0273-2289/1559-0291 (Online)/06/133/0263/$30.00

Effect of Aeration Rate on Production of Xylitol From Corncob Hemicellulose Hydrolysate

Xinghong Ding and Liming Xia*

Department of Chemical Engineering and Bioengineering, Zhejiang University, Hangzhou 310027, P.R.China, E-mail: xialm@zju.edu.cn

Received August 26, 2005; Revised November 18, 2005; Accepted November 21, 2005

Abstract

The effects of different aeration conditions on xylitol production from corncob hemicellulose hydrolysate by *Candida* sp. ZU04 were investigated. Batch fermentations were carried out in a 3.7-L fermentor at 30°C, pH 5.5, and agitation of 300 rpm. It was found that the two-phase aeration process was more effective than the one-phase aeration process in xylitol production. In the first 24 h of the aerobic phase, a high aeration rate was applied, glucose was soon consumed, and biomass increased quickly. In the second fermentation phase, aeration rate was reduced and an improved xylitol yield was obtained. The maximum xylitol yield (0.76 g/g) was obtained with an aeration rate of 1.5 vvm (*KLa* of 37 h^{-1}) for the first 24 h and 0.3 vvm (*KLa* of 6 h^{-1}) from 24 to 96 h.

Index Entries: Corncob; fermentation; hemicellulose hydrolysate; xylitol; aeration rate.

Introduction

Xylitol is a valuable pentitol currently produced at industrial scale by catalytic hydrogenation from xylose. Biotechnological production of xylitol from agro-industrial residues could be of economic interest and attractive because it occurs at lower temperatures and does not require pure xylose *(1–3)*. In this field, some *Candida* sp. and *Debaryomyces hansenii* have been employed in reported studies *(4,5)*. In particular, *Candida* sp. has been reported to ensure high xylitol yields and good productivities under proper cultivation conditions *(6)*.

*Author to whom all correspondence and reprint requests should be addressed.

Aeration rate plays an important role in the bioconversion of xylitol from xylose by yeasts, drastically affecting the xylitol yield as well as the xylitol production rate *(7)*. A high degree of aeration rate promotes cell growth, while being detrimental to xylitol accumulation. Xylitol is only produced with reduced oxygen availability. However, a severely restricted oxygen supply leads to a low production rate, even though the yields can be high *(8,9)*. A possible way to overcome this problem is to develop a two-phase bioprocess consisting of a first phase carried out under excess oxygen conditions, with the aim of obtaining rapid hexose consumption and biomass production, followed by an oxygen-limited phase *(10,11)*.

In the present work, the fermentation of corncob hemicellulose hydrolysate by *Candida* sp. ZU04 in a 3.7-L stirred-tank reactor was used to compare the effects of one- and two-phase aeration processes on xylitol production. The purpose of this research was to determine suitable aeration conditions to achieve an optimum xylitol yield in the fermentation.

Materials and Methods

Corncob

Corncob was from Shandong province, China. The composition was 38.5% cellulose, 35.0% hemicellulose, 17.5% lignin, and 9.00% other.

Preparation of Corncob Hemicellulose Hydrolysate

Acid hydrolysis of corncobs was carried out at 110°C with 1.0% H_2SO_4 for 3 h using a liquid/solid ratio of 8 g/g. The hydrolysate was then applied to a column packed with weak anion-exchange resins D301 (3 meq/mL, 16–50 mesh) for detoxification.

In selected experiments, the xylose concentration of hydrolysate was increased to the desired level by vacuum evaporation below 70°C, and the pH value was adjusted to 5.5 with 10% HCl.

Microorganism

Candida sp. ZU04 was maintained at 4°C on malt-extract agar slants.

Medium and Fermentation Methods

One loop of *Candida* sp. ZU04 cells grown on a maintenance slant was inoculated into 200 mL of medium containing 10 g/L of xylose, 10 g/L of glucose, 1.5 g/L of yeast extract, 2.5 g/L of peptone, and 3.0 g/L of malt extract. The culture was grown at 30°C, 180 rpm for 22 h in a 500-mL Erlenmeyer flask. Then it was used as inoculum.

