INTERNATIONAL JOURNAL OF RESEARCH AND SCIENTIFIC INNOVATION (IJRSI)
ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue IV April 2026

Production and Characterization of Cellulase from Penicillium Chrysogenum and Aspergillus Oxysporum

Ashiru A.W.[1], Kumoye E.A.[2], Famakinwa O.A.[3], OJIE B.N.[4]

Yaba College of Technology, Yaba-Lagos, Lagos, Nigeria

DOI: https://doi.org/10.51244/IJRSI.2026.1304000039

Received: 03 March 2026; Accepted: 11 March 2026; Published: 27 April 2026

ABSTRACT

Cellulase enzymes play an important role in the degradation of cellulose into simpler sugars and are widely used in several industrial applications such as biofuel production, food processing, textile processing, and waste management. This study investigated the production and characterization of cellulase from fungal isolates obtained from corn cob substrate. Agricultural wastes such as corn cobs are rich in cellulose and can serve as suitable substrates for cellulase-producing microorganisms. The fungal isolates were cultured and screened for cellulase production using cellulose agar medium. Identification of the isolates revealed the presence of *Penicillium chrysogenum* and *Aspergillus oxysporum*. Enzyme production was evaluated by measuring the diameter of hydrolysis zones formed around fungal colonies. The results showed that both fungal species were capable of producing cellulase; however, *Aspergillus oxysporum* demonstrated higher cellulolytic activity compared to *Penicillium chrysogenum*. The relatively higher enzymatic activity observed suggests strong potential for application in biofuel production and agricultural waste bioconversion industries, where efficient cellulose degradation is required. The findings highlight the potential of these fungi for cellulase production and their possible industrial applications.

Keywords: Cellulase, Fungi, Agricultural waste, Enzyme production, Corn cob substrate

INTRODUCTION

In recent decades, the global demand for enzymes has increased substantially, driven largely by industrial advancements and the growing preference for environmentally friendly technologies that align with the principles of green chemistry. Enzymes offer a sustainable alternative to harsh chemical catalysts, as they are biodegradable, highly specific, and operate under moderate conditions, thereby reducing energy consumption and environmental pollution (Maghraby *et al.,* 2023). For large-scale enzyme production to be economically viable, the use of affordable and readily available substrates is essential. Plant biomass, particularly plant cell walls, represents a major renewable carbon source due to its abundance and rich polysaccharide composition. These cell walls contain cellulose, hemicellulose, and other complex carbohydrates that can be hydrolyzed by microbial enzymes into simpler sugars. Microorganisms utilize these sugars as sources of energy and carbon, while the hydrolysis products also serve as valuable raw materials in several industrial processes. Consequently, microbial enzymes that degrade plant-derived polymers have attracted considerable scientific and commercial interest (Maghraby *et al.,* 2023).

Among microbial enzymes, cellulases have emerged as one of the most important and widely exploited enzyme groups. Cellulases are a complex system of enzymes responsible for the degradation of cellulose, the most abundant organic polymer on Earth, accounting for approximately 45 percent of all organic matter in the environment (Singhania *et al.,* 2017). The cellulase enzyme system consists primarily of three major components that act synergistically to achieve complete cellulose hydrolysis. These include endo-1,4-β-D-glucanases (EC 3.2.1.4), which randomly cleave internal β-1,4-glycosidic bonds within cellulose chains; exoglucanases or cellobiohydrolases (EC 3.2.1.91), which act on the newly formed chain ends to release cellobiose units; and β-

glucosidases (EC 3.2.1.21), which hydrolyze cellobiose and short oligosaccharides into free glucose molecules. The coordinated action of these enzymes converts insoluble cellulose into soluble sugars that can be assimilated by microorganisms or utilized in industrial fermentations (Shyaula *et al.,* 2023).

Cellulases occupy a prominent position in the global enzyme market due to their extensive industrial applications. They are ranked as the third largest class of industrial enzymes by market value and contribute approximately 20 percent of the total enzyme market worldwide. Their widespread demand is attributed to their diverse applications in industries such as pulp and paper, textiles, food and beverages, detergents, animal feed, pharmaceuticals, starch processing, and biofuel production (Behera and Ray, 2016). These applications highlight the economic importance of cellulases and the need for efficient production systems (Ejaz *et al.*, 2021).

Microorganisms are the primary producers of cellulases, with bacteria, fungi, and actinomycetes all reported to possess cellulolytic capabilities. However, fungi have emerged as the most effective and commercially preferred producers of cellulases due to their high enzyme yields, strong hydrolytic potential, and ability to secrete enzymes extracellularly. Species belonging to the genera *Trichoderma, Aspergillus,* and *Penicillium* are particularly notable for their cellulase-producing capacity and are widely used in both laboratory and industrial settings (Srivastava *et al.,* 2018). Among these, *Aspergillus niger* and *Penicillium chrysogenum* have gained attention due to their robustness, adaptability to diverse substrates, and capacity to produce stable and efficient cellulase systems. Agricultural wastes such as rice bran, wheat bran, sugarcane bagasse, cassava peels, banana peels, and other lignocellulosic residues have been successfully used as substrates for cellulase production, providing both an economic and environmental benefit by valorizing waste materials (Sulyman *et al.*, 2020).

Agricultural residues such as rice husk, wheat straw, and corn cobs are commonly used as substrates for cellulase production. Corn cob was selected in this study because of its high cellulose content, low lignin composition, availability, and cost-effectiveness compared to other lignocellulosic wastes such as rice husk and wheat straw. Additionally, corn cobs are often discarded as agricultural waste, making them environmentally sustainable substrates for enzyme production.

Fungi are among the most efficient producers of cellulase enzymes due to their ability to secrete large amounts of extracellular enzymes. Species such as *Aspergillus* and *Penicillium* have been widely studied for their cellulolytic capabilities.

