

Optimization of cultural conditions for cellulase production by *Aspergillus niger* (MTCC2196) using submerged fermentation

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(Received: January 28, 2009; Accepted: March 10, 2009)

ABSTRACT

Cellulases are complex enzymes and are capable of degrading lignocellulosic materials. These enzymes are produced by the fungus and bacteria. This work mainly focus on factors relevant for improvement of enzyme hydrolysis by using *Aspergillus niger* (MTCC2196) in submerged fermentation. Several cultural conditions were examined to access their effect in optimizing enzyme production. The *Aspergillus niger* (MTCC2196) could grow upto $28\pm 2^\circ\text{C}$ within a broad pH range of 3.0 to 6.0 with an optimal growth temperature and pH at 28°C and 5.0 respectively. For the *Aspergillus niger* (MTCC2196), cellulase production occurred between temperature 25°C to 30°C and pH 3.0 to 8.0. Maximum growth and enzyme production was observed at eighth day, when grown in 50ml of medium at pH 5.0, inoculum level 2ml and nitrogen sources like peptone 5mg, beef extract 5mg were added under shaking conditions at 28°C . The results showed that *Aspergillus niger* (MTCC2196) under study is a good producer of cellulases.

Key words: Cellulase, *Aspergillus niger* (MTCC2196), submerged fermentation, lignocellulosic material.

INTRODUCTION

Cellulose, being an abundant and renewable resource, is a potential raw material for the microbial production of food, fuel and chemicals (Coughlan, 1985). Various bacteria, actinomycetes and filamentous fungi produce extracellular cellulases (Pothiraj, C., *et al.*, 2006).

Cellulases are a complex enzyme system, comprising of endo-1,4- β -D-glucanase (EC-3.2.1.4), exo-1,4- β -glucanase (exo-cellobiohydrolase, EC-3.2.1.91) and β -D-glucosidase (β -D-glucoside glucanhydrolase, EC-3.2.1.21). They together with other related enzymes namely hemicellulases and pectinases, are among most important group of enzymes which

are employed in the processing of lignocellulosic materials for the production of feed, fuel and chemical feed stock (Ashok Pandey *et al.*, 2006).

Cellulases have a wide range of applications. Potential applications are in food, animal feed, textile, fuel, chemical industries, paper and pulp industry, waste management, medical/ pharmaceutical industry, protoplast production, genetic engineering and pollution/effluent treatment (Tarek, A.A. *et al.*, 2007; Beguin *et al.*, 1993; Mandels, 1985; Coughlan, 1985).

The objective of this investigation is to study the production of cellulases by *Aspergillus niger* MTCC 2196 obtained from MTCC of IMTECH,

Chandigarh, as well as the influence of different cultural conditions on enzyme production by this species in the laboratory. This organism is known as an excellent producer of cellulolytic enzymes (Tarek, A.A. *et al.*, 2007; El-Abyad *et al.*, 1996, 1997; Kurosawa *et al.*, 1989; Lachke *et al.*, 1988; Moussa, 1994).

MATERIAL AND METHODS

Chemicals

All chemicals and medium constituents used for the present study were procured from M/s Hi-media, Bombay (India).

Microorganism

Aspergillus niger MTCC2196 obtained from Culture collection of microbial type culture collection of IMTECH, Chandigarh, and was maintained on PDA slants at 4°C. Then it is subcultured once in a month. Spores were harvested from a 9 day old PDA slant in 5 ml of sterile distilled water.

Culture conditions

The organism was cultured by submerged fermentation in modified potato dextrose broth (MPD broth). Medium contains the following ingredients (g / 100 ml): Potato infusion, 20; Dextrose, 2.0; K₂HPO₄, 0.1; MgSO₄, 0.05; FeSO₄, 0.001 and NaNO₃, 0.3. Four sets of 100 ml each of MPD broth at pH 5.0 was inoculated by *Aspergillus niger* MTCC 2196. These were kept on a shaker at 120-150 rpm and 28±2°C for 8 days before harvesting. The fungal mycelia were harvested by centrifugation (REMI C24) at 5000 rpm for 20 minutes. The pellet was homogenized at 6000 rpm using acetate buffer (pH 4.8) and centrifuged subsequently at 6000 rpm for 30 minutes to extract the total fungal protein. The temperature during the course of extraction was maintained at 4°C. The supernatant was used as the crude enzyme preparation.

Effect of pH

The effect of pH was studied by adjusting the pH ranged from 3.0 to 8.0 of fermentation media. The pH of the medium was adjusted by using 1N HCl or 1N NaOH. After inoculating 2ml of spore solution of *Aspergillus niger* MTCC 2196, the flasks were kept in orbital shaker incubator (Lab Tech LSI-3016A2) at 28±2°C at 120-150 rpm.

Effect of temperature

Effect of temperature was determined by incubating fermentation medium at 25°C to 30°C in orbital shaker incubator (Lab Tech LSI-3016A2) at 120-150 rpm. After regular intervals, enzyme assay was performed.

Effect of inoculum level

Fermentation media inoculated with 1 ml to 5 ml of spore solution of *Aspergillus niger* MTCC 2196. After inoculation the flasks were incubated in orbital shaker incubator (Lab Tech LSI-3016A2) at 120-150 rpm. At regular intervals, enzyme assay was performed.

