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Cellulase Production from Spent Sulfite Liquor and Paper-Mill Waste Fiber

Scientific Note

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Index Entries: Cellulase; *Penicillium decumbens;* spent sulfite liquor; paper-mill waste fiber.

INTRODUCTION

Since a high proportion of the overall cost of the conversion of cellulosics to useful products is the expense of cellulase production (*1*), it is desirable to develop new processes for producing large amounts of cellulase inexpensively. So far, most of the research work on cellulase production has been carried out using milled cellulose powder and inorganic salts as substrates, which significantly increases the cost of enzyme production. In order to reduce the cost of raw materials, we tried to develop from industrial wastes a new medium for the production of cellulase. In this report, we describe a simple method by which an all-waste medium, which was composed of spent ammonium sulfite liquor and cellulosic waste of a paper mill, and a catabolite derepression mutant of *Penicillium decumbens* were used to produce the enzyme efficiently.

MATERIALS AND METHODS

Materials

Spent ammonium sulfite liquor (SASL) and cellulosic wastes (clarifier sludges and digester fines) were taken from a nearby paper mill. The

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spent liquor contained about 13% organic compounds (including lignosulfonate, hemicellulose, fine cellulose fiber, reducing sugars, and the like), inorganic salts (such as N, S, and K), and some compounds toxic to microorganisms (sulfur dioxide, furfural, organic acids, and so on). After adjusting the pH from 7.8 to 4.5 and supplying phosphorus by adding small amounts of phosphoric acid, the SASL was stripped by aeration at 80°C for 2 h to reduce its toxicity. The paper-mill waste fibers contained about 58% cellulose, 10% hemicellulose, and 10% lignin. After being delignified by sulfite at a high temperature, the waste fibers can be easily hydrolyzed by cellulase.

Microorganisms

A fast-growing cellulolytic fungus, *Penicillium decumbens*, was isolated from soil and mutated in our laboratory, and a catabolite-repression-resistant mutant, JU1, was isolated by a selective screening method (2). The mutant, JU1, produced high cellulase activity even in the presence of highly soluble sugars, such as 2% glucose. This catabolite derepression is very important for cellulase production in the SASL–waste-fiber medium, since the medium contains some reducing sugars and other easily metabolizable substrates that strongly repress cellulase synthesis in wild-type strains.

Cultivation

The stock culture of *P. decumbens* strains was maintained on the wheat bran extract agar slant. The fermentation experiments were performed in flasks or in a 50-L fermentor containing a pretreated and appropriately diluted SASL. The SASL was supplemented with the cellulosic waste as an additional carbon source and inducer for cellulase synthesis.

Analysis

Assays for cellulase and β-glucosidase (2) were made on the extracellular fluids.

RESULTS AND DISCUSSION

Small amounts of sulfur dioxide, furfural, methanol, and organic acids present in the SASL were inhibitory to growth of *P. decumbens* JU1; the strain could not grow even when the spent liquor was diluted twofold. However, by adjusting pH and stripping with compressed air at high temperature, some of the volatile toxics could be driven out, and the strain could then grow normally in the liquor after it was diluted to 3°Be'.

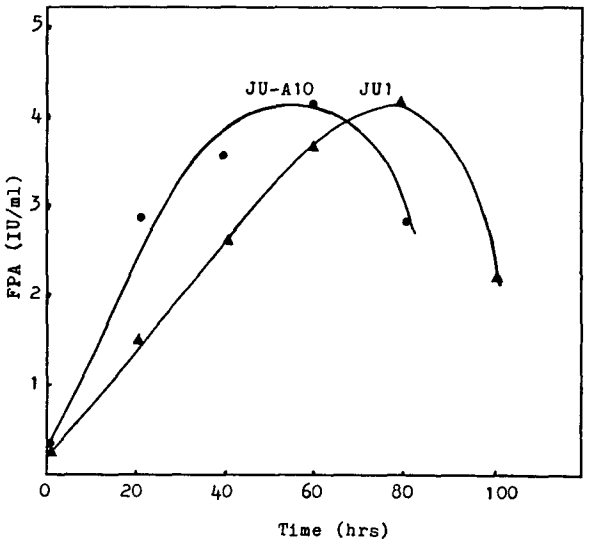

Fig. 1. Comparison of cellulase production by the parent strain, JU1, and the toxic-tolerant strain, JU-A10.

