Fed-Batch Culture for Xylitol Production by Candida boidinii

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Xylitol production by Candida boidinii NRRL Y-17213 was investigated in fed-batch fermentations with xylose (50, 100 g litre\(^{-1}\)) and a mixture of glucose (25 g litre\(^{-1}\)) and xylose (25 g litre\(^{-1}\)). All fermentations were initially batch processes with high levels of aeration and rapid production of biomass. Faster growth occurred when a mixture of glucose and xylose, instead of xylose, was used as a substrate; glucose was assimilate first and maximal xylitol production was 39-41 g litre\(^{-1}\), compared with 46.5 and 59.3 g litre\(^{-1}\) in the processes with xylose alone. Fed-batch cultures were characterized with higher xylitol yields (0.57-0.68 g g\(^{-1}\)) and production rates (0.32-0.46 g litre\(^{-1}\) h\(^{-1}\)) compared with a batch process. Ethanol was accumulated in all processes, in smaller quantities, varying from 11 to 21% of the xylitol concentration. The fed-batch process with the highest initial xylose concentration (100 g litre\(^{-1}\)) and the lowest level of aeration in the first phase, resulted in the highest yield of xylitol (75% of theoretical) and the highest productivity (approximately 35% higher than the productivities of the other two fed-batch experiments).

NOMENCLATURE

\(C_{cm}\) Dry cell mass (g litre\(^{-1}\))
\(C_e\) Ethanol concentration (g litre\(^{-1}\))
\(C_x\) Xylitol concentration (g litre\(^{-1}\))
\(Q_x\) Volumetric xylitol production rate (g litre\(^{-1}\) h\(^{-1}\))
\(R_{xe}\) Ratio xylitol/ethanol (g g\(^{-1}\))
\(Y_{x/s}\) Xylitol yield coefficient, g xylitol per g xylose consumed, (g g\(^{-1}\))
\(Y_{x/h}\) Percentage of the xylitol yield from the theoretical value (%)
\(\mu\) Specific growth rate (h\(^{-1}\))

INTRODUCTION

Xylitol, a five-carbon sugar alcohol and a ‘low calory’ non-cariogenic sweetener, is a constituent of many fruits and vegetables (raspberries, strawberries, yellow plum, lettuce, cauliflower). Although the concentration is usually less than 1% it has always been a minor part of the human diet. The human body produces 5-15 g of xylitol a day during normal metabolism. However, the low xylitol concentration in such sources as well as their high cost make the extraction process very uneconomical. Currently, it is obtained by hydrogenation of xylose which is produced from xylan-containing plant materials. As an alternative method, microbial production of xylitol is becoming more interesting and attractive.
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. pelliculosus, C. para-psilosis and C tropicalis.

Continuous and fed-batch culture techniques often provide better yields and productivities in the production of microbial metabolizes than batch cultures. Continuous fermentations often offer higher productivities of metabolizes only at low dilution rates (long residence times). For practical reasons, therefore, some continuous operations have been replaced by fed-batch processes.

In a previous study of xylitol production from xylose by Candida boidinii, we reported that high initial cell densities improved xylitol yields and specific production rates. An increase in initial xylose concentration induced xylitol formation in C. boidinii but at the same time acted as a growth inhibitory substrate leading to a long fermentation time. An attempt to overcome these problems has been made by applying a fed-batch culture in order to maintain the growth rate and substrate concentration at suitable levels throughout the whole period of cultivation.

MATERIALS AND METHODS

Microorganism
Candida boidinii NRRL Y-17213 was maintained at 4°C on agar slants (YPG), containing (g litre\(^{-1}\)): yeast extract, 10 g; Bactopeptone, 20 g; glucose, 20 g; and agar 20 g.

Culture medium and inoculum preparation
The fermentation medium contained (g litre\(^{-1}\)): yeast nitrogen base without amino acids and ammonium sulphate (Difco), 1.7 g; urea, 5 g; casamino acids (Difco), 5 g; and the sugars, xylose and glucose as required (Table 1). The medium was filter sterilized without the sugars. The sugar solution was sterilized separately by autoclaving and added aseptically to the medium.

The inoculum was prepared by transferring a loopful of cells from 3-day-old YPG slant into 50 ml of medium in a 125 ml Erlenmeyer flask plugged with foam and cultivated with shaking at 150 rpm for 48 h at 30°C. After a subsequent preculturing in flasks with larger volumes (500 and 2000 ml) the cultures were centrifuged, washed twice with distilled water and used as an inoculum.

