J. Microbiol. Biotechnol. (1998), **8**(3), 208–213

JOURNAL OF
MICROBIOLOGY AND
BIOTECHNOLOGY

Production of Cellulase by *Trichoderma reesei* Rut C30 in Wheat Bran-containing Media

YU, XIAO-BIN[1], HYUN SHIK YUN[2], AND YOON-MO KOO[2]*

[1]*School of Biotechnology, Wuxi University of Light Industry, Wuxi 214036, P. R. China*
[2]*Department of Biological Engineering, Inha University, Inchon 402-751, Korea*

Received: January 9, 1998

Abstract The effect of the addition of wheat bran to the growth medium on the production of cellulolytic enzymes of *Trichoderma reesei* Rut C30 was studied in batch culture using shake flasks. The activity of cellulase was enhanced by the addition of wheat bran to the cellulase production medium. KH_2PO_4-K_2HPO_4 buffer was used for pH control during cellulase production. As a result, high cellulase activities were obtained in shake flask culture; a CMC (carboxymethyl cellulose) activity of 125.78 U/ml was obtained from 2% Avicel- and 3% wheat bran-containing medium and an FP (filter paper) activity of 12.85 U/ml was obtained from 1% Avicel- and 5% wheat bran-containing medium after 6 days of cultivation.

Key words: Cellulase, *Trichoderma reesei*, wheat bran

Cellulase has many industrial applications. Its major application is for the hydrolysis of cellulose to produce glucose, which can be used for fuel, food, and chemical production. Other applications of cellulase can be found in many fields such as the food, feed, textile, detergent, and pulp industries. Recently, one of the most important usages of cellulase in the pulp and paper industry has been its application in the enzymatic deinking process of waste paper [11, 14].

The major impediment to the development of a practical process for enzymatic hydrolysis of cellulose is the high cost of enzyme. Cellulolytic enzyme systems can be produced by bacteria and fungi [10, 32]. However, most of the studies on cellulase have been conducted using fungal cellulolytic systems, and the most widely studied source of active extracellular fungal cellulases are those produced by *Trichoderma reesei*. The cellulase system consists of three enzymes: cellobiohydrolase, endoglucanase, and β-glucosidase. These enzymes work synergistically to hydrolyze cellulose to simple carbohydrates.

It has been shown that the rate and extent of cellulose hydrolysis are influenced by the structural characteristics of the cellulosic substrate [8, 26, 31]. Cellulose fibers consist of amorphous and crystalline regions. Amorphous cellulose is hydrolyzed much more rapidly than crystalline cellulose [13, 16]. Consequently, the fraction of crystalline cellulose in total cellulose is considered to be an important parameter affecting the rate and extent of enzymatic hydrolysis. Bertran and Dale [4] have shown that the lower the initial crystallinity of cellulose, the higher the extent of its conversion to soluble sugars. Wheat bran is an outer component of wheat kernel. It is used often in solid state fermentation for the production of cellulase. It has been proven to be a good substrate for cellulase production because its nutrients, starch, proteins, and lignocellulosic materials are readily available for the microbial growth and cellulase synthesis [5].

The effect of pH on enzyme production varies with the nature of the growth substrate. However, it is difficult to regulate the pH in shake flask cultures. The culture pH was maintained by increasing buffering capacity of the medium by adding KH_2PO_4 [6, 18, 19, 26, 29] or by adding cation exchange resins [7]. Knapp *et al.* [15] reported that by maintaining optimal pH during culture of *T. reesei* on Avicel, cellulase activity tripled compared with culture without pH control.

A high enzyme concentration is needed in cellulose hydrolysis due to the resistant character of the substrate. Much work has been done to increase cellulase production by *Trichoderma reesei*, by optimization of media compositions and fermentation conditions and isolation of high yielding mutants [3, 21, 23]. High cellulase production has usually been obtained in fed-batch and continuous cultivation using a fermenter [12,

*Corresponding author
Phone: 82-32-860-7513; Fax: 82-32-875-0827;
E-mail: ymkoo@inha.ac.kr

27, 30]. However, the use of crystalline cellulose as insoluble substrate in a culture system gives rise to difficulties in transferring the cellulosic material in continuous or fed-batch culture. Also, studies have demonstrated that the application of soluble sugar as substrate does not result in high cellulase productivity [1, 2].

