



CELLULASE AND PECTINASE PRODUCTION POTENTIALS OF *ASPERGILLUS NIGER* ISOLATED FROM CORN COB

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ABSTRACT

Production of pectinase and cellulase by *Aspergillus niger* from corn cob was examined. The organism was screened for enzymatic activity using Carboxyl Methyl Cellulose (CMC) and Pectin as substrate. The result revealed a clear zone of inhibition in the agar plates. The organism was subjected to different optimum conditions which include pH, temperature, biomass yield and enzymatic activity. The highest cellulose activity was obtained on the 4th day (1.9×10^{-4} $\mu\text{g/ml/sec}$) while the highest pectinase activity (1.5×10^{-4} $\mu\text{g/ml/sec}$) was obtained on the 4th and 5th day. The optimum pH for cellulase production was pH 4 with an activity of 2.70×10^{-4} $\mu\text{g/ml/sec}$ while the optimum pH for pectinase activity was pH 6 with an activity of 1.5×10^{-4} $\mu\text{g/ml/sec}$. The optimum temperature was at 50°C with an enzyme activity of 1.3×10^{-4} $\mu\text{g/ml/sec}$ for cellulase production while the optimum temperature of 60°C gave the highest pectinase activity of 1.6×10^{-4} $\mu\text{g/ml/sec}$. This study revealed that *Aspergillus niger* from corn cob has the ability to produce cellulase and pectinase, hence it may be considered as a source for the production of industrial cellulase and pectinase.

Keywords: Pectinase, cellulose, *Aspergillus niger*, enzymatic activity, corn cob

INTRODUCTION

Corn cobs contain 32.3-45.6% cellulose, 39.8% hemicelluloses - mostly composed of pentosan, and 6.7-13.9% lignin (Daron, 2008). Of the more than hundred or so enzymes being used industrially, over a half is from fungi and yeast and over a third from bacteria with the remainder divided between animal (8%) and plant (4%) sources. A larger number of enzymes find use in chemical analysis and clinical diagnosis (Chaplin, 2004; Oyeleke *et al.*, 2012).

Cellulase is an enzyme that has the ability to degrade cellulose. It has several commercial applications like malting, wood processing, preparation of denim fabrics in textile industries, maceration of protoplasts from plant tissues and de-inking process in recycling of printed papers. But the saccharification process has not reached to the level of commercialization in certain applications pertaining to production of biofuels. The major obstacle to the exploitation of cellulase is its high cost of production which includes other factors like complexity of cellulose structure, the type and source of cellulose employed for production and low amounts of cellulases production by cellulolytic organisms due to catabolite repression influence economics of cellulase production. One effective approach to reduce the cost of enzyme production is to replace pure cellulose by relatively cheaper substrates such as lignocelluloses materials (Oyeleke *et al.*, 2012).

Pectinase is also a well known term for commercial enzyme preparation that break down pectin; a polysaccharide substrate, found in the cell wall of plants (Oyewole *et al.*, 2011). This enzyme splits polygalacturonic acid into monogalacturonic acid by opening glycosidic linkages. Through this process,

it softens the cell wall and increase the yield of juice extract from the fruits. The two major sources of the enzyme pectinase are plant and microorganism. But for both technical and economic point of view microbial source of pectinase has become increasingly important. A great variety of strains of bacteria, yeast and mold are capable of producing pectic enzymes. The composition of pectic enzymes varies among species of microorganisms (Oyewole *et al.*, 2011). Many studies have been reported that the enzyme preparations used in the food industry are of fungal origin because fungi are the potent producers of pectic enzymes (Abe *et al.*, 2002). Now a days, pectinase is one of the most important enzymes in food processing industries mainly for extraction and clarification of fruit juices and wines (Oyewole *et al.*, 2011).

Despite the wide application of extracellular enzymes, they are not produced in Nigeria, but are imported. The local production of cellulase and pectinase from microorganism isolated from corn cob in the country will certainly help in conserving foreign exchange and in reducing environmental pollution caused by these waste products.

The high cost used in the importation of industrial extracellular enzymes has led to the high cost of finished industrial products. Besides, corn cob causes waste disposal problems since they are being thrown around indiscriminately.

This study is therefore aimed at the production of pectinase and cellulase from corn cob using *A. niger* and determine the optimum pH and temperature for cellulase and pectinase production by *A. niger*.

