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Effect of inoculum level on xylitol production from rice straw hemicellulose hydrolysate by *Candida guilliermondii*

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The effect of inoculum level on xylitol production by *Candida guilliermondii* was evaluated in a rice straw hemicellulose hydrolysate. High initial cell density did not show a positive effect in this bioconversion since increasing the initial cell density from 0.67 g L^{-1} to 2.41 g L^{-1} decreased both the rate of xylose utilization and xylitol accumulation. The maximum xylitol yield (0.71 g g^{-1}) and volumetric productivity (0.56 g L^{-1} h^{-1}) were reached with an inoculum level of 0.9 g L^{-1}. These results show that under appropriate inoculum conditions rice straw hemicellulose hydrolysate can be converted into xylitol by the yeast *C. guilliermondii* with efficiency values as high as 77% of the theoretical maximum.

Keywords: xylitol production; inoculum level; rice straw; hemicellulose hydrolysate; *Candida guilliermondii*

Introduction

Rice straw is an agricultural residue available in large quantities and has potential applications in different bioconversion processes. Hemicellulose is one of the major fractions in this residue that can be easily hydrolyzed to a mixture of reducing sugars containing a high proportion of D-xylose [11]. This pentose is a potential source for the production of useful chemicals and its efficient utilization is important in the overall conversion of plant biomass.

Xylitol, a valuable sweetener with anticariogenic properties and important clinical use as a sugar substitute for diabetics [15], can be produced by either chemical or biotechnological routes using D-xylose recovered from lignocellulosic materials [4,6]. Demand for its application in the food and pharmaceutical industries has created a strong market for low cost processes. An advantage expected for the biotechnological process over the chemical one is the reduction of the cost associated with the necessary purification steps in preparation of the starting material.

Many yeast strains produce high yields of xylitol from xylose [1,2,5,8]. The environmental conditions that affect this bioconversion, such as aeration, substrate concentration and inoculum level, are well documented for fermentations using synthetic media [2,7,9,13]. However, these conditions need to be established for xylose-rich hemicellulose hydrolysates. In previous studies we showed the influence of medium composition [10], pH and nitrogen source [11] on this bioconversion using rice straw hemicellulose hydrolysate. In the present work, the inoculum level was investigated in order to verify its effect on xylitol production by the yeast *Candida guilliermondii* in a rice straw hemicellulose hydrolysate.

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Materials and methods

Microorganism

The yeast *Candida guilliermondii* FTI 20037 described by Barbosa *et al* [1] was employed for this study. Stock cultures were maintained on malt extract agar slants stored at 4°C.

Inoculum preparation

The inoculum was prepared by growing cells in 125-ml Erlenmeyer flasks containing 50 ml of medium with the following nutrients (g L^{-1}): xylose, 20; $(NH_4)_2SO_4$, 3; $CaCl_2 \cdot 2H_2O$ 0.1 and rice bran, 20. The culture was cultivated on a rotary shaker at 30°C and 200 rpm for 24 h. The cells were separated by centrifugation at $1600 \times g$ for 10 min and used to prepare a suspension in sterile distilled water that was used to inoculate the fermentation medium.

Media and fermentation conditions

Rice straw hemicellulose hydrolysate obtained by acid hydrolysis was prepared as described by Roberto *et al* [11]. The hydrolysate (pH 5.3) containing (in g L^{-1}): xylose, 79.3; glucose, 22.6; arabinose, 13.4; furfural, 0.13; hydroxymethylfurfural, 0.51; and acetic acid, 1.82 was supplemented with 10 g L^{-1} of rice bran and 2 g L^{-1} of $(NH_4)_2SO_4$. The fermentation runs were carried out at 30°C in 125-ml Erlenmeyer flasks containing 50 ml of hydrolysate medium incubated on a rotary shaker at 200 rpm for 72 h. The initial inoculum concentration was varied from 0.67 g L^{-1} to 2.41 g L^{-1}.

Analytical methods

Xylose and xylitol concentrations were determined by high performance liquid chromatography (HPLC) using a model HP 1082B Hewlett-Packard apparatus under the following conditions: Aminex HPX87H column (Bio-Rad, Hercules, CA, USA) at 45°C, 0.01 N sulfuric acid as eluent, flow rate 0.6 ml min^{-1}, refraction index detector, and 20-μl sample volume. Cell growth was monitored by measuring absorbance at 600 nm using a Micronal model B34211 spectrophotometer (Micronal SA, São Paulo, Brazil). The cell concentration was calculated using a calibration curve (dry wt × absorbance) prepared with cells grown in hydrolysate supplemented with the same nutrients (except for xylose) and using the same conditions as those employed for inoculum preparation. Incubation time was 48 h.

