# Fungal Laccase Production; a Tool for Biodegradation of Maize Cobs

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**Abstract:** Laccases are increasingly being used in the biodegradation and utilization of biomass. This trend has led to increased need for development of efficient production systems. This research was carried out with the aim of optimizing the fermentation conditions of selected and consortia of four fungal species (*Lachnocladium flavidum, Aspergillus niger, Trichoderma reesei,* and *Lenzites betulina)* for production of laccase from biodegradation of corn cobs. Maize cobs were prepared, alkaline pretreated and fermented with various lignocellulolytic fungi. Single and mixed-culture solid state fermentation with various fungal species was carried out. Process parameters of pH, incubation time, moisture content and inoculum concentration were optimized and the effects of various carbon and nitrogen supplementation were determined. Changes in the fibre contents were analyzed using standard methods. *Lachnocladium flavidum* proved to be the most effective lignin degrader with optimal lignolytic activities. Optimal conditions were supported at incubation period of 8-9 days with culture conc. of 8 x $10^3$ spores/ml, pH 6-7, moisture at 65-70%, sucrose and yeast as best supporting carbon and nitrogen sources. Results also show variability in degradation of cellulose, hemicellulose and lignin. Cellulose and hemicellulose were most affected. Mixed culture of *T. reesei and A. niger* had the most significant reduction in cellulose (10.50±1.20%), *T. reesei* had the most significant reduction in hemicellulose (15.30±1.30%). Lignin was most significantly reduced by mixed culture of *Lach. flavidum/ A. niger* (7.20±0.80%).
Key words; Fungi, lignin, laccase, maize cob, mixed fermentation

## INTRODUCTION

Lignin a major component of maize cobs is an energy dense, three-dimensional amorphous polymer and its presence hinders the efficient extraction of cellulose and hemicellulose, which increases the costs associated with the conversion of lignocellulose into useful bioproducts (Wang *et al*., 2019). A lot of attention has been drawn to the approach on the use of a class of enzymes belonging to the oxidoreductases which catalyze the breakage of complex lignin simple oligomers. Laccases (benzenediol: oxygen oxidoreductases [EC 1.10.3.2]) like other industrial enzymes due to their importance in several industries have received significant attention. Laccase application in biotechnological processes has been limited because of high production costs resulting from low enzyme activity and low yield. Increasing research attention has been paid to achieve laccase production strategies associated with increased activity and reduced cost (Chia-wen *et al.,* 2015).

Maize is the most important cereal crop in sub-Saharan Africa and a major food crop in many parts of the world including Nigeria (International Institute for Tropical Agriculture, IITA 2015). Annually, vast amounts of agro-related residues or wastes produced are usually burned or left away to rot (Wang *et. al.,* 2019; Deming et al., 2022). However, the high amount of lignin content and level of crystallization of the residues underscores optimal utilization (Brenelli, 2018). The major steps involve the breakdown of lignocellulose; that is hydrolysis of polysaccharides and depolymerization of lignin have been identified and greatly studied (Chia-wen *et al.,* 2015). Poor and inconsistent results have been associated with enzymatic hydrolysis, in addition to time consumption during pretreatments (Brodeur *et al*., 2011) thus the combination of biological pretreatments with other pretreatments like chemical methods and the discovery of novel and more effective organisms for fast hydrolysis (Sanchez and Cardona, 2008). Several fungi but not all, secrete laccases in the course of their growth in the medium (Bourbonnais *et al*., 1995) during the secondary metabolism. A great number of organisms involve in degrading lignocellulolytic materials are continuously being been discovered and identified (Deming *et al*., 2022).

The use of two or more organisms as against a single organism for fermentation process stand to offer some benefits like an improved utilization of substrate because of possible increase in the number of enzymes produced, the product yield and rate of growth may be higher and also prone to lesser contamination. (Hesseltine, 1983). The effects of process parameters of fermentation on single and consortia of several fungi on laccase production in maize cobs to serve as an index of degradation is investigated in this study.

***Sample Collection and preparation***

Corn cobs residues were collected from several markets around Zaria metropolis and dried to constant weight at $50^0$C. This was stored in clean polythene bags after milling was carried out to a particle size of 1-2mm using a domestic blender. About 1kg of maize cob was mixed with 5% NaOH in a 5L solution at ambient temperature for 1hr and neutralized with hydrochloric acid. It was dried to a constant weight at $50^0$C after washing with distilled water.