In order to increase the tolerance of *P. decumbens* to the toxic compounds, the mutant JU1 was adapted repeatedly on the SASL-gradient agar plates with progressively increased SASL concentration. Finally, a strain tolerant to the toxins, JU-A10, was isolated. Using this strain, the cellulase activity of the cultured broth was similar to that of JU1, but the time needed to reach the maximum cellulase activity was 20 h less than was needed for the control (Fig. 1).

P. decumbens JU-A10 grew fast in the pretreated SASL without additional nutrient supply, but the cellulase activity of the broth was low. Therefore paper-mill cellulosic waste was used as an inducer for cellulase synthesis and as an additional carbon source to enhance the cellulase activity. The optimum concentration of the waste fiber was studied, and 30g/L was found to be most suitable for enzyme production (Fig. 2). Unlike Mandels' medium (3), wheat bran produced no obvious stimulation of cellulase production in this medium, suggesting that no additional nutrient supply was needed.

A previous study showed that the optimum temperature for growth and for cellulase production from strains of *P. decumbens* were different (4). To enhance cellulase productivity, a two-stage temperature program was used to shorten the enzyme production cycle. In the first 24 h of the fermentation, the temperature was controlled at 31°C to increase the growth rate of the fungus. It was then decreased to 28°C to improve cellulase synthesis. This method took 12 h less than cultivation at 28°C (Fig. 3). Consequently, the enzyme productivity was enhanced.

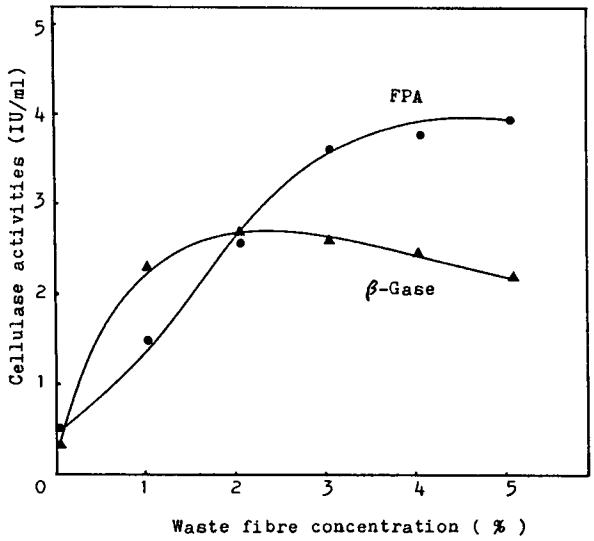

Fig. 2. Effect of waste-fiber concentration on the cellulase synthesis of *P. decumbens* JU-A10. β-Gase=β-glucosidase.

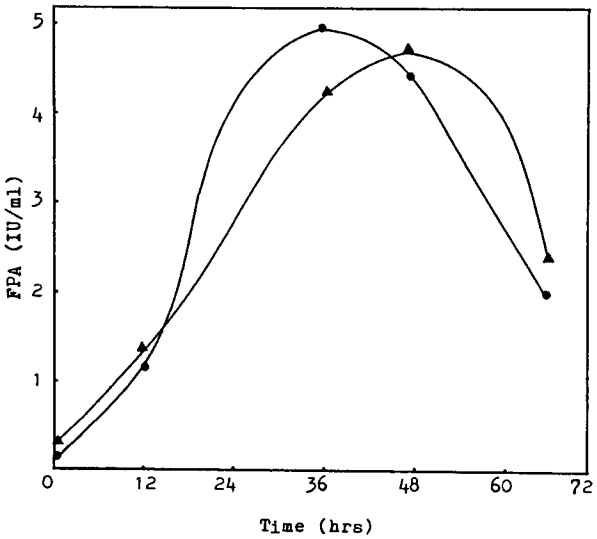

Fig. 3. Improvement of cellulase production by two-stage temperature profiling. -●- cultivation at 31°C for the first 24 h and then at 28°C; -▲- cultivation at 28°C only.

