Stimulatory Effect of Physicochemical Factors on the Expression of Cellulase by *Trichoderma viride* NSPRT23

Oladipo Oladiti Olaniyi and Yomi Victor Oyesiji  
Department of Microbiology, Federal University of Technology, P.M.B. 704, Akure, Nigeria

ABSTRACT
Nutritional and environmental factors have a stimulatory effect on extracellular cellulases production. The present study dealt with the control of these factors with a view to trigger the biosynthesis of cellulases in submerged state fermentation. The process parameters optimized include; carbon sources, nitrogen sources, pH, incubation temperature, incubation time and substrate concentrations. Different fungal strains were screened for the production of cellulases in mineral salt medium in which carboxymethyl cellulose (CMC) had been incorporated as the sole carbon source. All the tested fungal strains produced cellulases with differences in the amount of cellulases produced. Of all the selected fungal isolates screened, *Trichoderma viride* NSPRT23 was found to display highest cellulase activity. Among tested carbon sources, pineapple peels at a concentration of 5% was found to be the best carbon source for cellulases production. Different organic nitrogen sources were evaluated and cotton seeds were found to be the best for cellulases production. The optimum temperature, incubation time and pH for maximum cellulases production were 37°C, 72 h and 4.5, respectively.

Key words: Carboxymethyl cellulose, submerged state fermentation, cultural conditions, carbon sources

INTRODUCTION
Cellulose is the most abundant biopolymer in nature and constitutes a large pool of carbon source for microorganisms that responsible for the decomposition of organic matter in soil (Shankar *et al*., 2011). Cellulose together with hemicelluloses, pectin and lignin are mostly found in the cell wall of green plants and are important components of agricultural wastes. These agricultural wastes are disposed on the earth indiscriminately and cause environmental pollutions (Brijwani *et al*., 2010).

Enzymes which catalyze the hydrolysis of cellulose into disaccharides, glucose unit and simple sugars are generally known as cellulases or cellulolytic enzymes. Cellulase is a family of O-glycoside enzymes that hydrolyse β-1,4-glycosidic bonds of native cellulose and other related cello-oligosaccharide derivatives. Cellulases are among the most extensively studied enzymes. They are distributed throughout the biosphere such as plants, animals and microorganisms. Cellulases are chiefly produced by microorganisms and distributed throughout the world (Chinedu *et al*., 2011).

Cellulolytic enzymes are well known for their biotechnological potential in various industries including food industries, animal feed industries, brewing and wine making, agriculture biomass
refining, pulp and paper industries, textile, laundry industries and ethanol production (Bhat, 2000). However, the cost of cellulase production and optimization profoundly influence the economics of the entire production process. Currently, these enzymes account for approximately 20% of world enzyme market. Commercially speaking, *Trichoderma reesei* is the main producing organism (Murphy and Horgan, 2005). *Trichoderma* are filamentous fungi belonging to a group of largely asexually reproducing soil fungi. They are very effective soil and rhizosphere colonizers with high biodegradation potential (Kubicek, 2004). They are cellulase producers and their crude enzymes are commercially available. *Trichoderma* have received widespread industrial interest because of their ability to produce extracellular lignocelluloses degrading enzymes in large amounts (Pandey *et al*., 1999). *Trichoderma* sp. has been known to produce cellulases for a long time and many of the enzymes have been characterized: the endo-cellulase of *Trichoderma* is possibly the most studied enzyme (Allen, 1981).

The high cost of production of these enzymes has hindered the industrial application of cellulose bioconversion. One of the approaches to overcome this hindrance is to make continuous search for the organisms that can produce cellulase in copious amounts with high catalytic efficiency and to optimize cultural conditions. The study therefore, aimed at screening of selected fungal isolates sourced from the culture collection of Nigerian Stored Products Research Institute Ilorin, Kwara State, Nigeria and evaluated the stimulatory effect of process parameters that would result in optimum cellulase production.

**MATERIALS AND METHODS**

**Microorganisms:** *Trichoderma viride* NSPRT21, *T. viride* NSPRT22 and *T. viride* NSPRT23 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±2°C until the entire plates were covered by active mycelium and then stored at 4°C.

