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Research Article

Enhanced Cellulase Production for Improved Degradation of Maize Cob: A Mixed-Fungal Fermentation

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ABSTRACT

Cellulases are considered the most prominent of enzymes involved in the microbial breakdown of lignocellulosic waste. This study was designed to investigate the effect of various fermentation conditions on cellulase enzyme production in maize cobs from single and mixed fungal cultures as an index of good degradation and digestibility. Maize cobs were prepared and alkaline pretreated. Single and mixed-culture solid state fermentations with four degrading fungal species *Aspergillus niger, Trichoderma reesei, Lachnocladium flavidum* and *Lenzites betulina* were carried out and process parameters of incubation time, moisture content, inoculum concentration, pH, carbon source and nitrogen source were optimized from 10 groups of independent and mixed combinations of the fungi. Results indicated that *A. niger* and a co-culture of *T. reesei/ L. flavidum* were found to be most effective cellulase producers, with optimal conditions of: pH 3 - 7, moisture 70 - 75%, incubation period of 10 days, culture concentration of 5.5 x 103 spores/ ml. 1% glucose and peptone among several carbon and nitrogen supplements respectively supported optimal activities. Cellulose content was reduced by all the fermenting organisms to different degrees, however mixed culture of *T. reesei* and *A. niger* had the most significant reduction in cellulose (73.3 %). Optimal degradation of maize cob using mixed fungal cultures can be exploited for better utilization and improvement of nutritive value.

Keywords: *Cellulase, Cellulose, Fermentation, Fermenting fungi, Maize cob*

INTRODUCTION

Cellulolytic enzymes provide a key opportunity for achieving tremendous benefits of biomass utilization through the bioconversion into the simple digestible monomers (Østby *et al.*, 2020). Recent studies have shown that pretreatment technologies can greatly enhance enzyme yields by several folds (Ravindran *et al.*, 2018). Cellulases' potential in converting lignocellulosic waste material into useful end products has made them the focus of recent research. Patel *et al.* (2009) reviewed them and considered them the most prominent of the enzymes involved in the microbial breakdown of lignocellulosic waste. Chadha *et al.* (2019) looked at their production using recent technologies and from various microbial sources. Tiwari (2017) wrote on using metagenomics to find and explore novel cellulases for further application in biorefineries. Evaluation of lignocellulosic residues has shown that in their natural states, they are a poor-quality feed material due to low digestibility, low protein content, poor palatability and bulkiness (Ogbonna and Popoola, 1997). The nutritive value of agroresidues does not only depend on the availability of nutrient but on such attributes as crystallinity of cellulose which limit digestion. It was observed that digestibility of straw was inversely correlated to the amount of lignocellulose complex in substrates. Pretreatment affects the degree of utilization of cellulosic residues by making them more susceptible to enzymatic attack (Sun and Cheng 2002). The goal of the pretreatment process is to break down the structure and disrupt the crystalline structure of cellulose, so that the acids or enzymes can easily access and

hydrolyze the cellulose. The by-products of cellulose hydrolysis are sugars which act as carbon and energy sources for microorganisms.

Agrowastes which include husk, straw, leaves and cobs are highly underutilized in Africa, particularly Nigeria. A large quantity is left on the farmlands to be decomposed by microorganisms such as bacteria and fungi (Okafor, 1988). In Nigeria, large quantities of maize cobs are produced annually and these by-products are left to rot away or burned like other agro-industrial wastes (Oladeji, 2010). These wastes are promising feedstuffs for the production of energy, food and chemicals (Andriani *et al.*, 2011). A large quantity of maize residue is obtained during processing of maize which could be estimated at 18 - 20 % per ton (Hueze *et al*., 2017). In Nigeria, maize residues and by-product were estimated to be about 4.11 million tonnes in 1989, consisting mainly of straw, husks, skins and trimmings, cobs and bran (Adebowale, 1992). For every kilogram of maize grain, 3 kilograms of fibrous byproducts are produced (Kossila, 1984) indicating the production of about 2.25 million tonnes of fibrous byproducts annually. Maize cob has been reported by Alokan (1988) to constitute about 60 % of the maize "ear". It also has large cellulose and hemicellulose reserves. As a major component of plants, cellulose accounts for almost half of the plant dry weight. Therefore, there is a certain amount of cellulose contained in animal feed which reduces the digestibility of feed for most animals. The decomposition of cellulose can not only lead to the natural cellulose resources being fully used, but also reduce the anti-nutritional effect of crude fiber in feed.

