Biotechnology Letters **22:** 239–243, 2000.

Xylitol production from aspenwood hemicellulose hydrolysate by *Candida guilliermondii*

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Received 3 November 1999; Revisions requested 18 November 1999; Revisions received 17 December 1999; Accepted 17 December 1999

Key words: aspenwood, *Candida guilliermondii*, fermentation, hemicellulose hydrolysate, xylitol

Abstract

The production of xylitol by the yeast *Candida guilliermondii* was investigated in batch fermentations with aspenwood hemicellulose hydrolysate and compared with results obtained in semi-defined media with a mixture of glucose and xylose. The hemicellulose hydrolysate had to be supplemented by yeast extract and the maximum xylitol yield (0.8 g g^{-1}) and productivity (0.6 g l^{-1} h^{-1}) were reached by controlling oxygen input.

Introduction

Xylitol, a natural sugar alcohol, is an important substitute for sucrose and has found many applications in the food and drink industries. Commercial production of xylitol is based on the chemical reduction of xylose present in hemicellulosic hydrolysates from a variety of lignocellulosic raw materials, leading to a complex mixture of polyols and sugars that must be subjected to expensive refining treatments (Melaja & Hämäläinen 1977). In this field, biotechnological processes provide an interesting alternative for xylitol production depending on the efficiency of hemicellulosic sugar utilization and tolerance to the substances which are inhibitory to fermentation.

In previous papers, we reported that the yeast *Candida guilliermondii* ferments xylose with an appreciable xylitol production (Nolleau *et al.* 1993, 1995). Although its fermentative performance is improved in a semi-defined medium containing xylose as the only carbon source, *C. guilliermondii* could be an appropriate biocatalyst for xylitol production from lignocellulosic source such as aspenwood hemicellulose. Apart from xylose, steam explosion followed by acid hydrolysis of this natural feedstock yields two hexoses, glucose and mannose, in minor amounts. Promising results have been reported with *C. guilliermondii* FTI 20037 using sugar cane bagasse and rice straw hydrolysates (Roberto *et al.* 1995).

The purpose of the present paper was to study the ability of *C. guilliermondii* to produce xylitol from substrates of aspenwood hydrolysate in order to assess the feasibility of natural substrate utilization for xylitol production on an industrial scale. Semi-defined media were used as controls to show the influence of components present in the hemicellulose hydrolysate and particularly glucose on the fermentation parameters.

Materials and methods

Microorganism and inoculum preparation

Candida guilliermondii NRC 5578 (National Research Council) was supplied by the Foundation for Industrial Technology (Brazil) and was maintained on potato/dextrose/agar (PDA) slants at 4 °C. A loopful of cells grown on PDA slants was transferred to 100 ml of medium containing 10 g yeast extract l^{-1} (Biokar) with 20 g xylose l^{-1}. The culture was grown aerobically in a 1 l Erlenmeyer flask at 30 °C on a rotary shaker at 150 rpm. After 24 h, cells were harvested by centrifugation at 1300 *g* for 10 min, washed twice

with distilled water and suspended in 10 ml of distilled water. This suspension was used as inoculum.

Hemicellulose hydrolysate

Hemicellulose hydrolysate, kindly supplied by SAF-ISIS (France), was prepared from aspenwood chips by autohydrolysis steam explosion. The hydrolysate was diluted with distilled water to give the following average composition: 50 g xylose l^{-1}, 26 g glucose l^{-1}, 6 g mannose l^{-1}, 1.4 g acetic acid l^{-1}, 0.3 g furfural l^{-1}, 0.7 g hydroxymethylfurfural l^{-1}, 0.06 g vanillin l^{-1} and 0.16 g syringaldehyde l^{-1}. Its pH was 1.8.

Media and fermentation conditions

The semi-defined media were composed of 10 g yeast extract l^{-1} with 50 g xylose l^{-1} or with a simulated hydrolysate containing 26 g glucose l^{-1}, 50 g xylose l^{-1} and 6 g mannose l^{-1}. For use in experiments, the pH of the crude hemicellulose hydrolysate was adjusted to 6. To study the effect of the initial yeast extract concentration on yeast metabolism, the cells were grown in the hydrolysate in the presence of this extract at 0, 1, 2, 3, 5 and 10 g l^{-1}.