In the present study, the effects of the addition of wheat bran to various carbon sources were investigated and the optimal Avicel/wheat bran ratio in the medium for high cellulase activity was reported.

MATERIALS AND METHODS

Microorganism

Trichoderma reesei Rut C30 (ATCC 56765) was used in this study. It was grown on potato dextrose agar (Difco Lab., Detroit, U.S.A.) slants at 30°C for 3 days and then stored at 4°C until use. The organism was transferred to new agar slants every month.

Media and Growth Condition

T. reesei Rut C30 was grown in 250 ml Erlenmeyer flasks containing 50 ml of medium. The medium contained: 3 g/l peptone (Difco Lab.), 0.5 g/l yeast extract (Difco Lab.), 2 g/l $(NH_4)_2SO_4$ (Sigma Chemical Co., St. Louis, U.S.A.), 4 g/l KH_2PO_4 (Sigma Chemical Co.), 0.3 g/l $CaCl_2 \cdot 2H_2O$ (Sigma Chemical Co.), and 0.3 g/l $MgSO_4 \cdot 7H_2O$ (Sigma Chemical Co.) [17]. The media also contained either Avicel PH101 (Fluka Co., Buchs, Switzerland), Solka Floc BW200 (Fiber Sales and Development Co., Green Brook, U.S.A.), Filter papers, No. 1 (Whatman Lab., Hillsboro, U.S.A.), carboxymethyl cellulose, sodium salt, medium viscosity (Sigma Chemical Co.), starch (Yakuri Pure Chemical Co., Osaka, Japan), lactose (Merck Co., Darmstadt, Germany), and/or wheat bran (Donga Meal Co.). Carbon sources were sterilized separately. The flasks were incubated at 28°C in a shaking incubator (KMC-8480SF, Vision Scientific Co.) at 150 rpm for 6 days.

Analysis

Carboxymethyl cellulose (CMC) and filter paper (FP) activities were determined by the method of the International Union of Pure and Applied Chemistry (IUPAC) [9]. Reducing sugars liberated were determined by the dinitrosalicylic acid (DNS) method. One unit (U) of enzyme activity was defined as the amount releasing 1 μmole of reducing sugar per min. The dry cell mass of fungi was estimated by reacting the whole culture broth with $HClO_4$ solution and measuring the optical density at 260 nm (UV1601, Shimadzu Co., Tokyo, Japan) [21].

RESULTS AND DISCUSSION

Effect of Avicel Concentration on Cellulase Activity

Cellulose works as a carbon source for the growth of fungi and also acts as an inducer for the production of cellulases. The preferred substrates used by most researchers for cellulase production are pure celluloses such as Avicel, Solka Floc, and cotton. These pure cellulose substrates are good inducers and give comparable yields of cellulase. In the present study, Avicel was selected as a carbon source for cellulase production medium and the effect of Avicel concentration on cellulase activity in shake flask culture was examined. When *Trichoderma reesei* was grown on 1% Avicel-containing medium for 6 days, the CMC activity was highest (Table 1). When the concentration of Avicel increased to 2% or higher concentrations, a substantial decrease in CMC activity resulted. In batch culture, especially in shake flask culture where the control of medium pH is not easy, the medium pH decreased with Avicel concentration. As Avicel concentration increased, the pH of the medium decreased to less than 3.0 and resulted in the decrease in cellulase activity. Sternberg [24] reported that the loss of cellulase activity due to a decrease in medium pH was not recovable after the pH was adjusted upward.

Effect of Wheat Bran as a Supplementary Carbon Source

As shown in Table 1, the CMC activity decreased when Avicel concentration in the medium was higher than 1%. However, the addition of 1% wheat bran, a lignocellulosic material, to the medium containing 1% Avicel increased the CMC and FP activities. Shake flask cultures were performed for 6 days using various carbon sources with or without 1% wheat bran. The CMC and FP activities found with seven carbon sources are compared in Table 2. For all the carbon sources tested, the cellulase activities increased significantly when 1% wheat bran was added to the medium containing a cellulose source. Among them, the medium that contained 1% Avicel and 1% wheat bran showed the highest CMC activity and FP activity. As a result, the mixture of 1% Avicel and 1% wheat bran was used as a carbon source for further experiments. Wheat bran was found to be a good substrate for cellulase production

Table 1. Effect of Avicel concentration on CMC activity of cellulase produced by *Trichoderma reesei* Rut C30.