The use of fungi for degradation of lignocellulosic biomass lowers the thermo-mechanical energy requirements and this translates directly into lower temperature requirements, lower chemical consumption thus reduction in severity and capital costs and consequently lower inhibitor levels (Keller *et al*., 2003). Fungal solid-state fermentation is influenced by biological and physio-chemical factors (Rodriguez-Leon *et al*., 2008). Process parameters are relevant to production of enzyme by means of solid-state fermentation and are essential for better manipulation of the bioconversion process. Cellulase-producing microorganisms are widely identified over the years, but only a few have been studied extensively (Bhardwaj *et al.*, 2021). Mixed-culture fermentations offer a number of advantages over conventional single-culture fermentations as growth rate and product yield may be higher, may also permit better utilization of the substrate due to wider range of enzymes, and offer more protection against contamination (Hesseltine, 1983). The current study investigated the effect of various fermentation conditions on cellulase enzyme production as an index of good digestibility.

MATERIALS AND METHODS

Collection and preparation of sample

Air-dried sample of maize cob residues was collected in clean bags from markets around Zaria. Maize cob was dried to constant weight at 50 ^{0}C, milled using domestic blender 9 model (MX-391N Matsuhita Electric) to a particle size of 1 - 2 mm and stored in clean polythene bag for use. About 1 kg of maize cob was mixed in 4.5 L 5 % sodium hydroxide solution, at room temperature for an hour, neutralized with HCl, washed with distilled water and the residues dried to constant weight at 50 ^{0}C.

Test organisms and growth of inoculums

The test organisms, *Aspergillus niger*, *Lachnocladium flavidum*, *Trichoderma reesei* and *Lenzites betulina* were isolated, grown and maintained in potato dextrose agar (PDA) slants under refrigerated condition. The media for growth of culture contained: 0.3 % glucose, distilled water, 0.1 % potassium dehydrogenate sulphate, 0.1 % corn steep liquor and finally, 0.2 % sodium nitrate. The media was sealed tightly with aluminum foil and maintained at ambient temperature for 5 days. After substantial growth, the culture was washed with sterile distilled water and this served as the inoculums.

Solid-state fermentation

Biological treatment of maize cob followed solid-state fermentation and the fermented composition was as described by Ali *et al*. (1991). Organisms were cultivated in mineral salt- agrowastes media containing (g/L): 10.0 g/L of KH_2P0_4, 10.5 g/L of $(NH_4)_2SO_4$, 0.33 g/L of $MgS0_4.7H_20$, 0.5 g/L of $CaCJ_2$, 0.013 g/L of $FeS0_47H_2O$, 0.004 g/L of $MnSG_4$. H_2O, 0.004g/L $ZnS0_4.7H_2O$, 0.004 g/L $CoC1_2.6H_2O$, 0.5 g/L yeast- extract, and 40 - l00 g of the untreated and treated maize cobs. The initial pH was adjusted to 5.0 after autoclaving for 15 mins at 121 °C.

After sterilizing the medium, it was inoculated with 5 ml of spore suspension, and incubated at ambient temperature. Another 50 g was weighed and subjected to same conditions without addition of inoculums. This served as the control. Fermented cobs were harvested every two days (2) for a period of twenty-five (25) days.

Optimizing fermentation conditions

Effect of inoculum concentration was obtained by inoculating the fermentation media with spore suspension at various concentrations for each of the single and co-culture ranging between 2 - 8 x 10^3 spores/ml. Effect of incubation time was obtained by assaying enzyme activity at interval of fermentation between 3 to 15 days. Effect of carbon source was obtained by supplementing fermentation media with 1

% each of glucose, starch, maltose, fructose, carboxymethyl cellulose and sucrose. Effect of nitrogen supplementation was obtained by adding 1 % sodium nitrate, peptone, ammonium sulphate, yeast extract and urea to the fermentation media. Effect of moisture was determined by adjusting the moisture between 60 - 80 % using citrate phosphate buffer. Effect of pH on fermentation medium was obtained by adjusting the pH between 4.0, 5.0, 6.0, 7.0, and 8.5 using 1 M NaOH/1 M HCl.

Preparation of crude extract for enzyme analysis

All fermented cobs including their controls were analyzed for the production of lignocellulolytic enzymes to determine the best period for enzyme production and microbial enrichment.

Exactly 5 g of fermented samples was suspended in 10 ml of sterile distilled water. The mixture was homogenized thoroughly and centrifuged at 4000 rpm for 10 minutes. The supernatant obtained was used for chemical and enzymatic analysis and this was stored at 4 ^{0}C for several days without loss of enzyme activity.

Biomass determination

Fungal biomass population was determined by estimating the amount of protein in fermented samples, using the Lowry method as modified by Alexandra and Griffith (1993). Five milliliters of a mixture prepared from 4.9ml of 2% Na_2CO_3 in 0.1M NaOH, 0.05ml of 2.7% Na-K tartarate and 0.05ml 1% C_2SO4 solution were added to l ml of fungal extract. The mixture was incubated at room temperature for 10 minutes and 0.5 ml of 1:2 phenol reagent added. The absorbance was read at 700 nm after 30 minutes of addition of phenol. Amount of protein were extrapolated from Bovine serum albumin standard curve.