The hydrolysate was supplemented per liter with 3 g of yeast extract, 5 g of peptone, and 100 mL of inoculum. The fermentations were performed in a 3.7-L fermentor (Benchtop Fermenter KLF2000; Bioengineering AG, Wald, Switzerland) with agitation, aeration, temperature, pH, and dissolved oxygen control. Experiments were carried out at 30°C with 2.0 L of

culture medium (80 g/L of xylose, pH 5.5, and stirring rate of 300 rpm). Filter-sterilized air was supplied to the bioreactors during the fermentations. In the one-phase aeration process, aeration rate was constant. In the two-phase aeration process, the aeration rate was maintained by 1.5 vvm (volume of air per volume of medium per min) during the first phase. Then, during the second phase, lower aeration rates of 0.1 (A), 0.3 (B), 0.5 (C), and 0.7 vvm (D) were applied, respectively. In the two-phase aeration process, "A" refers to 1.5 vvm during the first phase, and 0.1 vvm in the second phase; "B" refers to 1.5 vvm during the first phase, and 0.3 in the second phase; "C" refers to 1.5 vvm during the first phase, and 0.5 in the second phase; "D" refers to 1.5 vvm during the first phase, and 0.7 vvm in the second phase.

Analytical Methods

Monosaccharides (xylose, glucose, and arabinose), xylitol, acetic acid, hydroxymethylfurfural (HMF), and furfural in the corncob hemicellulose hydrolysate and fermentation broth were determined by high-performance liquid chromatography (HPLC). The HPLC system consisted of a refractive index detector (Waters 2410), a UV detector (Waters 486), an Aminex HPX-87H column, and an H^+-guard column (Bio-Rad, Hercules, CA). The mobile phase was 5 m*M* H_2SO_4, and the flow rate was 0.4 mL/min.

Biomass concentration was measured by filtration (0.45-mm membrane filters), washing, and oven-drying to constant weight at 90°C.

The oxygen volumetric coefficient (*KLa*) was determined by the gassing-out method as described by Pirt *(12)*.

Results and Discussion

It is known that corncob hemicellulose hydrolysate fermentation is complex and critical, because the hydrolysate contains several chemical compounds that are toxic to the yeasts. Table 1 shows that the toxic compounds present in the raw hydrolysate were significantly reduced after detoxification with weak anion-exchange resins D301. The content of acetic acid, furfural, and HMF was reduced by 75.7, 53.3, and 50.0%, respectively. On the other hand, the loss of monosaccharides (xylose, glucose, and arabinose) was less than 3.22%.

Table 2 presents the results of xylitol production by *Candida* sp. ZU04 in the stirred-tank reactor with one-phase aeration rate. The increased aeration rate from 0.1 to 0.7 vvm resulted in decreases in xylitol yield; on the other hand, cell growth was enhanced and the fermentation time was shortened. In this case, an increase in the aeration rate led to higher cell growth but it was detrimental to xylitol production.

At an aeration rate of 0.1 vvm, a xylitol yield of 0.62 g/g was obtained. However, the residual xylose concentration was still high, and the xylitol concentration was lower than the results for 0.3, 0.5, and 0.7 vvm.

The low xylitol yield observed at an aeration rate of 0.7 vvm could probably be explained by the high levels of NAD^+ coenzyme derived from

Table 1
Concentration of Some Components in Corncob Hemicellulose Hydrolysate

Components	Original hydrolysate (g/L)	Hydrolysate treated with weak anion-exchange resins D301 (g/L)
Xylose	35.0	34.0
Glucose	3.10	3.00
Arabinose	4.46	4.32
Acetic acid	3.80	0.92
Furfural	0.30	0.14
HMF	0.10	0.05

the oxidative metabolism of xylose, and the high levels of NAD^+ could restrain the activity level of xylose reductase and result in low xylitol yield *(13)*.

KLa is an important parameter because it describes the aeration capacity of the fermentation system and supplies information for the scale-up of the process. Under our experimental conditions, the *KLa* varied from 2 to 18 h^{-1} (Table 2). However, many published data about *KLa* are contradictory. Some studies reported that optimum *KLa* values for xylitol production were close to 400 h^{-1} *(14)*, whereas others described more severe oxygen conditions corresponding to *KLa* values of 0.78 h^{-1} *(15)*.

To improve xylitol production, two-phase aeration rates were applied in the stirred-tank reactor. Figure 1 presents the time courses of xylitol production b*y Candida* sp. ZU04 with two-phase aeration.

In the first 24 h of the aerobic phase, there was vigorous cell growth and glucose concentration was drastically reduced in the fermented medium (Fig. 1). This can be explained by the fact that, during xylose bioconversion to xylitol, the glucose was used as a co-substrate preferentially to generate energy for cell growth and to regenerate NADPH, a fundamental cofactor in this bioconversion process. Kastner et al. *(16)* reported similar results in a study on the process of xylitol production using a mixture of xylose and glucose. Furthermore, it was found that the residual glucose could restrain the activity of xylose reductase and reduce the xylitol yield *(17)*.

