

## Full Length Research Paper

# Xylanase production by *Penicillium chrysogenum* (PCL501) fermented on cellulosic wastes

Okafor, U. A.<sup>1</sup>, Emezue, T. N.<sup>1</sup>, Okochi, V. I.<sup>1</sup>; Onyegeme-Okerenta, B. M.<sup>1</sup> and Nwodo-Chinedu, S.<sup>2\*</sup>

<sup>1</sup>Department of Biochemistry, College of Medicine, University of Lagos, PMB 12003 Idiaraba, Lagos, Lagos State, Nigeria.

<sup>2</sup>Department of Biological Sciences, College of Science and Technology, Covenant University, KM 10 Idiroko Road, Canaan Land, PMB 1023 Ota, Ogun State, Nigeria.

Accepted 20 August, 2007

Xylanase production by *Penicillium chrysogenum* PCL501, newly isolated from wood-wastes, was monitored at 24 h intervals for a period 168 h in media containing four different carbon sources (oat-spelt xylan, wheat bran, sawdust, and sugarcane pulp). The highest xylanase activity of 6.47 Units mL<sup>-1</sup> was obtained at 96 h in media containing wheat bran whereas media containing sugarcane pulp gave a peak value of 1.39 Units mL<sup>-1</sup> at 144 h. Sawdust and xylan gave a peak xylanase activity of 1.35 and 0.79 Units mL<sup>-1</sup> respectively at 120 h. Maximum protein released in xylan-containing media was 0.38 mg mL<sup>-1</sup>. Higher protein yield was obtained in media containing the agro-wastes, with wheat bran giving the highest value of 1.14 mg mL<sup>-1</sup>. The maximum specific xylanase activities were 2.59, 8.52, 16.06, and 9.36 Units mg Protein<sup>-1</sup> for sawdust, sugarcane pulp, wheat bran and xylan respectively. Out of the three agro-wastes used in this study, wheat bran holds the greatest promise for cost-effective production of the xylanase enzyme. The carbon source is the highest inducer of the enzyme in the fungus.

**Key words:** *Penicillium chrysogenum* PCL501, agro-wastes, wheat bran, sawdust, sugarcane pulp, xylanase.

## INTRODUCTION

Xylanolytic enzymes are receiving increasing attention because of their potential applications in improving digestibility of animal feed (Wong et al., 1988), pulp bleaching (Rifaat et al., 2005) and bioconversion of lignocelluloses into feed-stocks and fuels (Kim et al., 2000). Principal xylanolytic enzymes are endo- $\beta$ -xylanases (EC 3. 2. 1. 8), which attack the main chain of xylans, and  $\beta$ -xylosidases (EC 3. 2. 1. 37), which hydrolyze xylooligosaccharides into D-xylose. Several studies have shown that the xylanases are co-induced in response to xylan or natural substrates containing hemicellulose or even by pure cellulose (Atev et al., 1987; Ganju et al., 1989; Kadowaki and Souza, 1997). The use of waste plant materials as carbon sources in fermentation media is being studied as a cost-effective strategy for the production of the enzymes. Xylanases have been produced by *Candida utilis* using apple pomace (Villas-Boas et al.,

2002) and by *Pleurotus* species using banana wastes (Reddy et al., 2003). Xylan-rich natural substrates such as sawdust (Yu et al., 1987), corncob (Bennett et al., 1998), wheat bran (Seyis and Aksoz, 2005), sugar beet pulp (Tuohy et al., 1993), and sugarcane pulp or baggase (Prabhu and Maheshwari., 1999) have been used to stimulate xylanase production by different organisms.

