

Original article:

BIOETHANOL POTENTIALS OF CORN COB HYDROLYSED USING CELLULASES OF *ASPERGILLUS NIGER* AND *PENICILLIUM DECUMBENS*

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ABSTRACT

Corn cob is a major component of agricultural and domestic waste in many parts of the world. It is composed mainly of cellulose which can be converted to energy in form of bioethanol as an efficient and effective means of waste management. Production of cellulolytic enzymes were induced in the fungi *Aspergillus niger* and *Penicillium decumbens* by growing them in mineral salt medium containing alkali pre-treated and untreated corn cobs. The cellulases were characterized and partially purified. Alkali pre-treated corn cobs were hydrolysed with the partially purified cellulases and the product of hydrolysis was fermented using the yeast *saccharomyces cerevisiae* to ethanol. Cellulases of *A. niger* produced higher endoglucanase and exoglucanase activity (0.1698 IU ml⁻¹ and 0.0461 FPU ml⁻¹) compared to that produced by *P. decumbens* (0.1111 IU ml⁻¹ and 0.153 FPU ml⁻¹). Alkali pre-treated corn cob hydrolysed by cellulases of *A. niger* yielded 7.63 mg ml⁻¹ sugar which produced 2.67 % (v/v) ethanol on fermentation. Ethanol yield of the hydrolysates of corn cob by cellulases of *P. decumbens* was much lower at 0.56 % (v/v). Alkali pre-treated corn cob, hydrolysed with cellulases of *A. niger* is established as suitable feedstock for bioethanol production.

Keywords: Corn cob, endoglucanase, exoglucanase, sugar, ethanol

INTRODUCTION

Corn (maize) is a major food crop in many parts of the world. In Nigeria, corn is processed to a variety of diets including pap which is a major diet used for weaning. It is a major component of animal feeds and is consumed by many Nigerians as snack, either boiled or roasted. The capacity for corn production in Nigeria is high and efforts towards improvement are being made at various quarters. Currently, about eight million metric tonnes of corn are produced annually (Nwanma, 2009) and a production forecast for 2010-2015 envisaged a 23 % growth. The maize plant comprises of the stalks, husks, shanks, silks, leaf blades, leaf

sheaths, tassels and cobs. The corn cob carries the grain and together with associating husks, shanks and silks are harvested from the farm. The other parts are left on the farm to rot (Kludze et al., 2010).

Corn cobs form about 30 % of maize agro-wastes (Rangkuti and Djajanegara, 1983) of which application in bio-fuel industry are the focus of many researches aimed at achieving an effective and efficient waste management scheme. It contains 32.3-45.6 % cellulose, 39.8 % hemicelluloses - mostly composed of pentosan and 6.7-13.9 % lignin (Sun and Cheng, 2002) and can be converted to fermentable sugar for ethanol production. Enzymatic hydrolysis of corn cob to fermentable sugar

is however a difficult and rate limiting process due to the recalcitrance of lignin. Lignin interferes by acting as a physical barrier that prevents the contact of cellulase to cellulose (Umamaheswari et al., 2010). Overcoming the recalcitrance of natural cellulosic materials in the cellulosic ethanol process requires delignification to liberate cellulose and hemicelluloses from their complex with lignin (Mosier et al., 2005).

Pre-treatment processes using physical, physico-chemical, chemical or biological methods, leading to the removal of lignin and hemicellulose, reduction of cellulose crystallinity and increase of porosity significantly improve enzymatic hydrolysis. Organosolve and sulphite pre-treatment of recalcitrant lignocelluloses (SPORL) are very effective on materials with high lignin content (Zhu et al., 2009). Other techniques include steam explosion, ammonia fibre expansion (AFEX), ozone pre-treatment, dilute acid pre-treatment and alkaline wet oxidation (Sun and Cheng, 2002).

Hydrolysis of cellulose has been achieved with dilute acids under high temperature and pressure or with concentrated acids at low temperature and atmospheric pressure (Akpan et al., 2005). A major problem with acid hydrolysis however is the production of toxic degradation products such as furfural and hydroxyl methyl furfural which interfere with downstream enzymatic treatment in the bioethanol process. Alkali such as sodium hydroxide, ammonium hydroxide etc. also digests cellulose to yield sugar. Cellulases secreted by a number of fungi, bacteria and protozoa also hydrolyze cellulose to fermentable sugar. Among the thousands of fungal species that could utilize cellulose as sole carbon source, members of the genus *Trichoderma* (Herr, 1979), *Aspergillus* (Garg and Neelakantan, 1982), *Penicillium* (Borreti et al., 1972), *Fusarium* (Canevascini and Gattlen, 1981; Olishkevsk et al., 2009), *Phanerochaete chrysosporium* (Streamer et al., 1975), and *Sclerotium rolfsii* (Sadana et al., 1979; Shewale and Sadana, 1978) have been reported to elaborate high cellulase