MATERIALS AND METHODS

Collection of Corn Cob Samples

Corn cob was collected from a waste bin in Bosso market in Minna, Niger State, Nigeria and transported to the laboratory, prior to its use.

Isolation of *Aspergillus niger* from Corn Cob samples

The medium used was Sabouraud dextrose agar (Oxoid). The medium was prepared and sterilized using autoclave at 121°C for 15 minutes according to the manufacturer's instructions. Using a sterile swab stick the growth on the corn cob was picked and placed on already prepared Sabouraud dextrose agar (SDA) and were incubated for a period of 3- 5 days at 25 ±2°C. A small portion of the mycelia growth was carefully picked with the aid of a pair of sterile inoculating needles and placed in a drop of lactophenol cotton blue on a microscope slide and covered with a cover slip. The slide was examined under the microscope, first with (x10) and then with (x40) objective lens for morphological examination as described by Cheesbrough (2003). The isolates were identified by comparing their characteristics with those of known taxa using the schemes of Domsch and Gams (1970). The isolates were characterized based on the colour of aerial and substrate hyphae, type of hyphae, shape and kind of asexual spores, sporangiophore and conidiophores, and the characteristics of spore head. The growth of fungi colonies were observed after the incubation period. Sub-culturing was carried out until pure cultures of *Aspergillus niger* was obtained and the pure *A. niger* colony was used to produce cellulase and pectinase.

Screening of *A. niger* for Cellulase Activity

A loopful of grown culture of isolated colonies were inoculated on sabouraud dextrose agar (SDA) and amended with 0.1% Carboxymethyl cellulose (CMC). The SDA plates were incubated for 3 days at 25°C for fungi isolate and observed for growth. Following incubation, the plates showed zone of clearance around the line of growth (Singh *et al.*, 1988).

Screening *A. niger* for Pectinase Enzyme

Pectin, (1gm) was added into sabouraud dextrose agar. This medium was sterilized and distributed aseptically in Petri dishes *A. niger* was inoculated onto the plates. The plates were observed for a zone of clearance after 3-5 days as described by Palaniyappan *et al.* (2009).

Cellulase Enzyme Production and Assay

Aspergillus niger was placed in a basal medium used for cellulase production just like the modified medium of Deacon (1985) containing (in g/l), yeast extract, 2.0; NaNO₃, 5.0; KH₂PO₄, 1.0; MgSO₄.7H₂O, 0.5; and FeCl₃, 0.001. Carboxymethyl cellulose (CMC) was added at 1% concentration. The culture was grown for 7 days at 25°C. Culture filtrate was obtained by filtration through Whatman No. 1 filter paper and the culture filtrate served as the enzyme solution (Singh *et al.*, 1988).

Culture broth was sampled at different time during growth to determine cell density by

measurement of absorbance at 540 nm. Cellulase assay was done according to the method of Mandels (1985). One milliliter of 1% CMC in 0.1M citrate buffer (pH 5.5) was placed in a test tube and 1ml of culture filtrate was added. The reaction mixture was incubated at 50°C for 30 min and the reaction terminated by adding 1.5 ml 3,5-dinitrosalicylic acid (DNSA) reagent. The tubes were heated at 100°C in a boiling water bath for 15 min and then cooled at room temperature. The absorbance was read at 540 nm. Enzyme activity is expressed as mmol glucose released per sec-1ml-1 of culture filtrate as enzyme solution.

Pectinase Enzyme Production

Aspergillus niger was placed in a basal medium used for pectinase production, the medium consist of NaNO₃, 2 %; K₂HPO₄, 1 %; MgSO₄, 5 %; KCl, 5 %; FeSO₄, 0.001 %; Pectin 15 %. The culture was grown for 7 days at 25°C. Culture broth was sampled at different time during growth to determine cell density by measurement of absorbance at 575nm.

Pectinase Enzyme Assay

Pectinase assay was done according to the method of Mandels (1985). Half milliliter of 1% pectin in 0.1M citrate buffer (pH 5.8) was placed in a test tube and 0.5 ml of culture filtrate was added. The reaction mixture was incubated at 50°C for 30 min and the reaction terminated by adding 1.5 ml 3,5-dinitrosalicylic acid (DNSA) reagent. The tubes were heated at 100°C in a boiling water bath for 15 min and then cooled at room temperature. The absorbance was read at 575 nm. Enzyme activity is expressed as mmol glucose released per min-1ml-1 of culture filtrate as enzyme solution. Culture filtrate was obtained by filtration through Whatman No. 1 filter paper and the culture filtrate served as the enzyme solution (Singh *et al.*, 1988; El-Naghy *et al.*, 1991) and One unit (U) of pectinolytic activity was defined as the amount of enzyme that catalyzes the formation of 1µmol galacturonic acid under the assay conditions (Minjares-Carranco *et al.*, 1997).

