African Journal of Pure & Applied Sciences

DOI: 10.33886/ajpas.v5i1.494

Journal homepage: http://journal.ku.ac.ke/index.php/AJPAS

Research Article Open Access

ISOLATION AND CHARACTERIZATION AND SOME PROPERTIES OF CELLULASE FROM *FUSARIUM GRAMINEARUM* ISOLATED FROM CORN COB

*Ezima Esther Nkechi[1], Adegbesan Bukunola Oluyemisi[1], Osonuga Ifabunmi Oduyemi[2], Faponle Abayomi Samson[1], Abisoye, Segun Babatunde[1] and Odufuwa Kuburat Temitope[1]

[1]Department of Biochemistry, Faculty of Basic Medical Sciences, Sagamu Campus, Olabisi Onabanjo University, Ago-Iwoye, OgunState, Nigeria.

[2]Department of Physiology, Faculty of Basic Medical Sciences, Sagamu Campus, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria.

*Corresponding author's email: ezike.chi@oouagoiwoye.edu.ng Phone: +2348033685169

Received 15th February , 2024: Accepted 23rd April 2024; Published online 30th June 2024

Citation: Esther Nkechi, E., Bukunola Oluyemisi, A., Ifabunmi Oduyemi, O., Abayomi Samson, F., ,Segun Babatunde, A., & Kuburat Temitope, O. Isolation and Characterization and Some Properties of Cellulase from Fusarium Graminearum Isolated from Corn Cob. African Journal of Pure and Applied Sciences, 5(1). https://doi.org/10.33886/ajpas.v5i1.494

ABSTRACT

Breaking down lignocellulosic materials requires the use of cellulases which are recently been exploited for bioremediation. This study is aimed at isolating and characterizing cellulase from *Fusarium graminearum* isolated from corn cob for possible higher specific activities and greater efficiency for cellulose metabolism. Cellulase from *Fusarium graminearum* isolated from corn cob was partially purified using dialysis against 50 % glycerol and gel filtration on Sephacryl S-200 while characterization was carried out using standard methods. The purification process resulted in an overall yield of 10.9 % with a purification fold of 14.4. The partially purified enzyme obtained was of 6477.5 U/mg specific activity and native molecular weight of 35.8 kDa. Our study of the kinetic properties of the enzyme showed a cellulase with high affinity for cellulose with K_m and V_{max} as 0.1352 mg/ml and 17.86 Units/ml/min. The enzyme displayed its highest performance at 70 °C and pH 4. Mn^{2+} had little to no impact on *Fusarium graminearum* cellulase's activity whereas Mg^{2+} and Ca^{2+} somewhat decreased the activity at 5 mM, this was restored to almost 100% at 10 mM. However, Hg^{2+} and Ba^{+} had much impact on the activity of the enzyme. This work shows that corn cob *Fusarium graminearum* cellulase could be exploited as a potential industrial enzyme for targeted waste degradation process because of its high activity and thermostability.

Keywords: Bioconversion, waste degradation, cellulase, *Fusarium graminearum,* purification.

Introduction

The rising prices of crude oil and its supply and the environmental problems like global warming and air pollution has necessitated massive research in the development of alternative energy technology, among all the alternative energy strategies proposed and developed, bioconversion of biomass has been reported to be more significant than others because biomass is the most abundant and most renewable biomaterial on our planet (Dashtban et al., 2009; Nazir et al., 2024). Lignocellulosic biomass is made up of approximately 40–60 % cellulose, 10–40 % hemicelluloses and 15–30 % lignin and if well utilize can provide 14% of the world's total energy requirement, which is currently met by fossil fuels (Wu et al., 2020; Iram et al., 2021).

The degradation of lignocellulosic residues is mainly started by microorganisms such as fungi and bacteria which are capable of degrading lignocellulolytic materials using the cellulolytic enzyme system (Nazir et al., 2024). Cellulase is one of the world's most sought-after industrial enzymes because of its usefulness in the industries and in recent times for bioremediation and biofuel production (Bhardwaj *et al*., 2021).The major function of cellulase is the degradation of lignocellulose biomass into glucose monomers (Hoelker *et al.,* 2004, Chellapandi and Jani, 2008).

Cellulase is made up of three component enzymes which work in harmony to achieve the complete cellulosic degradation (Lo *et al,* 2010; Lin *et al*, 2009*)*. Cellulase enzyme is widely known to be produced during the growth of *Ascomycota*, *Basidiomycota*, and *Deuteromycota* members; because they are mainly rich in cellulose (Sajith*et al.,* 2016). Cellulase activity has also been reported in other genera of microbe such as *Trichoderma, Aspergillus and Penicillium* (Aweke et al., 2022). With regards to the hydrolysis of cellulose and cellulase synthesis, fungi appear to be the most researched of all the cellulolytic microorganisms. Fungal cellulases are used in many industries for textile manufacturing, waste-water treatment, food processing, feed preparation, and detergent formulation (Iram, 2023). Filamentous fungi has been reported as one of the top industrial producers of cellulases, preferring solid-state fermentation (SSF) as they have adapted to low-moisture environments, such as decaying wood, oat straw, sugar cane bagasses (Iram et al, 2021; Iram et al., 2023).

Fusarium graminearum is an important phytopathogen of crop plants that causes *Fusarium* head-blight, a destructive disease of cereals. Under favorable environmental conditions, *Fusarium* head-blight disease leads to serious economical losses in the agro-feeding industry. The damages caused by this fungus change according to the geographical location of the infection, affecting the quality parameters of grains, their weight, carbohydrate and protein composition (Kikot et al., 2010). The virulence of *Fusarium graminearum* has been link to the capacity of the fungus to produce extracellular enzymes involved in the fungal infection onto the host plant. These extracellular enzymes have also been suggested to be responsible for the degradation of the main components of plant cell wall (Kikot et al., 2010).

Among the various species of *Fusarium* isolated from diseased cereals *Fusarium graminearum* is the most frequent species and very aggressive toward cereals damage, especially in wheat, barley, maize, maize stem e.t.c. (Cosic & Jurkovic, 2000). *Fusarium graminearum* has been reported to efficiently exploit inexpensive and freely available carbon sources to produce vast amounts of extracellular enzymes which can effectively break down lignocellulosic wastes benefiting society's strategic and environmental goals. Although the isolation and characterization of Cellulase from *Fusarium graminearum* have not been extensively studied, there are reports of isolation and characterization of other enzymes like proteases, peptidases and amylases from *Fusarium graminearum* (Kumari et al., 2010; Rohan, et al., 2015; Abaildayev et al., 2022). This study is therefore aimed at isolating and characterizing an indigenous cellulase from *Fusarium graminearum* isolated from corn cob for possible higher specific activities and greater efficiency for cellulose metabolism.