The cultures were inoculated at an initial cell concentration of 1.5–2 g l^{-1} (dry weight). Fermentations were carried out under aerobic conditions using 2 l Erlenmeyer flasks containing 200 ml of media which were incubated at 30 °C in a rotary shaker at 150 rpm until total depletion of sugars. The initial pH of the fermentation broth was 6 and was uncontrolled during runs.

Fermentations under oxygen control were performed in a 2 l bioreactor of 1.5 l working volume. Cultures were run at 30 °C under saturation of oxygen (800 rpm, 2.5 vvm) then under O_2-limited conditions with an O_2 transfer rate of 2.2 mmol l^{-1} h^{-1} (300 rpm and 1 vvm). The pH was automatically ajusted at 6 with 2 M NaOH.

Analytical methods

Growth was monitored gravimetrically by drying washed samples to a constant mass at 105 °C. Xylose, glucose and xylitol concentrations were determined by HPLC and ethanol concentration was measured by gas chromatography as described previously by Preziosi-Belloy *et al.* (1997).

Results and discussion

The hemicellulose hydrolysate of aspenwood could be considered as potential substrate for growth and xylitol production by *C. guilliermondii* (Figure 1A). Hexoses and xylose were used concurrently and completely by the yeast. By comparison with the fermentation employing only xylose as substrate, it was obvious that fermentative performances were low although no lag phase was observed (Figure 1A–B): the xylitol yields were below 0.13 g g^{-1} with a productivity of 0.1 g l^{-1} h^{-1} against 0.4 g g^{-1} and 0.44 g l^{-1} h^{-1}, respectively with the semi-defined medium.

Hexoses, and particularly glucose, can affect xylose metabolism by a partial repression or inhibition of transport systems or catabolic enzymes (Webb & Lee 1990). However in semi-defined media, the presence of glucose at 20 g l^{-1} decreased the fermentation time from 50 to 30 h, thus improving the xylitol productivity (Figure 1B–C). We also observed an improvement of the ethanol yield from 0.04 to 0.23 g g^{-1} and, to a lesser extent, biomass yield from 0.1 to 0.2 g g^{-1}. The utilization of glucose enhanced ethanol formation from xylose since after a glucose depletion 0.09 g ethanol was produced per g xylose without changing the part of xylose converted in xylitol (0.4 g g^{-1} in absence of glucose and 0.45 g g^{-1} in its presence). This revealed a greater xylose participation since in absence of glucose only 80% of total carbon based on initial xylose was finally converted to cells, xylitol and ethanol.

Accordingly, low productivities obtained from the hemicellulose hydrolysate could be explained by a nutrient limitation. This was supported by ethanol and xylose being concurrently assimilated when glucose was depleted (Figure 1A). One way to show a limitation phenomenon is to add yeast extract to hemicellulosic hydrolysate since essential nutrients in semi-defined media were supplied by this crude extract.

As shown Figure 2A, yeast extract was fundamental to improve the rate of fermentation but above 2 g l^{-1} an increase of initial concentration did not really improve the xylitol production only glucose uptake and ethanol production rates were enhanced. The presence of yeast extract also stimulated ethanol, cell and xylitol productions in terms of yields, but the variations in yield values when the initial yeast extract concentrations were further increased, were minor (Figure 2B).

