*Scientia Africana, Vol. 23 (No. 2), April, 2024. Pp 1-12*
*© Faculty of Science, University of Port Harcourt, Printed in Nigeria* *ISSN 1118 – 1931*

# BIMOLECULAR OPTIMIZATION OF CELLULASE PRODUCTION BY *TRICHODERMA CITRINOVIRIDE* AND *ASPERGILLUS NIGER* ISOLATES ON CORN COB, RICE BRAN AND SORGHUM BRAN AS BIOMASS SUBSTRATES

**[1]Effiong, T.E., [2*]Benjamin, B., [3]Egbe, N.E.,[4]Abdulsalami, M.S., [5]Kereakede, E., and [6]Bakare, V.**

[1]Department of Biotechnology, Nigerian Defence Academy PMB, 2109, Kaduna-Nigeria
[2*]Department of Pharmaceutical Microbiology and Biotechnology, University of Jos, PMB 2084, Jos-Nigeria
[3]Department of Biotechnology, Nigerian Defence Academy PMB, 2109, Kaduna-Nigeria[4]Department of Biotechnology, Nigerian Defence Academy PMB, 2109, Kaduna-Nigeria[5]Department of Biotechnology, Nigerian Defence Academy PMB, 2109, Kaduna-Nigeria
[6]Department of Biological Sciences, Nigerian Defence Academy PMB, 2109, Kaduna-Nigeria
**Corresponding Authors mail:** thompsoneffiong2@gmail.com;benthaibenjamin@gmail.com ; nlegbe@nda.edu.ng;abdulsalamimosan@yahoo.com; ekereakede@nda.edu.ng; bakarevictoria2@gmail.com

*Received: 22-11-2023*
*Accepted: 19-12-2023*

https://dx.doi.org/10.4314/sa.v23i2.1

Journal Homepage: http://www.scientia-african.uniportjournal.info
Publisher: *Faculty of Science, University of Port Harcourt.*

## ABSTRACTS

*This work focuses on the assessment of the conditions relevant for the improvement of enzymes hydrolysis of pretreated corn cob, rice bran and sorghum bran by using Trichoderma Citrinoviride and Aspergillus niger. To achieve this, different fermentation conditions were applied to assess their effect in the optimization of cellulase production. Effects of fermentation duration, inoculation size, temperature and pH of fermentation on cellulase production were investigated. At 96 hrs of fermentation, maxim cellusale product was found to be at optimum in both organisms. Hence, 6% substrates concentration with 10 discs of 8mm inoculum size yielded maximum cellulase production in both A. niger and T. citrinoviride after 5 days of incubation. At 35ºC, A niger and T citrinoviride recorded maximum cellulase production 0.50 mg/ml in sorghum bran while 40ºC was optimum for maximum cellulase production for T, citrinoviride on corn cob. Whereas, pH 5.0 T. citrinoviride exhibits maximum cellulase production with sorghum bran 1.30 mg/ml compared to carboxymetyyl cellulose which served as control and sorghum bran 1.2mg/ml. These results highlight the potentials of T. citrinoviride as species of fungus for the industrial production of cellulase using Agricultural wastes as substrates. Cellulase yield was repressed in the presence of glucose and was induced in the presence of corn cob, rice bran and sorghum using T. citrinoviride. Cellulase yield from Corn cob, rice bran and sorghum bran differed significantly at (P<0.05) from glucose.*

**Keywords:** *Aspergillus niger.* Cellulase, Hemicellulase, Optimization, *Trichoderma citrinoviride*,

## INTRODUCTION

The demand for energy and its sources are on the increase due to growing population and industrialization. This implies that energy shortage will be a global challenge in the nearest future (Bakare *et al.*, 2019). The high cost of cellulase and hemicellulases contribute substantially to the price of bioethanol and as such, new studies aimed at understanding and

improving cellulases and productivity are of paramount importance.Cellulose is a linear polymer of glucose residue linked together by $\beta - 1 - 4$ glycosidic linkages (Muhammad *et al*., 2016). Glucose constitutes about 60% of the total sugars available in cellulosic biomaterials (de Souza *et al*., 2011), however fermentation of the available sugars in cellulosic biomass present a unique challenge because of the presence of other sugars such as Xylose and arabinose. Hence, the conversion of biomass to renewable energy independently is highly economically intensive unless hemicellulose is used in addition to cellulose as substrate (de Souza *et al.*, 2011). However, Agricultural wastes and residue including sugarcane bagasse (Siqueira *et al*., 2013), grass and corn cob (Pointner *et al*., 2014) have been recognized and employed as feasible substrates particularly for the production of fungal enzymes (Muhammed *et al*., 2016) and bioethanol in other to manage production cost.

Cellulases are a group of hydrolytic enzymes that act in a coordinated synergistic manner to hydrolyze the β-1, 4-glycosidic bonds in cellulose to release glucose units (Ezekiel *et al*., 2010; Nishida *et al*., 2007 and Lynd *et al.*, 2002). Current evolutionary studies based largely on measurements of sequence divergence among chronometric macromolecules, particularly small-subunit rRNAs (16S rRNA of prokaryotes and 18S rRNA of eukaryotes) reveal that the ability to digest cellulose is widely distributed among many genera in the bacteria and in the fungal groups (Woese, 2000 and Olsen *et al.*, 1994). Within the eubacteria, there are considerable concentration of cellulolytic capabilities among the predominantly aerobic order *Actinomycetales* (phylum *Actinobacteria*) and the anaerobic order *Clostridiales* (phylum *Firmicutes*). Fungal cellulose utilization is distributed across the entire kingdom, from the primitive, protist-like Chytridomycetes to the advanced Basidiomycetes. For microorganisms to hydrolyze and metabolize insoluble cellulose, extracellular cellulases must be produced that are either free or cell associated. Although a large number of microorganisms can degrade cellulose, only a few of them produce significant quantities of free enzymes capable of completely hydrolyzing crystalline cellulose (Koomnok, 2005).

Cellulase is a class of enzymes produced chiefly by fungi, bacteria and protozoans that catalyze cellulolysis (or hydrolysis of cellulose) (Abubakar and Oloyede, 2013). Although there are also cellulase producing plants and animals, a large number of microorganisms are capable of biodegrading cellulose, only a few of these microorganisms produce significant quantities of cell free enzymes capable of degrading cellulose in vitro.