Materials and Methods

Isolation of the fungal organism

Fusarium graminaerum used in this study was isolated from corn cob at the Department of Microbiology, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria. Organism enrichment was done using 1 g of Carboxymethyl-cellulose (CMC) in minerals salt solution containing (g/l): 0.2 K_2HPO_4; 0.4 $NaNO_3$; 0.01 $FeSO_4$; 0.1 $MgSO_4.7H_2O$ and 0.5 KCl. All the media were sterilized by autoclaving for 20 minutes at 121 ^oC and 15 lbs of pressure, incubation was done with shaking (150 rpm) for 5 days at 37 ^oC. The crude enzyme was obtained by10

minutes centrifugation of the media at 4,000 rpm to get the cell-free supernatant, this was then kept on 4 °C storage for further assays.

Assay of Enzyme

The action of cellulase was determined using Bernfeld, (1995) method. Exactly 0.1 ml of the enzyme was added to 0.9 ml of 1 % CMC in 0.4 M acetate buffer pH 4.5; and the resulting solution was incubated at 40 ºC for 20 mins with time-to-time agitation. After incubation, 0.4 ml of dinitrosalicylic acid was added to stop the reaction; after which the resultant mixture was boiled for 5mins; then allowed to cool at 27 ºC. Thereafter, 3 ml of distilled water was added and the absorbance of mixture was measured at 540 nm. Enzyme activity was then extrapolated from a standard glucose calibrated curve with glucose concentrations varied between 0 and 100 µg. A unit of cellulase activity was said to be the quantity of enzyme that liberated a micro-mole of reducing sugars (equivalent of glucose) in a minute.

Determination of Protein

The concentration of protein within the enzyme mixture at the various level of purification was accessed utilizing the procedure of Bradford, (1976); using 10 µg/ml BSA (Bovine serum albumin) as standard.

Cellulase Purification

Dialysis against 50% glycerol

Fusarium graminearum's crude enzyme extract was dialyzed three times against 50% glycerol to concentrate the enzyme. The 50% glycerol was made by combining equal volumes (50 ml) of glycerol and 0.4 M acetate buffer (pH 4.5).

Filtration on Sephacryl S-200 Gel

The sephacryl S-200 used for this work was equilibrated using 0.4 M acetate buffer (pH 4.5) and packed in (1.5 X 25 cm) column. The dialysate (6.2 ml) obtained from the procedure before was loaded, the proteins were eluted by washing the column with 0.4 M acetate buffer (pH 4.5); thereafter, 5ml fractions were taken at the rate of 10 ml per hour. Afterwards, concentration of protein was observed spectrophotometrically at 280 nm while cellulase activity was routinely assessed using the method previously mentioned. All fractions indicating presence of enzymatic were combined "pooled" together and concentrated via dialysis against 80 % glycerol.

Cellulase Characterization

Apparent Molecular Weight Estimation

Gel filtration chromatography which used Sephacryl S-200 resin in 1.5 cm×70 cm column was employed to estimate the molecular mass of *Fusarium graminearum* cellulase. Also, 1 mg/ml blue dextran was used to determine the void volume. Standard protein used at 2 mg/ml include; Lysozyme (13 kDa), Peroxidase (44 kDa), α-chymotrypsinogen (25 kDa), and BSA (66 kDa). The proteins were individually run one after the other and then 100 mM Tris-HCl buffer "pH 8.5" was used to wash the chromatographic column. The proteins were surveyed spectrophotometrically at 280 nm in 5 ml fractional eluents collected at 20 ml per hour flow rate after which the elution volume of estimated proteins was computed. Then, 5 ml of the partially purified cellulase from *Fusarium graminearum* was ran through the same column. The graph showing logarithm of molecular weight versus partition coefficient (K_{av}) for the benchmark proteins was plotted and the molecular mass of cellulase obtained from *Fusarium graminearum* was interpolated from the curve.

Kinetic properties of cellulase from Fusarium graminearum

Kinetic parameters such as (K_m and V_{max}) of the enzyme were measured adopting the double reciprocal plot proposed by Line-weaver and Burk (1934). Substrate (CMC) concentrations between 0.1 and 1.0 mM were used for the enzyme assay. The graph of the reciprocal of the enzyme activity (1/V) versus the reciprocal of the various substrate concentrations [1/CMC] was plotted to generate the K_m and V_{max} values while the plot of the enzyme activity (V) versus the various substrate concentrations [CMC] was plotted to generate the Michaelis-Menten plot .

pH Effects on cellulase from Fusarium graminearum

The influence of pH on the action of cellulase from *Fusarium graminearum* was analyzed using Bakare et al. (2005)'s method by conducting an enzyme assay at varying pH ranging from 3 to 9 utilizing citrate buffer (pH 3-5), phosphate buffer (pH 6-7) and Tris buffer (pH 8-9). Graph of enzyme activity versus the various pH values was plotted to generate the pH profile.

Temperature influence on cellulase from Fusarium graminearum

The influence of temperature on the action of cellulase obtained from *Fusarium graminearum* was assessed at varying from 30 ºC to 80 ºC temperatures following the method of Bakare *et al.* (2005). An initial incubation of the assay mixture was conducted at the stated temperature for 10 minutes before the subsequent reaction proceeded following the addition of an aliquot of the enzyme already prepared at the exact temperature. The activity of cellulase was determined using the method previously mentioned; the result was then plotted against the different temperature values to generate the temperature profile.

Influence of metal ions on Fusarium graminearum cellulase

The study of the impact of metal ions on corn cob *Fusarium graminearum* cellulase was carried out using $MnCl_2$, $MgCl_2$, $CaCl_2$, $HgCl_2$ and $BaCl_2$ till an end concentration of 5.0 mM and 10.0 mM. The enzyme was incubated for 1 hour in the presence of each of the various metals and the residual cellulase activity was recorded. The residual cellulase activity in the absence of metal ions was considered as control (100).

Results and Discussion

Isolation and partial purification of cellulase from Fusarium graminearum

Summarized results of the purification procedures for this study are shown in Table 1. The cellulase produced by *Fusarium graminearum* from corn cob exhibited a specific activity of 6477.5 U/mg proteins, 10.4% yield, and 14.4 purification-fold. Dialysis of the crude enzyme against 50 % glycerol greatly increased the activity of the enzyme while gel filtration on Sephacryl S-200 gave two cellulase peaks, a prominent one and a weak one as shown in Figure 1. The cellulase with the prominent peak was used for this work. The specific activity of 6477.5 U/mg proteins obtained for the cellulase from *Fusarium graminearum* in this study is higher than

that obtained from the culturing of *Aspergillus niger* on *Arachis hypogaea* shells with specific activity of 484.3 U/mg as reported by Sulyman *et al*., (2020), *Aspergillus niger* (Van tieghem, 1867) having a specific activity of 176.2 U/mg (Ahmed and colleagues, 2015) and *Penicillium decumbens* showing a specific activity of 46.6 U/mg protein (Nehad and colleagues, 2019). Pachauri *et al.,* (2017) reported 30 U/mg as specific activity for *Trichoderma longibrachiatum.* Moreover, cellulase with lower specific activities has been reported; a specific activity of 6.63 U/mg was the result of cellulase from *Pseudomonas aeruginosa IZ* grown on corn cob (Itani *et al.,* 2017) while *Pseudomonas sp.* Cellulase from dump site waste has specific activity of 0.757 U/mg (Goel and colleagues, 2019).

