http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 4 (7): 875-876, 2001

Kinetics of Cellulase and Xylanase of *Chaetomium thermophile* with Respect to Aeration

Kishwar Hayat, Haq Nawaz, [1]Farooq Latif and M. Asghar
Department of Chemistry, University of Agriculture, Faisalabad, Pakistan
[1] NIBGE, Jhang Road, Faisalabad, Pakistan

Abstract: The purpose of present study was to determine the effect of dissolved O_2 on cellulase and xylanase production by *Chetomium thermophile* in growth medium of wheat straw. The concentration of dissolved oxygen was optimized through stirring. Kinetic parameters including substrate utilization (g substrate L/h) and enzyme production (IU) was studied from the maximum slope in plots of substrate utilized (g/L) and enzyme produced (IU/L) vs time of fermentation. Specific rate of product formation (QP) and substrate utilization (Qs) was also determined.

Key words: Cellulase, kinetics, aeration, xylanase

Introduction

Microbial degradation of the biological wastes is a natural process that has occurred since the on set of life on earth. In fermentation processes micro-organisms utilize the wastes as potential energy source for synthesis of very useful products such as enzymes and biomass/single cell proteins. Cellulases are carbohydrases that hydrolyze the B-1, 4 linkages of cellulose or its chemically modified forms, in addition to degrading cellodextrin or cellobiose. Typically they are multienzyme complexes bearing endo-l, 4-β-glucanase, cellobihydrolase and β-glucosidase activity (Goyal *et al.*, 1991).

Fungal cellulases are used alone or in combination with pectinase, β-glucanases and amylases in brewing, cereal processing, fruit juice extractions and wine production and alcoholic, fermentation. Cellulases also have been used to improve the palatability of low-quality vegetables, increase the flavour of mushrooms, promote the extraction of natural products and alter the texture of foods (Coughlan, 1985; Maerk *et al.*, 1990). Xylanases are the key enzymes for breakdown of xylan since they depoymerize the backbone structure. They have potential applications in biopulping, nutritional improvement of lignocellulosic feedstock, production of ethanol and methane, and in the processing of food (Wong *et al.*, 1988). Hemicellulases also are used to improve the properties of doughs used in the production of based goods (Kulp, 1968).

Keeping in view the significance of cellulase and xylanase the project was undertaken to study the kinetics of cellulase and xylanase production by culturing *Chaetomium thermophile* on wheat straw substrate.

Materials and Methods

Substrate: Wheat straw collected from NIBGE was dried in hot air at 70°C. It was ground in an electric grinder (1 mm mesh) and stored in polythene bags for subsequent use as fermentation medium.

Growth Medium: Growth medium was prepared using ingredients from Sigma company, following the method of Eggins and Pough (1962).

Microorganisms: Pure culture of *Chaetomium thermophile* was obtained from NIBGE fungal stocks and maintained on growth medium.

Inoculum: Inoculum medium was prepared and its pH was adjusted at 4.5. It was sterilized and spores of fungus were transferred into it. The medium was then placed on orbital shaker (120 rpm) for 24 hours at 45°C. Then it was aseptically transferred to the fermenter containing optimum growth medium (Table 1) of wheat straw for production of cellulase and xylanase. The fermentation conditions of bioreactor were: working volume of 20L, aeration, 0.25-0.75 vvm, pH, 4.5-5.5, stirring 300 rpm and temperature 45°C. After optimum fermentation period the biomass was filtered and filtrate was centrifuged for 15 minutes. The supernatant was then filtered through milipore filter to get a clear filterate. This filterate thus obtained was used for enzyme assays.