Fermentation conditions for fed-batch culture
The fermenter used was a 2 litre bench top fermenter (New Brunswick Scientific Co.) with a working volume of 1 litre.

Fed-batch fermentations were operated as batch processes for up to 72 h at 30°C, pH 5.5, using xylose or a mixture of xylose and glucose as substrate. The fermentation conditions for both phases, batch and fed-batch, are given in Table 1. Different oxygenation levels were obtained by controlling the air supply by a flowmeter. Substrate solution was fed to the fermenter continuously, with different feeding rates, using a peristaltic pump (LKB Bromma, 2120 Varioperpex). Samples were withdrawn once a day while maintaining the working volume at 1 litre.

Analytical methods
Samples were taken daily and centrifuged. After washing the residues twice with distilled water, they were dried for cell mass determination at 102°C. Xylose, glucose and xylitol were determined by high-performance liquid chromatography.

<p>| Table 1. Fermentation conditions for the fed-batch culture of Candida boidinii for xylitol production |
|---------------------------------------------------|---------------------|---------------------|---------------------|---------------------|</p>
<table>
<thead>
<tr>
<th>Experiment</th>
<th>Initial substrate concentration (g litre(^{-1}))</th>
<th>Aeration (v v(^{-1}) min(^{-1}))</th>
<th>Agitation (rev min(^{-1}))</th>
<th>Feeding rate (g litre(^{-1}) h(^{-1}))</th>
<th>Aeration (v v(^{-1}) min(^{-1}))</th>
<th>Agitation (rev min(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Xylose, 50</td>
<td>2</td>
<td>400</td>
<td>Xylose, 25</td>
<td>0.75</td>
<td>150</td>
</tr>
<tr>
<td>2</td>
<td>Xylose, 25</td>
<td>2</td>
<td>400</td>
<td>Glucose, 25</td>
<td>0.75</td>
<td>150</td>
</tr>
<tr>
<td>3</td>
<td>Xylose, 100</td>
<td>0.3</td>
<td>370</td>
<td>Xylose, 8</td>
<td>0.25</td>
<td>250</td>
</tr>
</tbody>
</table>

*Batchwise mode.
*Fed-batch mode.
graphy while ethanol was analysed by gas chromatography as described elsewhere. 

RESULTS AND DISCUSSION

For effective xylitol production, the first critical step is the rapid production of cell mass in the culture medium. This may be achieved by maintaining a relatively high level of aeration in the culture. However, maintaining a high level of aeration in the medium throughout the fermentation would lead to the production of D-xylulose instead of xylitol. Therefore, the fed-batch fermentations were operated initially as batch processes with high levels of aeration, and thereafter the oxygen supply was lowered to the level suitable for xylitol production.

Comparative profiles of two fed-batch fermentations are shown in Figs 1 and 2. In the first phase, batchwise mode, when oxygen supply was relatively high, the biomass concentration increased 4.8 and 7.6-fold in the first and second experiment, respectively. Faster growth occurred when a mixture of glucose and xylose was used as a substrate (Experiment 2). Glucose and xylose were consumed sequentially with complete depletion of the glucose during the first day of cultivation (batch mode). Xylose was also consumed but in smaller amounts, 4.48 g compared to 25 g of glucose (Fig. 2). The remainder of the available xylose, 21 g, was completely consumed by the end of the batchwise mode, accumulating only a small amount of xylitol, 1.72 g litre$^{-1}$.

In the experiment with only xylose as a substrate, growth was slower but the total cell mass produced at the end of a batchwise mode was 17.84 g litre$^{-1}$. This was similar to that when a mixture of glucose and xylose was used, 18.30 g litre$^{-1}$. In this case, xylitol accumulated from the beginning of cultivation, supporting the findings that its formation is induced by xylose but not by glucose.

In the second phase, fed-batch mode, when oxygen supply was lowered, the yeast responded by ceasing growth and increasing xylitol formation up to 46.5 g litre$^{-1}$ (Experiment 1) and 39.41 g litre$^{-1}$ (Experiment 2). Ethanol accumulation in both experiments followed the same pattern as xylitol production but with much lower quantities (Fig. 1).

Comparing the substrate consumption in both experiments, it can be observed that the daily substrate consumption was usually higher in Experiment 2. All the glucose as well as a portion of the xylose was used. In both cases, in the fed-batch phase, the highest amounts of substrate were consumed during day 4. Of the xylose quantities fed to the fermenter, 67.54% were consumed by the yeast in the first experiment, and 81.25% in the second one.