Determination of cellulase activity

Cellulase activity was determined colorimetrically by measuring changes in reducing sugar by the hydrolysis of carboxymethylcellulose (high viscosity, Sigma), substrate (Ali *et al.*, 1991). The reaction mixture (3 ml) which contained 1 ml each of 2 % (w/v) Carboxymethylcellulose (CMC) solution, 0.1 M acetate buffer (pH 5.0) and culture supernatant; was incubated at 30 ^{0}C for 10 minutes. The liberated glucose in the reaction mixture was estimated by the dinitrosalicylate method. One unit of cellulase activity was defined as the amount of enzyme which released 1 µmole glucose min^{-1} mg^{-1} protein at specified assay conditions.

RESULTS AND DISCUSSION

Results

Effect of fermentation conditions on cellulase enzyme production

Effect of incubation period on cellulase production using mono and co-cultures of different fungal strains for SSF (Solid State Fermentation) of corn cobs is shown in Table 1 Fermentation over the course of 15 days showed higher cellulase activities after nine (9) days of incubation, with a co-culture of *A. niger* and *L. flavidum* showing highest activity (730.00 ± 5.40 U/L) at 10 days of incubation. Single culture of *Lachnocladium flavidum* also showed similar activity.

The effect of inoculum size on cellulase production using mono and co-cultures of different fungal strains for SSF of corn cobs is shown in Table 2. Higher cellulase activities (811.00 ± 5.00 U/L) were experienced with mixed cultures at higher inoculum concentrations (8 × 10^3 spores /ml), while the mono cultures of *A. niger, and L. flavidum* with higher cellulase activities thrived better at lower inoculum concentrations. (4 × 10^3 spores /ml).

Effect of pH on cellulase production using mono and co-cultures of different fungal strains for SSF of corn cobs is shown in Table 3. Cellulase activities were significantly higher at lower pH (4 - 6) among the co- culture fermentations. At pH of 4, the co- culture of *A. niger* and *L. flavidum* had highest cellulase activity of 711.00 ± 4.20 U/L. The mono cultures had higher activities within a higher pH range (6-8).

Table 4 shows the effect of moisture content on cellulase production using mono and co-cultures of different fungal strains for SSF of corn cobs. The mono cultures exhibited higher activities at lower moisture contents; *A. niger, L. flavidum, T. reesei and L. betulina* (60 % - 70 % moisture), while the mixed cultures exhibited better activities at higher moisture contents (75 % - 80 %). The co-culture of *A. niger* and *L. flavidum*. had the highest Cellulase activity of 790.00 ± 5.0 U/L.

The effect of 1 % carbon sources (sucrose, glucose, maltose, fructose, carboxymethyl cellulose and starch) on cellulase production using mono and co-cultures of different fungal strains for solid state fermentation of corn cobs is shown in Table 5. Glucose, starch, carboxymethyl cellulose and maltose were seen to have the most significant effect on cellulase activities. Fermentation with co-culture of *T. reesei/L. flavidum* had the highest activity (784.00 ± 6.20 U/L). The co-cultures *A. niger/L. flavidum, T. reesei/ A. niger* also showed significant difference ($P < 0.05$) in

activities compared to other single and co-culture fermenting setups.

The effect of 1 % nitrogen source (yeast, peptone, urea, sodium nitrate, ammonium nitrate and potassium nitrate) on cellulase enzyme production from mono and co-culture solid state fermentation of corn cobs is shown in Table 6. Potassium nitrate, peptone, and urea were seen to have induce the most significant effect on cellulase activities and production. Fermentation with co-culture of *A. niger/Lach. sp*. had the highest cellulase activity (824 ± 5.9 U/L). The co-culture of *T. reesei /Lach. flavidum, T. reesei/ A. niger* also showed significantly different ($P < 0.05$) activities as compared to other single and co-culture fermenting setups.

Table 1. Effect of Incubation Period on Cellulase Enzyme Production from Solid State Fermentation of Corn Cobs

