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Solid-State Fermentation with *Aspergillus niger* for Cellobiase Production

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Abstract

Aspergillus niger NRRL3 was cultivated in a moist wheat bran and ground corncob solid medium supplemented with inorganic minerals for the production of cellobiase (β-1,4-glucosidase, EC 3.2.1.21). With this method, *A. niger* NRRL3 was able to produce a high concentration of cellobiase (215 IU/g of solid substrate) after 96 h of incubation. Temperature and moisture content affected final cellobiase titers. The best conditions for cellobiase production from solid substrate by *A. niger* NRRL3 were determined to be 70% moisture and 35°C.

Index Entries: *Aspergillus niger*; cellobiase; solid-state fermentation.

Introduction

Lignocellulosic biomass is the most plentiful of all the naturally occurring organic compounds. It serves as the carbon dioxide sink. Because lignocellulosic materials are plentiful and renewable, extensive research in recent years has been undertaken to convert those materials into food, feeds, chemicals, and fuels.

In general, conversion of cellulosic polymers into useful products by fermentation involves two stages: (1) conversion of cellulose to glucose by cellulase, and (2) microbial conversion of the resulting glucose to products. When there is a significant concentration of glucose, the enzymatic activity of cellobiase will be affected, which results in the accumulation of cellobiose. The accumulation of cellobiose in turn inhibits cellulase activities *(1)*. To overcome the problems caused by the cellulose hydrolysis product feedback inhibition, a process known as simultaneous saccharification and fermentation (SSF) was developed *(2)*.

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SSF is the process in which production of bioproducts from cellulosic materials is achieved by utilizing cellulose, cellulase, microbes, and nutrients in the same reactor. This process is attractive because the continuous removal of sugars by fermentative organisms alleviates the end-product inhibition of saccharification. The process is also simplified because only one reactor is used for SSF.

Many different microorganisms, including fungi, bacteria, and protozoa, are capable of producing cellulase enzymes. *Trichoderma reesei* (formally *T. viride*) is the better known cellulase-producing microbe; it produces an active cellulase complex toward insoluble cellulose. Unfortunately, *Trichoderma* enzyme preparations are low in cellobiase activity. To enhance the saccharification of cellulose, cellobiase derived from *Aspergillus* species is often supplemented during the SSF process *(3)*.

In this article, we report results of the study on the production of cellobiase by *Aspergillus niger* NRRL3 in solid medium.

Materials and Methods

Microorganisms

Strains of *Aspergillus* were kindly supplied by Dr. Kurtzman of Northern Regional Research Center, US Department of Agriculture, Peoria, IL. The strains were maintained by successive subculture on potato dextrose agar (Difco, Detroit, MI).

Inoculum

Conidiospores were produced in YMP agar consisting of yeast extract (0.3%), malt extract (0.3%), peptone (0.3%), and agar (1.5%) in Erlenmeyer flasks after 7 d of incubation at ambient temperature. Spores were collected by first flooding the culture surface with 0.02% Tween-80 solution and collected as spore suspension.

Solid-State Fermentation

Equal weights (100 g) of wheat bran and ground corncob were placed in 500-mL Erlenmeyer flasks, moistened with distilled water to the required moisture content, supplemented with mineral salts (0.5% KH_2PO_4, 0.5% $MgSO_4$, and 0.1% $CoCl_2$), and mixed thoroughly. The cotton-plugged flasks were autoclaved at 121°C for 30 min, allowed to cool to room temperature, and inoculated with spore suspension. The contents were then mixed thoroughly, and the flasks were incubated in a slanting position at the desired temperature.

Enzyme Extraction

To obtain enzyme, 5 g of the fermented solid medium was suspended in 50 mL of acetate buffer (0.05 *M*, pH 4.7). After 30 min of shaking, solution was collected through a glass fiber and centrifuged at 1000*g* for 15 min at 5°C to obtain clear crude enzyme solution.

Table 1
Cellobiase Production by *Aspergillus* spp.[a]

Organism	Cellobiase (IU/g)
A. clavatus NRRL 1980	25
A. niger NRRL 3	91
A. niger NRRL 2001	29
A. niger NRRL 9142	65
A. oryzae ATCC 56747	76
A. phoenicis ATCC 14332	72
A. sojae ATCC 46250	38

[a]Incubation temperature, 30°C; for medium composition, *see* ref. *1*.

Enzyme Activity

Cellobiase activity was determined by measuring the amount of glucose produced from cellobiose (Sigma, St. Louis, MO), which was used as the substrate. The following procedure was used. Enzyme extract (0.1 mL) was mixed with 2% cellobiose (0.1 mL) in 0.05 *M* acetate buffer (pH 4.7). This mixture was then incubated at 50°C for 5 min. The concentration of glucose released was determined by high-performance liquid chromatography (HPLC). The cellobiase activity was expressed as international units per gram of initial dry solid. One unit of cellobiase activity is defined as the amount of cellobiase required to release 2 μmol of glucose/min from 1 μmol of cellobiose at 50°C.

