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PRODUCTION AND CHARACTERIZATION OF CELLULASES OF *ASPERGILLUS NIGER* BY USING RICE HUSK AND SAW DUST AS SUBSTRATES

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Abstract

The production of cellulases by *Aspergillus niger* in submerged fermentation with two substrates rice husk and saw dust was compared. The highest cellulase activity was observed at 3rd day in pretreated rice husk media (13.702±0.1) and at 2nd day in pretreated saw dust media (9.683±0.2). The optimum temperature of the enzyme was observed to be around 40ºC. It was found that the enzyme activity has a broad pH range between 3-9 and 40% of the original activity was retained after heat treatment at 90ºC for 15 min. Maximum cellulase activity obtained when 2.5% substrate was used above this concentration no regular increase in enzyme activity was noticed. Among two lignocellulosic wastes tested in this study rice husk prove to be a good substrate for cellulase enzyme production by the organism *Aspergillus niger*.

Introduction

Cellulose is the most abundant and renewable natural product in the biosphere with its estimated synthesis rate of 10^{10} tonnes per year (Schlesinger, 1991; Singh and Hayashi, 1995; Lynd *et al*., 2002). It is the major constituent of plant cell walls providing their rigidity (Beguin & Aubert, 1994). It is a biopolymer consisting of insoluble, linear chains of β-(1-4)-linked glucose units linked with glucosidic bonds and the resulting biopolymer are assossiated by means of H-bonding. A matrix of lignin and hemicellulose encrusts and protects the cellulose of the plant cell wall (Hammel, 1997).

The agricultural wastes are composed essentially of cellulosic or lignocellulosic matter. These are considered to be the cheapest source for the production of different utilizable products throughout the world (Ali *et al*., 1991). Cellulose is commonly degraded by enzyme called cellulase. Complete enzymatic hydrolysis of cellulose requires synergistic action of 3 types of enzymes, namely cellobiohydrolases, endoglucanases or carboxymethylcellulase (CMCase) and B-glucosidases (Bhat, 2000).

Many fungi and bacteria secrete a multicomponent enzyme system called cellulase that exhibits the ability to saccharify cellulose (Wood *et al*., 1986). Bacterial cellulases are constitutively produced, whereas fungal cellulase is produced only in the presence of cellulose (Suto & Tomita, 2001). Filamentous fungi particularly *Aspergillus* and *Trichoderma* spp., are well known efficient producers of cellulases (Peij *et al.*, 1998). All four classes of enzymes have been identified in *Aspergillus* (de Vries & Visser, 2001).

It is estimated that approximately 20% of the >1 billion US dollars of the world's sale of industrial enzymes consists of cellulases, hemicellulases and pectinases. Since the production of cellulase enzyme is a major process and economically viable, much work has been done on the production of cellulases from lignocellulosics. The bioconversion of various complex cellulosic waste materials such as baggase (Kanosh *et al.*, 1999), corncob (Ojumu *et al.*, 2003); saw dust (Solomon *et al.*, 1999 Immanuel *et al*., 2007), rice husk (Milala *et al.*, 2005) have been reported. The crystallinity and lignification limit the accessibility and susceptibility of cellulose to cellulolytic enzymes and other hydrolytic agents (Fan *et al*., 1987). Therefore pretreatment of these materials is necessary to increase the rate of hydrolysis of cellulose to fermentable sugars (Galbe & Zacchi, 1993). Pretreatment of cellulose opens up the structure and removes secondary interaction between glucose chains (Fan *et al*., 1987; Tang *et al*., 1996) and make more accessible to cellulase producing microorganisms.

Production of cellulase depends on the type of substrate, pretreatment and strain of microorganism used (Ali *et al*., 1991) and presence of inducer in cultivation medium. In the present study, two different substrates such as rice husk and saw dust were used as substrates and the effect of environmental factors such as pH and temperature were also studied on cellulase production.

Materials and Methods

Fungal strain: Pure culture of *Aspergillus niger* was obtained from fungal culture bank, department of Microbiology and Plant Pathology, University of the Punjab, Lahore and was used throughout the study.

Substrates: The lignocellulosic biomass used for the study were rice husk and sawdust. The rice husk was obtained from a farmer of Shaikhupura, Pakistan and the saw dust was obtained from a local carpenter-shop, Lahore, Pakistan.