Concentration Avicel (%, w/v)	1	2	3	5
CMC activity (U/ml)	52.9	34.4	12.5	2.23
Final pH	2.93	2.71	2.56	2.44

because its nutrients, starch, proteins, and lignocellulosic materials are readily available for the microbial growth and cellulase synthesis [5]. However, wheat bran itself did not result in high cellulase activity as shown in Table 2, partly due to the presence of starch and protein [28]. When the final pH's of Avicel- and wheat bran-containing media were compared, Avicel-containing medium showed a much lower pH than that of wheat bran-containing medium. Cellulase production during growth on Avicel is highly susceptible to changes in pH and the cellulase from *T. viride* is not stable below pH 3 at 28°C, losing 30% of the activity at pH 2.5 [25]. The addition of wheat bran probably alleviated a significant pH decrease during growth on Avicel and resulted in the increase in cellulase activity.

Effect of Glucose Concentration on Cellulase Activity

High concentrations (0.5~1.0%) of rapidly metabolized carbon sources such as glucose, cellobiose, or glycerol strongly repress cellulase formation. However, *T. reesei* Rut C30 is known to be resistant to carbon catabolite repression by glucose and cellobiose [20, 22]. When 1% glucose was contained in the medium as a carbon source, there were a considerable amount of CMC and FP activities after 6 days of cultivation (Table 2). Since glucose can be metabolized easily and stimulate cell growth, it was added to the cellulase production medium that contained 1% Avicel and 1% wheat bran. If the medium contained more than 1% cellulose, it is possible that the medium pH might have decreased to much lower than pH 3, resulting in possible cellulase inactivation. To minimize the pH effect, KH_2PO_4-K_2HPO_4 buffer was used for pH control. 0.3% K_2HPO_4 was added to the medium and the results are shown in Fig. 1 and Fig. 2.

Table 2. Effect of wheat bran as an additional carbon source on the activity of cellulase by *Trichoderma reesei* Rut C30.

Carbon source	Concentration*	CMC**	FP**	pH***
Avicel	1.0	58.31	5.15	3.05
Avicel + bran	1.0/1.0	79.62	5.61	2.99
Solka Floc	1.0	63.78	4.60	2.95
Solka Floc + bran	1.0/1.0	73.27	5.08	2.87
Pulp	1.0	53.38	4.10	5.90
Pulp + bran	1.0/1.0	69.28	4.96	5.22
Lactose	1.0	16.15	1.16	4.57
Lactose + bran	1.0/1.0	59.06	4.73	3.03
Starch	1.0	4.69	0.22	4.88
Starch + bran	1.0/1.0	33.29	2.58	3.22
CMC	1.0	4.29	0.25	6.70
CMC + bran	1.0/1.0	20.78	2.18	6.52
Wheat bran	1.0	22.68	1.93	6.45
Wheat bran	2.0	39.87	2.88	6.34
Glucose	1.0	19.85	0.92	3.46

*% concentration in w/v.
**activity in U/ml.
***final pH.

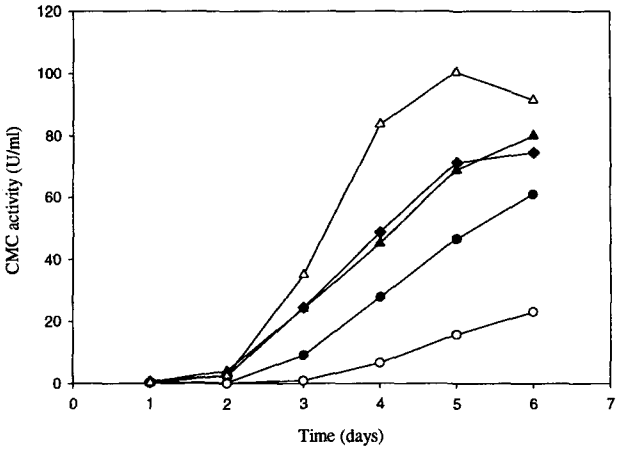

Fig. 1. Variations in cellulase activity of *Trichoderma reesei* Rut C30 in shake flask culture with 1% Avicel and various concentrations of glucose. 0.3% K_2HPO_4 was added to the medium.
○: 1% Avicel, △: 1% Avicel + 1% wheat bran, ●: 1% Avicel + 0.2% glucose, ▲: 1% Avicel + 0.6% glucose, ◆: 1% Avicel + 1% glucose.

