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Effect of Inorganic Nutrients on Endoglucanase Production from a Lignocellulosic Substrate

M. Asgher, Y. Saleem, M. Yaqub and M.A. Sheikh
Department of Chemistry, University of Agriculture, Faisalabad-38040, Pakistan

Abstract
Fermentation studies were carried out to investigate the effect of fermentation period, substrate level and micronutrients on the production of endoglucanase by *Arachniotus* sp. Maximum enzyme activity (1.13 b IU/mL/min) was recorded, when the fungus was cultured in the medium containing 7.5 per cent corn stover as substrate along with 0.2 per cent $(NH_4)_2SO_4$, 0.05 per cent $CaCl_2$, $2H_2O$, 0.01 per cent $MgSO_4.7H_2O$ and 0.2 per cent KH_2PO_4 under continuos shake conditions at pH 4 and $30 \pm 2°C$ for 24 hours. Addition of inorganic nutrients like $(NH_4)_2SO_4$, $CaC1_2.2H_2O$, $MgSO_2.7H_20$ and KH_2PO_4 enhanced the fungal growth and endoglucanase production upto certain (optimum) levels and further higher concentrations had negative effect on enzyme production.

Key words: Endoglucanase production, Lignocellulosic substrate, *Arachniotus* sp.

Introduction
Utility of waste lignocellosics can be tremendously increased if these are first hydrolyzed chemically or enzymatically to glucose which can be subsequently used for making biomass protein, fuel (ethanol) and other fermentation products. Increasing knowledge regarding the mode of action of cellulases and their recent applications has greatly increased the prospects of enzymatic hydrolysis over acid and alkali hydrolysis because of its high saccharification efficiency and avoidance of pollution (Cochet, 1991; Kubicek *et al*., 1993). However, the cost of production and low yields of cellulases are the major problems in the economics of process and influence their utilization on industrial scale (Macris, 1984).

The efficiency of enzymatic hydrolysis of cellulose to glucose depends upon the source of cellulose, pretreatment methods and mode of fermentation (Gusakov *et al*., 1987). The present communication reports the studies on the effect of fermentation period, substrate level and inorganic nutrients on the secretion of endoglucanase (a member of cellulase complex) by a filamentous fungus *Arachniotus* sp. using corn stover as lignocellulosic substrate.

Materials and Methods
Substrate: Corn stover was sun dried, oven dried at 70°C to constant weight and ground to 40 mm mesh size.

Organism and inoculum: *Arachniotus* sp. procured from Department of Plant Pathology, University of Agriculture, Faisalabad, was maintained on substrate agar slants and inoculum was prepared by the method described by Bajwa *et al*. (1991).

Culture Cultivation: Duplicate flasks cotnaining different culture media employed for optimization of fermentation period, substrate level and inorganic nutrients were inoculated (5%) aseptically and subjected to continuous shake culture fermentation at pH4 and $30 \pm 2°C$ temperature on orbital shaker (100-120 rpm).

Harvesting: After the stipulated incubation period, the fermented biomass samples were filtered and filtrate was centrifuged at 400 rpm. The supernatant was ultrafiltered and the filtrate thus obtained was subjected to endoglucanase assay by spectrophotometric method described by Wood and Bhat (1988).

Optimization of Culture Conditions

Incubation period: Culture media of corn stover (5%) were fermented for 12, 24, 36 and 48 h to find out the optimum fermentation time.

Substrate level: Four levels of corn stover i.e. 2.5, 5.0, 7.5 and 10 per cent were used in duplicate fermentation media.

Inorganic nutrients: Four different concentrations each of $(NH_4)_2SO_4$, $CaCl_2.2H_2O$, $MgSO_4.7H_2O$ and KH_2PO_4, were tested to get the optimum concentrations of the nutrients for maximum endoglucanase production. The experiments were conducted in such a way that condition optimized in one experiment was used in the subsequent studies.

Results and Discussion
The study was undertaken to optimize various culture conditions for the production of endoglucanase by *Arachniotus* sp. and the results have been discussed as under:

Fermentation Period: It was observed that maximum endoglucanase (0.25 IU mL^{-1}) was secreted by the fungus after 24 h of incubation which decreased thereafter and reached a minimum (0.20 IU mL^{-1}) after 48 h (Fig. 1). Statistical analysis revealed a highly significant difference ($p < 0.01$) between different time intervals. Santos *et al*. (1978) used synthetic liquid medium for the growth of *P. italicurn* and observed maximum endoglucanase activity

Table 1: Effect of varying concentrations of inorganic nutrients on endoglucanase production by *Arachniotus* sp. under optimum conditions*

