



SCREENING AND OPTIMIZATION OF CULTURE CONDITIONS FOR CELLULASE PRODUCTION BY *ASPERGILLUS NIGER* NSPR012 IN SUBMERGED FERMENTATION

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ABSTRACT

This study aimed at screening of selected fungal strains and optimization of process parameters for cellulases production in submerged fermentation. *Aspergillus niger* NSPR012 was selected for further studies as the most potent in producing cellulase of high activity. Utilization of various agro-wastes as substitute to carboxy methyl cellulose (CMC) for cellulase production was also investigated. Among tested carbon sources, banana peels at a concentration of 5% was found to be the most effective carbon source. The cellulase production by *Aspergillus niger* NSPR012 in mineral salt medium attained maximum after 96 h of incubation. Maximum cellulase activity (0.466 μmol/min/mL) was obtained with locust beans as the best organic nitrogen source. The optimum incubation temperature and initial pH were 37°C and 5.5, respectively. With this information, banana peels could have good biotechnological potential for bio-products formation in which cellulase is one.

Keywords: *Aspergillus niger* NSPR012, carboxymethylcellulose, cellulases, submerged fermentation, culture conditions

INTRODUCTION

Cellulose is the most abundant polymer in the biosphere with its estimated synthesis rate of 10^{10} tonnes per year (Singh and Hayashi, 1995; Lynd *et al.*, 2002). Cellulose rich plant biomass is one of the foreseeable and sustainable sources of fuel, animal feed and feed stock for chemical synthesis (Bhat, 2000). The utilization of cellulosic biomass continues to be a subject of worldwide interest in view of fast depletion of our oil reserves and food shortages (Gong *et al.*, 1999). The conversion of cellulosic mass to fermentable sugars through biocatalyst cellulase derived from cellulolytic organisms has been suggested as a feasible process and offers potential to reduce use of fossil fuels and reduce environmental pollution (Lynd *et al.*, 1999). Complete enzymatic hydrolysis of enzymes requires synergistic action of 3 types of enzymes, namely cellobiohydrolase, endoglucanase or carboxymethylcellulase (CMCase) and 6-glucosidases (Bhat, 2000). However, the high cost of production of these enzymes has hindered the industrial application of cellulose bioconversion. One of the different approaches to overcome this hindrance is to make continuous search for organisms with secretion of cellulase enzymes in copious amounts and to optimize enzyme production with them.

Cellulases are among the industrially important hydrolytic enzymes and they have a great significance in biotechnology (Gilna and Khaleel, 2011). Cellulases are widely used in the food, feed, textile and pulp industries (Ojumu *et al.*, 2003; Hafiz *et al.*, 2010). Cellulose hydrolysis is accomplished with the aid of cellulase enzyme complex which is made up of three classes of enzymes namely exoglucanases, endoglucanases and β-glucosidase (Gautam *et al.*, 2010; Hafiz *et al.*, 2010). Cellulases are synthesized by fungi belong to the *Chaetomium*, *Aspergillus*, *Penicillium*, *Fusarium*, *Myrothecium* and *Trichoderma* species (Akinyele *et al.*, 2013) and bacteria belong to *Ruminococcus*, *Bacillus*, *Pseudomonas* species (Ohara *et al.*, 2000; Kotchoni *et al.*, 2003; Bakare *et al.*, 2005).

The aim of this study was to screen selected fungal strains provides from culture collection of the Nigerian Stored Products Research Institute Ilorin, Kwara State, Nigeria as active producers of cellulases and evaluated the influence of some culture conditions upon yield of enzymes production.

MATERIAL AND METHODS

Microorganisms

Aspergillus niger NSPR012, *A. niger* NSPR013 and *A. parasiticus* NSPR018 strains were obtained from culture collection of the Nigerian Stored

Products Research Institute Ilorin, Kwara State, Nigeria. The pure cultures were maintained on Potato Dextrose Agar (PDA) medium and subcultured once in a month. They were incubated at $30 \pm 2^\circ\text{C}$ until the entire plates were covered by active mycelium and then stored at 4°C .