This study therefore aims to isolate cellulase-producing fungi from corn cob substrates and characterize their enzyme production potential.

MATERIALS AND METHODS

Study Area

The study was carried out at the Microbiology Laboratory of Yaba College of Technology (YABATECH), Lagos, Nigeria.

Materials / Apparatus

Corn cobs, Distilled water, Ethanol (70%), Beakers, Measuring cylinders, Potato Dextrose Agar (PDA), CMC (Carboxymethylcellulose), Congo red, Sterile distilled water, Sodium Chloride, Bradford reagent, DNSA reagent, Chloramphenicol, Autoclave, Incubator, Petri dishes, Inoculating loop, Conical flasks, Bunsen burner, Marker, Lactophenol cotton blue stain, Glass slides, Cover slips, Microscope, pH meter, Spectrophotometer, Water bath, Test tubes, Pipettes, Thermometer, Curvette, Centrifuge, Refrigerator, Oven, Cotton wool, Gloves, Sample bottles.

Sample Collection and Preparation

Corn cobs were used as the substrate for this study. The samples were obtained from three different sources within Lagos State, which include Ketu Market, a domestic waste dump site, and a residential household. Then,

the samples obtained were kept at ambient room temperature for seven days to allow natural fungal growth. All the corn cob samples from the different locations were then transported to the laboratory for further microbiological analysis.

Media Preparation

The solid Media used for isolation of microorganisms in this study was Potato Dextrose Agar (PDA). It was weighed and prepared according to the manufacturer's specification and sterilized in an autoclave at a temperature of 121°C for 15 minutes. After preparation of PDA, chloramphenicol was added to it in order to inhibit the growth of bacteria. The glassware used for the experiment was properly washed, rinsed with distilled water, and sterilized in a hot air oven at 160°C for 2 hours.

Dilution Of Samples

Serial dilution was carried out to obtain a suitable microbial load for fungal isolation. One gram of the fungal-colonized portion of the corn cob was aseptically transferred into 9 mL of sterile distilled water in a test tube and thoroughly mixed to obtain a 10^{-1} dilution. From this suspension, 1 mL was aseptically transferred into another test tube containing 9 mL of sterile distilled water to produce a 10^{-2} dilution. This process was continued in the same manner until a final dilution factor of 10^{-5} was achieved.

Isolation of Fungi

The samples were processed using standard microbiological techniques. Serial dilution was performed, and aliquots were plated on Potato Dextrose Agar (PDA). The plates were incubated at 28 ± 2°C for 5–7 days to allow fungal growth.

Sub-Culturing and Purification of Fungi

Subculturing and purification of the isolated fungi were carried out to obtain pure fungal cultures. Distinct fungal colonies from the primary isolation plates were selected based on their morphological appearance. Using a sterile inoculating needle, a small portion of mycelium from the edge of each selected colony was aseptically transferred onto freshly prepared Potato Dextrose Agar (PDA) plates. The inoculated plates were incubated at room temperature for 3 days.

Identification Of Fungi

The purified fungal isolates were identified using standard macroscopic and microscopic techniques.

Macroscopic Identification:

Macroscopic identification was carried out by observing the cultural characteristics of the fungal colonies on Potato Dextrose Agar (PDA). Parameters such as colony colour, texture, margin, elevation, surface appearance, and rate of growth were carefully examined and recorded after incubation

Microscopic Identification:

Microscopic identification was performed using the lactophenol cotton blue staining technique. A small portion of fungal mycelium was aseptically placed on a clean glass slide, stained with lactophenol cotton blue, and gently covered with a coverslip. The stained preparation was examined under a microscope at ×10 and ×40 magnifications to observe the characteristics.

Screening of Cellulase Activity

Screening for cellulase production was carried out using Carboxymethyl Cellulose (CMC) agar medium. The inoculated plates were incubated at 30°C for 72 hours. After incubation, plates were flooded with Congo red solution and rinsed with sodium chloride solution. Clear zones around colonies indicated cellulase production.

Analysis of Enzyme Activity

Effect of pH on Turbidity

The effect of pH on turbidity was examined to evaluate the influence of hydrogen ion concentration on fungal growth and biomass formation during cellulase production. CMC (Carboxymethylcellulose) broth was prepared and dispensed into sterile test tubes. The pH of the media was adjusted across a range of pH 3 to 9 using appropriate buffer systems, including citrate buffer for acidic conditions, phosphate buffer for near-neutral pH, and Tris-HCl buffer for alkaline conditions. A calibrated pH meter was used to ensure accurate pH adjustment before sterilization. Each test tube containing the adjusted broth was inoculated with the fungal inoculum and incubated at room temperature for 3 days. After incubation, fungal growth was assessed by measuring turbidity, which served as an indirect indicator of biomass concentration. The cultures were gently mixed, and aliquots were transferred into clean cuvettes for optical density measurement. Turbidity readings were obtained using a spectrophotometer. Uninoculated broth adjusted to corresponding pH values served as blanks to eliminate background interference. All measurements were performed in triplicate to ensure reproducibility and accuracy. The turbidity values obtained were compared across the different pH levels to determine the pH range that best supported fungal growth.