S.No.	1	2	3	4	5
1	0.1	-	-	-	0.63
	0.2	-	-	-	0.77
	0.3	-	-	-	0.54
	0.4	-	-	-	0.38
2	0.2	0.1	-	-	0.82
	0.2	0.05	-	-	0.91
	0.2	0.10	-	-	0.74
	0.2	0.15	-	-	0.69
3	0.2	0.05	0.01	-	0.99
	0.2	0.05	0.015	-	0.87
	0.2	0.05	0.020	-	0.77
	0.2	0.05	0.025	-	0.69
4	0.2	0.05	0.01	0.1	1.13
	0.2	0.05	0.01	0.2	1.10
	0.2	0.05	0.01	0.3	0.96
	0.2	0.05	0.01	0.4	0.89

*Corn stover, 7.5 per cent, pH4 and 30 ± 2°C temperature. 1 - $(NH)_2SO_4$ (%); 2 = $CaCl_2.2H_2O$ (%); 3 = $MgSO_4.7H_2O$ (%) = KH_2PO_4, (%); 5 = Endoglcanase activity after 24 hours (IU/mL/min).

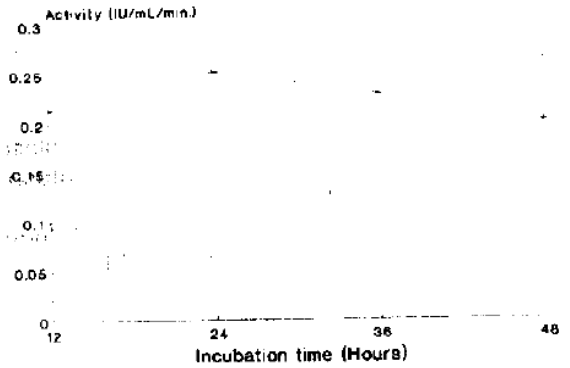

Fig. 1: Effect of different incubation periods on endoglucanase production.

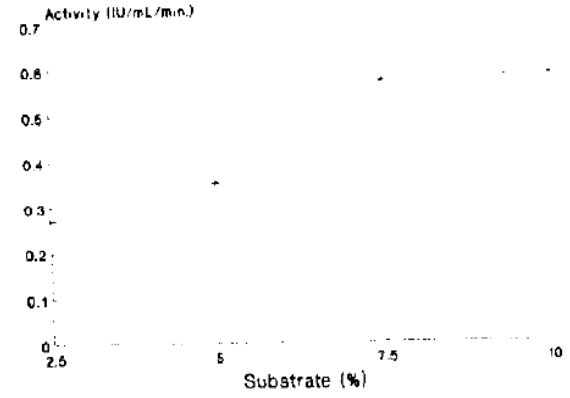

Fig. 2: Effect different levels of substrate on endoglucanase production.

after 12 hours. Maximum endoglucanase activity after 48 hours fermentation of alkali treated wheat straw by *P. Janthinellum*. The difference in optimum fermentation period may be attributed to difference of substrate and organism.

Substrate Level: Culture media containing 2.5, 5.0, 7.5 and 10 per cent corn stover were fermented for 24 hours and 7.5 per cent substrate level yielded optimum endoglucanase (0.57 IU mL^{-1}) activity (Fig. 2). All the substrate levels produced significantly different ($p<0.01$) endoglucanase except 7.5 and 10 per cent substrate which showed a non significant difference. The results are supported by those of Ortega (1985) who found out 6 per cent CMC as the optimum substrate level for endoglucanase production.

Inorganic Nutrients

$(NH_4)_2SO_4$: Four different media containing 0.1, 0.2, 0.3 and 0.4 per cent $(NH_4)_2SO_4$ were fermented at 7.5 per cent substrate level for 24 h. Results showed optimum production of endoglucanase (0.78 IU mL^{-1}) with 0.2 per cent $(NH_4)_2SO_4$ whereas 0.1, 0.3, 0.4 per cent $(NH_4)_2SO_4$ produced 0.63, 0.54 and 0.38 IU mL^{-1} enzyme activities respectively (Table 1). All the treatments showed a highly significant ($p<0.01$) difference with respect to endoglucanase production as compared to control (with no $(NH_4)_2SO_4$). The findings are supported by those of (Illanes and Schaffeld (1982) who fermented leached heat pulp as substrate for *T. reesei* and observed an increase in enzyme activity by the addition of $(NH_4)_2SO_4$ as nitrogen source. There are 0.2 per cent $(NH_4)_2SO_4$ as optimum nitrogen source for cellulase production.

$CaCl_2.2H_2O$: Addition of 0.01, 0.05, 0.1 and 0.15 per cent $CaCl_2.2H_2O$ along with optimum concentration of $(NH_4)_2SO_4$ (0.2%) yielded 0.82, 0.92, 0.74 and 0.69 IU mL^{-1} endoglucanase activity respectively. It was observed that 0.05 per cent $CaCl_2.2H_2O$ gave higher enyzme yield as compared to other levels tested (Table 1). Statistical analysis revealed a highly significant ($p<0.01$) effect of $CaCl_2.2H_2O$ on endoglucanase production. Macris (1984) studied the effect of $CaCl_2.2H_2O$ on the production of cellulases and observed maximum endoglucanase activity in the presence of 0.03 per cent $CaC1_2.2H_2O$ in glucose and wheat bran medium during fermentation with *Alternaria alternata*. Mubeen (1997) observed 0.01 per cent $CaCl_2.2H_2O$ as the optimum level for endoglucanase production from wheat bran by *Arachniotous* sp.