Agro-wastes treatment

Palmkernel wastes, banana peels and rice bran were procured from farm fields, domestic source and market in Akure, Ondo State, Nigeria which serve as substrates. The substrates were washed and oven-dried at 70°C with DHG Heating Drying Oven (Jianqyin Linqing Machinery Co., Limited, China) for 2 h, and then milled and sieved to 40 mm mesh size and stored in air tight transparent plastic containers to keep it moisture free (Hafiz *et al.*, 2010). Agricultural wastes used as substitutes of carboxyl methyl Cellulose (CMC) (10g) were treated separately with 1000 mL of 4% NaOH solution for 24 h in Petri dishes at room temperature prior to autoclaving. The substrates were washed with distilled water until it is neutral to litmus paper and dried at 70°C in DHG Heating Drying Oven (Jianqyin Linqing Machinery Co., Limited, China) to constant weight. The alkaline effect was further neutralized with diluted HCl and then the mixture was autoclaved at 121°C for 15 min (Muthuvelayudham and Viruthagiri, 2006).

Fermentative media preparation and submerged cultivation for cellulases production

Medium composition described by Mandles and Weber (1969) was used for submerged fermentation. The media contained (g/L): peptone 1.0, urea 0.3, $(\text{NH}_4)_2\text{SO}_4$ 1.4, KH_2PO_4 2.0, CaCl_2 0.3, $\text{MgSO}_4 \cdot \text{H}_2\text{O}$ 0.3, $\text{FeSO}_4 \cdot \text{H}_2\text{O}$ 5.0 mg, $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ 1.6 mg, $\text{ZnSO}_4 \cdot \text{H}_2\text{O}$ 0.0014 g, CoCl_2 0.002 g and CMC 10g. pH of the media were adjusted to 6.5 with a pH/conductivity meter Model 20 Denver Instrument, (Systronics Limited, India) prior sterilization. Then, 100 mL of the liquid medium was placed in 250 mL Erlenmeyer flask and sterilized by autoclaving 121°C for 15 min. This was cooled and inoculated with 10 discs of 8 mm diameter of the biomass obtained by cultivation on PDA using sterile cup borer. The flasks were incubated at $30 \pm 2^\circ\text{C}$ for 5 days on a rotary shaker (Gallenkamp, Model RS-12, Singhla Scientific Industries, India) at 120 rpm. Sterile basal medium supplemented with CMC without organism served as the control. Crude enzyme preparation was obtained by centrifugation at 5000 rpm for 10 min at 4°C using refrigerated ultracentrifuge (Model KBM-70, Centurion Scientific Limited, Germany). The supernatant was used as the crude extracellular enzymes source (Gautam *et al.*, 2010).

Screening of agro-wastes (carbon sources) for cellulases production

Effects of various carbon compounds namely: palmkernel wastes, banana peels and rice bran were screened in this study with CMC serving as control. The broth was distributed into 250 mL flasks containing 50 mL optimized medium and 0.5 % of each carbon sources were then added before inoculation of the strain and after culture inoculation, the flasks were incubated for 3 days and after culture inoculation, the flasks were incubated at 30 ± 2 °C, for 3 days, at 120 rpm, in submerged conditions of cultivation on rotary shaker (Gallenkamp, Model RS-12, Singhla Scientific Industries, India) (Gautam et al., 2010).

Effect of type and concentrations of carbon and nitrogen sources on cellulases production

Soybeans, locust beans and cotton seeds at 0.2 % concentration were replacing the prescribed inorganic nitrogen source (ammonium sulphate) of the fermentation medium. Different concentrations of the banana peels ranged from 1.0 % (w/v) to 5.0 % (w/v) were added to the basal salt medium for cellulases production replacing the prescribed carbon source of the fermentation mediums (Hafiz et al., 2010).

Effect of pH and temperature on cellulases production

The pH of the fermentative medium was varied from 4.5 to 7.5. The cultivation took place in submerged conditions at 30 ± 2 °C, for 3 days, at 120 rpm. In order to determine the optimum incubation temperature for cellulases production, fermentation was carried out at 28 °C, 32 °C and 37 °C respectively (Gautam et al., 2010).

Effect of cultivation time on cellulases production

In optimal conditions before established the production of enzyme was studied during 120h of cultivation. The cellulases activity was measured at regular intervals of 24h, and the period of maximum enzyme production was determined (Milala et al., 2005).

Cellulase assay

Enzyme activity was determined using the method recommended by Acharya et al. (2008). The reaction mixture contained 0.5 mL of 0.5% of CMC as substrate prepared in 0.5 M sodium acetate buffer pH 5.5 and 0.5 mL of enzyme extract. The control sample contained the same amount of substrate and 0.5 mL of the enzyme solution heated at 100 °C for 15 min. Both the experimental and control samples were incubated at 50 °C for 30 min. At the end of the incubation period, tubes were removed from the water bath (Lamfield Medical England Model DK-600, Labnics Equipment, United Kingdom), and the reaction was terminated by addition of 3 mL of 3, 5-dinitrosalicylic acid (DNSA) reagent per tube (Shazia et al., 2010). The tubes were incubated for 5 min in a boiling water bath for color development and then were cooled rapidly. The activity of reaction mixture was measured against a blank sample at wavelength of 540 nm. The concentration of glucose released by enzymes was determined by comparing against a standard curve constructed similarly with known concentration of glucose. Unit enzyme activity was defined as the amount of enzyme required for liberating 1 μ M of glucose per millilitre per minute, in analysed conditions of reaction and was expressed as μ M/mL/min.