Results and discussion

The bioconversion of xylose into xylitol by *Candida guilliermondii*, using rice straw hemicellulose hydrolysate as substrate, was greatly influenced by the level of inoculum. Increasing the initial inoculum concentration from 0.67 to 2.41 g L^{-1} did not enhance xylose consumption. As shown in Figure 1, similar xylose consumption of about 90% was obtained for fermentations carried out with inoculum levels of 0.67 and 0.90 g L^{-1}, after 72 h. Using higher inoculum concentrations (1.78 or 2.41 g L^{-1}), only 75% of the xylose was utilized. This behaviour can possibly be attributed to low oxygen availability since a higher inoculum concentration tends to reduce the oxygen level in the medium. Oxygen level plays an important role on xylose uptake rate by pentose-fermenting yeasts. Sreenath *et al* [12] showed that the yeast *Candida shehatae* fermented xylose under strictly anaerobic conditions and in the presence of oxygen both cellular growth and xylose consumption were favoured. Chan *et al* [3] using *Schizosaccharomyces pombe*, a genetically transformed yeast, also noted a decrease in xylose consumption when the aeration level was reduced. The glucose present in the medium was fully consumed and arabinose was not utilized, independently of the inoculum concentration (data not shown).

The increase in the initial inoculum concentration also negatively affected both biomass formation (Figure 2) and xylitol production (Figure 3). When the medium containing hydrolysate was inoculated with an initial cell concentration of 0.67 g L^{-1} the values of xylitol concentration and cell mass were 39.46 g L^{-1} and 6.74 g L^{-1}, respectively, after 72 h of fermentation. These results decreased to 21.74 g L^{-1} and 4.79 g L^{-1}, respectively, when the initial inoculum concentration was increased to 2.41 g L^{-1}. Wood and Millis [14], working with *Pachysolen tannophilus*, also showed that xylitol production was reduced when the initial cell density of the medium was increased. This behaviour can also be related to a decrease in oxygen concentration. As observed by Nolleau *et al* [9], under strictly oxygen-limited conditions, *C. guilliermondii* growth was severely restricted, and this led to low xylitol production rates.

With respect to the fermentative parameters (Table 1), the maximum specific growth rate (μ_m), xylitol yield ($Y_{p/s}$) and volumetric productivity (Q_p) were sharply decreased by increasing the cell density. Earlier work on xylose-fermenting yeasts have demonstrated different behaviour in relation to the effect of inoculum level for this bioconversion. Sreenath *et al* [12] reported that, in *Candida shehatae*, the xylitol yield dropped from 0.20 to 0.14 g g^{-1} with an increase in cell density from 0.42 to 3.6 g L^{-1}. On the other hand, the xylitol yield from *Candida boidinii* increased from 0.12 to 0.24 g g^{-1} when inoculum level was increased from 1.3 to 5.1 g L^{-1} [13]. This different behaviour could be attributed to the use of different species of microorganisms and also to the experimental conditions utilized.

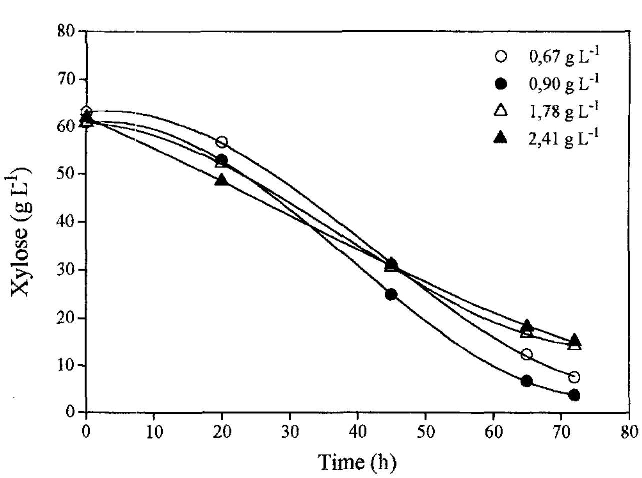

Figure 1 Effect of initial inoculum concentration on xylose consumption by *Candida guilliermondii* from rice straw hemicellulose hydrolysate.