The commercial cellulases are mainly extracellular enzymes produced by mesophilic or thermophilic fungi (Ki1m *et al*., 2005). Naturally, lignocellulosic biomass can undergo spontaneous fermentation by native microorganisms that inhabit them but the resulting products and rates of activities might be very low and inefficient. Harvesting products that result from such natural fermentation processes could be problematic. For maximum performance and product recovery however, these fermentations must be carried out under carefully controlled conditions with selected microorganisms while eliminating possible inhibitors of microbial activities. Two major methods used are the submerged and solid state fermentation methods (Vintilla *et al.*, 2009 and Jacob and Prema, 2006). Glucose produced from cellulosic substrate could be further used as substrate for subsequent fermentation or other processes which could yield valuable end products such as ethanol, butanol, methane, amino acids, single cell protein among others (Walsh, 2002). Cellulase has been used for several years in food processing, feed preparation, waste paper treatment, detergent formulation, textile production among other applications.

Additional potential application includes the production of wine, beer and fruits juice

*Scientia Africana, Vol. 23 (No. 2), April, 2024. Pp 1-12*
*© Faculty of Science, University of Port Harcourt, Printed in Nigeria* *ISSN 1118 – 1931*

(Walsh, 2002). Therefore, this work focuses on factors relevant for improvement of enzymatic hydrolysis of lignocelluloses material such as, Corn cob, Rice bran, and Sorghum bran. It is also aimed to provide better understanding of conditions for the production of cellulase by *A. niger* and *Tcitrinoviride* isolates.

## MATERIALS AND METHODS

### Description of Study Site

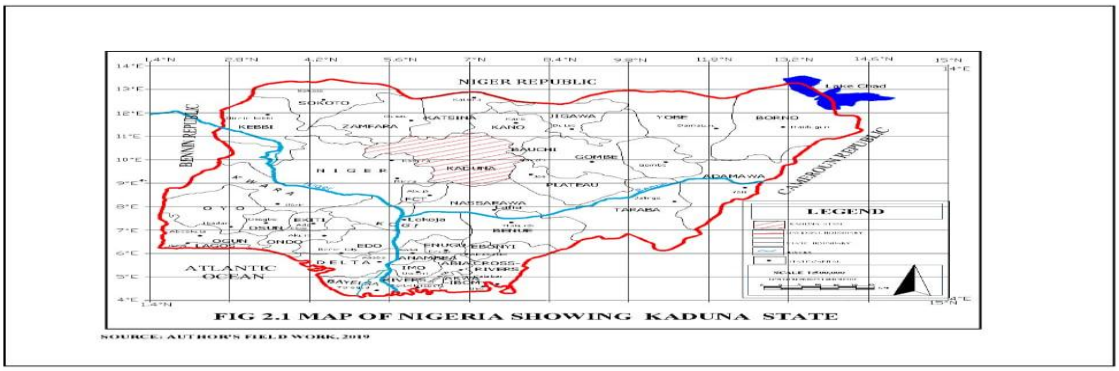



FIG 2.1 MAP OF NIGERIA SHOWING KADUNA STATE

SOURCE: AUTHOR'S FIELD WORK, 2019

The study was conducted at Nigerian Defence Academy Biology Lab, Kaduna State. The state has a total land mass of 48473.25Km$^2$ and is located West Geographical Zone of Nigeria. It lie between longitude 6° and 9° E and latitude 9° and 11° N. the state has distinct Wet Season (April –October) and Dry Season November-March) Vegetation type is the Guinea Savannah (Dilli *et al.*, 2021 and Ajaero *et al.*, 2023).

### Sources of Biomass

Three selected agricultural biomass; corn cob, rice bran and sorghum bran were utilized. Carboxymethyl cellulose (purified substrate) served as the control.

### Source of Microorganisms

Two different strains, *Trichoderma citrinoviride* and *Aspergillus niger*. previously cultured and reported by *Effiong et at.* (2019) for cellulose production were retrieved from the stored stocked culture.

### Biomass Processing and Delignification

The pre-treatment of substrates were carried out using the method (Damisa *et al*., 2015) described. Sun-dried materials were grounded and further pulverized into smaller particles. The pulverized substrates were washed with hot water several times to remove sugar residues and subsequently oven-dried at 70$^{\circ}$C for 24 hours and were further broken into pieces in preparation to alkaline treatment (Ali *et al*., 1991).

Each pulverized sample (20g) was soaked in 0.5 M solution of sodium hydroxide (100 mL) in a conical flask which served as digester for 1 hour. After the soaking period, copious amount of water was added to the flask content to dilute the alkali and the sample was recovered through filtration. Digested sample was washed repeatedly with distilled water until neutral a pH was recorded for the wash water and thereafter oven-dried at 60$^{\circ}$C overnight. Hence, all dried samples were packaged in polythene bags and labeled for subsequent studies.

### Preparation of Conidial Suspension of Isolates for Fermentation

Spore suspension of the *Aspergillus niger* and *Trichoderma citrinoviride* was prepared by serial dilusion of 5─day old culture with phosphate buffer (7.0). Into a 5 days old slant culture, 5 mL of buffer was introduced with aid of sterile inoculating needle; spores were dislodged from mycelia and mixed thoroughly by shaking. Inoculum size was determined with haemocytometer as described by Ekundayo and Juwon (2015). Five millimeters of homogenous spore solution was pipetted into one of the two counting chambers of the haemocytometer.

Therefore, spores in each of the four $0.1m^2$ corner square of the haemocytometer were counted, recorded and average was calculated.

**Media Preparation for Enzyme Production/Biomass Fermentation**

The basal medium according to Mendel *et al.*, (1981) which consist of (per liter of distilled water) $KH_2PO_4$, 2.0g, $(NH_4)_2$ $SO_4$, 4g; urea 0.3; $MgSO_4.7H_20$ 0.3; CaCl2 0.3g; peptone, 1.0g, tween 80 0.1%; $FeSO_4.7H_2O$, 0.5g and $COCl_2$ $6H_2O$ 2g, supplemented with 1% of various carbon source Carboxymethyl cellulose (control), corn cob, rice bran, sorghum bran as agricultural biomass was prepared accordingly. The pH was adjusted to 6.0, and subsequently medium was divided into 100 mL into 250 mL conical flask and cotton plugged. It was then further sterilized at 121°C for 15 minutes and cooled at room temperature before 10% inoculum spore suspension that had been diluted to $1.0x10^6$ cells per mL was aseptically seeded into the content of the flask. All fermentations were carried out in an Orbital shaker (model OS21), 350rpm at room temperature. During the fermentation processing, samples were taken aseptically at 24hours interval for 5days throughout the period for cellulase analysis using enzyme supernatant as previously described by Damisa *et al*. (2015).