However, the specific activity of cellulase of *Fusarium graminearum* isolated from corn cob compares well with that produced by *Trichoderma longibrachiatum* which gave specific activity of 6680.8 U/mg (Noor and Hameed, 2018) but lower than that of *Penicillium verruculosum* cellulase having a specific activity value of 23098.95 U/mg (Sajith *et al.,* 2015). Thus, higher specific activity of corn cob *Fusarium graminearum* cellulase indicates a very active enzyme that could be promising in industrial application.

Table 1: Summation of the purification procedures for *Fusarium graminearum* cellulase

Purification Procedures	Volume (ml)	Activity (U/ml)	Protein (mg/ml)	Total activity (units)	Total protein (mg)	Specific activity (U/mg)	Yield (%)	Purification fold
Crude Enzyme	20	296.6	0.657	5932	13.14	451.44	100	1
Dialysis against 50% glycerol	6.2	697.9	0.2237	4326.98	1.38	3119.80	72.94	6.91
Gel-filtration on Sephacryl S-200								
Pool A	30	32.62	0.016	978.6	0.48	2038.8	16.5	4.5
Pool B	20	1.92	0.027	38.4	0.54	71.1	0.65	0.16
Dialysis against 80% glycerol (Pool A)	12.50	51.82	0.008	647.8	0.1	6477.5	10.9	14.4

A unit of cellulase activity was said to be the quantity of enzyme that liberated a micro-mole of reducing sugars (equivalent of glucose) in a minute under standard assay condition (20 min incubation at 40 °C with 1% CMC in 0.4 M acetate buffer pH 4.5)

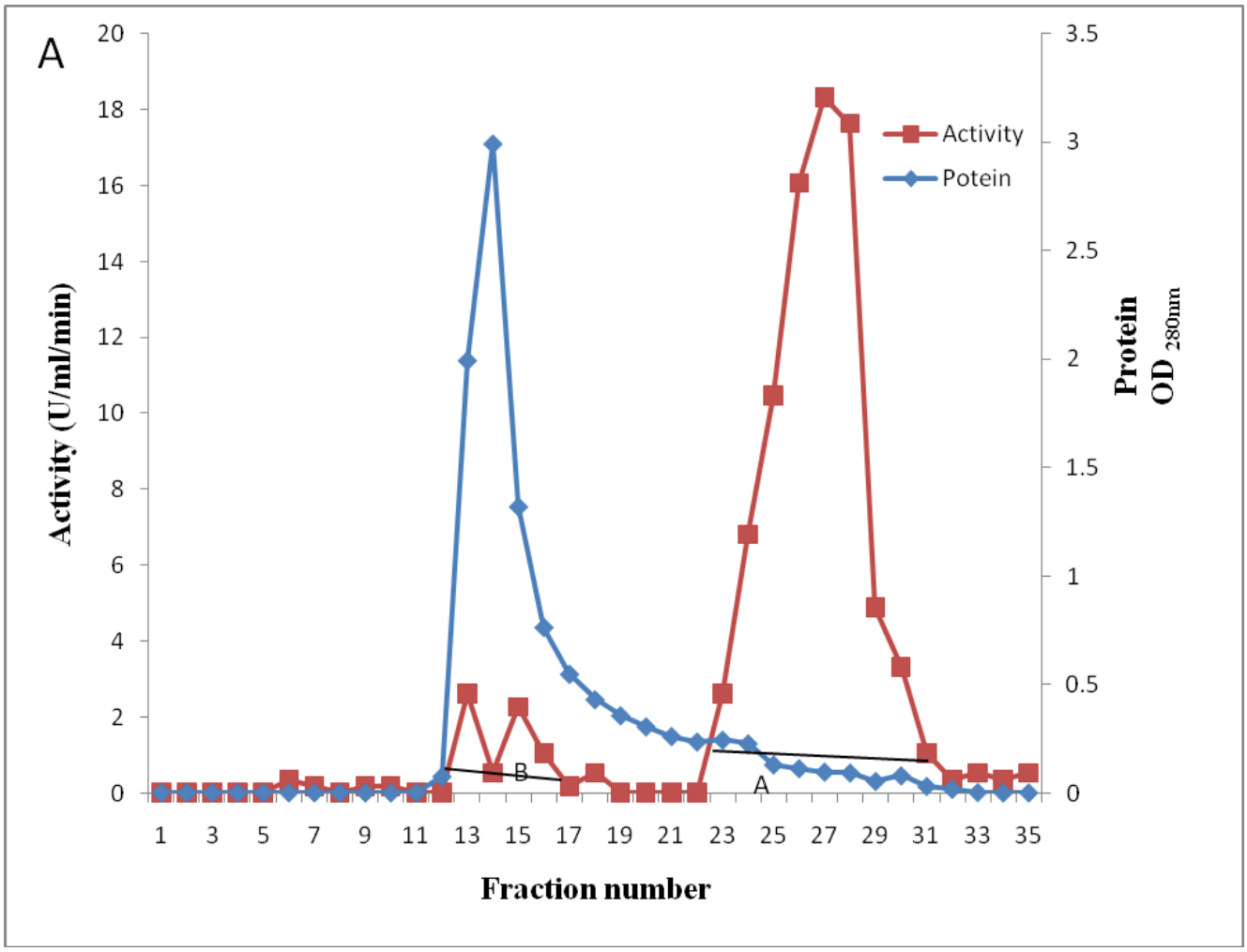

Figure 1: Gel filtration chromatography of corn cob *Fusarium graminearum* cellulase on Sephacryl S-200 resin equilibrated with 0.4 M acetate buffer (pH 4.5) and packed in (1.5 X 25 cm) column, 5ml fractions were taken at the rate of 10 ml per hour.

Native molecular weight

The molecular weight estimated for *Fusarium graminearum* cellulase was 35.8 kDa as shown in Figure 2. This value is similar to that for *Aspergillus niger* (van Tieghem 1867) reported by Ahmed *et al.,* 2015, with an estimated mass of 34.0 KDa, Bakare *et al.,* (2005) for the wild-type, CRRmt24 and CRRmt4 of *Pseudomonas fluorescens* (36.0, 26.0 and 36.0 kDa respectively). Bai *et al.,* (2013) reported 33.2 kD for glucanases from *Penicillium simplicissimum H11* while Zhao *et al.,* (2015) reported 31.0 kDa for *consortium XM70* cellulase in terrestrial hot spring.

