Increase of Xylitol Production Rate by Controlling Redox Potential in Candida parapsilosis

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Abstract: The effect of redox potential on xylitol production by Candida parapsilosis was investigated. The redox potential was found to be useful for monitoring the dissolved oxygen (DO) level in culture media, especially when the DO level was low. An increase in the agitation speed in a 5 L fermentor resulted in an increased culture redox potential as well as enhanced cell growth. Production of xylitol was maximized at a redox potential of 100 mV. As the initial cell concentration increased from 8 g/L to 30 g/L, the volumetric productivity of xylitol increased from 1.38 g/L h to 4.62 g/L h. A two-stage xylitol production strategy was devised, with stage 1 involving rapid production of cells under well-aerated conditions, and stage 2 involving cultivation with reduced aeration such that the culture redox potential was 100 mV. Using this technique, a final xylitol concentration of 180 g/L was obtained from a culture medium totally containing 254.5 g/L xylose in a 3,000 L pilot scale fermentor after 77 h fermentation. The volumetric productivity of xylitol during the fermentation was 2.34 g/L h. © 1998 John Wiley & Sons, Inc. Biotechnol Bioeng 58: 440–444, 1998.

Keywords: xylitol production; redox potential; dissolved oxygen; Candida parapsilosis

INTRODUCTION

Xylitol, a five-carbon sugar alcohol, is anticariogenic, does not cause acid formation, has a sweetness equal to sucrose, and can replace sucrose on a weight-weight basis (Mäkinen, 1979). Xylitol has substantially low viscosity and negative heat effect when dissolved in a solution. With these properties, xylitol has found increasing use in the food industry as a sweetener (Pepper and Olinger, 1988).

Xylitol is currently manufactured by the chemical reduction of xylose present in hemicellulose hydrolysates. As the hemicellulosic fraction contains polymers of other sugars, the chemical process includes expensive separation and purification steps to remove these byproducts from xylose or xylitol (Hyvönen and Koivistoinen, 1983). It can also be produced by microbiological methods with xylose utilizing yeasts (Du Preez, 1994; Du Preez et al., 1989a; Gong et al., 1981; Gong et al., 1983; Horitsu et al., 1992; Lee et al., 1988; Meyrial et al., 1991; Nishio et al., 1989; Smiley and Bolen, 1982; Vongsuvanlert and Tani, 1989; Yahashi et al., 1996).

In xylitol-producing yeasts, oxygen supply affects the rate and yield of xylitol production, and determines the partitioning of the carbon flux from xylose between cell growth and xylitol formation (Hahn-Hägerdal et al., 1994). Excessive oxygen conditions lead to NADH being oxidized to NAD⁺, and a high NAD⁺/NADH ratio leads to oxidation of xylitol to xylulose, which is further metabolized to cell material, and as a result, less xylitol and more cells are accumulated. Under a limited oxygen supply, the electron transfer system is unable to reoxidize all of the produced NADH by respiration and/or fermentation; the intracellular NADH level increases and the reaction of xylitol to xylulose decreases, and consequently xylitol accumulates (Du Preez et al., 1989a; Furlan et al., 1994; Furlan et al., 1991). For effective xylitol production, oxygen must thus be carefully controlled at a low dissolved oxygen (DO) level. Dissolved oxygen can be monitored by both a redox (reduction-oxidation) potential electrode and a DO electrode. The redox potential values are known to be more useful for monitoring the DO level in a culture media, especially when the DO level is low (Chung and Lee, 1986; Du Preez et al., 1989b; Kjaergaard, 1977). Therefore, the redox potential can be used as a control parameter of oxygen supply by monitoring a low DO level during xylitol production.

Here, the effects of redox potential on the xylitol production by Candida parapsilosis KFCC 10875 were investigated and the optimum range of redox potential was also determined. For effective production of xylitol, we tried to get as high a content of cell mass as possible during the aerobic phase. Thereafter, the redox potential was reduced to the optimum range for xylitol production. By using this technique, the high rate production of xylitol from a high concentration of xylose was carried out in a pilot-scale fermentor.
MATERIALS AND METHODS

Microorganism and Media

The strain used throughout this study was *Candida parapsilosis KFCC 10875*, a mutant of ATCC 22019. Cultures were maintained frozen at −70°C. The growth medium was YM medium containing 20 g/L glucose, 5 g/L peptone, 3 g/L yeast extract, and 3 g/L malt extract. The fermentation medium consisted of industrial-grade reagents including xylose (400 g/L solution, Sampoong Chemical Co., Korea), 5 g/L yeast extract, 5 g/L KH$_2$PO$_4$, 2 g/L (NH$_4$)$_2$SO$_4$, and 0.4 g/L MgSO$_4$·7H$_2$O.

Culture Conditions for Fermentor Experiments

A frozen cell suspension was inoculated into a 250 mL flask containing 50 mL growth medium, and was cultivated at 30°C and 240 rpm for 14 h, then the culture broth of 150 mL was transferred to a 5 L fermentor (Korea Fermentor Co.) containing 3 L of fermentation medium. Temperature and pH of the fermentor were controlled at 30°C and 5.0, respectively. Aeration rate was fixed to 0.5 vvm and agitation speed was adjusted to change redox potential. Culturing was performed until xylose was completely consumed.

