Research Journal of Agriculture and Biological Sciences 1(4): 325-328, 2005

Studies on the Use of Agricultural Wastes for Cellulase Enzyme Production by *Aspegillus niger*

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Abstract: The production of cellulolytic enzymes by *Aspergillus niger* in submerged culture with millet, guinea corn straw, rice husks and maize straw as substrates was studies. Effects of some factors, such as pH and substrate concentrations were reported. Optimal cellulase secretion by A*spergillus niger* was achieved at a time (growth period) of 72 hours in maize straw and rice husk media respectively. 96 hours and 120 hours were the growth period in millet and guinea corn straws respectively. Substrate concentration of five percent (5%) w/v and pH3 resulted in optimal enzyme secretion. The importance of cellulase enzyme in industries cannot be over emphasized. The crude enzyme when purified may serve the importance of this enzyme in both refining and de-inking of recycled papers.

Key words: *Aspergillus niger*, cellulase enzyme, agricultural wastes, factors, enzyme secretion

INTRODUCTION

Agricultural and industrial wastes are among the causes of environmental pollution. Their conversion into useful products may ameliorate the problems they cause. These wastes which include cereals, straw, leaves, corncobs etc are highly underutilized in Africa, particularly Nigeria. In most parts of the country, these materials are mainly used as animal feeds. A large quantity is left on farmlands to be decomposed by microorganism such as bacteria and fungi[1].

Economically, the most important industrial material other than foodstuffs affected by microorganisms are cellulose and wood products including the wood itself. Production of wood products such as pulp, paper, textiles from natural fibres such as cotton flax and jutes are enhanced by microorganisms specifically fungi[2]. Cellulose which forms about 40-50% of plants' composition is the most abundant organic matter on earth. Proper biotechnological utilization of these wastes in the environment will eliminate pollution and convert them into useful by-products.

Cellulase (a complex multienzyme system) acts collectively to hydrolyze cellulose from agricultural wastes to produce simple glucose units[3]. Celluloses are synthesized by cellulolytic fungi such as the C*haetomium, Funsarium Myrothecium* and T*richoderma* species[4,5]. Other species include the *Penicillium* and *Aspergillus* species.

Cellulase with its immense importance is being imported for use in Nigeria at very high cost. The local production of such enzymes using locally available agricultural wastes which can serve as substrates may therefore reduce the cost of importation and encourage self-reliance. It is against this background that this study is aimed at isolating *Aspergillus niger* from the leaves and stalk of guinea corn infested with the organism, evaluating its cellulolytic activity using some cereals as substrate. The study is also aimed at determining some factors that would result in optimal production of cellulase by the fungus.

MATERIALS AND METHODS

Samples were collected locally in Maiduguri, Nigeria. The samples were guinea corn leaves and stalk, maize cobs, Millet and rice husks, which were pick from farmlands after harvest. The samples were then taken into the laboratory and labelled for further treatment/processing in order to isolate the fungi required for this research work.

For the purpose of this study potato Dextrose Agar was used. At the on set, the agar medium was sterilized using an autoclave. The already prepared and sterilized

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culture medium was then poured into sterilized petri dishes. During this stage, aseptic technique was seriously observed, because any small contamination could render all the work useless. Three drops of 50% lactic acid were dropped into the petri dishes to inhibit bacterial growth.

Processing of Sample for Fungal Isolation: The collected samples were cut into smaller fragments. Fragments of the sample material were placed into two separate petri dishes. In the first petri dish, 10% commercial bleach was poured on the leaves and stirred using sterilized stirring rod, and left for about 5 minutes. After 5 minutes, the commercial bleach was discarded and the leaves and stalks were washed with sterilized distilled water for about three times. This method is termed surface sterilization (SS). The plant materials in the second petri dish were only washed with sterilized distilled water to provide moist environment for the fungi. This method is termed surface unsterilized (SU). The plant materials in the first petri dish was transferred into a culture plate containing culture medium. Five pieces of the sample materials were transferred, placing one in the middle and surrounding it with the other four. This culture plate was laballed surface sterilized (SS). The same method was used for the second culture plate containing the unsterilized leaves and stalk. This was labeled unsterilized surface (US). The above procedure was repeated to have 5-6 different parent cultures labeled SS_1-SS_6 and US_1-US_6. The same process was repeated for maize, millet and rice husks.