Lower molecular weights have also been reported, *Penicillium verruculosum* BS3 cellulase with molecular weight of 17.0 kDa was reported by Sajith *et al.,* 2015 while Sulyman and colleagues, 2020 reported 13.5 KDa for *Aspergillus niger* from *Arachis hypogaea* shells culture. However, heavier cellulases have been reported, in 2018, Kumar and co-workers reported a molecular weight of 60.0 KDa for *Schizophyllum commune* "NAIMCC-F-03379" from degraded *Lantana camera* leaf sample, Nisar et al, (2022) also reported 60.0 KDa for *Thermomyces dupontii* cellulase.

Enzyme kinetics

Figure 3 shows the estimated V_{max} and K_m of the Corn cob *Fusarium graminearum* cellulase (17.85 U/ml/min and 0.135 mg/ml respective). The low K_m obtained indicates that the cellulase from *Fusarium graminearum* has a high affinity towards its substrate (CMC) and may be a good candidate for the hydrolysis of cellulose rich waste especially in waste treatment and management. The study by Kumar *et al*., (2018) highlighted cellulase with very high affinity for CMC with K_m of 0.0909 mg/ml from *Schizophyllum commune* "NAIMCC-F-03379". Other K_m as well as V_{max} values has been reported by researchers. According to Liu *et al.,* (2011) *A. fumigatus* Z5 produced two cellulases (Egl2 and Egl3) with V_{max} and $K_m$of437.3 µmol/min/mg and 37.8 mg/ml and652.7 µmol/min/mg and 51.8 mg/ml respectively. Similarly, Li *et al.,* 2012 reported V_{max} of 4.6 U/min/mg and K_m of 134 mg/ml for *A. niger* BCRC31494 produced cellulase while Bakare *et al*. (2005) reported 3.3 U/ml and 3.6 mg/ml for cellulase obtained from *Pseudomonas fluorescens*. The Michaelis-Menten plot depicting enzyme activities versus substrate concentrations shows that the corn cob *Fusarium graminearum* cellulase is a simple enzyme that obeys the Michaelis-Menten equation as shown in Figure 4.

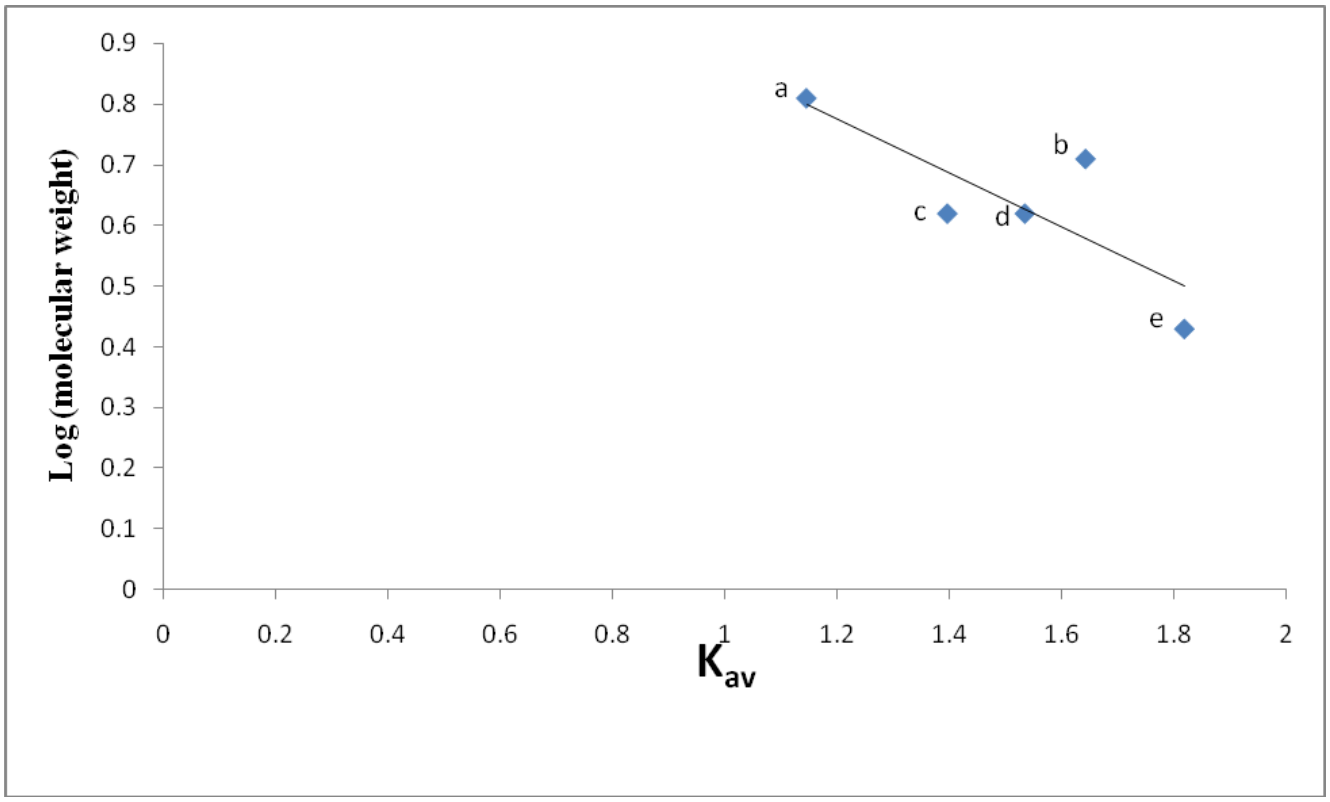

Figure 2: Calibration curve for the native molecular weight determination of cellulase from *F. graminearum* by gel filtration using Sephacryl S-200 resin in 1.5 cm×70 cm column.
a. BSA, b. Peroxidase, c. α-chymotrypsinogen, d. *F. graminearum* cellulase and e. Lysozyme