Table 1: Composition of inoculum medium (pH of the medium 4.5-5.5) stirring 300rpm and temperature 45°C

Ingredients	Quantity (g/L)
Carbon source (wheat straw 2% w/v)	-
KH_2PO_4	1.0
$(NH_4)_2SO_4$	0.5
KCl	0.5
$MgSO_4$	0.2
L-Asparagin	0.5
Yeast extract	0.5
α-Arginine	0.5
$CaCl_2$	0.1

Table 2: Maximum cellulase activities at different aeration rates

Aeration Rate (vvm)	FPase activity (U/ml)	CMCase activity (U/ml)	β-glucosidase activity (U/ml)
0.25	1.2	1.6	1.3
0.50	1.1	1.5	1.15
0.75	1.0	1.4	1.00

Enzyme Assays: Xylanase activity was assayed by spectrophotometer (Miller, 1959) using crystalline oatspelt xylan as substrate and DNS as coupling reagent.

β-glucosidase activity was determined according to the method of Roy *et al.* (1991).

Filter paperase and endoglucanase (CMCase) activities were determined as described by Gadgil *et al.* (1995) using filter paper strips and carboxymethyl cellulose (CMC) as substrates, respectively.

Bradford (1976) method was followed for the determination of extracellular protein in biomass.

Potential kinetic parameters such as volumetric rate of substrate utilization (QP) and volumetric rate of enzyme production (Qs) by *C. thermophile* were also determined (Rajoka and Shahid, 1998).

Results and Discussion

The results relating to effect of different aeration rates on cellulase and xylanase production have been presented and discussed here under:

Effect of Fermentation time and aeration on *Xylanase* activity: It was observed that xylanase activity started increasing at 16

Table 3: Kinetic values of different enzymes of *Chaetomium thermophile* in 20L fermenter

Kinetic Parameter	Aeration	Xylanase	FPase	CMCase	ß-glucosidase	Extra-cellular protein
	0.25	278.13	13.9	20.83	17.19	11.11
QP (I/U/L.h)	0.50	273.44	13.9	20.83	17.19	11.25
	0.75	278.13	16.7	19.44	17.19	11.4
	0.25	0.22	0.22	0.22	0.22	0.22
Qs (g/L.h)	0.50	0.21	0.21	0.21	0.21	0.21
	0.75	0.20	0.20	0.20	0.20	0.20

hours and became maximum at 72 hours. Enzyme activity was also determined at three aeration rates but 0.25 vvm gave maximum activity (18.2 u/ml) for the breakdown of it's xylan (Fig. 1). The results are supported by Palma *et al.* (1996) who studied the growth of the *P. janthiellum* and reported the highest enzymic activity (8.90 ml^{-1}) at a very low oxygen supply and ka of 1.24 h^{-1}.

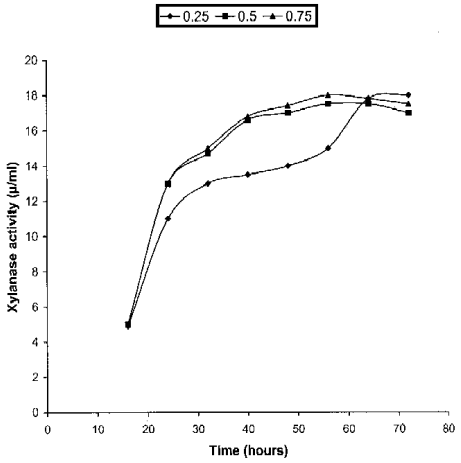

Fig. 1: Xylanase activity (U/ml) at different aeration rates

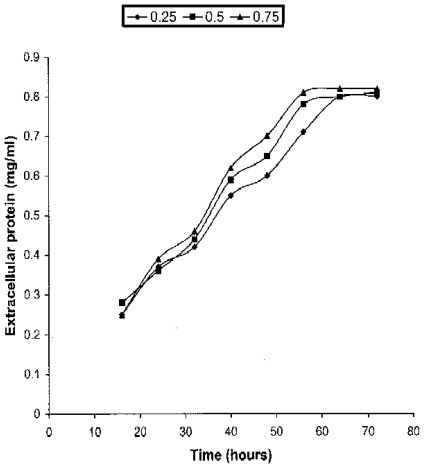

Fig. 2: Extracellular protein (mg/ml) at different aeration rates

Effect of aeration on cellulase activity: Three major components of extracellular (free) cellulase (FPase, endoglucanase and β-glucosidase) showed maximum (1.2, 1.3, 1.6 U/ml) respectively activities at aeration rate of 0.25 vvm, while 0.50 and 0.75 vvm gave somewhat less activity (Table 2). It was observed that enzyme activity was also effected by fermentation period. It was maximum at 72 hours (Fig. 2). The results are in line with those of Umikalsom *et al.* (1998).