Prtreatment of substrates: The raw substrates were sun dried individually to reduce the moisture content and then crushed into bits. Then the substrates were soaked individually in 1% sodium hydroxide solution (NaOH) in the ratio 1: 10 (substrate: solution) for two hours at room temperature and autoclaved at 121°C for one hour. The treated substrates were then filtered and washed with distilled water until the wash water become neutral (Gharpuray *et al.*, 1983; Solomon *et al.*, 1999) then dried overnight at 60°C. The dried substrates were packed in polypropylene bags until use.

Proximate analysis of substrate samples: Each of the pulverized samples passed through sieve and was analyzed proximately using the AOAC (1990) method for moisture, fat and ash contents.

Screening: For plate screening, Caboxymethylcellulose-Agar (CMC-Agar) medium was used. Conidia from one week old PDA plates were suspended in sterile distilled water. A small well created in the middle of the plates and 100 ul of conidia suspension was inoculated into the wells. Plates were incubated at 28ºC for two days. For cellulolytic activity observations, plates were stained with 1% CongoRed dye for 0.5-1 h followed by destaining with 1 M NaCl solution for 15-20 min. (Onsori *et al*., 2005).

Fermentation medium and enzyme production: The fungal strain *Aspergillus niger* was cultivated at temperature of 30ºC in Vogel's medium consisting of (g/100ml): Trisodium citrate, 0.25; KH_2HPO_4, 0.50; NH_4NO_3, 0.20; $(NH_4)_2SO_4$, 0.40; $MgSO_4$. $7H_2O$, 0.02; Peptone, 0.10; yeast extract, 0.20; and 2 g of the appropriate lignocellulosic substrate. pH was adjusted to 5.5 by using 0.05 M NaOH and 1M HCl solution. The medium was then autoclaved for 15 min at 121ºC, allowed to cool and fungal spores were aseptically transferred into the flask and was kept at 30ºC on an orbital shaker for enzyme production.

Determination of mycelial weight: Biomass of the culture was removed by filtration, dried at 70°C in an oven until weight becomes constant and then measured. Fungal growth was expressed in terms of dry weight of mycelial mat (mg/flask) (Narashima *et al*., 2006;, Oshoma & Ikenebomeh, 2005).

Enzyme assays: During fermentation period , samples were withdrawn for analysis of cellulase activity at every 24 h until enzyme activity peaks off.

Filter paper activity (FPA) was determined for both the substrates by using filter paper as the substrate as proposed by Ghose (1987). It was assayed by incubating 0.5 ml of each culture supernatant with a rolled '1 by 6 cm' filter paper strip (Whatman No. 1) in one millilitre (1 ml) of 0.05 M citrate buffer (pH 4.8) contained in test tube at 50ºC for 30 min. 3.0 ml of DNS reagent was added mixed well, boiled for exactly 5.0 min. in a vigorously boiling water bath. The color formed, is measured against the spectro zero at 540 nm. Cellulase activity was calculated and expressed in International Units (IU). One unit of cellulase corresponded to the amount necessary to form 1 milligram (1 mg) of glucose per minute at 50ºC.

Endoglucanase activity was measured as described previously (Ghose, 1987). 0.5 ml of sample was taken in a test tube, 0.5 ml of 1% carboxymethylcellulose (CMC) substrate was added to the test tube, and tube was covered with aluminium foil and incubated at 50ºC for 30 min. 3.0 ml DNS was added, mixed well, boiled for exactly 5.0 min in a vigorously boiling water bath containing sufficient water. After boiling, transfered immediately to a cold water bath and measurerd the absorbance at 540 nm.

For determination of reducing sugar concentration 1 ml of culture filtrate was taken in a test tube and 3 ml of DNS reagent was added. Test tubes were placed in boiling water for 10 minutes. Cooled to room temperature and absorbance was measured at 540 nm.

Charcterization of enzyme extrtact: For determination of the optimum temperature of the enzyme, the enzyme was incubated with substrate for 30 min at various temperatures between 30 to 90ºC and then activity was measured. For determination of optimum pH, the enzyme was mixed with substrates prepared in two buffer solutions: citrate phosphate buffer (0.1 M citric acid, 0.2 M Na2HPO4, pH 3.0 to 7.0), and Tris buffer (0.08 M Tris, 0.1 M HCl, pH 8 to 9.0) and then activity was measured . The thermostability of the enzyme was studied by heating the enzyme at different temperatures (40-90ºC) for 15 min then the activity was assayed under the standard conditions. To check the effect of substrate concentration on enzyme activity, crude enzyme was incubated with different concentrations (0.5-4%) of CMC substrate and then activity was assayed like the CMC-ase assay.

Results

Proximate analysis of substrate sample: Proximate composition of substrates is presented in the Table 1.

