SENRA Academic Publishers, Burnaby, British Columbia
Vol. 2, No. 2, pp. 357-362, 2008
ISSN: 1715-9997

Canadian Journal of pure&applied sciences

GROWTH AND CELLULASE ACTIVITY OF WILD-TYPE *ASPERGILLUS NIGER* ANL301 IN DIFFERENT CARBON SOURCES

*Chinedu, Nwodo S[1], Nwinyi, Obinna C[1] and Okochi, Veronica I[2]
[1]Department of Biological Sciences, College of Science and Technology
Covenant University, KM 10 Idiroko Road, Canaan Land, PMB 1023 Ota, Ogun State, Nigeria
[2]Department of Biochemistry, College of Medicine, University of Lagos, PMB 12003 Idiaraba

ABSTRACT

A wild-type *Aspergillus niger* (ANL301) isolated from wood-waste in Lagos, Nigeria, produces extracellular proteins with cellulase (EC 3. 2. 1. 4) activity. Three different carbon sources (Glucose, Cellulose and Sawdust) influenced the organism's growth and the production of extracellular cellulase enzymes. Best growth was obtained with glucose at 72 hours of incubation. The peak mycelia weight of 1.56 mg/ mL obtained with glucose was about 3 times the maximum weight of 0.58 and 0.49 mg/ mL respectively obtained with cellulose and sawdust at 96 hours. The peak protein contents of the culture filtrates were 0.02, 0.15 and 0.69 mg/ mL respectively in the media containing glucose, cellulose and sawdust. There was no significant cellulase activity in the filtrates from glucose-containing media. The culture filtrates of the organism from cellulose- and sawdust-containing media yielded significant cellulase activities with maximum values of 105.6 Units /L (at 72 hours for cellulose) and 101.9 Units /L (at 144 hours for sawdust). There is a correlation between the protein content and cellulase activity of the culture filtrates. Sawdust can serve as a low-cost substrate for cellulase production by the organism.

Keywords: *Aspergillus niger* ANL301, growth, cellulase activity, cellulosic materials.

INTRODUCTION

There is a growing interest in the conversion of lignocelluloses into bulk chemicals and biofuels as a means of alleviating energy shortages and reducing pollution-load (Howard *et al*., 2003). One method being intensively studied is the hydrolysis of cellulosic materials into simple sugars and subsequent fermentation into ethanol (Fan *et al*., 1987; Spano *et al*., 1978). Cellulase is the generic name for the group of enzymes which catalyze the hydrolysis of cellulose and related cellooligosaccharide derivatives. The enzyme is potentially useful for industrial saccharification of cellulosic biomass. An economic process for its production is thought to be critical for successful utilization of cellulosic materials (Solomon *et al*., 1999; Wu and Lee, 1997). Cellulase is adaptive in most fungi; substances such as cellulose and sophorose are known to induce the enzyme production (Berry and Paterson, 1990; Ryu and Mandels, 1980). Major limiting factors on the commercial use of the enzyme are low activity and high cost of the available enzyme preparations (Spano *et al*., 1978). This has necessitated a renewed search for cellulolytic organisms with novel cellulase properties and strategies for low-cost enzyme production.

In search of viable cellulolytic organisms, we isolated different cellulolytic microfungi from decomposing wood-wastes in Lagos, Nigeria, which included *Aspergillus niger* AN301 (Nwodo-Chinedu *et al.*, 2005). *Aspergillus niger* group is particularly noted for the secretion of extracellular enzymes which can hydrolyze the β-glycosidic bonds of native cellulose and associated hemicelluloses (de Vries and Visser, 2001). *Aspergillus niger* AN301grows rapidly on media containing sugarcane pulp and sawdust as sole carbon sources (Nwodo-Chinedu *et al*., 2007) and produces celulase (Nwodo-Chinedu *et al.*, 2005) and xylanase enzymes (Okafor *et al.*, 2007) in such media. In the present study, growth and cellulase production by the wild strain of *Aspergillus niger* AN301 cultured in submerged liquid media containing glucose, cellulose and sawdust were investigated. Our data shows rapid growth but no significant cellulase activity in glucose-containing media. In contrast there was low growth and very significant cellulase production in the media containing cellulose and sawdust. There appears to be a correlation between the protein content and cellulase activity of the cell-free filtrates.