Many filamentous fungi secrete high levels of plant cell wall hydrolyzing enzymes such as cellulases and xylanases into their culture media, and are employed for the hydrolysis of lignocellulosic materials (Berry and Paterson, 1990). In a search for microorganisms capable of degrading lignocelluloses in our environment, cellulolytic microfungi, including a strain of *Penicillium chrysogenum* (PCL501), were isolated from decomposing wood-wastes and characterized (Nwodo-Chinedu et al., 2005). The fungus grows effectively and produces extracellular proteins with cellulase activities in basal media containing cellulose, sawdust and sugarcane pulp (Nwodo-Chinedu et al., 2005; Nwodo-Chinedu et al., 2007a; Nwodo-Chinedu et al., 2007b). In this study, the effect of different agro-wastes on the production of xylanase enzyme (EC 3. 2.

\*Corresponding author. E-mail: [sncresearch@yahoo.com](mailto:sncresearch@yahoo.com). Tel: +234-(0)802- 862-6605.

**Table 1.** Proximate composition of the waste cellulosic materials.

Cellulosic Waste	Moisture (%)	Ash (%)	Fat (%)	Crude Protein (%)	Crude Fiber (%)	Carbohydrate (%)
Sawdust	6.1 ± 0.6	1.1 ± 0.1	6.4 ± 0.4	3.2 ± 0.2	61.0 ± 3.4	22.3 ± 1.8
Sugarcane pulp	4.3 ± 0.5	1.5 ± 0.1	5.2 ± 0.5	4.3 ± 0.4	46.1 ± 2.9	38.6 ± 2.4
Wheat Bran	10.9 ± 0.8	5.1 ± 0.3	5.7 ± 0.6	15.8 ± 0.7	27.4 ± 1.5	35.1 ± 2.3

1. 8) by the wild strain of *P. chrysogenum* PCL501 was investigated. The aim is to evaluate the potential use of the wastes materials as cost-effective substrates for xylanase production by the wild strain of *P. chrysogenum* PCL501. Our data show that of the three agro-wastes used in this study, wheat bran has the greatest prospect to serve as low-cost substrate for xylanase production using the wild strain of *P. chrysogenum* PCL501.

## MATERIALS AND METHODS

### Chemicals

All chemicals and reagents were of analytical grade. Potato Dextrose agar and crystalline cellulose were obtained from Merck, Germany. Oat spelt xylan was obtained from Fluka, Biochemika, Germany. All other chemicals and reagents were obtained from Sigma Chemicals Company Limited, England.

### Cellulosic materials

Sawdust of Abora wood (*Mitragyna ciliata*) was collected from Okobaba Saw-mills, Ebute-Metta, Lagos, Nigeria. Mature Sugarcane (*Saccharum officinarum*) was purchased from Oshodi market in Lagos, Nigeria. Sugarcane pulp was obtained by crushing and washing the pulp repeatedly in water to remove all residual sugars. Wheat bran was obtained from Mushin market in Lagos, Nigeria. The cellulosic materials were identified by the Botany unit of the Department of Botany and Microbiology, University of Lagos, Nigeria. The samples were dried in the oven at 80°C for 2 h, ground with Marlex Exceller Grinder (Mumbai, India), and passed through a sieve (about 0.5 mm pore size) to obtain the fine powder used for the study.

### Composition of the cellulosic materials

The standard method of the Association of Official Analytical Chemists (A. O. A. C) was used to determine the proximate composition of the cellulosic materials (AOAC, 1990).

### Organism

The strain of *P. chrysogenum* (PCL 501) used for this study was isolated from wood-waste dump at Okobaba sawmill, Ebute-Metta, Lagos and identified in the Department of Botany and Microbiology, University of Lagos and Federal Institute of Industrial research, Oshodi (FIIRO), Lagos, Nigeria as described previously (Nwodo-Chinedu et al., 2005). The organism was maintained at 4°C on Potato Dextrose Agar (PDA) slants. Fresh cultures were obtained

by sub-culturing on fresh sterile PDA plates and incubating at 30°C for 72 h.