activities. Most commercial cellulases are produced by members of the genus *Trichoderma* and *Aspergillus* (Cherry and Fidantsef, 2003; Kirk et al., 2002). *Aspergillus niger* secretes copious amounts of cellulolytic enzymes and together with *Trichoderma* have been studied intensively for industrial production of the enzymes (Acharya et al., 2008; Coral et al., 2002; Kader and Omar, 1998; Omojasola et al., 2008; Sridevi et al., 2009).

The use of corn cobs as a biomass feedstock offers promising possibilities for renewable energy production. A feedstock used in bio-energy conversion must have adequate energy content. While corn cobs are not as energy dense as the fossil fuels that society is familiar with, they have a similar energy density to other biomass feedstock and less energy dense coals, both of which are successfully utilized as energy feedstocks around the world. The chemical properties and physical characteristics of corn cobs make it suitable as a feedstock for energy generation. This study examines the potentials of corn cob hydrolysed with cellulases of *A. niger* and *P. decumbens* in the bio-energy industry particularly in the production of bioethanol.

MATERIALS AND METHODS

Organism

The fungi *Aspergillus niger* and *Penicillium decumbens* were obtained from the culture collection centre of the Department of Microbiology, University of Ilorin, Nigeria. The organisms were grown on potato dextrose agar plates and maintained on agar slants at 4 °C till required.

Screening

The agar diffusion method of Hankin and Anagnostakis (1977) was employed to screen for cellulolytic activities of the fungi. The medium comprised of 1 % carboxyl methyl cellulose (CMC) incorporated into Mandels mineral salt (Mandels et al., 1974) and solidified with agar (15 g/l). Medium was autoclaved and distributed into petri dishes. Each plate was inoculated by streak-

ing once across the middle of the plate and incubated at 28 ± 2 °C. Cellulolytic activity was detected after growth, by flooding the plates with 1 % congo red solution for 15 min. The dye was drained and plates were flooded with 1 N sodium chloride solution for another 15 minutes. Clearance around growth of isolate represents cellulase production. The diameter zone of clearance was measured at five different locations and the mean was used to represent cellulase activity of the organism.

Viscosity reduction test

A method which measures a reduction in viscosity based on rate of flow of culture from the nozzle of a syringe was employed (SAPS, 2010). The fungi was inoculated into Mandels mineral salt medium (Mandels et al., 1974) containing 2 % (w/v) CMC in an Erlenmeyer flask and incubated on a shaker incubator (150 rpm) at room temperature for 7 days. Control was set up with the same medium which was not inoculated. After the incubation period, mycelia were removed from culture by running it through a 100 µm sieve. Culture filtrate and control were then treated as follow.

Culture filtrate was filled into the barrel of a syringe held in vertical position with nozzle pointing downward. The rate of flow was measured by taking the time for 8 ml of the fluid to drain through the nozzle with the aid of a stop watch. Measurement was taken 5 times for each sample and the mean was recorded as the flow rate. Enzyme activity, expressed as percentage reduction in viscosity was obtained using the following formula.

$$V = \frac{T_0 - T}{T_0 - T_{\text{water}}} \times 100$$

V = percent loss in viscosity, T_0 = flow time in seconds of uninoculated medium, T = flow time of culture filtrate and T_{water} = flow time of water (ePlantscience.com).

Collection and pre-treatment of corn cob

Corn cob obtained from the waste bin near a grains market was washed in water to remove dust and sun dried for two days. The cobs were broken with the aid of a wooden mortar and pestle, ground in an electric grinder and sieved through a mesh sieve (pore size, 100 µm). The resulting powder was treated with alkali solution to swell the cellulose and make it available for enzyme hydrolysis following a method described elsewhere (Vyas et al., 2005). The dried powder obtained was stored in sealed polythene bags at room temperature till required.