Effect of pH on Cellulase Activity

The effect of pH on cellulase activity was investigated to determine the optimum pH at which the enzyme exhibited maximum catalytic efficiency. Crude enzyme extract obtained from the fungal cultures was used as the enzyme source, while carboxymethyl cellulose (CMC) served as the substrate. Reaction mixtures were prepared in test tubes containing CMC substrate dissolved in buffer solutions of varying pH values. Different buffer systems were employed to cover a wide pH range, ensuring enzyme stability and consistent reaction conditions. Equal volumes of crude enzyme extract were added to each reaction mixture to maintain uniform enzyme concentration. The mixtures were incubated in a water bath at a constant temperature for a fixed reaction time to allow enzymatic hydrolysis of cellulose. Following incubation, the enzymatic reaction was terminated by the addition of DNS reagent (3,5-dinitrosalicylic acid), which reacts with reducing sugars released during cellulose breakdown. The test tubes were then heated in the water bath to develop a reddish-brown color indicative of reducing sugar formation. After cooling, absorbance was measured using a spectrophotometer at 540 nm. Appropriate blanks containing substrate and buffer without enzyme were prepared to correct for background absorbance. Cellulase activity was calculated based on the amount of reducing sugar produced at each pH level. The pH corresponding to the highest enzyme activity was identified as the optimum pH.

Effect of Temperature on Cellulase Activity

The influence of temperature on cellulase activity was evaluated to establish the thermal conditions under which the enzyme functioned most effectively. Reaction mixtures containing crude enzyme extract, CMC substrate, and appropriate buffer were prepared in test tubes. The pH of all reaction mixtures was kept constant to isolate the effect of temperature. The prepared reaction tubes were incubated in a thermostatically controlled water bath set at different temperatures, typically ranging from low to moderately high values. A thermometer was used to confirm the accuracy of the water bath temperature throughout the incubation period. Each reaction was allowed to proceed for a fixed time to ensure comparability. At the end of incubation, the enzymatic reactions were stopped using DNS reagent, followed by heating in the water bath to facilitate color development. The reaction mixtures were cooled, and absorbance readings were taken using a spectrophotometer at 540 nm. Control reactions without enzyme were included to account for non-enzymatic substrate degradation. Cellulase activity was calculated based on reducing sugar concentration released at each temperature. The temperature that yielded the highest enzyme activity was identified as the optimum temperature.

Effect of Carbon Sources on Cellulase Activity

The effect of different carbon sources on cellulase production was studied to assess how substrate availability influences enzyme synthesis. A basal salt medium was prepared and supplemented separately with different

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue IV April 2026

carbon sources, including glucose, sucrose, lactose, and corn cob powder. The media were dispensed into conical flasks and sterilized before inoculation. Each flask was inoculated with the fungal culture and incubated under controlled conditions in an incubator to ensure proper aeration and nutrient distribution. Measuring cylinders were used to ensure accurate preparation of media components. The cultures were incubated for a defined period to allow sufficient enzyme production. After incubation, the cultures were centrifuged to obtain crude enzyme extracts. Cellulase activity was then assayed using standard enzymatic methods involving CMC substrate and DNS reagent. Absorbance readings were obtained using a spectrophotometer. The cellulase activities recorded for each carbon source were compared to determine which substrate most effectively induced enzyme production.

Effect of Metal Ions on Cellulase Activity

The influence of metal ions on cellulase activity was investigated to determine their role as enzyme activators or inhibitors. Metal salt solutions of $MgSO_4$, $MnSO_4$, $FeSO_4$, $CuSO_4$, and $ZnSO_4$ were prepared at appropriate concentrations. Reaction mixtures containing crude enzyme extract, CMC substrate, buffer, and individual metal ions were prepared in test tubes. Control reactions without metal ions were also set up to serve as references. The reaction mixtures were incubated in a water bath at a constant temperature for a fixed duration. Pipettes were used to ensure precise measurement of all reagents. Following incubation, the reactions were terminated using DNS reagent, and the tubes were heated to develop color. Absorbance was measured using a spectrophotometer at 540 nm. Cellulase activity in the presence of each metal ion was calculated and compared with the control.

Kinetic Behaviour of the Enzymatic Reaction

The kinetic behaviour of cellulase was studied to understand the relationship between substrate concentration and reaction rate. Reaction mixtures were prepared using crude cellulase enzyme and CMC substrate at varying concentrations, while buffer solution maintained constant pH conditions. Each reaction was carried out in test tubes and incubated in a water bath at a predetermined optimal temperature. A stopwatch was used to control reaction time precisely. After incubation, DNS reagent was added to stop the reaction and facilitate color development. The mixtures were heated, cooled, and absorbance readings were taken using a spectrophotometer at 540 nm. The rate of reaction was calculated based on the amount of reducing sugar released per unit time.

Statistical Data Analysis

All experiments were conducted in triplicates, and the results were expressed as mean ± standard deviation. Statistical analysis was performed using IBM SPSS Statistics version 25. One-way analysis of variance (ANOVA) was used to determine significant differences between the mean values of cellulase activity obtained from the fungal isolates. Differences were considered statistically significant at $p < 0.05$. Graphical representations of the data were prepared using Microsoft Excel 2016.

RESULTS

Identification of Fungal Isolates

Microscopic examination (Table 2) revealed distinct morphological features that enabled presumptive identification of the fungal isolates. Septate, branched hyphae with brush-like conidiophores terminating in chains of conidia were observed in one isolate, consistent with *Penicillium chrysogenum*. Other isolates exhibited septate hyphae with long conidiophores ending in swollen vesicles surrounded by phialides bearing chains of conidia, characteristic of *Aspergillus niger*. Broad, non-septate hyphae with spherical sporangia were also observed in some isolates, consistent with Rhizopus species. Based on cellulolytic potential, P. *chrysogenum* and A. *niger* were selected for further enzyme characterization.