**Cellulase Assay using Dinitrosalicylic (DNS) Method**

The culture filtrate was collected from fermentation medium containing different substrates by centrifugation. One milliliter of culture filtrate was taken in a test tube and was equalized with 2ml of distilled water. To the prepared culture filtrate, 3ml of DNS reagent was added. The content in the test tube was heated in boiling water bath for 5 minutes. After heating, the contents were allowed to cool at room temperature. At the time of cooling, 7ml of freshly prepared 40% potassium sodium tartarate solution was added. After cooling, the samples were read at 540nm using colorimeter (model CIBA-CORNING 252). The amount of reducing sugar was determined using the standard graph.

**Filter Paper Assay (FPA) of Cellulase Determination**

Cellulase activity was determined by a method of Xiaoxiao *et al*., (2016). An aliquot 0.5 mL of cell-free culture supernatant of the various substrates was transferred to clean test tubes and 1 mL of Na-citrate buffer (PH 4.8) was added. Whatman No 1 filter paper strip (6cmx1cm) was added to each tube. Tubes were votexed to coil the filter paper in the bottom. Tubes were incubated in a water bath at 50°C for 1hour followed by an addition of 3ml DNS reagent. Tubes were then immersed in an ice-bath, followed by the addition of 15ml distilled water to each tube. Content of the tube was mixed and absorbance was read at 540 nm (wavelength). Cellulase activity was expressed in terms of filter paper unit (FPU) per ml of undiluted culture filtrate. Filter paper unit (FPU) is defined as mg of reducing sugar librated in one hour under standard assay condition (Sadaf *et al*., 2005), reducing sugar produced in one hour was calculated by comparing $A_{540}$ with that of standard curve.

**Effect of Substrate Concentration on Cellulase Production**

This was determined according to Millala *et al*. (2005). Different concentrations of substrates ranging from 2%, 4%, 6% and 8% (w/v) were added to 100ml of the basal medium in separate cornical flasks, cotton pluged and autoclaved at 121°C for 15 minutes. After cooling, the basal salt solution in different flask were inoculated with *T. citrinoviride* and the other flask with *A. niger* and incubated at $30^0$C in orbital shaker at 350 rpm for 96hours. The cellulase activity was determined and recorded using Dinitrosalicylic (DNS) method.

**Effect of Varying Temperatures on Cellulase Production**

The optimal temperature of enzyme production was determined by growing the fungi in sterile

*Scientia Africana, Vol. 23 (No. 2), April, 2024. Pp 1-12*
*© Faculty of Science, University of Port Harcourt, Printed in Nigeria* *ISSN 1118 – 1931*

media containing various substrates (CC, CMC, RB and SB) incubated at temperature 30ºC, 35ºC, 45ºC, 50ºC for five days. Cellulase activity was determined and recorded (Millala *et al*., 2005).

**The effects of Fungi Inoculum Size on Cellulose Production**

The inoculum size determination was carried out using the method described by (Acharya *et al*., 2013). This was done by culturing the biomolecules of fungi strains (*Aspergillus niger* and *Trichoderma citrinoviride*) on Potatoe Dextrose Agar (PDA). Plate containing *Aspergillus niger* and *Trichoderma citrinoviride* were punched by using sterile cup borer of 8mm size. The substrate containing fermentation media was inoculated with 5, 10, 15 and 20 discs of *Aspergillus niger* and another set with *Trichoderma citrinoviride* aseptically. After inoculation, the flasks were incubated in orbital shaker at $30^0$C at 150rpm for five days. Enzyme assay was performed and recorded.

**Effect of varying pH on Cellulase Production**

This was performed according to the method described by (Immanuel *et al*., 2006). The optimized media was prepared using the individual substrate (corn cob, Rice bran, sorghum bran, and CMC (control) and the PH was set at 5, 6, 7, 8, and 9 respectively by adding 1% NaOH and concentrated Hydrochloric acid. Then the media was autoclaved and later inoculated with *Trichoderma citrinoviride* and *Aspergillus niger* separately and incubated in a shaker (150rpm) set at 30°C for 5 days. Enzyme assay was carried out at the end of the 5days incubation

**Effect of Fermentation Duration**

This was conducted according to the method of Acharya *et al*. (2013). Fermentation medium in flask containing Corn cob, Rice bran, Sorghum and CMC were inoculated with *A. niger* and *T. citrinoviride* and incubated 120 hours at 30°C in orbital shaker incubator at 120rpm. Enzyme assay was carried out at 24hours intervals.