MATERIALS AND METHODS

Chemicals

All chemicals and reagents were of analytical grade. Potato Dextrose agar and crystalline cellulose (Avicel) were obtained from Merck, Germany. Carboxymethyl-Cellulose (CM52) was obtained from Whatman Ltd,

*Corresponding author email: sncresearch@yahoo.com

England. All other chemicals and reagents were obtained from Sigma Chemicals Co. Ltd, England.

Sawdust

Sawdust of Abora wood (Mitragyna ciliata) was collected from Okobaba Saw-mills, Ebute-Metta, Lagos, Nigeria. The sample was dried in the oven at 80 ^{O}C for 2 hours, 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.

Media preparations

The liquid media contained (per liter of distilled water): $NaNO_3$, 3.0 g; KCl, 0.5 g; KH_2PO_4, 1.0 g; $MnSO_4$. $7H_2O_4$, 0.5 g; $FeSO_4$. $7H_2O$, 0.01 g; and 10.0 g of the carbon source (Glucose, Crystalline cellulose or sawdust). One liter (1 L) of the media was supplemented with 1.0 mL of trace solution containing (per liter of distilled water) $ZnSO_4$, 1.0 g and $CuSO_4$. $5H_2O$, 0.5 g. The pH of each media was adjusted to 5.6.

Organism

The strain of *Aspergillus niger* (ANL301) was isolated from wood-wastes in Lagos, Nigeria and identified as described previously (Nwodo-Chinedu *et al*., 2005). The organism was maintained at 4^{o}C on Potato Dextrose Agar (PDA) slants.

Growth Studies

Fresh sub-culture of the organism was made on sterile PDA plates and incubated at 30^{o}C for 72-120 hours. The colonies on PDA plates were covered with 10 mL of 1.0% Tween 80 [Polyoxyethylene (20) Sorbitan Monooleate]. Conidia were harvested using sterile cotton swab and transferred into a sterile test tube. Serial dilutions of the suspension were made using 1.0% Tween 80 to obtain the spore suspension of 2.0-4.0 X 10^6 spores/ mL used as inoculum for the growth and other analyses. Two (2.0) mL of the spore suspension was inoculated into 100 mL of the respective sterile liquid medium placed in 250 mL Erlenmeyer flask. The flask was covered with sterile cotton wool and incubated at 30^{o}C for 24 – 168 hours with continuous agitation at 100 osc min^{-1} using the Griffin flask shaker. Cultures were harvested at 24 hour intervals by filtration over a 168-hour period. The mycelium was washed and dried in the oven at 80 ^{O}C for 2 hours. The cell-free filtrates were used as crude protein and enzyme source.

Protein assay

Protein content of the culture filtrates was determined by the folin ciocalteau method described Lowry *et al*. (1951) using Bovine Serum Albumin (BSA) as standard.

Cellulase (Endo-1, 4-β-Glucanase; EC 3. 2. 1. 4) assay

A modification of the reducing sugar method described by Khan (1980) was used to for the assay of Endo-1, 4-β-Glucanase (EC 3. 2. 1. 4) activity. Carboxylmethyl-cellulose (CMC) was used as enzyme substrate. The reaction mixture contained 2.0 mL of 0.1% (w/v) substrate in 0.1M sodium acetate buffer (pH 5.0) and 2.0 mL of cell-free culture supernatant (or 0.5 mL of partially purified enzyme). The mixture was incubated at 37^{o}C in water bath with shaking for 30 minutes. The reducing sugar released was measured using 3, 5-dinitrosalicylic acid and read at 540nm using a spectrophotometer (Miller, 1959). The released reducing sugar was expressed in glucose equivalent and expressed in Units mL^{-1}. A unit of activity was defined as amount of enzyme required to liberate 1µmol of Glucose per minute under the assay conditions.