Production of enzyme

a) Inoculums development

The inoculums medium comprised of Mandels mineral salt medium (Mandels et al., 1974), 0.2 % (w/v) carboxyl methyl cellulose (CMC), in Erlenmeyer flasks (250 ml). Spores of *A. niger* and *P. decumbens* were harvested by flooding 1 week old stock culture on agar slants with sterile distilled water (5 ml). The inoculums medium (100 ml), was inoculated with the entire spore suspension and incubated on a gyratory shaker (150 rpm) at room temperature for 72 h.

b) Fermentation

Each 250 ml flask contains 100 ml of Mandels mineral salt medium (Mandels et al., 1974), into which was incorporated 10 g of cellulosic materials i.e. alkali pre-treated and untreated corn cob; and carboxyl methyl cellulose (CMC). The medium was inoculated with inoculums culture (5 ml) containing approximately 4.6×10^6 spores/ml of each of the fungi in separate flasks and incubated at 28 ± 2 °C on a gyratory shaker (150 rpm) for 10 days (Omojasola and Jilani, 2008). Enzyme filtrates were obtained after cultures were centrifuged at $13,000 \times g$ for 20 min in a table top high speed refrigerated centrifuge H1850R at 4 °C and stored at -10 °C until required.

Assay for cellulase activities

a) Endoglucanase activity- carboxyl methyl cellulase (CMCase)

Carboxyl methyl cellulase activity was determined following the method of the International Union of Pure and Applied Chemistry (Ghose, 1987). Reaction mixture comprised of 0.5 ml carboxyl methyl cellulose (2 % w/v) in 0.05 M citrate buffer, pH 4.8 and 0.5 ml culture filtrate in test tubes. Mixture was incubated at 50 °C for 1 h. After incubation 1ml of dinitro-salicylic acid (DNS) reagent was added to stop the reaction. The reactants in test tubes were boiled for 5min in a boiling water bath and transferred to cold water bath. Distilled water (10 ml) was added and absorbance measured at 540 nm in a Genesys-20 Thermo Scientific Spectrophotometer. Enzyme blank, spectro zero and glucose standards were prepared and treated as above with boiled enzyme; citrate buffer and varying concentration of glucose solution replacing the enzyme filtrate respectively. Amount of reducing sugar was read off from a curve obtained by plotting value of absorbance against concentration of glucose. One unit of enzyme activity was defined as the amount of enzyme that released 1 µm of reducing sugar.

b) Exoglucanase activity - Filter paper assay (FPase)

Exoglucanase activity was determined according to the method of Mandels et al. (1976). The reaction mixture comprised of 1.0 ml citrate buffer pH 4.8, containing filter paper strip (1 x 6 cm²) and 1.0 ml of culture filtrate in a test tube. The mixture was incubated at 50 °C for 1 h. Di-nitro-salicylic acid reagent (3 ml) was added to the reactant to stop the reaction. Mixture was boiled for 15 min in rigorously boiling water bath and cooled immediately by transferring to cold water bath. Distilled water (10 ml) was added to each tube and content mixed properly. Absorbance was read at 540 nm and amount of reducing sugar obtained from the standard curve. One unit of enzyme activity was defined as

the amount of enzyme that released 1 µm of reducing sugar.

Optimization of conditions for the activity of crude enzyme filtrates

Temperature

Culture filtrates (0.5 ml) was added to 0.5 ml carboxyl methyl cellulose (2 % w/v) in 0.05 M citrate buffer, pH 4.8 in a test tube. Mixture was incubated in water bath at temperatures ranging between 20 to 80°C for 1 h. Endoglucanase activity was then determined following the DNSA method recommended by International Union of Pure and Applied Chemistry (Ghose, 1987).

pH

Endoglucanase activity was also determined at varying pH values ranging from pH 3.0 to 7.0. The enzymes were assayed in 0.05 M citrate buffer in the pH range of 3.0 to 6.0 and in 0.05 M phosphate buffer in the pH range 6.5 to 7.0 (Macris, 1984). All the tubes were incubated at 50 °C.

Substrate concentration

The effect of substrate concentration on the activity of the cellulases of the fungal isolates was also determined. Different concentrations of carboxyl methyl cellulose ranging between 1 and 12 % in 0.05 M citrate buffer, pH 4.8 was prepared in 100 ml Erlenmeyer flask and inoculated with culture filtrates at 10 % (v/v). This was incubated at 50 °C for 8 h. Samples (1 ml each) were withdrawn hourly and analysed for reducing sugar following DNSA method of Mandels et al. (1976).

Partial purification of enzyme filtrates with ammonium sulphate

Solid (NH₄)₂SO₄ was added to clarified culture fluid to obtain 20 % saturation. After centrifugation (10,000 x g, 4 °C, 10 min) the sediment was discarded, and ammonium sulphate was added to the supernatant to 80 % saturation. The mixture was again centrifuged, and the precipitate was dissolved in 0.05 M phosphate buffer at pH 7.0 (Macris, 1984). Endoglucanase and

exoglucanase activity was determined following the DNSA methods described earlier.