Table 1: Microscopic characteristics of fungal isolates

Isolate Code	Hyphal Characteristics	Reproductive Structures	Presumptive Identification
HCC2	Septate, thin and branched hyphae (2–5 µm thick)	Branched conidiophores ending in brush-like clusters (penicilli) of phialides bearing chains of round conidia	Penicillium chrysogenum
DCC6	Septate and branched hyphae	Long, smooth conidiophores ending in round vesicles covered with phialides bearing chains of conidia	Aspergillus niger
DCC4	Septate and branched hyphae	Erect, unbranched conidiophores ending in swollen vesicles surrounded by phialides producing conidia	Aspergillus niger
HCC4	Broad, coenocytic (non-septate) hyphae	Long, unbranched sporangiophores ending in spherical sporangia containing spores; rhizoids present at base	Rhizopus stolonifer
HCC6	Broad, non-septate hyphae	Long unbranched sporangiophores ending in round sporangia filled with sporangiospores; columella visible	Rhizopus spp.
DCC1	Broad, non-septate hyphae	Large spherical sporangium at hyphal tip; sporangiospore visible	Rhizopus spp
DCC2	Septate, branched hyaline	Long conidiospore ending in a swollen vesicle surrounded by phialides bearing chains of conidia	Aspergillus niger

Key words: RCC = Residential Corn cob, DCC = Dumpsite Corn cob

Screening for Cellulase Production

Qualitative screening using the Congo red assay (Table 2) demonstrated varying cellulolytic capacities among the isolates. Clear zones around colonies confirmed extracellular cellulase secretion. The largest hydrolysis zones were observed in selected *Aspergillus* and *Penicillium* isolates, indicating higher cellulase production. Isolates with smaller clearing zones exhibited comparatively weaker cellulolytic activity. These results justified the selection of high-performing isolates for quantitative enzyme analysis.

Glucose Standard Curve

Table 2: Screening for cellulase

Isolate code	Clearing zone diameter (mm)
DCC4	17.00±2.00
DCC6	16.33±4.62
HCC4	13.33±10.21
HCC6	8.00±5.20
HCC2	13.00±4.36
DCC1	3.00±1.73

Keywords: RCC = Residential Corn cob, DCC = Dumpsite Corn cob

The glucose calibration curve (Figure 1) demonstrated a strong linear relationship between glucose concentration and absorbance at 540 nm. The regression analysis confirmed the reliability and accuracy of the DNS method for quantifying reducing sugars released during enzymatic hydrolysis. The linearity of the curve validates the subsequent enzyme activity calculations.

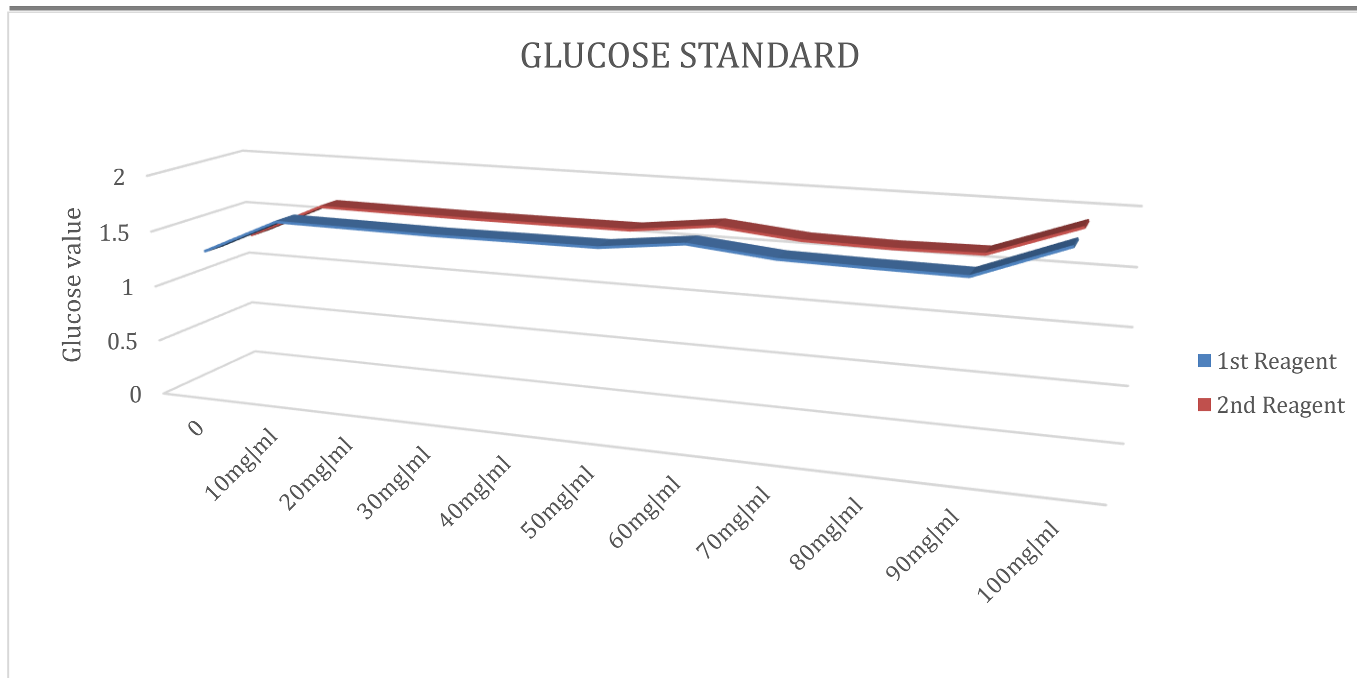

Figure 1: line plot representing glucose standard at1st and 2nd reagents at different concentrations

Effect of pH on Cellulase Activity

Cellulase activity varied significantly across the tested pH range (Figure 7). Maximum activity was observed under mildly acidic conditions, after which activity declined at both lower and higher pH values. The sharp decrease outside the optimum range suggests sensitivity of enzyme conformation and catalytic efficiency to hydrogen ion concentration.

