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Properties of *Aspergillus flavus* Cellulase Produced from Solid State Fermentation of Brewers' Spent Grain (BSG) as Substrate

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Authors' contributions

This work was carried out in collaboration between all authors. Author FAO designed the study, performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript and managed the literature searches. Authors FAO, END, AKL, AOS, FF, YLS, ACF, AIU, BI, AOA, EEI and GNE managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The production of cellulase from *Aspergillus flavus* isolated from Brewers' Spent Grain (BSG) undergoing bio-deterioration was carried out using solid state fermentation method. Out of a total of four fungal isolates with cellulase producing potentials, *Aspergillus flavus* with a clearance zone of 5.0cm was the best hyper-producer and was selected for optimization and cellulase production.

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Two different enzyme production media containing either yeast extract or potassium nitrate (KNO_3) served as sources of nitrogen to the *Aspergillus flavus.* The crude activity of the potassium nitrate (KNO_3) option (28,170 Units) was significantly higher than the crude cellulase from yeast extract option (18,600 Units). Ammonium sulphate precipitation at 60%, 80%, and 100% had cellulase activities 14600 units, 4400 unit, and 2800uits in yeast extract amended experimental option. In addition, ammonium sulphate precipitation at 60%, 80%, and 100% gave cellulase activities of 21130 units, 1700 units, and 1110 units, respectively in the KNO_3 amended experimental option. Thus, 60% ammonium sulphate precipitation gave the best cellulase activity. The optimum pH of *Aspergillus flavus* cellulase was 6.0, while the optimum temperatures were between 50℃ to 60℃. Tolerance to some alkali and alkaline–earth metals was also established, and probable co-factor behaviour of manganese ion to this cellulase was also established. Good government policies that support high import duties on foreign enzymes, emphasis on local production are advocated for in order to stamp out the economic leakages accruing through enzyme importation into Nigeria.

Keywords: Cellulase; solid state fermentation; Aspergillus flavus; Brewers' Spent Grain (BSG).

1. INTRODUCTION

Cellulases are the inducible bioactive compounds produced by microorganisms during their growth on cellulosic matters [1]. Cellulase is an enzyme that has the ability to degrade cellulose. It has several commercial applications like malting, wood processing, and preparation of denim fabrics in textile industries, maceration of protoplasts from plant tissues and de-inking process in recycling of printed papers [2]. 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 [3].

High cost of cellulase is mainly due to the substrates used in production, and also the slow growth rate of fungi. Bacteria which have high growth rate as compared to fungi have good potentials to be used in cellulase production. However, the application of bacteria in producing Cellulase is not widely used. Bacterial cellulase usually lacks one of the three cellulase activities [4].

However, cellulases produced by bacteria are often more effective catalysts. They may also be less inhibited by the presence of material that has already been hydrolyzed (feedback inhibition).

Brewers Spent Grains (BSG) are by products of the brewing industry containing about 17% cellulose, 28% non-cellulosic polysaccharides, mostly arabinoxylans and 28% lignin, and they have very little or no economic use [5].

The two major methods of fermentation that have been successfully used in microbial enzyme production include submerged and solid state fermentation. The submerged fermentation method is achieved fundamentally in the presence of free water using mainly bacterial species. The solid state fermentation is usually done in the absence of free water using moulds. Several arguments over the efficiency of each of the two fermentation methods in enzyme production exist. However, for fungal enzymes, the solid state fermentation methods have been widely accepted. Since the natural habitats of these filamentous fungi are solid media, the solid state fermentation (SSF) procedure offers considerable advantages over submerged fermentation (SmF). These include high volumetric productivity; higher concentrations of the enzymes produced, and lower energy consumption [6]. Another important feature of SSF is that it can use agro-industrial residues as carbon sources, and lower cost of equipment [6-8].

The cost of enzyme production by submerged fermentation is higher compared to SSF. This has been scientifically proved by [9] which compared cellulase production costs in SSF and SMF. The amount (volume) of cellulase produced by bacterial strain *Bacillus subtilis* on banana fruit stalk wastes was 12 times higher in solid state fermentation than in submerged fermentation under similar experimental condition [10].

The objectives of this study were to screen for cellulase hyper producing strains of moulds,

produce cellulase by solid state fermentation of Brewers' Spent Grains (a cheap agro-based waste), characterize the fungal cellulase based on temperature, pH, and metallic ion tolerance.