RESULTS

The growth of *Aspergillus niger* ANL301in liquid media containing glucose, cellulose and sawdust is shown in figure 1. Very rapid growth was obtained in the media containing glucose where the mycelia weight reached a peak of 1.56 mg/mL after 96 hours. In cellulose containing media, the mycelia weight attained a peak of 0.58 mg/ mL after 72 hours. A maximum weight of 0.49 mg/mL was obtained after 96 hours in sawdust-containing medium. A sharp decline in mycelial weight was noted in all the media after the organism attained the peak growth. Figure 2 shows the cellulase activities of *A. niger* ANL301 in media containing cellulose and sawdust respectively. The cellulase activity of the organism cultured in cellulose-containing medium gave a peak of 105.6 Units/mL (X10^3) after 96 hours while a peak of 101.9 Units/mL (X 10^3) was obtained after 144 hours in sawdust-containing medium.

The Protein yield of the organism cultured in the media containing cellulose and sawdust respectively is shown in figure 3. The organism produced much more protein in medium containing sawdust compared to that containing cellulose. The protein content of culture filtrates in cellulose-containing medium was highest (1.5 mg/mL) after 96 hours and maximum (6.9 mg/mL) in sawdust-containing medium after 72 hours.

Plots of the protein contents and cellulase activities of culture filtrates of *A. niger* ANL301 cultivated in modified Czapek-Dox Broth containing cellulose and sawdust respectively are shown in figure 4. The plots show a direct relationship between the protein content and cellulase activity of the culture filtrates. In cellulose-containing medium, the protein content and cellulase activity of the cell-free filtrates peaked at the same period and declined afterwards (Fig. 4 a). In the medium containing sawdust, the protein content of the culture filtrates peaked before the cellulase activity. The highest protein content was obtained at 72 hours while the

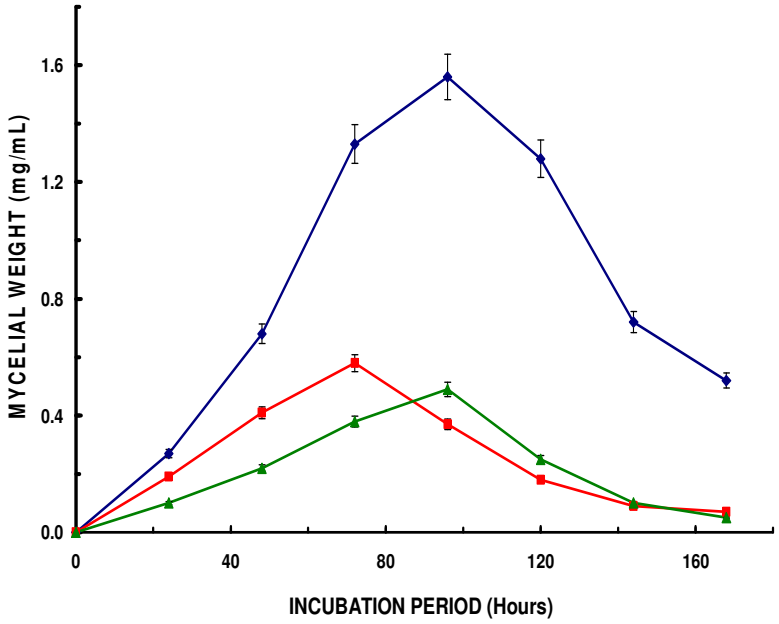

Fig. 1. Mycelia weights of *Aspergillus niger* ANL301cultured at 30°C in liquid media containing Glucose (♦), Cellulose (■) and Sawdust (▲) as sole carbon source for 24-168 hours.