Protein content determination

Protein content was determined by the method of Lowry et al. (1951) using bovine serum albumin (BSA) as standard (Ghose, 1987).

Statistical analysis

Microsoft excel (Microsoft corporation, USA) was used to analyze data on the average of three replicates (\pm SE) obtained from three independent experiments.

RESULTS

Screening of fungal strains based on the ability for cellulases production on CMC

Among the tested fungal strains (*Aspergillus niger* NSPR012, *A. niger* NSPR013 and *A. parasiticus* NSPR018) on CMC as the sole carbon source in submerged fermentation, *A. niger* NSPR012 gave the highest cellulase activity of 0.161 μ mol/min/mL, and it was therefore selected for further studies (Figure 1).

Screening of agro-wastes (carbon sources) for cellulases production

The agricultural by-products were separately supplemented in mineral salt medium, and of all the agro-wastes tested, banana peels were found to be the best

carbon source for cellulases production which gave maximum cellulase activity of 0.412 μ mol/min/mL after 5 day of submerged fermentation (Figure 2). The cellulase activity of banana peel was 20.6 fold higher than what was obtained for CMC.

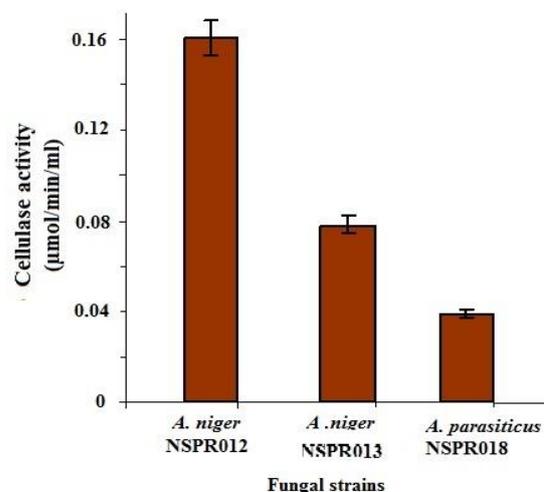


Figure 1 Microbial screening of selected fungal strains for cellulases production on CMC

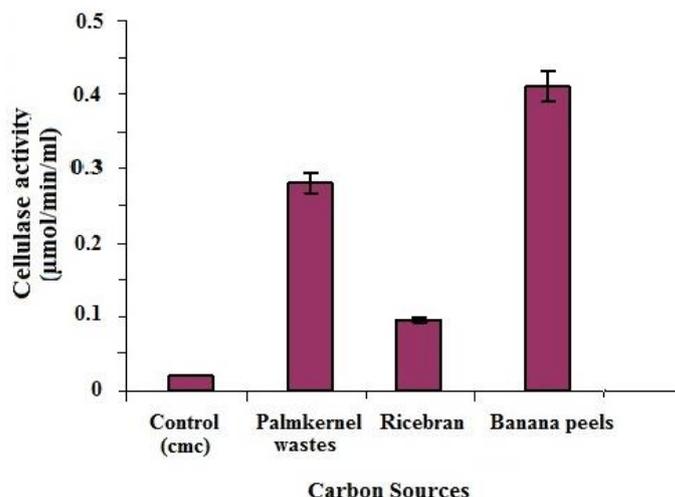


Figure 2 Effect of different agro-wastes used as carbon source on the production of cellulases by *A. niger* NSPR012

Effect of different substrate concentrations on cellulases production

Cellulase activity was studied by varying the concentration of banana peels from 1% to 5% with the exclusion of 4% (Figure 3). Of these, 5% pretreated banana peels gave maximum cellulase activity of 0.244 μ mol/min/mL. Thus, the optimum substrate concentration for maximum cellulases production was obtained at 5%.

