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EFFECT OF CULTURE CONDITIONS ON CELLULASE PRODUCTION BY *ARACHNIOTUS* sp.

M. Asgher, M. Yaqub, M.A. Sheikh & A.R. Barque
Departments of Biochemistry & Animal Nutrition University of Agriculture, Faisalabad

Optimization studies on cellulase production from corn stover by Arachniotus sp. under continuous shaking conditions showed that addition of (NH4)2S04'MgS04.7$H_2$0, CSCr, 2HP and KH_zP0_4 to the fermentation medium enhanced cellulose production. Maximum endoglucanase (1.15IU/ml/min), exoglucanase (O.64IU/ml/min) and 13-glucosidase(1.12IU/mVmin) activity was recorded after 24 hours continuous shaking fermentation (120rpm) in the culture medium of 7.5% corn stover containing (NH_4)zS04'0.2%; $CaCl_z$.2Hp, 0.05%; MgS04.7Hp, 0.01% and KH_zP04, 0.2% at pH 4 and 32^{Q}C.

Key words: *Arachniotus sp.*, cellulase optimization, continuous shaking fermentation

INTRODUCTION

Over the past decade, emphasis has been on the enzymatic hydrolysis of cellulosic residues into glucose to meet the future energy demands (Gadgil et al., 1995). The efficiency of the hydrolytic process depends upon many factors governed by the source, composition and structure of cellulosic substrate, pre-treatment methods and reactor design (Gusakov et al., 1987). Cellulases are generally considered to be synthesized in the presence of inducers like cellulose, lignocellulose derivatives, cellobiose, etc. Regulation of microbial synthesis of cellulase complex is under control led by induction and catabolite repression. Various organisms behave differently with different inducers and growth environment (Bahkali, 1992). However, considerable progress has been made in overcoming these inherent constraints by the isolation of hyper-producing microbial strains whose enzymes are less vulnerable to catabolite repression and end product inhibition. The present paper reports the optimization of culture conditions to establish the relationship between *Arachniotus* sp. and corn stover for cellulase production.

MATERIALS AND METHODS

Arachniotus sp. was obtained from the Department of Plant Pathology, University of Agriculture, Faisalabad. It was maintained (pH 4 and 32 QC)on slants of potato-dextrose agar (PDA) and subcultured at monthly intervals. Conical flasks with 100 ml of corn stover medium containing different concentrations of micro-nutrients were inoculated with 5 ml of homogeneous spore suspension (3 x 10^6/m!) prepared from PDA slant cultures. The flasks were incubated at pH4 and 32 QC(Bajwa et al., 1991) on a shaker (120 rpm) for optimum fermentation period. The fermented biomass was filtered and the filtrate was centrifuged. The supernatent was ultra-filtered through Millipore filter and the filtrate was assayed for cellulolytic enzymes.

Optimization of Culture Conditions: In the first experiment, culture medium of corn stover (5%) was fermented for 12, 24, 36 and 48 hours for the optimization of fermentation period. Fermentation medium containing different levels of substrate was incubated for optimum time period in the second experiment. Varying concentrations of (NH_4)zS04' MgS04.7H_zO, $CaCl_z$.2$H_2$0 and KH_zP04 were then used in four different experiments, respectively to determine their optimum levels for cellulase production by *Arachniotus* sp. Studies were carried out in such a way that concentration of a nutrient optimized in one experiment was used in subsequent investigations.

Enzyme Assays: An appropriately diluted culture filtrate was used to determine endoglucanase (CMCase) and 13-glucosidaseactivity in O.lM citrate buffer (pH 4.8) at 50^{Q}C (Gadil et al., 1995). Exoglucanase assay was performed according to the method of Deshpande et al. (1984). One unit of enzyme activity in each case was defined as the amount of enzyme which released one nmol of respective reducing sugar equivalent to per ml per second.

RESULTS AND DISCUSSION

Culture conditions were optimized for maximum cellulase production.

Fermentation Period: Culture filtrates harvested after 24 hours showed maximum endoglucanase

Table 1: Activity of cellulases produced by *Arachniotus* sp, at different fermentation periods under optimum conditions*

Fermentation period (Hours)	Cellulase' activity (IU/mllmin)		
	Endoglucanase	Exoglucanase	.8-glucosidase'
12	0.31 ± 0.001	0.19 ± 0.003	0.32 ± 0.003
24	0.35 ± 0.002	0.23 ± 0.006	0.34 ± 0.003
36	0.32 ± 0.001	0.19 ± 0.002	0.33 ± 0.001
48	0.30 ± 0.002	0.17 ± 0.003	0.29 ± 0.000

*pH 4, 30±2 °C temperature (Bajwa et al,, 1991); (± SE).