The addition of glucose to 1% Avicel increased the cellulase activity. However, the cellulase activity was lower than that with the 1% Avicel and 1% wheat bran mixture. This is probably due to the fact that *T. reesei* Rut C30 is partially relieved from catabolite repression but repression by glucose still exists during cellulase production. When 0.3% K_2HPO_4 was added to 1% Avicel- and 1% wheat bran-containing medium (K_2HPO_4-KH_2PO_4 buffer medium), CMC activity (100.4 U/ml) was higher than in the medium that did not contain K_2HPO_4-KH_2PO_4 buffer (79.6 U/ml) (Fig. 1 and Table 2). When 2% Avicel was used as a carbon source and

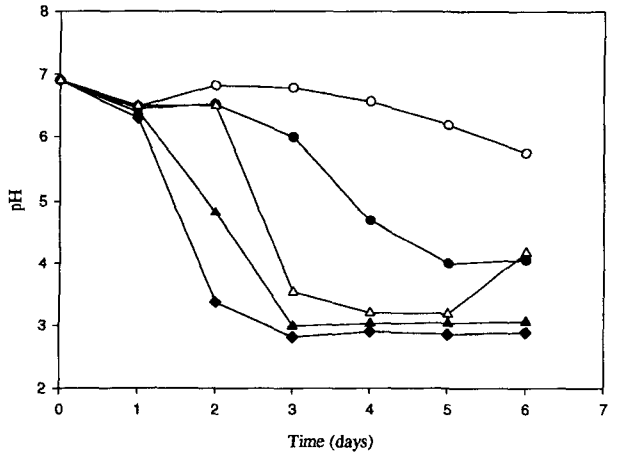

Fig. 2. Variations in pH during shake flask culture of *Trichoderma reesei* Rut C30 for the production of cellulase with 1% Avicel and various concentrations of glucose. 0.3% K_2HPO_4 was added to the medium.
○: 1% Avicel, △: Avicel + 1% wheat bran, ●: 1% Avicel + 0.2% glucose, ▲: 1% Avicel + 0.6% glucose, ◆: 1% Avicel + 1% glucose.

Table 3. The effects of relative concentration ratio of Avicel to wheat bran on cellulase activity.

Avicel/wheat bran*	CMC**	FP**	pH***
1.0/0.0	58.31	5.15	3.05
1.0/1.0	79.06	6.21	3.06
1.0/2.0	95.98	7.46	2.98
1.0/3.0	100.83	8.25	2.97
1.0/4.0	112.33	9.23	2.99
1.0/5.0	115.50	12.85	3.22
1.0/6.0	113.15	11.87	3.26
2.0/0.0	60.44	5.33	3.04
2.0/1.0	95.32	7.88	3.76
2.0/2.0	120.36	9.33	4.08
2.0/3.0	125.78	10.58	4.48
2.0/4.0	120.74	9.88	4.91
3.0/0.0	32.30	2.98	2.72
3.0/1.0	36.35	3.12	2.74
3.0/2.0	34.14	3.01	2.78

K_2HPO_4 was added to the media for pH control when Avicel concentration was higher than 1%.
*% concentration in w/v.
**activity in U/ml.
***final pH.

0.3% K_2HPO_4 was added to the medium (K_2HPO_4-KH_2PO_4 buffer medium), the CMC activity was 60.44 U/ml and FP activity was 5.33 U/ml (Table 3). Both CMC activity (42.11 U/ml) and FP activity (3.68 U/ml) were lower when 0.3% K_2HPO_4 was not added to the medium. These facts show that the K_2HPO_4-KH_2PO_4 buffer medium contributes to maintain the medium pH, though it does not sufficiently buffer the acid produced. However, CMC activity (23.0 U/ml) with 1% Avicel-containing K_2HPO_4-KH_2PO_4 buffer was much lower than that (58.3 U/ml) without K_2HPO_4-KH_2PO_4 buffer. This might be due to the high pH (6.90~5.74) of the culture medium which is not favorable for fungal growth and cellulase production (Fig. 2).

In order to study the effect of the addition of glucose to the medium on cellulase activity, 0.2%, 0.4%, and 0.6% (w/v) glucose were added to the medium that contained 1% Avicel and 1% wheat bran. The medium that contained 1% Avicel and 1% wheat bran was used as a control and K_2HPO_4-KH_2PO_4 buffer was used for all experiments. The results are shown in Fig. 3 and Fig. 4.