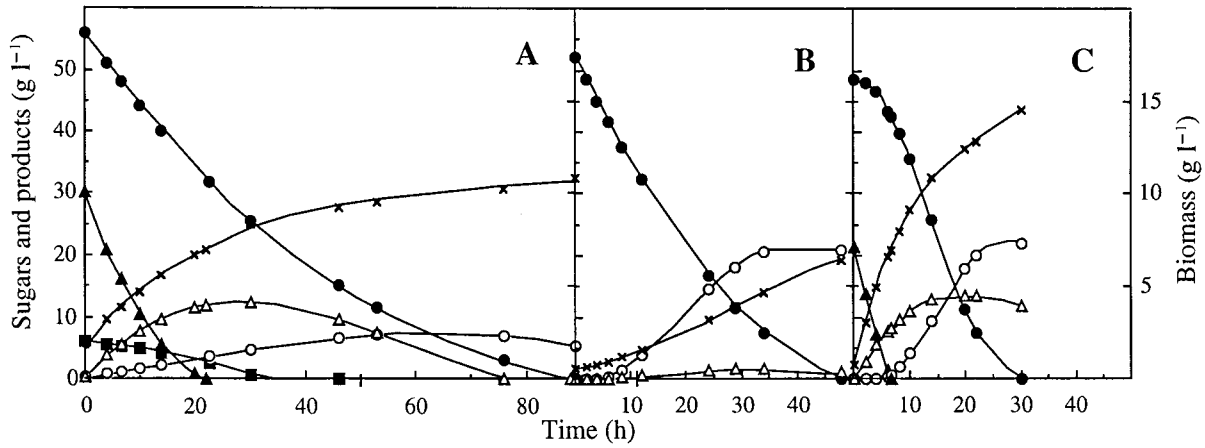

Fig. 1. Fermentations by *Candida guilliermondii* at 30 °C, pH 6, under aerobic conditions in different media: aspenwood hemicellulose hydrolysate (A), semidefined medium with only xylose (B) and semidefined medium with a sugar mixture of glucose and xylose (C). Symbols: (×) biomass, (▲) glucose, (■) mannose, (●) xylose, (△) ethanol and (◯) xylitol.

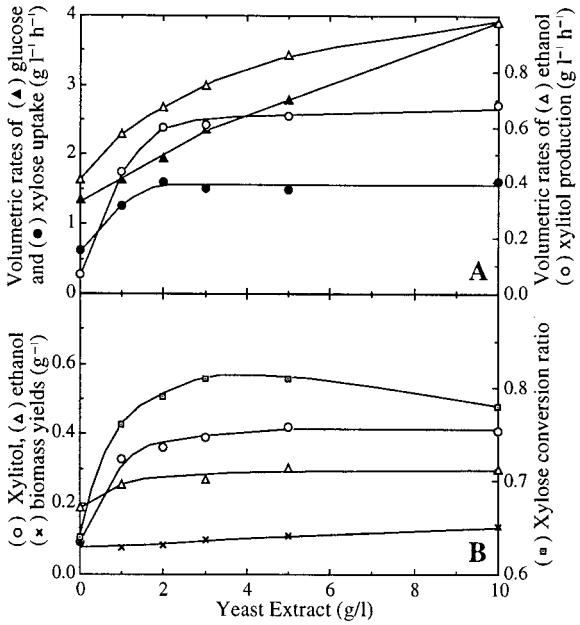

Fig. 2. The influence of initial concentrations of yeast extract on sugar assimilation and production rates (A), on yields and on xylose conversion ratio ((xylose remaining + xylitol produced)/initial xylose concentration) estimated at the shortage of glucose (B).

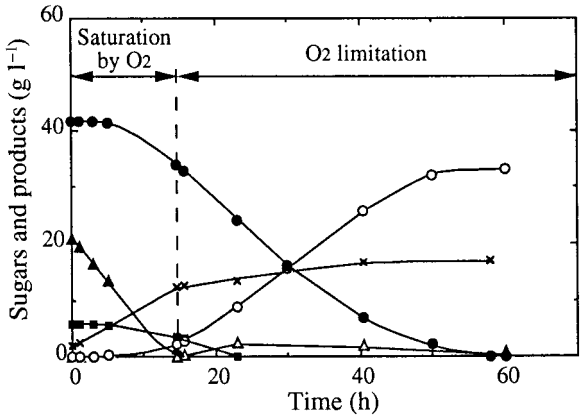

Fig. 3. The influence of oxygen control on hemicellulosic sugars fermentation and xylitol production; the first step was conducted under saturation of oxygen (800 rpm, 2.5 vvm) and the second step under oxygen-limited conditions with OTR 2.2 mmol l^{-1} h^{-1} (300 rpm, 1 vvm). Symbols: (×) biomass, (▲) glucose, (■) mannose, (●) xylose, (△) ethanol and (◯) xylitol.

The initial concentration of yeast extract seems to influence xylose utilization, estimated by xylose conversion ratio, as observed at the end of the glucose assimilation phase: the conversion of xylose into xylitol was improved by increase of initial yeast extract concentration, reached a maximum at 3 g l^{-1} (Figure 2B). This ratio was measured at the shortage of glucose since after a total depletion of glucose, a twofold decrease was observed for all fermentations, xylose supporting both xylitol production and cell growth (data not shown).