The already prepared plant materials (surface sterilized and unsterilized surface) were inoculated into the culture plates to observe growth from the plant samples contaminated by the fungi. The inoculated culture plates were then incubated at room temperature to observe fungal growth. This was followed by sub culturing of the different fungal colonies in order to isolate a pure colony.

Fungi were identified by their colony characteristics as well as their vegetative and reproductive structures as observed under the electronic microscope[6]. Some macroscopic characteristics include, colour of the colony, patterns of growth of colony and the bye products released by the organisms. Some of the microscopic characteristics as viewed under the microscope include, the shape of the conidia head, pattern of arrangement of spores on the conidia, shape of the spores and shape of the conidiophores[7].

The substrates used for the production of cellulases by *Aspergillus niger* are millet, guinea corn, rice husks and maize straw. The substrates were ground to get the powdered form. For the pre-treatment of the substrate, a modification of Ali *et al*[8] method was used. The pre-treated media were sterilized in the autoclave and then inoculated with pure colony of *Aspergillus niger* using a sterilized needle. The inoculated media were then incubated on a flask sharker for subsequent determination of cellulase activity.

Determination of Time Course for Enzyme Production: *Aspergillus niger* was inoculated into mineral salt millet husk (MSM), mineral salt guinea corn husk (MSG), minerals salt rice husk (MSR) and mineral salt maize straw (MSMa) media in separate conical flasks and incubated at 30°C for a period of 0-216hrs. The cellulase activity was measured at regular intervals, and the period of maximum enzyme production determined.

Effect of Substrate Concentration: Different concentrations of the substrates ranging from 1.0% (W/V) to 6.0% (W/V) were added to the basal salt solution in separate conical flasks thereby serving as the fermentation media. *Aspergillus niger* was inoculated into these fermentation media and incubated at 30°c for 96hrs.

Effect of pH: *Asperigillus niger* was inoculated into a series of MSM, MSG, MSR and MSMa media separately with the pH varied form 3.0 to 8.0. The inoculated media were incubated at 30°c for 96hrs.

Enzyme Assay: The cellulase enzyme was assayed by measuring the amount of glucose released from the substrates following the secretion of cellulase by the organism. The determination of glucose liberated from the substrate was done using the modification of Asatoor and King method[9]. One unit of cellulase activity was defined as the amount of enzyme which will release 10µg of glucose in 30 minutes under specified conditions.

RESULTS AND DISCUSSIONS

Table 1 shows the extent of production (yield) of cellulase enzyme for a period of 192 hours measured as enzyme activity and also the production rate of the enzyme measured as the ratio of yield to time. The fermentation lasted for 192 hours with enzyme activity and production rates measured as the ratio of yield to time. Cellulase was maximum at 72 hours for maize straw and rice husk, while millet husk and guinea corn straw had 96 and 120 hours respectively. The trend continued from 72hrs to 120hrs for all other substrates except millet that had a slight decrease form 96 to 168hrs. This is in line with the findings of Ali *et al*[8] who reported that the enzyme could be harvested at about 72 hours of fermentation when the activity is highest.

Maize substrate gave the highest cellulase activity of 102 (i.U/mL). The decrease in activity in maize, rice and

Table 1: Time course for cellulase enzyme production by the organism *Aspergillus niger*.