Effect of organic nitrogen sources on cellulases production

Among the organic nitrogen sources tested at the level of 0.2% in place of ammonium sulphate, locust beans gave the maximum cellulase activity of 0.466 μ mol/min/mL while other organic nitrogen sources yielded cellulases with all liberating more than 0.140 μ mol/min/mL (Figure 4). The lowest cellulase production was obtained for ammonium sulphate ($(\text{NH}_4)_2\text{SO}_4$). The cellulase activity obtained from locust beans was approximately 3.0 higher than the ammonium sulphate (control).

Effect of pH and temperature on cellulases production

The effect of pH value on the specific cellulase activity of *A. niger* NSPR012 was investigated at various pH values ranging from 4.5 to 7.5 (Figure 5). Maximum specific cellulase activity of 0.506 μ mol/min/mg was achieved when the pH of basal medium was kept a 5.5. At pH 7.5, the specific activity decreased by 17.98% of that obtained at pH 5.5. The maximum cellulase activity of

0.457 μmol/min/mL was achieved at an incubation temperature of 37°C. At lower incubation temperatures, the enzyme activity in the culture showed slower enzyme activity.

Effect of incubation period on cellulases production

A. niger NSPR012 was inoculated into a basal medium in fermentation flasks and incubated for a period of 120 hours (Figure 6). The cellulase activity was measured at regular intervals. The maximum cellulase yield (0.850 μmol/min/mL) expressed in term of percentage relative activity was obtained after 96 h of fermentation. The production of cellulase increased progressively with increase in fermentation period and beyond the optimum incubation period (96 h) a decline in enzyme production was observed.

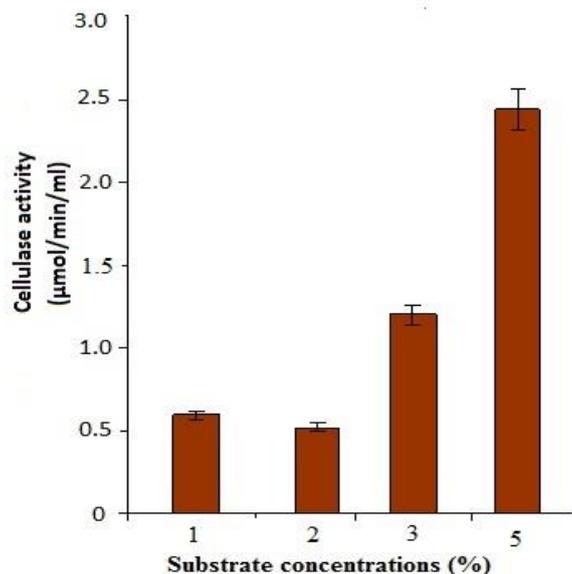


Figure 3 Effect of varying of banana peels concentrations on cellulases production by *A. niger* NSPR012

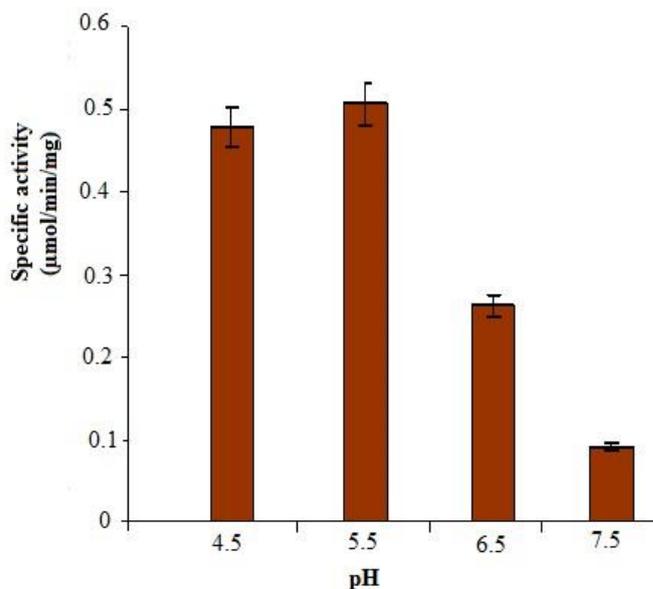


Figure 5 Theeffect of pH on cellulases production by *A. niger* NSPR012

Table 1 Variation on cellulases biosynthesis by *A. niger* NSPR012in different temperatures

Temperature (0°C)	Cellulase activity (μmol/min/m L)	Protein content (mg/mL)	Specific activity (μmol/min/mg)	Percentage relative activity (%)
28°C	0.152±0.01	4.495±0.005	0.034	33.26
32°C	0.227±0.04	4.086±0.010	0.056	49.67
37°C	0.457±0.01	6.188±0.03	0.074	100