Fermenting organism	3 days	5 days	7 days	9 days	11 days	15 days
T. reesei	179.00±1.20^{a}	242.00±1.60^{a}	299.00±2.10^{a}	301.00±2.60^{a}	321.00±2.70^{a}	300.00±3.00^{c}
Lenzites betulina	214.00±1.02^{b}	243.00±1.30^{a}	276.00±2.30^{a}	321.00±3.10^{b}	399.00±3.00^{b}	201.00±4.00^{a}
A. niger	243.00±1.50^{c}	444.00±2.80^{d}	467.00±2.65^{e}	498.00±3.40^{d}	502.00±4.00^{d}	499.00±3.00^{e}
L. flavidum	321.00±2.90^{d}	673.00±4.80^{g}	699.00±4.50^{f}	788.00±3.60^{h}	810.00±5.20^{h}	819.00±5.00^{i}
T. reesei & L. betulina	261.00±2.00^{c}	284.00±2.80^{b}	311.00±2.30^{ab}	346.00±3.00^{b}	398.00±2.00^{b}	201.00±3.00^{a}
T. reesei & A. niger	449.00±3.40^{f}	654.00±4.20^{f}	699.00±3.60$_{f}$	695.00±4.10^{f}	701.00±5.60$_{f}$	655.00±5.50^{g}
T. reesei & L. flavidum	333.00±1.30^{d}	399.00±2.10^{c}	426.00±2.60^{d}	551.00±3.00^{e}	607.00±4.00^{e}	610.00±3.90^{f}
L. betulina & A. niger	259.00±2.40^{c}	267.00±2.00^{a}	289.00±2.60^{a}	299.00±3.00^{a}	301.00±2.00^{a}	250.00±3.00^{b}
L. betulina & L. flavidum	295.00±3.10^{e}	301.00±2.90^{b}	395.00±4.00^{c}	428.00±2.40^{c}	487.00±4.00^{c}	406.00±3.50^{d}
A. niger & L. flavidum	565.00±5.00^{g}	592.00±4.30^{e}	698.00±6.20^{f}	701.00±4.70^{g}	729.00±6.50^{g}	700.00±5.40^{h}

Values are Mean ± SD, Values with different superscript letter down the column are significantly different at $p < 0.05$ using Duncan Multiple range test (DMRT)

Table 2. Effect of Inoculum Concentration on Cellulase Enzyme Production from Solid State Fermentation of Corn Cobs

Inoculum size	2×10^3	4×10^3	5.5×10^3	6.5×10^3	8×10^3
T. reesei	299.00±2.15^{ab}	331.00±4.02^{b}	339.00±3.00^{c}	281.00±1.98^{b}	211.00±2.18^{a}
Lenzites betulina	361.00±2.50^{c}	378.00±2.00^{c}	311.00±3.00^{b}	301.00±2.00^{c}	245.00±3.00^{c}
A. niger	657.00±4.90^{h}	725.00±2.60^{h}	810.00±3.40^{j}	649.00±3.70^{g}	555.00±4.50^{f}
L. flavidum	401.00±4.00^{d}	491.00±5.00^{d}	436.00±4.00^{e}	404.00±4.00^{e}	391.00±4.00^{e}
T. reesei & L. betulina	301.00±2.50^{b}	333.00±1.68^{b}	394.00±2.95^{d}	401.00±2.65^{e}	356.00±2.12^{d}
T. reesei & A. niger	6.00±4.00^{e}	595.00±5.00^{f}	566.00±4.00^{f}	345.00±3.00^{d}	355.00±2.00^{d}
T. reesei & L. flavidum	601.00±2.99^{g}	643.00±3.50^{g}	698.00±2.40^{h}	701.00±6.00^{h}	711.00±5.00^{h}
L. betulina & A. niger	701.00±7.50^{i}	731.00±5.34^{h}	798.00±6.00^{i}	801.00±4.00^{i}	811.00±5.00^{i}
L. betulina & L. flavidum	288.00±7.50^{a}	308.00±4.00^{a}	299.00±1.00^{a}	257.00±3.00^{a}	233.00±2.00^{b}
A. niger & L. flavidum	487.00 ±4.00^{f}	578.00±4.60^{e}	610.00±5.80^{g}	634.00±6.70^{f}	635.00±5.00^{g}

Values are Mean ± SD, Values with different superscript letter down the column are significantly different at $p < 0.05$ using Duncan Multiple range test (DMRT)

Table 3. Effect of pH on Cellulase Enzyme Production from Solid State Fermentation of Corn Cobs

Fermenting organism	pH 4	pH 5	pH 6	pH 7	pH 8
T. reesei	257.00±3.00^{b}	297.00±3.00^{b}	296.00±2.00^{b}	301.00±2.50^{b}	289.00±3.00^{b}
Lenzites betulina	221.00±1.50^{a}	230.00±2.00^{a}	225.00±1.50^{a}	284.00±2.50^{a}	279.00±2.56^{a}
A. niger	459.00±3.20^{e}	468.00±2.90^{f}	491.00±4.00^{f}	486.00±3.00^{g}	477.00±2.50^{h}
Lach. flavidum	461.00±1.98^{e}	423.00±2.40^{d}	467.00±3.00^{e}	498.00±3.50^{h}	441.00±2.60^{f}
T. reesei & L. betulina	311.00±3.20^{c}	341.00±3.00^{c}	301.00±2.80^{b}	324.00±3.70^{c}	296.00±2.70^{c}
T. reesei & A. niger	444.00±3.50^{d}	465.00±2.90^{ef}	431.00±3.50^{d}	399.00±2.85^{d}	378.00±2.00^{d}
T. reesei & L. flavidum	486.00±2.90^{f}	694.00±4.30^{h}	675.00±4.15^{h}	664.00±4.35^{j}	669.00±4.80^{j}
L. betulina & A. niger	443.00±3.16^{d}	461.00±2.90^{e}	423.00±2.50^{c}	411.00±2.00^{e}	409.00±1.50^{e}
L. betulina & L. flavidum	569.00±3.50^{g}	548.00±3.45^{g}	555.00±3.00^{g}	506.00±2.00^{i}	499.00±3.00^{i}
A. niger & L. flavidum	711.00±4.20^{h}	708.00±3.70^{i}	687.00±3.50^{i}	459.00±3.00^{f}	455.00±2.50^{g}