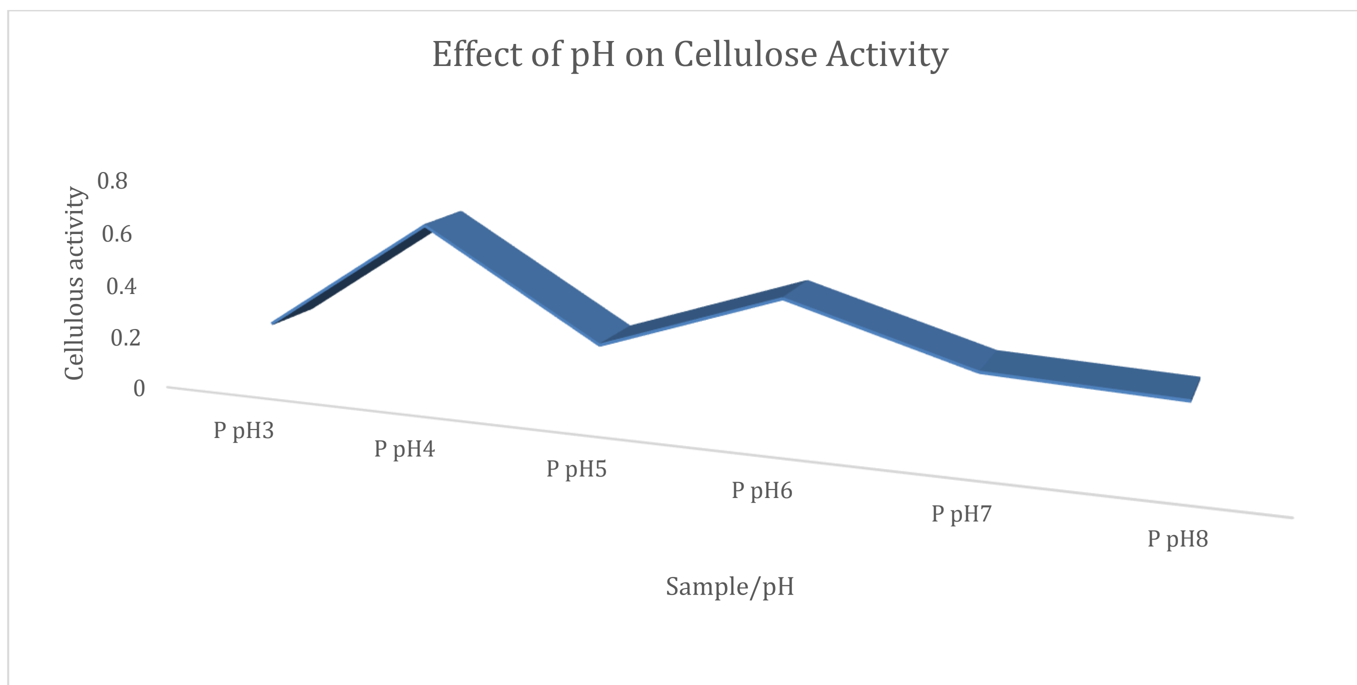

Figure 7: Line plot representing effect of pH on cellulous activity

Effect of Temperature on Cellulase Activity

Temperature influenced enzyme performance markedly (Figure 6). Enzyme activity increased progressively with temperature up to an optimum point, beyond which activity declined. The decrease at higher temperatures suggests possible thermal instability or partial denaturation of the enzyme structure.

