Applied Biological Research 16 (2): 223-231; (2014)
DOI: 10.5958/0974-4517.2014.00014.7

BIOPROCESSING OF BAJRA STRAW USING LOCALLY ISOLATED *Aspergillus niger* HD-6 FOR ENDOCELLULASE PRODUCTION

D.K. Acharya*, G.R. Bhojani, P.R. Prajapati, H.M. Patel and R.M. Shukla[1]

Biotechnology Department, Anand Mercantile College of Science, Management and Computer Technology, Near New S.T. Station, Bhalej Road, Anand, Gujarat – 388 001 (India)
[1]Department of Microbiology and Biofertilizer Project, B.A. College of Agriculture, Anand Agriculture University, Anand, Gujarat - 388 001 (India)
*e-mail: *dkacharya07@yahoo.com*

(Received 4 July, 2014; accepted 31 October, 2014)

ABSTRACT

***Aspergillus niger* strain HD-6, isolated from Anand, Gujarat (India), was evaluated for endocellulase enzyme production through solid state fermentation (SSF) using bajra straw as main carbon source. Various cultural conditions such as temperature, pH, moisture content, incubation period, nitrogen source, inoculum age and size, etc. were standardized for optimum enzyme production. Using 3 g bajra straw as sole carbon source maximum endocellulase production from 96 hr old *A. niger* HD-6 was achieved at pH 5.0, incubation temperature 28°C and moisture content 65% after incubation for 4 days.**

Key words: Bajra straw, endocellulase, optimization, solid state fermentation

INTRODUCTION

The major components of plant cell walls are cellulose, hemicellulose and lignin, with cellulose as the most abundant component. Plant biomass, on average, comprises of 23% lignin, 40% cellulose and 33% hemicellulose by dry weight (Ahmed *et al*., 2009). Cellulose is the most abundant renewable natural resource in the world and a potential source for the production useful materials such as fuels and chemical. Lignocellulosic biomass in the form of wood and agricultural residues is virtually inexhaustible, since their production is based on the photosynthetic process which is about 60% of the total biomass produced (Kuhad *et al*., 1997). Terrestrial plants, as per estimates, produce about 1.3×10^{10} metric tons of cellulose annum^{-1} which is energetically equivalent to about two-thirds of the world's energy requirement (Kim and Yun, 2006). Moreover, agricultural residuals or byproducts are annually renewable, abundantly available and account for >180 million tons year^{-1} (Kapdan and Kargi, 2006). However, agricultural and agro-industrial wastes require safe disposal to avoid environmental pollution (Da Silva *et al*., 2005).

Bajra or pearl millet (*Pennisetum glaucum*) is an important coarse grain crop and is considered to be poor man's food. It is believed to be the native of Africa; where from it spread to India and other countries. It is one of the important crops of South East Asia, China, India, Pakistan, Arabia, Sudan, Russia and Nigeria. In India, it is grown in the states of Haryana, Gujarat, Maharashtra,

Rajasthan and Uttar Pradesh. Bajra crop survives under adverse climatic conditions. India is the largest producer of pearl millet, both in terms of area (9.3 million ha) and production (7.97 million tonnes), with an average productivity of 856 kg ha^{-1}. It contributes 7.8% to the total food grain area of the country and 3.9% to the total food grain production (Manga and Kumar, 2011).

Agricultural wastes including bajra wastes can be converted into soluble sugars by chemical or enzymatic hydrolysis. Cellulose is degraded by enzyme cellulase produced mainly by microbes such as fungi, bacteria, seaweeds, protozoa, gastropods and arthropods. Fungi have great ability to produce cellulose degrading enzymes (Kim *et al*., 2003). For the production of cellulose degrading enzyme, microorganism must be grown on cellulosic material which has inducer effect on enzyme synthesis (Lee and Koo, 2001). Microbial enzymes are produced by using either submerged or solid-state fermentation (SSF) techniques. Currently the production of cellulose degrading enzymes is extensively studied in SSF as compared to submerged fermentation by using different microbes. The present study was aimed to assess endocellulase production by SSF of bajra straw as a model agricultural waste using *Aspergillus niger* HD-6 strain. The study was aimed to optimize various production parameters like pretreatment of bajra straw, incubation time, inoculum age and size, moistening agent, moisture content, temperature, pH and nitrogen source.

MATERIALS AND METHODS

All the chemicals used were of analytical grade. The media and chemicals used were purchased from Hi-Media, Merck, SD Fine and SRL, India. Bajra straw was collected from agricultural fields in Anand, Gujarat (India). The substrate was sun-dried for 4 days to reduce moisture content to 5% level, milled into smaller pieces (3-5 mm) (Abo-State *et al*., 2010) and stored at room temperature in polythene bags. The milled straw was used for solid state fermentation.