Hydrolysis of waste biomass with the partially purified cellulases of isolated fungi

The method of Mandels et al. (1974) was employed. Citrate buffer (100 ml) containing alkaline treated corn cob, (10 %w/v) in 250 ml Erlenmeyer flask was inoculated with 10 ml of the partially purified enzyme filtrate and incubated at 50 °C for 7 h. Samples (1 ml) was withdrawn aseptically from each flask at 1hr interval and analysed for reducing sugar by the DNSA method to determine the optimum time in hours for cellulase digestion of corn cob. The hydrolysate was then used for fermentation to produce ethanol.

Fermentation of the products of waste biomass digestion to alcohol

Organism

Baker's yeast, *Saccharomyces cerevisiae* was purchased from a local retailer in Ilorin and cultured on yeast extract agar. Dried yeast sample (1 g) was measured into 10 ml sterile distilled water in MacCartney bottle. Bottle was shaken rigorously for even distribution of the cells. An aliquot (0.1 ml) of the cell suspension was then used to inoculate yeast peptone dextrose agar medium. Plates were incubated at 28 ± 2 °C for 48 h. Colonies were purified by sub culturing on YPDA and pure cultures maintained on agar slants at 4 °C. (Zakpaa et al., 2009)

Inoculum preparation:

Yeast peptone dextrose broth (100 ml) in 250 ml Erlenmeyer flask was inoculated with pure colonies of yeast from agar slant with the aid of an inoculating loop. This was incubated at 28 ± 2 °C on a gyratory shaker at 150 rpm for 48 h. Cell population was enumerated with the aid of a haemocytometer (Zakpaa et al., 2009).

Fermentation:

The fermentation broth (100 ml) comprised of (%w/v), peptone, 2; yeast extract, 2 and the product of hydrolysis of corn cob as the fermenting sugar. The broth (80 ml) was filled into a 100 ml sealable bottle, sterilized in an autoclave and inoculated with 10 ml of the inoculums culture of yeast at 3.8×10^6 cells ml⁻¹. The bottles were sealed with the aid of an adhesive tape and incubated at 28 ± 2 °C for a period of 8 to 48 h. Bottles were removed at 8 h interval to determine the amount of ethanol produced and the residual sugar in the medium.

Determination of ethanol concentration by the acidified dichromate/thiosulfate titration method

The fermented broth was assayed for ethanol using the acidified dichromate/thiosulfate titration method www.outreach.canterbury.ac.nz/chemistry/documents/ethanol.pdf with some modifications. Ten millilitre acid dichromate solution (0.01 M in 5.0 M sulphuric acid) was placed in 250 ml Erlenmeyer flask. This was connected to 10 ml of fermented broth in another 250 ml Erlenmeyer flask which was placed in water bath set at 80 °C. The set up was allowed to stand for 3 h during which ethanol produced by fermentation of the broth would have evaporated into the acid dichromate solution. All the flasks were fixed with rubber stopper and sealed with wax to avoid leakages After the incubation period, the setup was dismantled, 100 ml distilled water and 1.0 ml potassium iodide (1.2 M) was added to the dichromate solution. This was titrated with sodium thiosulphate (0.03 M) until the brown colour turned to yellow, at which point 1 % starch solution (1 ml) was added as indicator of iodine, and further titrated until the blue colour fades. Three flasks consisting of 10ml acid dichromate were setup as blanks and titrated first so as to monitor the volume of thiosulphate required. The difference between the volume of thiosulphate used in the titration of the blank and that of

the sample was used in calculating the amount of ethanol produced.

Determination of residual sugar in the fermentation medium

The amount of sugar in the fermentation medium after each period of fermentation was determined following the DNSA method of Miller (1959). Dinitro-salicylic acid (DNSA) reagent (1 ml) was added to an aliquot (1 ml) of the fermentation medium in a test tube and properly mixed. The mixture was boiled for 5 min and cooled under running tap water. Five millilitres of 40 % Rochelle salt solution was added to the mixture and absorbance was read in a Genesys-20 Thermo Scientific Spectrophotometer at 540 nm. Amount of reducing sugar was read off a curve of glucose standard and expressed as mg ml^{-1} .

Statistical analysis

All data were subjected to analysis of variance and the sample means tested for significant differences using the Duncan multiple intervals test. This was carried out using the statistical package SPSS 15.0.