**Cellulase production induction test on Glucose, Corn cob, Rice bran and Sorghum bran using *T. citrinoviride***

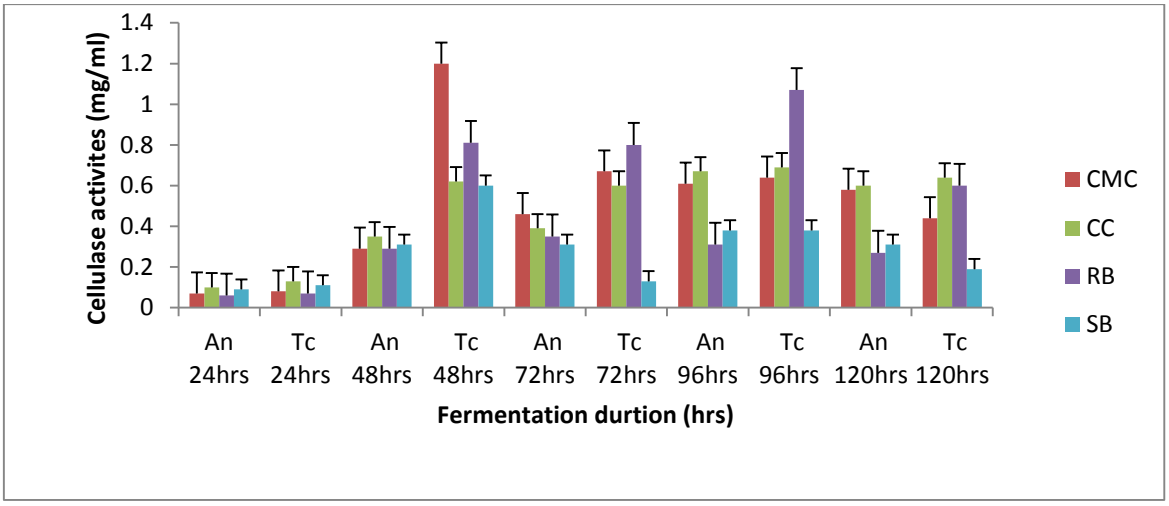
*T. citrinoviride* was activated in basal complete medium (BCM) at 30°C for five days and harvested by adding 20ml of sterile distilled water .Then 100mls of basal medium was transferred into 250ml conical flask and was seeded with $1\times10^6$ spore per 30ml of fungal culture. The spore was grown in basal medium with 1% glucose (W/V) as the carbon source at 30°C for 48 hours. The mycelia from the glucose culture were washed and transferred to flask containing fermentation medium supplemented with 1% (W/V) Glucose, Corn cob, Rice bran and Sorghum bran media as sole carbon sources. The flasks were incubated for 24 hours. Mycelia were harvested by filtration through whatman high-grade filter paper, washed with sterile water followed by determination of enzyme production in the cell free culture supernatant by DNS method.

**Statistical Analysis**

Data obtained from optimization for cellulase production by *Aspergillus niger* and *Trichoderma citrinoviride* using corn cob, rice bran, sorghum bran and carboxymethyl cellulose (control) were analysed by descriptive statistics methods and results presented in tables and graphs with respective error bars. Hence, data obtained through induction test were analysed using one way analysis of variance (ANOVA) to verify differences between the fungi isolates as well as enzyme yield with different substrates.

## RESULT

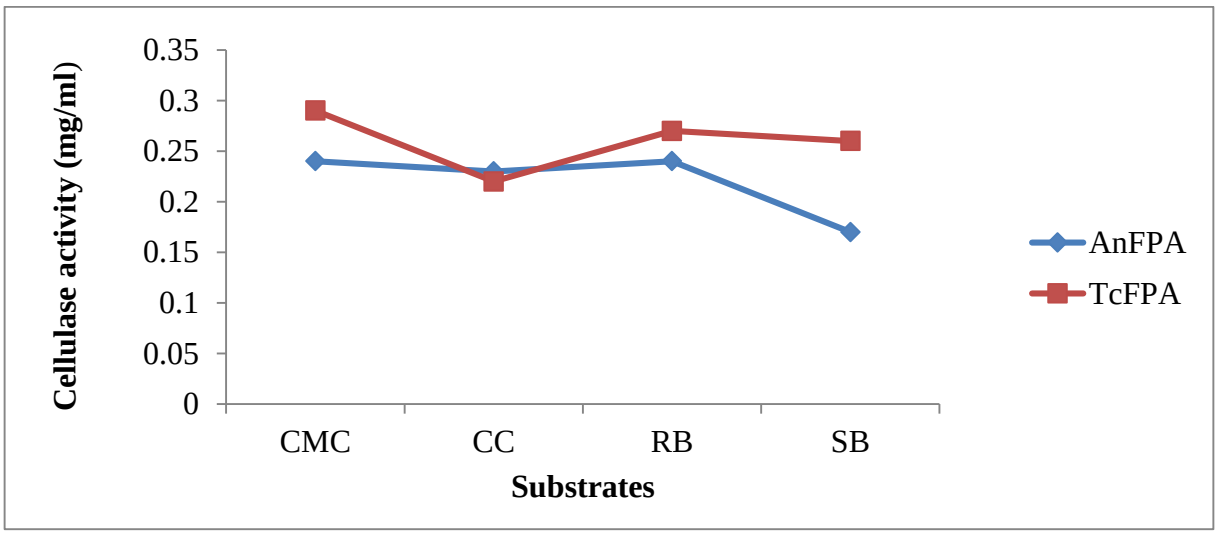
**Figure 1: Effect of Fermentation Duration on Cellulase Production**




**KEY:** CMC= carboxymethyl cellulose (control)**,** CC= corn cobs**,** RB= rice bran**,** SB= sorghum bran**,** An= *Aspergillus niger***,** Tc= *Trichoderma citrinoviride*

*Aspergillus niger*recorded maximum cellulose activity at 96 hrs. (0,67mg/ml) on corn cob and least cellulase activity at 24hrs (0.06mg/ml) on rice bran as shown in Figure 1. *T. citrinoviride* showed maximum cellulase activity at 48hrs with CMC (1.2mg/ml), 96 hours with RB (1.07mg/ml) and least at 24 hours on Sorghum bran as shown in Figure 1.