Isolation of cellulolytic fungi

The soil samples were collected from various sites in Anand, Gujarat. The isolation of fungi was carried out using serial dilution technique. Soil sample (1 g) was aseptically transferred to 9 mL sterile distilled water and mixed thoroughly. Then 0.1 mL soil extract was transferred to potato dextrose agar (PDA) plates and plates incubated at 28±2ºC for 96 hr. The well isolated single colonies were picked up and subculture on PDA slants periodically. The fungi were identified on culturo-morphological characters and pattern of growth on PDA (Shah *et al*., 2014) and identity got confirmed at Agharkar Research Institute, Pune (India). The strain was maintained on PDA slants by monthly transfer and stored at 4°C.

Determination of enzyme production and activity

Enzyme production was carried out in 250 mL conical flask using 3 g untreated bajra straw as carbon source by SSF technique. The moisture content was maintained between 60-70% using mineral salt medium (Ekperigin, 2007), which contained 0.01% $MgSO_4$, 0.1% $(NH_4)_2SO_4$, 0.2% KH_2PO_4, 0.7% K_2HPO_4 and 0.05% Na-citrate. The medium was adjusted to pH 5.0±0.2 and autoclaved at 121ºC for 20 min., followed by cooling and inoculation with isolated fungi using 8 mm dia. sterile cork borer. After inoculation, the flasks were incubated at 28±2ºC for 96 hr under static condition. After incubation, the enzyme was extracted with 50 mM citrate buffer (pH 4.8) by shaking the mixture at 180 rpm for 30 min. at room temperature on environmental shakes. All the contents were filtered through muslin cloth to remove mycelia. The filtrate was centrifuged at 10,000 rpm for 10 min. and the supernatant used as enzyme source (Acharya *et al*., 2010).

Endocellulase activity was determined by dinitrosalicylic acid (DNS) method (Ghose, 1986). Culture filtrate, obtained after centrifugation, was used for endocellulase assay to which 0.5 mL crude

enzyme, 1.0 mL 1% CMC and 1.0 mL 0.05 M (pH 4.8) sodium citrate buffer (w/v) was added and incubated at 50°C for 30 min. The reaction was terminated by adding 1 mL DNS reagent. The reducing sugars were measured at 540 nm (Miller, 1959). One international unit (IU) is defined as the enzymatic activity needed for the release of 1 μ mol glucose equivalents $unit^{-1}$ volume $minute^{-1}$ of reaction.

'One-variable-at-a-time' optimization for endocellulase production

The conventional 'one-variable-at-a-time' approach was followed for endocellulase production wherein the nutritional/cultural factors are optimized by changing one factor at a time and keeping other variables constant. The lignocellulosic materials used as carbon source were jawar straw, bajra straw and rice straw, where 3 g of each agro-waste was placed in each 250 mL Erlenmeyer flask with 60-70% moistening agent (mineral salt medium). After autoclaving, 5 fungal discs of 8 mm size were inoculated in each flask. All the flasks were incubated in 28±2ºC for 96 hr. The enzyme was assayed at regular intervals.

The selection of cellulolytic fungi was carried out by inoculating various isolated fungi to various flasks containing 3 g bajra straw in 60-70% moistening agent (mineral salt medium). Inoculation was carried out by inoculating 5 discs of different fungi into different flasks. After inoculation the flasks were incubated at 28±2ºC for 96 hr and enzyme assayed at regular intervals.

Pretreatment assay was carried out by soaking 10 g bajra straw in 100 mL solution ranging from 1 to 5 N NaOH and HCl in different flasks for 24 hr at room temperature. After 24 hr, bajra straw was washed with tap water, dried in oven at 50ºC and used as carbon source for fermentation.

The inoculum size was optimized by preparing the inoculum of *A. niger* HD-6 on PDA plate using sterile cork borer of 8 mm size. Different flasks, containing fermentation media, were inoculated with 3, 5, 8, 10, 13 and 15 numbers of discs of *A. niger* HD-6, respectively. Discs were transferred aseptically to the flasks and incubated at 28±2ºC. To assess the effect of age of culture on endocellulase production, 5 discs of 8 mm size of *A. niger* HD-6, with different age i.e. 72, 96, 120, 144, 168 and 192 hr were aseptically inoculated in different flask using sterile cork borer. After inoculation all the flasks were incubated in static condition at 28±2ºC.

Incubation time was optimized by incubating various flasks at 28±2ºC for 72 to 264 hr at static conditions by inoculating 5 discs of 8 mm size of *A. niger* HD-6. Enzyme assay was carried out at each 24 hr interval after 72 hr of incubation.