***Fungi and growth of culture***

The test organisms *Lenzites betulina and Lachnocladium flavidum* were obtained from dept of Crop Protection, while *Aspergillus niger and Trichoderma reesei* were obtained from dept of Microbiology Ahmadu Bello University Zaria. They were maintained under refrigerated condition in potato dextrose agar (PDA) slants. The medium for growth of culture contained: 0.3% glucose, distilled water, 0.1% potassium dehydrogenate sulphate, 1% corn steep liquor and finally 0.2% sodium nitrate. The medium was sealed tightly with aluminum foil and maintained at ambient temperature for 3-5 days. After substantial growth, the culture was washed with sterile distilled water and this served as the inoculums.

***Solid-state fermentation***

Solid state fermentation carried out followed method described by Ali *et al.* (1991). The fermenting organisms (*Aspergillus niger*, *Lachnocladium flavidum*, *Trichoderma reesei* and *Lenzites betulina*) were cultivated in mineral salt- agrowastes media containing (g/L): $KH_2PO_4$,10.0; $(NH_4)_2SO_4$,10.5; $MgSO_4.7H_2O$, 0.33; $CaCl_2$ 0.5; $FeSO_47H_2O$, 0.013; $MnSG_4$. $H_2O$, 0.004; $ZnS0_4.7H_2O$; 0.004; $CoC1_2.6H_2O$ 0.0067; yeast- extract, 0.5 and 40-l00g of the untreated and treated maize cobs. The initial pH was adjusted to 5.0 after autoclaving for 15min at 121 °C.

After sterilizing the medium, they were inoculated with 5 ml of spore suspension, and incubated at ambient temperature. Another 50g 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 period of twenty-five days (25).

***Optimizing fermentation conditions***

Effect of inoculum concentration were obtained by inoculating the fermentation media with spore suspension at various concentrations for each of the single and co-culture ranging between 3, 5, 7, 9, 11 and 15 x $10^3$ spores/ml. Effect of incubation time was obtained by assaying enzyme activity at interval of fermentation between 3 to 15 days. Fermentation media was supplemented with 1% carboxymethyl cellulose, maltose, fructose, glucose, starch and sucrose to determine their how various carbon sources affect laccase production. while 1% selected organic and inorganic nitrogen source (yeast, peptone, urea, ammonium sulphate and sodium nitrite) were used to determine the effect of nitrogen source. 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 1M NaOH/1M 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. The thoroughly homogenized mixture was centrifuged for 10min at 4000rpm. The supernatant was

separated and stored at $4^0$C for analysis. Exactly 5 g of fermented samples were suspended in 10 ml of sterile distilled water.

***Determination laccase activity***

Laccase activity was determined at 37°C using 2,2'-azino-bis- ethylbenzothiazoline sulphate (ABTS) as substrate in reaction mixtures (3ml) containing 1 ml each of 0.1 M sodium acetate buffer (pH 5.0), 0.03% (w/v) ABTS and culture supernatant (Buswell *et al.,* 1995). Rate of ABTS oxidation was measured at 420nm for 10min.One unit of enzyme activity was defined as the amount of enzyme required to oxidize 1 mole of ABTS/min.

***Determination lignin***

Neutral detergent fiber (NDF) was extracted from the milled agrowastes using a neutral detergent solution based on the method of (Georing and Vansoest (1970). Klasson lignin in NDF was determined as the residue after treatment in $H_2SO_4$ (720gkg$^{-1}$) at $30^0$ C for I hour followed by autoclaving at $121^0$C in $H_2SO_4$ (30gkg$^{-1}$) for 1 hour. Thereafter glass fiber filters were used in 30ml Goack crucibles of fine porosity to filter Klasson lignin (Kaar *et al.*, 1991).

***Determination of cellulose and hemicellulose***

This was carried out according to the method described by Guhagarkar (1997). Exactly 0.5g of the sample was dissolved in 5mI of 72% (v/v) $H_2SO4$ maintained at $30^0$C in a water bath for 45 min and washed with 140ml of distilled water into a round bottom flask, then boded lor 5hours. The solution was cooled and made up to 250ml with distilled water. Ten milliliters (10ml) of the digest were neutralized with 10% (w/v) $BaCO_3$ solution and then filtered for the estimation, of sugars. The filtrate was made up to 25ml with distilled water and total reducing sugar was analyzed by using the DNS method (Miller, 1959). The estimation of hemicellulose was carried out using N-$H_2SQ_J$. Glucose in the 72% $H_2SO_4$ hydrolysate was regarded as coming from cellulose while that in N-$H_2SC$. hydrolysate was regarded as coming from non- cellulose β-glucan (hemicellulose) (Guhagarkar, 1997).