**Figure 2: Filter Paper Assay (FPA) Of Exoglucanase and Endoglucanase**

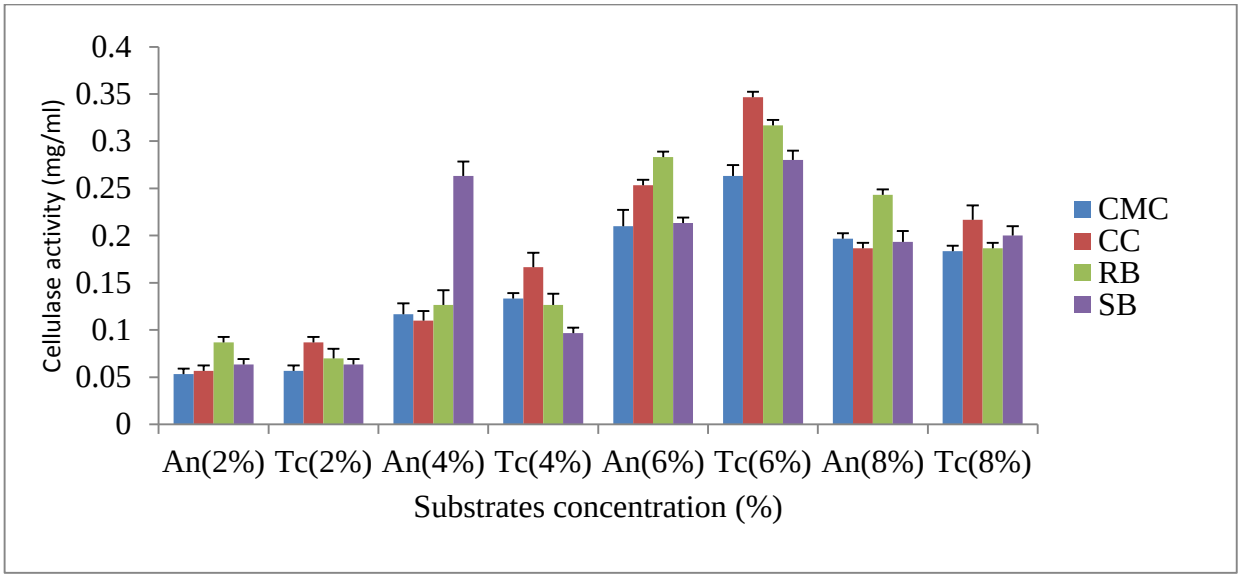



**KEY:** CMC= Carboxymethyl cellulose (control)**,** CC= Corn cobs**,** RB= Rice bran**,** SB= Sorghum bran, An = *Aspergillus niger***,** Tc = *Trichoderma citrinoviride*

*Trichoderma citrinoviride* produced maximum exo glucanase and endo glucanase activity on RB (0.24mg/ml) and least on CC (0.22mg/ml) compared to the control CMC (0.29 mg/ml). With *Aspergillus niger*, maximum endo and exo glucanase activity was obtained on RB and CMC (0.24mg/ml) and least on SB (0.17mg/ml) as shown in Figure 2.

*Scientia Africana, Vol. 23 (No. 2), April, 2024. Pp 1-12*
*© Faculty of Science, University of Port Harcourt, Printed in Nigeria* *ISSN 1118 – 1931*

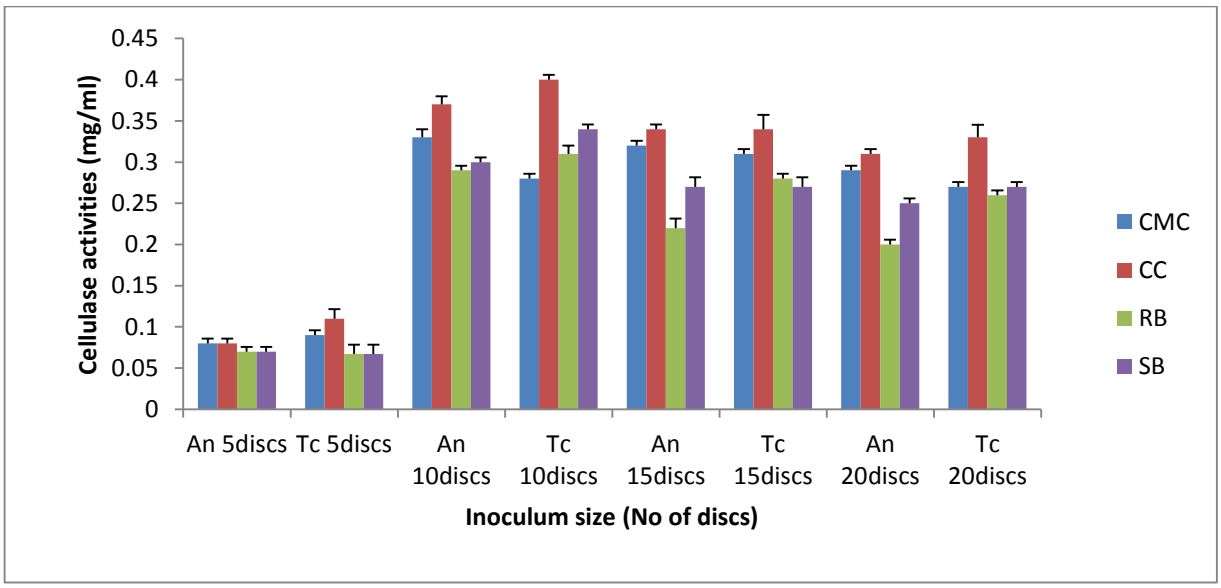
**Figure 3: Effect of Substrate Concentration on Cellulase Production**




**KEY:** CMC= Carboxymethyl cellulose (control), CC= Corn cobs, RB= Rice bran, SB= Sorghum bran, An= *Aspergillus niger*, Tc = *Trichoderma citrinoviride*

Substrates concentrations were varied as 2%, 4%, 6%, and 8%. *T. citrinoviride* recorded maximum cellulase activity at 6% concentration of corn cob (0.35mg/ml,) while *A. niger* recorded maximum cellulase activity at 6% substrate concentration of Rice bran (0.28mg/ml) as shown in Figure 3. Fungal enzyme cellulase activity reduced in all the substrates above 6% substrate concentration.

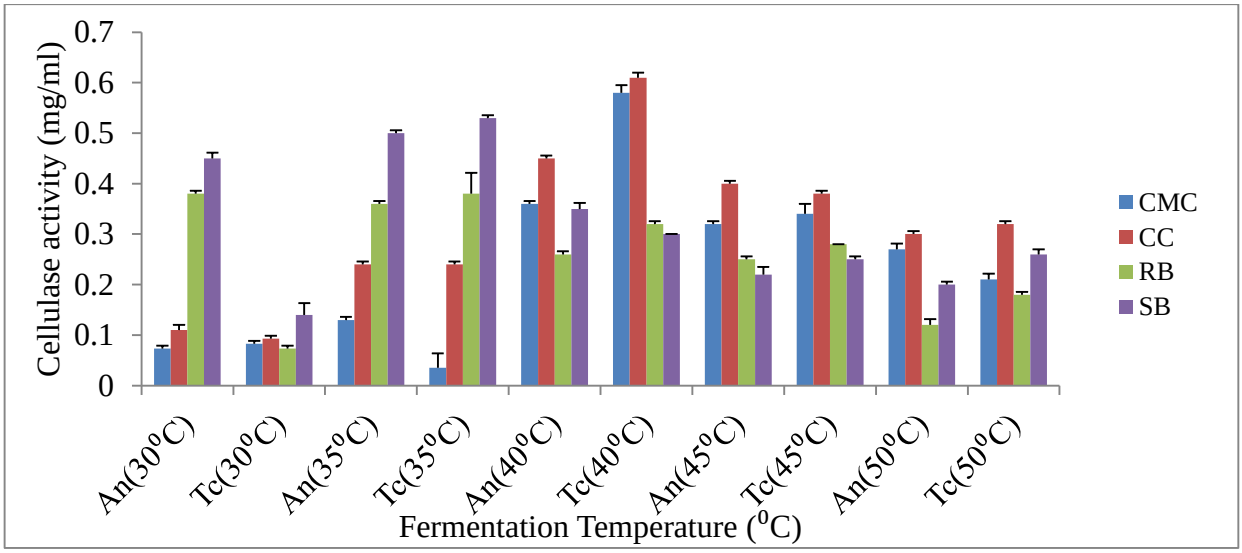
**Figure 4: The Effects of Fungal Inoculum Size on Cellulase Production**