Moistening agent was optimized by adding various moistening agents like mineral salt medium (Ekperigin, 2007), basal medium (Kirk *et al*., 1990), Berg's medium (Berg *et al*., 1972), Mandel and Weber medium (Mandel and Weber, 1969) and Mandel and Sternberg's medium (Mandels and Sternburg, 1976); to the flasks containing 3 g bajra straw. All the flasks were autoclaved at 121ºC for 20 min. The flasks were inoculated with 5 discs of 8 mm size of the organism from PDA plate using sterile cork borer and then incubated at 28±2ºC. The moisture concentration was optimized by adding moistening agent in different amounts to the flasks containing bajra straw. The moisture levels tested were 55, 60, 65, 70, 75 and 80%. After autoclaving the media, each flask was inoculated with 5 discs of *A. niger* HD-6 and incubated at 28±2ºC.

To determine optimum pH, a set moistening agent with variable pH viz., 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, and 7.0 were used. The pH of medium was adjusted by using 1 N HCl and 1 N NaOH. After autoclaving, the medium was inoculated with 8 mm size 5 discs of *A. niger* HD-6. All the flasks were incubated in static condition at 28±2ºC. In order to determine optimum temperature, the flasks were incubated at 20, 28, 37 and 45ºC, respectively, in static condition.

The mineral salt medium was supplemented with different organic and inorganic nitrogen sources (peptone, ammonium sulfate, urea and yeast extract) at 0.5% level for optimization purpose. All the flasks were incubated at 28±2ºC in static condition for 96 hr.

The data were presented as means ± standard deviation (SD). The data were analyzed for standard deviation as per the method of Rangaswami (1995).

RESULTS AND DISCUSSION

Seventeen fungal cultures were isolated on PDA by serial dilution technique. Four fungi HD 1, HD 4, HD 6 and HD 9 were found potent cellulose degraders and screened out on the basis of their cellulase activity (Table 1). These were identified as *Aspergillus* spp., *Mucor* spp., *A. niger* and *Rhizopus* spp. The identification was got confirmed at Agharkar Research Institute, Pune (India). *A. niger* HD-6 was used for further study on the basis of higher cellulase activity.

Table 1: Cellulolytic activity of isolated fungal strain

Strain	Name of fungi	Cellulase activity (IU g^{-1})
HD 1	*Aspergillus* spp.	1.065
HD 4	*Mucor* spp.	0.308
HD 6	*A. niger*	1.183
HD 9	*Rhizopus* spp.	0.473

Selection of lignocellulosic wastes

The nature of carbon source plays important role in the production of cellulases. The use of pure substrate is costly for large scale production of enzyme so lignocellulose can be used as cost-effective substrates for lignocellulolytic enzyme production (Beg *et al*., 2000). Amongst the three agricultural wastes examined as carbon source bajra straw gave maximum endocellulase activity 2.124 IU g^{-1} (Fig. 1).

Pre-treatment

The presence of lignin and hemicelluloses make the accessibility of cellulase enzymes to cellulose difficult and reduces the efficiency of the hydrolysis, so pretreatment is necessary. NaOH was used for pretreatment purpose. Maximum endocellulase activity (3.43 IU g^{-1}) was achieved when 1 N NaOH-treated bajra straw was used (Fig. 2). Ojumu *et al.* (2003) reported that *A. flavus* grown on saw dust gave highest cellulase activity of 0.0743 IU mL^{-1}. Alkali treatment causes swelling of straw particles thereby increases its internal surface area. Swellings are caused by inter medullar ester bonds and separation of hydrogen bond holding cellulose in straw which causes depolymerization of cellulose (Ong *et al*., 2010). This results in better accessibility of cellulase enzyme to cellulose. Acid hydrolysis causes pore size distribution effect on bajra straw. Acid pretreatment was given to bajra straw with 1, 2, 3, 4 and 5 N HCl. Maximum endocellulase activity was observed at 1 N HCl (0.709 IU g^{-1}) (Fig. 3).

Incubation time

Time course plays a very critical role in fungal metabolic activity and growth. The incubation time necessary for optimal biosynthesis varied between different enzymes produced from one substrate. Therefore, enzyme production was determined after every 24 hr incubation upto 11 days. Maximum