Table 1. Proximate composition of the substrates.

Parameter %	Rice husk %	Saw dust %
Age moisture	7.5	7.8
Age ash	17.35	2.2
Age fat	8.2	2.3

Screening: A clear zone of cellulase was evident around the growing colonies as shown in Fig. 1.

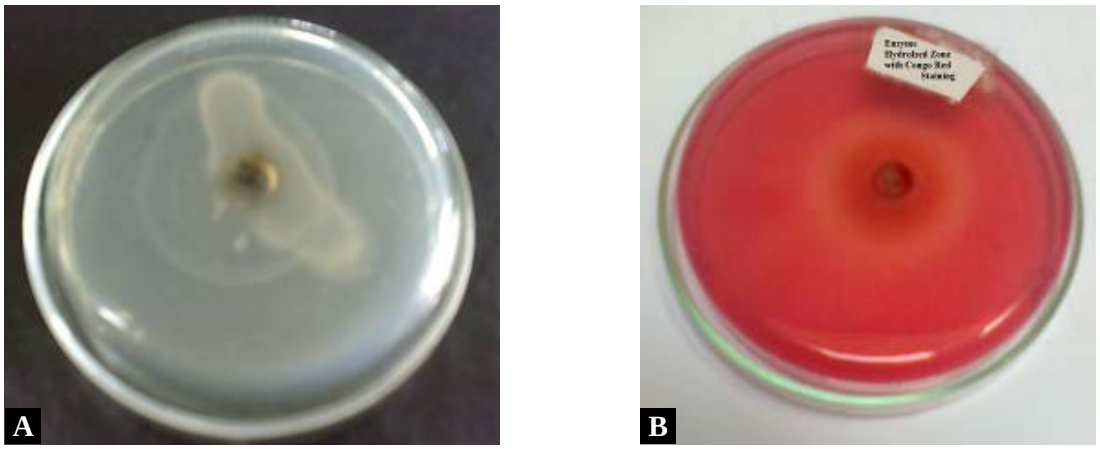

Fig. 1. Cellulase zone on CMC agar plate (A) without and (B) with stain.

Culturing of *Aspergillus niger* on substrates: During fermentation , growth and cellulolytic activities (carboxy methyl cellulase activity and filter paper activity) were monitored for 7 days and presented in Tables 2-5. Tables 2 and 3 shows pH values, the growth of *A. niger* in terms of mycelial weights, sugar concentrations and cellulase activities on untreated and pretreared rice husk. pH first decreases as the mycelial weight increases upto 5th day and then slightly increases. Mycelial weight increases first and reached at maximum on 4th day for both pretreated and untreated substrates. Maximum glucose conc. was recorded of about 0.191 ± 0.4 mg/ml on pretreated and 0.1465±0.1 mg/ml on untreated rice husk at 2nd day of fermentation shown in Table 2 and 3. Maximum CMC-aseactivity of about 13.702 ± 0.1IU/ml/min on pretreated rice husk and 7.484 ± 0.2 IU/ml/min on untreated rice husk were recorded respectively at 3rd day of fermentation. Maximum filter paper activity of about 8.028 ± 0.1 IU/ml/min on pretreated rice husk and 7.135 ± 0.6 IU/ml/min on untreated rice husk were recorded at 3rd day of fermentation (Tables 2 and 3).

In case when saw dust used as substrate value of pH first decreases as the mycelial weight increases upto 5th day and then slightly increases in both pretreated and untreated substrate. Maximum glucose conc. was recorded of about 1.423±0.2 mg/ml on pretreated and 0.178±0.8 mg/ml on untreated saw dust presented in Tables 4 and 5. Maximum CMC-ase activities of about 9.683 ± 0.2 IU/ml/min on pretreated saw dust and 5.865 ± 0.1

IU/ml/min on untreated saw dust were recorded respectively at 2nd day of fermentation shown in Tables 4 and 5. Maximum FP-ase activity of about 6.802±0.4 IU/ml/min on pretreated saw dust and 6.247 ± 0.3 IU/ml/min on untreated saw dust culture filtrate was observed respectively at 2nd day of fermentation shown in Tables 4 and 5.

Table 2. Activity of *A. niger* on untreated rice husk media.