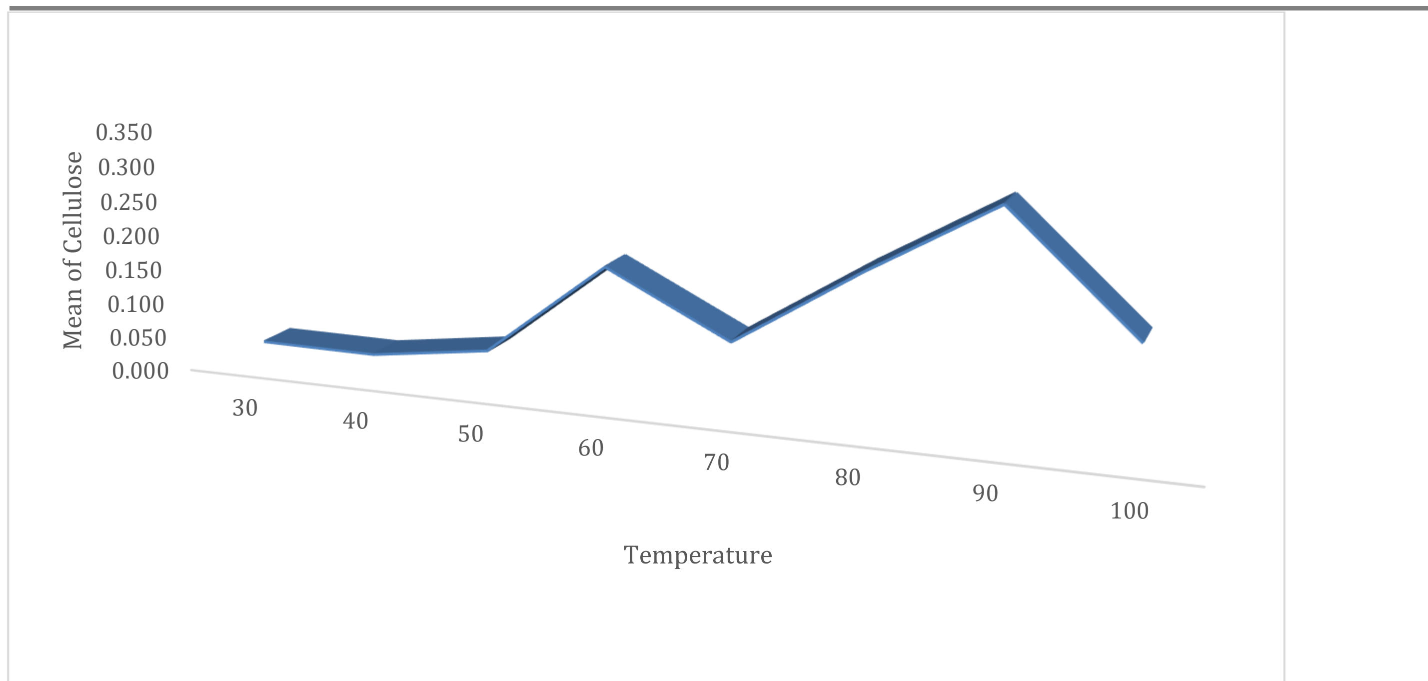

Figure 6: Line plot representing effect of temperature on cellulose

Effect of Carbon Sources on Cellulase Production

Different carbon sources significantly affected cellulase production (Figure 5). Complex lignocellulosic substrates supported higher enzyme activity compared to simple sugars. Enzyme production was comparatively reduced in media containing easily metabolizable sugars, indicating possible catabolite repression effects.

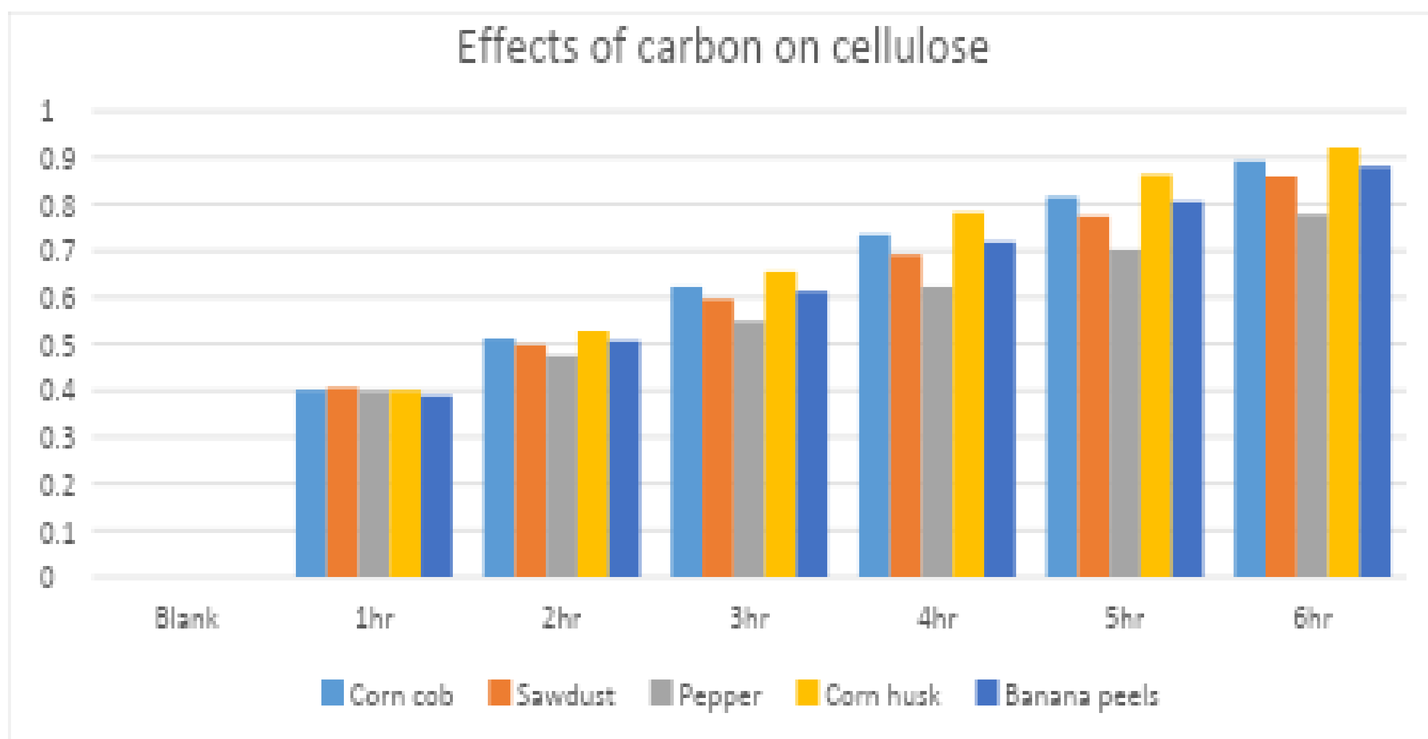

Figure 5: Bar chart representing effect of carbon on cellulose from 1hr to 6hr

Effect of Metal Ions on Cellulase Activity

Metal ions exhibited varying effects on enzyme activity (Figure 4). Some ions enhanced cellulase activity, while others caused noticeable inhibition. The stimulatory effects suggest possible cofactor roles or stabilization of enzyme structure, whereas inhibitory effects may be due to interference with the enzyme's active site.