The first two fed-batch experiments were operated initially with a substrate concentration of 50 g litre$^{-1}$ and a high level of aeration (Table 1), aiming at a rapid accumulation of biomass capable of generating substantial amounts of xylitol.

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Fig. 1. Time course of fed-batch cultures of C. boidinii NRRL Y-17213. (□, ■) Xylitol, (▲, ▼) ethanol and (○, ●) cell mass. Open symbols are for Experiment 1 and solid symbols are for Experiment 2 (Table 1). Negative values of time indicate days prior to the beginning of the fed-batch mode.

Fig. 2. Substrate consumption in fed-batch cultures of C. boidinii NRRL Y-17213. (□) Xylose consumption in Experiment 1; (■) glucose and (■) xylose consumption in Experiment 2. (Table 1).
However, the amounts of xylitol produced in both cultures were not as expected, especially when glucose was used as a co-substrate.

In an attempt to establish conditions under which xylitol was produced earlier, another fed-batch experiment was designed. It was based on higher initial cell and xylose concentrations and consequently a lower feeding rate and lower level of aeration in the batchwise mode in order to achieve high biomass concentration and early induction of the necessary enzymes for xylitol production (Experiment 3, Table 1). Indeed, the cells began to accumulate xylitol immediately and at the end of a batch phase its concentration was 29.93 g litre$^{-1}$, which was 2.7 and 11.6 times more than in the first and second experiments respectively. Xylitol production continued in the fed-batch phase reaching a maximum value of 59.3 g litre$^{-1}$ (Fig. 3). Concurrently with xylitol production, ethanol was accumulated. Its maximum concentration of 6.7 g litre$^{-1}$ was between the values obtained in the first and second experiments respectively. Xylose concentration, in the first phase dropped from 97.7 to 43.5 g litre$^{-1}$ (consumption of 55.54%), whereas the biomass content increased from 8.7 to 18.3 g litre$^{-1}$. At the end of the experiment, xylose content increased up to 110 g litre$^{-1}$. Obviously, the feeding rate exceeded xylose consumption, although the feeding policy was based on previously obtained data on xylose consumption rate.

Figure 4 shows specific growth rates and xylitol productivities in all three experiments. The maximum specific growth rate in the third experiment was considerably lower ($\mu = 0.013 \ h^{-1}$) compared to those in the first ($\mu = 0.023 \ h^{-1}$) and second ($\mu = 0.067 \ h^{-1}$) experiments. However, the value of the maximum productivity, 0.46 g litre$^{-1}$ h$^{-1}$, was approximately 35% higher compared to the productivities of the other two fed-batch experiments.

Apart from productivity, the second significant parameter for evaluating the process efficiency is yield. The xylitol yield from xylose consumed is given in Fig. 5. In all three cases the highest yield...
Xylitol production by Candida boidinii was achieved on the third day of the fed-batch fermentation. After which the yields declined gradually with time. Debaryomyces hansenii achieved a maximum xylitol yield of 0.53 g g⁻¹, and Candida guilliermondii, 0.74 g g⁻¹. Nolleau et al., working with Candida parapsilosis reported the highest xylitol yield of 0.74 g g⁻¹ with a corresponding production rate of 0.14 g litre⁻¹ h⁻¹ and the highest production rate of 1.23 g litre⁻¹ h⁻¹ with a corresponding yield of 0.5 g g⁻¹. Unfortunately, a real comparison of these parameters is not possible since all these data are for batch fermentations. To our knowledge, no published data are available on xylitol production in a fed-batch mode.

Table 2 summarizes the maximum fermentation parameters of the three fed-batch cultures and one batch process. The batch process was carried out under the most suitable conditions for xylitol production regarding the initial xylose concentration and the level of aeration, which was the same as applied in the second phase of the fed-batch cultures (unpublished data).

All the fed-batch processes were characterized with higher yields and productivities, especially Experiment 3 when the xylitol yield was almost 75% of the theoretical yield. The xylitol/ethanol ratio was also higher. Although Experiment 2 had a higher yield and productivity than the batch one, the absolute xylitol concentration was rather low.

Vongsuvanlert and Tani who worked with C. boidinii, but in a batch mode, reported that the yeast produced 0.44 g xylitol/g xylose with a productivity of 0.25 g litre⁻¹ h⁻¹ when 100 g litre⁻¹ xylose was initially present in a medium. The inference from these summarized data is that the best results were obtained under the conditions in Experiment 3. However, changing the substrate feeding rate might improve yield and productivity further.

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REFERENCES