## RESULTS

***Effect of fermentation conditions on laccase enzyme production***

Effect of incubation period on laccase production using mono and co-cultures of different fungal strains for SSF of corn cobs is shown in table 1. After 15days of incubation, highest laccase activities were observed between 7-9 days of fermentation., with *Lach. flavidum* showing highest activity at about 7days of incubation. Co-culture of *Lach. flavidum /Lenzites betulina* and *Lach. flavidum* also showed similarly higher laccase activities as compared to other fermenting cultures.

Table 2. shows the effect of various inoculum size on laccase production higher laccase activities were experienced with mono- cultures of *Lach. flavidum* at lower inoculum concentrations ($5.5 \times 10^3$ spores /ml), while the mixed cultures of *A. niger*, and *Lach. flavidum* showed higher activities at higher concentrations. The activities were however increasing with increasing inoculum concentrations in most of the fermenting cultures before decreasing.

Table 3 shows the effect of a range of pH on laccase production. Laccase 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. Table 4 shows the effect of different percentages of moisture content on Laccase. Mixed cultures of *Lach. flavidum* with *T. reesei* and *L. betulina* showed better laccase activities at 60 – 70% moisture contents. The effect of 1% carbon sources on laccase production is shown in table 5. Sucrose, fructose, CMC, and maltose were seen to have the most significant effect on laccase enzyme activities. Fermentation with *Lach. flavidum* had the highest activity. The co-culture of *Lach. flavidum/ L. betulina* showed significantly different (<0.05) activities as compared to other single and co-culture fermentations.

The effect of 1% nitrogen source on laccase enzyme production from mono and co-culture solid state fermentation of corn cobs is shown in Table 6. Laccase activities were most significantly affected by 1% sodium nitrite, urea and yeast. Fermentation with *Lach. flavidum* had the highest cellulase activity. The co-culture of *Lach. flavidum/ A. niger* and *T. reesei/ A. niger* also showed significantly higher (P<0.05) activities as compared to other single and co-culture fermenting setups.

The effects of fermentation on the fibre components in corn cobs is shown in figure 4. Cellulose, hemicellulose and lignin were seen to have been significantly degraded by all the fermenting organisms. Mixed culture of *T. reesei* and *A. niger* had the most significant reduction in cellulose, *T. reesei* had the most significant reduction in hemicellulose. Lignin was most significantly reduced by *Lach. flavidum* and its mixture with *A. niger.*