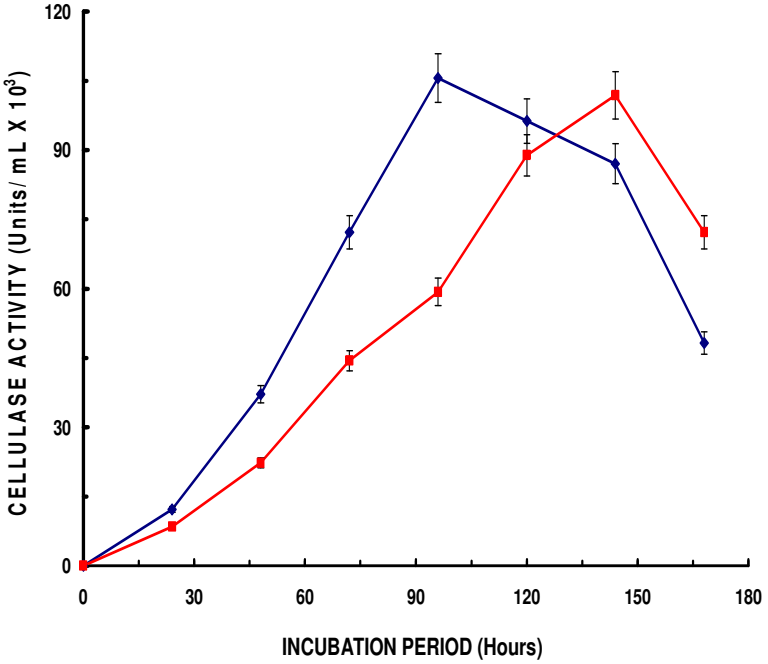

Fig. 2. Cellulase activities of the cell-free culture filtrates of *Aspergillus niger* ANL301 incubated at 30°C in liquid media containing Cellulose (♦), and Sawdust (■) for 24-168 hours.

maximum cellulase activity was attained at 144 hours of incubation (Fig. 4b).

DISCUSSION

The liquid media containing glucose yielded higher amounts of mycelia compared to the modified media containing cellulose or sawdust as the respective carbon source (Fig. 1). The peak growth period varied for the different carbon sources. It was 72 hours with cellulose as sole carbon source whereas the peak mycelial weight was at 96 hours for the media containing glucose or sawdust as sole carbon source. The differences in the carbon sources of the media could account for the disparity in the