Effect of Temperature on the Production of Cellulase

The optimum temperature of Cellulase from *Aspergillus niger* was determined by incubating 1ml appropriately diluted enzyme with 2 ml 1% CMC in citrate buffer pH 4.8 (Bertrand *et al.*, 2004) at different temperature (30 – 80°C) for 2 hours. Reducing sugars were estimated by the dinitrosalicylic acid reagent method (Miller, 1959).

Effect of pH on the Production of Cellulase

The optimum pH was determined by incubating the 1 ml appropriately diluted enzyme mixed with 2 ml 3% CMC in 1 ml citrate buffer, pH 4.8 (Bertrand *et al.*, 2004) buffer of different pH (4 – 8) for 2 hours at room temperature (40°C). Reducing sugars thus released were estimated by the dinitrosalicylic acid reagent method (Miller, 1959).

Effect of Temperature on the Production of Pectinase

The optimum temperature of pectin was determined by incubating 1 ml appropriately diluted enzyme with 2 ml 3% pectin in citrate buffer pH 5.8 (Bertrand *et al.*, 2004) at different temperature (40 – 90°C) for 2 hours. Reducing sugars were estimated by the dinitrosalicylic acid reagent method (Miller, 1959).

Effect of pH on the Production of Pectinase

The optimum pH was determined by incubating the 1 ml centrifuged enzyme mixed with pectin in 1 ml citrate buffer, pH 5.8 (Bertrand *et al.*, 2004) buffer of different pH (4 – 9) for 30 minutes at

room temperature (50°C). Reducing sugars thus released were estimated by the dinitrosalicylic acid reagent method (Miller, 1959) and then boiled at 100°C for 15 minutes.

RESULTS

Cellulase activities of *A. niger*

Fig. 1 shows the cellulase activities of *A. niger* for an incubation period of 7 days. The highest cellulase activity was obtained on the 4th day ($1.9 \times 10^{-4} \mu\text{g/ml/sec}$) and the least ($0.7 \times 10^{-4} \mu\text{g/ml/sec}$) on the 7th day. This corresponds to the day with the highest and lowest biomass activities.

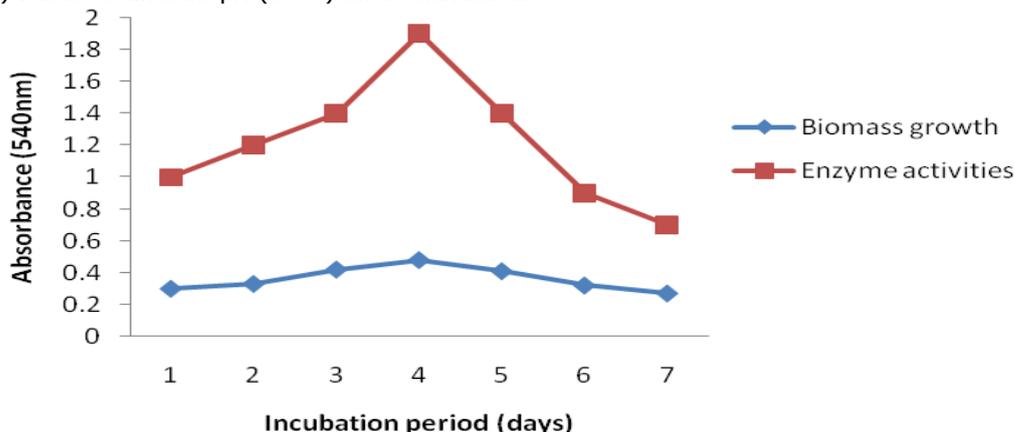


Fig. 1: Cellulase activity ($\mu\text{g/ml/sec}^{-4}$) of *A. niger*

Effect of pH on Cellulase production by *A. niger*

Fig. 2 shows the effect of pH on cellulase production by *A. niger*. The optimum pH was obtained at pH 4 with a cellulase activity of $2.70 \times 10^{-4} \mu\text{g/ml/sec}$ and the lowest at pH 9 with an activity of ($0.31 \times 10^{-4} \mu\text{g/ml/sec}$).