RESULTS AND DISCUSSION

Cellulase activity of fungi

The cellulolytic activities of the fungi were demonstrated on agar plates. Clearance zones measuring 30.0 ± 0.6 mm and 15.2 ± 0.3 mm were obtained after staining the cultures of *Aspergillus niger* and *Penicillium decumbens* on agar medium containing carboxyl methyl cellulose with congo red solution respectively (Plate 1 and 2). Ability to reduce viscosity when grown in medium containing carboxyl methyl cellulose was taken as a measure of cellulase activity of the fungi. This method has been used to measure the initial hydrolysis rate for endoglucanases using soluble cellulose derivatives (Zhang et al., 2006) and is strongly recommended along with determination of reducing sugar (Ghose, 1987). Activity was determined for the two fungi. Highest activity represented by a reduction in viscosity (97.24 %) was produced by *P.*

decumbens while *A. niger* reduced viscosity by 96.75 %. A viscosity reduction of 98 to 99 % within 5 min was reported with cellulase hydrolysis of carboxyl methyl cellulose while acid hydrolysis took 1 h to achieve 93 % reduction in viscosity (Sreenath, 1993).



Plate 1: Cellulase activity of *A. niger* as zone of clearance on potato dextrose agar incorporated with 0.5 % CMC

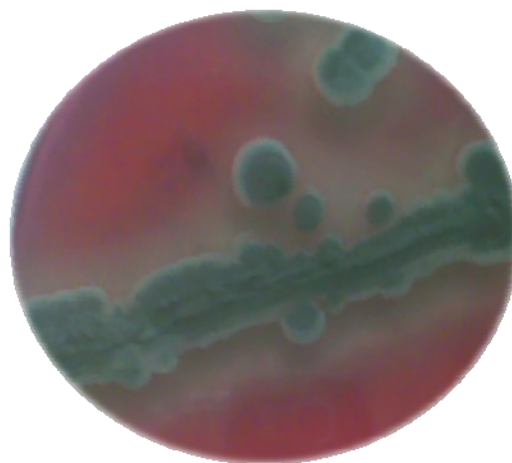


Plate 2: Cellulase activity of *P. decumbens* as zone of clearance on potato dextrose agar incorporated with 0.5 % CMC

Effect of alkali pre-treatment of substrate on cellulase activity

The effect of alkali pre-treatment of corn cob on cellulase production by the fungi was determined (Table 1). Cellulases are considered to be non-constitutive and require cellulose substrates as inducers (Hulme and Stranks, 1970). Alkali treated corn cob induced production of cellulase in

A. niger significantly ($p < 0.05$). The endoglucanase activity produced was higher by over 20 % while over 100 % more exoglucanase activity was obtained when compared to that obtained in the culture filtrate of untreated corn cob. Activity also compared favourably with that obtained with culture filtrates grown in carboxyl methyl cellulose. Cellulase production by *P. decumbens* was also higher significantly in the alkali pre-treated corn cob with 88 % more endoglucanase activity. Treatment also enhanced production of exoglucanase by *P. decumbens* with over 40 % higher activity than that of untreated substrate. Carboxyl methyl cellulose however, significantly induced higher exoglucanase production (94 %) compared to the alkali treated corn cob. Bagasse, corn cob and sawdust were found to induce production of cellulase enzyme in *Aspergillus flavus* after ballmilling and pretreatment with caustic soda (Ojumu et al., 2003).

Optimal conditions for cellulase activity

Cellulases produced by the studied fungi were optimized at 50 °C and a pH between 4.5 and 5.5 (Figures 1 and 2). A diverse temperature and pH optima have been reported for cellulases of the studied fungi. Cellulases of *A. niger* produced optimum activity at temperature of 40 °C and pH range between 3.0 to 9.0 (Coral et al., 2002), while Acharya et al. (2008) obtained best activity at pH 4.0 to 4.5 and temperature of 28 °C. Optimum activity was also reported at pH 3.5 and 40 °C by Omojasola

and Jilani (2008). Cellulase activity of *Penicillium* sp. was optimum at 65 °C and pH 4.5 (Picart et al., 2007). Optimum temperature of 60 °C was also recorded for endoglucanase activity of *P. decumbens* (Liu et al., 2010). This is an indication that optimum condition for the activity of cellulases vary among organisms and within different strains of a particular organism.