2. MATERIALS AND METHODS

2.1 Isolation and Identification of Fungal

Mould isolation for cellulase was initiated by plating out serially diluted Brewers Spent Grain undergoing bio-deterioration in a Potato Dextrose Agar (PDA) using the pour plate technique. The serial dilution procedure, Pour plating followed the procedures previously described [11]. Incubation at room temperature was done for five (5) days. The moulds were sub-cultured using spot-inoculation to obtain pure strains. Pure cultures were identified by colonial appearance on Potato Dextrose Agar and wet mount procedures using Lactophenol Blue stain as previously described [12]. Isolates were identified based on colonial appearance, microscopic appearance and comparison with standard mycological atlas.

2.2 Screening for Hyper Producing Strains of Fungi and Identification of Moulds

The screening method previously described with some modification was adopted [8]. Potato Dextrose Agar (Oxoid) was prepared according to the manufacturer's instruction, and 2% Crystalline Cellulose (Merck) was incorporated into the Potato Dextrose Agar (PDA). In an aseptic condition in a laminar air flow, a sterile cork borer was used to bore 6mm holes on the centre of the Sterile PDA (containing 2% cellulose). Another sterile Cork borer was used to bore a 6mm hole on PDA containing the test organism (*Aspergillus flavus*), and with the help of an inoculating needle the 6 mm agar containing the *Aspergillus flavus* mycelia was transferred into the 6mm hole of the sterile PDA (with 2% Cellulose). Incubation was done for 3days at room temperature, after which the surface of the agar was flooded with Grams Iodine for 10 minutes. The excess Gram's iodine was drained off, and the zone of clearance of the cellulose was measured using meter rule.

2.3 Enzyme Production Medium and Experimental Design

Composition of mineral media for cellulase production followed previous studies with modifications [3,7]. BSG (300 grams) was amended with 300mls of mineral medium containing KH_2P04 3.0 g/l, $MgSO_4.7H_2O$ 1.0 g/l, $CaCl_2.H_2O$ 0.5 g/l, $ZnSO_4.7H_2O$ 1.6 g/L, $FeSO_4.7H_20$ 0.05 g/l, $CoCl_2.H_20$ 0.5 g/l. Two different experimental set-ups were established in duplicates such that one group was supplemented with 2.0 g/L of $KN0_3$, and another group supplemented with 2.0 g/L of yeast extract as different sources of nitrogen. Each set-up had 1% Crystalline cellulose (Merck) was used as inducer. The experiment was set-up in a rectangular fermentation tray of 6.6 cm x 95.5 cm dimension. The pH of the amended substrates was adjusted to pH 6.0 with freshly prepared neutral Phosphate buffer. The amended substrates were placed into different muslin bags and labeled according to the source of nitrogen. Autoclaving was done at 121℃, 15 psi, and a holding time of 60minutes. Thereafter autoclaving, the cooled amended substrates were transferred to Steel fermentation trays previously sterilized using hot air oven. Solution of sterile Tween 80 (10 ml) solution was used to wash off spores of seven days old *Aspergillus flavus* (FIIRO M15 strain) in to the sterile substrates in trays. The fungal mycelia (5.6 x 10^5spores/ml of tween 80) were mixed under laminar air flow. The trays were covered with sterile foil paper and incubation was done at room temperature for seven (7) days. The moisture content at the initial point of incubation was 55%.

2.4 Enzyme Extraction and Preservation

The cellulase was extracted after fermentation (7 days incubation) using sterile 0.05 M acetate buffer of pH 5.0. In order to extract the extracellular enzyme, 3000 mls of the 0.05 citrate buffer were sterilised, and delivered into each fermentation trays containing 300 grams of fermented BSG substrate, and after gentle agitation for 30 minutes, the enzyme –buffer solution was filtered using sterile muslin cloth. Centrifugation at 1,000 g was carried to remove existing spores, and concentrate the cellulase further, as the crude cellulase was decanted carefully into enzyme bottles. The cellulase extract was preserved using 5grams of sodium meta-bisulphite under 4℃ until required for assay.

2.5 Partial Purification using Ammonium Sulphate Precipitation

Analytical grade of ammonium sulphate (Baker Inc, USA), was used. The ammonium sulphate

was weighed (100 grams, 80 grams, 60 grams) into different 500 mls capacity conical flasks and 100 mls of cellulase extracts were gently added into the flasks to give 100%, 80%, and 60% precipitation, respectively. The mixture was left overnight (12 hours) at 4℃. The mixture was centrifuged at 10,000 rpm for 15minutes. Pellet was dissolved in 10 ml of 50mM sodium acetate buffer (pH – 5.5) for further purification. Thereafter the remaining cellulase was gently decanted into enzyme bottles for further analyses. These procedures were adopted from previously reported experiments [13,14].