Table 1. Effect of Incubation Period on Laccase Enzyme Production from Corn Cobs

| Incubation Period | 3days | 5 days | 7 days | 9 days | 11 days | 15 days |
|---|---|---|---|---|---|---|
| *T. reesei* | 150.00 ±7.60$^{a}$ | 469.00 ±6.50$^{b}$ | 777.00 ±9.60$^{c}$ | 799.00 ±11.20$^{d}$ | 701.00 ±9.80$^{e}$ | 534.00 ±6.10$^{f}$ |
| *Lenzites betulina* | 210.00 ±4.50$^{a}$ | 524.00 ±8.60$^{b}$ | 662.00 ±12.30$^{c}$ | 546.00 ±11.40$^{d}$ | 531.00 ±10.50$^{d}$ | 501.00 ±9.00$^{e}$ |
| *A. niger* | 215.00 ±6.90$^{a}$ | 291.00 ±8.10$^{b}$ | 485.00 ±9.20$^{c}$ | 501.00 ±12.40$^{d}$ | 406.00 ±10.20$^{e}$ | 326.00 ±5.40$^{f}$ |
| *Lach. flavidum* | 287.00 ±7.10$^{a}$ | 995.00 ±10.60$^{b}$ | 1345.00 ±16.30$^{c}$ | 1509.00 ±19.30$^{d}$ | 1498.00 ±18.90$^{d}$ | 1089.00 ±18.20$^{e}$ |
| *T. reesei & L. betulina* | 215.00 ±4.50$^{a}$ | 575.00 ±13.20$^{b}$ | 675.00 ±14.50$^{c}$ | 775.00 ±17.30$^{d}$ | 455.00 ±7.80$^{e}$ | 399.00 ±7.30$^{f}$ |
| *T. reesei & A. niger* | 255.00 ±6.80$^{a}$ | 577.00 ±16.30$^{b}$ | 601.00 ±13.40$^{c}$ | 698.00 ±17.90$^{d}$ | 554.00 ±12.20$^{b}$ | 453.00 ±7.80$^{e}$ |
| *T. reesei & L. flavidum* | 789.00 ±19.50$^{a}$ | 989.00 ±22.50$^{b}$ | 1059.00 ±24.10$^{c}$ | 1482.00 ±25.30$^{d}$ | 1007.00 ±21.80$^{c}$ | 994.00 ±19.70$^{ce}$ |
| *L. betulina & A. niger* | 323.00 ±6.40$^{a}$ | 348.00 ±6.50$^{b}$ | 789.00 ±20.10$^{c}$ | 821.00± 18.40$^{d}$ | 567.00 ±10.70$^{e}$ | 498.00 ±12.50$^{f}$ |
| *L. betulina & L. flavidum* | 346.00 ±5.00$^{a}$ | 1203.00 ±15.90$^{b}$ | 1342.00 ±19.40$^{c}$ | 1502.00 ±20.20$^{d}$ | 1201.00 ±23.10$^{e}$ | 1433.00 ±21.60$^{f}$ |
| *A. niger & L. flavidum* | 358.00 ±6.20$^{a}$ | 661.00 ±10.60$^{b}$ | 699.00 ±11.70$^{b}$ | 798.00 ±13.40$^{c}$ | 893.00 ±20.30$^{d}$ | 589.00 ±14.50$^{e}$ |

Values are Mean ± SD, values with different superscript on same row are significantly different at p <0.05

Table 2: Effect of Inoculum Concentration on Laccase Enzyme Production from Corn Cobs

| Inoculum Conc. | **2** | **4** | **5.5** | **6.5** | **8** |
|---|---|---|---|---|---|
| *T. reesei* | 124.00±4.20$^{a}$ | 324.00±5.60$^{b}$ | 543.00±13.40$^{c}$ | 661.00±15.30$^{d}$ | 601.00±17.00$^{e}$ |
| *Lenzites betulina* | 169.00±6.50$^{a}$ | 342.00±8.70$^{b}$ | 568.00±12.20$^{c}$ | 640.00±17.80$^{d}$ | 599.00±13.50$^{e}$ |
| *A. niger* | 103.00±5.50$^{a}$ | 199.00±7.60$^{b}$ | 435.00±10.30$^{c}$ | 501.00±14.70$^{d}$ | 419.00±13.40$^{e}$ |
| *Lach. flavidum* | 897.00±18.60$^{a}$ | 1045.00±20.40$^{b}$ | 1654.00±24.70$^{c}$ | 1845.00±26.20$^{d}$ | 1899.00±27.20$^{d}$ |
| *T. reesei & L. betulina* | 567.00±17.30$^{a}$ | 872.00±21.90$^{b}$ | 985.00±23.50$^{c}$ | 1002.00±22.00$^{c}$ | 996.00±19.50$^{c}$ |
| *T. reesei & A. niger* | 546.00±10.85$^{a}$ | 721.00±16.70$^{b}$ | 798.00±15.30$^{c}$ | 985.00±18.20$^{d}$ | 869.00±21.80$^{e}$ |
| *T. reesei & L. flavidum* | 703.00±15.60$^{a}$ | 762.00±17.40$^{b}$ | 895.00±20.30$^{c}$ | 876.00±19.54$^{c}$ | 895.00±20.50$^{c}$ |
| *L. betulina & A. niger* | 447.00±9.40$^{a}$ | 568.00±11.90$^{b}$ | 871.00±15.76$^{c}$ | 995.00±21.05$^{d}$ | 909.00±22.40$^{c}$ |
| *L. betulina & L. flavidum* | 679.00±18.60$^{a}$ | 1005.00±23.50$^{b}$ | 1249.00±26.10$^{c}$ | 1562.00±28.20$^{d}$ | 1499.00±21.40$^{e}$ |
| *A. niger & L. flavidum* | 789.00±20.90$^{a}$ | 947.00±23.70$^{b}$ | 1239.00±26.90$^{c}$ | 1432.00±19.40$^{d}$ | 1441.00±21.80$^{d}$ |