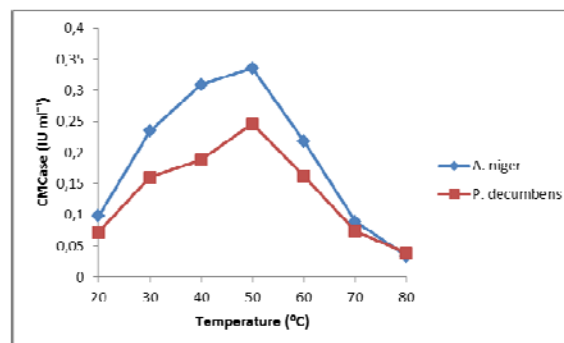


Figure 1: Effect of temperature on endoglucanase activity of the crude enzyme produced by the studied fungi

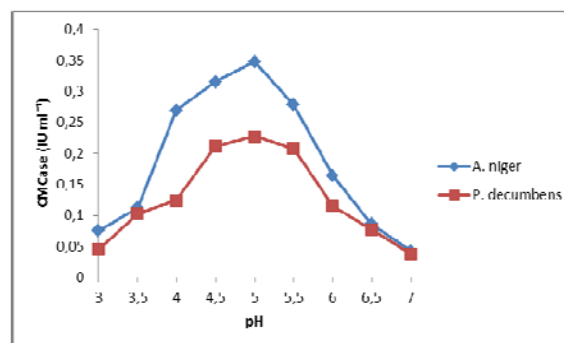


Figure 2: Effect of pH on the endoglucanase activity of the crude enzyme produced by the studied fungi

Table 1: Effect of alkali pre-treatment of corn cob on cellulase production by the studied fungi

Substrate	Fungi	Cellulase activity	
		CMCase (IU ml ⁻¹)	FPase (FPU ml ⁻¹)
ATCC	<i>A. niger</i>	0.1698 ± 0.0047 ^a	0.0461 ± 0.0056 ^a
	<i>P. decumbens</i>	0.1111 ± 0.0048 ^a	0.0216 ± 0.0012 ^d
UTCC	<i>A. niger</i>	0.1401 ± 0.0077 ^d	0.0153 ± 0.0006 ^d
	<i>P. decumbens</i>	0.0592 ± 0.0112 ^d	0.0192 ± 0.0003 ^f
CMC	<i>A. niger</i>	0.1680 ± 0.0056 ^a	0.0382 ± 0.0046 ^e
	<i>P. decumbens</i>	0.0381 ± 0.0010 ^{bc}	0.0296 ± 0.004 ^a

Key – ATCC = Alkali treated corn cob; UTCC = Untreated corn cob; CMC = Carboxyl methyl cellulose; CMCase = Endoglucanase activity; FPase = Exoglucanase activity
Each value represents mean of three independent tests. Means displayed with homogenous superscript within the same column are insignificantly different ($P < 0.05$)

Concentration of substrate was also studied as another important factor that may be optimized for improved enzyme activity. The optimum time for endoglucanase activities of the fungal cellulases was also determined. Cellulases of the two fungi used in this study were optimized at 10 % substrate concentration (Figure 3) and optimum activity was obtained after 4 hours of incubation (Figures 4 and 5). Activity of the cellulases at lower concentration of substrate was however not significantly different ($p < 0.05$). Vyas et al. (2005) also recorded an insignificant difference in endoglucanase activity while ranging substrate concentration from 1 to 5 %. Exoglucanase activity was however significantly higher at high concentration of substrate (Vyas et al., 2005). Endoglucanase and exoglucanase activity of culture filtrates of *A. niger* were optimized at 2 % concentration in most of the substrate tested by Jaafaru and Fagade (2007).

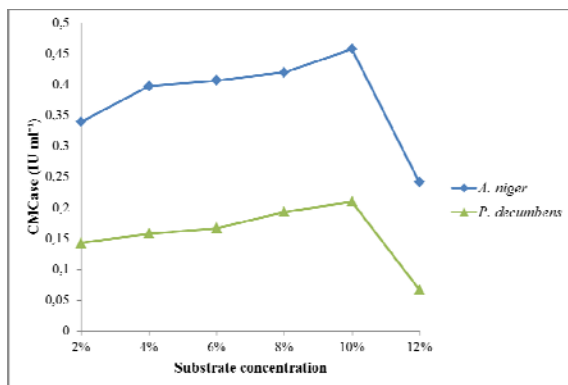


Figure 3: Effect of substrate concentration on the endoglucanase activity of the crude enzyme produced by the studied fungi

