OPTIMIZATION OF FERMENTATIVE XYLITOL PRODUCTION FROM AGRO-INDUSTRIAL RESIDUES.

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1. INTRODUCTION

The difficulty of the agro-industrial waste degradation has driven to the study of different processes in order to recover these substrates with a limited environmental impact. In particular, biotechnological transformations can ensure the production of high value products, which can also be considered as natural and thus utilized in the food industry according to the international food additives guidelines.
Woody materials are made by three main parts: cellulose, hemicellulose and lignin; the first two are rich in sugar polymers that can be converted into monosaccharides by enzymatic or acid hydrolysis and fermented in a subsequent step to fuels or chemicals. The hemicellulosic fraction has a high xylose composition when mild acid treatments are performed (1).
The main xylose fermentative product is xylitol, a polyalcohol currently manufactured by substrate hydrogenation, which is of particular interest owing to its dietetic and clinical properties (2).
The best xylitol producers are yeasts, mainly belonging to the species *Candida guilliermondii, Pachysolen tannophilus* and *Debaryomyces hansenii* (3), which metabolize xylose through a two-step oxido-reductive route. At first, xylose reductase (XR), in the presence of NADH and/or NADPH as cofactor, reduces D-xylose to xylitol (4). In a subsequent reaction, this pentitol is oxidized to D-xylulose by either NAD^+-linked or $NADP^+$-linked xylitol dehydrogenase (XDH), depending on the microorganism (5).
A certain number of studies have stressed the importance of oxygen to ensure high yield fermentations. Under both anaerobic and oxygen-limited conditions, *Candida guilliermondii* and other yeast strains with XR activity linked both to NADH and NADPH give ethanol as main product and no xylitol is accumulated, owing to the redox balance between the cofactors of XR and XDH (6). On the contrary, under semi-aerobic conditions, in yeasts with XR activity linked only to NADPH, a redox imbalance limits the rate of the oxidative step, leading to xylitol accumulation (7).

Microorganisms growing on wood hydrolyzates show lower productivities than those growing on commercial xylose solutions, owing to the presence of inhibitory byproducts released during the hydrolysis (8).
The experimental results of microaerobic fermentations of synthetic and complex media are used in this work to investigate the influence of both the inhibitors and the operative conditions.

2. MATERIALS AND METHODS

2.1 Microorganisms and growth media

The experiments were carried out with the yeasts *Debaryomyces hansenii* NRRL Y-7426, *Pachysolen tannophilus* NRRL Y-2460 and *Candida guilliermondii* NRC 5578. The cells were maintained on slants of PDA additioned as described by Barbosa (9). The cells were cultivated in a rotatory shaker at 30°C and 250 rpm for 72 h, then collected by sterile centrifugation (5800 rpm for 15 min) and finally used for inoculum.

2.2 Fermentation conditions

The fermentations were carried out in a 3-L Applikon Z61103CT04 fermentor containing 1 L of medium at 30 ± 0.2°C, agitation of 250 rpm, oxygen flow rate of 4.98 mg s^{-1} (corresponding to 0.2-0.5% of the saturation value) and pH 5.5. The corresponding oxygen levels were reported as the optimum microaerobic conditions for xylitol accumulation in yeasts (4,10). The operative conditions were controlled through the electronic device Applikon ADI 1030.
Batch tests were performed on synthetic medium containing: 5 g l^{-1} $(NH_4)_2SO_4$, 0.5 g l^{-1} $MgSO_4 \cdot 7\ H_2O$, 1 g l^{-1} KH_2PO_4, whereas the initial xylose concentration was varied from 50 to 300 g l^{-1}.

2.3 Analytical methods

Xylose and xylitol concentrations were determined at 35°C by HPLC (Hewlett Packard 1100); a 0.1% of H_3PO_4 water solution was used as eluent at a flow rate of 0.5 ml min^{-1}.
Acetic acid, furfural and phenolic substances were determined by gas chromatography.
The cell mass concentration was determined by dry weight after filtration of known-volume aliquots of the fermentation broth through 0.45 μm membrane filters.

3. RESULTS AND DISCUSSION

The experimental analysis evidenced the presence of several microbial inhibitors in wood hydrolyzates, such as: 1) metals and minerals present in the wood and soil or released by the hydrolysis equipment; 2) substances formed by decomposition of carbohydrates (including furfural and hydroxymethylfurfural); 3) phenolic compounds and acetic acid derived from lignin degradation. It was noticed that the microbial activity is strongly affected by the concentration of these substances and depends on both the yeast strain and the operative conditions.
Batch tests performed at variable temperature and pH evidenced as optimum fermentation conditions for all tested strains T = 30°C and pH = 5.5.