2.6 Determination of Protein Content

Protein content of the enzyme extracts were determined by following the method of Lowry and colleagues [15] using Bovine serum albumin as standard. Protein extract (0.2 ml) was measured into tubes and 0.8 ml distilled water was added to it. Distilled water was used as blank while BSA standard curve. Alkaline copper reagent (5.0 ml) was added to all the tubes, mixed thoroughly and allowed to stand for 10 minutes, and thereafter 0.5 ml of Folin- C solution was added to all the test tubes and left for 30 mins. The optical density was read at 600 nm wavelength in a spectrophotometer (T70 PG Instrument UV model). The protein concentration was estimated using values extrapolated from standard graph of protein. Protein content was determined by the formula below;

$$Protein\ Content = \frac{\text{Absorbance}}{\text{Gradient}}$$

2.7 Determination of Crude and Specific Activities

Crude activity in centrifugal supernatant was determined according to the method previously reported. The reducing sugar released was estimated by the Dinitro Salicyclic Acid (DNSA) method [16-18]. One unit of filter paper (FPU) activity was defined as the amount of enzyme releasing 1 µmole of reducing sugar. Specific activity was determined using the formula below;

$$\text{Specific activity } \left(\frac{U}{Mg}/ml\right) = \frac{\text{Crude activity (U)}}{\text{Protein content (Mg/ml)}}$$

2.8 Determination of Optimum Temperature

The filter paper strip (0.5 gram) was used as the substrate. Thereafter dilution of the enzyme, 0.5 ml of the diluted cellulase was pipetted into test tube containing 0.5 grams of what man no 1 filter paper. The tubes were labelled according to the source of nitrogen ($KN0_3$ and yeast extract). The enzyme –substrate mixture was incubated at 40℃, for 60 minutes. Other tubes were prepared in the way but incubated at 50℃, 60℃, 70℃, and 80℃ for 1 hour in each case. Dinitro-salicyclic Acid (DNSA) (5 mls) was added into all tubes to stop the reaction. The tubes were boiled for 5minutes at 50℃ further to develop the colour, and 0.2 ml was taken into cuvette and absorbance read at 540 nm wavelength.

2.9 Determination of Optimum pH

The activity of the cellulase was measured at various pH using different pH values of citrate buffer. Technically, 0.5 ml of the citrate buffer at different pH (4.0 to 8.0) was mixed with 0.5 ml of the diluted enzyme in a well labelled test tube containing 0.5 gram of filter paper strip. The assay was done with DNSA method, and water bath incubation was carried out at 50℃ (optimum temperature).

2.10 Determination of Effect of Metal Ions

The reaction of the enzyme and filter paper substrate was allowed to proceed at 50℃ with duplicate test tubes containing 10mM $MnSO_4$, $CaSO_4$, $MnSO_4$, $ZnSO_4$, and $FeSO_4$. Test tubes were labelled according to the source of nitrogen (KNO_3 and yeast extract), and above mentioned salts. The cellulase extract was diluted accordingly, and 0.5 ml of the diluted enzyme was pipetted into the labelled test tube already loaded with 0.5 gram of shredded filter paper. Thereafter, 0.5 ml of 10 mM solution of each of the above mentioned salts (one salt to one test tube) was pipetted into a test tube. The incubation of the reaction mixture was done at 50℃, and the assay done using previously described DNSA Method [18]. Tubes containing distilled water and substrates (without the enzyme) but treated in the same way as sample served as the blank.

2.11 Statistical Analyses

The data generated in this study were subjected to Duncan multiple range test (DMRT) to determine various points of significance.