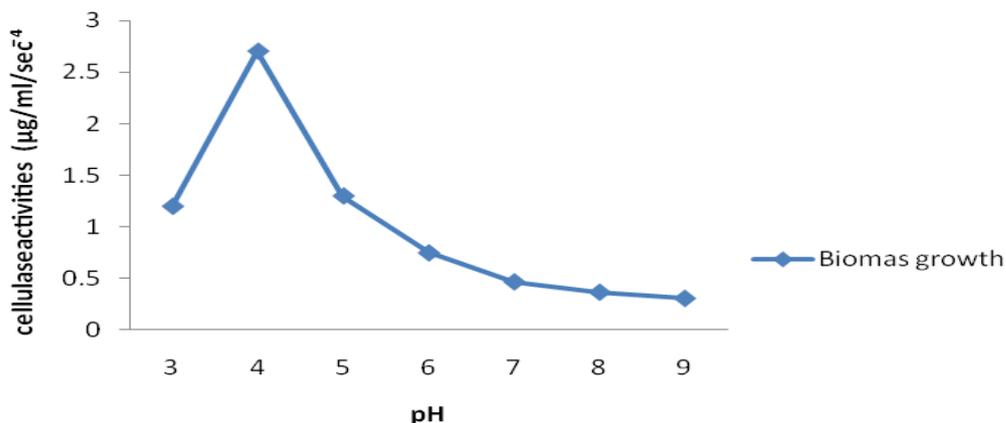


Fig. 2: Effect of pH on cellulase activities of *A. niger*

Effect of Temperature on Cellulase production by *A. niger*

Fig. 3 shows that the effect of temperature on cellulase produced by *A. niger*. The optimum temperature was at 50°C with an enzyme activity of $1.3 \times 10^{-4} \mu\text{g/ml/sec}$ and the lowest enzymatic activity of $0.21 \times 10^{-4} \mu\text{g/ml/sec}$ was obtained at 90°C.

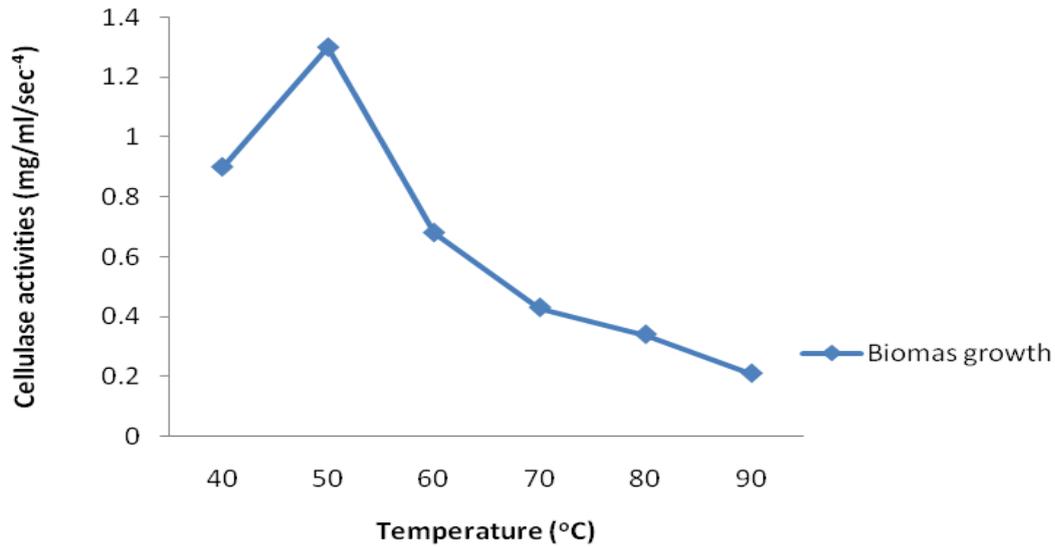


Fig. 3: Effect of temperature (°C) on Cellulase activity of *A. niger*

Fig. 4 shows pectinase activities of *A. niger* for an incubation period of 7 days. The highest pectinase activity ($1.5 \times 10^{-4} \mu\text{g/ml/sec}$) was obtained on the 4th and 5th day and the least ($0.93 \times 10^{-4} \mu\text{g/ml/sec}$) on the 6th day.