Culture Conditions for Pilot Plant Experiments

Pilot plant experiments were carried out in a pilot-scale fermentor system (Korea Fermentor Co.). A frozen cell suspension was inoculated into a 500 mL flask containing 75 mL growth medium and cultivated at 30°C and 240 rpm for 15 h, and then the culture broth was transferred to a 2.5 L fermentor containing 1.5 L growth medium and was cultivated for 9 h. This seed culture was inoculated into a 50 L fermentor containing 30 L fermentation medium and was cultivated for 12 h, then the culture broth of 30 L was transferred to a 400 L fermentor containing 200 L fermentation medium and was cultivated for 8 h. The seed culture of 200 L was inoculated to a 3000 L main fermentor containing 1400 L fermentation medium. Temperature and pH of fermentors were the same as culture conditions for fermentor experiments. The DO of seed fermentors was maintained above 20%. Aeration rate was fixed to 0.5 vvm. Agitation speed in a 3000 L pilot fermentor was gradually increased from 40 to 130 rpm to maintain DO level above 20% during growth phase and was shifted in the range of 65 to 75 rpm during production phase, which corresponded to the redox potential of 100 mV.

Analytical Methods

The oxygen transfer coefficient ($k_{L\alpha}$) was calculated from the rate of DO concentration recovery from air after nitrogen degassing of the liquid phase (dynamic gassing-out method). The DO concentration in liquid phase was continuously monitored with an Ingold polarographic electrode. The redox potential was measured using an Ingold platinum redox electrode and Horizon pH/MV controller.

The cell mass was estimated by using a calibration curve made from a relationship between optical density at 600 nm and dry cell weight. Xylitol and xylose were determined by high performance liquid chromatography (Shimadzu LC-6A, Japan) using a Shodex Sugar-Pak I column (Waters, USA) with a refractive index detector (Shimadzu RID-6A). The column was eluted with distilled water at a column temperature of 90°C and a flow rate of 0.5 mL/min.

RESULTS AND DISCUSSION

Effect of Redox Potential on Xylitol Production

Profiles of xylitol fermentation by *Candida parapsilosis KFCC 10875* accompanying DO and redox potential are shown in Figure 1. Fermentation in a 5 L fermentor was performed under fixed conditions such as 0.5 vvm aeration rate, 120 rpm agitation speed. High redox potential favored cell production over xylitol production, while low redox potential stimulated xylitol production. Dissolved oxygen values decreased to near zero during xylitol production, and became very difficult to measure. By contrast, redox potential could be measured even after DO fell to zero, and was found to be more useful than DO for monitoring at the low DO levels.

In order to evaluate the effect of redox potential on the cell growth and xylitol production, several fermentation runs were made under the controlled redox potential condition during the production phase (Fig. 2). After the cell...
concentration in each fermentation run reached a level of 3 g/L, the redox potential was controlled by adjusting the agitation speed in the range of −60 mV to 250 mV. To use the redox potential as a substitute for DO without any interference, several factors such as pH, temperature, aeration, and medium composition were kept constant during the xylitol production. The xylitol production was maximum at redox potential of 100 mV, above which there were marked increases in cell growth rather than xylitol production. At a high redox potential, oxygen critically changed the participation of xylose carbon flux from the xylitol production to cell growth.

The effect of redox potential on the specific and volumetric productivities of xylitol, calculated from Figure 2 was also studied (Fig. 3). The specific and volumetric productivities varied substantially with the redox potential and were maximum at the redox potential of 100 mV. This suggested that the redox potential must be controlled to be 100 mV for effective xylitol production.

**Effect of Xylose Concentration and Cell Concentration on Xylitol Production**

Cells grown on 30 g/L xylose medium in a 10 L fermentor were centrifuged and concentrated. The concentrated cells of 20 g/L were inoculated to a 2.5 L fermentor containing 1.5 L medium. The initial xylose concentration in the fermentor was varied from 50 to 270 g/L in order to evaluate the effect of xylose concentration on the specific productivity of xylitol, volumetric productivity of xylitol, and the xylitol yield from xylose (Table 1). The constant redox potential of 100 mV was obtained by adjusting the agitation speed. Higher initial xylose concentration reduced the increased time for xylose consumption and xylitol production. The specific productivity of xylitol, volumetric productivity of xylitol, and the xylitol yield from xylose were maximum at an initial xylose concentration of 170 g/L, above which the xylitol production decreased.