Effect of partial purification of fungal cellulases on its activity

The endoglucanase and exoglucanase activities of the partially purified cellulases of the two fungi were determined. Partial purification of culture filtrates by ammonium sulphate solution significantly enhanced

cellulase activity of *A. niger* and *P. decumbens* (Table 2). In a comparative analysis of the crude and partially purified cellulases, a significant increase in activity ($p < 0.05$) was obtained from the partially purified enzyme. Cellulases of *A. niger* produced in medium containing alkali treated corn cob was improved by 62.84 % and 36.45 % while that of *P. decumbens* were enhanced by 136.73 and 36.45 % for the endoglucanase and exoglucanase activities respectively. Chen et al. (2004) obtained a significant improvement in the activity of cellulases precipitated with 40–60 % ammonium sulphate prior to purification.

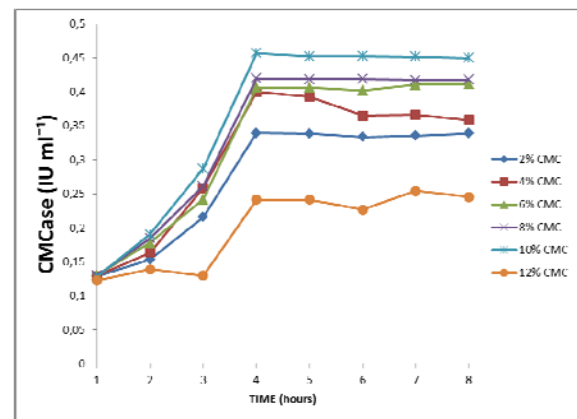


Figure 4: Time course for the endoglucanase activity of *A. niger*

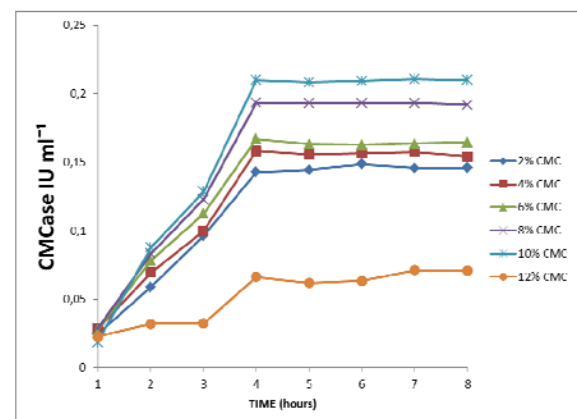


Figure 5: Time course for the endoglucanase activity of *P. decumbens*

Table 2: Cellulase activity of partially purified enzyme filtrates of fungal isolates

Substrate	Fungi	Cellulase activity	
		CMCase (IU ml ⁻¹)	FPase (FPU ml ⁻¹)
ATCC	<i>A. niger</i>	0.2765±0.0360 ^e	0.0629±0.0008 ^a
	<i>P. decumbens</i>	0.2630±0.0401 ^c	0.0179±0.0009
UTCC	<i>A. niger</i>	0.1516±0.0088 ^c	0.0285±0.0008 ^d
	<i>P. decumbens</i>	0.0772±0.0084 ^a	0.0199±0.0006 ^d
CMC	<i>A. niger</i>	0.1742±0.0185 ^{bc}	0.0552±0.0013 ^e
	<i>P. decumbens</i>	0.2204±0.0237 ^b	0.0381±0.0016 ^a

Key – ATCC = Alkali treated corn cob; UTCC = Untreated corn cob; CMC = Carboxyl methyl cellulose; CMCase = Endoglucanase activity; FPase = Exoglucanase activity
Each value represents mean of three independent tests. Means displayed with homogenous super-script within the same column are insignificantly different (P < 0.05)

Sugar yield from hydrolysis of alkali pre-treated corn cob with partially purified fungal cellulases

A yield of 7.63 mg ml⁻¹ of sugar was produced on hydrolysis of corn cob with cellulases of *A. niger* within 48 h compared to 1.15 mg ml⁻¹ obtained with the use of cellulases of *P. decumbens* (Figure 6). A varying yield of sugar from corn cob has been reported. A yield of 90.4 % (Liming and Xuelang, 2004) and 49.99 % glucose (Ouyang et al., 2009) was obtained on hydrolysis of corn cob residue with cellulases of *Trichoderma reesei* Rut C-30. Alkali treated corn cob was reported to produce 5.55 mg ml⁻¹ of reducing sugar on hydrolysis with culture filtrates of *Aspergillus niger* as against 4.25 mg ml⁻¹ from untreated corn cob (Jaafaru and Fagade, 2007).