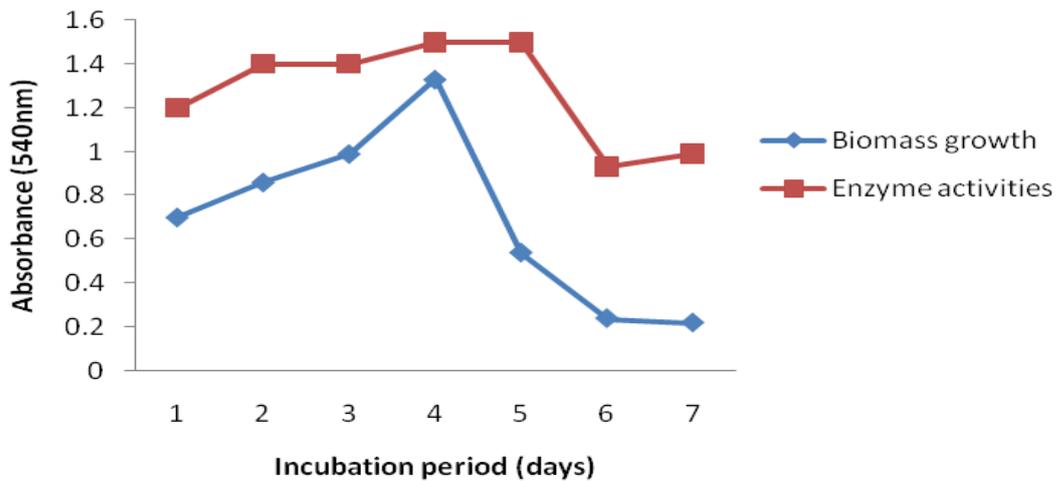


Fig. 4: Pectinase activity ($\mu\text{g/ml/sec}^{-4}$) of *A. niger*

Effect of pH for Pectinase production

Fig. 5 shows the effect of pH on pectinase production by *A. niger*. The optimum pH was obtained at pH 6 with an activity of $1.5 \times 10^{-4} \mu\text{g/ml/sec}$ and the least was obtained at pH 4 with an activity of $0.37 \times 10^{-4} \mu\text{g/ml/sec}$.

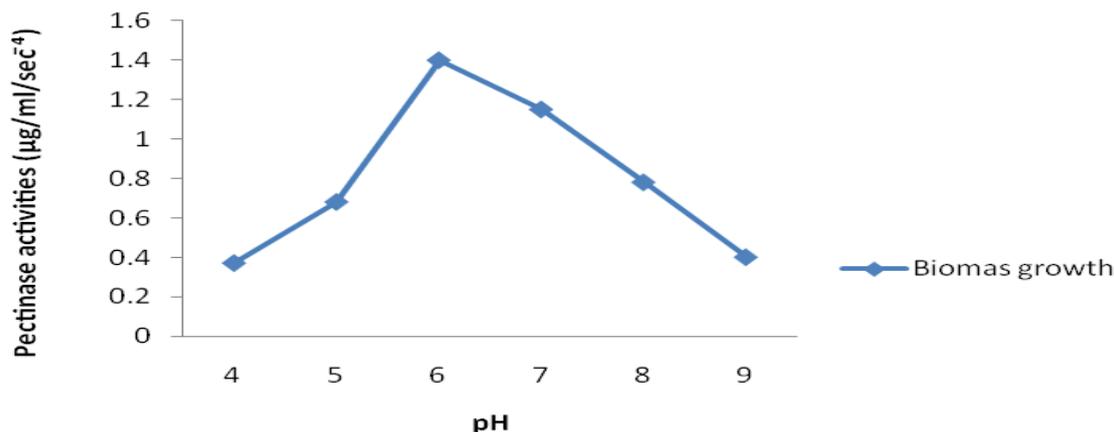


Fig. 5: Effect of pH on pectinase activities of *A. niger*

Effect of Temperature on pectinase activities

Fig. 6 shows the effect of temperature on pectinase production with temperature ranging from 40°C-90°C. The optimum temperature of 60°C gave the highest enzyme activity of 1.6×10^{-4} µg/ml/sec while the least activity (0.5×10^{-4} µg/ml/sec) was recorded at 90°C.