Effect of aeration on extracellular protein: The maximum (0.88 mg/ml) protein production was observed in the fermentation medium which was controlled at 0.25 vvm for 72 hours (Fig. 2). These results are in agreement with those of Illanes *et al.* (1992) who performed solid state fermentation for cellulase production on leach beet pulp by native and mutant strains of *Trichoderma aurociride* in column type solid substrate fermenters. Protein enrichment of residual solid was significant in all cases.

The results of all enzyme assays showed maximum activity at 72 hours (Fig. 1).

Kinetic values of different enzymes have been presented in Table 3. These values seemed to be in dependent of aeration rates, for all the enzymes.

References

Bradford, N.M., 1976. A rapid and sensitive method for quantitation of microgram qualities of proteins utilizing the principle of proteins dyebinding analysis. Biochem., 72: 248-254.

Coughlan, M.P., 1985. The properties of fungal and bacterial cellulases with comment on their production and application. Biotechnol. Genet. Eng. Rev., 3: 39-11.

Eggins, H. and G.J.F. Pough, 1962. Isolation of cellulose decomposing fungi from the soil. Nature, 193: 94-95.

Gadgil, N.J., H.F. Daginawala, T. Chakarbarti and P. Khanna, 1995. "enhanced cellulase production by a mutant of *Trichoderma reesci* Enz.Microbiol.Technol., 17: 942-946. Elsevier Science Inc. New York.

Goyal, A., B. Ghosh and D. Eveleigh, 1991. Characteristics of fungal cellulases. Bioresource Technol., 36: 37-50.

Illanes, A., G. Aroca, L. Cabello and F. Acevedo, 1992. "Solid substrate fermentation of leached beet pulp with *Trichoderma aureoviride*". World J. Microbiol. Biotechnol., 8:488-493.

Kulp, K., 1968. Enzymolysis of pentosanase of wheat flour. Cereal Chem., 45: 339-350.

Marek, E., E. Schalinatus, E. Weigelt, G. Mieth and J. Kude, 1990. On the application of enzymes in the production of vegetable oil. Prog. Biotechnol., 6: 471-474.

Miller, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugars. Anal. Chem., 31:426-428.

Palma, M.B., A.M.F. Milagres, A.M. Prata and I.M. Mancilha, 1996. Influence of aeration and agitation rate on the xylanase activity from *Pencillium janthinellum*. Process Biochem. 31 No. 2, pp: 141-145.

Rajoka, M.I. and R. Shahid, 1998. Kinetics of microbial activity in solid state fermentation (SSF) of cellulosic materials for production of endo, β-D xylanase and β-xylosidase using *Aspergillus niger*. Biologia, 44: 275-289.

Roy, S., K. Raha, R.K. Sadhukhan and S.L. Chaktabarty, 1991. Purification and characterization of Myceliophthora thermophila. World J. Microbiol. Biotechnol., 7: 613-618.

Umikalsom, M.S., A.B. Ariff, M.A. Hassan, M.I.A. Karim, 1998. Kinetics of cellulase production by *Chaetomium globosum* at different levels of dissolved oxygen tension. World. J. Microbiol Biotechnol., 149: 491-498.

Wong, K.K.Y., L.U.L. Tan and J.N. Saddler, 1988. Multiplicity of β-1, 4-xylanase in microorganisms. Functions and applications. Microbiol. Rev., 52: 305-317.