Values are Mean ± SD, Values with different superscript letter down the column are significantly different at $p < 0.05$ using Duncan Multiple range test (DMRT)

Table 4. Effect of Moisture Content on Cellulase Enzyme Production from Solid State Fermentation of Corn Cobs

Moisture Content	60%	65%	70%	75%	80%
T. reesei	271.00±2.30^{b}	342.00±3.00^{a}	491.00±3.50^{d}	301.00±2.90^{a}	399.00±2.75^{a}
Lenzites betulina	211.00±1.85^{a}	432.00±2.65^{e}	550.00±4.00^{f}	401.00±3.45^{b}	422.00±2.60^{b}
A. niger	543.00±4.50^{g}	499.00±3.70^{g}	520.00±4.10^{e}	498.00±3.20^{f}	502.00±4.80^{e}
Lach. flavidum	443.00±3.30^{f}	467.00±4.10^{f}	689.00±5.20^{h}	721.00±5.60^{h}	734.00±5.80^{g}
T. reesei & L. betulina	321.00±2.90^{c}	361.00±2.40^{b}	382.00±3.20^{a}	421.00±3.10^{c}	418.00±3.40^{b}
T. reesei & A. niger	641.00±3.90^{h}	598.00±4.00^{h}	655.00±5.10^{g}	692.00±4.50^{g}	643.00±5.20^{f}
T. reesei & L. flavidum	642.00±4.00^{h}	731.00±6.00^{j}	765.00±5.00^{j}	799.00±6.50^{j}	801.00±6.50^{i}
L. betulina & A. niger	392.00±2.00^{e}	421.00±3.00^{d}	455.00±3.50^{b}	461.00±3.30^{d}	454.00±4.00^{c}
L. betulina & L. flavidum	349.00±2.10^{d}	401.00±3.00^{c}	465.00±3.40^{c}	472.00±3.00^{e}	479.00±3.50^{d}
A. niger & L. flavidum	665.00±5.10^{i}	692.00±4.50^{i}	753.00±6.70^{i}	787.00±6.00^{i}	790.00±5.00^{h}

Values are Mean ± SD, Values with different superscript letter down the column are significantly different at $p < 0.05$ using Duncan Multiple range test (DMRT)

Table 5. Effect of 1 % Carbon Source on Cellulase Enzyme Production from Solid State Fermentation of Corn Cobs

Fermenting organism	Sucrose	Glucose	Maltose	Fructose	CMC	Starch
T. reesei	175.00±1.50^{a}	129.00±1.25^{a}	199.00±1.80^{b}	347.00±3.30^{c}	157.00±2.00^{b}	412.00±3.00^{c}
Lenzites. Betulina	121.00±2.00^{b}	140.00±1.80^{b}	100.00±1.00^{a}	310.00±3.20^{a}	145.00±1.40^{a}	201.00±2.50^{a}
A. Niger	542.00±4.00^{j}	443.00±3.10^{f}	354.00±2.90^{e}	501.00±4.20^{g}	451.00±3.90^{g}	336.00±2.70^{b}
L. flavidum	442.00±3.20^{f}	273.00±2.00^{c}	469.00±4.10^{g}	399.00±3.40^{e}	298.00±2.00^{d}	423.00±2.00^{d}
T. reesei & L. betulina	223.00±2.30^{c}	343.00±3.20^{e}	269.00±2.90^{c}	333.00±2.70^{b}	224.00±1.80^{c}	339.00±2.60^{b}
T. reesei & A. niger	456.00±3.50^{g}	601.00±5.50^{h}	566.00±5.00^{i}	345.00±4.00^{c}	295.00±2.50^{d}	491.00±4.00^{e}
T. reesei & L. flavidum	498.00±4.00^{h}	784.00±6.20^{j}	445.00±3.00^{f}	391.00±2.70^{d}	555.00±3.90^{h}	701.00±5.00^{g}
L. betulina & A. niger	251.00±2.00^{d}	311.00±2.50^{d}	298.00±2.30^{d}	399.00±3.00^{e}	421.00±3.30^{f}	549.00±4.40^{f}
L. betulina & L. flavidum	389.00±2.70^{e}	461.00±3.40^{g}	511.00±4.90^{h}	492.00±3.60^{f}	394.00±3.50^{e}	550.00±4.20^{f}
A. niger & L. flavidum	523.00±2.00^{i}	701.00±5.00^{i}	610.00±4.00^{j}	548.00±3.00^{h}	699.00±4.00^{i}	750.00±4.50^{h}