Substrates	Day One	Day Two	Day Three	Day Four	Day Five	Day Six	Day Seven	Day Eight
G/Corn	23.67±1.51^{d}	56.00±2.00^{b}	77.33±2.08^{a}	76.00±2.00^{a}	86.00±3.51^{a}	38.67±2.51^{c}	38.67±2.51^{c}	37.33±3.05^{c}
Maize	41.67±3.05^{d}	63.33±2.51^{c}	102.30±2.52^{a}	84.67±2.52^{b}	89.67±2.52^{b}	54.00±3.00^{c}	43.66±2.51^{d}	46.00±3.00^{d}
Millet	27.00±2.00^{d}	54.00±3.00^{c}	93.00±3.00^{a}	93.33±3.05^{a}	81.33±2.52^{b}	74.00±3.61^{b}	71.33±2.51^{b}	41.67±3.05^{c}
Rice	43.66±2.51^{d}	63.33±2.51^{b}	91.00±3.16^{b}	67.66±2.52^{b}	76.00±2.00^{b}	51.67±3.05^{c}	37.00±3.00^{d}	23.67±1.52^{d}

Values are presented as mean ± standard deviation (n=3)
All groups are compared to each other at p<0.05.
Values with different super scripts along a vertical column are statistically different.

Table 2: Effect of substrate concentration on the production of cellulase enzyme by *Aspergillus niger*.

Substrates Concentration	1%	2%	3%	4%	5%	6%
G/Corn	29.67±8.62^{c}	38.67±5.51^{c}	81.33±8.62^{b}	133.33±5.51^{a}	155.67±5.51^{a}	146.66±5.50^{a}
Maize	42.33±2.89^{c}	55.67±9.81^{c}	122.33±14.36^{b}	158.50±3.54^{b}	225.00±4.24^{a}	200.00±4.20^{a}
Millet	58.50±3.54^{d}	64.00±4.24^{b}	114.00±4.24^{c}	161.50±7.78^{b}	221.50±16.26^{a}	181.50±16.20^{a}
Rice	53.00±16.26^{d}	55.57±16.26^{b}	103.00±4.24^{c}	72.50±7.78^{b}	205.50±7.78^{a}	195.50±7.75^{a}

Values are presented as mean ± standard deviation (n=3)
All groups are compared to each other at p<0.05.
Values with different super scripts along a vertical column are statistically different.

Table 3: Effect of pH on the production of cellulase enzyme by *Aspergillus niger*.

	pH 3	**pH 4**	**pH 5**	**pH 6**	**pH 7**	**pH 8**
G/Corn	68.00±1.41^{a}	55.50±3.54^{a}	48.00±1.41^{b}	32.00±1.41^{c}	30.00±1.41^{c}	25.50±2.12^{c}
Maize	103.00±1.41^{a}	94.50±2.12^{a}	70.00±1.41^{b}	36.00±1.41^{c}	36.00±0.50^{c}	31.00±0.50^{c}
Millet	108.00±1.41^{a}	104.50±3.54^{a}	76.50±4.95^{b}	39.00±4.24^{c}	39.00±1.41^{c}	38.00±02.83^{c}
Rice	80.00±2.83^{a}	81.00±1.41^{a}	61.00±1.41^{b}	32.00±1.41^{c}	29.00±2.83^{c}	25.00±4.24^{c}

Values are presented as mean ± standard deviation (n=3)
All groups are compared to each other at p<0.05.
Values with different super scripts along a vertical column are statistically different.

guinea corn substrates after a fermentation period of the highest activity may be attributed to cumulative effects of cellobiose[10]. Cellobiose, a dimmer of glucose is known to inhibit both endoglucanase and $-glucosidase. It may also suggest that delignification produces aromatic water soluble products that repress the cellulolytic action of the enzyme[8].

Substrate concentrations of 1%-6% w/v were considered. Enzyme activity increased for all substrates used for concentrations 1-5% as presented in table 2. The increase which was not statistically significant can be explained to be as a result of availability of more cellulose of 5.0%. A decrease in enzyme activity beyond maximum (5%) substrate concentration may be due to inlubitors.