**KEY:** CMC= carboxymethyl cellulose (control)**,** CC= corn cobs, RB= rice bran**,** SB= sorghum bran**,** An= *Aspergillus niger***,** Tc= *Trichoderma citrinoviride*

As shown in Figure 4, 10disc of 8mm diameter of *T. citrinoviride* produced maximum cellulose activity of 0.40mg/ml on corn cob and 0.37mg/ml on rice bran for *A. niger*.

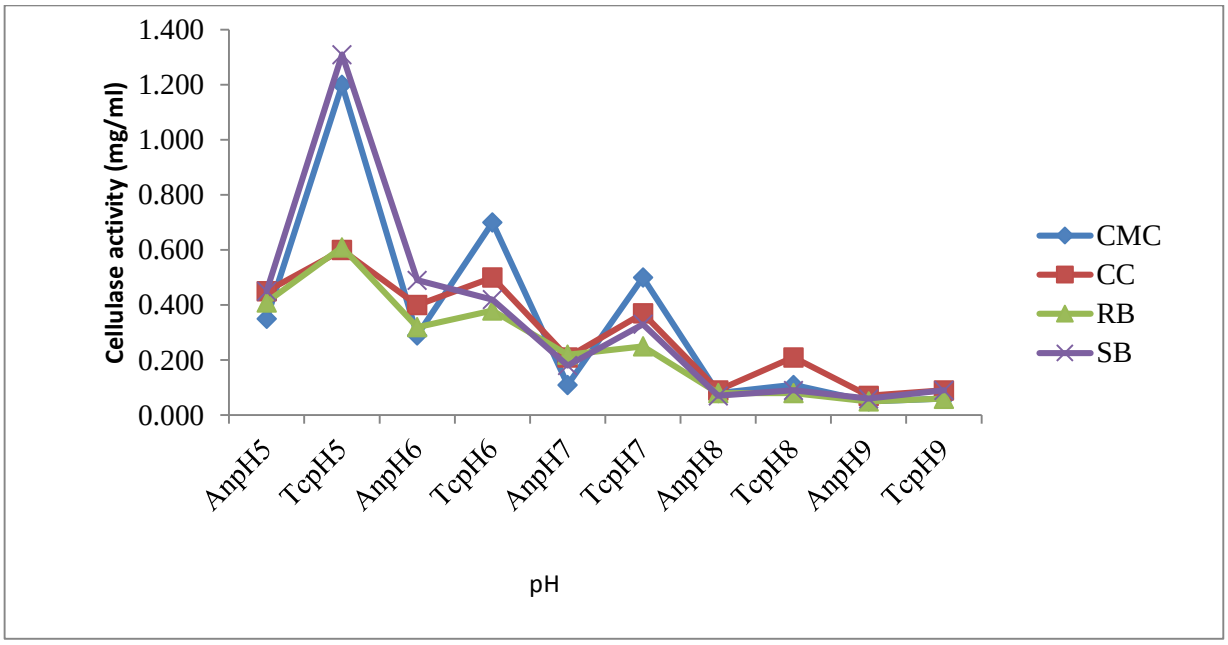
**Figure: 5 Effect of Varying Temperature on Cellulase Production**




**KEY:** CMC= Carboxymethyl cellulose (control)**,** CC= Corn cobs**,** RB= Rice bran**,** SB= Sorghum bran**,** An= *Aspergillus niger***,** Tc = *Trichoderma citrinoviride*

The optimum temperature for cellulase activity for *T. citrinoviride* was 40ºC using corn cobs as substrate, while 35ºC optimum was obtained for *A. niger* on sorghum substrate.

**Figure 6: Effect of Varying Ph on Cellulase Production**

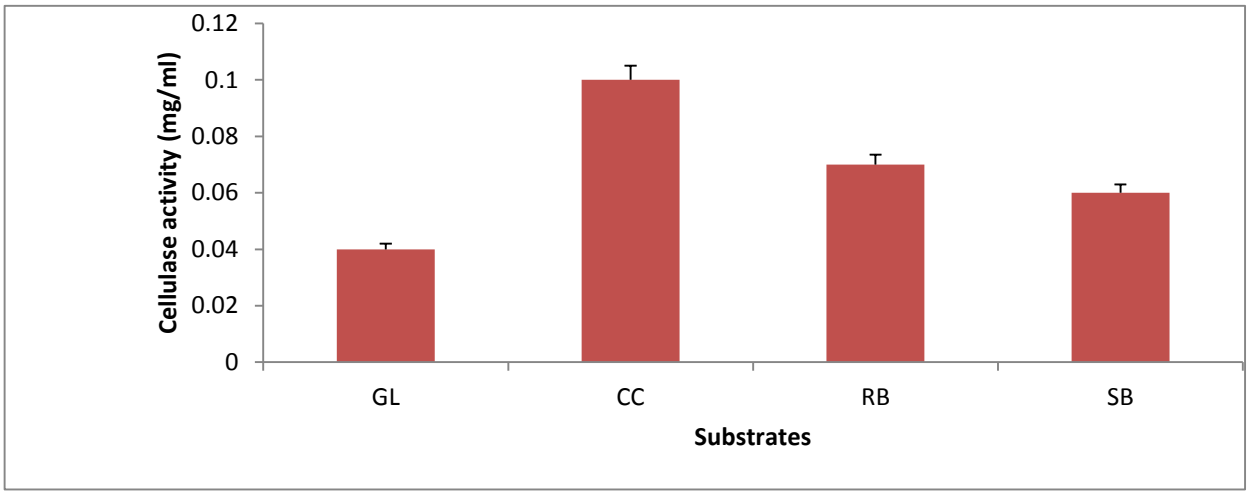



**KEY:** CMC= Carboxymethyl cellulose (control), CC= Corn cobs, RB= Rice bran, SB= Sorghum bran, An= *Aspergillus niger*, Tc = *Trichoderma citrinoviride*

With *A. niger*. Cellulase activity reached maximum at pH5 on CC, (0.45mg/ml) while T. *citrinoviride*, exhibited highest cellulase activity at pH5 on SB (1.31mg/ml). Cellulase activity by both organisms declined in all the substrates as pH increase from 6-9 as shown in Figure 6.