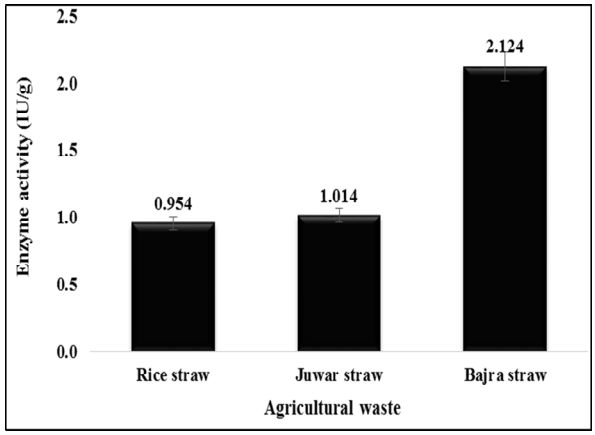

Fig. 1: Endocellulase enzyme activity using different agricultural waste

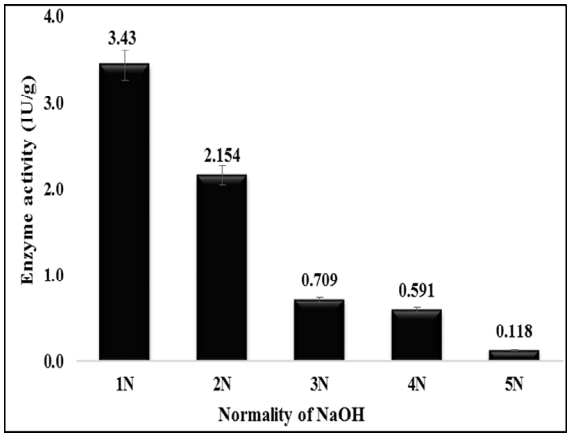

Fig. 2: Effect of alkaline pretreatment to bajra straw on endocellulase activity

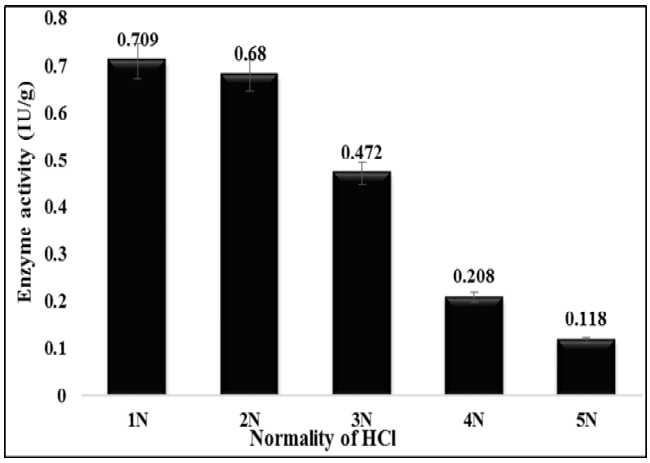

Fig. 3: Effect of acidic pretreatment to bajra straw on endocellulase enzyme production

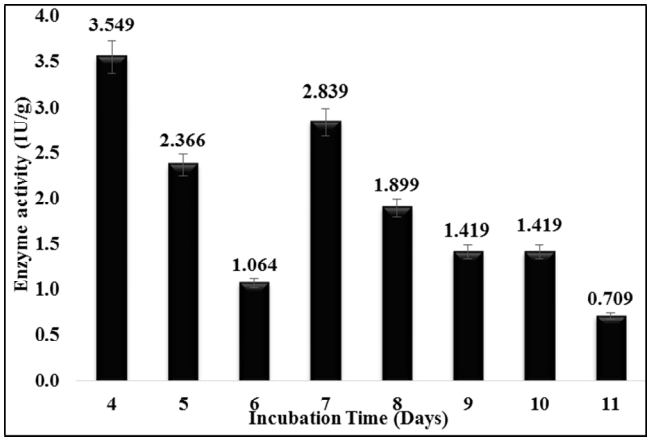

Fig. 4: Effect of incubation time on endocellulase enzyme production

Enzyme production was observed on 4th day (3.549 IU g^{-1}) (Fig. 4). Similar results have been reported by Khan *et al.* (2007). The observed decrease in enzyme activity may be attributed to the denaturation of enzymes as a result of variation in pH values and cellular metabolism during fermentation (Xin and Geng, 2010). The subsequent decrease in enzyme synthesis could probably be due to the exhaustion of nutrients and inactivation of enzyme due to interaction with constituents present in the medium. This may have stressed the fungal physiology and inactivated secretary machinery of the enzymes (Nochure *et al.*, 1993).

Age and size of inoculum

A. niger HD-6 of different ages, from 48 to 168 hr, were selected to assess their endocellulase production ability. The data revealed that initial inoculum age affected the endocellulase production (Fig. 5). *A. niger* HD-6 gave maximum activity at age of 96 hr which was 2.014 IU g^{-1}. The substances are initially more susceptible, making a rapid rise in biosynthesis of enzymes. But with the prolongation of cultural time, the susceptible portions are completely hydrolyzed by microbes which inhibit the enzyme secretion pathways (Haq *et al.*, 2006). Optimization of inoculum size is one of the important parameter which influences enzyme activity by affecting substrate utilization rate. variable inoculum sizes of *A. niger* HD-6 from 3 to 13 discs were added to culture medium. Maximum endocellulase activity (1.419 IU g^{-1}) was found when 5 discs of fungus were inoculated (Fig. 6). With increase in inoculum size gradual decrease in enzyme production was observed.