This is supported by the findings of Gbekeloluwa and Moo-young[9] who reported the inhibitory effect of accumulated cellobiose and cellodextrin of low degree of polymerisation. The decrease may also be due to depletion of the other nutrients (mineral-salt) other than the energy source or due to the specific binding of the enzymes with the substrate[11,12].

The instability of these enzymes at very low or very high pH values (table 3) is due to the fact that they are proteins which are generally denatured at extreme pH values[13]. This is in agreement with the work of Ali *et al* 1991 in which pH of 3 and 4 were reported as favouring higher yields of cellulase enzyme[8].

Conclusion: Agricultural waste in the form of cellulose which is the most abundant renewable biomass in the biosphere have been shown to be used in the production of valuable products by microorganism. Maize straw, millet, guinea corn and rice husks which are some of these agricultural wastes used in this work as fermentation substrate produced a large amount of cellulase enzymes by *Asperigillus niger*. These results highlight the industrial potentials of the substrates as possible raw materials for cellulase enzyme production by *Aspergillus niger*.

ACKNOWLEDGEMENT

We wish to thank Mr. Joshua of Plant pathology Laboratory Department of Crop Science, University of Maiduguri Nigeria for his assistance.

REFERENCES

1. Okafor Nduka, 1987. Industrial Microbiology. University of Ife Press Ltd., Ile-Ife, Nigeria, pp: 32-33.
2. Wainright M., 1992. An introduction to fungal biotechnology. John Wiley & Sons Ltd., West Sussex, England, pp: 36-40.
3. Smith, E. John, 1996. "An Industrial Application of Cellulases", Journal of Biotechnology and Bioengineering. Press syndicate of the University of Cambridge, Third Edition. pp: 68-83.
4. Neway O. Justin, 1989. "Fermentation process development of Industrial organisms". World Journal of Microbial Biotechnology, Vol. 4, pp: 38-46.
5. Cobley, L.S and W.M. Steele, 1976. An introduction to the Botany of Tropical Crops, 2nd Edition, Longman London, pp: 34-38.
6. Raper, K.B. and D.I. Fennel 1965. The genus *Aspergillus*, Williams and Wilkins Company Baltimore, M.D., pp: 72-76.
7. Bennet, J.W., 1985b. Taxonomy of fungi and biology of the *Aspergilli*. In A. L, Demain and N.A Solomon (eds), Biology of Industrial Microorganisms. Benjamin Cumming, Pub. Co. Manlo Park C.A., pp: 102-120.
8. Ali, S., A. Sayed, R.T. Sarker and R. Alau, 1991. Factors affecting cellulase production by *Aspergillus niger* and *Aspergillus terrus* using water Hyacinth, World Journal of Microbial Biotechnology, 7: 62-66.
9. Asatoor, A.M. and E.J. King, 1954. Blood sugar and its Determination in partial clinical Biochemistry (4th edition) Arnorld Heinemann Publishers Limited India, 86-88.
10. Gbekeloluwa, B.O. and Moo-young, 1991. Production and properties of $-glucosidase by *Neurospora sitophila.* World Journal of Microbial Biotechnology, 7: 4-11.
11. Grey, S.K. and S. Neclakantan, 1983. Effect of nutritional factors on cellulase enzyme and microbial protein production by *Asperigillus niger* and *Aspergillus terrus* and its evaluation, Journal of Biotechnology and Bioengineering, 24: 109-125.
12. Gilkes, N.R, M.T. Langsford, W.W. Wakarchuk, D.G. Kilbum, R.C. Miller and R.A.J. Warren, 1984. The cellulase system of *cellulomunas finni*. Journal of General Microbiology, 130: 1367-1376
13. Steiner, J., C. Sodia and J. Enzaguirre, 1994. Culture condition for enhanced cellulase production by *Aspergillus niger*. World Journal of Microbial Biotechnology, 10: 280-284.