$MgSO_4.7H_2O$: Four different concentrations (0.01, 0.015, 0.02 and 0.025%) of $MgSO_4.2H_2O$ were added to the fermentation medium of corn stover (7.5%) along with optimum concentrations of $(NH_4)_2SO_2$ (0.2%) and $CaCl_2.2H_2O$ (0.05%) and results have been depicted in Table 1. Addition of 0.01 per cent $MgSO_4.7H_2O$ produced significantly ($p<0.05$) higher endoglucanase (0.99 IU mL^{-1}) than all other levels tested. In general $MgSO_4.7H_2O$ increased the enzyme production. Results are in line with Kuhad and Singh (1993) who reported 0.05 per cent $MgSO_4.7H_2O$ as optimum concentration for the production of endoglucanase by *P. citrinum* using rice husk as substrate. Bahkali (1994) produced maximum endoglucanase in CMC medium

containing 0.05 per cent $MgSO_4.7H_2O$ along with other micro nutrients.

KH_2PO_4: Effect of 0,1, 0.2, 0.3 and 0.4 per cent KH_2PO_4 on endoglucanase production was studied under preoptimized culture conditions. Maximum endoglucanase activity (1.13 IU mL^{-1}) was recorded in the medium containing 0.2 per cent KH_2PO_4 and there was a non-significant difference between endoglucanase activities with 0.2 and 0.3 per cent KH_2PO_4 (Table 1).

Macris *et al*. (1985) observed 0.2 per cent KH_2PO_4 as the optimum concentration with cotton fibre and wheat straw as substrates and *T. harziamum* as organism. Results are in accordance with those of Bahkali (1994) who also reported maximum endoglucanase production with the addition of 0.1 per cent KH_2PO_4 in cellulose medium fermented with *Verticillium tricorpus*.

References

Bahkali, A.H., 1994. Production of cellulase, xylanase and polygalacturonase by *Verticillium tricorpus* on different substrates. Bio-Source Technol., 26: 171-174.

Bajwa, M.A., T. Aziz and A.S. Hashmi, 1991. Production of fungal biomass protein from alkali-treated rice straw by *Arachniotus* sp. J. Am. Plant Sci., 1: 79-81.

Cochet, N., 1991. Cellulases of *Trichoderma reesei*: Influence of culture conditions upon the enzymatic profile. Enzyme Microb. Technol., 13: 104-109.

Gusakov, A.V., A.P. Sinitsyn and A.A. Klyosov, 1987. Factors affecting the enzymatic hydrolysis of cellulose in batch and continuous reactors: Computer simulation and experiment. Biotechnol. Bioeng., 29: 906-910.

Illanes, A. and G. Schaffeld, 1982. Protein enrichment of sugar beet pulp for fermentation. Rev. Argent. Microbiol., 14: 97-104.

Kubicek, C.P., R. Messner, F. Gruber, R.L. Mach and E.M. Kubicek-Pranz, 1993. The *Trichoderma cellulase* regulatory puzzle: From the interior life of a secretory fungus. Enzyme Microb. Technol., 15: 90-99.

Kuhad, R.C. and A. Singh, 1993. Enhanced production of cellulases by *Penicillium citrinum* in solid state fermentation of cellulosic residue. World J. Microbiol. Biotechnol., 9: 100-101.

Macris, B.J., 1984. Enhanced cellulase and β-glucosidase production by a mutant of *Alternaria alternata*. Biotechnol. Bioeng., 26: 194-196.

Macris, B.J., M. Paspaliari and D. Kekos, 1985. Production and cross-synergistic action of cellulolytic enzymes from certain fungal mutants grown on cotton and straw. Biotechnol. Lett., 7: 369-372.

Mubeen, F., 1997. Production of cellulases and hemicellulases from wheat bran by *Arachniotus* sp. and their bioassay. M.Phil Thesis, Department of Chemistry, University of Agriculture, Faisalabad.

Ortega, J., 1985. Some characteristics of the cellulase of *Aspergillus candidus*. Biotechnol. Lett., 7: 109-112.

Santos, T., M. Sanchez, J.R. Villanueva and C. Nombela, 1978. Regulation of the β-1,3-glucanase system in *Penicillium italicum*: Glucose repression of the various enzymes. J. Bacteriol., 133: 465-471.

Wood, T.M. and K.M. Bhat, 1988. Methods for Measuring Cellulase Activities. In: Methods in Enzymology, Volume 160, Wood, W.A. and S.T. Kellogg (Eds.)., Academic Press, London, pp: 87-112.