Fermentation period (days)	pH	Dry mycelial weight (mg/flask)	Reducing sugar conc. (mg/ml)	Enzyme activity	
				CMC-ase (IU/ml/min)	FP-ase (IU/ml/min)
1	5.16 ± 0.1	198 ± 0.9	0.086 ± 0.6	2.064 ± 0.9	4.382 ± 0.4
2	4.72 ± 0.6	342 ± 0.3	0.146 ± 0.1	3.482 ± 0.5	5.447 ± 0.2
3	4.45 ± 0.2	469 ± 0.4	0.1043 ± 0.7	7.484 ± 0.2	7.135 ± 0.6
4	4.13 ± 0.3	521 ± 0.6	0.093 ± 0.2	5.427 ± 0.1	6.389 ± 0.2
5	3.97 ± 0.7	486 ± 0.1	0.0732 ± 0.4	5.396 ± 1.4	5.243 ± 0.3
6	4.27 ± 0.4	453 ± 0.2	0.069 ± 0.02	5.548 ± 0.2	4.732 ± 0.5
7	4.31 ± 0.3	398 ± 0.3	0.0335 ± 0.2	5.328 ± 1.6	4.133 ± 0.1

Results are expressed as means ± SD of triplicate analysis

Table 3. Activity of *A. niger* on pretreated rice husk media.

Fermentation period (days)	pH	Dry mycelial weight (mg/flask)	Reducing sugar conc. (mg/ml)	Enzyme activity	
				CMC-ase (IU/ml/min)	FP-ase (IU/ml/min)
1	5.22 ± 0.1	252 ± 0.8	0.109 ± 0.4	3.105 ± 0.6	5.082 ± 0.3
2	4.48 ± 0.6	394 ± 0.2	0.191 ± 0.3	9.397 ± 0.2	5.643 ± 0.1
3	3.83 ± 0.4	497 ± 0.3	0.128 ± 0.9	13.702 ± 0.1	8.028 ± 0.1
4	3.78 ± 0.2	613 ± 0.6	0.113 ± 0.1	8.887 ± 0.4	6.308 ± 0.5
5	3.47 ± 0.1	574 ± 0.1	0.053 ± 0.8	9.786 ± 0.6	5.842 ± 0.4
6	3.55 ± 0.3	536 ± 0.1	0.046 ± 0.2	10.192 ± 0.03	4.684 ± 0.1
7	3.64 ± 0.5	482 ± 0.9	0.048 ± 0.4	10.069 ± 0.2	4.615 ± 0.6

Results are expressed as means ± SD of triplicate analysis

Table 4. Activity of *A. niger* on untreated saw dust.

Fermentation period (days)	pH	Dry mycelial weight (mg/flask)	Reducing sugar conc. (mg/ml)	Enzyme activity	
				CMC-ase (IU/ml/min)	FP-ase (IU/ml/min)
1	5.41 ± 0.4	54 ± 0.2	0.535 ± 0.3	3.037 ± 0.4	3.367 ± 0.2
2	5.27 ± 0.1	71 ± 1.0	0.707 ± 0.2	5.865 ± 0.1	6.247 ± 0.3
3	5.13 ± 0.3	130 ± 0.8	0.341 ± 1.1	4.3567 ± 0.9	4.992 ± 1.1
4	4.85 ± 0.1	260 ± 0.5	0.178 ± 0.8	1.0924 ± 1.1	4.342 ± 1.2
5	4.38 ± 0.6	460 ± 0.3	0.226 ± 0.5	0.868 ± 0.6	4.738 ± 0.9
6	4.78 ± 0.5	630 ± 0.9	0.206 ± 1.0	1.734 ± 0.1	3.926 ± 0.3
7	4.98 ± 0.7	540 ± 0.3	0.206 ± 0.7	1.484 ± 0.3	3.554 ± 0.1

Results are expressed as means ± SD of triplicate analysis

Table 5. Activity *A. niger* on pretreated saw dust.

Fermentation period (days)	pH	Dry mycelial weight (mg/flask)	Reducing sugar conc. (mg/ml)	Enzyme activity	
				CMC-ase (IU/ml/min)	FP-ase (IU/ml/min)
1	5.32 ± 0.3	261 ± 0.1	1.214 ± 0.3	5.304 ± 0.5	3.502 ± 0.6
2	4.81 ± 0.1	457 ± 0.5	1.423 ± 0.2	9.683 ± 0.2	6.802 ± 0.4
3	4.64 ± 0.8	786 ± 0.7	1.131 ± 1.1	7.535 ± 0.5	5.233 ± 0.2
4	3.92 ± 0.2	951 ± 0.4	0.732 ± 0.8	5.385 ± 0.4	4.617 ± 0.1
5	3.85 ± 0.4	871 ± 0.3	0.384 ± 1.3	5.546 ± 0.3	3.344 ± 0.3
6	3.93 ± 0.7	697 ± 0.1	0.253 ± 0.1	6.232 ± 0.1	3.314 ± 0.1
7	4.02 ± 0.9	668 ± 0.3	0.226 ± 0.7	5.786 ± 0.2	3.117 ± 0.4

Results are expressed as means ± SD of triplicate analysis

Characterization of enzymes

Optimum temperature of the enzyme: The optimum temperature for the enzyme was observed to be around 40ºC as shown in Figs. 2 and 3.