Values are Mean ± SD, values with different superscript on same row are significantly different at p <0.05

Table 3: Effect of pH on Laccase Enzyme Production from Fermentation of Corn Cobs

| pH | pH 4 | pH 5 | pH 6 | pH 7 | pH 8 |
|---|---|---|---|---|---|
| *T. reesei* | 175.00±4.50$^{a}$ | 446.00±7.20$^{b}$ | 784.00±10.90$^{c}$ | 706.00±12.10$^{d}$ | 540.00±9.65$^{e}$ |
| *Lenzites betulina* | 221.00±7.50$^{a}$ | 524.00±9.35$^{b}$ | 650.00±10.50$^{c}$ | 450.00±8.60$^{d}$ | 531.00±9.75$^{b}$ |
| *A. niger* | 203.00±5.40$^{a}$ | 291.00±8.20$^{b}$ | 531.00±9.70$^{c}$ | 501.00±7.80$^{d}$ | 406.00±9.40$^{e}$ |
| *Lach. flavidum* | 337.00±5.70$^{a}$ | 1024.00±21.40$^{b}$ | 1345.00±23.50$^{c}$ | 998.00±13.20$^{bd}$ | 879.00±11.40$^{e}$ |
| *T. reesei & L. betulina* | 365.00±7.00$^{a}$ | 577.00±9.50$^{b}$ | 612.00±10.10$^{c}$ | 555.00±8.90$^{b}$ | 501.00±10.00$^{e}$ |
| *T. reesei & A. niger* | 255.00±4.50$^{a}$ | 577.00±6.80$^{b}$ | 601.00±11.80$^{c}$ | 698.00±12.70$^{d}$ | 554.00±11.50$^{cd}$ |
| *T. reesei & L. flavidum* | 789.00±16.90$^{a}$ | 989.00±19.50$^{b}$ | 1059.00±20.70$^{c}$ | 1482.00±22.40$^{d}$ | 1007.00±21.50$^{b}$ |
| *L. betulina & A. niger* | 298.00±7.70$^{a}$ | 301.00±8.45$^{a}$ | 669.00±12.30$^{c}$ | 559.00±11.70$^{d}$ | 561.00±13.10$^{d}$ |
| *L. betulina & L. flavidum* | 665.00±15.40$^{a}$ | 1456.00±23.50$^{b}$ | 1663.00±24.50$^{c}$ | 1550.00±25.10$^{d}$ | 1324.00±23.60$^{e}$ |
| *A. niger & L. flavidum* | 345.00±9.50$^{a}$ | 551.00±13.40$^{b}$ | 702.00±19.20$^{c}$ | 698.00±18.90$^{c}$ | 600.00±17.50$^{d}$ |

Values are Mean ± SD, Values with different superscript on same row are significantly different at p <0.05

Table 4: Effect of Moisture Content on Laccase Enzyme Production from Fermentation of Corn Cobs

| Moisture Content | 60% | 65% | 70% | 75% | 80% |
|---|---|---|---|---|---|
| *T. reesei* | 515.00±12.20$^{a}$ | 725.00±13.40$^{b}$ | 778.00±17.30$^{c}$ | 980.00±21.40$^{d}$ | 557.00±10.90$^{e}$ |
| *Lenzites betulina* | 666.00±16.80$^{a}$ | 420.00±8.50$^{b}$ | 909.00±16.20$^{c}$ | 457.00±5.90$^{d}$ | 601.00±9.80$^{e}$ |
| *A. niger* | 450.00±14.30$^{a}$ | 351.00±7.20$^{b}$ | 651.00±9.50$^{c}$ | 309.00±5.00$^{d}$ | 512.00±8.90$^{e}$ |
| *Lach. flavidum* | 1006.00±20.40$^{a}$ | 1199.00±21.50$^{b}$ | 1530.00±24.30$^{c}$ | 990.00±19.50$^{d}$ | 841.00±18.60$^{e}$ |
| *T. reesei & L. betulina* | 601.00±14.50$^{a}$ | 575.00±9.80$^{b}$ | 758.00±12.40$^{c}$ | 801.00±14.20$^{d}$ | 457.00±13.50$^{e}$ |
| *T. reesei & A. niger* | 455.00±8.90$^{a}$ | 777.00±15.30$^{b}$ | 701.00±15.10$^{c}$ | 845.00±12.50$^{d}$ | 565.00±15.40$^{e}$ |
| *T. reesei & L. flavidum* | 898.00±17.20$^{a}$ | 1554.00±23.40$^{b}$ | 1550.00±25.00$^{b}$ | 1001.00±23.00$^{c}$ | 890.00±19.80$^{d}$ |
| *L. betulina & A. niger* | 444.00±7.90$^{a}$ | 556.00±13.40$^{b}$ | 854.00±19.50$^{c}$ | 858.00±19.80$^{c}$ | 499.00±10.60$^{d}$ |
| *L. betulina & L. flavidum* | 888.00±17.40$^{a}$ | 1001.00±20.60$^{b}$ | 1220.00±22.80$^{c}$ | 990.00±19.90$^{b}$ | 1201.00±21.50$^{c}$ |
| *A. niger & L. flavidum* | 459.00±11.00$^{a}$ | 601.00±12.00$^{b}$ | 705.00±16.00$^{c}$ | 994.00±15.30$^{d}$ | 902.00±13.80$^{e}$ |