3. RESULTS AND DISCUSSION

The cellulase hyper-producing potentials of some moulds isolated from BSG under-going bio-

deterioration was studied using PDA-Cellulose medium (Fig. 1). *Aspergillus niger* (M11), *Aspergillus niger* (M13), *Trichoderma harzianum* (M13B), *Aspergillus flavus* (M15) had zone of clearance of 2.5 cm, 2.2 cm, 2.7 cm, and 3.0 cm, respectively (Table 1). Statistical analyses showed that the highest zone of clearance among all the isolates was established by *Aspergillus flavus* (M15 strain-our reference), and it was further used for cellulase production. The activities of crude cellulase were 18600U and 28170 U when yeast extract and KNO_3 served as nitrogen sources respectively. Statistics showed that at 95% confidence level, the KNO_3 option had its cellulase activity greater than the yeast extract option (Table 2). The partial purification studies on the Cellulase showed a progressive decrease in the activities of partially purified cellulase from 60% to 100% ammonium sulphate precipitation (Table 2). Thus on comparison, the activity of the crude remained significantly different from the activity of the partially purified cellulase. This indicates that the partial purification process may have precipitated out some proteins including the cellulase. However, this slight decrease in activity does not depict the needless nature of partial purification by precipitation rather it depicts the need for incorporation of dialyses membrane filtration using appropriate dialyses sac and buffer. The process of partial purification is still incomplete without dialyses. This could be achieved by determining the molecular weight of the *Aspergillus flavus* cellulase, and thereafter, the right size of the dialysis membrane would be used to sieve –off all non-enzymic protein, and salts. This salting out process will definitely enhance specific activities of the cellulase. In a related study, *Trichoderma reesii* was used in a solid state fermentation method to produce cellulase and activities of the produced cellulase ranged between 20 to 180 Units/gds (gram per dry substrate) [19]. The production of cellulase by other strains of *Trichoderma* with endoglucanase activities ranging between 20 UI/g to 70 UI/g has also been reported [8].

Protein content of the enzyme extract was studied. The protein contents of the extracts were 22.40 mg/ml and 28.20 mg/ml for yeast extract and KNO_3 treatment options, respectively. Observation and statistics ($p>0.05$) showed that the specific activity of the crude cellulase from the KNO_3 option (998.90 U/mg/ml) was significantly higher than the cellulase activity of the yeast extract option (Table 3). The existence of 0.45 to 0.46 soluble proteins in cellulase produced through submerged fermentation system, and 6.20 to 6.80 mg/ml of soluble protein in cellulase from *Pestalotiopsis versicolor* under solid state fermentation has been reported [20]. This observation is consistent with our present study where proteins produced by fungal isolates during solid state fermentation is significantly higher than the soluble protein from fungal isolates during submerged fermentation of substrates.

The decrease in protein content after precipitation with ammonium sulphate at 60%, 80%, and 100% impacted greatly on the specific activities of cellulase purified with 60%, 80%, and 100% ammonium sulphate. It was observed that beyond 60% partial purification, the specific cellulase activities reduced significantly in each of the sources of the nitrogen (Table 3). This implies that partial purification of this *Aspergillus flavus* cellulase gives optimal activity when not more than 60% ammonium sulphate precipitation is employed.

The effect of pH on the activities of cellulase from both treatments (yeast extract and KNO_3) was studied at different pH (4.0 to 8.0). Cellulase activities were very low at high acidic pH of 4.0 and 5.0. In addition, at neutral and alkaline pH of 7.0 and 8.0, cellulase activities were significantly higher than the activities at 4.0 and 5.0. However, the optimum pH was 6.0 for both options. The cellulase activities of 1098.41 Units and 1148.35 Units were observed for cellulase for the yeast extract and KNO_3 fermentation media, respectively (Table 4). Notably, the KNO_3 option was the best source of nitrogen considering the activity of cellulase from the fermentation trays amended with KNO_3 as source of nitrogen. The optimum pH indicates that for the best of activity of *Aspergillus flavus* (FIIRO M15 strain) to be harnessed, the pH of the enzyme-substrate medium will have to be adjusted to 6.0. A critical observation which is also note worthy is the fact that the *Aspergillus flavus* cellulase in this present study tolerated wide pH from acidic to alkaline. Thus it is suitable for industrial processes at various pH. Comparatively, the cellulase of *Streptomyces* strain produced in a solid state fermentation was reported to have an optimum pH of 5.0 [21]. There are previous reports on the optimum pH of 5.5 for endoglucanase from a strain of *Streptomyces lividans* which [22], while it was also shown that cellulase from *Streptomyces* sp. (strain J2) was active over a pH range of 4 - 7 with maximum activity at pH 6.0 [23].

