INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/99/01–4–342–344

A Study of Optimum Conditions for Exoglucanase Production by *Arachniotus sp.*

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ABSTRACT

Maize stover was used as carbon source for the production of Exoglucanase (cellobiohydrolase) an important component of cellulase system by *Arachniotus sp.* Optimum production of enzyme was investigated by measuring it's activity in the culture filtrates at different fermentation periods, substrate water ratios and varying concentrations of $(NH_4)_2SO_4$, $CaCl_2.2H_2O$, $MgSO_4.7H_2O$, and KH_2PO_4 used as micro-nutrients. Maximum exoglucanase was produced in the continuous shaking medium containing 7.5% maize stover after 24 hours incubation in the presence of 0.2% $(NH_4)_2SO_4$, 0.05% $CaCl_2.2H_2O$, 0.01% $MgSO_4.7H_2O$ and 0.1% KH_2PO_4.

Key Words: Enzyme production; Exoglucanase; Maize stover; *Arachniotus sp.*

INTRODUCTION

The microbial conversion of cellulosic/ lignocellulosic biomass into useful products is a complex process involving combined action of three enzymes namely endoglucanase, exoglucanase and β-glucosidase converting it into glucose. Exoglucanase converts higher oligosaccharides produced by Endoglucanase into cellubiose units to be acted upon by β-glucosidase to complete the hydrolysis, (Marsden & Gray, 1986). Mixture of endoglucanase and cellobiohydrolase (CBH) account for most of the cellulase activity.

Arachniotus sp. was selected for this study because it acts as an antagonist to other microbes and prevents contamination (Alexopoulos & Mims, 1985).

The purpose of present work was to optimize conditions for the production of exoglucanase from maize stover by *Arachniotus sp.* and to propose methodology for the control of agro-industrial based environmental pollution in Pakistan.

MATERIALS AND METHODS

All chemicals used were of analytical grade. Substrate maize stover obtained from local market of Faisalabad was dried at 65 °C up to constant weight and ground to 40 mesh size.

Microorganism and its nutritional requirements. *Arachniotus sp.* procured from the Department of Animal Nutrition, University of Agriculture Faisalabad Pakistan, was maintained on maize stover agar slants at 32 °C. Sporulation media and inoculum media were developed for the growth of fungus and preparation of inoculum respectively, using maize stover as substrate (Bajwa *et al.*, 1991).

Enzyme production and assay. Various experiments were conducted to optimize fermentation conditions through continuous shaking fermentation trials of 24 hours with varying levels of substrate, $(NH_4)_2SO_4$, $CaCl_2.2H_2O$, $MgSO_4.7H_2O$ and KH_2PO_4 per cent.

In each experiment duplicate growth media containing varying concentrations of specific micro nutrients were autoclaved for 15 minutes at 1.1 kg cm^{-3} pressure and 121 °C temperature. The pH of the media was adjusted to 4 with N HCl/N NaOH. Spore inocula (5 ml) containing 107–108 spore/ml (Zarofonetis, 1959) were added under aseptic conditions to each flask. The flasks were incubated at 100–120 rpm on rotary shaker at 30±2 °C temperature.

After the expiry of incubation period the fermented materials were filtered, centrifuged at 4000 rpm for 15 minutes and ultra-filtered through milli-pore assembly. Filtrates were then subjected to enzyme assay spectrophotometrically (Deshpand *et al.*, 1984).

RESULTS AND DISCUSSION

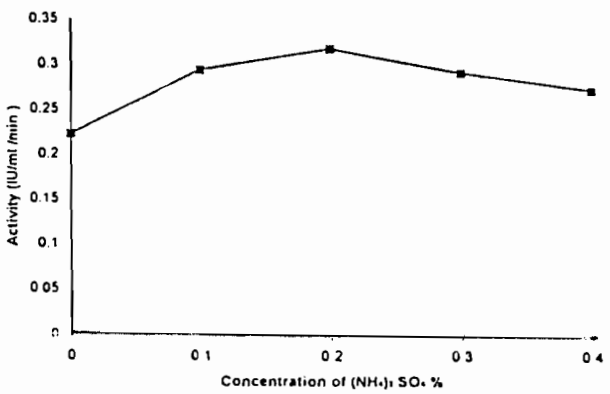
The study was aimed to optimize conditions for maximum production of exoglucanase enzyme (i.e. fermentation period, substrate: water ratio and concentrations of $(NH_4)_2SO_4$, $CaCl_2.2H_2O$, $MgSO_4.7H_2O$ and KH_2PO_4.

First experiment was carried out to investigate optimum fermentation period by using 5% maize stover as substrate. After 12 hrs, 24 hrs, 36 hrs and 48 hrs of incubation the activity was measured and found 0.156 IU/ml/min, 0.195 IU/ml/min, 0.151 IU/ml/min

and 0.136 IU/ml/min, respectively. The enzyme activity after 24 hrs (0.195 IU/ml/min) was found maximum. Our results are supported by Ortega (1985) who observed maximum cellulases production after 36 hours of incubation using CMC as substrate for the growth of *Aspergillus candids*. Steiner *et al.* (1994) also observed enzymes stability after 24 hours which hydrolyze a wide range of substrates including cellobiose.

Second experiment was meant to observe enzyme activity at varying levels of substrates i.e. 2.5%, 5%, 7.5% and 10% substrate was used and found 0.138 IU/ml/min. 0.181 IU/ml/min, 0.226 IU/ml/min and 0.199 IU/ml/min activities, respectively. The enzyme activity at 7.5% was observed highest (0.226 IU/ml/min) than all. The results of our study are supported by Chahal (1987) who fermented 5% wheat straw for exoglucanase production. Farid and Shahed (1993) also reported 6% cellulose as optimum substrate for maximum cellualses production.

