0273-2289/95/5302—0101$05.20

Production of Cellulase and Xylanase in a Bubble Column Using Immobilized *Aspergillus Niger* KKS

SEONG-WOO KANG,[1]
SEUNG-WOOK KIM,*[,1] AND JIN-SUK LEE[2]

[1]*Department of Genetic Engineering, The University of Suwon, Suwon, P. O. Box 77, Suwon, 445-743, Korea; and* [2]*Korea Institute of Energy Research, Daejeon, Korea*

Received June 13, 1994; Accepted August 2, 1994

ABSTRACT

Aspergillus niger KKS, isolated from a farmland near Suwon, was immobilized on Celite and polyurethane foams. Enzyme activities produced by the immobilized cell system in a bubble column were higher than that of shake-flask culture. The enzyme productivities were twice as high. β-Glucosidase, β-xylosidase, and xylanase activities obtained in a bubble column were significant when the ground rice straw was used as a substrate.

Index Entries: *Aspergillus niger;* immobilization; cellulase; xylanase; bubble column.

INTRODUCTION

Ethanol produced from renewable biomass is attracting attention as an alternative energy source. Among the various biomass materials, lignocellulosic materials have been looked on as a promising feedstock for ethanol production because of their abundance and cheapness. Wood, which is the most abundant lignocellulosic material, consists mainly of cellulose and hemicellulose, together with lignin and minor components. To reduce the cost of ethanol production, it is essential to utilize all components of wood.

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

Although there are many microorganisms in nature that can degrade cellulose, the major component of wood, very few of these secrete extracellular cellulase in quantities capable of hydrolyzing cellulose at desired rates. *Trichoderma reesei* was the most extensively studied microorganism as a fungal cellulase. *T. reesei*, however, produces cellulase of very little cellobiase activity (*1*), which is a disadvantage from the point of view of practical saccharification.

In a search for alternatives to *T. reesei*, Kang et al. (2) have found *Aspergillus niger* to be promising. In shake-flask studies, this organism secreted extracellular cellulase with reasonable levels of activity against carboxymethyl-cellulose (CMC) and filter paper. The organism also produced high levels of β-glucosidase, xylanase, and β-xylosidase.

Previously, we reported on the optimization of fermentation medium and culturing conditions for cellulase and xylanase production by *A. niger* KKS (2). To enhance the productivity of cellulase, an immobilized cell system that has potential advantages over free-cell culture (*3*) has been examined in this work. The objective of the present investigation was to study the effects of support materials on the cellulase productivity. Following the shake-flask culture experiments, attempts were made to increase the production of cellulase and xylanase by immobilized cells in a bubble column reactor.

MATERIALS AND METHODS

Microorganism and Media

A. niger KKS (*4*) isolated in our laboratory was maintained by transferring the organism monthly on a malt extract agar slant. Basic fermentation medium consisted of 2% ground rice straw, 0.5% bacto peptone, 0.5% KH_2PO_4, 0.05% yeast extract, and 0.05% $MgSO_4 \cdot 7H_2O$. The initial pH of the medium was adjusted to 7.

Immobilization Procedure

Celite R-633 (Manville Co., USA) and polyurethane foams (Bridgestone Co., Japan) were used as support materials for the immobilization of *A. niger* (Tables 1 and 2). The spore-entrapment technique reported by Gbewonyo and Wang (*5*) was adopted and modified as follows: a spore suspension was prepared by adding sterile distilled water to the spores previously developed on an agar slant. Spore concentration of the suspension was adjusted to about 10^7 spores/mL. Various foams were cut into particles of four sizes (0.125, 1, 4, 8 cm^3), then submerged in a shake flask containing 2% (w/v) malt extract, and autoclaved. Five milliliters of spore suspension (10^7 spores/mL) were inoculated into the shake flask.

Table 1
Physical Characteristics of Celite R-633

Type of Celite	Mesh size	Pore size, μm	Pore volume, cm^3/g
R-633	Sphere(30/50)	6.5	1.47

Table 2
Physical Characteristics of Polyurethane Foams

Polyurethane foam, No.	Pore size, pore/in.	Hardness, Kg_f/cm^2
HR 08	6–10	7–13
HR 13	11–16	7–13
HR 20	17–23	7–13
HR 30	27–33	11–17
HR 40	47–53	11–17

Shake-Flask Cultivation

The spores immobilized on Celite and polyurethane foams were activated in 2% (w/v) malt extract medium for 3 h and 3 d, respectively. After activation, the spore carriers were washed thoroughly with sterile distilled water to remove free spores and/or cells and then added to 250-mL Erlenmeyer flasks containing 100 mL fermentation medium. The inoculated flasks were incubated at 30°C and 200 rpm.