### Media preparations and enzyme production

The media contained (g L<sup>-1</sup>): NaNO<sub>3</sub>, 3.0; KCl, 0.5; KH<sub>2</sub>PO<sub>4</sub>, 1.0; MnSO<sub>4</sub>.7H<sub>2</sub>O, 0.5; FeSO<sub>4</sub>. 7H<sub>2</sub>O, 0.01; and 1 % (w/v) carbon source (Sawdust, Sugarcane pulp, Wheat bran and Oat spelt xylan). One liter (1 L) of the media was supplemented with 1.0 mL of trace solution containing ZnSO<sub>4</sub>, 1.0 g L<sup>-1</sup>, and CuSO<sub>4</sub>.5H<sub>2</sub>O, 0.5 g L<sup>-1</sup>. The pH was adjusted to 5.6 before autoclaving at 121°C for 15 min. Then, 100 mL of the sterilized liquid media in 250 mL Erlenmeyer flask was inoculated with 10 discs of the organism (5.0 mm diameter) from PDA culture plates using a sterile cork borer. The cultures were incubated at 30°C with continuous agitation at 100 Osc/ min using Griffin flask shaker and harvested in triplicates at 24 h intervals by centrifugation at 1000 X g over a period of 168 h. The culture supernatants were the crude extracellular enzyme source.

### Protein assay

Protein content of the culture supernatants was determined by Lowry (1951) method using Bovine Serum Albumin (BSA) as standard.

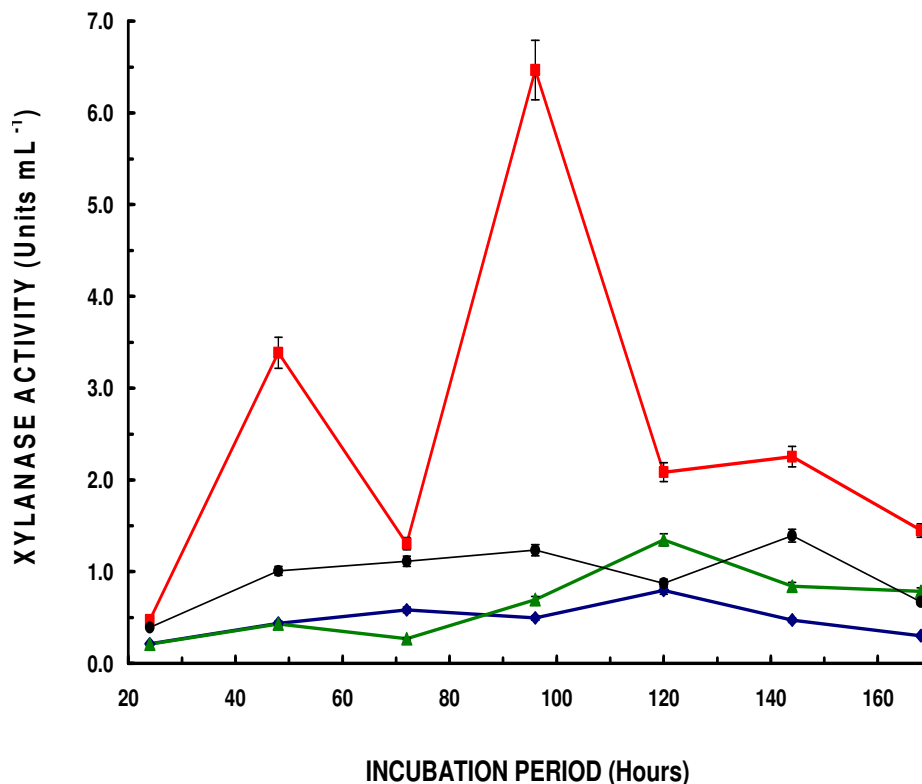
### Xylanase assay

Xylanase (EC 3. 2. 1. 8) activity was assayed by a modification of the reducing sugar method described by Khan (1980) using oat spelt xylan (Fluka) as enzyme substrate. The reaction mixture contained 0.5 mL of 0.1% (w/v) substrate in 0.1 M sodium acetate buffer (pH 5.0) and 0.1 mL of the culture supernatant. The mixture was incubated at 40°C in water bath with shaking for 30 min. Released reducing sugar was measured using 3, 5-dinitrosalicylic acid (Miller, 1959) and xylose as standard. Colour was developed by boiling in water bath for 5 min and read, using spectrophotometer (Spectronic Genesys TMS, USA), at 540 nm. One unit of activity was defined as amount of enzyme required to liberate 1 μmol of xylose per minute under the assay conditions.