Analytical Methods

Glucose and cellobiose concentrations were determined and quantified by HPLC (L-6200A; Hitachi Instrument) using a Bio-Rad Aminex HPX-87H ion exclusion column (300 × 7.8 mm) (Bio-Rad, Hercules, CA) with a refractive index detector (L-3350 RI; Hitachi Instrument, Tokyo, Japan). The column was eluted with dilute sulfuric acid (0.005 *M*) at a column temperature of 80°C and a flow rate of 0.8 mL/min over a 10-min period.

Results and Discussion

Screening for Better Cellobiase Producer

Many *Aspergillus* species have been examined with respect to their ability to produce extracellular cellobiase for potential industrial application *(4,5)*. In the present study, eight selected cellobiase-producing *Aspergillus* strains were tested in order to select the best cellobiase producer. Of the eight strains examined, strain NRRL3 was found to produce the greatest amount of cellobiase from liquid medium with cellobiose as the sole carbon source (Table 1). As a result of the screen test, *A. niger* NRRL3 was chosen for further studies for cellobiase production under solid-state fermentation conditions.

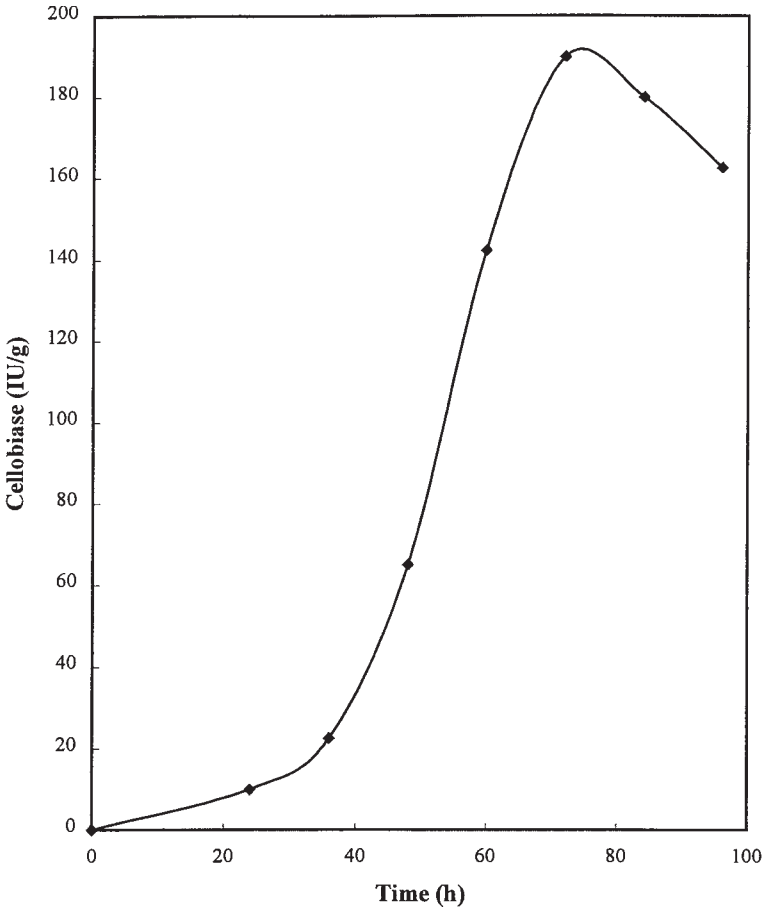

Fig. 1. Kinetics of cellobiase production by *A. niger* NRRL3 in solid substrate. Moisture content = 70%; temperature = 35°C; pH = 5.5.

Kinetics of Cellobiase Production by A. niger *NRRL3*

Solid-state fermentation has been studied as a method for the production of extracellular microbial enzymes *(6–9)*. In general, solid-state fermentation is less expensive and yields higher enzyme titers than liquid fermentation.

For the production of cellobiase in solid medium, spores of *A. niger* NRRL3 were inoculated and incubated at 35°C. Figure 1 demonstrates the time course of cellobiase production by *A. niger* NRRL3 grown on solid substrate. The production of cellobiase was not obvious until 24 h after incubation, which coincides with the rapid establishment of vegetative growth throughout the solid substrate. Cellobiase production increased rapidly thereafter and reached the maximum level at 72 h. A similar observation has been reported by Smits et al. *(10)* with *T. reesei* using wheat bran as the solid substrate for cellulase production.