Bubble Column Fermentor Operation

Bioparticles (12 g Celite) were transferred to the reactor under sterile condition. The reactor used in the study of the immobilized particles was constructed as follows. The central body of a pyrex tube was 160 × 70 mm id. A 1.5-mm thick sintered glass filter with an average pore size of 20 μm was located above a lower end of the reactor. At the top end of the reactor was a silicone rubber bung. Sterile air was continuously supplied to the bottom of the reactor. The air flow rate was maintained at 0.5 v/v min throughout the fermentation. Temperature was maintained at 30°C by circulating water through the jacket of the fermentor. For a batch operation of the fermentor, the working volume of culture broth was kept at 300 mL. Silicone oil (Dow Chemical Corp., USA) was used as an antifoam agent if necessary.

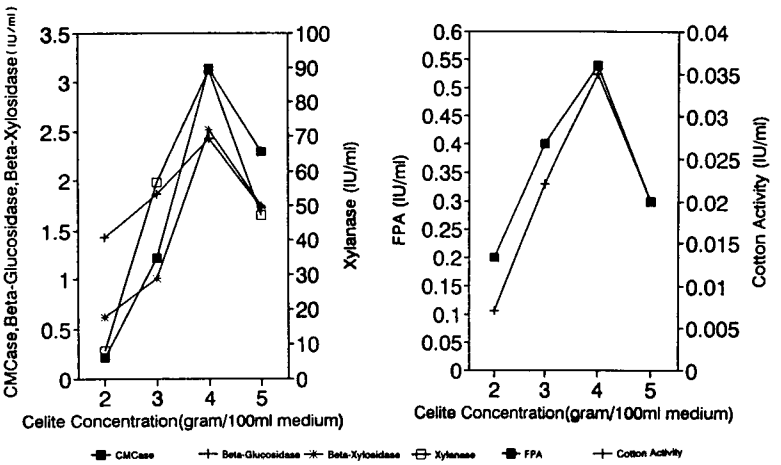

Fig. 1. Effect of Celite concentrations on the enzyme production.

Analytical Methods

Endoglucanase, cotton, filter paper, β-glucosidase, β-xylosidase, and xylanase activities were assayed using 1% (w/v) CMC, 50 mg cotton, filter paper (Whatman No. 1), 1 m*M* *p*-nitrophenyl-β-D-glucopyranoside, 10 m*M* *p*-nitrophenyl-β-D-xylopyranoside, and 2% (w/v) birch wood xylan suspension, respectively, in a 50-m*M* citrate buffer of pH 4.8, as described by Mandels and Weber (*6*) and Yu et al. (*7*). Reducing sugars were determined by using the dinitrosalicylic acid (DNS) method (*8*). *p*-Nitrophenol production was determined from the assays of β-glucosidase and β-xylosidase. Spore concentration was determined by using a hemocytometer (AO Brightline).

RESULTS AND DISCUSSION

To investigate the effect of Celite concentrations on the cellulase productivities, 2–5 g Celite were added to each flask containing 100 mL fermentation medium. A set of typical results of the experimental works are shown in Fig. 1. Four percent of Celite gave the highest cellulase activity. Although the highest enzyme activities obtained in immobilized cell fermentation were more or less low compared with those obtained in free-cell culture (2), the productivities of enzymes in immobilized cell system were two times greater than in free-cell culture. It was also found that free cells did not grow in this immobilized system.

Similar results have been obtained in fermentations with immobilized *T. reesei* Rut C-30 on Celite (*9*). The results suggest that Celite is a potential carrier for immobilized to improve the enzyme productivity.

Table 3
Effect of the Physical Characteristics of Foams on the Enzyme Production

Foam code	Size, cm^3	Enzyme activities, IU/mL					
		CMCase	FPA	Cotton activity	β-Glucosidase	β-Xylosidase	Xylanase
RH08	0.125	0.83	0.41	0.017	2.14	2.92	33.6
	0.1	0.76	0.45	0.019	1.42	2.45	47.8
	0.4	0.99	0.50	0.020	1.79	3.19	39.6
	0.8	0.78	0.42	0.021	1.52	2.36	51.5
RH13	0.125	1.76	0.52	0.020	2.06	2.74	31.5
	0.1	0.71	0.41	0.019	1.21	1.87	23.5
	0.4	0.8	0.51	0.021	1.47	2.63	67.0
	0.8	0.65	0.34	0.010	0.83	1.43	24.9
RH20	0.125	0.89	0.45	0.020	1.65	2.27	35.1
	0.1	0.86	0.40	0.018	0.92	1.36	29.1
	0.4	0.99	0.40	0.015	0.73	1.18	46.0
	0.8	0.42	0.35	0.009	0.60	1.21	51.3
RH30	0.125	0.81	0.44	0.018	0.85	1.18	22.4
	0.1	0.72	0.44	0.023	1.13	1.53	23.9
	0.4	0.75	0.37	0.018	1.06	1.30	29.0
	0.8	0.11	0.21	0.005	0.46	0.38	13.6
RH40	0.125	0.83	0.42	0.014	0.79	1.10	23.9
	0.1	0.59	0.38	0.016	0.79	1.11	26.8
	0.4	0.99	0.40	0.018	1.05	1.18	29.2
	0.8	0.59	0.28	0.013	0.74	1.11	21.8