## RESULTS

The proximate composition of the cellulosic waste materials (sawdust, sugarcane pulp and wheat bran) is shown in Table 1. Generally, the materials contained low levels of moisture, ash and fat. High crude protein content of 15.8 ± 0.7% was obtained for wheat bran.

Percentage crude fiber contents of 61.0 ± 3.4, 46.1 ± 2.9 and 27.4 ± 1.5 respectively was recorded for sawdust, sugarcane pulp and wheat bran.



**Figure 1.** Xylanase activity of *Penicillium chrysogenum* PCL501 cultured at 30 °C in liquid media containing sawdust (▲), sugarcane pulp (●), wheat bran (■), and oat spelt xylan (◆) as sole carbon sources. (1 Unit of xylanase activity = 1  $\mu\text{mol Xylose min}^{-1}$ ).

Figure 1 shows the xylanase activity of the culture supernatant of *P. chrysogenum* (PCL501) from the different media. Xylanase activity of the culture supernatants increased as incubation progressed to a peak value, after which it declined. Xylanase activity peak of 6.47 Units  $\text{mL}^{-1}$  was obtained at 96 h from media wheat bran-containing culture. The media containing sugarcane pulp gave two xylanase activity peaks of 1.23 and 1.39 Units  $\text{mL}^{-1}$  respectively at 96 and 144 h. Peak xylanase activity of 1.34 and 0.79 Units  $\text{mL}^{-1}$  respectively was obtained at 120 h from the culture containing sawdust and xylan.

The protein content of the culture supernatants is shown in Figure 2. Higher protein levels were obtained from cultures containing the agro-wastes compared to the culture containing oat spelt xylan. Highest protein value of 1.14  $\text{mg mL}^{-1}$  was obtained at 96 h from the culture containing wheat bran. Peak protein values of 0.52 (at 120 h) and 0.51  $\text{mg mL}^{-1}$  (at 96 h) were obtained from cultures containing sawdust and sugarcane pulp respectively. The peak protein value obtained from xylan-containing cultures was 0.38  $\text{mg mL}^{-1}$  at 120 h.

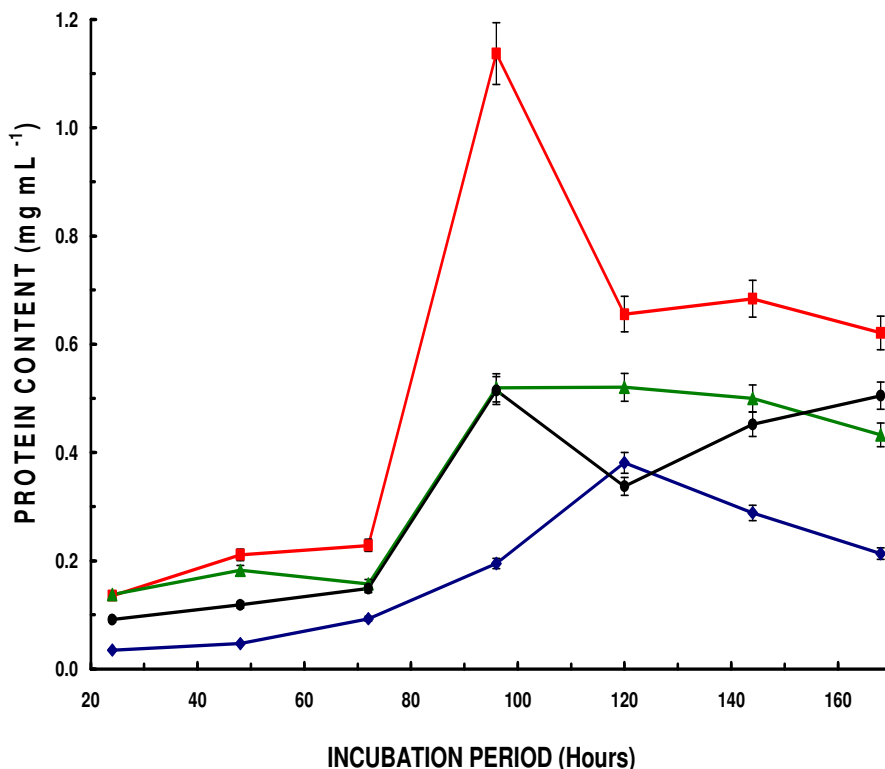
Figure 3 shows the specific xylanase activity of the cultures of *P. chrysogenum* (PCL501) using the different carbon sources. The highest specific xylanase activity value of 16.06 Units  $\text{mg Protein}^{-1}$  was obtained at 48 h from the culture containing wheat bran. This was higher

