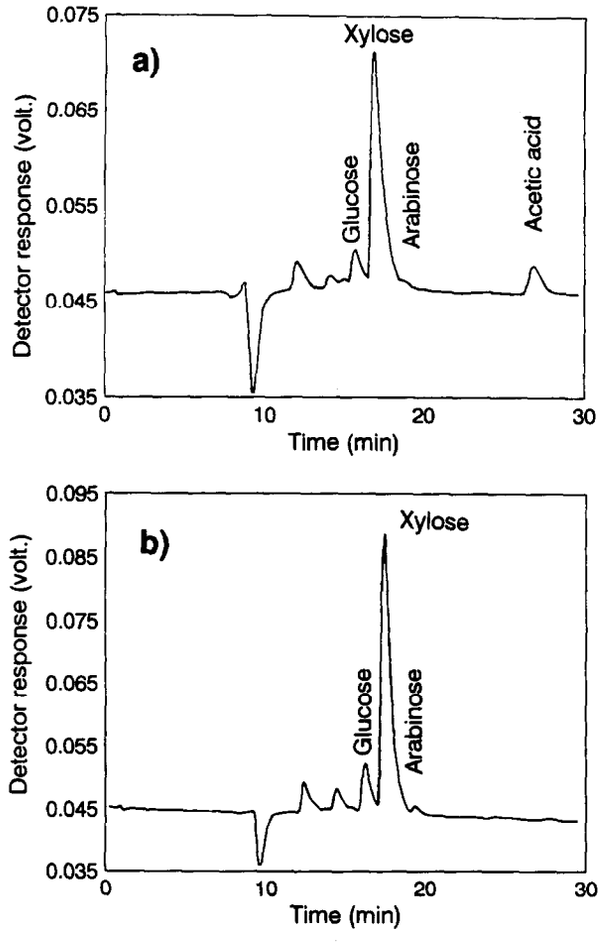

Fig. 3. HPLC chromatograms of (a) raw hydrolysates, (b) hydrolysates obtained from alkali-treated wood.

Sulphitation of hydrolysates

As a possible way to improve the results obtained with concentrated hydrolysates, sulphite and bisulphite were added to the hydrolysates. The goal was to modify the chemical structure of phenolic compounds acting as inhibitors, as reported in the literature (Tran & Chambers,

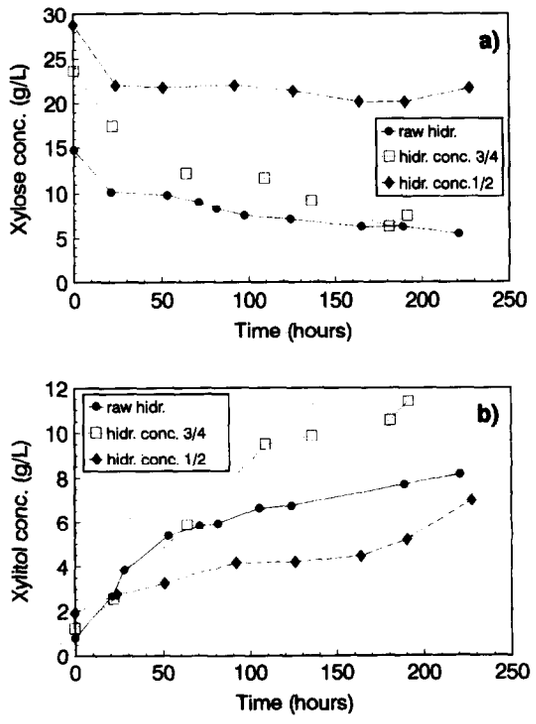

Fig. 4. Dependence of xylose and xylitol concentrations on the fermentation time observed for culture media made from raw hydrolysates or hydrolysates concentrated to [final volume]/[initial volume] ratios of 3/4 and 1/2.

1985). For this purpose, the hydrolysates concentrated by 1/2 were sulphited and used for making culture media. The experimental results demonstrated that no significant improvements were obtained with this strategy: both the xylitol productivity (0.02 g litre^{-1} h^{-1}) and the product yield (0.45 g g^{-1}) were of the same order as those observed in the absence of sulphite and bisulphite.

CONCLUSION

The concentration of hydrolysates by evaporation was the most promising alternative for enhancing the biotechnological production of xylitol. An alkaline pretreatment was useful to almost quantitatively remove acetic acid from the fermentation media, but lignin-derived inhibitors led to slow fermentation kinetics. In contrast, concentration by evaporation of hydrolysates resulted in improved product yields (up to 0.79 g g^{-1}), with xylitol productivities up to 0.033 g litre^{-1} h^{-1}. When the hydrolysates were concentrated in ratios higher than 3/4, the corresponding increases in concentration of inhibitors hindered the bioconversion, and a further sulphitation stage did not improve the overall process.

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