Optimum pH for the enzyme: Enzyme maintained its stability over a wide range of pH 6-8, but show maximum activity at pH 5 as shown in Figs. 4 and 5.

Heat stability: Thermal stability of the CMCs-ase was studied and it was found that 40 % of the original activity was retained after heat treatment at 90°C shown in Figs. 6 and 7.

Effect of Substrate concentration on CMC-ase activity: The enzyme showed maximum activity when 2.5% substrate was used. Upto 1% there is a linear increase in the rate of reaction. Above this no linear increase was observed as shown in Figs. 8 and 9.

Discussion

Cellulase is one of the most important industrial enzyme, it is used in paper, textile, pharmaceutical, food industries, brewing and chemical industries. To reduce cost of production, the lignocellulosic substrates are used instead of synthetic cellulase due to their reasonable cost, high enzyme production capacity. In the present study, the lignocellulosic waste materials, rice husk and saw dust have been used as major carbon source for the production of cellulase enzyme by fungal strain.*Aspergillus niger* was cultured in Vogel' s medium with 2% of the substrate and incubated at 30ºC under shaking conditions for seven days and enzyme activities were monitored after every 24 hr. The study shows that the rate of utilization of cellulose in rice husk media by *Aspergillus niger* is rapid as compared to sawdust. Rice husk medium show maximum yield of cellulase at 3rd day of fermentation (growth period). Milala *et al.*, (2005), reported that optimal cellulase secretion by A*spergillus niger* was achieved at a time (growth period) of 72 hours in maize straw and rice husk media respectively.

A*spergillus niger* exhibited higher cellulolytic activitiy on 1% NaOH pretreated substrates than the untreated substrates. Pretreating the substrates with sodium hydroxide causing easy removal of the lignin and cellulose depolymerization by the separation of hydrogen bonds of cellulose (Damisa *et al*., 2008). In the present study it is revealed that pH decreases as the enzyme activity and mycelial weight increases.

In the present study maximum enzyme activity observed around pH 5 and the optimum temperature was found to be around 40 ^{0}C . According to Parry *et al.*, (1983), optimal pH for CMC-ase from *A. niger* was found to be 6.0 to 7.0. Akiba *et al*., (1995) reported that the production was high at pH 4 and 4.5 by *A. niger*. Coral *et al*., (2002) reported maximum CMCase activity at pH 4.5 and pH 7.5 by *Aspergillus niger* (Z10, wild type strain) among the tested pH range between 4.0 and 9.0.

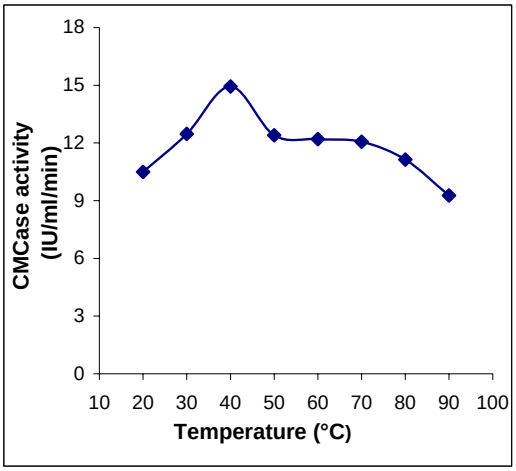

Fig. 2. Effect of temperature on CMC-ase activity in pretreated rice husk media.

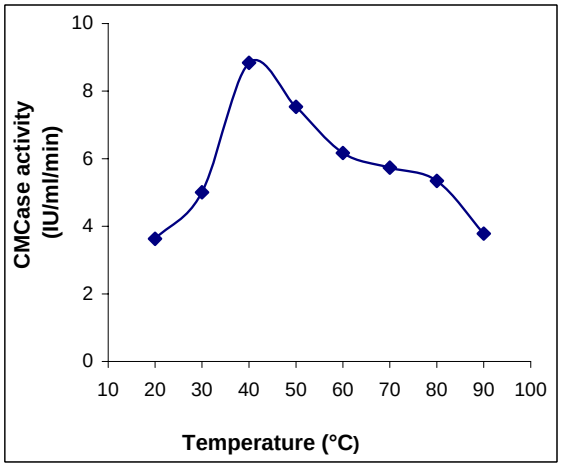

Fig. 3. Effect of temperature on CMC-ase activity in pretreated sawdust media.