**Treatment of agricultural wastes:** Pineapple peels, sorghum wastes and cotton seeds were procured from farm fields, domestic source and market in Akure, Ondo State, Nigeria. The substrates were washed and oven-dried at 70°C with DHG heating drying oven (Jianqyin Linqlinq Machinery Co., Limited, China) for a period of 2 h and then milled and sieved with 40 mm mesh size. The sieved substrates were stored in air tight transparent plastic containers to keep it moisture free (Iqbal *et al*., 2010). Agricultural wastes used as substitutes to Carboxyl Methyl Cellulose (CMC) 10 g 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 was neutral to litmus paper and dried at 70°C in DHG Heating Drying Oven (Jianqin Linqlinq Machinery Co., Limited, China) to constant weight. The alkaline effect was further neutralized with diluted HCl and the mixture was autoclaved at 121°C for 15 min (Muthuvelayudham and Viruthagiri, 2006).

**Fermentative media preparation and submerged cultivation for cellulase production:** Medium composition described by Mandel and Weber (1969) was used for submerged fermentation. The medium contained (g L⁻¹): peptone 1.0, urea 0.3, (NH₄)₂SO₄ 1.4, KH₂PO₄ 2.0, CaCl₂ 0.3, MgSO₄•H₂O 0.3, FeSO₄•H₂O 5.0 mg, MnSO₄•H₂O 1.6 mg, ZnSO₄•H₂O 0.0014 g, CoCl₂ 0.002 g and CMC 10 g. Hydrogen ion concentration (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 fungal strain. The flasks were incubated at 30±2°C for 5 days on a rotary shaker (Gallenkamp, Model RS-12, Singhla Scientific Industries, India) at 120 rpm. 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 agricultural wastes (carbon sources) for cellulase production:** Carbon sources namely: pineapple peels, sorghum wastes and cotton seeds were screened as substrates for cellulase production in submerged fermentation. The broth was distributed into 250 mL flasks containing 50 mL basal medium and 0.5% of each carbon sources were added before inoculation of the strain. After culture inoculation, the flasks were incubated for 3 days at 30±2°C on rotary shaker (Gallenkamp, Model RS-12, Singhla Scientific Industries, India) (Gautam et al., 2010).

**Effect of different nitrogen sources on cellulase production:** The best organic nitrogen source for cellulase production was determined by supplementing the fermentation medium with different nitrogen sources (soybeans, locust beans and cotton seeds) at 0.2% level, replacing the prescribed ammonium sulphate in the fermentation medium (Iqbal et al., 2010).

**Effect of different substrate concentrations on cellulase production:** Different concentrations of pineapple peels ranged from 1.0-5.0% (w/v) were added to the basal medium for cellulase production replacing the prescribed carbon source in the fermentation mediums (Iqbal et al., 2010). Effect of pH and temperature on cellulase production: The pH of the fermentation medium was varied from 4.5-7.5 by the use of NaOH and HCl. The fermentation took place in submerged condition at 30±2°C for 3 days. In order to determine the optimum incubation temperature for cellulase production, fermentation was carried out at 28, 32 and 37°C, respectively (Gautam et al., 2010).

**Effect of cultivation time on cellulase production:** In this study, the fermentation was carried out up to 120 h and production rate was measured at 24 h intervals to determine the best cultivation time for maximum enzyme production (Milala et al., 2005).

**Cellulase assay:** Enzyme activity was determined using the method of 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 the addition of 3 mL of 3, 5-dinitrosalicylic acid (DNSA) reagent per tube (Malik 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 μM of glucose per minute under standard experimental conditions and was expressed as μM min⁻¹ mL⁻¹.

**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 (±SE) obtained from three independent experiments.

**RESULTS**

**Screening of fungal strains for cellulase production:** The results indicated potential differences between the fungal strains to produce cellulase on CMC (Fig. 1). In shaken condition, *T. viride* NSPRT23 (0.212 μmol min⁻¹ mL⁻¹) displayed highest cellulase activity followed by *T. viride* NSPRT22 (0.104 μmol min⁻¹ mL⁻¹) while the lowest activity was recorded for *T. viride* NSPRT21 (0.09 μmol min⁻¹ mL⁻¹).