than the peak value of 9.36 Units  $\text{mg Protein}^{-1}$  obtained from xylan-containing culture. Maximum specific activity of 2.59 Units  $\text{mg Protein}^{-1}$  was obtained with sawdust at 120 h while sugarcane pulp gave a maximum activity of 8.52 Units  $\text{mg Protein}^{-1}$  at 48 h.

## DISCUSSION

Extracellular proteins with significant xylanase activity were obtained from the cultures of all the carbon sources. This shows that the xylanase enzyme (EC 3. 2. 1. 8) of *P. chrysogenum* PCL501 is induced by xylan as well as the agro-wastes (sawdust, sugarcane pulp and wheat bran).

The xylanase activity of the organism varied with the carbon source of the culture medium. Maximum xylanase activity of 0.79, 1.35, 1.39, and 6.47 unit  $\text{mL}^{-1}$  respectively were obtained with xylan, sawdust, sugarcane pulp and wheat bran. This is quite significant considering the fact that the activities obtained with the agro-wastes were higher than that obtained with xylan as sole carbon source. Several workers have reported various levels of xylanase activities by different microorganisms. Comparing of this activity value is difficult due to differences in the cultural and assay conditions employed by the different researchers. For instance, in the study of xylanase production by *Aspergillus japonicus* cultivated on wheat bran, xyla-



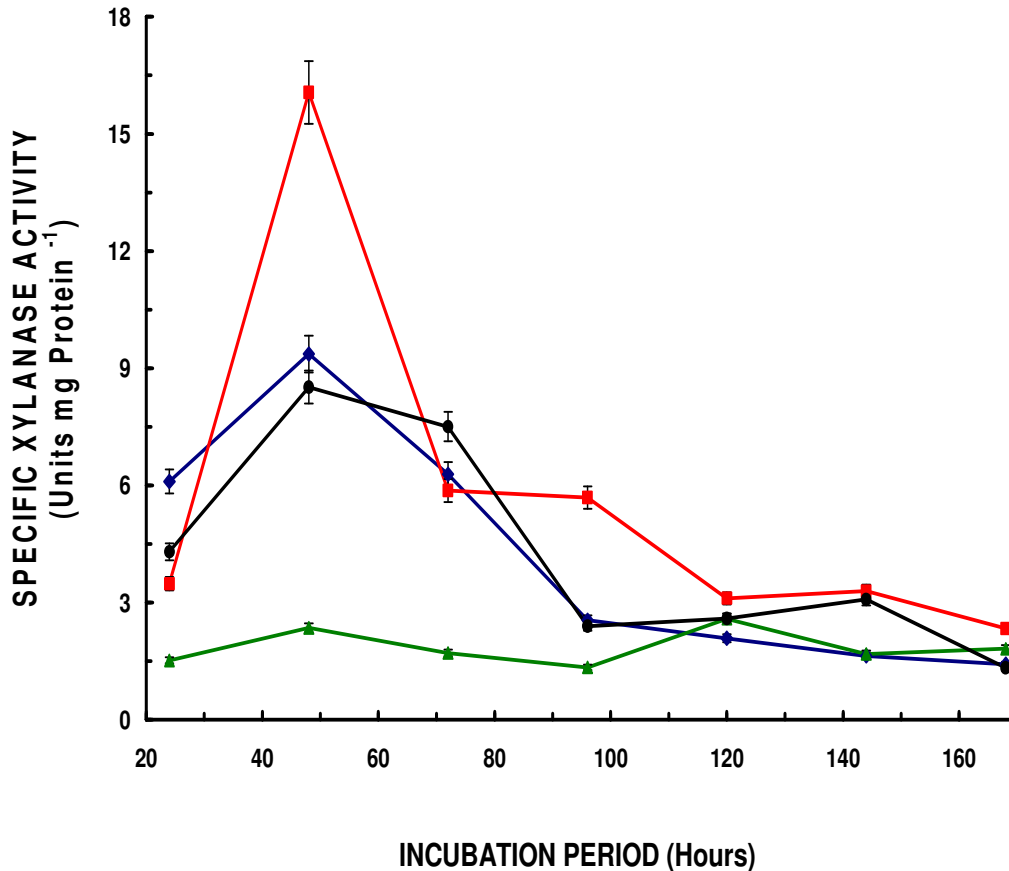
**Figure 2.** Protein content of culture supernatant of *Penicillium chrysogenum* PCL501 incubated at 30 °C in liquid media containing sawdust(▲), sugarcane pulp (●), wheat bran (■), and oat spelt xylan (◆) as sole carbon sources.