Table 2. Activity of cellulases produced by *Arachniotus sp.* with varying substrate levels under optimum conditions*

Substrate level (%)	Cellulase activity after 24 hours (IU/mllmin)		
	Endoglucanase	Exoglucanase	8-glucosidase'
12	0.27 ± 0.001	0.17 ± 0.001	0.29 ± 0.004
24	0.35 ± 0.000	0.21 ± 0.002	0.34 ± 0.001
36	0.58 ± 0.004	0.26 ± 0.003	0.49 ± 0.002
48	0.57 ± 0.001	0.24 ± 0.000	0.49 ± 0.005

* pH 4, 30±2 °C temperature; (± SE).

(0.35 IU/mllmin), exoglucanase (0.23 IU/mllmin) and B-glucosidase (0.34 IU/mllmin) activity which declined thereafter through 36 to 48 hours (Table 1). All the three cellulases showed maximum activity after the same time of incubation because all the components of cellulase complex are secreted simultaneously and act synergistically to hydrolyze cellulose into glucose (Kim et al,, 1994). These results are in line with those of Ortega (1985) who observed maximum cellulase production by *Aspergillus candid us* after 36 hours. Keskar (1992) noted maximum cellulase activity in optimum growth medium of alkali-treated rice straw cultured with *Penicillium janthinellum* after 48 hours.

Substrate Level:. Culture medium containing 2.5, 5.0, 7,5 and 10% corn stover was subjected to fermentation for 24 hours. Results showed maximum production of endoglucanase (0.57 IU/mllmin), exoglucanase (0.25 IU/mllmin) and B-glucosidase (0.49 IU/ml/min) with 7.5% substrate (Table 2). A further increase in substrate level (10%) caused a decrease in cellulase production due to comparatively poor aeration and mixing (Chahal *et al.,* 1985). Ortega (1985) observed maximum cellulase productivity by *Aspergillus candidus* with 6% carboxymethylcellulose (CMC) in the fermentation medium.

(NH4)2S04: Four different concentrations of ammonium sulphate viz. 0.1, 0.2, 0.3 and 0,4% were used as an additional nitrogen supplement in the fermentation medium of corn stover (7.5%). Results after 24 hours indicated that secretion of cellulases by *Arachniotus* sp. increased with the addition of (NH4)2S04and 0.2% concentration gave optimum production of endoglucanase (0.77 Il,l/ml/rnin), exoglucanase (0.35 IU/mllmin) and B-glucosidase (0.72 IU/mllmin). Further increase in $(NH_4)2S04$ concentration up to 0.4% caused a decrease in the yield of cellulase (Fig. 1). Singh *et al.* (1992) also found out 0.2% (NH4)2S04as the optimum nitrogen source for cellulase production from 2.0% alkali-treated corn cobs by *Aspergillium niger* AS101, whereas Brimer et al. (1994) reported 0.5% (NH4)2S04as the optimum nitrogen source for the production of cellulase by *Penicillium* sp.

$CaCI2 \cdot 2H20$: The experiment was carried out to study the effect of varying concentrations of $CaCI2 \cdot 2Hp$ in the presence of preoptimized substrate and (NH4)2S04levels. Secretion of all the three cellulases increased with the addition of $CaCI2 \cdot 2H20$ to the fermentation medium and maximum endoglucanase (0.90 IU/mllmin), exoglucanase (0.45 IU/mllmin) and B-glucosidase (O.89IU/mllmin) activity was recorded with 0.50% $CaCI2 \cdot 2H20$. Further increase in $CaCI_2.2H_20$ caused a decrease in the production of cellulases by *Arachriiotus* sp. (Fig. 2). Macris (1984) supplemented the growth medium of glucose with 0.03% $CaCI2.2Hp$ along with optimum concentrations of other micronutrients for enhanced production of cellulase complex by a mutant of *Alternaria alternata.* Prasertsan et al. (1997) also