**Screening of agricultural wastes (carbon sources) for cellulase production:** In this present study, pineapple peels, sorghum wastes and cotton seeds were separately incorporated into mineral salt medium as substitutes to CMC for cellulase production by *T. viride* NSPRT23 (Fig. 2). Highest cellulase activity was observed when pineapple peels were used as the carbon source.

---

Fig. 1: Microbial screening of selected fungal strains for cellulase production on CMC

Fig. 2: Effect of different agricultural wastes (carbon sources) on cellulase production by *Trichoderma viride* NSPRT23
cellulase activity of 0.698 µmol min\(^{-1}\) mL\(^{-1}\) was observed with pineapple peels while, sorghum wastes and cotton seeds had cellulase activities of 0.155 and 0.185 µmol min\(^{-1}\) mL\(^{-1}\), respectively. The cellulase activity of *T. viride* NSPRT23 when pineapple peels was used as carbon source was observed to be 3-fold higher than CMC. However, *T. viride* NSPRT23 gave higher cellulase activity on CMC than sorghum wastes and cotton seeds.

**Effect of different substrate concentrations on cellulase production:** Different concentrations (1, 2, 3 and 5%) of pineapple peels were individually supplemented in basal medium. The optimum pineapple peels concentration was 5%, yielding the highest cellulase activity (2.829 µmol min\(^{-1}\) mL\(^{-1}\)) (Fig. 3). It was observed that enzyme activity increased with increase in substrate concentration up to 5%. Therefore, 5% substrate concentration was considered as the optimum substrate concentration for cellulase production by *T. viride* NSPRT23. The cellulase activity at 1% substrate concentration was 73.42% lower than the value obtained at 5%.

**Effect of organic nitrogen sources on cellulase production:** The effect of different nitrogen sources on the production of cellulase was studied in enzyme production medium supplemented with 5% pineapple peels (Fig. 4). Ammonium sulphate used as inorganic nitrogen source in the basal medium was replaced by soybeans, locust beans and cotton seeds, each at a time. Of the entire organic nitrogen sources tested, cotton seeds were observed to yield maximum cellulase activity (0.489 µmol min\(^{-1}\) mL\(^{-1}\)), followed by locust beans while the lowest cellulase activity was recorded with soybeans.

![Fig. 3: Effect of different pineapple peels concentrations on cellulase production by *Trichoderma viride* NSPRT23](image1)

![Fig. 4: Effect of different nitrogen sources on cellulase production by *Trichoderma viride* NSPRT23](image2)
Effect of pH and temperature on cellulase production: *Trichoderma viride* NSPRT23 selected for further studies was allowed to grow in different pH ranged from 4.5-7.5 in order to determine the optimum pH for cellulase production (Fig. 5). At pH 4.5, the isolate showed highest specific enzyme activity of 0.358 μmol min⁻¹ mg⁻¹ while further increase in the pH of the fermentation medium brought down the specific enzyme activity. At pH 7.5, the specific enzyme activity was 64.8% lower than the value obtained at pH 4.5. Thus, the optimum pH for cellulase production was 4.5. Optimization process was carried out by incubating the fermentation flasks at 28, 32 and 37°C and the cellulase activities were 0.361, 0.328, 0.528 μmol min⁻¹ mL⁻¹, respectively. Thus, as shown in Table 1, maximum cellulase production was observed at 37°C.

Effect of incubation period on cellulase production: Figure 6 shows the rate of cellulase production by *T. viride* NSPRT23 in shake flasks. The cellulase production increased with increase in incubation period and reached maximum (0.922 μmol min⁻¹ mL⁻¹) at 72 h of incubation. Further increase in the incubation period however resulted in gradual decrease in cellulase production. Therefore, 72 h of incubation was found to be the best for cellulase production by *T. viride* NSPRT23.