Culture was carried out at 30°C for 7 d in the fermentation medium.

Shake-flask cultures were carried out to determine the effects of pore sizes and foam sizes on the cellulase production. The results are summarized in Table 3. The 13-ppi particles (RH13) had higher cellulase activities than other foams, and the optimum size for the bioparticle was determined to be 4 cm^3. However, the enzyme activities were quite low, only 30–50% of those obtained in shake-flask culture with free cell. This result might be the result of poor mass-transfer efficiency in polyurethane foams.

Since Celite appeared to be good support material for cellulase production, enzyme production with Celite immobilized *A. niger* KKS in a bubble column reactor was carried out. Figure 2 shows a typical time-course for cellulase production in bubble column reactor experiments. Activity against carboxymethylcellulose and filter paper, obtained from the fermentation medium with 10 g/L ground rice straw, consistently reached values of around 5.07 and 1.39 IU/mL, respectively. These values were remarkably high compared with those obtained in shake-flask culture. Filter paper activity was increased up to 100% as compared to that of

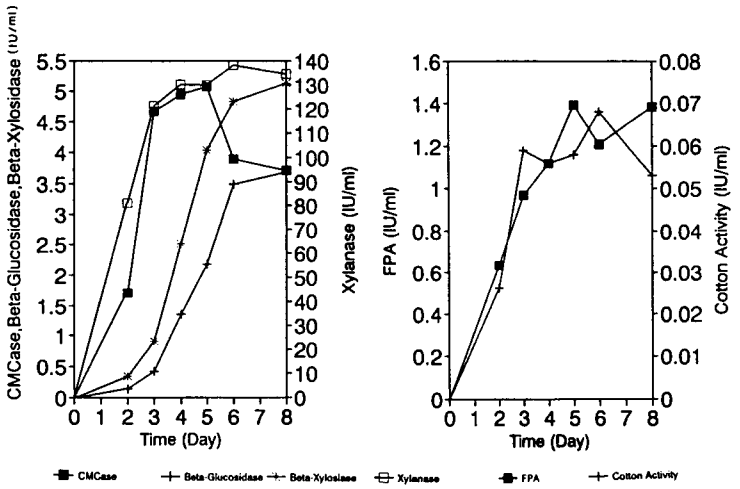

Fig. 2. Cellulase production by Celite immobilized *A. niger* KKS in a bubble column reactor operated in batch culture.

shake-flask cultures. Cotton, xylanase, β-glucosidase, and β-xylosidase activities were also increased compared with those of shake-flask cultures. The most noticeable thing was the broad spectrum of enzyme activities produced when agricultural waste was used as the carbon source. This is a significant advantage from the viewpoint of a practical saccharification reaction. These results clearly show that *A. niger* KKS immobilized on Celite has potential for cellulase production.

REFERENCES

1. Wood, T. M. and McCrae, S. D. (1975), in *Symp. Enz. Hydro. Cellulose*, SITRA, Aulanko, Finland, pp. 231–254.
2. Kang, S. W., Kim, S. W., and Kim, K. (1994), *J. Microbiol. Biotechnol.* **4**, 49–55.
3. McHale, A. P. (1988), *Biotechnol. Lett.* **10**, 361–364.
4. Kang, S. W. (1993), MSc. Thesis, The University of Suwon, Suwon, Korea.
5. Gbewonyo, K. and Wang, D. I. C. (1983), *Biotechnol. Bioeng.* **25**, 967–983.
6. Mandels, M. and Weber, J. (1969), *Adv. Chem. Ser.* **95**, 391–413.
7. Yu, E. K. C., Tan, L. U. L., Chan, M. K. H., and Saddler, J. (1987), *Enzyme Microb. Technol.* **9**, 16–24.
8. Miller, G. L. (1959), *Anal. Chem.* **31**, 426–428.
9. Frein, E. M. (1986), Ph.D. Thesis, Rutgers University, New Brunswick, NJ.