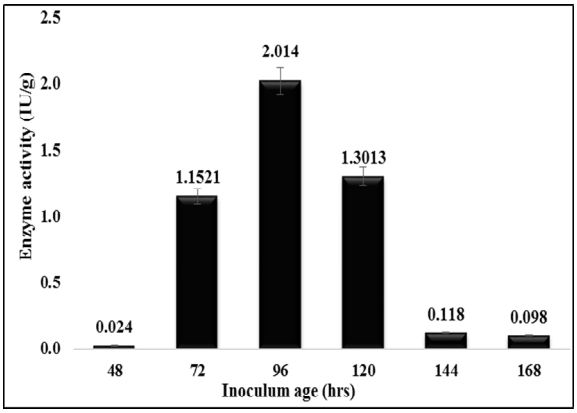

Fig. 5: Effect of inoculum age on endocellulase enzyme activity

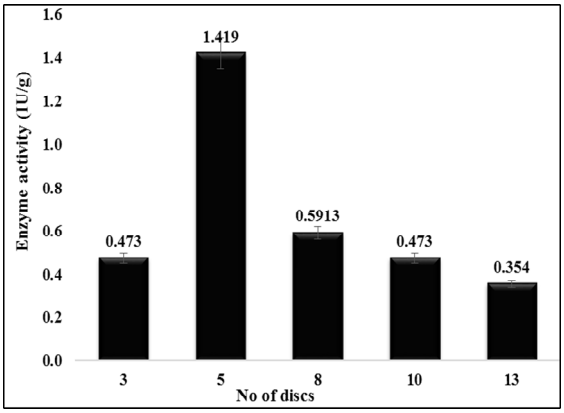

Fig. 6: Effect of inoculum size on endocellulase enzyme activity

The results revealed that the initial microbial load also affects the growth and primary metabolite production. The smaller inoculum size may extend the lag phase of fungal growth (Sharma *et al.*, 1996). An increase in inoculum size generally improves the growth and growth related activities of fungal culture to a certain level, but could cause reduction in microbial activity due to nutrient limitation. This requires longer growth time to yield optimum number to utilize the substrate and form the desired product.

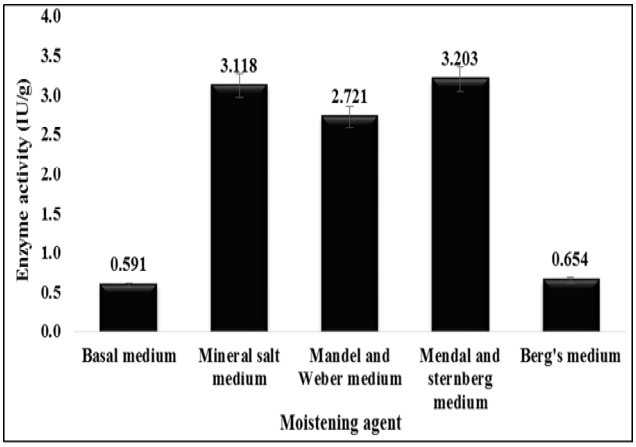

Fig. 7: Effect of moistening agent on endocellulase enzyme production

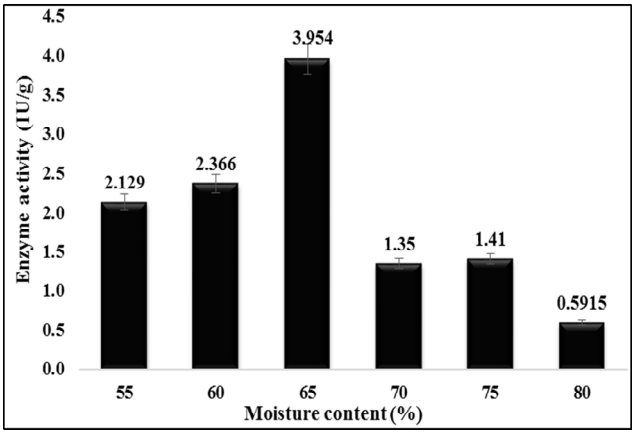

Fig. 8: Effect of moisture content on endocellulase enzyme production

Moistening agent and moisture content

The proper nutrient and moisture content in substrate is one of the critical factors affecting solid state fermentation. Moisture content in solid substrates affects both aeration and nutrient solubility. The optimum enzyme activity of 3.203 IU g^{-1} was noticed in Mendal and Sternberg medium (Fig. 7) followed by mineral salt medium (3.18 IU g^{-1}). It indicated that providing direct organic carbon along with inorganic salt gives maximum cellulase activity. From the economy point of view and for optimum enzyme production, there is need to ascertain cheap and effective fermentation medium for lignocellulolytic fungi. Ekperigin (2007) described mineral salt medium cost-effective for enzyme production.