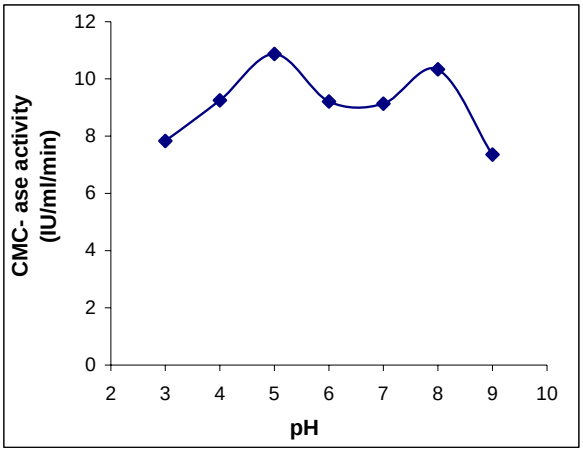

Fig. 4. Effect of pH on CMC-ase activity in pretreated rice husk media.

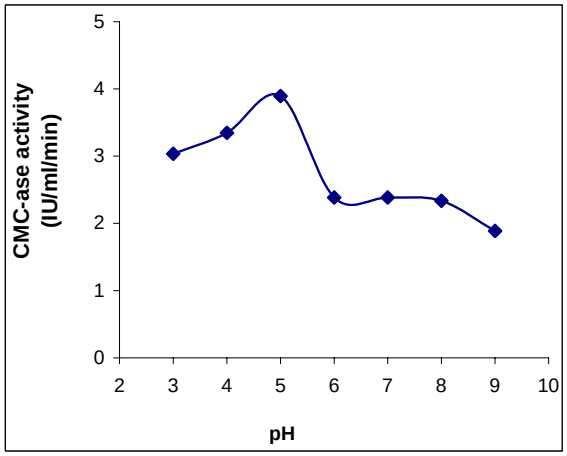

Fig. 5. Effect of pH on CMC-ase activity in pretreated sawdust media.

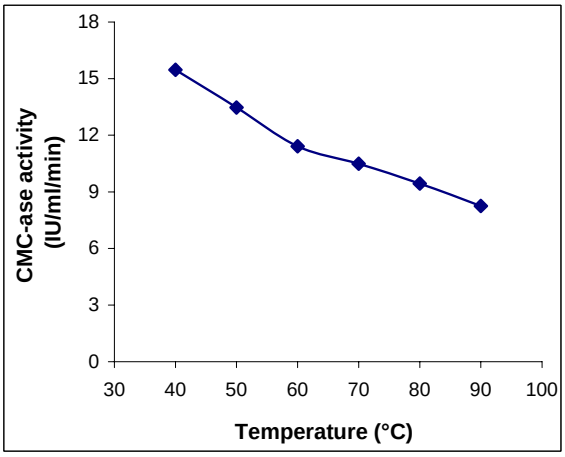

Fig. 6. The effect of heat shock on CMC-ase activity in rice husk media.

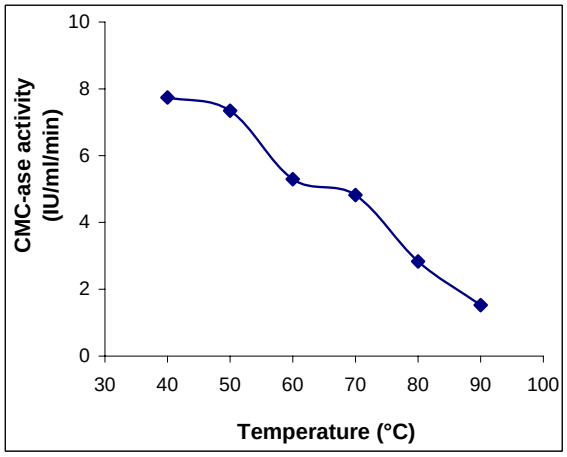

Fig. 7. The effect of heat shock on CMC-ase activity in sawdust media.