Table 1: Variation of cellulase biosynthesis by *T. viride* NSPRT23 in different temperatures

| Temperature (°C) | Cellulase activity (μmol min⁻¹ mL⁻¹) | Protein content (mg mL⁻¹) | Specific activity (μmol min⁻¹ mg⁻¹) | Percentage relative activity (%) |
|-----------------|--------------------------------------|---------------------------|--------------------------------------|----------------------------------|
| 28°C            | 0.361±0.02                           | 3.444±0.04                | 0.105                                | 68.37                            |
| 32°C            | 0.328±0.012                          | 3.269±0.05                | 0.100                                | 62.12                            |
| 37°C            | 0.528±0.10                           | 4.203±0.01                | 0.126                                | 100.00                           |

Fig. 5: Effect of different pH on cellulase production by *Trichoderma viride* NSPRT23

Fig. 6: Time course of cellulase production by *Trichoderma viride* NSPRT23 using 5% pineapple peels as a carbon source
DISCUSSION
Regardless of the fermentation process that is used to grow cells, it is necessary to monitor and control parameters starting from the selection of optimum carbon and nitrogen sources and including inoculum volume to moisture content, pH, temperature, incubation period and so on (Ray et al., 2007; Malik et al., 2010). Changes in one of these parameters can have a dramatic effect on the yield of cells and the stability of protein product (Ray et al., 2007). The high rate of metabolism supports the critical period of metabolite production. Consequently, adequate and timely supply of carbon and nitrogen can be key factors affecting peak productivity levels and their duration. The meaning of optimization in this context needs careful consideration of the environmental and nutritional parameters for growth and production. Medium formulation is the foremost step for designing successful laboratory experiments for yield enhancement. The medium constituents must satisfy the elemental requirement for cell biomass and metabolite production, hence, there must be adequate energy supply for biosynthesis and cell maintenance (Ray et al., 2007).

Substrate selection for an enzyme production under submerged state fermentation process depends upon several factors, mainly related with substrate cost and availability and thus may involve screening several agro-industrial residues (Howard et al., 2003). In the course of this study, pineapple peels, sorghum wastes and deteriorated cotton seeds were considered as substrates for fermentation. Therefore, they were subjected to mechanical and alkaline treatment. Pretreatment of agro-industrial wastes for cellulases production had been reported by many workers (Iqbal et al., 2010; Gilna and Khaleel, 2011). It was observed that pretreatment cause the removal of lignocellulosic contents including lignin and hemicelluloses successfully while at the same time it also causes the loosening in structure of lignin and destroy the crystallinity of cellulose that improves the porosity of substrates (Muthuvelayudham and Viruthagiri, 2006). In view of the above facts, the natural waste materials were effectively utilized as novel substitutes to carboxymethylcellulose 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 (Iqbal et al., 2010). The important cellulolytic fungus like Trichoderma sp. (Daoud and Alam, 2010; Gautam et al., 2010), Sporotrichium sp. (Sukumaran et al., 2005), Aspergillus sp. (Iqbal et al., 2010) and so on have been reported to have cellulolytic activity. In the screening program conducted for the selected fungal strains on CMC as the standard carbon source for cellulases production in submerged state fermentation, T. viride NSPRT23 showed the highest cellulase activity while others displayed lesser. The variation in the amount of cellulase revealed by individual isolate suggests that the difference in the rate of cellulase production might depend on the genetic composition of the microorganisms (Gautam et al., 2010).

The agricultural by-products were supplemented with mineral salt medium for cellulase production and of all the substrates tested, pineapple peels were found to be the most potent substrate for the production of cellulase. It might be due to the fact that the pineapple peels provided adequate amount of nutrients like proteins, carbohydrates, fat, fibers, ash, trace elements, various amino acids and porosity for oxygen supply (Bakri et al., 2003; Ikram-ul-Haq et al., 2006). Although, many investigators used CMC as a sole carbon source for the cultivation of some fungi as well as substrate for cellulase production (Nguyen and Quyen, 2010), yet few investigators used pineapple peels as a substrate for cellulase production (Omojasola et al., 2008). Reduction in the cellulases yield beyond the optimum fermentation time might be due to the depletion of nutrients and accumulation of other by-products like proteases in the fermentation
medium, resulting in the inactivation of secretory machinery of the enzymes (Liu and Yang, 2007). This is in agreement with the findings of Malik et al. (2010).

In this investigation, the optimum temperature for cellulase production was found to be 37°C. The temperature of the fermentation medium is one of the vital factors that have deep influence on the end product (Ahmed et al., 2009; Iqbal et al., 2010). Many workers have reported different temperatures for maximum cellulases production either in submerged or solid state fermentation using Trichoderma sp. This suggests that the optimal temperature for cellulases production also depends on the strain variation of the microorganisms (Gautam et al., 2010). The incubation temperature is a factor regulating the enzyme synthesis. Liu and Yang (2007) found out that maximum growth and cellulase production by Trichoderma sp. were at 25-35°C. The temperature maintained in the submerged state fermentation by Trichoderma sp., in general is in the range of 27-33°C and it depends on the growth kinetics of the microorganisms rather than on the enzyme produced (Liu and Yang, 2007).