Solid substrates used in SSF are as such insoluble in water. However; the water on the surface of substrate particles is used by microorganisms for their growth and metabolic activity. Optimum enzyme production of 3.954 IU g^{-1} was observed at 65% moisture content (Fig. 8). Similar findings have been reported by Rudravaram *et al.* (2006); Topakas *et al.* (2003); Bakri *et al.* (2003) and Beg *et al.* (2000). Lower moisture content resulted in decline in enzyme yield. Perhaps this may be due to sub-optimal growth, less substrate swelling and high water tension under low moisture conditions. The rate of water absorption varies from one substrate to another which also affects the enzyme production. Thus it appears that the degree of substrate hydration plays an important role on the growth of fungi and subsequent enzyme production (Kheng and Omar, 2005).

Effect of temperature and pH

Incubation temperature is an important physical variable affecting the performance of SSF because both cell growth and production of enzymes and metabolites are usually sensitive to temperature. *A. niger* HD-6 preferred 28ºC temperature for maximum endocellulase production (4.138 IU g^{-1}) [Fig. 8]. Higher temperatures cause changes in membrane composition and protein catabolism and inhibit fungal growth. The deleterious effect of high temperature on spore germination, cell growth, product formation, sporulation and overall productivity of fermentation process has been reported by Haq *et al.* (2005) who found 28ºC as optimum temperature for enzyme synthesis using *Trichoderma harzianum* KMO7. Similarly cellulase enzyme was produced by *Aspergillus niger*

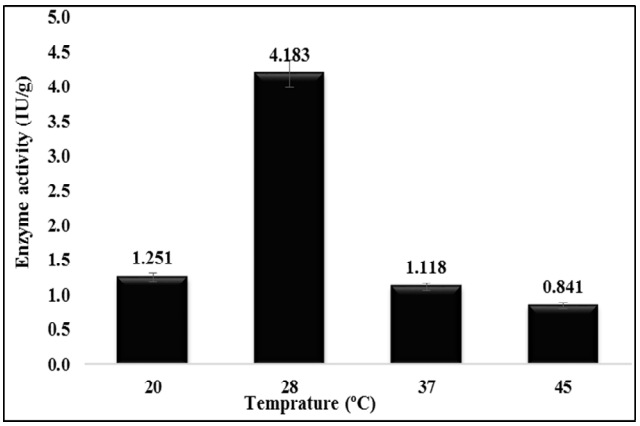

Fig. 9: Optimization of temperature for endocellulase enzyme production

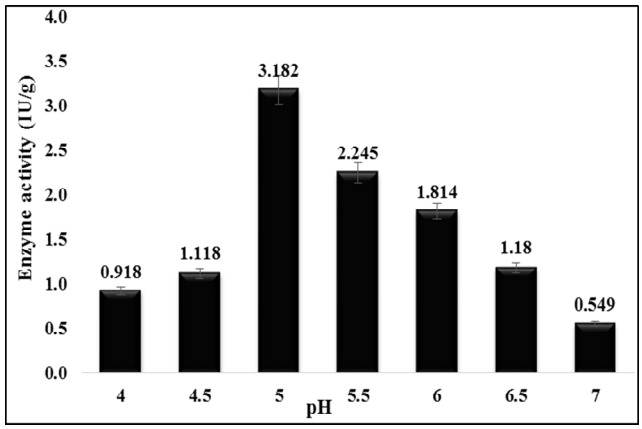

Fig. 10: Optimization of pH for for endocellulase enzyme production

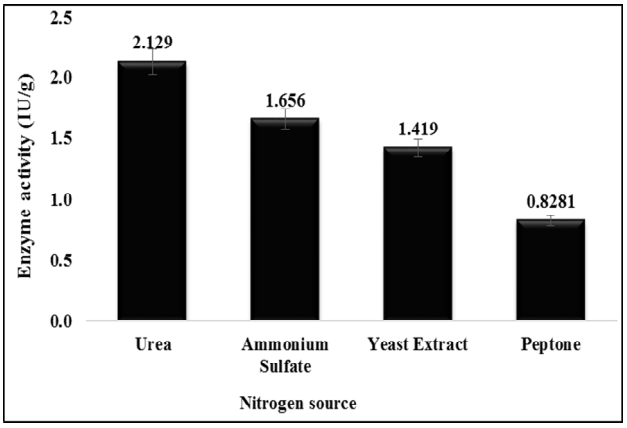

Fig. 11: Optimization of nitrogen source for endocellulase enzyme production

KK2 at 28ºC (Kang *et al.,* 2004).