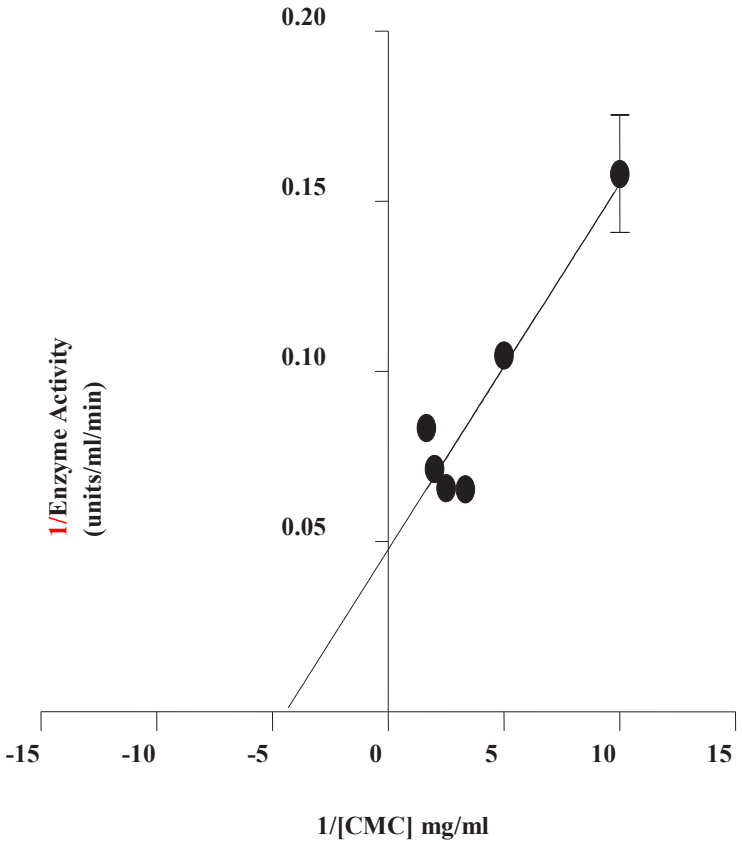

Figure 3: Line weaver-Burk plot of corn cob *Fusarium graminearum* cellulase for the determination of Km and vmax. The values of 1/V was plotted against 1/[CMC] at varying concentration of CMC between 0.1 and 1.0 mM.

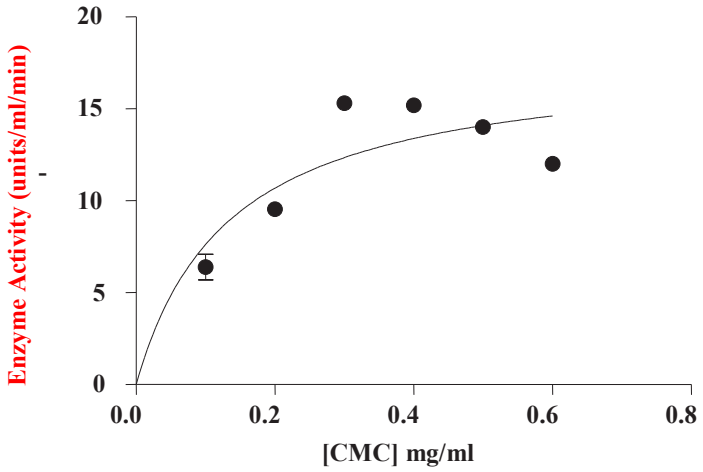

Figure 4: Michaelis-Menten plot of activities of cellulase from corn cob *Fusarium graminarium.* The values of V was plotted against [S] at varying concentration of CMC between 0.1 and 1.0 mM .

pH Effects on cellulase from Fusarium graminearum

As presented in Figure 5, cellulase from *Fusarium graminearum* was most active at pH 4; however, further increment in pH led to decrease in enzyme activity. Similar pH optima have been reported for cellulases from other sources. Noor and Hameed, (2018), Nehad and associates, (2019), and Sulyman and colleagues, (2020) reported pH optima of 4 for *Trichoderma longibrachiatum* from Iraqi soil, *Penicillium decumbens* and culturing of *Aspergillus niger* on *Arachis hypogaea* shells respectively. Optimum pH of 5 has also been reported by Rajesh *et al.,*(2012) for *Trichoderma* resse, Ahmed at al., (2015) for *Aspergillus niger (Van tieghem*, 1867) and Zeng *et al.,*(2016) for *Trichoderma virens* while Soliman *et al.,* (2013) reported a relative stability in pH range of between 3-6 for the CMCase II. Higher optimum pH of 6.5 has been reported by Bakare and Co-workers, (2005) for *Pseudomonas fluorescens* cellulase. The result of this work corresponds to the findings of Bhikhabhai *et al*.,

(1984) that fungal cellulases are stable over a pH range of 3–8 and are often active across a pH range of 3.5–7 in phosphate, citrate, or acetate buffers. *Fusarium graminearum* cellulase was active within pH range of 3 to 8.

Temperature influence on cellulase from Fusarium graminearum

Although different temperature optima have been reported for cellulases from different sources, our result showed a highly thermostable enzyme with estimated optimum temperature of 70 ºC (Figure 6). This value is among the highest temperature optima reported so far, Akiba *et al.,* (1995) reported an optimal temperature of 70 ºC at pH 6 with 2hr stability for the cellulase produced by *A. niger* IFO31125, Nisar et al, (2022) also reported temperature optimum of 70 ºC for *Thermomyces dupontii*. Lower Temperature optima of 50 ºC has been reported by Rajesh *et al.,*(2012) for *Trichoderma* ressei, Rahnama *et al.,*(2016) for *Trichoderma harzianum* SNRS3 cellulase and Zeng *et al.,*(2016) also reported for *Trichoderma virens*. Noor and Hamdan, (2018) reported 40 ºC for cellulase from *Trichoderma longibrachiatum* in soil from Iraq; while Ahmed and colleagues, (2015) and Kumar and associates, (2018) published temperature optima of 30 ºC and 25 ºC respectively for cellulases from *Aspergillus niger (Van tieghem*, 1867) and *Schizophyllum commune.*