*Scientia Africana, Vol. 23 (No. 2), April, 2024. Pp 1-12*
*© Faculty of Science, University of Port Harcourt, Printed in Nigeria* *ISSN 1118 – 1931*

**Figure 7: Cellulase production induction test on Glucose, Corn cob, Rice bran and sorghum bran using *T. citrinoviride***




**KEY:** GL= glucose, C= corn cobs, RB= rice bran**,** SB= sorghum bran

Cellulase activity by *T. citrinoviride* increased when lignocellulosic substrates were used as carbon source (CC, 0.1mg/ml, RB, 0.07mg/ml, SB, 0.06mg/ml). Glucose gave least cellulase activity when used as carbon source (0.04mg/ml) as shown in Figure 7. There was significant difference at $\alpha = 0.05$ confidence limit ($p = 0.0001$) in cellulase produced from corn cob compared with that of other substrates as shown in Figure 7. Soluble enzyme inducers like glucose are weaker in inductive capability when compared to insoluble substrates like corn cob, rice bran and sorghum bran.

## DISCUSSION

The study of the effects of incubation duration on cellulase production by *T. citrinoviride* and *A. niger* showed that maximum cellulase production on different cellulosic substrates was obtained at 96hours incubation. Higher incubation duration above 96hours resulted in decreased level of enzyme production. This result is in agreement with the findings of Abubakar *et al.* (2000)who obtained highest level of cellulase activity at 96 hrs using *Aspergillus niger* on rice bran. Ekundayo and Arotupin (2015)reported highest cellulase production at 72 and 96 hours of fermentation respectively on carboxymethyl cellulose. Thedecrease in cellulase activity of both organisms beyond 96hours of incubation may be attributed to cumulative effect of cellobiose (Lee *et al*., 2007). Cellobiose, a dimer of glucose is known to inhibit both endo glucanase and glucosidase. It may also suggest that delignification produce aromatic water soluble products that repress the cellulolytic action of the enzyme. This is supported by the findings of Emmanuel *et al.* (1991) who reported the inhibitory effects of accumulated cellobiose and cellodextrin of low degree of polymerisation. The decrease may also be due to depletion of other nutrients (mineral salt) other than the energy source.

In filter paper (FPA) method of enzyme analysis, *T. citrinoviride* showed high enzyme yield on carboxymethyl cellulose (0.29mg/ml) while *Aspergillus* showed activity of 0.24mg/ml on CMC and rice bran, (Figure 2). This result is contrary to findings of Muhammad *et al*. (2009) who reported that *Trichoderma citrinoviride* and *Penicillum janthinellum revealed* the higher level of B-glucanse and xylanase when bagase was used with purified cellulose. This could be as a result of differences in fermentation duration or other cultural conditions.

Enzyme production by *A. niger* and *T. citrinoviride* increased to maximum at 6% substrate concentration in all the substrates. This is in agreement with findings of Abubakar and Oleyede (2013)who recorded maximum

enzyme activity at 6% substrate concentration using rice bran and orange peel with *A. niger.*

The effect of fungal inoculum size on enzyme production was investigated. For *A. niger*and *T. citrinoviride* maximum cellulase activity was obtained with 10 discs of 8mm inoculum size but reduced with increase inoculum discs. This data supports the findings of Acharya *et al.* (2008) which reported that when high fungal mass are used cellulose activity decreased but when low fungal mass are used cellulase activity increased.

Cellulase activities on CMC and the substrates peaked at temperature of 30°C to 40°C for *A. niger*and *T. Citrinoviride* respectively. This is expected as the optimum growth temperature for fungi falls within the range. Immanuel *et al.* (2000)and Abubakar (2013)also recorded temperature of 40°C as favourable for maximum enzyme production.

At pH 5, *A. niger* and *T. citrinoviride* recorded maximum enzyme activity on sorghum bran and corn cob *as* shown in Figure 6. This is in line with the findings of Immanuel *et al.* (2013) and Abubakar *et al*. (2010) which recorded maximum enzyme activity for *A. niger*at pH 5 using coir waste and corn cob as substrate. The temperature range and pH range recorded could be the optimum for enzyme production and enzyme stability for the isolates tested.

Peak in the cellulase enzyme production was observed when *T. citrinoviride* was grown on cellulosic substrate as sole carbon source (Corn cob, 0.10mg/ml, Rice bran, 0.07 mg/ml, Sorghum bran, 0.06mg/ml while a very low rate of enzyme production was noticed when glucose was used as sole carbon substrate (0.04mg/ml). Corn cob, Rice bran and Sorghum bran differed significantly at ($P<0.05$) from glucose. These results are in agreement with other results obtained by Ledeberg (1992), and Lynd *et al*. (2002)where residual enzyme activity was noted when cellulosic fungi were grown in the presence of glucose and high in enzyme yield reported in the presence of cellulosic substrates. The enzyme expression in the cellulosic substrates could be as a result of genes that code for cellulosic enzymes occasioned by the starvation experienced by the organism in the medium lacking easily metabolizable carbon sourse. Mouse and Thawat, (2007) reported that the production of low level of cellulase in the presence of other carbon sources such as glucose is related to glycolytic mechanism known as catabolite repression.

## CONCLUSION

Among the substrates used in this study, Corn cob was found to be the substrate for high cellulase production in submerged fermentation during optimization and induction studies. This result highlights the industrial potentials of corn cob as possible raw materials for cellulase production by *T. citrinoviride*. The potential of *T. citrinoviride* to produce high titre of cellulase on substrate such as corn cob, rice bran and sorghum bran makes it a good candidate for industrial cellulosyic enzymes production.