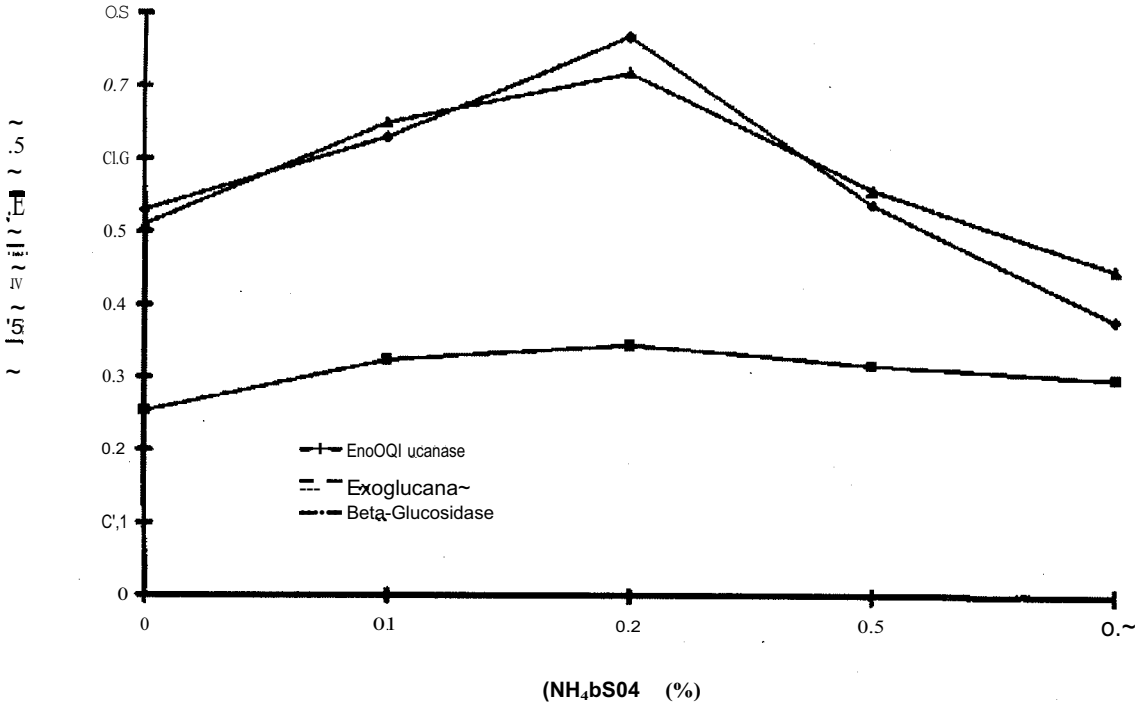

Fig 1. Effect of varying concentrations of $(NH_4)_2SO_4$ on the production of cellulase by *Arachniotus* sp.