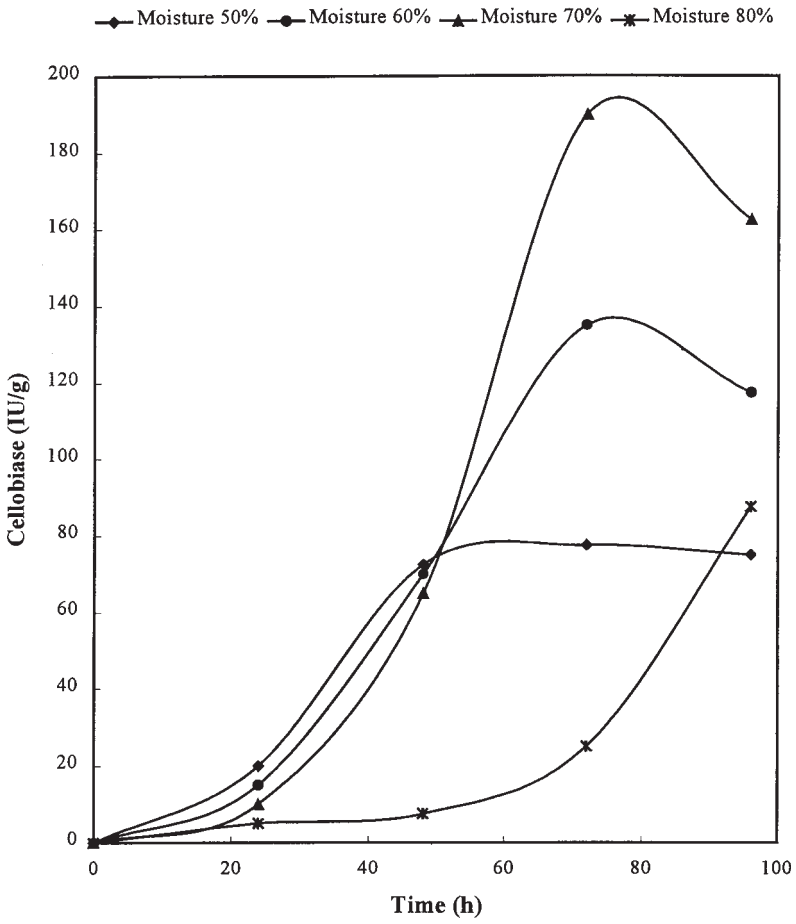

Fig. 2. Effect of moisture content on cellobiase production by *A. niger* NRRL3 in solid substrate. Temperature = 35°C; pH = 5.5.

Effect of Moisture Content

Moisture content has a profound influence on the production of cellobiase by *A. niger* NRRL3 in the solid-state fermentation system. Figure 2 shows the yields of cellobiase under four different moisture contents. A moisture content of 70% provides the best environment for cellobiase production, which is similar to the extracellular phytase production by *A. ficuum* with canola meal as the solid substrate *(8)*.

Effect of Temperature

When grown on solid substrate at different temperatures, *A. niger* NRRL3 produced less cellobiase at a higher temperature (Fig. 3). However, the sporulation of the fungus was less noticeable at a lower incubation temperature.

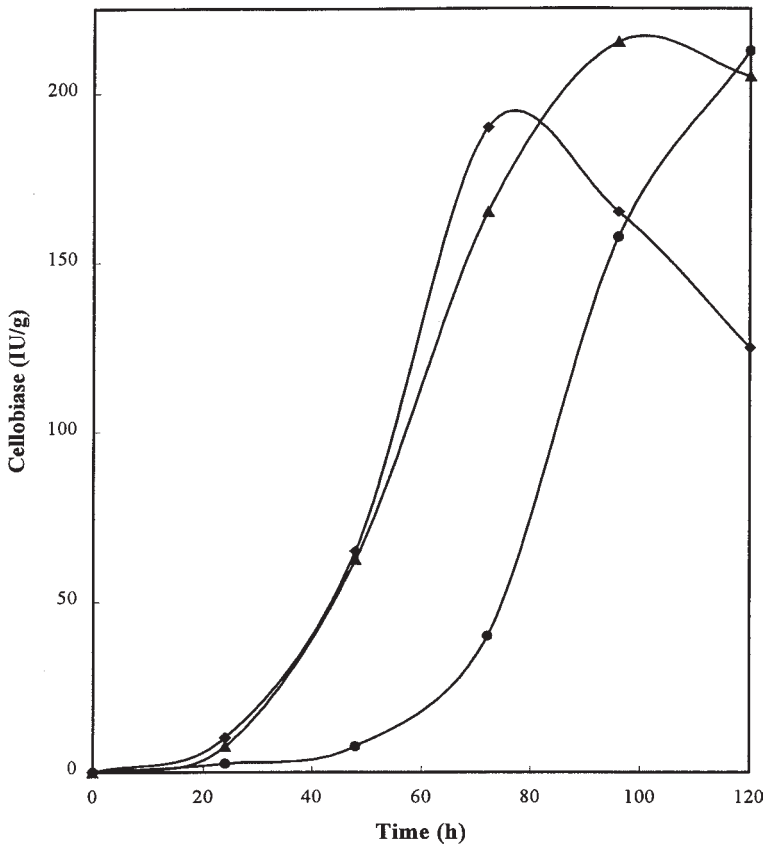

Fig. 3. Effect of temperature on cellobiase production by *A. niger* NRRL3 in solid substrate. Moisture content = 70%; pH = 5.5. (—●—), 25°C; (—◆—), 30°C; (—▲—), 35°C.

Conclusion

Under the optimal moisture content and incubation temperature, *A. niger* NRRL3 is able to produce a high titer of extracellular cellobiase when grown in solid substrate consisting of ground corncob, wheat bran, and mineral salts. Cellobiase produced under these fermentation conditions can be recovered readily and used as the supplement for SSF of cellulose for bioconversion to valuable products.

Acknowledgment

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