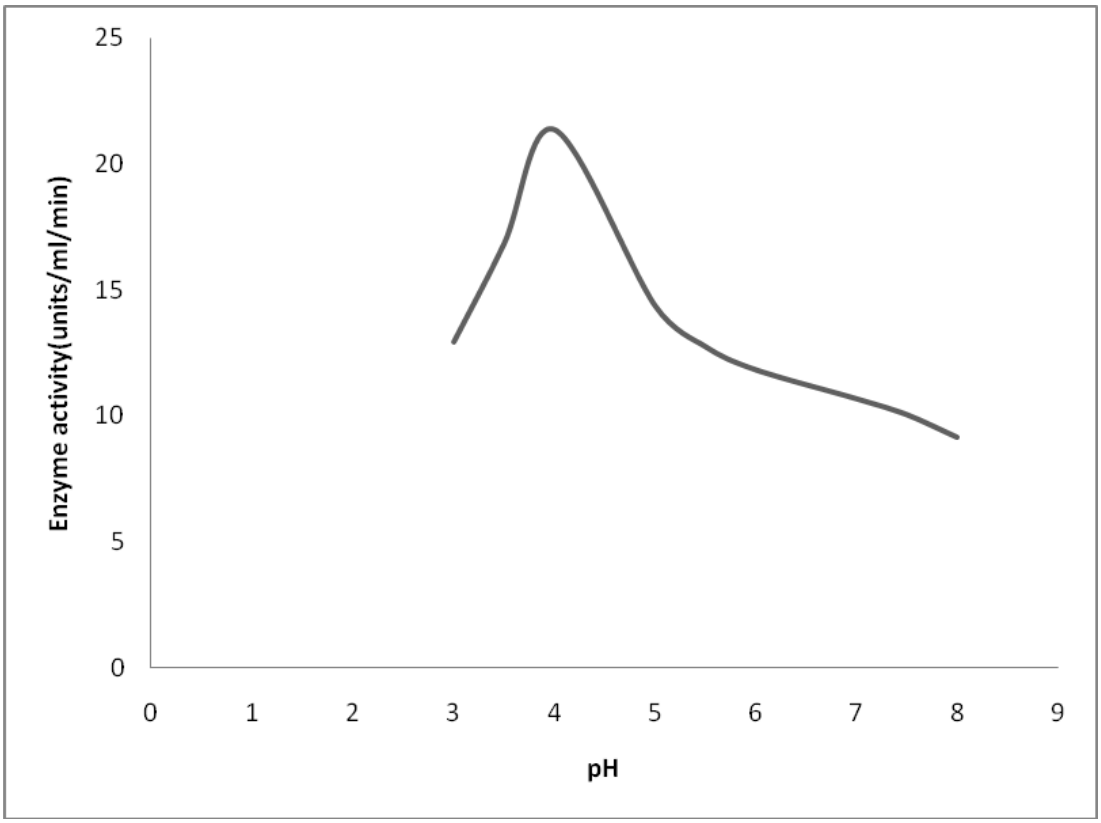

Figure 5: The profile of the influence of pH on cellulase from corn cob *Fusarium graminearum*. The enzyme was assayed at varying pH ranging from 3 to 8 utilizing citrate buffer (pH 3-5), phosphate buffer (pH 6-7) and Tris buffer (pH 8-9)

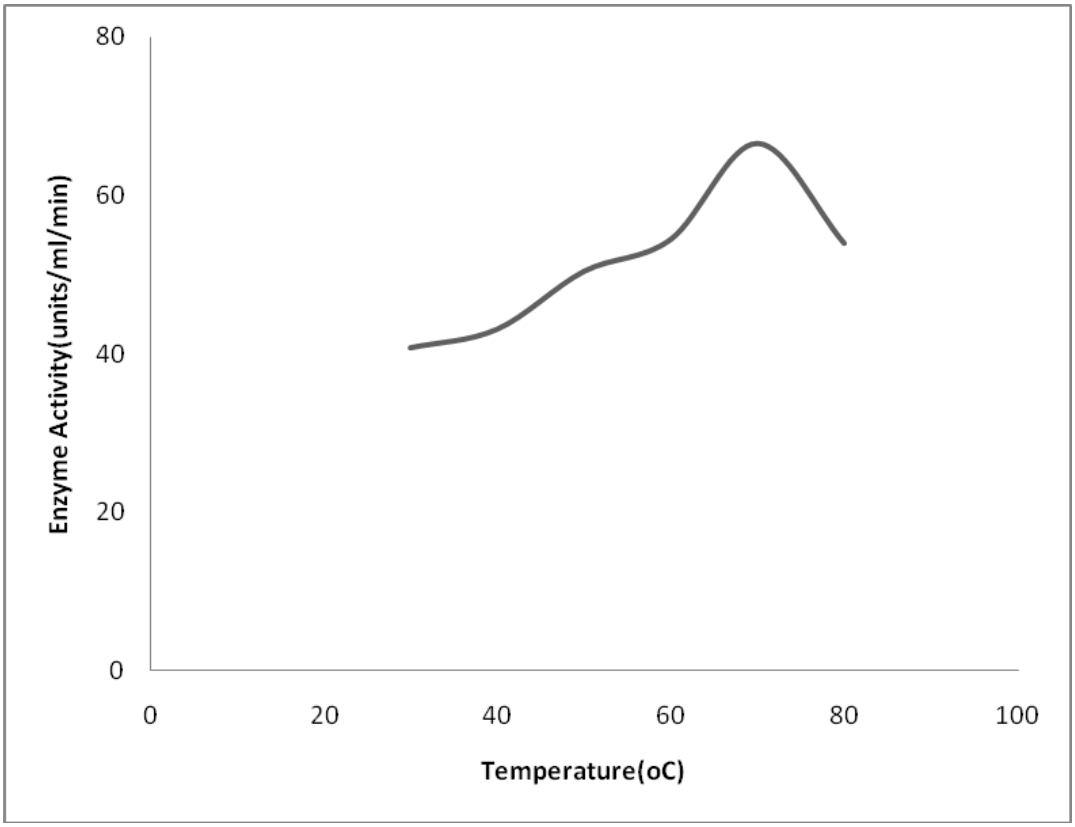

Figure 6: The profile of the impact of temperature on the activity of cellulase from corn cob *Fusarium graminearum*. The enzyme was assayed at varying temperatures between 30 ºC and 80 ºC.

Influence of metallic ions on* cellulase *from Fusarium graminearum

Result from this current study on the influence of metallic ions on cellulase activity of *Fusarium graminearum* from corn cob are shown in Table 2. The findings revealed that Mn^{2+} had little or no impact on the enzyme at10mM and 5mM concentrations whereas Mg^{2+} and Ca^{2+} slightly decrease enzyme activity at 5 mM, but the activity was restored to almost 100% at 10 mM concentration. However, at both 5 mM and 10 mM concentrations, Hg^{2+} and Ba^{+} inhibited the enzyme's activity with Ba^{+} inhibiting the enzyme the more. There are reports of the influence of metal ions on cellulase activities, Ahmed *et al*., (2015) reported $Mn^{2+}$and CO^{2+} as having a positive effect on *Aspergillus niger (van Tieghem* 1867) cellulase while Hg^{2+}, Cd^{2+}, Fe^{2+} and Fe^{3+} inhibited the enzyme at different degrees. In another study, Fe^{2+} was reported to activate the cellulase from *Aspergillus flavus* whilePb^{2+}, Hg^{2+}, Ca^{2+}, Cu^{2+}, Mg^{2+}, CO^{2+}, Zn^{2+} andMn^{2+}all inhibited the enzyme (Okonkwo, 2019). Onyia *et al*., (2021) reported that cellulolytic activities of palm biomass isolated fungi was positively influenced by Ca^{2+} while Pb^{2+}, Hg^{2+}, K^{+}, Cu^{2+}, $Mn^{2+}$and Fe^{3+} negatively affected the enzyme. The activities of cellulase obtained from *Aspergillus niger* with the use of *Arachis hypogaea* shells was reported by Sulyman *et al*. (2022) to be activated by $Mn^{2+}$and CO^{2+} while Mg^{2+}, Zn^{2+}, Ca^{2+},$Cu^{2+}$and Fe^{2+} inhibited the enzyme at concentration above 1 mM.

These various accounts seem to show that metal ion effects on the activities of cellulase are dependent on the sources of the enzyme which may affect the amino acids sequence of the enzyme. One major correlation in most of the reports is that Hg^{2+} ion is an inhibitor of the enzyme.