nase activity of 143.9 Unit mL<sup>-1</sup> was obtained when solid-state fermentation (SSF) was employed compared to 18.3 Unit mL<sup>-1</sup> obtained using submerged fermentation (Simoes and Tank-Tornisielo, 2006). Maximal xylanase activity as high as 1447 Unit mL<sup>-1</sup> have been reported for *Streptomyces sp.* (strain 1b 24D) using tomato pomace as culture substrate (Rawashdeh et al., 2005). Isil and Nilufer (2005) reported a xylanase activity values of 480.1 Unit mL<sup>-1</sup> and 185 Units g<sup>-1</sup> for *Trichoderma harzianum* 1073 D3 using liquid and semi-solid state media respectively whereas Rezende et al. (2002) reported a recovery of 15.0, 10.4, and 6.6 Unit mL<sup>-1</sup> at first, second and third extractions from cultures of *T. harzianum* RIFAI cultivated on milled sugarcane bagasse using solid state fermentation. A peak xylanase activity of up to 27 Unit mL<sup>-1</sup> was reported for anaerobic fungus, *Neocallimastix frontalis*, (Mountfort and Asher, 1989). Submerged fermentation was used in the present study. The maximum specific xylanase activity for the organism is 9.36, 2.36, 8.52, and 16.06 Unit mg protein<sup>-1</sup> respectively with xylan, sawdust, sugarcane pulp and wheat bran.

The ability of the organism to produce xylanase enzyme as shown by the present result also validates the use of *P. chrysogenum* mycelium in animal feed pretreatment (Nuero and Reyes, 2002). The organism has been shown to produce cellulases (Nwodo-Chinedu et al., 2007b). This is in line with the reports of several workers using

different agro-wastes to produce xylanase (Atev et al., 1983; Kadowaki and Souza, 1997; Simoes and Tank-Tornisielo, 2005).

The period for optimum xylanase activity varied with the different carbon sources. The time of optimum xylanase activity was 96 h for wheat bran, 120 h for xylan and sawdust, and 144 for sugar cane pulp. In terms of enzyme activity levels, the optimum activity obtained with wheat bran was over 4 times the amount obtained with sawdust and sugarcane pulp and over 8 times that obtained with xylan (Figure 1). The protein and xylanase activity profiles of the crude enzyme preparations obtained from the different carbon sources also differed significantly. Higher levels of protein were secreted by the organism in media containing wheat bran compared to the media containing any of the other carbon sources (Figure 2). The lowest protein level was found in media containing xylan. This is the basis for the high specific xylanase activity obtained in xylan containing media (Figure 3). In spite of the high protein level of the media containing wheat bran, the maximum specific xylanase activity of 16.06 Units mg Protein<sup>-1</sup> obtained was about twice that obtained with xylan and sugarcane pulp, and over 6 times that obtained with sawdust. This implies that of all the materials used in the present study, wheat bran is the best prospective carbon source for the production of the enzyme using the wild strain of *P. chrysogenum*