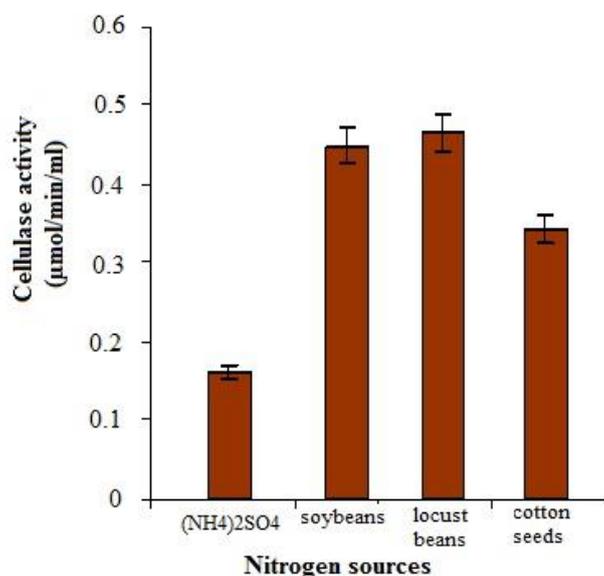


Figure 4 Effect of different nitrogen sources on the cellulases production by *A. niger* NSPR012

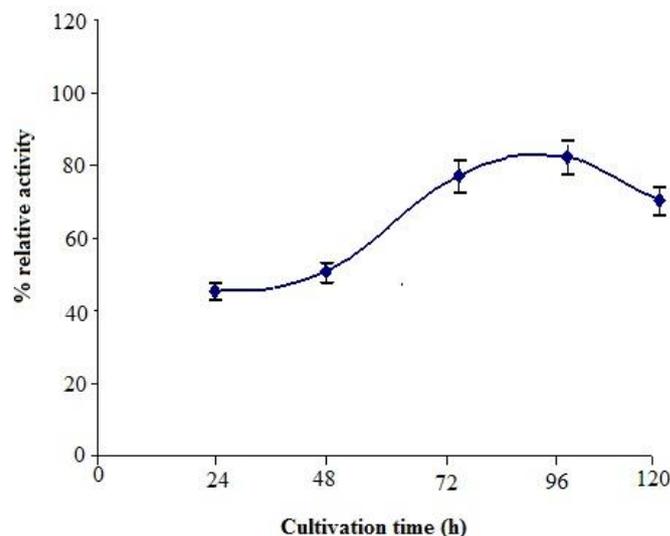


Figure 6 Time course of the cellulases production by *A. niger* NSPR012 using 5% banana peels as single carbon source

DISCUSSION

In this present work, the optimization of culture medium components and some environmental parameters could improve the cellulase activity in *A. niger* NSPR012. Major impediments to exploit the commercial potential of cellulases are the yield stability and cost of cellulase production (Sukumaran et al., 2005). Agricultural by-products such as corn cob, wheat straw, rice straw, bagasse and many others had been screened as substrates for cellulases production (Ojumu et al., 2003; Omojola et al., 2008). Although, the raw materials are cheaper, pretreatment is generally required to improve the utilizability of lignocellulosic materials and the cost is considerable (Godliving, 2009). To study the effect of pretreatment of substrates for cellulases production, alkaline pretreatment was

given to all the wastes with 2N NaOH at 4%, autoclaved at 121°C for 15 minutes in an Erlenmeyer flask 250ml to ensure proficient deprivation of lignocellulosic contents of wastes to get optimum cellulase production (Muthuvelayudham and Viruthagiri, 2006). Chemicals ranging from oxidizing agents, alkali, acids and salts had been used for pre-treatment of low cost wastes by many researchers (Godliving, 2009). When these are performed by the aforementioned chemicals, they substantially facilitate saccharification and improve enzymatic hydrolysis of low cost wastes (Godliving, 2009).

In view of the above facts, the natural waste materials such as palmkernel waste, banana peels and rice bran were effectively utilized as novel substitutes to CMC which is known to be expensive. A capacity to degrade cellulose is a character distributed among a wide variety of aerobic, facultative aerobic, anaerobic bacteria and fungi. The characters are restricted to a few species among several major taxa (Hafiz et al., 2010). The important cellulolytic fungus like *Trichoderma* sp. (Jamal and Alam, 2010; Gautam et al., 2010), *Sporotrichium* sp (Sukumar et al., 2005); *Aspergillus* sp. (Hafiz et al., 2010) and so on have been reported to have cellulolytic potential.