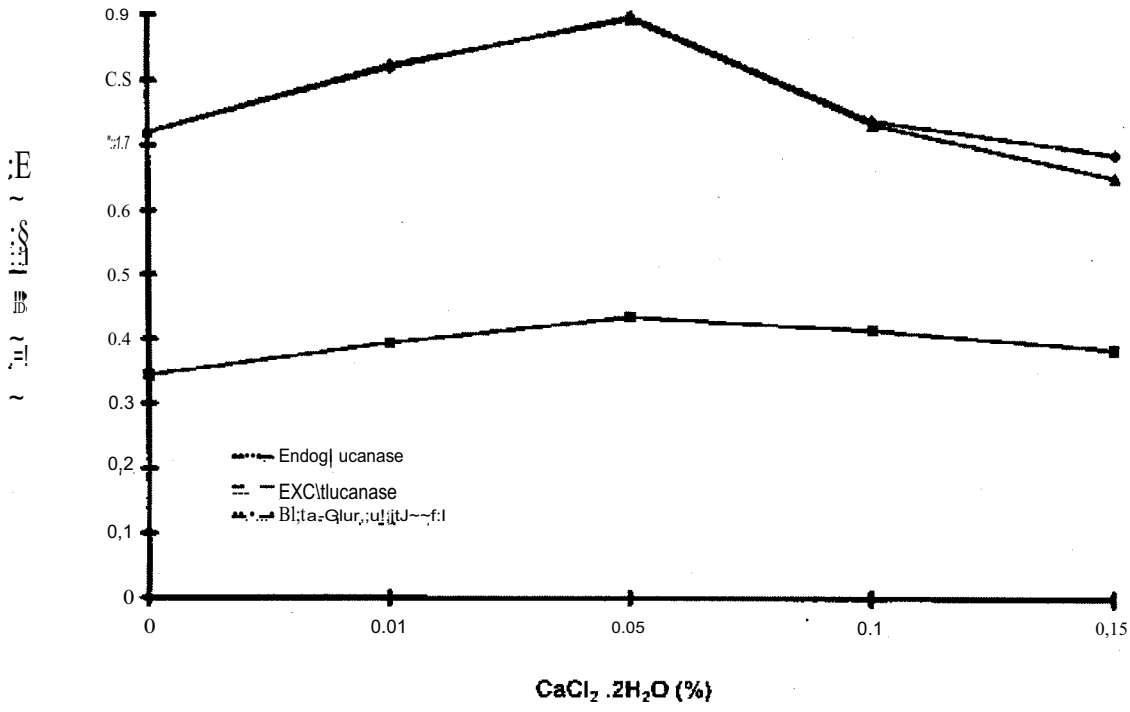

Fig 2. Effect of varying concentrations of $CaCl_2.2H_20$ on the production of cellulase by *Arachniotus* sp.

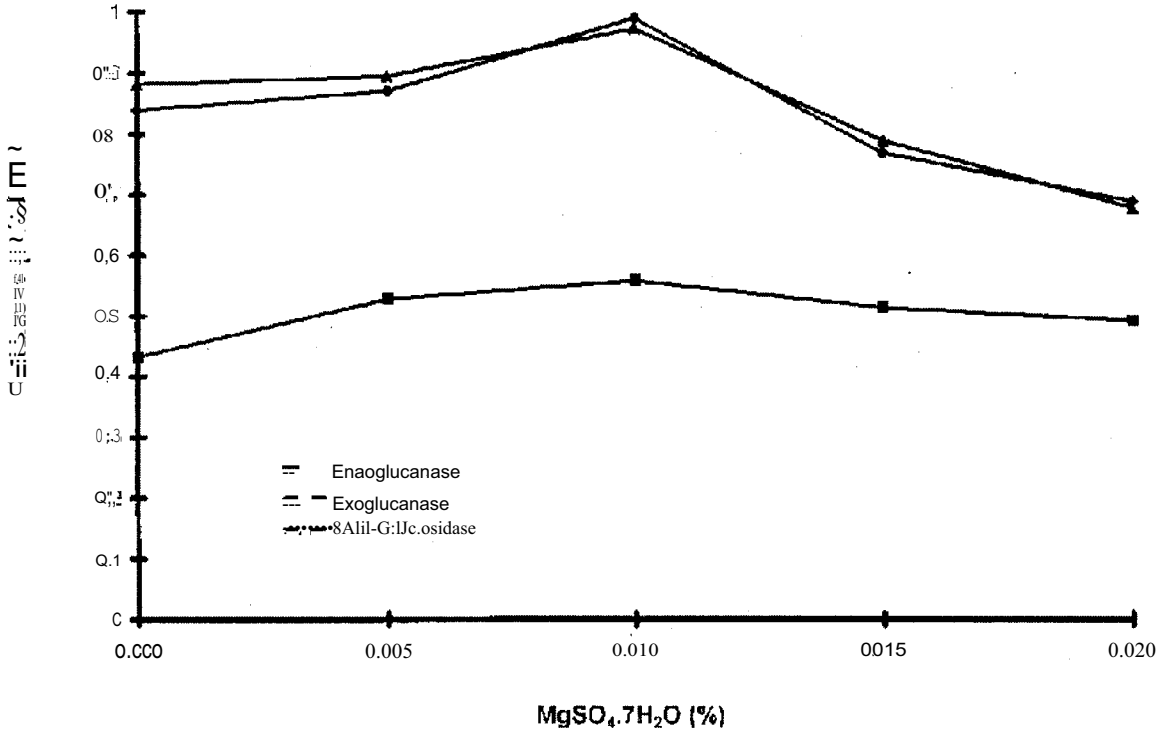

Fig 3. Effect of varying concentrations of $MgSO_4.7H_2O$ on the production of cellulase by *Arachniotus* sp.