Effect of nitrogen source

For optimization, different nitrogen sources used were peptone and beef extract. These sources were used in different concentrations. Peptone was used in range from (mg/10ml) 1 to 5. The beef extract was used in range from (mg / 10 ml) 1 to 5 in fermentation medium. All the flasks were incubated at 28±2°C in orbital shaker incubator (Lab Tech LSI-3016A2) at 120-150 rpm. At regular intervals enzyme assay was performed.

Enzyme assay

The cellulase estimation was done by the method of Mendel's and Weber (1969). 50 mg of Whatman No.1 filter paper strips were incubated with 1 ml acetate buffer (pH 4.8) and 0.5 ml of undiluted sample of the extracted enzyme at 50°C for 1 hour. The reducing sugars released were estimated by the dinitrosalicylic acid (DNSA) reagent (Miller, 1959). The cellulase enzyme activity was expressed in terms of milligrams of reducing sugars released per milliliter of the enzyme extract.

Protein Collection

The protein was measured in the culture supernatant, and estimated by Modified Lowry's method (1951).

RESULTS AND DISCUSSION

The *Aspergillus niger* MTCC 2196 was selected for the production of cellulases in submerged fermentation since the *Aspergillus niger* MTCC 2196 produced cellulase over the entire range of pH (3.0-8.0). However, maximum cellulase

production was observed at pH 5.0. While at pH 5.5 the cellulase production was about 70% (Fig.1). Most commercially available cellulases have been reported from the genus *Aspergillus* (Narasimha, G., et al.,2006).

Based on the exhibited cellulase activity and growth temperature range of 25 - 30°C, and the effect of temperature on cellulase production was determined. The *Aspergillus niger* MTCC 2196 exhibited maximum cellulase activity at 28°C. While, at temperature 28.5°C the cellulase production was about 75% (Fig.2).

The results in Fig.3 showed the effect of inoculum level on cellulase production. The flasks were inoculated with 1ml to 5ml of spore solution, where the maximum cellulase production was observed at 2 ml inoculum level. While at 3ml inoculum level the cellulase activity was about 80%. Among the various nitrogen sources tested for the cellulase production, peptone proved to be the best single nitrogen source for the enzyme production followed by the beef extract, yeast extract etc. (Fig.4).

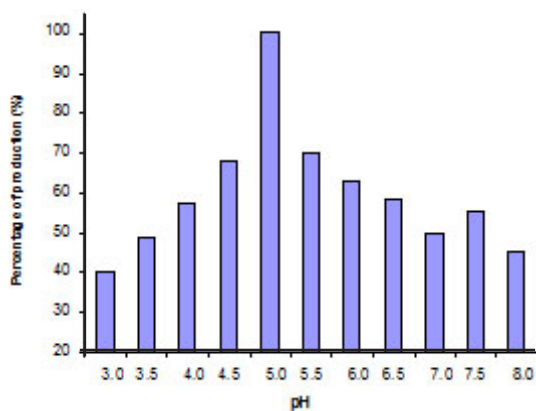


Fig. 1: Effect of pH on cellulase production

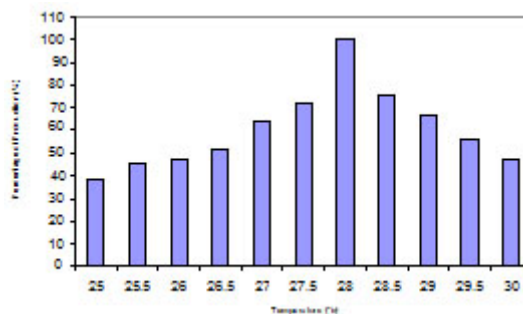


Fig. 2: Effect of temp. on cellulase production

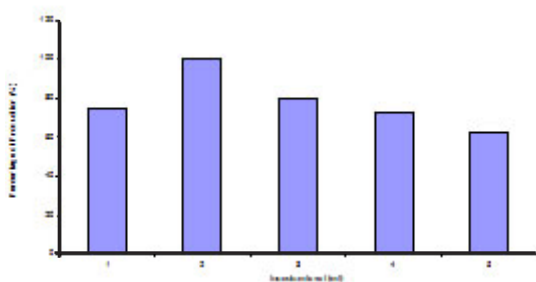


Fig. 3: Effect of inoculum level on Cellulase production

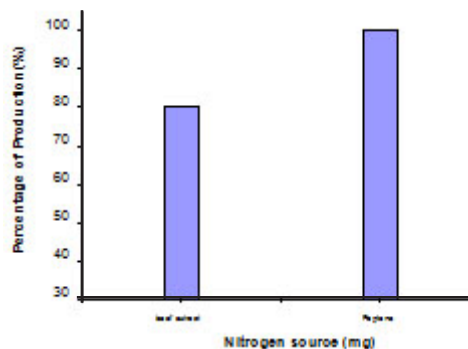


Fig.4: Effect of nitrogen source on cellulase production

ACKNOWLEDGEMENTS

The author wish to thank research scholars J. Swarnalatha, Y. Neeraja & M. Anamika at Centre

for Biotechnology, University College of Technology, Osmania University, Hyderabad, for their friendly cooperation.

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