To maintain constant and maximum the xylose conversion ratio during all fermentation time, i.e., to limit biomass formation from xylose, fermentations under controlled aeration conditions were employed: a first step without oxygen limitation followed by a second one under limited oxygen conditions. The first step using excess of oxygen, is aimed to improve the active transport of glucose and its utilization *via* cell production (Barnett & Sims 1982). The second step under limited oxygen conditions, is intended to

Table 1. Fermentation parameters attained with or without oxygen control.

Growth conditions	**Yx/s** (g g^{-1})	**Ye/s** (g g^{-1})	**Yp/s** (g g^{-1})	**Qxose** (g l^{-1} h^{-1})	**Qglu** (g l^{-1} h^{-1})	*Qxol* (g l^{-1} h^{-1})
Without O_2 control	0.10	0.27	0.4	1.5	2.4	0.60
With O_2 control	0.32	0.05	0.8	0.7	1.4	0.58

Yx/s, cell yield coefficient (g dry cell mass per g sugar); **Ye/s**, ethanol yield coefficient (g ethanol per g sugar); **Yp/s**, xylitol yield coefficient (g xylitol per g xylose); **Qxose** and **Qglu**, xylose and glucose uptake rates; **Qxol**, xylitol volumetric productivity

increase the xylose conversion into xylitol (Nolleau *et al*. 1995).

As shown in Figure 3 and Table 1, saturating oxygen conditions favoured growth (0.32 g g^{-1} against 0.1 g g^{-1}) while the accumulation of ethanol and xylitol were negligible. The preferential glucose utilization was enhanced since only 21% of xylose was simultaneously assimilated with a net lag phase of 5 h. After the glucose depletion, the metabolic pathway from growth to xylitol production was driven by decreasing the oxygen transfer rate to 2.2 mmol l^{-1} h^{-1}. Biomass remained relatively constant whereas a real improvement of the xylose conversion into xylitol was observed since the xylitol yield increased twofold. This yield was even 1.2 times higher than the one obtained with sugar mixture in semi-defined medium. Xylitol productivity was not improved (0.58 against 0.6 g l^{-1} h^{-1}), this fact was due in part to the glucose uptake rate that is twice lower than the rate measured in fermentations without oxygen control. This inverse relationship between glucose uptake rate and oxygen supply rate was also observed with another yeast like *Candida parapsilosis* (Nolleau *et al.* 1995).

The process used in this study with *C. guilliermondii* seems to be very interesting with a xylitol production rate of 0.58 g l^{-1} h^{-1} and a yield of 0.8 g g^{-1}. Moreover, we have observed a rapid adaptability to by-products of hemicellulose hydrolysis since each of them disappears rapidly 25 to 50 h after the onset of growth independently to oxygen supply (data not shown). The potential of the yeast *Candida guilliermondii* for microbiological production of xylitol using hemicellulosic hydrolysates of sugarcane bagasse, rice straw (Roberto *et al*. 1995) and eucalyptus wood (Felipe *et al*. 1996) have been reported with sugars and by-products amount close to those used in the aspenwood hydrolysate. Xylitol yield and productivity measured from sugar cane bagasse hydrolysate were around 0.7 g g^{-1} and 0.64 g l^{-1} h^{-1}, respectively. Similar values were obtained for rice straw hydrolysate since a yield of 0.71 and a productivity of 0.56 were reported. With the eucalyptus hemicellulose hydrolysate, the xylitol yield and productivity were low 0.3 g g^{-1} and 0.03 g l^{-1} h^{-1}, this could be attributed to acetic acid concentration that is about 17 times higher than those present in the other hydrolysates cited.

Further improvements in kinetics of fermentation seem to be possible by increasing the initial cell concentration. Cao *et al.* (1994) with *Candida* sp B-22 and Roberto *et al.* (1996) with *C. guilliermondii* FTI 20037 have shown the importance of cell mass concentration on xylitol production rate. The increasing initial xylose concentration should then be tested since the correspondent increase in the concentration of potential inhibitors will be counterbalanced by higher cell mass.

Acknowledgement

The authors wish to thank the society SAF-ISIS (Soustons, France) for supply of hemicellulose hydrolysate of trembling aspenwood.

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