High acidic and high basic pH, both showed negative effect on endocellulase production. At lower or higher pH, may be the affect stability of extracellular enzyme values and causes the rapid denaturation. Result indicated that at pH 5 endocellulase activity found maximum (3.182 IU/g). Enzyme activity increased up to pH 5 than it was decreased (Figure 10). Similar type of observations were recorded by Haq *et al*., (2006) who found optimal pH 5.5 for maximal CMCase production after incubation of 72 h for simultaneous co-culture of *A. niger* and *Trichoderma viridae*.

Nitrogen source

In this experiment, peptone, urea, ammonium sulfate and yeast extract were used to optimize nitrogen sources for maximum enzyme production. Urea gave maximum endocellulase activity which was 2.129 IU/g, followed by ammonium sulfate, yeast extract and peptone (Figure 11). It indicates that addition of nitrogen source resulted in increased growth and enzyme production, but they were not an effective replacement of inorganic nitrogen sources because of their higher cost. It also indicates that production of endocellulases is sensitive to the nitrogen source.

Conclusion: From total 17 isolates, HD 6 was identified as a potent cellulolytic micro-organism and morphologically identified at Agharkar Research Institute, Pune, Maharashtra, India, as *Aspergillus niger* HD-6. After parametric optimization, maximum endocellulase activity of *Aspergillus niger* HD-6 was observed, when pH of mineral salt medium was 5.0, incubation temperature of 28°C, by maintaining moisture content of 65%, after incubation of 96 hrs (4 days) by using 3 g bajra straw as sole carbon source with 5 discs of 96 hrs old *A. niger* HD-6.

REFERENCES

Abo-State, M.A.M, Hammad, A.I., Swelim, M. and Gannam, R.B. 2010. Enhanced production of cellulase(s) by *Aspergillus* spp. isolated from agriculture wastes by solid state fermentation. *American-Eurasian Journal of Agricultural and Environmental Sciences*, **8**: 402-410.

Acharya, D.K., Chabhadiya, S.B., Shah, A.J., Acharya, P.B., Shilpakar, P. and Modi, H.A. 2010. Enzyme profiling of lignocellulolytic fungi. *International Journal of Biological and Chemical Science*, **4**: 443-449.

Ahmed, S., Bashir, A., Saleem, H., Saadia, M. and Jamil, A. 2009. Production and purification of cellulose degrading enzymes from a filamentous fungus *Trichoderma harzianum*. *Pakistan Journal of Botany*, **41**: 1411-1419.

Bakri, Y., Jacques, P. and Thonart, P. 2003. Xylanase production by *Penicillium canescens* 10-10c in solid-state fermentation. *Applied Biochemistry and Biotechnology*, **105**: 737-747.

Beg, Q.K., Bhushan, B., Kapoor, M. and Hoondal, G.S. 2000. Enhanced production of thermostable xylanase from *Streptomyces* sp. QG-11-3 and its application in biobleaching of eucalyptus kraft pulp. *Enzyme and Microbial Technology*, **27**: 459-466.

Berg, B., Hofstan, B.V. and Petterson, B. 1972. Growth and cellulase formation by *Cellvibrio fulvus*. *Journal of Applied Bacteriology*, **35**: 201-214.

Da Silva, R., Lago, E.S., Merheb, C.W., Macchione, M.M., Park, Y.K. and Gomes, E. 2005. Production of xylanase and CMCase on solid state fermentation in different residues by *Thermoascus aurantiacusmiehe*. *Brazilian Journal of Microbiology*, **36**: 235-241.

Ekperigin, M.M. 2007. Preliminary studies of cellulase production by *Acinetobacter anitratus* and *Branhamella* sp. *African journal of Biotechnology*, **6**: 28-33.

Ghose, T.K. 1986. Measurement of cellulase activities. *Pure and Applied Chemistry*, **59**: 257-268.

Haq, I., Hameed, U., Shahzadi, K, Javed, M.M., Ali, S. and Qadeer, M.A. 2005. Cotton saccharifing activity of cellulases by *Trichoderma harzianum* UM-11 in shake flask. *International Journal of Botany*, **1**: 19-22.

Haq, I., Javed, M.M. and Khan, T.S. 2006. An innovative approach for hyperproduction of cellulolytic and hemicellulolytic enzymes by consortium of *Aspergillus niger* MSK-7 and *Trichoderma viride* MSK-10. *African journal of Biotechnology*, **5**: 609-614.