## REFERENCES

Abubakar, F.A. and Oloyede, O.B. (2013). Production and activity of cellulase from *Aspergillus niger*using rice bran and orange peel as substrates. *International Journal of Scientific Research and Management.* 1(5):285-291.

Acharya, P.B., Acharya, D.K. and Modi, H.A. (2008). Optimization for cellulose production by *Aspergillus niger* using saw dust as substrate. *African Journal of Biotechnology.* 7(22):4147-4152.

Ajaero, U.C, Yusuf, O.S., Abdulsalami, S.M, Benjamin, B., Ndibe, T.O., Bakare, V. and Sani, J. (20203). Assessment of Nutritional chemicals and Antioxidant activities of some food Components for their Potential as Functional Foods and Prebiotics. *Scientia Africana,* 22(3):1-10.

Bakare, V., Abdulsalami, M.S., Onusirioka, B.C., Appah, J., Benjamin, B. and Ndibe, T.O. (2019). Ethanol production from lignocellulosic materials by fermentation

*Scientia Africana, Vol. 23 (No. 2), April, 2024. Pp 1-12*
*© Faculty of Science, University of Port Harcourt, Printed in Nigeria* *ISSN 1118 – 1931*

process using yeast. *Applied Journal of Science and Environmental Management.* 23(5):875-882.

Damisa, D., Kuta, F.A. Abioye, O.P, Bala, J.D. and Egbe, N.E, (2015). Cellulase producing potential of *Aspergillus terreus* Uv2 on cellulosic wastes pre-treated with acid and alkali. *British Biotechnology Journal*. 9(2):1-10.

De souza, W.R, De Gouvea, P.F., Savoldi, M., Malavazi, I., De souza, B., Maria, S.G., Ronald, P., Dervries, julina., V., De castro, O. and Gustavo, H.G. (2011). Transcriptiome analysis of *Aspergillus niger* grown on sugar cane basses. *Biotechnology for Biofuels and Bioproducts*. 4: 40.

Dilli, D.D., Benjamin, B., Ochuko, O., Moltong, V.Y. and Markus, T.P. (2019). Molecular characterization of dermatophyte isolated from horses of Nigerian Defence Academy Equitation Stable, Kaduna. *Journal of Veterinary Advances.* 2(2):19-25.

Effiong, T.E, Abdulsalami, M.S, Egbe, N.E, and Bakare, V. (2019). Screening of fungi isolates from soil, pulp waste water and rotten wood for cellulase producing potentials. *Journal of Applied Science and Environmental Management.* 23(8): 1051 ─ 1055.

Ekundayo, T.C., Arotupin, D.J. (2015). Isolation and identification of cellulytic fungi from agro wastes and Sawmill soils. *British Biotechnology Journal*. (7): 147-159.

Ezikiel, C.N., Odebode, A.C, Omenka, R.O. and Adesioye, F.A. (2010). Growth response and comparative cellulose induction in soil fungi growth on different cellulose media. *Acta SATECH* 3(2): 52 ─ 59.

Jacob, N. and Prema, P. (2006). Influence of Mode of Fermentation on Production of Polygalacturonase by a Novel Strain of Streptomyces lydicus. *Food Technology and Biotechnology*. 44(2):263-267.

Koomnok, C. (2005). *Selection of cellulase producing thermophilic fungi*. 31st Congress on Science and Technology of Thailand; Suranaree University: Nakhonratchasima Thailand.

Ledeberg, J. (1992).Cellulases. *Encyclopedia of Microbiology.* (Vol.1; A−C) Academic Press Incorporation.

Lynd, L.R., Weimer, P.J., Vanzyl, W.H. and Pretorious. I.S. (2002). Microbial cellulose utilization: Fundamentals and Biotechnology. *Microbiology and Molecular Biology Review*. 66:506-577.

Milala, M.A., Shugaba, Gidado, A., Ene, A.C. and Wafer, J.A. (2005). Studies on the case of Agricultural wastes for cellulase Enzyme production by *Aspergillums niger*. *Journal of Agriculture and Biological Science*. 1(4): 325-328.

Moussa, T.A. and Tharwat, N.A. (2007). Optimization of cellulose and β ─ glucosidae induction by sugerbeet pathogen *Sclerotium rolfsii*. *African Journal of Biotechnology.* 6: 1048 ─ 1054.

Mohammed, I.J. and Obasola. E.F. (2010). Optimization studies on cellulase enzyme production by an isolation strain of *Aspergillus niger* YL128. *African Journal of Microbiology Research.* 4(24): 2635-2639.ISSN 1996 ─ 0808.

Nishida, Y., Suzuki, K.I., Kumagai, Y., Tanaka, H., Inoue, A. and Ojima, T. (2007). Isolation and primary structure of a cellulase from the Japanese sea urchin *Strongylocentrotus nudus*. *Biochimie.* 1-10.

Olsen, G.J., Woese, C.R. and Overbeek, R. (1994). The winds of (evolutionary) change: breathing new life into microbiology. *Journal of Bacteriology*. 176:1–6.

Pointner, M., Kuttner, P., Obrlik, T., Jager, A., and Kahr, H. (2014). Composition of corn cobs as a substrate for fermentation of Biofuels. *Agronomy Research.*12:391-396.

Siqueira, G., Varnai, A., Farraz, A. and Milagres, A.M.F. (2013). Enhancement of cellulose hydrolysis in sugarcane bagasse by the selective removal of Lignin with sodium chlorite. *Applied Energy.*102:399-402.

Walsh, G. (2002). *Industrial enzyme proteases and carbohydrate*. In protein Biochemistry

and Biotechnology John Wiley and sons Ltd.

Woese, C.R. (2000). Interpreting the universal phylogenetic tree. Proc. Natl. Academic Science. USA 97: 8392–8396.

Xiaoxiao, Y., Yan, L., Yuxio, C., Qiyue, C., Zaixio, Z., Jia, H.L., Qingfan, M., Lirong, T. and Xiodon, R. (2016). Measurement of filter paper activities of cellulase with microplate-based assay. *Saudi Journal of Biological Sciences*, 23(1):S93-S98.