Values are Mean ± SD, Values with different superscript letter down the column are significantly different at p <0.05 using Duncan Multiple range test (DMRT)

Table 6. Effect of Nitrogen Sources (1%) on Cellulase Production Using Mono and Co-cultures of Different Fungal Strains for SSF of Corn Cobs

Fermenting organism	Yeast	Peptone	Urea	Ammonium nitrate	Sodium nitrate	Potassium Nitrate
T. reesei	150.00±1.20^{a}	134.00±1.40^{a}	85.00±0.50^{a}	251.00±2.10^{a}	101.00±1.10^{b}	310.00±2.70^{c}
L. betulina	191.00±1.70^{b}	210.00±1.90^{b}	200.00±1.50^{b}	333.00±2.20^{d}	81.00±0.90^{a}	303.00±2.00^{b}
A. niger	641.00±4.00^{h}	543.00±3.60^{g}	454.00±3.20^{e}	301.00±2.00^{b}	261.00±2.30^{c}	559.00±4.80^{g}
L. flavidum	542.00±4.00^{f}	642.00±5.00^{h}	701.00±4.90^{g}	330.00±3.10^{d}	598.00±4.60^{h}	243.00±2.50^{a}
T. reesei & L. betulina	569.00±3.80^{g}	225.00±2.00^{c}	439.00±3.00^{d}	555.00±4.50^{f}	391.00±3.90^{d}	539.00±4.50^{f}
T. reesei & A. niger	566.00±4.80^{g}	499.00±2.10^{e}	765.00±5.90^{i}	701.00±5.00^{g}	658.00±4.00^{i}	591.00±3.00^{h}
T. reesei & L. flavidum	699.00±5.00^{i}	824.00±5.90^{j}	591.00±4.70^{f}	487.00±3.40^{e}	655.00±5.20^{i}	691.00±4.50^{i}
L. betulina & A. niger	465.00±2.70^{c}	511.00±4.30^{f}	421.00±2.90^{c}	319.00±2.30^{c}	444.00±3.80^{f}	490.00±4.00^{e}
L. betulina & L. flavidum	481.00±3.40^{d}	349.00±2.60^{d}	711.00±5.40^{h}	321.00±2.50^{c}	401.00±3.00^{e}	392.00±2.50^{d}
A. niger & L. flavidum	533.00±4.10^{e}	691.00±5.20^{i}	710.00±6.10^{h}	558.00±3.50^{f}	495.00±4.30^{g}	810.00±5.50^{j}

Values are Mean ± SD, Values with different superscript letter down the column are significantly different at p <0.05 using Duncan Multiple range test (DMRT)

Table 7. Fiber Components from Fungal Mono and Co-culture Solid State Fermentation of Corn Cobs (%)

Fungi	Cellulose
Control	39.40 ± 4.30^{g}
Unfermented	35.30 ± 2.90^{f}
Trichoderma reesei	12.60 ± 0.84^{ab}
Lenzites betulina	20.40 ± 2.10^{de}
Aspergillus niger	15.70 ± 1.60^{bc}
Lachnocladium flavidum	18.20 ± 1.80^{cd}
T. reesei & L. betulina	22.50 ± 1.72^{e}
T. reesei & A. niger	10.50 ± 1.20^{a}
T. reesei & L. flavidum	11.90 ± 1.50^{ab}
L. betulina & A. niger	18.70 ± 1.40^{cd}
L. betulina & L. flavidum	20.90 ± 2.10^{de}
A. niger & L. flavidum	15.20 ± 1.50^{bc}

Discussion

The effect of incubation time on cellulase production by using mono and co-cultures of different fungal strains for SSF of corn cobs showed that with fermentation over the course of 15 days, highest cellulase activities were recorded after eight (8) to nine (9) days of incubation. Co-culture of *A. niger* and *Lach. flavidum* and single culture of *Lach. flavidum* showed highest activity**.** Other study using rice straw also showed that incubation period longer than 7 days i.e., 11 to 12 days, resulted in lower final cellulase activity (Wonoputri, 2018)**.** The duration of fungal fermentation in enzyme production is closely related to other parameters, such as inoculum preparation, type and nature of substrate, and conditions that favor the growth and enzyme production of the fungus. (Arnau, 2020) It might seem that longer incubation duration is favorable for solid-state fermentation as there is sufficient time for the fungus to colonize and produce desired enzyme products. However, prolong fermentation duration is not beneficial for enzyme production which might be owing to exhausted substrate nutrient and limited space for growth (Ramachandran *et al*., 2004). Cellulase production was also generally characterized by an initial logarithmic phase, associated with rapid increase in enzyme activity, followed by a declining activity with the period of incubation. The subsequent decline in activity may have been due to enzyme repression as a result of high level of reducing sugar encountered in the medium or increase in medium acidity which might be inhibitory to the fermenting media.