Profiles of the xylitol production from xylose with various cell concentrations are shown in Figure 4. The concentrated cells were obtained with the same method as the previous experiments. As the cell concentration was increased, the \( k_{la} \) increased due to the increase of oxygen uptake by cells. It was also found that the volumetric productivity of xylitol increased with increasing initial cell concentration. Fermentation time was shortened from 23 h to 7 h by increasing the initial cell concentration from 8 g/L to 30 g/L. The higher the cell concentration used, the higher the rate of conversion from xylose to xylitol obtained. However, the yield of xylitol from xylose was almost constant regardless of the initial cell concentration. The effect of cell concentration on the specific and volumetric productivities of xylitol (calculated from the data of Fig. 4) is shown in Figure 5. The volumetric productivity of xylitol increased as the cell concentration was increased. Specific productivity of xylitol, however, was almost constant regardless of the cell concentration. In particular, the volumetric productivity

<table>
<thead>
<tr>
<th>Initial xylose (g/L)</th>
<th>Final xylitol (g/L)</th>
<th>( Q_P^a ) (g/L·h)</th>
<th>( q_P^b ) (g/g·h)</th>
<th>( Y_{PS}^c ) (g/g)</th>
<th>Time ( d ) (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>34</td>
<td>3.09</td>
<td>0.15</td>
<td>0.68</td>
<td>11</td>
</tr>
<tr>
<td>90</td>
<td>63</td>
<td>3.15</td>
<td>0.16</td>
<td>0.70</td>
<td>20</td>
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<tr>
<td>140</td>
<td>103</td>
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<td>0.17</td>
<td>0.74</td>
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<tr>
<td>170</td>
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<td>0.18</td>
<td>0.74</td>
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<tr>
<td>220</td>
<td>159</td>
<td>3.06</td>
<td>0.15</td>
<td>0.72</td>
<td>52</td>
</tr>
<tr>
<td>270</td>
<td>189</td>
<td>2.22</td>
<td>0.11</td>
<td>0.70</td>
<td>85</td>
</tr>
</tbody>
</table>

\( ^a \) Volumetric productivity of xylitol.

\( ^b \) Specific productivity of xylitol.

\( ^c \) Xylitol yield from xylose.

\( ^d \) Time that xylose was completely consumed.
of xylitol at the cell concentration of 30 g/L increased up to 4.62 g/L·h.

High cell concentration can be obtained either by centrifuging cells grown on xylose or by inducing rapid growth during the aerobic phase. Centrifugation has several disadvantages with respect to cells, xylose, and time losses. Therefore, rapid growth during the aerobic phase was studied further in a pilot fermentor to enhance volumetric productivity.

Xylitol Production from High Xylose Concentration in a Pilot Fermentor

The rapid growth technique for an effective xylitol production was introduced by Horitsu et al. (1992) as follows.

First, a high concentration of cell mass was rapidly obtained by cultivation with a high DO. Thereafter, the DO level in the same culture was reduced to the redox potential of 100 mV and the fermentation process continued. In this research, xylitol production at a high rate from high concentration of xylose was performed by using this technique.

A fed-batch culture was grown with the initial xylose concentration of about 170 g/L because the xylitol production rate decreased above this value (Fig. 6). The volume of fermentor was increased from 1400 L containing 240 kg xylose to 2200 L containing 560 kg xylose by feeding xylose twice (160 kg at 31 h and 47 h, respectively). During the aerobic phase, the DO concentration was maintained at a high level. After the cell concentration reached 20 g/L, the redox potential was controlled at 100 mV by adjusting the agitation speed. The total amount of added xylose, corresponding to 254.5 g/L, was completely consumed at 77 h. A final xylitol concentration of 180 g/L was obtained with a 71% xylitol yield from xylose. The volumetric productivity of xylitol during the fermentation was 2.34 g/L·h. The average specific productivity of xylitol was almost the same as that obtained from the fermentation that used 50 g/L xylose at the same controlled redox potential. This result confirmed that the specific productivity of xylitol was constant regardless of the cell concentration, and that the volumetric productivity of xylitol could be increased by raising the cell concentration.

Comparison to Other Studies

Candida guilliermondii grown on 300 g/L xylose produced 221 g/L xylitol over a period of 406 h in a flask culture (Meyrial et al., 1991). This is the highest reported xylitol productivity obtained in the absence of cell concentration. The volumetric productivity of xylitol with C. parapsilosis in this study was 4.3 times higher than that previously at-
tained with *C. guilliermondii*, 0.54 g/L·h. It has recently been reported that a final xylitol concentration of 94 g/L from 150 g/L xylose by *C. tropicalis* can be obtained in a 3 L fermentor in 32 h. The volumetric productivity of xylitol with *C. tropicalis* was 2.94 g/L·h, which was the highest volumetric productivity of xylitol (Yahashi et al., 1996). However, the cells used for the xylitol production were concentrated by centrifugation after growth on a xylose medium.

As compared with the previous reports, the xylitol production by *C. parapsilosis* in this study was improved in several aspects. First, the concentrated cells were obtained not by centrifuging but by inducing the rapid growth during the aerobic phase. Second, the high rate of xylitol production was obtained by using the redox potential as a control parameter. Third, this was the first attempt to apply this technique on the pilot plant basis. This results will contribute greatly to the xylitol production by microbiological processes in a scaled-up production.

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**References**