For this reason, additional fermentations were carried out, under these conditions, to evaluate the influence of the different hemicellulose hydrolyzate detoxification treatments on xylitol yield.
Among the selected detoxification techniques, overliming (11) was firstly investigated to neutralize furfural and hydroxymethylfurfural and to precipitate heavy metals and inorganic ions; this technique consists of $Ca(OH)_2$ addition until pH = 10, cold filtration, and final acidification to 5.5 with H_2SO_4 addition.
The second step was one hour adsorption on active charcoal in order to remove the phenolic substances; tests performed at different hydrolyzate/carbon ratios demonstrated for all three strains the highest removal yields at 1/10 ratio. The results of fermentations carried out on hydrolyzates detoxified combining these detoxification techniques confirmed those of previous works (7) and showed the best xylitol yields for *P. tannophilus* and *D. hansenii*.
Batch runs, carried out with these yeast strains, showed an outstanding increase in xylitol yield when a preliminary three hours evaporation step was used to eliminate acetic acid, which had a starting concentration in the raw materials of about 25 g l^{-1}. A decrease of acetic acid concentration under 1 g l^{-1} was able to ensure a xylitol yield of 78.5% for the yeast *P. tannophilus* and of 64.2% for the yeast *D. hansenii*.
Finally, the influence of xylose initial concentration (S_0) was investigated in batch runs on a xylose synthetic medium by *D. hansenii*. Optimum xylitol yields (75-83%) and volumetric productivities (0.48-0.69 g_p $l^{-1}h^{-1}$) were found in a range of S_0 between 90 g l^{-1} and 200 g l^{-1} (Fig. 1).

- **Insert figure 1**

At low xylose concentration a relative lack of substrate for excess biomass (high X_0/S_0 ratio) could be responsible for an increase of the respiratory activity to ensure enough energy to the system. On the contrary, at high S_0 values a substrate inhibitory effect was stressed, thus sliding the metabolic activity, once again, to the respiration.
Additional tests on synthetic media by *D. hansenii*, which were performed to optimize the oxygen flow rate, showed the best yield (83%), at an oxygen flow rate of 4.98 mg s^{-1}.
These results were finally used to perform a study on the metabolism of *D. hansenii*, which allowed to estimate the percentages of xylose consumed and of different products formed during the three cell growth phases (Lag, Exponential, and Stationary) by metabolic activities which simultaneously take place in this yeast (xylitol formation, biomass growth and respiration).
As reported in Figs. 2 and 3, this study points out a high xylose consumption for xylitol formation during the exponential phase which in the above S_0 range, evidencing an outstanding activity of the yeast, especially at 90 g l^{-1}, where respiration is negligible. On the contrary, at low S_0 levels, the carbon source is mainly used to ensure energy to the system in the form of ATP through the respiratory activity.

- **Insert figures 2 and 3**

4. CONCLUSIONS

This study has evidenced a high xylitol yield at S_0 = 90 g l^{-1} in xylose fermentation by *D. hansenii*. On the other hand at lower S_0 enough carbon source is not available for xylitol formation; at higher S_0 a substrate inhibiting effect can be stressed. A complete detoxification process is necessary to obtain high yields of conversion from hydrolyzates media.

REFERENCES

(1) Parajó JC, Domínguez H, Domínguez JM (1995) Production of xylitol from raw wood hydrolyzates by *Debaryomyces hansenii* NRRL Y-7426. *Bioproc. Eng.*, **13**: 125-131.

(2) Emodi A (1978) Xylitol. Its properties and food application. *Food Technology*, **1**: 28-32.

(3) Parajó JC, Domínguez H, Domínguez JM (1998) Biotechnological production of xylitol. Part 2: operation in culture media made with commercial sugars. *Biores. Techn.* **65**: 203-212.

(4) Taylor KB, Beck MJ, Huang DH, Sakai TT (1990) The fermentation of xylose: studies by carbon-13 nuclear magnetic resonance spectroscopy. *J. Ind. Microbiol.* **6**:29-41.

(5) Gírio FM, Peito MA, Amaral-Collaço MT (1990) Xylitol production by fungi. An enzymatic test for screening good xylitol producer fungi. In: Grassi G, Gosse G, dos Santos G, editors. *Biomass for Energy and industry*, Vol. **2**. Amsterdam: Elsevier.

(6) Converti A, Perego P, Domínguez JM (1999) Xylitol production from hardwood hemicellulose hydrolysates by *Pachysolen tannophilus, Debaryomyces hansenii* and *Candida guilliermondii*. *Appl. Biochem. Biotechnol.* **82**: 141-151.

(7) Domínguez JM, Gong CS, Tsao GT (1997) Production of xylitol from D-xylose by *Debaryomyces hansenii*. *Appl. Biochem. Biotechnol.* **63/65**: 117-127.

(8) Watson NE, Prior BA, Lategan PM (1984) Factors in acid treated bagasse inhibiting ethanol production from D-xylose by *Pachysolen tannophilus*. *Enzyme Microb. Technol.*, **6**: 451-456.