In the present study, three fungal strains were screened in minimal salt medium supplemented with CMC showed differences in the amount of enzyme produced. The differences suggest that the rate of cellulase production depends on the genetic composition of the microorganisms (Gautam et al., 2010). *Aspergillus niger* NSPR012 was therefore selected for further studies because of its high cellulase activity. There was variation in the amount of cellulase produced when agro-wastes were substituted in culture medium. The large variation in cellulase yield may be due to the nature of cellulose or hemicellulose, presence of some components (activators or inhibitors) in these materials and variations in the substrate accessibility (Mabrouk and Ahwany, 2008). The difference in the production of cellulolytic enzymes on a variety of lignocelluloses by different organisms could be attributed to various factors such as variable cellulose content in lignocellulose derived from different plant sources, heterogeneity of structure and cellulolytic abilities of the organisms at different degree (Narasimha et al., 2006). Among these, banana peels were found to be the most suitable waste for cellulase production and it was therefore selected for optimization studies as a carbon source replacing CMC.

Time course profile for cellulases production was studied from 24-120 h. The cellulases production increased with increase in fermentation period and reached maximum after 96 h of fermentation. Further increase in the incubation period resulted into a decline in cellulase production. Reduction in the enzyme production might be because of rapid digestion of susceptible portion of the substrate, which is required for the production and leaving crystalline portions, which cannot be used by the tested isolate for its growth and ultimate enzyme production (Omojasola et al., 2008). This result was coincident with other reports. The maximum production of cellulase was reported at 96 h of fermentation for *Aspergillus humicola* (Nipa et al., 2006).

The optimum temperature of cellulase enzyme was found to be 37°C. Many authors have reported different temperatures for maximum cellulase production either in submerged or solid state fermentation using *Aspergillus* sp. suggesting that the optimal temperature for cellulase production also depends on the strain variation of the microorganisms (Lu et al., 2003; Gautam et al., 2010). The pH values were found to be important parameters that influenced enzyme activity (Odeniyi et al., 2009). In the present study, pH 5.5 was obtained to be the best. Different optimal pH values had been reported for different microorganisms by many scientists. The Pham et al. (2010) showed that the optimum pH for cellulases production from strain of *Aspergillus niger* VTCC-F021 was 5.0. Acharya et al. (2006) also found that cellulase enzyme from *A. niger* was optimum at pH 4.0-4.5. Coral et al. (2002) reported pH optimal for a cellulase production by an *A. niger* strain was 4.5 and 7.5.

In this study, different concentrations of substrate (banana peels) ranging from 1-5% with exclusion of 4% were used and maximum cellulase activity was achieved at 5% substrate concentration. A dynamic influencing feature that affects the yield and initial hydrolysis rate of cellulose is substrate concentration (Hafiz et al., 2010). Low substrate concentration results in an increase in yield and reaction rate of the hydrolysis while, high substrate concentration can cause substrate inhibition, which substantially lowers enzymes formation (Liu and Yang, 2007; Singhania et al., 2007). This result was matched with other reports that the optimum substrate concentration for cellulase production by a strain of *Trichoderma* spp. was 5% (Gautam et al., 2010) and 5% optimum substrate concentration was also reported by Abo-State et al. (2010) for *Aspergillus* spp. Although, different optimal substrate concentrations had been reported by many researchers and this could be attributed to the chemical nature and nutrient availability of the used substrates (Gautam et al., 2010).

In this study, locust beans were selected as the best organic nitrogen source. It might be due to the fact that locust beans provided the ammonium for conidial cell growth and enzyme production (Mekala et al., 2008).

CONCLUSION

This study reports a biotechnological method for proper wastes management of agro-wastes through bioconversion processes with a filamentous fungus *A. niger* NSPR012 for cellulases production that could be used in the industrial

applications such as glucose production. The results of this study indicate the remarkable cellulases production potential of *A. niger* NSPR012 using banana peels as a substrate. The optimal culture factors for cellulase production were proposed at incubation period of 96 h, pH 5.5, 37°C, 5% banana peels and 0.2% locust beans.