A 50-L fermentor was used for the batch fermentation study. *P. decumbens* JU-A10 grew very fast in the SASL–waste-fiber medium, and the maximum cellulase activity was obtained in <48 h (Fig. 4). Filter-paper activity of 4.6 IU/mL, productivity of 100 IU/Lh, and yield of 264 IU/g cellulose were reached in the batch fermentation. These results were much higher than those obtained with the Mandels' medium using cellulose powder as carbon source (4).

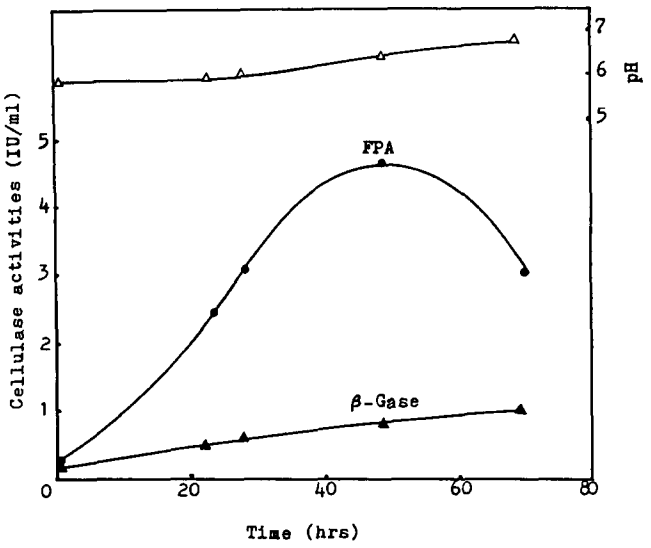

Fig. 4. Results of batch fermentation in a 50-L fermentor.

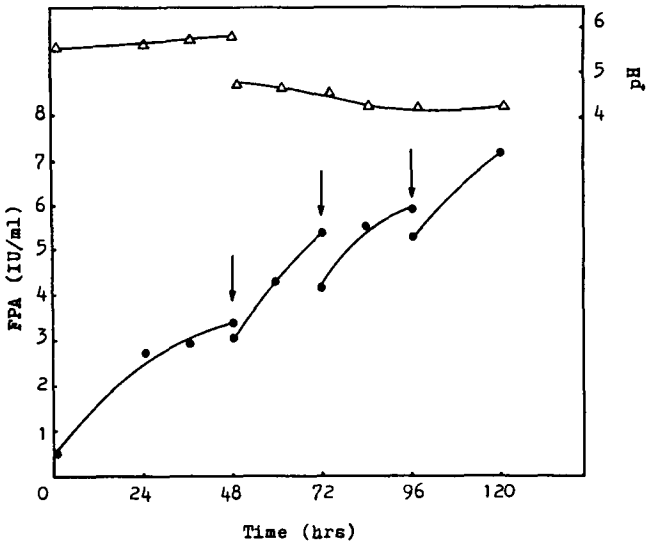

Fig. 5. Preliminary results of fed-batch fermentation under the feeding policy 10g/L every 24 h starting after 48 h; 2g/L of phosphoric acid was added with the first feed to control the pH of broth.

A preliminary study on fed-batch fermentation was carried out (Fig. 5). After feeding 10 g/L of the waste fiber three times at intervals of 24 h, 7.2 IU/mL of FPA was obtained, but the productivity of cellulase was obviously not increased as a result of the additional time. Further research is needed to improve this process.

In China, most of the paper is made from agricultural residues. Those processes produce more fine-fiber waste than making paper from wood,

especially when high-quality paper is needed. The waste fibers are currently discarded to rivers, causing severe pollution. Therefore, recovery and reuse of the fine-fiber waste is important to the development of the pulp and paper industry in China. Since all the raw materials used in this study were waste from a paper mill, the process described here may be a promising technique for cellulase and ethanol production, and for reducing water pollution by paper mills.

ACKNOWLEDGMENTS

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