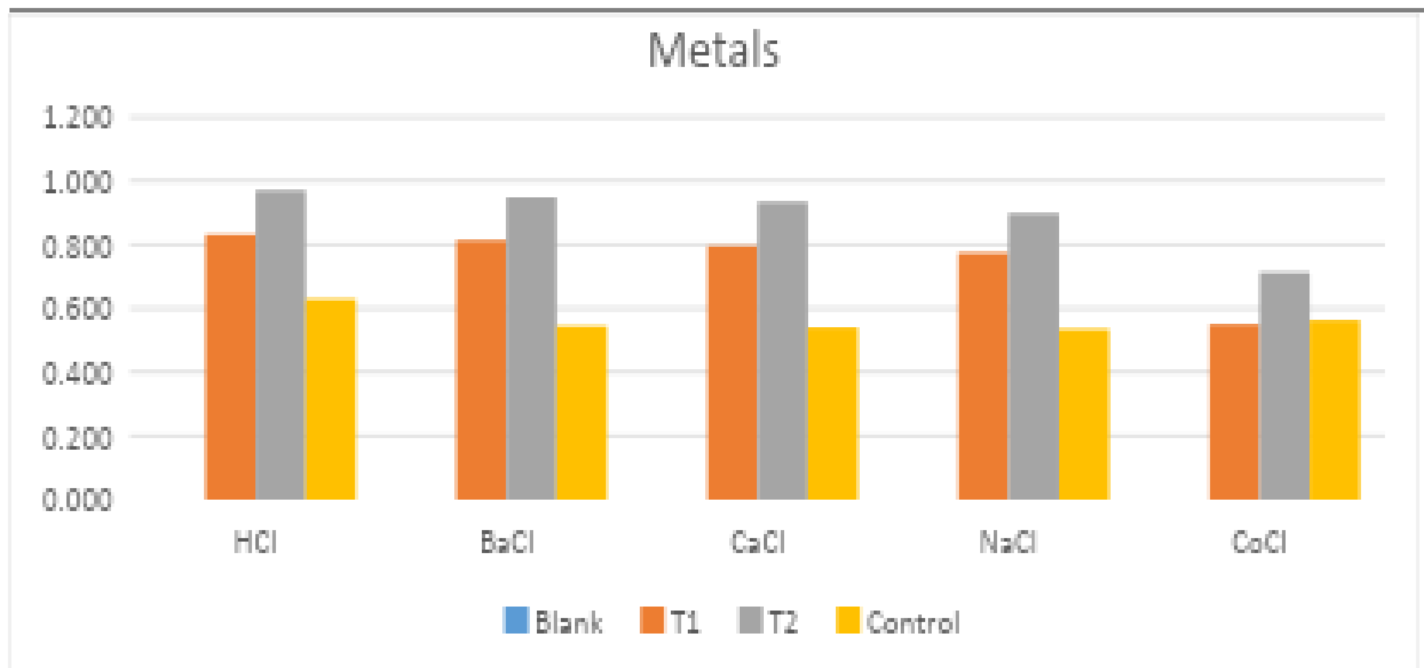

Figure 4: Bar chart representing metals results

Kinetic Behavior of Cellulase

The kinetic profile (Figure 3) demonstrated an increase in reaction rate with increasing substrate concentration until a plateau was reached, indicating enzyme saturation. This behavior is consistent with classical Michaelis–Menten kinetics and confirms that the cellulase exhibits predictable substrate-dependent catalytic activity.

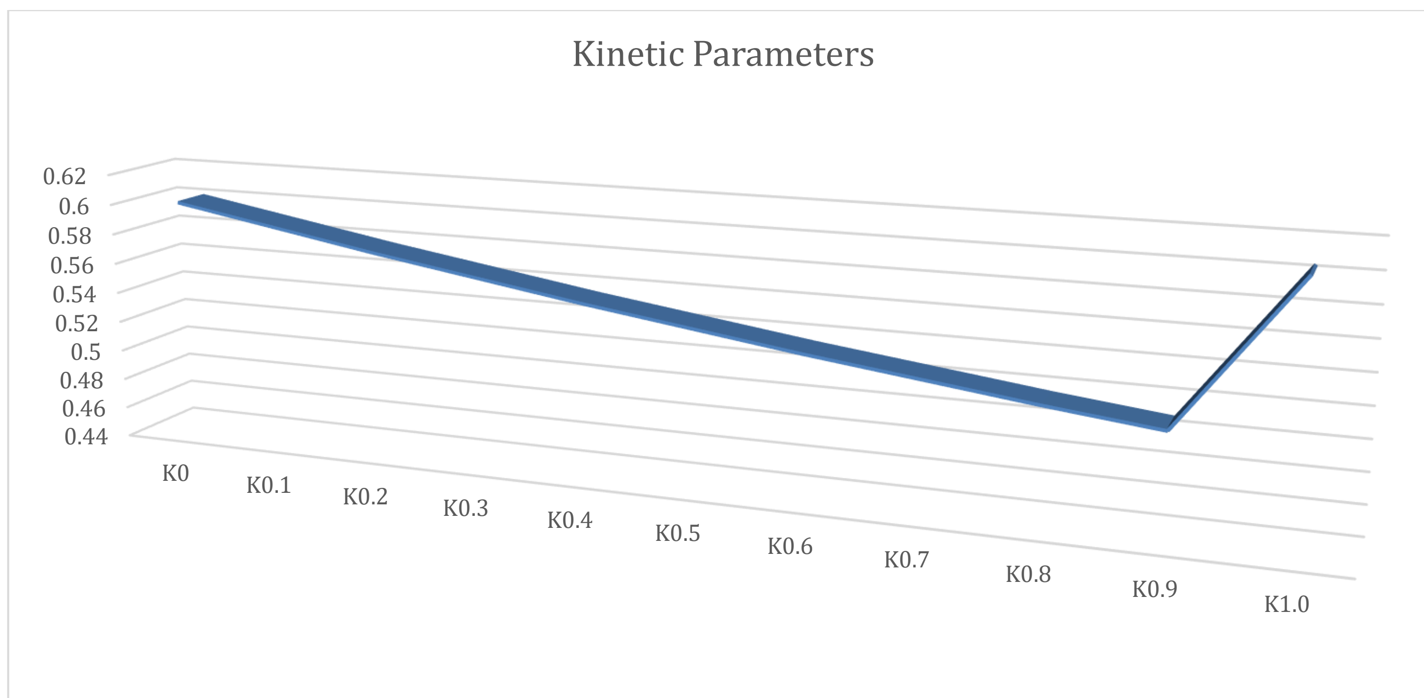

Figure 3: Line plot representing kinetic parameters results

DISCUSSION

The present study demonstrates the successful isolation, screening, and characterization of cellulase-producing fungi from corn cob substrates. Microscopic identification confirmed the presence of *Penicillium chrysogenum* and *Aspergillus niger*, both widely reported as efficient cellulase producers in previous studies (Singhania et al., 2017; Behera & Ray, 2016).