The effect of inoculum size on cellulase production using mono and co-cultures of different fungal strains showed higher cellulase activities with mixed cultures at higher inoculum concentrations, while most of the mono cultures thrived better at lower inoculum concentrations. The determination of an optimum inoculum loading is one of the crucial steps in solid state fermentation. A low inoculum loading may be insufficient to initiate the growth of microbe and longer time is required for the microbe to multiply to adequate amount for substrate utilization and production of desired product. On the other hand, a high inoculum loading may result in competitive inhibition on microbial growth (Ellaiah *et al*., 2002), and decrease microbial metabolic activity due to fast depletion of nutrients (Patel *et al*., 2009). Thus, a suitable loading of inoculum was needed to ensure a rapid proliferation and synthesis of microbial biomass by striking a balance between growth and nutrient availability (Ramachandran *et al*., 2004). Mixed cultures of *Lach. flavidum* and *A. niger* showed better compatibility and thrived together, producing higher cellulase activities. There was also a general increase in cellulase activities with increase in inoculum size, probably due to initial high availability of microorganism.

Optimal pH is very important for the growth of microorganism and its metabolic activities. (Dave *et al.*, 2021). Effect of pH on cellulase production using mono and co-cultures of different fungal strains for SSF of corn cobs showed that cellulase activities were significantly higher at lower pH (4 - 6) among the co- culture fermentations. At pH of 4, the co- culture of *A. niger* and *Lach. flavidum* had highest cellulase activity. The pH of substrate may change during solid-state fermentation due to fungal metabolic activities. During the process, fungus secretes organic acids such as citric, acetic or lactic acid, which causes a decrease in pH, and thus, the kinetics of pH variation is depended on the fungal strain (Rodriguez-Leon *et al.,* 2008). It was observed that an initial pH of 4 – 6 was ideal for cellulase production (Latifian *et al*., 2007; Wen *et al*., 2005) as there may be rapid denaturation at lower or higher values.

The effect of moisture content on cellulase production using mono and co-cultures of different fungal strains for SSF of corn cobs showed that mono cultures exhibited higher activities at lower moisture contents; *A. niger, Lach. flavidum, T. reesei and L. betulina* (60 – 70 % moisture), while the mixed cultures exhibited better activities at a slightly higher moisture content, with the co-culture of *A. niger* and *Lach. flavidum* having the highest cellulase activity. Hence, moisture content of solid substrate in solid-state fermentation is a critical parameter for growth, biosynthesis and secretion of metabolites like enzymes (Ellaiah *et al*., 2002; Ramachandran *et al*., 2004). Moisture level is governed by the nature of substrate, type of end-product and the requirement of the microbe (Lonsane *et al.*,

1985). It was observed that further increase in moisture in the current research affects enzyme production negatively. Ahmed (2008) and Sodhi *et al.* (2005) reported that moisture content of the substrate is one of the critical factors influencing the outcome of SSF, lower moisture content causes a reduction in solubility of nutrients provided to organism by SSF, a lower degree of swelling and higher water tension. On the other side, reduction in enzyme production at high moisture may be due to the reduction in substrate porosity, changes in the structure of substrate particles reduction of gas volume and decreasing microbial growth (Baysal *et al*., 2003). Water causes the swelling of the substrate and facilitates good utilization of substrates by the microorganisms. Low moisture may also have reduced the solubility and swelling capacity of the corn cobs substrate causing high water stress, and consequently decrease in growth and enzyme production (Raimbault and Alazard, 1980). In fungal solid-state fermentation, the moisture content of solid matrix oscillates between 20 to 70 % (Pandey, 2003). The wide range of applicable moisture content observed in this work is advantageous for the development of a specific solid-state fermentation process and enzyme production.

The effect of carbon sources on cellulase production using mono and co-cultures of different fungal strains for SSF corn cobs shows glucose, starch, carboxymethyl cellulose and maltose having the most significant effect on cellulase activities. Fermentation with co-culture of *T. reesei/ Lach. flavidum* had the highest activity with the various supplementations. The production of enzymes by microbes in fermentations can be stimulated by the addition of a variety of inducing substances depending on the targeted enzymes (Osman, 2011). Supplementations of carbon and nitrogen encourage the adaptation of fungus in fermentation and they are useful for the production of ligninolytic enzymes (Patel *et al*., 2009). The addition of carbon source such as simple sugars promotes fungal growth and allow rapid establishment of the fungus on the solid substrate. Investigations on filamentous fungi showed that the carbon catabolic repression by easily metabolized carbon sources such as glucose and sucrose, repress genes coding cellulases and hemicellulases even in the presence of the lignocellulosic substrate (Znameroski *et al.*, 2012). Production of extracellular cellulase has been shown to be sensitive to repression by different carbohydrate and nitrogen sources. However, the rate of the cellulases synthesis is generally slow when insoluble sugars in the lignocellulosic biomass are used as substrates (Wayman and Chen, 1992). Cellulases are inducible enzymes, and their production can be enhanced in the presence of other carbon sources (Jun *et al*., 2011). Cellobiose induces cellulolytic enzymes in *Neurospora crassa*, *T. reesei*, and *Aspergillus* species but not in *P. chrysosporium* (Znameroski *et al*., 2012), in which, instead, cellotriose and maltose are excellent stimulants (Suzuki *et al*., 2010). In *Polyporus arcularius*, glucose, cellobiose, cellotriose, and starch repress enzyme production, while maltose has the opposite effect (Ohnishi *et al*., 2007). This variability shows the absence of a general rule and each specie relies on specific inducers.