Most microorganisms grow optimally within a wide pH range. In the present study, maximum cellulase activity was recorded at pH 4.5. Malik et al. (2010) reported maximum cellulase activity at pH 5.5 by T. viride among the tested pH range between 4.5 and 6.5, Malik et al. (2010) also reported a similar result of maximum CMCase and FPase activity at pH 5.5, Coral et al. (2002) reported maximum CMCase activity at pH 7.5 by Aspergillus niger (Z10, wild type strain) among the tested pH range between 4.0 and 9.0. Similarly, the optimal pH of 6.0 to 7.0 for maximum protease-resistant cellulase activity in A. niger was reported by Akiba et al. (1995).

In this present study, pineapple peels at 5% level proved to be the best for cellulase production by T. viride NSPRT23. This result was in conformity with other reports, that the optimum substrate concentration for cellulase production by a strain of Trichoderma spp. was 5% (Gautam et al., 2010), 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 substrate used (Gautam et al., 2010).

In this present study, cotton seeds at 0.2% level proved to be the best for T. viride NSPRT23. It might be due to the fact that cotton seeds provided both the ammonium as well as sulfate ions for conidial cell growth and enzyme production (Mekala et al., 2008).

CONCLUSION
This study dealt with the screening of different fungal strains and stimulatory effects of process parameters for maximum cellulase production. The cellulase production with T. viride NSPRT23 is effective by cultivation on medium contain 5% pineapple peels and 0.2% cotton seeds as nitrogen source, after 72 h of cultivation at pH 4.5 and 37°C.

REFERENCES
Abo-State, M.A.M., M. Swelim, A.I. Hammad and R.B. Gannam, 2010. Some critical factors affecting cellulase(s) production by Aspergillus terreus Mam-F23 and Aspergillus flavus Mam-F35 under solid-state fermentation of wheat straw. World Appl. Sci. J., 9: 1171-1179.
Acharya, P.B., D.K. Acharya and H.A. Modi, 2008. Optimization for cellulase production by Aspergillus niger using saw dust as substrate. Afr. J. Biotechnol., 7: 4147-4152.
Ahmed, S., A. Bashir, H. Saleem, M. Saadia and A. Jamil, 2009. Production and purification of cellulose-degrading enzymes from a filamentous fungus Trichoderma harzianum. Pak. J. Bot., 41: 1411-1419.
Akiba, S., Y. Kimura, K. Yamamoto and H. Kumagai, 1995. Purification and characterization of a protease-resistant cellulase from Aspergillus niger. J. Ferment. Bioeng., 79: 125-130.

Allen, A.L. and R.E. Mortensen, 1981. Production of cellulase from Trichoderma reesei in fed-batch fermentation from soluble carbon sources. Biotechnol. Bioeng., 23: 2641-2645.

Bakri, Y., P. Jacques and P. Thonart, 2003. Xylanase production by Penicillium canescens 10-10c in solid-state fermentation. Applied Biochem. Biotechnol., 108: 737-748.

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

Brijwani, K., H.S. Oberoi and P.V. Vadlani, 2010. Production of a cellulolytic enzyme system in mixed-culture solid-state fermentation of soybean hulls supplemented with wheat bran. Process Biochem., 45: 120-128.

Chinedu, S.N., V.I. Okochi and O. Omidiji, 2011. Cellulase production by wild strains of Aspergillus niger, Penicillium chrysogenum and Trichoderma harzianum grown on waste cellulosic materials. Ife J. Sci., 13: 57-62.

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

Daoud, J.I. and M.Z. Alam, 2010. Statistical optimization of fermentation conditions for cellulase production from palm oil mill effluent. Am. J. Environ. Sci., 6: 66-70.