Table 2: Influence of metal ion on cellulase from corn cob *F. graminearum*

	Residual enzyme activity	
Salt	5 mM	10 mM
Control	100.00	100.00
Mn^{2+}	102.24	86.05
Mg^{2+}	76.58	90.65
Ca^{2+}	60.37	89.79
Hg^{2+}	50.34	49.45
Ba^{2+}	23.07	9.53

The cellulase from corn cob *F. graminearum* was incubated for 1 hour in the presence of each of the various metal ions in overall concentration of 5.0 mM and 10.0 mM and the residual cellulase activity recorded. The residual cellulase activity in the absence of metal ions was considered as control (100).

Conclusion

In conclusion, our work has shown that *Fusarium graminearum* from corn cob can produce cellulase with high specific activity and affinity for its substrate (CMC). The enzyme is thermostable with an optimal temperature of 70 ºC and is stable on pH ranging from 3-8. The enzyme is strongly inhibited by Hg^{2+} and Ba^{+}. *Fusarium graminearum* cellulase has demonstrated good characteristics that may be exploited for possible use in biofuel productions, animal feeds, paper industries as well as in bioremediation.

Acknowledgments

We acknowledge Dr. Agu G. C of the Microbiology Department of Faculty of Sciences, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, for her assistance and guidance during the isolation of *Fusarium graminearum* from corn cob.

References

Abaildayev, A.O., Kuzovlev, V. A., Sharipov, K. K & Khakimzhanov, A. A. (2022). Purification and characterization of α-amylase and protease of the phytopathogenic fungus *Fusarium graminearum.* EJAB, 3-13. 10.11134/btp.3.2022.1.

Ahmed, K., Munawar, S. and Naz, S. (2015). Purification and characterization of cellulase from *Aspergillusniger (Van Tieghem*, 1867). *Sci. Int. (Lahore),* 27(5): 4341-4344.

Akiba, S., Kimura, Y., Yamamoto, K. and Kumagai, H. (1995) Purification and characterization of a protease-resistant cellulase from Aspergillusniger. *J. ferment. **B**ioeng*., 79: 125-130.

Aweke, Y. K., Tasisa, B. Y., Garoma, W. D., & Denbi, W. B. (2022). Extraction and characterization of cellulase from forest and compost soil fungal isolates for the application of straw degradation. Int. J. Health Sci., 6(3):7146– 7162.

Bakare, M., Adewale, I. and Ajayi, A. (2005). Purification and characterization of cellulase from the wild type and two improved mutants of *Psuedonomas flourescens*. *Afr. J. Biotechnol.,* 4:898-904.

Bai, H., Wang, H., Junde-Sun, M.I., Han, M., Huang, Y., Han, X., Yang, Q.(2013). Production, purification and characterization of novel beta glucosidase from newly isolated *Penicillium simplicissimum* H-11 in submerged fermentation. EXCLI Journal. 12:528-540

Bernfeld, P. (1995), in *Methods in Enzymology*, vol. 1, Collowick, S. P. and Kaplan, N. O., eds., Academic, New York, p. 149.

Bhardwaj, N., Kumar, B. and Agrawal, K. (2021). Current perspective on production and applications of microbial cellulases. *Bioprocess*.8:95.

Bhikhabhai, R., Johansson, G., and Pettersson, G. (1984). Isolation of cellulolytic enzymes from *Trichoderma reesei* QM 9414. *J. Appl. Biochem.,* 6: 336-345.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of Protein-Dye Binding. *Analy. Biochem*., 72:248-254

Chellapandi, P and Jani, HM. (2008). Production of endoglucanase by the native strains of *Streptomyces* isolates in submerged fermentation. Braz. J. Microbiol., 39: 122-127.

Cosic, J., & Jurkovic, D. (2000). Biological characterization of Fusarium graminearum Schw. Isolated from different host plants. In *6th European Fusarium Seminar* (pp. 79-80).

Dashtban, M., Schraft, H., & Qin, W. (2009). Fungal bioconversion of lignocellulosic residues; opportunities & perspectives. *International journal of biological sciences*, *5*(6), 578-594.

Elakkiya, P., and Muralikrishnan, V. (2014). Cellulase production and purification of mutant strain *Trichoderma viride*, *Int. J. Curr. Microbiol. App. Sci,* 3(9): 720–727.

Fagbohunka, B. S, Babasanya – Craig, O. O., Adenuga, G. A., and Agboola, F. K (1997). Partial purification and characterization of cellulase from heamophyl and the gut of the Giant African Snail, *Achachatina maginata*. *Nig. J. Nutri. Sci.*, 18(2):28-34.

Gaur, R. and Tiwari, S. (2015). Isolation, production, purification and characterization of an organic-solvent thermostable alkalophilic cellulase from Bacillus vallismortis RG-07.*BMC. Biotechnol*., 15:1-19.

Goel, N., Patra, R. and Verma, S. K. (2019). Purification and characterization of cellulase from *Pseudomonas* sp. isolated from waste dumping site soil. *J. Appl. Biotechnol. Bioeng*., 6(3):118-124

Holker, U., Hofer, M. and Lenz, J. (2004): Biotechnological advantages of laboratory-scale solid state fermentation with fungi. Appl. Microbiol. Biotechnol., 64(2):175-186.

Itani, A., Olama, Z. and Holail, H. (2017). Purification and characterization of *aeruginosa* IZ cellulase grown on corn cob. *Int. J. MicroBiol. Gent.Mono. Biol. Res.* 1:20–28.

Iram, A., Berenjian, A. & Demirci, A. (2021). A Review on the Utilization of Lignin as a Fermentation Substrate to Produce Lignin-Modifying Enzymes and Other Value-Added Products. Molecules. 26: 2960

Iram, A., Cekmecelioglu, D. & Demirci, A.(2021).Ideal Feedstock and Fermentation Process Improvements for the Production of Ligno˘ cellulolytic Enzymes. Processes. 9: 38.

Iram, A., Cekmecelioglu, D. & Demirci, A. (2023). The Effects of Dilution, Aeration, and Agitation on Fungal Cellulase and Xylanase Production in the Fermentation Media Based on Distillers Dried Grains with Solubles Using Stirred Tank Bioreactors. Ind. Biotechnol. 2023; 19: 229–236.

Kang, S.W., Park, Y.S., Lee, J.S., Hong, S.I., Kim, S.W., (2004). Production of cellulases and hemicellulases by *Aspergillus niger*KK2 from lignocellulosic biomass. *Biores. Technol*. 91:153-156.

Kikot, G. E., Hours, R. A. & Alconada, T. M. (2010). Extracellular enzymes of *Fusarium graminearum* isolates. Braz. arch. biol. technol. 53 (4):779-783

Kumar, B., Bhardwaj, N., Alam, A., Agrawal, K., Prasad, H. and Verma, P. (2018). Production, purification and characterization of an acid/ alkali and thermo tolerant cellulase from *Schizophyllum commune* NAIMCC-F-03379 and its application in hydrolysis of lignocellulosic wastes. *AMB Expr.,* 8:173(1-16).