As shown in Fig. 3, there was not much difference in final CMC activity when various concentrations of glucose were added to the medium that contained 1% Avicel and 1% wheat bran. In the early growth stage, cell mass and CMC activity of glucose-containing media were higher than those of the control. However, when glucose was added to the medium, the pH decrease was more significant and the final pH was lower than the medium without glucose addition (Fig. 4). The addition of glucose did not significantly enhance cellulase activity

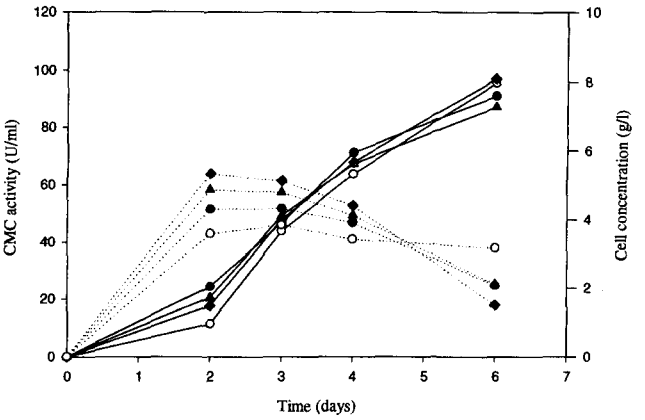

Fig. 3. Variations in cell growth (·····) and cellulase activity (——) of *Trichoderma reesei* Rut C30 in shake flask culture with 1% Avicel and 1% wheat bran with various concentrations of glucose. 0.3% K_2HPO_4 was added to the medium.
○: 1% Avicel + 1% wheat bran (control), ●: 1% Avicel + 1% wheat bran + 0.2% glucose, ▲: 1% Avicel + 1% wheat bran + 0.4% glucose, ◆: 1% Avicel + 1% wheat bran + 0.6% glucose.

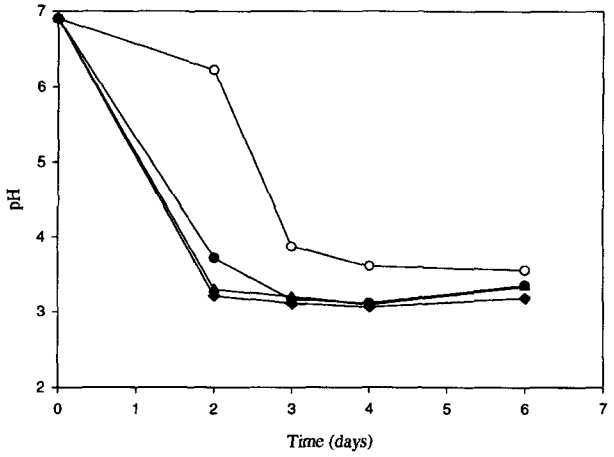

Fig. 4. Variations in pH during shake flask culture of *Trichoderma reesei* Rut C30 for the production of cellulase with 1% Avicel and 1% wheat bran with various concentrations of glucose. 0.3% K_2HPO_4 was added to the medium.
●: 1% Avicel + 1% wheat bran + 0.2% glucose, ▲: 1% Avicel + 1% wheat bran + 0.4% glucose, ◆: 1% Avicel + 1% wheat bran + 0.6% glucose, ○: 1% Avicel + 1% wheat bran (control).

in the end, though it could increase the cell mass and reduce the lag period for cellulase synthesis.

Effect of the Concentration Ratio of Wheat Bran to Avicel

In order to obtain the optimal Avicel/wheat bran ratio for the activity of cellulase, *T. reesei* Rut C30 was grown on various compositions of Avicel- and wheat bran-containing media for 6 days (Table 3). When 1% Avicel was contained in the medium, CMC and FP activities increased with wheat bran concentrations of up to 5%.

Table 4. Cellulase activities obtained from fermentation of different *Trichoderma reesei* strains [23].

Strain	CMC activity (U/ml)	FP activity (U/ml)
QM6a	88	5
QM9414	109	10
MCG77	104	11
C30	150	14
NG14	133	15

Cultures grown 14 days in 10 l fermenters on 6% 2 roll-milled cotton. pH was controlled above 3.0 using 2N NH_4OH.