Fig. 1. Zone of clearance of cellulose in cellulase screening agar by different mould strains

Table 1. Zone of clearance of cellulase on screening with different fungal species

S/no	Our reference	Fungal identity	Zone of clearance (CM)
1	M11	*Aspergillus niger*	2.5[b]
2	M13	*Aspergillus niger*	2.2[b]
3	M13B	*Trichoderma harzianum*	2.7[a]
4	M15	*Aspergillus flavus*	3.0[a]

Means followed by the same letter in the column are not significantly different (p<0.05) (Duncan multiple range test)

Table 2. Cellulase activities at different source of nitrogen and partial purification levels

Ammonium sulphate concentration	Cellulose activities(unit) yeast extract + S.D	Cellulase activities KNO_3 + S.D
Crude (0%)	18,600±0.02[a]	28170±0.01[b]
60%	14,600±0.03[b]	21130±0.07[b]
80%	4400±0.02[c]	1700±0.02[b]
100%	2800±0.01[d]	1110±0.01[b]

Values with different alphabetical superscripts show significant different at p<0.05
Means followed by the same letter in the column are not significantly different (p<0.05) (Duncan multiple range test)

Table 3. Specific activities of *Aspergillus flavus* cellulase at different levels of purification and sources of nitrogen

Ammonium sulphate concentration	Protein content (mg/ml) Yeast extract ± S.D	Specific activity (u/mg/ml) Yeast extract ± S.D	Protein content (mg/ml) KNO_3 ± S.D	Specific activity KNO_3 ± S.D
Crude (0%)	22.40±0.05[a]	830.40±4.67[a]	28.20±0.09[a]	998.90±4.60[a]
60%	18.90±0.07[b]	772.50±4.15[a]	17.00±0.04[b]	1242.90±2.71[b]
80%	10.70±0.07[ab]	411.20±4.75[ab]	24.00±0.36[a]	70.80±3.91[ab]
100%	9.20±2.60[ab]	309.80±6.19[c]	12.80±0.97[ab]	86.70±2.97[ab]

Means followed by the same letter in the column are not significantly different (p<0.05) (Duncan multiple range test)

Table 4. Effect of pH on the specific activities of cellulase produced with different sources of nitrogen

pH	Specific activity (u/mg/ml) yeast extract ± S.D	Specific activty (u/mg/ml) KNO_3 ± S.D
4.0	655.79(0.443)±O.013[d]	892.40(0.781)±0.271[b]
5.0	840.84(0.568)±0.019[c]	659.30(0.577)±0.271[d]
6.0	1098.41(0.742)±0.206[b]	1148.35(1.005)±0.008[a]
7.0	895.61(0.605)±0.007[c]	759.85(0.665)±0.046[c]
8.0	1336.75(0.905)±0.048[a]	609.02(0.533)±0.051[d]

Numbers in brackets are absorbance values, S.D; Standard deviation. Means followed by the same letter in the column are not significantly different (p<0.05) (Duncan multiple range test)

The effect of temperature on the activities of the *Aspergillus flavus* cellulase obtained in two different fermentation conditions (yeast extract and KNO_3) was studied. The cellulase activities at 30℃, 40℃, were not as high as the activity at 50℃. An activity of 986.09 Units was observed on cellulase obtained on using KNO_3 as nitrogen source. This shows that 50℃ is the optimum temperature of the cellulase produced on utilizing KNO_3 as nitrogen source during Brewers' Spent Grain (BSG) cellulose fermentation. On the other hand, 60℃ was the optimum temperature of cellulase obtained from yeast extract fermentation medium. In addition, the *Aspergillus flavus cellulase* showed some level of thermo-tolerance between the temperature of 70℃ and 80℃ (Table 5). This thermo-tolerance property is of great industrial importance as it will be beneficial to industrial processes like bio-ethanol/biogas production, and textile material production etc. Considering previous related studies, it was reported that cellulase from *Streptomyces* strain produced using solid state fermentation method had an optimum temperature of 40ºC, which related to our observation for *Aspergillus flavus* (M15) strain [21]. In a similar direction to our findings, there are reports of optimal temperature for cellulase activity in the range of 40 - 55℃ for cellulase from *Streptomyces lividans, Streptomyces flavogrisus*, and *Streptomyces nitrosporus* [24]. The optimum temperature for cellulase from *Aspergillus niger* produced using cheap corn cob was established to be 50℃ with an enzyme activity of 1.3x10-4 µg/ml/sec [3].

Generally, enzyme activities are affected by presence of metal ions. Thus the ability of some enzymes to withstand a renewable concentration of some metal ions encountered in Industrial processes will be highly appreciable. With particular reference to this study, the presence of 10 mM calcium and Magnesium had very little impact on the cellulase activities. The specific activities in the absence of the metal ions were 830 U/mg/ml and 998.90 U/mg/ml respectively for yeast extract and KNO_3 options respectively (Table 6). The enzyme-substrate reaction in the presence of 10 mM calcium solution showed reduced value of activity, which is not statistically significant (α =0.05) (Table 6). Similar observation holds for Magnesium ion, except the cellulase obtained on using KNO_3 had its specific activity reduced. Thus the tolerance of *Aspergillus flavus* cellulase to calcium and magnesium ions is hereby established.