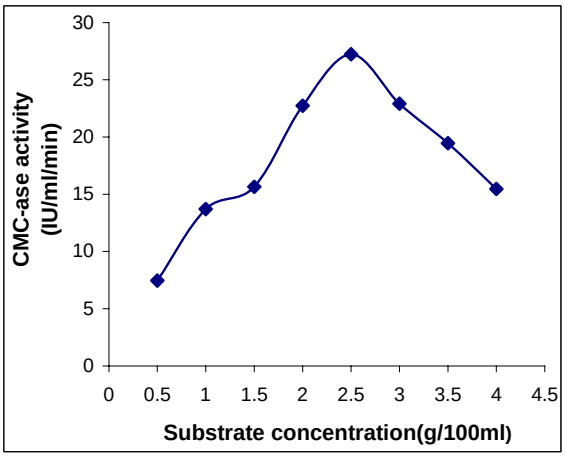

Fig. 8. Effect of substrate concentration on CMC-ase activity in rice husk media.

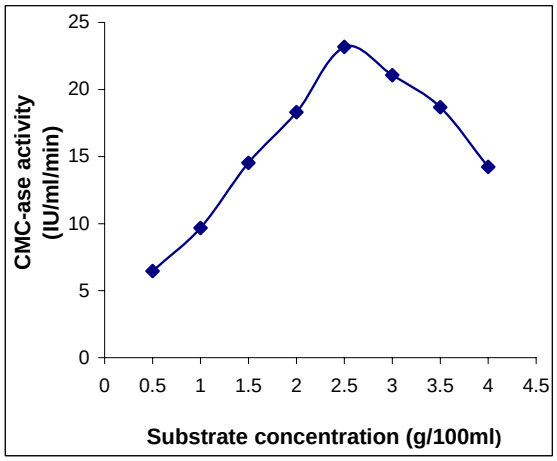

Fig. 9. Effect of substrate concentration on CMC-ase activity in sawdust media.

Conclusion

Fungal strain *A. niger* was successfully screened for cellulase enzyme production and maximum activities recorded were 13.702I ±0.1U/ml/min (rice husk) and 9.683±0.2 IU/ml/min (saw dust) for CMC-ase and 8.028±0.1U/ml/min (rice husk) and 6.802±0.4 IU/ml/min (saw dust) respectively for FPase, The optimum temperature of maximum activity was found to be around 40°C and pH was found to be around 5.0. Enzyme retained its activity over a wide pH and temperature range. The pretreatment of substrate with 1% NaOH enhanced the biosynthesis of cellulases by *A. niger*. Higher enzyme activity observed at 2.5% concentration of substrate. Among two carbon sources tested in this study, rice husk proved a good substrate for cellulase production by *A. niger* as compared to saw dust.

References

Akiba, S., Y. Kimura, K. Yamamoto and H. Kumagap. 1995. Purification and characterization of a protease-resistant cellulase from *Aspergillus niger*. *J.Ferment. Bioengin*, 79: 125-130.

Ali, S., A. Sayed, R.T. Sarker and R. Alam. 1991. Factors affecting cellulose production by *Aspergillus terreus*. *World J. Microbiol and Biotechnol.*, 7: 62-66.

Anonymous. 1990. Association of Official Analytical Chemists (AOAC). Official Methods of Analysis of Official Analytical Chemists. 15th ed. AOAC, Arlington.

Benguin, P. and J.P. Aubert. 1994. The biological degradation of cellulose. *FEMS Microbiol. Rev.*, 13: 25-58.

Bhat, M.K. 2000. Research review paper: Cellulases and related enzymes in biotechnology. *Biotechnol. Adv.*, 18: 355-383.

Coral, G., B. Arikan, M.N. Unaldi and H. Guvenmes. 2002. Some properties of crude carboxymethyl cellulase of *Aspergillus niger* Z10 wild-type Strain. *Turk J. Biol.*, 26: 209-213.

Damisa, D., J.B. Ameh and V.J. Umoh. 2008. Effect of chemical pretreatment of some lignocellulosic wastes on the recovery of cellulase from *Aspegillus niger*. *Afr. J. Biotechnol*., 7(14): 150-152 : 2444-2450.

de Vries, R.P. and J. Visser. 2001. *Aspergillus* enzymes involved in degradation of plant cell wall polysaccharides. *Microbiol Mol. Biol. Rev*., 65: 497-522.

Fan, L.T., M.M. Gharpuray and Y.H. Lee. 1987.Cellulose Hydrolysis. Berlin, Germany: *Springer-Verlag.*, 3: 1-68.

Galbe, M. and G. Zacchi. 1993. Simulation of processes for conversion of lignocellulosics. In: Bioconversion of Forest and Agricultural Plant Residues, (Ed.): Saddler. CAB International, UK, 291-342.