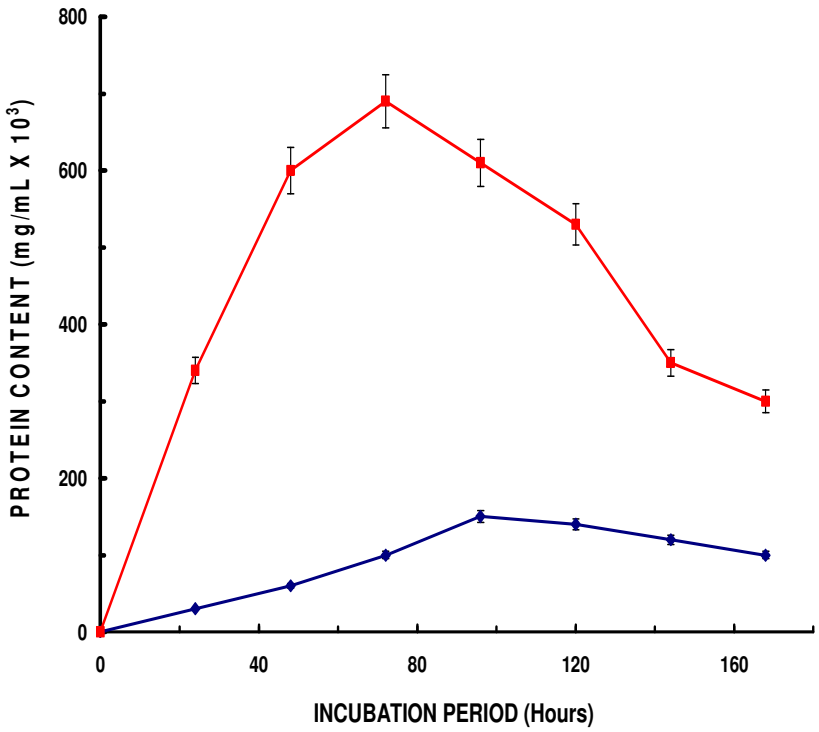

Fig. 3. Protein content of the cell-free culture filtrates of *Aspergillus niger* ANL301 incubated at 30°C in liquid media containing Cellulose (♦), and Sawdust (■) for 24-168 hours.

growth of the organism in the different media. Since glucose is more readily assimilated and metabolized by cells, there is greater tendency for organisms to grow very rapidly in media containing the simple sugar compared to that which contain cellulose or sawdust. Cellulose is a polymer of β-D-glucose while sawdust (wood) is composed of complex plant cell wall components which include cellulose, hemicelluloses and lignin (Grant and Long, 1981). In order to obtain simple sugars from cellulose or sawdust, the organism have to synthesize the enzymes required for the hydrolysis of the macromolecules. This may account for the much slower growth in media containing cellulose and sawdust.

The protein content and cellulase activity of filtrates from glucose containing media were extremely low and thus considered insignificant. This is expected because the organism already has the simple sugar, glucose, in its media and hence do not need to produce the hydrolytic enzymes (proteins). Cellulases of most fungi are inducible and are also regulated by catabolite repression (Berry and Paterson, 1990). Absence of cellulose or other inducers as well as the high concentration of glucose in the medium will thus turn off the production of the enzymes. This may account for the low protein content and insignificant cellulase activity recorded in glucose-containing media, and goes on to suggest that the production of cellulase enzyme by the wild-type *A. niger* ANL301 is induced by cellulose and sawdust in the absence of glucose.

There were different periods for peak cellulase activity for the two carbon sources (cellulose and sawdust). The time was shorter in cellulose compared to sawdust. Cellulose is a homo-polysaccharide containing only β-D-glucose monomers whereas sawdust contains other polymers such as hemicelluloses, in addition to cellulose. When cellulose is the sole carbon source, the production of cellulases will be more rapid since glucose molecules needed for the organism's metabolism must come from cellulose hydrolysis. This may not be the case when sawdust is the sole carbon source. Most microorganisms generally have far greater ability to depolymerize hemicelluloses compared to cellulose due to the greater solubility of the former (Grant and Long, 1981). *Aspergillus niger* ANL301 was found to produce high levels of xylanases when cultured on media containing sawdust and other agro-wastes (Okafor *et al*., 2007).

In terms of protein yield, higher values were obtained in media containing sawdust compared to that containing crystalline cellulose (Fig. 3). The high protein released in the sawdust suggests the presence of other proteins (beside the cellulase enzyme) which may include other cell-wall hydrolyzing enzymes. Fungi such as *Aspergillus species* are known to produce many plant cell-wall hydrolyzing enzymes (de Vries and Visser, 2001). Hemicellulases particularly xylanases are also required for the hydrolysis of natural cellulose (Khan, 1980).