This probably resulted from the fact that wheat bran-containing media maintain relatively high pH and the cellulose content of wheat bran is not so high as Avicel (the cellulose content of wheat bran is usually approximately 50% of the dry substance). The CMC activity was highest when 2% Avicel was used with 3% wheat bran. However, the FP activity was highest when 1% Avicel was used with 5% wheat bran. When 3% Avicel was contained in the medium, the enzyme activities were lower than those in the medium that contained 1 or 2% Avicel, regardless of the concentration of wheat bran in the medium.

In conclusion, 1% cellulose was optimal for the activity of cellulase produced by *T. reesei* Rut C30 in shake flask culture without pH control. The addition of wheat bran to 1% cellulose medium increased the cellulase production. The cellulase activity was enhanced further when a K_2HPO_4-KH_2PO_4 buffer medium was used. Addition of glucose to the medium could promote cell growth, but it did not increase cellulase titers. The effects of the addition of wheat bran on cellulase activities are probably due partially to the pH of the culture and partly to the quality of the substrate as a cellulase inducer. The highest cellulase activities obtained in this study were CMC activity of 125.78 U/ml and FP activity of 12.85 U/ml and those were comparable to other cellulase activities presented in the literature [26] using pH controlled-batch fermenter (Table 4).

Acknowledgments

This research work was supported by the Project Developments for the Clean Production Technology of the Korea Ministry of Trade, Industry and Energy and Inha University (1996).

References

1. Allen, A. L. and R. E. Andreotti. 1982. Cellulase production in continuous and fed-batch culture by *Trichoderma reesei* MCG80. *Biotechnol. Bioeng. Symp.* **11:** 451–459.
2. Allen, A. L. and C. D. Roche. 1989. Effects of strain and fermentation conditions on production of cellulase by *Trichoderma reesei. Biotechnol. Bioeng.* **33:** 650–656.
3. Andreotti, R. E., J. E. Medeiros, C. Roche, and M. Mendels. 1981. Effects of strain and substrate on production of cellulase by *Trichoderma reesei* mutants, pp. 373–388. *In* T. K. Ghose (ed.), *Bioconversion and Biochemical Engineering*, vol. **1.** IIT, New Delhi, India.
4. Bertran, M. S. and B. E. Dale. 1985. Enzymatic hydrolysis and recrystallization behavior of initially amorphous cellulose. *Biotechnol. Bioeng.* **27:** 177–181.
5. Chaabouni, S. E., H. Belguith, I. Hassairi, K. M'Rad, and R. Ellouz. 1995. Optimization of cellulase production by *Penicillium occitanis. Appl. Microbiol. Biotechnol.* **43:** 267–269.
6. Doppelbauer, R., H. Esterbauer, W. Steiner, R. M. Lafferty, and H. Steinmuller. 1987. The use of lignocellulosic wastes for production of cellulase by *Trichoderma reesei. Appl. Microbiol. Biotechnol.* **26:** 485–494.
7. Duff, S. J. B., D. G. Cooper, and O. M. Fuller. 1987. Effect of media composition and growth conditions on production of cellulase and β-glucosidase by a mixed fungal fermentation. *Enzyme Microb. Technol.* **9:** 47–52.
8. Fan, L. T., Y. H. Lee, and D. H. Beardmore. 1980. Mechanism of the enzymatic hydrolysis of cellulose: Effects of major structural features of cellulose on enzymatic hydrolysis. *Biotechnol. Bioeng.* **22:** 177–179.
9. Ghose, T. K. 1987. Measurements of cellulase activities. *Pure Appl. Chem.* **59:** 257–268.
10. Gilkes, N. R., D. G. Kilburn, Miller, Jr. R. C., and R. A. J. Warren. 1991. Bacterial cellulases, *Biores. Technol.* **36:** 21–35.
11. Heise, O. U., J. P. Unwin, J. H. Klungness, Fineran, Jr. W. G., M. Sykes, and S. Abubakr. 1996. Industrial scale-up of enzyme-enhanced deinking of nonimpact printed toners. *Tappi J.* **79:** 207–212.
12. Hendy, N. A., C. R. Wilke, and H. W. Blanch. 1984. Enhanced cellulase production in fed-batch culture of *Trichoderma reesei* C30. *Enzyme Microb. Technol.* **6:** 73–77.
13. Huang, A. A. 1975. Kinetic studies on insoluble cellulose-cellulase system. *Biotechnol. Bioeng.* **17:** 1421–1433.
14. Jeffries, T. W., J. H. Klungness, M. S. Sykes, and K. R. Rutledge-Cropsey. 1994. Comparison of enzyme-enhanced with conventional deinking of xerographic and laser printed paper. *Tappi J.* **77:** 173–179.
15. Knapp, J. S. and M. Legg. 1986. The effect of different cellulosic growth substrates and pH on the production of cellulolytic enzymes by *Trichoderma reesei. J. Appl. Bacteriol.* **61:** 319–329.
16. Ladisch, M. R., K. W. Lin, M. Voloch, and G. T. Tsao. 1983. Process considerations in the enzymatic hydrolysis of biomass. *Enzyme Microb. Technol.* **5:** 82–102.
17. Mandels, M. 1975. Microbial sources of cellulases in cellulose as a chemical and energy resource. *Biotechnol. Bioeng. Symp.* **5:** 81–105.