Fig. 1. Effects of varying concentrations of $(NH_4)_2SO_4$ on exoglucanase activity

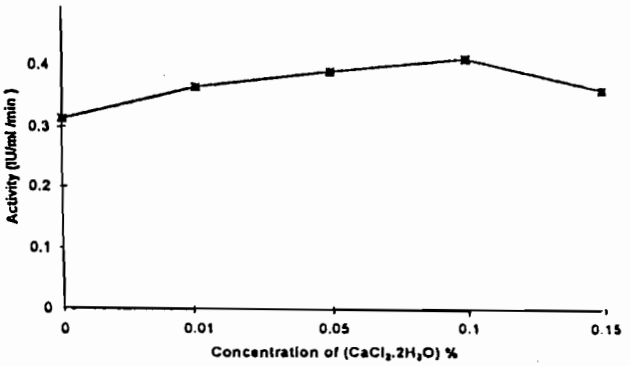


The maximum activity 0.315 IU/ml/min of exoglcanase was observed with 0.2% $(NH_4)_2SO_4$ as shown in the Fig. 1.

When different concentrations of ammonium sulphate were added with previously optimized conditions. Results of this study are supported by Singh *et al.* (1992) who observed 0.2% $(NH_4)_2SO_4$ is optimum Nitrogen source for maximum cellulase production by *Aspergillus niger*. AS 101. Using alkali treated corn cobs as substrate. Levin and Forchiassin (1995) also reported that there is proportional relationship between concentrations of $(NH_4)_2SO_4$ and enzyme activity .

Next experiment was performed to find out optimum concentration of $CaCl_2.2H_2O$ with previously

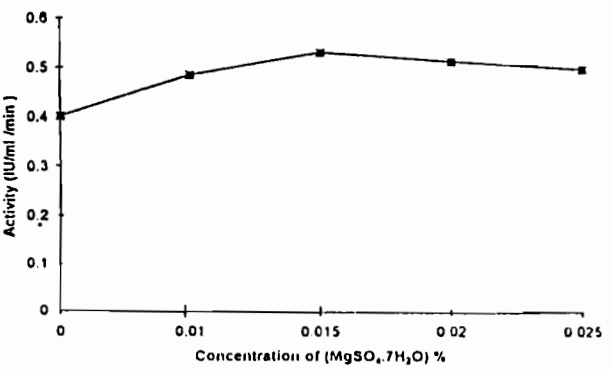
Fig. 2. Effects of varying concentrations of $CaCl_22H_2O$ on exoglucanase activity



optimized conditions and maximum enzyme activity was noted at 0.1% $CaCl_2.2H_2O$ as shown in Fig. 2.

Our results are inline with Ahmed (1991) who used 0.05% $CaCl_2.2H_2O$ for the growth of *Arachinots sp.* on gram bran. Prasertsan *et al.* (1997) also observed 0.03% $CaCl_2.2H_2O$ in corn cobs and palm oil mill effluents, fermentation media used for cellulase poduction by *Asperigillus niger* ATTC6275.

Fig. 3. Effects of varying concentrations of $MgSO_4.7H_2O$ on exoglucanase activity

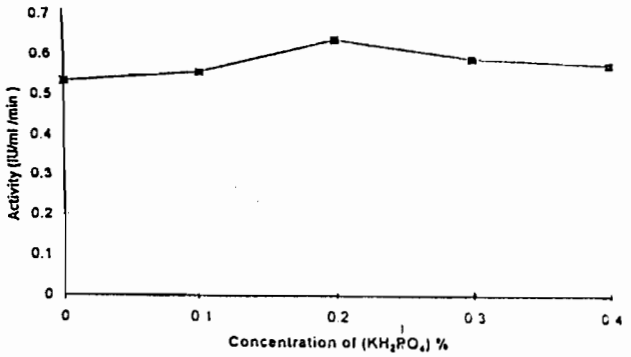


Optimum concentration of $MgSO_4.7H_2O$ was also determined in an other experiment after applying its different concentrations along with previously optimized conditions and maximum activity of the exogluca ase enzyme was noted at 0.015% as shown in Fig. 3.

Bakhali (1994) used 0.05% of $MgSO_4.7H_2O$ for the production of cellulases by *Veriticilium tricorpus* on pectin. Variation of organism and substrate is responsible for the difference in results of both studies. Macris (1985) used 0.03% $MgSO_4$ as optimum

concentration in the growth media of wheat straw for cellulase production by mutant of *Alternaria altranta*.

Fig. 4. Effects of varying concentrations of KH_2PO_4. on exoglucanase activity



In the last experiment different concentrations of KH_2PO_4 were used with previously optimized conditions (fermentation period 24 hours, substrate water ratio 7.5%, concentrations of $(NH_4)_2SO_4$ 0.2%, $CaCl_2.2H_2O$ 0.1% and $MgSO_4.7H_2O$ 0.015%) and maximum activity of exoglucanase was observed with 0.2% concentration of KH_2PO_4 as shown in the Fig. 4. The results of present study were coincided with Mubeen (1997) who observed maximum activity of cellulases by using 0.2% KH_2PO_4 produced by *Arachniots sp.* using wheat bran as the inducer. Prasertsan *et al.* (1997) used 0.1% KH_2PO_4 in optimum growth medium for the production of cellulases by *Aspergillus niger*. ATTC6275.

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(Received 13 July 1999; Accepted 01 October 1999)