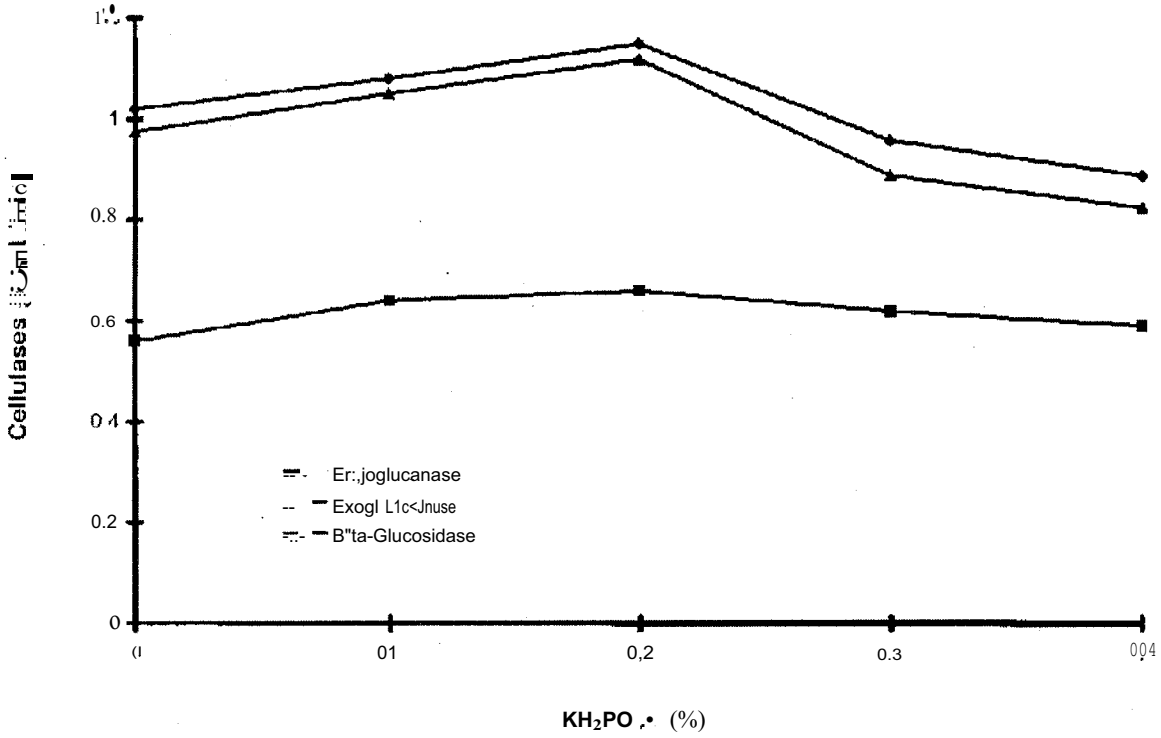

Fig 4. Effect of varying concentrations of $KHIO_4$ on the production of cellulase by *Arachniotus* sp.

observed 0.03% as the optimum concentration of $CaCl_2.2H_20$ in palm oil mill effluents medium used for cellulase production by *Aspergillus niger* ATCC6275.

$MgSO_4.7H_20$: Addition of $MgSO_4.7H_20$ to the growth medium enhanced the production of cellulases and 0.01% $MgSO_4.7H_20$ facilitated higher endoglucanase, exoglucanase and B-glucosidase production than all other concentrations tested. Macris (1984) used 0.03% $MgSO_4.7H_20$ in the optimum growth media of wheat bran, cotton fibres and wheat straw employed for cellulase production by different organisms. *Aspergillus niger* produced maximum cellulases with 0.03% $MgSO_4.7H_20$ in the optimum culture medium of alkali-treated corn cobs (Singh et al., 1992).

KH_2PO_4: Secretion of cellulases by *Arachniotus* sp. increased with the addition of KH_2PO_4 to the optimum growth medium. Maximum endoglucanase (1.15 IU/ml/min), exoglucanase (0.64IU/ml/min) and B-glucosidase (1.12 IU/ml/min) activity was noted in the medium containing 0.2% KH_2PO_4 (Fig 4). Further increase in KH_2PO_4 resulted in a slow but gradual decrease in enzyme production. Bahkali (1994) observed 0.1% KH_2PO_4 as its optimum level for cellulase production by *Verticillium tricarpus* on cellulosic substrates. These results agree with those of Brimer *et al.* (1994) who also used 0.2% KH_2PO_4 in the optimum culture medium of citrus pectin for maximum production of cellulases by *Penicillium* sp.

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