Kang, S.W., Park, Y.S., Lee, J.S., Hong, S.I. and Kim, S.W. 2004. Production of cellulase and hemicellulase by *Aspergillus niger* KK2 from lignocellulosic biomass. *Bioresource Technology*, **91**: 153-156.

Kapdan, L.K. and Kargi, F. 2006. Biohydrogen production from waste materials. *Enzyme and Microbial Technology*, **38**: 569-582.

Khan, M.D., Ali, M.H., Fakhru'l-Razi, A.S. and Alam, M.D.Z. 2007. Use of fungi for bioconversion of rice straw into cellulase enzyme. J*ournal of Environmental Science and Health Part B*, **42**: 381-386.

Kheng, P.P. and Omar, I.C. 2005. Xylanase production via solid state fermentation. *Journal of Science and Technology*, **27**: 332.

Kim, J. and Yun, S. 2006. Discovery of cellulose as a smart material. *Macromolecules*, **39**: 4202-4206.

Kim, K.C., Yoo, S.S., Oh, Y.A. and Kim, S.J. 2003. Isolation and characteristics of *Trichoderma harzianum* FJ1 producing cellulases and xylanase. *Journal of Microbiology and Biotechnology*, **13**: 1-8.

Kirk, T.K., Tien, M., Kersten, P.J., Kalyanaraman, B., Hammel, K.E., Farrell, R.L. 1990. Lignin peroxidase from fungi: *Phanerochaete chrysosporium*. *Methods in Enzymology*, **188**: 159-171.

Kuhad, R.C., Singh, A. and Eriksson, K.E.L. 1997. Microorganisms enzymes involved in the degradation of plant fiber cell walls. *Advances in Biochemical Engineering/Biotechnology,* **57**: 45-125.

Lee, S.M. and Koo, Y.M. 2001. Pilot-scale production of cellulase using *T. reesei* rut C-30 in fed-batch mode. *Journal of Microbiology and Biotechnology*, **11**: 229-233.

Mandels, M. and Sternburg, D. 1976. Recent advances in cellular technology. *Journal of Fermentation Technology*, **54**: 267-286.

Mandels, M. and Weber, J. 1969. Production of cellulases. *Advances in Chemistry Series*, **95**: 391-414.

Manga, V.K. and Kumar, A. 2011. Cultivar options for increasing pearl millet productivity in arid regions. *Indian Journal of Fundamental and Applied Life Sciences,* **1**: 200-208.

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

Nochure, S.V., Roberts, M.F. and Demain, A.L. 1993. True cellulase production by *Clostridium thermocellum* grown on different carbon sources. *Biotechnology Letters,* **15**: 641-646.

Ojumu, T.V., Solomon, B.O., Betiku, E., Layokun, S.K. and Amigun, B. 2003. Cellulase production by *Aspergillus flavus* Linn isolate NSPR 101 fermented in sawdust, bagasse and corncob. *African journal of Biotechnology,* **2**: 150-152.

Ong, L.G.A., Chuah, C. and Chew A.L. 2010. Comparison of sodium hydroxide and potassium hydroxide followed by heat treatment on rice straw for cellulase production under solid state fermentation. *Journal of Applied Sciences,* **10**: 2608-2612.

Rangaswami R. 1995. *A Text Book of Agricultural Statistics.* New Age International Pub. Ltd., New Delhi, India.

Rudravaram, R., Chandel, A.K., Linga, V.R. and Pogaku, R. 2006. Optimization of protein enrichment of deoiled rice bran by solid state fermentation using *Aspergillus oryzae* MTCC 1846. *International Journal of Food Engineering,* **2(4)**: 1-14.

Shah, I.J., Gami, P.N., Shukla, R.M. and Acharya, D.K. 2014. Optimization for α-amylase production by *Aspergillus oryzae* using submerged fermentation technology. *Basic Research Journal of Microbiology,* **1(4)**: 1-10.

Sharma, D.K., Tiwari, M. and Behere, B.K. 1996. Solid state fermentation of new substrates for production of cellulase and other biopolymer hydrolyzing enzymes. *Applied Biochemistry and Biotechnology,* **15**: 495-500.

Topakas, E., Katapodis, P., Kekos, D., Macris, B.J. and Christakopoulos, P. 2003. Production and partial characterization of xylanase by *Sporotrichum thermophile* under solid-state fermentation. *World Journal of Microbiology and Biotechnology*, **19**: 195-198.

Xin F. and Geng A. 2010. Horticulture waste as the substrate for cellulase and hemicellulase production by *Trichoderma reesei* under solid state fermentation. *Applied Biochemistry and Biotechnology,* **162**: 295-306.