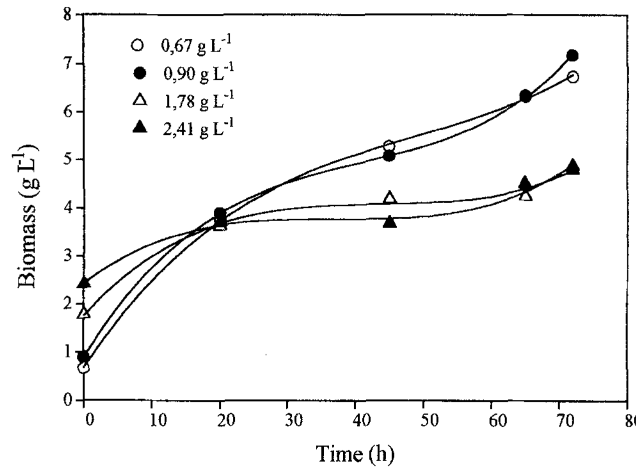

Figure 2 Effect of initial inoculum concentration on biomass formation by *Candida guilliermondii* from rice straw hemicellulose hydrolysate.

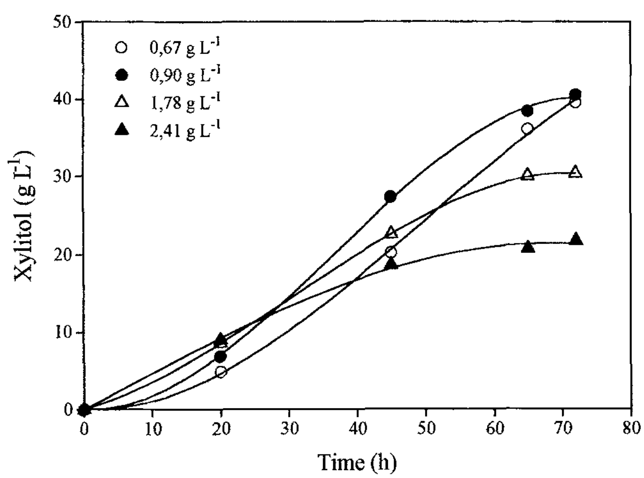

Figure 3 Effect of initial inoculum concentration on xylitol production by *Candida guilliermondii* from rice straw hemicellulose hydrolysate.

Table 1 Effect of the initial inoculum concentration on the fermentation parameters of *Candida guilliermondii*

Parameters	Inoculum level ($g\ L^{-1}$)			
	0.67	0.90	1.78	2.41
Time (h)	72	72	65	65
Maximum xylitol ($g\ L^{-1}$)	39.46	40.48	29.98	20.76
Residual xylose ($g\ L^{-1}$)	7.59	3.83	16.96	18.25
$Y_{p/s}$ ($g\ g^{-1}$)	0.71	0.71	0.68	0.47
$Y_{x/s}$ ($g\ g^{-1}$)	0.080	0.082	0.038	0.015
Q_p ($g\ L^{-1}\ h^{-1}$)	0.55	0.56	0.46	0.32
μ_m (h^{-1})	0.114	0.105	0.050	0.023
η (%)	77	77	74	51

$Y_{p/s}$: xylitol yield coefficient (g xylitol per g xylose utilized).
$Y_{x/s}$: cell yield coefficient (g dry cell mass per g xylose and glucose utilized).
Q_p: xylitol volumetric productivity ($g\ L^{-1}\ h^{-1}$).
μ_m: maximum specific growth rate (h^{-1}).
η: efficiency of the bioconversion calculated from the theoretical value of 0.917 $g\ g^{-1}$ described by Barbosa *et al* [1].

The best fermentative performance of *C. guilliermondii* in a rice straw hemicellulose hydrolysate was obtained with an initial cell density of 0.9 $g\ L^{-1}$. Under these conditions, a yield of 0.71 $g\ g^{-1}$, corresponding to 77% of the theoretical bioconversion and a volumetric productivity of 0.56 $g\ L^{-1}\ h^{-1}$, were attained. The results exhibited by *C. guilliermondii* in a rice straw hydrolysate are similar to those reported by other authors employing the same yeast strain cultivated in synthetic media [7,9]. According to Meyrial *et al* [7] *C. guilliermondii* was characterized by a high potential for xylitol production from xylose-rich material since its specificity for xylose reduction is high. Our results agree with this observation since under appropriate inoculum conditions *C. guilliermondii* produced xylitol from rice straw hydrolysate with high yields.

Rice straw hemicellulose hydrolysate represents an attractive substrate for industrial application with respect to xylitol production using biotechnological routes. Compared to other microorganisms, *C. guilliermondii* appears to be a promising xylitol-producing yeast from xylose-rich material since it can convert about 77% of xylose from rice straw hemicellulose hydrolysate into xylitol. Further studies referring to the aeration level of this conversion will be necessary to optimize this process.

Acknowledgements

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