No glucose was detected in the fermented medium after 24 h of fermentation. At that point, the aeration rates were reduced to a fixed value of 0.1 (Fig. 1A), 0.3 (Fig. 1B), 0.5 (Fig. 1C), and 0.7 vvm (Fig. 1D), respectively, and the oxygen dissolved in the medium was reduced from 30 to 0%, which marked the beginning of the oxygen-limited phase in the fermentation process.

In the second phase of 0.1 vvm (*KLa* of 1 h^{-1}), lower biomass formation occurred and xylitol yield and volumetric productivity values were lower than those of 0.3 vvm (*KLa* of 6 h^{-1}) and 0.5 vvm (*KLa* 12 h^{-1}). However, in the second phase of 0.7 vvm (*KLa* of 20 h^{-1}), greater xylose consumption

Table 2
Results of Xylitol Fermentation on Corncob Hemicellulose Hydrolysate by *Candida* sp. ZU04 Under Different Aeration Rates

Aerobic rate (vvm)	K_La (h^{-1})	Fermentation time, t (h)	Residual xylose concentration, S (g/L)	Xylitol concentration, P (g/L)	Cell mass (dry wt), X (g/L)	Volumetric productivity, Qp (g/[L·h])	Xylitol yield, Yp/s (g/g)
0.1	2	132	48.0	20.0	5.30	0.15	0.62
0.3	7	108	22.5	34.5	7.80	0.32	0.60
0.5	10	72	3.00	40.0	13.8	0.55	0.52
0.7	18	66	1.50	39.2	21.5	0.59	0.50

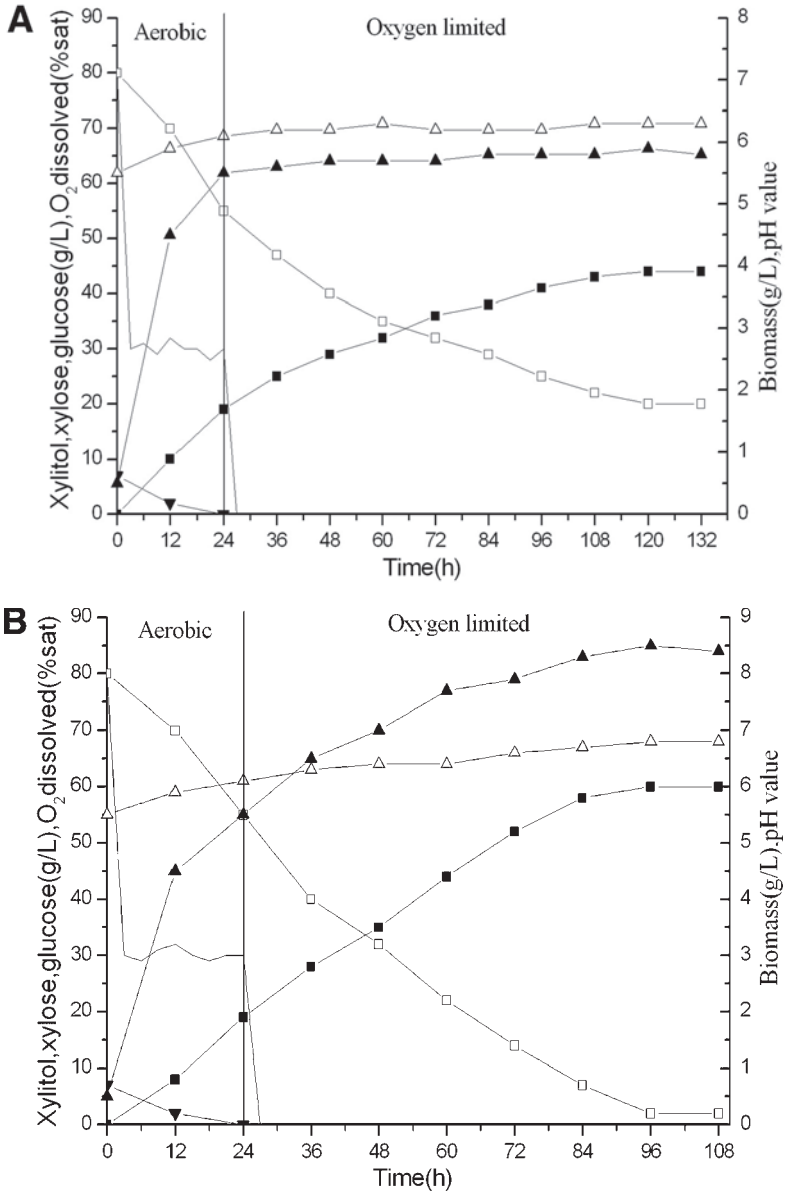

Fig. 1. Time courses of xylitol production from corncob hemicellulose hydrolysate by *Candida* sp. ZU04 under two-phase aeration process. **(A–D)** Different two-phase aeration processes, respectively. (■) xylitol; (□) xylose; (▼) glucose; (▲) biomass; (—) dissolved O_2; (△) pH value. **Parts A–D** represent the four kinds of aeration methods mentioned in Materials and Methods.