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue IV April 2026

The Congo red assay results revealed varying hydrolysis zone diameters among isolates, reflecting differences in extracellular enzyme secretion efficiency. Similar variability in cellulolytic potential among fungal strains has been reported in comparative studies of *Aspergillus* and *Penicillium* species (Khokhar et al., 2013). Larger clearing zones observed in selected isolates indicate stronger cellulose degradation capacity and correlate with enhanced enzyme production.

The glucose standard curve confirmed the precision of the DNS assay method, consistent with the widely adopted protocol described by Miller (1959). Accurate linearity of the standard curve ensures reliable quantification of reducing sugars released during enzymatic hydrolysis.

The observed optimum cellulase activity under mildly acidic conditions aligns with previous reports that fungal cellulases typically exhibit peak performance at pH 4–6 (Singhania et al., 2017). Acidic conditions favor proper ionization of catalytic amino acid residues at the enzyme's active site. Deviations from this optimum likely altered enzyme conformation, reducing substrate binding efficiency.

Temperature profiling revealed enhanced activity at moderate temperatures followed by a decline at elevated temperatures. This trend agrees with earlier studies showing that fungal cellulases are generally mesophilic, with optimal activity between 30°C and 50°C (Behera & Ray, 2016). The reduction in activity at higher temperatures may be attributed to protein denaturation and structural instability.

Carbon source evaluation demonstrated higher enzyme production in the presence of complex substrates compared to simple sugars. This observation supports existing literature indicating that lignocellulosic materials induce cellulase gene expression, whereas glucose may suppress enzyme synthesis through catabolite repression mechanisms (Singhania et al., 2017). The use of corn cob substrate in this study further supports the potential of agricultural waste valorization for cost-effective enzyme production.

Metal ion analysis revealed both stimulatory and inhibitory effects on cellulase activity. Similar modulatory effects have been documented, where divalent cations such as Mg^{2+} and Mn^{2+} enhance enzyme stability, while heavy metals may inhibit catalytic function (Ejaz et al., 2021). These findings highlight the biochemical sensitivity of cellulases to ionic environments.

The kinetic study confirmed classical Michaelis–Menten behavior, demonstrating enzyme saturation at higher substrate concentrations. Such predictable kinetic behavior is advantageous for industrial scale-up, as it allows determination of optimal substrate loading conditions and reactor design parameters.

Overall, the findings of this study are consistent with established literature and reinforce the industrial potential of cellulases derived from *Penicillium chrysogenum* and *Aspergillus niger*. The combination of acidic pH preference, moderate thermal stability, substrate inducibility, and predictable kinetics supports their suitability for applications in biomass conversion, biofuel production, and waste management.

CONCLUSION

This study successfully isolated, screened, and characterized cellulase-producing fungi from corn cob substrates collected within Lagos State. Microscopic examination confirmed the presence of *Penicillium chrysogenum* and *Aspergillus niger* among the isolates, both of which demonstrated significant extracellular cellulolytic activity.

Qualitative screening using the Congo red assay revealed clear hydrolysis zones, indicating effective cellulose degradation. Quantitative analysis further confirmed measurable enzyme activity, supported by a reliable and linear glucose standard curve. The characterization studies demonstrated that cellulase activity was strongly influenced by environmental factors. Maximum enzyme activity occurred under mildly acidic conditions, confirming the pH sensitivity typical of fungal cellulases. Temperature profiling revealed optimal performance at moderate temperatures, with decreased activity observed at higher temperatures due to possible thermal instability.

Carbon source analysis showed that complex lignocellulosic substrates enhanced cellulase production more effectively than simple sugars, suggesting induction mechanisms and reduced catabolite repression.

Additionally, metal ions exhibited both stimulatory and inhibitory effects, indicating that enzyme activity is modulated by ionic conditions. The kinetic study confirmed that the enzyme follows classical Michaelis–Menten behavior, demonstrating predictable substrate-dependent catalytic activity.

Overall, the findings highlight the biotechnological potential of cellulases produced by *Penicillium chrysogenum* and *Aspergillus niger* isolated from agro-waste substrates. The ability to utilize corn cob waste for enzyme production further supports sustainable bioprocessing and waste valorization strategies.

RECOMMENDATIONS

Based on the findings of this study, the following recommendations are proposed:

1. Molecular Identification: Future studies should employ molecular techniques such as ITS rDNA sequencing to confirm species-level identification and enhance taxonomic accuracy.
2. Optimization Studies: Further optimization using statistical approaches such as Response Surface Methodology (RSM) is recommended to maximize enzyme yield under controlled fermentation conditions.
3. Purification and Characterization: Partial or complete purification of the cellulase enzyme should be carried out to determine detailed biochemical properties such as molecular weight, stability profile, and specific activity.
4. Industrial Application Trials: The enzyme should be tested under industrially relevant conditions, particularly in biomass hydrolysis and bioethanol production systems.
5. Scale-Up Studies: Pilot-scale fermentation studies are recommended to evaluate economic feasibility and commercial viability.
6. Genetic Improvement: Strain improvement through mutagenesis or recombinant DNA technology may enhance cellulase productivity and stability.
7. Comparative Substrate Evaluation: Additional agricultural residues should be evaluated to identify the most cost-effective and high-yielding substrates for enzyme production.

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