The reverse was the case for zinc ion, as its presence at 10 mM concentration reduced the activities of the cellulase. This is not surprising as the heavy metals binds competitively into the active site of the enzyme, and inhibits the binding of the substrate (cellulose). On incorporation of sodium ion into the reaction medium, the yeast extract option had its cellulase activity significantly increased (P=0.05), whereas the KNO_3 option had its cellulase activity reduced from 998.90 U/mg/ml to 948.38 U/mg/ml, but this variance is not statistically significant (p=0.05) (Table 5). Interestingly, manganese ion in reaction with cellulase from both treatments, showed a geometric increase the activities of the cellulase. Manganese is a metal, and cellulase activity is somewhat dependent on its presence. This shows that manganese ion could possibly be a co-factor to this *Aspergillus flavus* cellulase. The co-factor will bind to the cellulase active sites, and this binding will in turn assists in bringing the substrates in close proximity with the active sites of the enzyme. This reaction will reduce the activation energy of the reaction, thus creating another pathway for bond breaking and formation of product(s), which is glucose in this case. There are also reports that showed that the production of cellulase from *Cellulomonas* was stimulated by Co^{2+} and Mn^{2+} while Hg^{2+} and Fe^{2+} reduced the activities of the enzyme [25]. The observation that manganese has co-factor property is consistent with the observations of Irfan *and* co-wokers [25].

Tables 5. Effect of temperature on specific activity of the cellulase based on different sources of nitrogen at pH 5.0

Temperature (0°c)	Specific activity (u/mg/ml) yeast extract ± S.D	Specific activities (u/mg/ml) KNO_3 ±S.D
30	340.48(0.230)±0.331[a]	571.32(0.500)±0.005[a]
40	396.48(0.207)±0.341[a]	453.63(0.397)±0.027[a]
50	741.65(0.501)±0.001[b]	986.09(0.863)± 0.315[b]
60	826.08(0.558)±0.015[b]	817.54(0.768)±0.271[ab]
70	614.34(0.415)±0.180[ab]	802.13(0.702)± 0.319[ab]
80	482.59(0.326)±0.119[a]	538.18(0.471)±0.001[a]

Numbers in brackets are absorbance values, S.D; Standard deviation. Means followed by the same letter in the column are not significantly different (p<0.05)(Duncan multiple range test). KNO_3 and yeast extract are the different sources of Nitrogen

Table 6. Effect of metallic ions on the activity of cellulase produced with different sources of nitrogen

Metallic ions (10 Mm)	Specific activities yeast extract ± S.D	Specific activities KNO_3 ±S.D
Ca^{2+}	861.56(0.582)±0.031[a]	828.41(0.725)±0.227[a]
Mg^{2+}	808.27(0.546)±0.027[b]	615.86(0.539)±0.202[b]
Zn	771.18(0.525)±0.047[b]	406.78(0.356)±0.080[ab]
Na	1000.70(0.676)±0.008[ab]	948.38(0.830)±0.147[c]
Mn	1652.06(1.116)±0.090[c]	1331.71(1.165)±0.070[cd]
K	802.34(0.542)±0.241[b]	609.02(0.533)±0.314[b]

Specific activities of the crude cellulase without the metal ions = 830U/mg/ml and 998.90 U/Mg/ml respectively for yeast extract and options respectively. Numbers in brackets are absorbance values. S.D; Standard deviation. Means followed by the same letter in the column are not significantly different (p<0.05) (Duncan multiple range test)

4. CONCLUSION

The production of cellulase from local sources such as Brewers Spent Grain (an agro-based waste), and local strain of *Aspergillus flavus* will bridge the gap between enzyme utilisation and cost of importation [26]. Good government policies that support high import duties on foreign enzymes, emphasis on local production are advocated for, in order to stamp out the economic leakages accruing through enzyme importation into Nigeria. Most local and foreign industries can now access cellulase produced in Nigeria at cheaper cost. This saves foreign exchange earnings for Nigeria, creates jobs for the unemployed and contributes in moving Nigeria to achieving its vision of being among the first 20 fastest growing economy in 2020 [26].

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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