(9) Barbosa MFS, Medeiros MB, Macilha I, Schneider H, Lee H (1988) Screening of yeast for production of Xylitol from D-xylose and some factors which affect xylitol yield in *Candida guilliermondii*. *J. Ind. Microbiol.,* **3**: 241-251.

(10) Converti A, Domìnguez JM (2001) Influence of temperature and pH on xylitol production from xylose by *Debaryomyces hansenii. Biotechnol. Bioeng.*, accepted for publication.

(11) Perego P, Converti A, Zilli M, Del Borghi M (1994) Acid hemicellulose hydrolyzates: Physical treatments and continuos immobilized-cell fermentations. *Bioproc. Eng.*, **10**: 35-41.

Fig.1 . Xylose conversion and xylitol yield of batch fermentations by *D. hansenii* at different S_0 values.

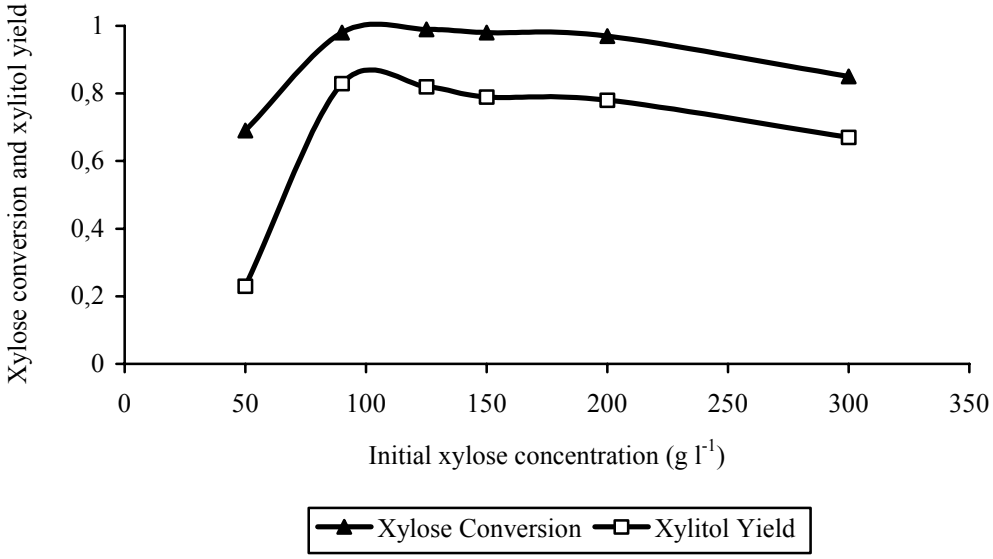

Fig.2. Influence of starting xylose concentration on the percentage of xylose consumed for biomass growth (▲), xylitol production (■) and respiration (○) during batch fermentations by *Debaryomyces hansenii*

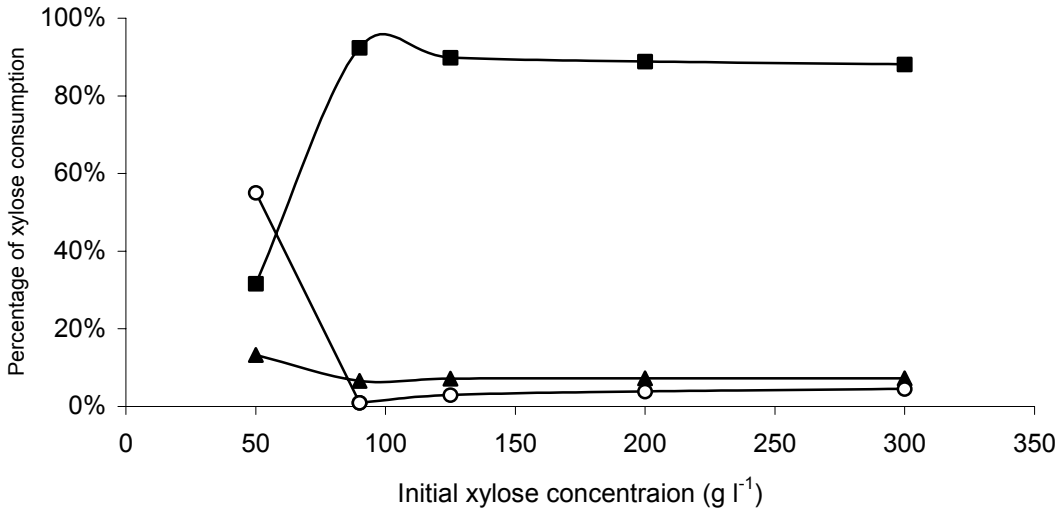

Fig. 3. Xylose consumption value during batch run at S_0 = 90 g l^{-1} in xylitol fermentation by *Debaryomyces hansenii* in each cell growth phase.