18. Mandels, M. and R. E. Andreotti. 1978. Problems and challenges in the cellulose to cellulase fermentation. *Proc. Biochem.* **12:** 6–13.
19. Mandels, M., L. Hontz, and J. Nystrom. 1974. Enzymatic hydrolysis of waste cellulose. *Biotechnol. Bioeng.* **16:** 1471–1493.
20. Mandels, M. and E. T. Reese. 1960. Induction of cellulase in fungi by cellobiose. *J. Bacteriol.* **79:** 816–826.
21. Morikawa, Y., M. Kawamori, Y. Ado, Y. Shinsha, F. Oda, and S. Takasawa. 1985. Improvement of cellulase production in *Trichoderma reesei*. *Agric. Biol. Chem.* **49:** 1869–1871.
22. Persson, I., F. Tjerneld, and B. Hahn-Hagerdal. 1991. Fungal cellulolytic enzyme production. *Proc. Biochem.* **26:** 65–74.
23. Ryu, D. D. Y. and M. Mandels. 1980. Cellulase: Biosynthesis and application. *Enzyme Microb. Technol.* **2:** 91–102.
24. Sternberg, D. 1976. Production of cellulase by *Trichoderma*. *Biotechnol. Bioeng. Symp.* **6:** 35–53.
25. Sternberg, D. and S. Dorval. 1979. Cellulase production and ammonium metabolism in *Trichoderma reesei* on high levels of cellulose. *Biotechnol. Bioeng.* **20:** 181–191.
26. Stone, J. E., A. M. Scallan, E. Donefer, and E. Ahlgren. 1969. Digestibility as a simple function of molecule of similar size to a cellulase enzyme. pp. 219–241. *In* G. J. Hajny and E. T. Reese (eds.), *Advances in Chemistry*, American Chemical Society, Washington DC, U.S.A.
27. Tangnu, S. K., H. W. Blanch, and C. R. Wilke. 1981. Enhanced production of cellulase, hemicellulase, and β-glucosidase by *Trichoderma reesei* (Rut-C30). *Biotechnol. Bioeng.* **23:** 1837–1849.
28. Toyama, N. 1976. Feasibility of sugar production from agricultural and urban cellulosic wastes with *Trichoderma viride* cellulase. *Biotechnol. Bioeng. Symp.* **6:** 207–219.
29. Toyama, N. and K. Ogawa. 1981. Cellulase production of *Trichoderma viride* in solid and submerged culture methods, pp. 249–267. *In* T. K. Ghose (ed.), *Bioconversion and Biochemical Engineering*, vol. **1**. IIT, New Delhi, India.
30. Watson, T. G., I. Nelligan, and L. Lessing. 1984. Pilot scale production of cellulase by *Trichoderma reesei* (Rut-C 30) in fed-batch culture. *Biotechnol. Lett.* **6:** 667–672.
31. Wood, T. M. and S. I. McCrae. 1979. Synergism between enzymes involved in the solubilization of native cellulose, pp. 181–209. *In* R. D. Brown Jr. and L. Jurasek (eds.), *Advances in Chemistry*, vol. **181.** American Chemical Society, Washington DC, U.S.A.
32. Wood, T. M., S. I. McCrae, C. A. Wilson, K. M. Bhat, and L. M. Gow. 1988. Aerobic and anaerobic fungal cellulases, with special reference to their mode of attack on crystalline cellulose. *FEMS Symp.* **43:** 31–52.