and biomass formation occurred, but it was detrimental to xylitol accumulation. This fact is probably related to the switch of the xylose metabolism to the formation of byproducts such as ethanol. As a result, it is essential to reduce the aeration rate to some degree for optimum xylitol production in the second phase.

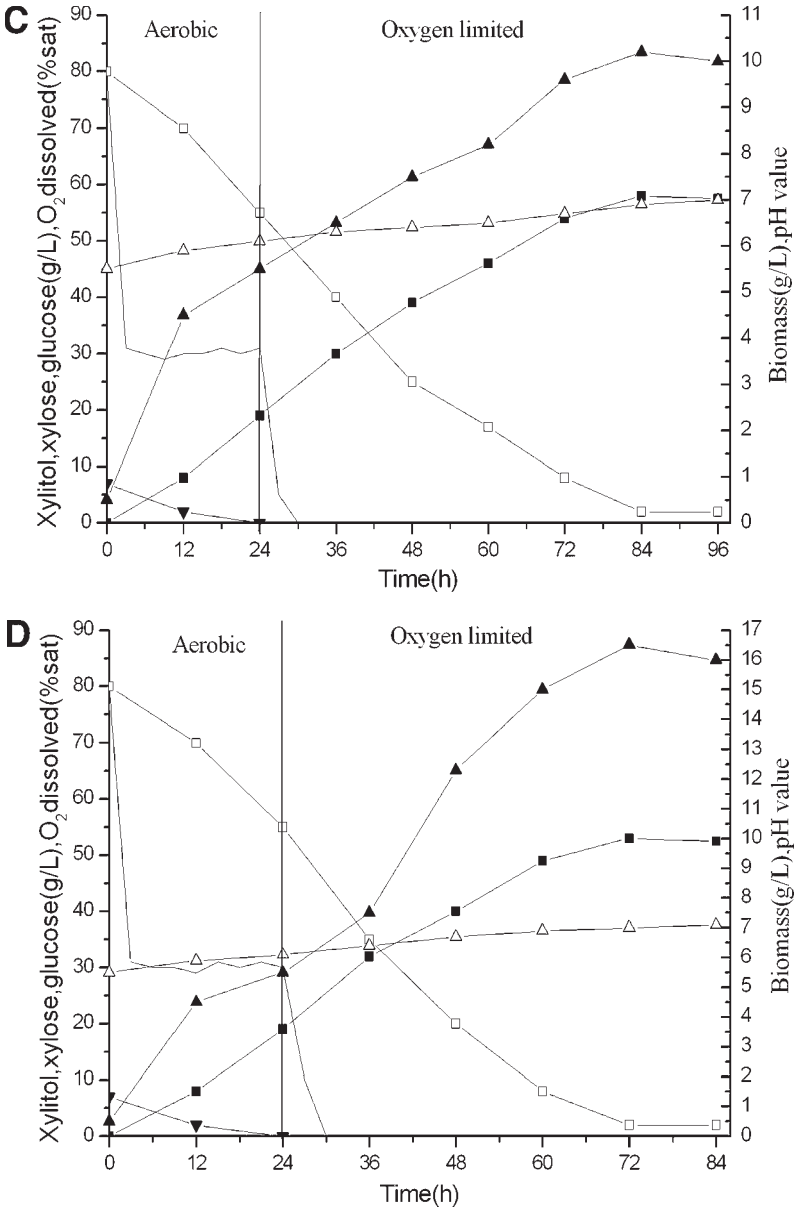

Fig. 1. *(continued)*.

In the second phase of 0.3 and 0.5 vvm, xylitol was formed mostly as the aeration rate decreased from the first phase of 1.5 vvm, whereas these oxygen limitation caused slower biomass growth and xylitol volumetric productivity lower than those of the second phase of 0.7 vvm. This is because the lower aeration rates in the second phase resulted in low oxygen uptake, and the electron transfer system present in the tricarboxylic acid cycle becomes unable to regenerate the complete NAD^+ from the NADH.