**Figure 3.** Specific xylanase activity of culture supernatant of *Penicillium chrysogenum* PCL501 cultured at 30 °C in liquid media containing sawdust (▲), sugarcane pulp (●), wheat bran (■), and oat spelt xylan (◆) as sole carbon sources. (1 Unit of xylanase activity = 1  $\mu\text{mol Xylose min}^{-1}$ ).

PCL501. In a study for the optimization of xylanase biosynthesis using wheat bran and soybean bran, Simoes and Tank-Tornisielo (2005) reported that the best xylanase activity by *A. japonicus* was achieved when the organism was cultivated in wheat bran. In a similar work carried out in our laboratory using a wild strain of *Aspergillus niger* also isolated from wood-wastes, wheat bran was also found to be the preferred substrate for xylanase production (Okafor et al., 2007). This work therefore supports the report of high xylanase production using wheat bran as sole carbon source.

### Conclusion

In conclusion, our data has shown that the strain of *P. chrysogenum* (PCL501) is capable of producing extracellular proteins with significant xylanase activity when cultivated in media containing the different agro-wastes as sole carbon sources. It also reveals that of the three agro-waste materials studied, wheat bran is the best substrate for xylanase production. Wheat bran is an agro-industrial waste produced in large quantities in Nigeria by brewing industries and many flour mills (Abu et al., 2000).

The use of the agro-wastes in the production of such enzymes as xylanases will ultimately bring down their production cost and at the same time reduce pollution-load due to the waste material.

### REFERENCES

- Abu EA, Onyenekwe PC, Ameh DA, Agbaji AS, Ado SA (2000). Cellulase (E. C. 3. 2. 1. 3) production from sorghum bran by *Aspergillus niger* SL 1: An assessment of pretreatment methods. Proceedings of the International Conference on Biotechnology: Commercialization and Food security, Abuja, Nigeria. pp. 153 – 159.
- Atev AP, Panoyotov CA, Bobareva LG, Damyanova LD, Nicolava D (1987). Studies of biosynthesis of hydrolysis by *Trichoderma sp m7* on submerged and solid-state cultivation conditions. Acta Biotechnologica. 7: 9-16.
- Ganju RK, Vithayathil PJ, Murthy SK (1989). Purification and characterization of two xylanase from *Chaetomium thermophile* var. *coprophile*; Can. J. Microbiol. 35: 836–842.
- Isil S, Nulifer A (2005). Investigation of factors affecting xylanase activity from *Trichoderma harzianum* 1073 D3. Braz. Arch. Biol. Technol. 48 (2): 187-194.
- Kadowaki MK, Souza CGM (1997). Xylanase production by *Aspergillus tamarii*. Appl. Biochem. Biotechnol. 66: 97-106.
- Kim J-H, Kim SC, Nam SW (2000). Constitutive overexpression of the endoxylanase gene in *Bacillus subtilis*. J. Microbiol. Biotechnol. 10: 551-553.

- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951). Protein measurement with the folin-phenol reagent. *J. Biol. Chem.* 193: 265-275.
- Miller GL (1959). Use of dinitrosalicylic reagent for the determination of reducing sugars. *Analytical Chemistry* 31: 426-428.
- Mountfort DO, Asher RA (1989). Production of xylanase by ruminal anaerobic fungus, *Neocallimastix frontalis*. *Appl. Environ. Microbiol.* 55 (4): 1016-1022.
- Nuero OM, Reyes F (2002). Enzymes for animal feeding from *Penicillium chrysogenum* mycelial wastes from penicillin manufacture. *Lett. Appl. Microbiol.* 34: 413-416.
- Nwodo-Chinedu S, Okochi VI, Smith HA, Omidiji O (2005). Isolation of cellulolytic microfungi involved in wood-waste decomposition: Prospect for enzymatic hydrolysis of cellulosic wastes. *Int. J. Biomed. Hlth Sci.* 1(2): 41-51.
- Nwodo-Chinedu S, Okochi VI, Omidiji O, Omowaye OO, Adeniji BR, Olukaju D, Chidozie F (2007 a). Potentials of cellulosic wastes in media formulation. *Afr. J. Biotechnol.* 6 (3): 243-246.
- Nwodo-Chinedu S, Okochi VI, Smith HA, Okafor UA, Onyegeme-Okerenta BM, Omidiji O (2007 b). Effect of carbon sources on cellulase (EC 3. 2. 1. 4) production by wild-type *Penicillium chrysogenum* PCL501. *Afr. J. Biochem. Res.* 1 (1): 6-10.
- Okafor UA, Okochi VI, Onyegeme - Okerenta BM, Nwodo – Chinedu S (2007). Xylanase production by *Aspergillus niger* ANL301 using agro wastes. *Afr. J. Biotechnol.* 6(14): 1710-1714.
- Prabhu KA, Maheshwari R (1999). Biochemical properties of xylanases from a thermophilic fungus, *Melanocarpus albomyces*, and their action on plant cell walls. *J. Biosci.* 24:461-470.
- Rawashdeh R, Saadoun I, Mahasneh A (2005). Effect of cultural conditions on xylanase production by *Streptomyces* sp. (strain 1b 24D) and its potential to utilize tomato pomace. *Afr. J. Biotechnol.* 4 (3): 251-255.
- Reddy G, Babu PR, Komaraih P, Roy KRRM, Kothari IL (2003). Utilization of banana waste for the production of lignolytic and cellulolytic enzymes by solid substrate fermentation using two *Pleurotus* species (*P. ostreatus* and *P. sajorcaju*). *Process Biochem.* 381: 457–1462.
- Rezende MI, Barbosa AM, Vasconcelos AFD, Endo AS (2002). Xylanase production by *Trichoderma harzianum* FIFAI by solid state fermentation on sugarcane bagasse. *Braz. J. Microbiol.* 33: 67-72.
- Rifaat HM, Nagieb ZA, Ahmed YM (2005). Production of xylanases by *Streptomyces* species and their bleaching effect on rice straw pulp. *Applied ecology and environmental research.* 4(1): 151-160.
- Seyis I, Aksoz N (2005). Xylanase Production from *Trichoderma harzianum*. *Food Technol. Biotechnol.* 43 (1): 37–40.
- Simoes MLG, Tank-Tornisielo SM (2005). Optimization of xylanase biosynthesis by *Aspergillus japonicus* isolated from “caatinga” area in the Brazilian state of Bahia. *Afri. J. Biotechnol.* 5 (11):1135-1141.
- Tuohy MG, Puls J, Claeysens M, Vrsanska M, Coughlan MP (1993). The xylan-degrading enzyme system of *Talaromyces emersonii*: novel enzymes with activity against aryl  $\beta$  -D-xylosides and unsubstituted xylans. *Biochem. J.* 290: 515–523.
- Villas-Boas SG, Esposito E, de Mendonca MM (2002). Novel lignocellulolytic ability of *Candida utilis* during solid-substrate cultivation on apple pomace, *World J. Microbiol. Biotechnol.* 18: 541–545.
- Wong KKY, Tan LUL, Saddler, JN (1988). Multiplicity of  $\alpha$ -1, 4 xylanase in microorganisms' functions and applications. *Microbiol. Rev.* 52: 305-317.
- Yu EKC, Tan LUL, Chan MK-H, Deschatelets L, Saddler JN (1987). Production of thermostable xylanase by a thermophilic fungus, *Thermoascus aurantiacus*; *Enzyme Microb. Technol.* 9: 16–24.