Gautam, S.P., P.S. Bundela, A.K. Pandey, Jamaluddin, M.K. Awasthi and S. Sarasaiya, 2010. Optimization of the medium for the production of cellulase by the Trichoderma viride using submerged fermentation. Int. J. Environ. Sci., 1: 656-665.

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

Gilna, V.V. and K.M. Khaleel, 2011. Cellulase enzyme activity of Aspergillus fumigatus from mangrove soil on lignocellulosic substrate. Recent Res. Sci. Technol., 3: 132-134.

Howard, R.L., E. Abotsi, E.L.J. van Rensburg and S. Howard, 2003. Lignocellulose biotechnology: Issues of bioconversion and enzyme production. Afr. J. Biotechnol., 2: 602-619.

Ikram-ul-Haq, M.M. Javed, Z. Siddiq and T. Saleem, 2006. Triggering of β-glucosidase production in Trichoderma viride with nutritional and environmental control. J. Applied Sci. Res., 2: 884-889.

Iqbal, M.N.T., M. Asgher, I. Ahmed and S. Hussain, 2010. Media optimization for hyper-production of carboxymethyl cellulase using proximally analyzed agro-industrial residue with Trichoderma harzianum under SSF. Int. J. Agro Vet. Med. Sci., 4: 47-55.

Kubicek, C.P., 2004. Molecular Biology of Bio-Control Trichoderma. In: Fungal Biotechnology in Agricultural, Food and Environmental Applications, Arora, D.K. (Ed.). Institute of Chemical Engineering, Vienna.

Liu, J. and J. Yang, 2007. Cellulase production by Trichoderma koningii AS3.4262 in solid-state fermentation using lignocellulosic waste from the vinegar industry. Food Technol. Biotechnol., 45: 420-425.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193: 265-275.

Malik, S.K., H. Mukhtar, A.A. Farooqi and Ikram-ul-Haq, 2010. Optimization of process parameters for the biosynthesis of cellulases by Trichoderma viride. Pak. J. Bot., 42: 4243-4251.

Mandel, M. and J. Weber, 1969. Exoglucanase activity by microorganisms. Adv. Chem., 95: 391-414.

Mekala, N.K., R.R. Singhania, R.K. Sukumaran and A. Pandey, 2008. Cellulase production under solid-state fermentation by Trichoderma reesei RUT C30: Statistical optimization of process parameters. Applied Biochem. Biotechnol., 151: 122-131.
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 Aspergillus niger. Res. J. Agric. Biol. Sci., 1: 325-328.

Murphy, R.A. and K.A. Horgan, 2005. Antibiotics, enzymes and chemical commodities from Fungi. In: Fungi: Biology and Applications, Kavanagh, K. (Ed.). John Wiley and Sons Ltd, Atrium, pp: 113-143.

Muthuvelayudham, R. and T. Viruthagiri, 2006. Fermentative production and kinetics of cellulase protein on Trichoderma reesei using sugarcane bagasse and rice straw. Afr. J. Biotechnol., 5: 1873-1881.

Nguyen, V.T. and D.T. Quyen, 2010. Optimizing culture conditions for the production of endo-β-1,4-glucanase by Aspergillus awamori strain Vietnam Type Culture Collection (VTCC)-F099. Afr. J. Biotechnol., 9: 6337-6344.

Omojasola, P.F., O.P. Jilani and S.A. Ibiyemi, 2008. Cellulase production by some fungi cultured on pineapple waste. Nat. Sci., 6: 64-79.

Pandey, A., P. Selvakumar, C.R. Soccol and P. Nigam, 1999. Solid state fermentation for the production of industrial enzymes. Curr. Sci., 77: 149-162.

Ray, A.K., A. Bairagi, K.S. Ghosh and S.K. Sen, 2007. Optimization of fermentation conditions for cellulase production by Bacillus subtilis CY5 and Bacillus circulans TP3 isolated from fish gut. Acta Ichthyologica Piscatoria, 37: 47-53.

Shankar, T., V. Mariappan and L. Isaiarasu, 2011. Screening cellulolytic bacteria from the mid-gut of the popular composting earthworm, Eudrilus eugeniae (Kinberg). World. J. Zool., 6: 142-148.

Sukumaran, R.K., R.R. Singhania and A. Pandey, 2005. Microbial cellulases-production, applications and challenges. J. Scient. Ind. Res., 64: 832-844.