As a consequence, an increase in intracellular NADH levels occurs, reducing the enzymatic reaction rate of the NAD^+-dependent xylitol dehydrogenase and allowing xylitol to accumulate *(18)*.

The pH value of fermented medium was also affected by the aeration rate (Fig. 1). At the second phase of 0.1 vvm, the increase in pH was not pronounced. However, at the second phase of 0.7 vvm, the pH increased from 5.5 to 7.0. These results are in agreement with those found by Silvio et al. *(19)*. According to these investigators, the xylose and acetic acid present in hydrolysate were consumed simultaneously, whereas under the second phase of 0.1 vvm there was little acetic acid consumption, and the residual acetic acid may be toxic to biomass formation and xylitol accumulation.

Conclusion

The treatment of hemicellulose hydrolysate with anion-exchange resins D301 could remove most of the inhibitory compounds and improved the fermentability of the hydrolysate. The optimum aeration rate for xylitol production was 0.5 vvm under the one-phase aeration process, and the xylitol concentration was 40 g/L under that condition. The two-phase aeration process was more effective in xylitol fermentation. In the first phase, glucose in the fermented medium was soon consumed and the cells grew quickly. In the second phase, the lower aeration rate promoted xylitol yield. The maximum xylitol yield (0.76 g/g) was obtained with an aeration rate of 1.5 vvm (*KLa* of 37 h^{-1}) for the first 24 h and 0.3 vvm (*KLa* of 6 h^{-1}) from 24 to 96 h.

References

1. Buhner, J. and Agblevor, F. A. (2004), *Appl. Biochem. Biotechnol.* **119(1),** 13–30.
2. Fogel, R., Rodrigues Garcia, R., Rebeca, D. A., et al. (2005), *Appl. Biochem. Biotechnol*. **121–124,** 741–752.
3. Santos, J. C., Garvalho, W., Silva, S. S., and Converti, A. (2003), *Biotechnol. Prog*. **19(4),** s1210–1215.
4. Sreenivas Rao, R., Prakasham, R. S., Krishna Prasad, K., Rajesham, S., Sarma, P. N., and Venkateswar Rao, L. (2004), *Process Biochem*. **39,** 951–956.
5. De Faveri, D., Torre, P., Perego, P., and Converti, A. (2004), *J. Food Eng*. **65,** 383–389.
6. Huang, W., Fang, X., and Xia, L. (2004), *J. Zhejiang Univ. Eng. Sci*. **38(10),** 1366–1370.
7. Walther, T., Hensirisak, P., and Agblevor, F. A. (2001), *Bioresour. Technol*. **76(3),** 213–220.
8. Barbosa, M. F. S., Medeiros, M. B., Mancilha, I. M., Schneider, H., and Lee H. (1988), *J. Ind. Microbiol.* **3,** 241–251.
9. Granstrom, T., Ojamo, H., and Leisola, M. (2001), *Appl. Microbiol. Biotechnol*. **55(1),** 36–42.
10. Preziosi-Belloy, L., Nolleau, V., and Navarro, J. M. (2000), *Biotechnol. Lett.* **22(3),** 239–243.
11. Saha, B. C. and Bothast, R. J. (1999), *J. Ind. Microbiol. Biotechnol*. **22(6),** 633–636.
12. Pirt, S. J. (1975), *Principles of Microbe and Cell Cultivation*, Blackwell, Oxford, UK.
13. Prior, B. A., Kilian, S. G., and de Preez, J. C. (1989), *Process Biochem*. **24,** 21–32.
14. Horitsu, H., Yahashi, Y. Y., Takamizawa, K., Kawai, K., Suzuki, T., and Watanabe, N. (1992), *Biotechnol. Bioeng*. **40,** 1085–1091.
15. Chung, I. S. and Lee, Y. Y. (1986), *Enzyme Microb. Technol.* **8,** 503–507.
16. Kastner, J. R., Jones, W. J., and Roberts, F. S. (1998), *J. Ind. Microbiol. Biotechnol*. **20,** 339–343.
17. Bicho, P. A., Runnals, P. L., Cunnighan, J. D., Lee, H. (1988), *Appl. Environ. Microbiol*. **54,** 50–54.
18. Gimenes, M.A., Carlos, L. C., Faria, L. F., Pereira, N. Jr. (2002), *Appl. Biochem. Biotechnol.* **98(2),** 1049–1059.
19. Silva, S. S., Ribeiro, J. D., Felipe, M. G. A., Vitolo, M. (1997), *Appl. Biochem. Biotechnol*. **63–65,** 557–564.