Choice of an appropriate nitrogen source for the cultivation of cellulolytic microbes is an important factor determining the yield of the process. Like carbon sources, the nitrogen sources have an inducible effect on the cellulases. Many previous studies have proven that both the nature and concentration of nitrogen sources are powerful nutritional factors regulating lignocellulolytic enzyme production by fungi (Zakariashvili and Elisashvili, 1993; Sun *et al*., 2004). It is worth noting that the effect of these compounds depends not only on the fungi physiology but also on the cultivation medium. In this study, the effect of 1 % nitrogen source showed that potassium nitrate, peptone, and urea have the most significant induction effects on cellulase activities and production. Fermentation with co-culture of *A. niger*/ *Lach. flavidum* had the highest cellulase activity (824 ± 5.9 U/L). The co-culture of *T. reesei / Lach. flavidum*, *T. reesei/ A. niger* also showed significantly different (<0.05) activities as compared to other single and co-culture fermenting setups. Different studies have indicated the enhancing effect of different ammonium potassium salts. Shankar and Isaiarasu (2011) have found ammonium molybdate as the best nitrogen source for cellulase production by *Bacillus pumilus*. Nitrogen is one of the major cell proteins and stimulation of cellulase activity by ammonium and potassium salts might be due to their direct entry in protein synthesis. Microorganisms are able to use both inorganic and organic nitrogen sources, although one or the other is sometimes preferred by individual microorganisms. A preference for an organic source may simply indicate a requirement for one or other amino acid or vitamin contained in the organic source.

One of the major aims of treating lignocellulosics (corn cobs) is to reduce the cellulolytic, hemicellulolytic and lignolytic components for better utilization. Results of fiber component analysis from fungal mono and co-culture solid state fermentation of corn cobs showed that, cellulose have been degraded by all the fermenting organisms to different degrees. Mixed culture of *T. reesei and A. niger* had the most significant reduction in cellulose (10.50 ± 1.20 %). Species/strain genetical basis as well as cultivation conditions have been seen to affect the cellulolytic enzyme production and consequent degradation of the cellulose as a fiber component. Numerous reports also showed

considerable diversity among species and strains in cellulose degradation capacity and polymer degradation selectivity (Stajic *et al.*, 2009, Dong *et al.*, 2013, Knežević *et al.*, 2013). Significant decreases were seen in the cellulose content in the fungal treated corn cobs, suggesting utilization of these components by the fermenting organisms. A decrease in cellulose content (37.71 %) similar to the one used in the present study was evidenced with the fermentation of corn cob by *Bacillus stearothermophilus* (Ugwuanyi *et al*., 2008). Effect of fermentation in reducing cellulose (51 %), hemicellulose (51%) was obtained from *Saccharomyces cerevisiae* and the fermentation of corn stalk with *Pleurotus ostreatus* (Darwish *et al*., 2012**).** The enzymatic hydrolysis of lignocellulose has been reported to be affected by many factors including porosity (accessible surface area) of materials, cellulose fiber crystallinity, proportion of hemicelluloses and lignin content. (Nguyen, 1993; Grethlein and Converse, 1991).

CONCLUSION

The present study demonstrated the effect of solid state fermentation of single and mixed cultures of four fungal species (*Aspergillus niger*, *Trichoderma reesei*, *Lachnocladium flavidum* and *Lenzites betulina*) and the optimization of their fermentation conditions for improved degradation maize cobs. All fungi showed ability to produce cellulase and degrade cellulose. Mixed fermentation of *T. reesei/L. flavidum* was found to be most effective in cellulase production under determined optimal conditions. The mixed culture of *T. reesei* and *A. niger* had the most significant reduction in cellulose. The increased cellulase activity and cellulose degradation can greatly enhance the nutritive value and subsequent utilization of maize cob.

AUTHORS' CONTRIBUTIONS

All authors contributed to the study conception and design. The material preparation, data collection and analysis were performed by author AO. The first draft of the manuscript was written by author AO and the design was done by author EO and DAA. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST

The authors declared no conflict of interest

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