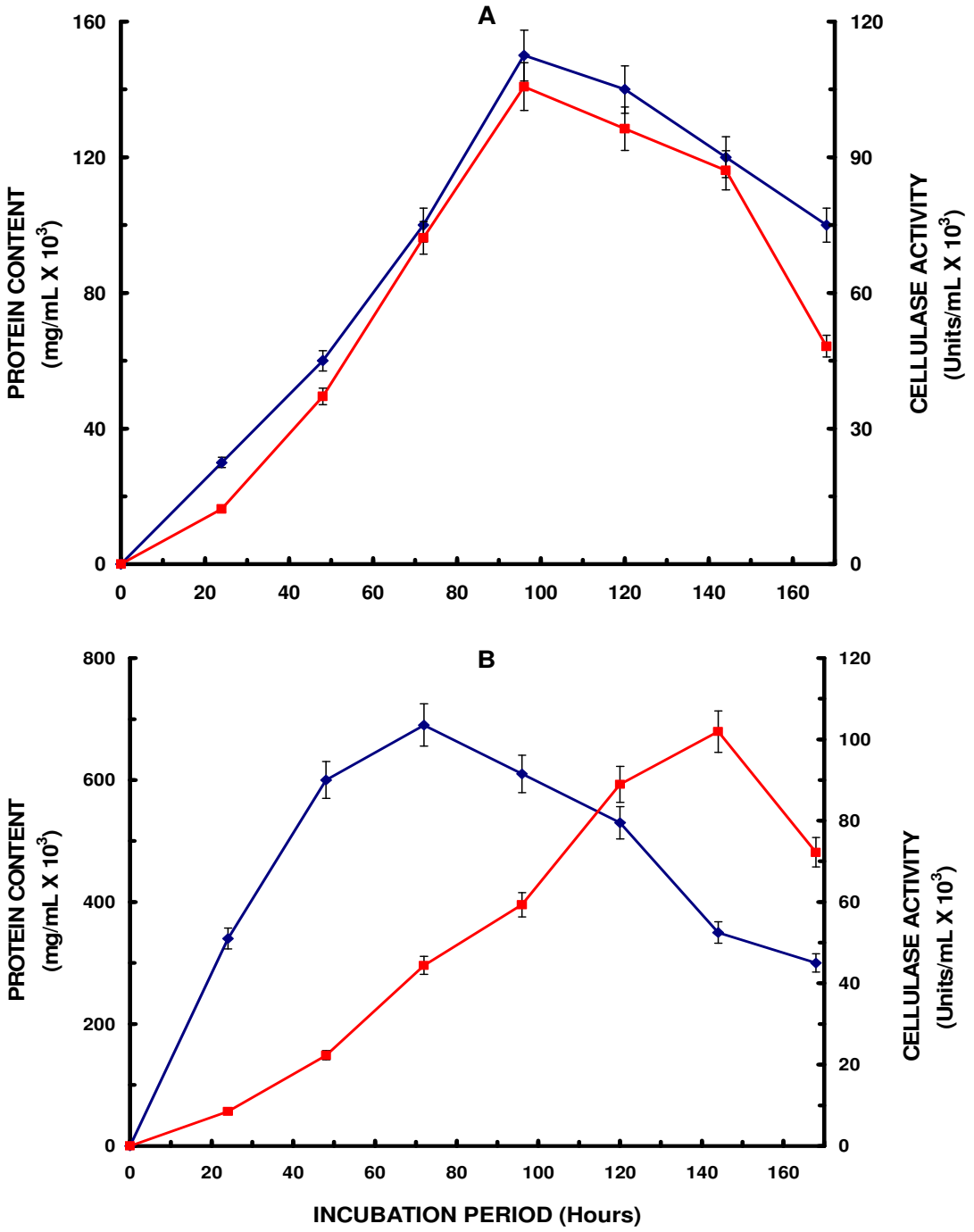

Fig. 4. Protein contents (♦) and cellulase activities (■) of culture filtrates of *Aspergillus niger* ANL 301 incubated at 30°C in liquid media containing (A) Cellulose and (B) Sawdust for 24-168 hours.

There appears to be a correlation between the protein content and cellulase activity of the crude enzyme obtained at the different period of incubation (Fig. 4). The organism seems to secrete the hydrolytic enzymes for the breakdown of the polymers into the growth media which largely accounts for the protein contents of the cell-free filtrates. The amounts of protein released appear to be a function of the complexity of the carbon sources. The more complex the carbon source, the greater the amount of proteins secreted.