Gharpuray, M.M., Lee, Y.H and L.T. Fan. 1983. Structural modification of lignocellulosic by treatment to enhance enzymatic hydrolysis. *Biotechnol. Bioeng.*, 25: 157-170.

Ghose, T.K. 1987. Measurement of cellulase activities. *Pure Appl. Chem.*, 59: 257-268.

Hammel, K.E. 1997. Fungal degradation of lignin. In: Driven by Nature: Plant Litter Quality and Decomposition. (Eds.): G. Cadisch and K.E. Giller. *CAP International Wallingford*, 33-45.

Immanuel, G., C.M.A. Bhagavath, P.I. Raj, P. Esakkiraj and A. Palavesam. 2007. Production and Partial Purification of Cellulase by Aspergillus niger and A. fumigatus Fermented in Coir waste and Sawdust. *The Int. J. of Microbiol.* 3: 1-7.

Kansoh, A.L., S.A. Essam and A.N. Zeinat. 1999. Biodegradation and utilization of bagasse with *Trichoderma reesie*. *Polym. Degrad. Stab*., 63(2): 273-278.

Lynd, L.R., P.J. Weimer, W.H. van Zyl and I.S. Pretorius. 2002 Microbial cellulose utilization:fundamentals and biotechnology. *Microbiol. Mol. Biol. Rev*., 66: 506-577.

Milala, M.A., A. Shugaba, A., Gidado, A.C. Ene and J.A. Wafar. 2005. Studies on the Use of Agricultural Wastes for Cellulase Enzyme Production by *Aspegillus niger*. *Research Journal of Agriculture and Biological Sciences*, 1(4): 325-328.

Narasimha, G., A. Sridevi, V. Buddolla, C.M. Subhosh and B.R. Reddy. 2006. Nutrient effects on production of cellulolytic enzymes by Aspergillus niger. *Afr. J. Biotechnol*., 5(5): 472-476.

Ojumu, T.V., B.O. Solomom, E. Betiku, S.K. Layokun and B. Amigun. 2003. Cellulase production By *Aspergillus niger* Linn isolate NSPR 101fermented in sawdust, baggasse and corncob. *Afr. J. Biotechnol*., 2(6): 150-152.

Onsori, H., M.R. Zamani, M. Motallebi and N. Zarghami. 2005. Identification of over producer strain of endo-B-1-4-glucanase in *Aspergillus* species: Characterization of crude carboxy methyl cellulase. *Afr. J. Biotechnol.*, 4(1): 26-30.

Oshoma, C.E. and M.J. Ikenebomeh. 2005. Production of *Aspergillus niger* Biomass from Rice Bran. *Pakistan Journal of Nutrition*, 4(1): 32-36.

Parry, J.B., J.C. Stewart and J. Heptinstall. 1983. Purification of the major endoglucanase from *Aspergillus fumigatus* Freseius. *Biochem. J*., 213: 437-444.

Peij, N., M.M.C. Gielkens, R.P. Verles, K. Visser and L.H. Graff. 1998. The transcriptional activator XinR regulates both xylanolytic endoglucanase gene expression in *Aspergillus niger*. *Appl. Environ. Microbial*., 64(10): 3615-3617.

Schlesinger, W.H. 1991. Biogeo chemistry: an analysis of global change.*Academic*, *San Diego*. pp: 443.

Singh, A. and K. Hayashi. 1995. Microbial Cellulases. Protein Architecture Molecular Properties and Biosynthesis. *Adv. Appl. Microbiol*., 40: 1-35.

Solomon, B.O., B. Amigun, T.V. Betikue, T. Ojumu and S.K. Layokun. 1999. Optimization of cellulase production by *Aspergillus flavus* Linn. isolates NSPR 101 grown on baggase. *JNSChE*, 18: 61-68.

Suto, M. and F. Tomita. 2001. Induction and Catabolite repression mechanisms of cellulase in fungi. *J. Biosci. Bioeng*., 92(4): 305-311.

Tang, L.G., D.N.S. Hon, S.H. Pan, Y.Q. Zhu, Z. Wang and Z.Z. Wang. 1996. Evaluation of microcrystalline cellulose changes in ultrastructural characteristics during preliminary acid hydrolysis. *J. Appl. Polym. Sci.*, 59: 483-488.

Wood, T.M., C.A. Wilson, S.I. McCrae and K.N. Joblin. 1986. A highly extracellular cellulase from anaerobic rumen fungus *Neocallimastix frontalis*. *Microbiol. Lett*., 34: 37-40.

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