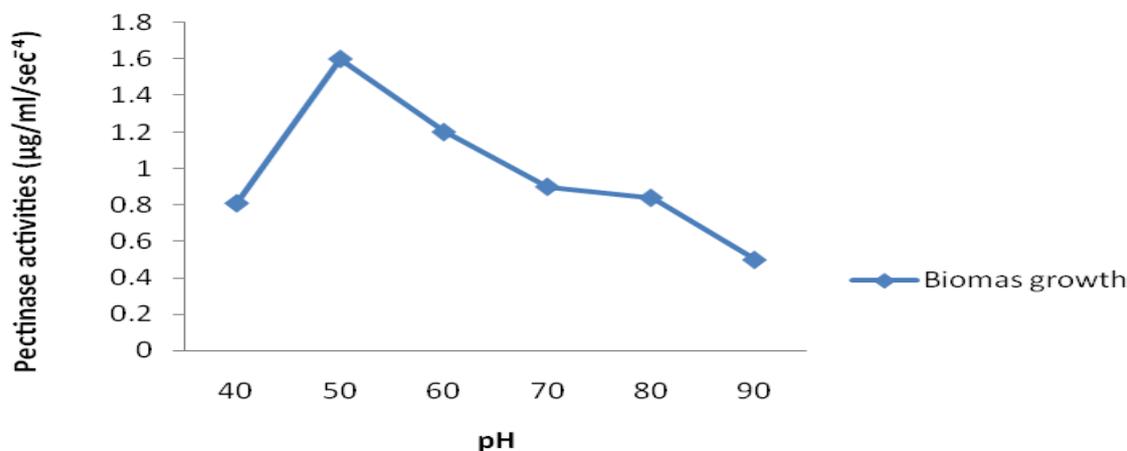


Fig. 6: Effect of temperature (°C) on pectinase activities of *A. niger*

DISCUSSION

For cellulose production, *A. niger* was cultivated in mineral salt medium for 7 days at room temperature, and it showed its highest enzymatic activity after 4 days with an activity of 1.9×10^3 mg/ml/sec. This is similar to the findings of Umbrin *et al.*, (2011) who recorded maximum cellulase productivity after 4 days in the solid state fermentation of *A. niger* by *Vigna mungo*.

For pectinase enzyme, *A. niger* exhibited its highest enzymatic activity on the 4th day of incubation with an activity of 1.5×10^3 µg/ml/sec and biomass production of 1.33 µg/ml/sec after cultivating in mineral salt medium for 7 days. Although, this is different from findings by Wellington *et al.* (2004) that had maximum enzymatic activity after 9 hours for *Bacillus sp.* *A. niger* had its highest enzymatic activity in 6 days, with an activity of 3.14×10^3 µg/ml/sec after cultivation in mineral salt medium for 7 days.

The optimum temperature for pectinase and cellulase produced by *Aspergillus niger* was 50°C and 60°C (with pectinase activity of 0.497 µg/ml) and

A. niger (with cellulolytic activity of 0.43 µg/ml). Temperatures beyond 50°C led to decrease in pectin and cellulase yield. This is in contrast to the findings of Devi *et al.* (2008) and Palaniyappan *et al.* (2009) who reported optimum temperatures of 45°C for *Aspergillus* species for pectinase production. Oyeleke *et al.* (2010) reported that increase in temperature led to increase in enzyme activity but that there was limit to the increase in activity because higher temperatures led to a sharp decrease in activity. This could be due to the denaturing of protein structure.

The effect of pH on pectinase yield by *A. niger* (Fig. 6) revealed that as pH increased, the production of pectinase also increased until optimum pH for pectinase production by *A. niger* (pH 6) with pectinolytic activity of 0.45 µg/ml/min was reached, then the production of enzyme decreased till pH 9. Low or high pH values inactivate the enzyme and may affect its production.

The effect of pH on bioenzyme synthesis has been reported by many authors. pH 4.0 to 5.5 was reported for *A. terreus* and *A. niger* (Garg and Neelakantan, 1981), pH 4.5-5.0 for *Sclerotiumrolfsii* (Darmwal, 1986) and pH 5.0 to 6.0 for *Rhizopusoryzae* (Amadioha, 1993).

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- It is evident from this study that there is a possibility of utilizing *A. niger* isolated from corn cob to produce cellulase and pectinase enzymes in a cost effective and eco-friendly method, which can be used in production of ethanol, detergent, weave, textile, coffee, pulp and paper and pharmaceutical industries.
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