In conclusion, the strain of *Aspergillus niger* ANL301 produces extracellular proteins with significant cellulase activity when cultured on media containing cellulose and

sawdust as sole carbon sources. On the other hand, *Aspergillus niger* ANL301did not yield significant extracellular protein or cellulase activity when cultured on media containing glucose as sole carbon source. Much more protein was produced on sawdust compared to cellulose, but the maximum cellulase activities of the filtrates from both culture media were about the same. Sawdust is indicated as a good inducer of cellulase activity in *Aspergillus niger* ANL301. The waste cellulosic material is available in abundance and can be used as low-cost carbon source for the production of commercial cellulases. Such use could help reduce the pollution due to wood-wastes.

REFERENCES

Berry, DR. and Paterson, A. 1990. Enzymes in Food Industry. In: Enzyme Chemistry, Impact and applications, 2nd Edition. eds. Suckling C.J. 306-351.

De Vries, RP. and Visser, J. 2001. Aspergillus enzymes involved in degradation of plant cell wall polysaccharides. Microbiology and Molecular Biology Review. 65: 497-552.

Fan, LT., Gharpuray, MM. and Lee, YN. 1987. Cellulose Hydrolysis Berlin, Germany: Springer-Verlag. 3: 1-68.

Grant, WD. and Long, PE. 1981. The carbon cycle. In: Environmental Microbiology, Tertiary level Biology. Thomas Litho Ltd., Scotland. 91-116.

Howard, RLI, Abotsi, E., Jansen Van Rensburg, ELI. and Howard, S. 2003. Lignocellulose biotechnology: issues of bioconversion and enzyme production. Review. African Journal of Biotechnology. 2 (12): 602-619.

Khan, AW. 1980. Cellulolytic enzyme system of Activibrio cellulolyticus, a newly isolated Anaerobe Journal of General Microbiology. 121: 499-502.

Lowry, OH., Rosebrough, N.J., Farr, AL. and Randall, RJ. 1951. Protein measurement with the folin-phenol reagent. Journal of Biological Chemistry. 193: 265-275.

Miller, GL. 1959. Use of dinitrosalicyclic reagent for the determination of reducing sugars. Analytical Chemistry. 31: 426-428.

Nwodo-Chinedu, S. Okochi, VI., Smith, HA. and Omidiji, O. 2005. Isolation of cellulolytic microfungi involved in wood-waste decomposition: Prospect for enzymatic hydrolysis of cellulosic wastes. International Journal of Biomedical and Health Sciences. 1(2): 41-51.

Nwodo-Chinedu, S., Okochi, VI., Omidiji, O., Omowaye, O. O., Adeniji, B. R., Olukoju, D. and Chidozie, F. 2007. Potentials of cellulosic wastes in media formulation. African Journal of Biotechnology. 6 (3): 243-246.

Okafor, UA., Okochi, VI., Onyegeme-Okerenta, MB. and Nwodo-Chinedu, S. 2007. Xylanase production by Aspergillus niger ANL301using agro-wastes. African Journal of Biotechnology. 6 (14): 1710-1714.

Ryu, DD. and Mandels, M. 1980. Cellulases: Biosynthesis and Applications. Enzyme Microbiology and Technology. 2: 92-102.

Solomon, BO., Amigun, B., Betiku, E., Ojumu, TV. and Layokun, SK. 1999. Optimization of cellulase production by Aspergillus flavus Linn Isolate NSPR101 Grown on Bagasse. *JNSCHE*. 16: 61-68

Spano, L., Alien, A., Tarssinane, T., Mandels, M. and Ryu, DD. 1978. Reassessment of economics of Technology for production of ethanol Proceedings in Industrial fuel from biomass symposium. Troy, New York, USA. 671-674.

Wu, Z. and Lee, YY. 1997. Inhibition of the enzymatic hydrolysis of cellulose by ethanol. Biotechnology Letters. 19: 977-979.