Kumari, B. L., Vijetha, P & Sudhakar. P.(2010). Optimization of Physio-Chemical Properties for Production of Alkaline Protease from *Fusarium graminearum*. Rec. Res. Sci. Technol., 2: 2076-5061.

Li, C.H., Wang, H. R. and Yan, T. R. (2012). Cloning, purification, and characterization of a heat- and alkaline-stable endoglucanase B from *Aspergillus niger* BCRC31494. *Molecules* 17: 9774-9789.

Lin, L., Meng, X., Liu, P., Hong, Y., Wu, G., Huang, X., Li, C., Dong, J., Xiao, L., Liu, Z. (2009). Improved catalytic efficiency of endo-beta-1,4-glucanase from *Bacillus subtilis* BME-15 by directed evolution. *Appl. Microbiol. Biotechnol*. 82:671-679.

Lineweaver, H. and Burk, D. (1934). The Determination of Enzyme Dissociation Constants. JACS. 56: 658.

Liu, D., Zhang, R., Yang, X., Xu, Y. and Tang, Z. (2011). Expression, purification and characterization of two thermostable endoglucanases cloned from a lignocellulosic decomposing fungi Aspergillus fumigates Z5 isolated from compost. Protein Expr. Purif. 79: 176-186.

Lo, C. M., Zhang, Q., Callow, N.V., Ju, L.W. (2010). Cellulase production by continuous culture of *Trichoderma reesei* RUT C30 using acid hydrolysate prepared to retain more oligosaccharides for induction. *Biores. Technol.,* 101:717-723.

Lowe, R. G., McCorkelle, O., Bleackley, M., Collins, C., Faou, P., Mathivanan, S. & Anderson, M. (2015). Extracellular peptidases of the cereal pathogen *Fusarium graminearum*. Front. Plant Sci. 6(6):962.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951). Protein measurement with the Folin Phenol reagent. *J. Biol. Chem.,* (193):265–275.

Nehad, E. A., Yoness, M. F. and Reem, A. A.(2019). Optimization and purification of cellulase produced by *Penicillium decumbens* and its application. *Egypt Pharmaceut. J.,* 18:391-402.

Nisar, K., Abdullah, R., Kaleem, A., Iqtedar, M., Aftab, M., Saleem F. (2022). Purification, characterization and thermodynamic analysis of cellulases produced from *Thermomyces dupontii* and its industrial applications. PJBS. 29(12):103483.

Nazir, M., Iram, A., Cekmecelioglu, D., & Demirci, A. (2024). Approaches for Producing Fungal Cellulases Through Submerged Fermentation. *Frontiers in Bioscience-Elite*, *16*(1), 5.

Noor, T. H. and Hamed, M. J. (2018). Purification and Characterization of Cellulase enzyme from Trichoderma longibrachiatum isolated in Iraqi soil. IOSR-JBB 4(1): 32-41.

Okonkwo, I. F. (2019). Effect of metal ion and enzyme inhibitor on the activity of cellulase enzyme of *Aspergillus flavus. IJEAB*; 4(3): 727-734.

Onyia, D. C., Onyeneke, E. C., Okunwaye, T., Asiriuwa, N. U.,Okogbeni, E.O., Obibuzor, J. U. and AnemeneH. (2021) The influence of metal ions on cellulolytic activities of fungal isolates from palm biomass. *Nig. J. Biotech*.; 38(2): 67-72.

Pachauri, P., V. More, A., S., Sullia, S. B., and Deshmukh, S. (2017). Purification and characterization of cellulase from a novel isolate of *Trichoderma longibrachiatum*. *Biofuels*, 1–7.

Rahnama, N., Shah, U. K. M., Foo, H. L., Rahman, N. A. A., and Ariff, A. B. (2016). Production and characterisation of cellulase from solid state fermentation of rice straw by Trichoderma harzianum SNRS3. Pertanika J. Trop. Agric. Sci., 399(4), 507-531.

Rajesh, J. M., Rajesh L., and Wondimu, L. (2012). Optimization of Solid-State Fermentation Conditions for the Production of Cellulase by Using *Trichoderma reesei* Scholars Research Library.*Eur. J. Applied Engr. Sci. Res.*, 1(4), 196–200.

Sachdeva, V. (2023). An Insight into Microbial Cellulases. IJCRT, 11(4):h609-626
Schiffer H. W.(2008). WEC energy policy scenarios to 2050. Energy Policy 36:2464-2470.

Sajith, S., Priji, P., Sreedevi, S. and Benjamin, S. (2016). An overview of fungal cellulases with an industrial perspective. *J. Nutr. Food. Sci*., 6:641.

Sajith, S., Sreedevi, S., Priji, P., Unni K. N. and Benjamin, S. (2015). Production and partial purification of cellulase from a new isolate, penicilliumverrulosum BS3. *Br. Microbial. Res. J.* 9(1):1-12.

Singhania, R. R., Sukumaran, R. K., Pillai, A., Prema, P., Szakacs, G. and Pandey, A. (2006). Solid-state fermentation of lignocellulosic substrates for cellulase production by Trichoderma*reesei*NRRL 11460. *Indian J. Biotechnol*. 1:332-336.

Soliman, S. A., El-zawahry, Y. A. and El-mougith, A. A. (2013). Fungal Biodegradation of Agro-Industrial Waste. In Cellulose Biomass Conversion, *InTech*, pp75-99.

Sulyman, A.O. Igunnu, A. and Malomo, S.O. (2020). Isolation, purification and characterization of cellulase produced by *Aspergillus niger* cultured on *Arachis hypogaea* shells, *Heliyon* 6 (e05668):1-10.

Sulyman, A., Igunnu, a. and Malomo S.O. (2022). Effect of metal ions on the activities of cellulase produced *Aspergillus niger* using *Arachis hypogaea* shells. *NJBMB*., 37(1): 58-63.

Steiner, W., Nidetzky, B., Haynt, M., and Claeyssenst, M. (1994). Cellulose hydrolysis by the cellulases from *Trichoderma reesei*: a new model for synergistic interaction. *Biochem. J.* 298: 705-710.

Wu, X., Luo N., Xie, S., Zhang, H., Zhang, Q. & Wang, F. 2020). Photocatalytic transformations of lignocellulosic biomass into chemicals. Chem. Soc. Rev. 49: 6198–6223.

Xia, L. and Cen, P. (1999). Cellulase production by solid-state fermentation on lignocellulosic waste from the xylose industry. *Process Biochem.*34:909-912.

Zhao, C., Lu, X., Deng, Y., Huang, Y. and Liu, B (2015). Purification and characterization of thermostable cellulase from Consortium XM70 in terrestrial hot spring with sugarcane bagasse; Trop. J. Pharm. Res. 14 (4): 591-598

Zeng, R., Yin, X., Ruan, T., Hu, Q., Hou, Y., Zuo, Z., and Huang, H. (2016). A Novel Cellulase Produced by a Newly Isolated *Trichoderma virens*, *Bioengineering,* 3(2):1–9.