REFERENCES

- ABO-STATE, M.A.M., SWELIM, M., HAMMAD, A.I., GANNAM, R.B. 2010. Some critical factors affecting cellulases production by *Aspergillus terreus* Mam-F23 and *Aspergillus flavus* Mam-F35 under solid state fermentation of wheat straw. *World Applied Science Journal*, 9(10), 1171-1179.
- ACHARYA, P.B., ACHARYA, D.K., MODI, H.A. 2008. Optimization for cellulase production by *Aspergillus niger* using saw dust as substrate. *African Journal of Biotechnology*, 7(22), 4147-4152.
- ACHARYA, P.B., ACHARYA, D.K., MODI, H.A. 2006. Optimization for cellulase production by *Aspergillus niger* using saw dust as substrate. *African Journal of Biotechnology*, 7(22), 4147-4152.
- AKINYELE, B.J., FABUNMI, A.O., OLANIYI, O.O. 2013. Effect of variations in growth parameters on cellulase activity of *Trichoderma viride* NSPR006 cultured on different wood-dusts. *Malaysian Journal of Microbiology*, 9(3), 193-200.
- BAKARE, M.K., ADEWALE, I.O., AJAYI, A.O., OKOH, A.I., SHONUKAN, O.O. 2005. Regulatory mutations affecting the synthesis of cellulase in *Pseudomonas fluorescens*. *African Journal of Biotechnology*, 4(8), 838-843.
- BHAT, M.K. 2000. Cellulases and related enzymes in biotechnology. *Biotechnology Advances*, 18, 355-383.
- CORAL, G., ARIKAN, B., UNALDI, M.N., GUVENMES, H. 2002. Some properties of crude carboxymethyl cellulase of *Aspergillus niger* Z10 wild-type strain. *Turkey Journal of Biology*, 26, 209-213.
- GAUTAM, S.P., BUDELA, P.S., PANDEY, A.K., JAMALUDDIN, A.M.K., SARSAIYA, S. 2010. Optimization of the medium for the production of cellulase by the *Trichoderma viride* using submerged fermentation. *International Journal of Environmental Sciences*, 4(1), 656-665.
- GHOSE, T.K., 1987. Measurement of cellulase activities. *International Union of Pure and Applied Chemistry*, 59(2), 257-268. <http://dx.doi.org/10.1351/pac198759020257>
- GILNA, V. V., KHALEEL, K. M. 2011. Cellulase enzyme activity of *Aspergillus fumigatus* from mangrove soil on lignocellulosic substrates. *Recent Research in Science and Technology*, 3(1), 132-134.
- GODLIVING, Y.S.M. 2009. Recent advances in pre-treatment of lignocellulosic wastes and production of value added products. *African Journal of Biotechnology*, 8(8), 1398-1415.
- GONG, C.S., CAO, N.U., DU, J., TSAO, G.T. 1999. Ethanol production by renewable sources. *Advance Biochemistry and Engineering Biotechnology*, 65, 207-241. http://dx.doi.org/10.1007/3-540-49194-5_9
- HAFIZ, M.N.T., MUHAMMAD, A., ISHTIAQ, A., SHAHBAZ, H. 2010. Media optimization for hyper-production of carboxymethylcellulase using proximally analysed agro-industrial residues with *Trichoderma harzianum* under SSF. *International Journal for Agro Veterinary and Medical Sciences*, 4(2), 47-55.
- JAMAL, I.D., ALAM, M.Z. 2010. Statistical optimization of fermentation conditions for cellulase production from palm oil mill effluent. *American Journal of Environmental Sciences*, 6(1), 66-70. <http://dx.doi.org/10.3844/ajessp.2010.66.70>.
- KOTCHONI, O.S., SHONUKAN, O.O., GACHOMO, W.E. 2003. *Bacillus pumilus* BpCRI 6, a promising candidate for cellulase production under conditions of catabolite repression. *African Journal of Biotechnology*, 2(6), 140-146.
- LIU, J., YANG, J. 2007. Cellulase production by *Trichoderma koningi* AS3.4262 in solid-state fermentation using lignocellulosic waste from vinegar industry. *Food Technology and Biotechnology*, 45, 420-425.
- LOWRY, O.H., ROSEBROUGH, N.J., FARV, A.L., RANDALL, R.J. 1951. Protein measurement with Folin Phenol reagent. *Journal of Biology and Chemistry*, 193, 265-275.
- LU, W., LI, D., WU, Y. 2003. Influence of water activity and temperature on xylanase biosynthesis in pilot scale solid-state fermentation by *Aspergillus sulphuros*. *Enzyme Microbiology and Technology*, 32, 305-311. [http://dx.doi.org/10.1016/S0141-0229\(02\)00292-2](http://dx.doi.org/10.1016/S0141-0229(02)00292-2)
- LYND, L. R., WEIMER, P. J., VAN ZYL, W. H., PRETORIOUS, I. S. 2002. Microbial cellulase utilization: Fundamentals and biotechnology. *Microbiology and Molecular Biology Review*, 66, 506-577. <http://dx.doi.org/10.1128/mmb.66.3.506-577.2002>
- LYND, L.R., WYMAN, C.E., GERNGROSS, T.U. 1999. Biocommodity engineering. *Biotechnology Programme*, 15, 777-793. <http://dx.doi.org/10.1021/bp990109e>
- MABROUK, E.M., AHWANY, M.D. 2008. Production of mannanase by *Bacillus amylolequifaciens* 10A1 cultured on potato peels. *African Journal of Biotechnology*, 7(8), 1128.
- MANDLES, M., WEBER, J. 1969. Exoglucanase activity by microorganisms. *Advanced Chemistry*, 95, 391-414. <http://dx.doi.org/10.1021/ba-1969-0095.ch023>