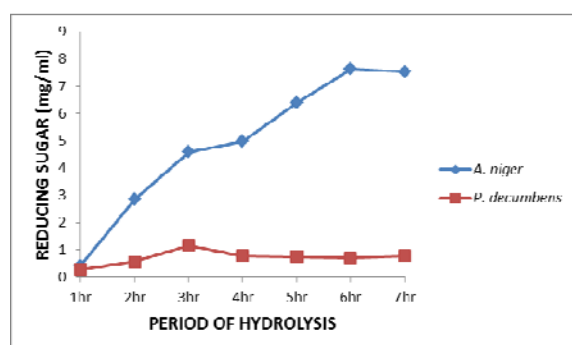


Figure 6: Time course for the hydrolysis of corn cob with partially purified cellulases of produces by fungi

Ethanol yield

Fermentation of the sugar (product of enzymatic hydrolysis of corn cob) by the yeast, *Saccharomyces cerevisiae* yielded ethanol in accordance with the sugar concentration obtained. Corn cob hydrolyzed by cellulases of *A. niger*, yielded 2.67 % ethanol which was 5.93 fold the amount produced by the hydrolysate of corn cob with cellulases of *P. decumbens* (Figures 7 and 8). The fermentation efficiency was about 35 % in the two products. Punnapayak et al. (1999) obtained 0.30 g/g ethanol in a solid state fermentation involving *T. reesei* and *S. cerevisiae*; and 0.11 g/g with *Acrophialophora* sp. combined with *S. cerevisiae*. The conversion rate obtained from fermentation with the yeasts, *Candida wickerhami*, *Pachysolen tannophilus*, *S. cerevisiae*, *Kluyveromyces marxianus* var. *marxianus*, and *K. fragilis* were 20, 27, 30, 40 and 45 % respectively (Umamaheswari et al., 2010). A high ethanol yield (49 %) was achieved in the fermentation of biomass hydrolysate at 60 g/l by the bacterium *Zymomonas mobilis* compared to the yield obtained with *S. cerevisiae* which was 43 % (Lawford and Rousseau, 2003). Theoretically, the maximum conversion efficiency of glucose to ethanol is 51 % on a weight basis (http://www.appropedia.org/Understanding_Ethanol_Fuel_Production_and_Use).

Fermenting yeast, however, consumes sugar for growth and production of other metabolic products. Moreover since

growth commences during the aerobic phase, some amount of sugar gets used up before the anaerobic stage which is characterized by ethanol production (<http://homedistiller.org/ferment.htm>).

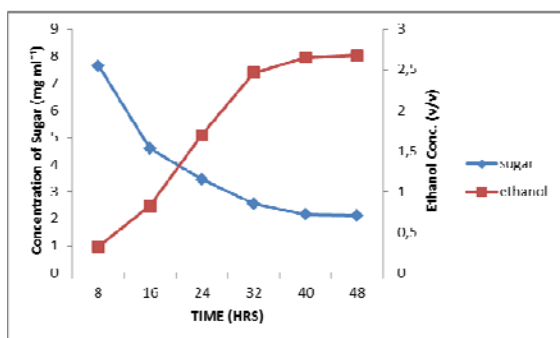


Figure 7: Time course for ethanol production and fermentation of sugar produced by hydrolysis of corn cob with cellulases of *A. niger*

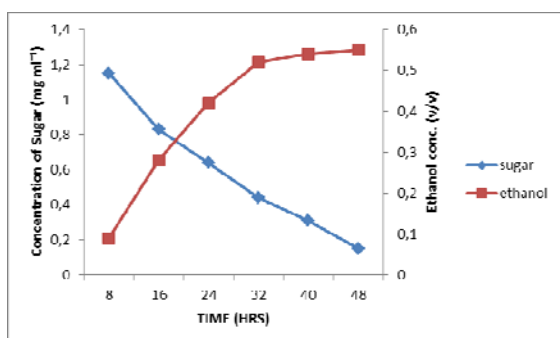


Figure 8: Time course for ethanol production and fermentation of sugar produced by hydrolysis of corn cob with cellulases of *P. decumbens*

CONCLUSION

Corn cob was affirmed as viable feed-stock for energy production particularly in the production of bioethanol. While *P. decumbens* demonstrates a high cellulase activity, the supremacy of the cellulases of *A. niger* in hydrolysing biomass wastes to fermentable sugars was established. Overall, corn cob hydrolyzed by cellulases of *Aspergillus niger* was most productive in terms of ethanol yield and can therefore be harnessed in biofuel production.

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