- MEKALA, N.K., SINGHANIA, R.R., SUKUMARAN, R.K., PANDEY, A. 2008. Cellulase production under solid-state fermentation by *Trichoderma reesei* RUT C30: Statistical optimization of process parameters. *Applied Biochemistry and Biotechnology*,151(2-3), 122-131. <http://dx.doi.org/10.1007/s12010-008-8156-9>
- MILALA, M.A., SHUGABA, A., GIDADO, A., ENE, A.C., WAFAR, J.A. 2005. Studies on the use of agricultural wastes for cellulase enzyme production by *Aspergillus niger*. *Research Journal of Agriculture and Biological Sciences*, 1(4), 325-328.
- MUTHUVELAYUDHAM, R., VIRUTHAGIRI, T. 2006. Fermentative production and kinetics of cellulase protein on *Trichoderma reesei* using sugarcane bagasse and rice straw. *African Journal of Biotechnology*, 5(20), 1873-1881.
- NARASIMHA, G., SRIDEVI, A., BUDDOLLA, V., SUBHOSH, C.M., RAJASEKHAR, R.B. 2006. Nutrient effects on production of cellulytic enzymes by *Aspergillus niger*. *African Journal of Biotechnology*, 5, 472-476.
- ODENIYI, O. A., ONILUDE, A. A., AYODELE, M. A. 2009. Production, characteristic and properties of cellulase/polygalacturonase by a *Bacillus coagulans* strain from a fermenting palm-fruit industrial residue. *African Journal of Microbiology Research*,3(8),407-417.
- OHARA, H., KARITA, S., KIMURA, T., SAKKA, K., OHMIYA, K. 2000. Characterization of the cellulolytic complex (cellulosome) from *Ruminococcus albus*. *Bioscience, Biotechnology and Biochemistry*, 64, 254-260. <http://dx.doi.org/10.1271/bbb.64.254>
- OJUMU, T.V., SOLOMON, B.O., BETIKU, E., LAYOKUN, S.K., AMIGUN, B. 2003. Cellulase production by *Aspergillus flavus* Linn isolate NSPR 101 fermented in sawdust, bagasse and corn cob. *African Journal of Biotechnology*,2, 150-152.
- OMOJASOLA, P.F., JILANI, O.P., IBIYEMI, S.A. 2008. Cellulase production by some fungi cultured on pineapple waste. *Nature and Science*, 6(2), 64-79.
- PHAM, T.H., QUYEN, D.T., NGHIEM, N. M. 2010. Optimization of endoglucanase production by *Aspergillus niger* VTCC-FO21. *Australian Journal of Basic and Applied Sciences*, 4(9), 4151-4157.
- SHAZIA, K. M., HAMID, M., AMMAD, A. F., IKRAM, U. H. 2010. Optimization of process parameters for the biosynthesis of cellulose by *Trichoderma viride*. *Pakistan Journal of Botany*, 6(42), 4243-4251.
- SINGH, A., HAYASHI, K. 1995. Microbial cellulase, protein architecture, molecular properties and biosynthesis. *Advance Applied Microbiology*, 40, 1-44. [http://dx.doi.org/10.1016/S0065-2164\(08\)70362-9](http://dx.doi.org/10.1016/S0065-2164(08)70362-9)
- SINGHANIA, R.R., SUKUMARAN, R.K., PANDEY, A. 2007. Improved cellulase production by *Trichoderma reesei*RUT C30 under SSF through process optimization. *Applied Biochemistry and Biotechnology*,142, 60-70. <http://dx.doi.org/10.1007/s12010-007-0019-2>
- SUKUMARAN, R.K., SINGHANIA, R.R., PANDEY, A. 2005. Microbial cellulases production, applications and challenges. *Journal of Sciences and Industrial Research*, 64, 832-844.