Values are Mean ± SD, Values with different superscript on same row are significantly different at p <0.05 using

Table 5: Effect of Carbon Source on Laccase Enzyme Production from Fermentation of Corn Cobs

| Caron source | Sucrose | Glucose | Maltose | Fructose | CMC | Starch |
|---|---|---|---|---|---|---|
| *T. reesei* | 815.00 ±11.20$^{a}$ | 723.00 ±10.50$^{b}$ | 888.00 ±12.30$^{c}$ | 786.00 ±9.80$^{d}$ | 666.00 ±8.20$^{e}$ | 910.00 ±15.60$^{f}$ |
| *Lenzites betulina* | 966.00 ±17.40$^{a}$ | 813.00 ±14.70$^{b}$ | 601.00 ±9.60$^{c}$ | 782.00 ±13.20$^{d}$ | 795.00 ±11.90$^{d}$ | 915.00 ±18.00$^{e}$ |
| *A. niger* | 450.00 ±6.80$^{a}$ | 661.00 ±8.50$^{b}$ | 665.00 ±12.50$^{b}$ | 559.00 ±10.40$^{c}$ | 711.00 ±12.10$^{d}$ | 867.00 ±13.70$^{e}$ |
| *Lach. flavidum* | 1643.00 ±23.40$^{a}$ | 992.00 ±19.50$^{b}$ | 1150.00 ±24.30$^{c}$ | 1408.00 ±18.50$^{d}$ | 998.00 ±14.6$^{b}$ | 890.00 ±13.40$^{e}$ |
| *T. reesei & L. betulina* | 901.00 ±15.00$^{a}$ | 885.00 ±13.00$^{b}$ | 758.00 ±10.50$^{c}$ | 991.00 ±17.00$^{d}$ | 950.00 ±16.20$^{e}$ | 766.00 ±9.80$^{b}$ |
| *T. reesei & A. niger* | 675.00 ±12.50$^{a}$ | 777.00 ±19.00$^{b}$ | 801.00 ±11.30$^{b}$ | 985.00 ±14.70$^{c}$ | 895.00 ±21.00$^{d}$ | 694.00 ±16.00$^{a}$ |
| *T. reesei & L. flavidum* | 1006.0 0±25.00$^{a}$ | 995.00 ±22.00$^{a}$ | 895.00 ±23.00$^{b}$ | 876.00 ±16.00$^{b}$ | 895.00 ±17.40$^{b}$ | 799.00 ±13.50$^{c}$ |
| *L. betulina & A. niger* | 555.00 ±9.50$^{a}$ | 675.00 ±9.70$^{b}$ | 753.00 ±13.60$^{c}$ | 858.00 ±15.40$^{d}$ | 699.00 ±17.30$^{e}$ | 701.00 ±14.00$^{f}$ |
| *L. betulina & L. flavidum* | 1103.00 ±12.80$^{a}$ | 1005.00 ±21.50$^{b}$ | 998.00 ±16.70$^{b}$ | 1550.00 ±23.40$^{c}$ | 1401.00 ±17.20$^{d}$ | 1001.00 ±19.80$^{b}$ |
| *A. niger & L. flavidum* | 992.00 ±12.70$^{a}$ | 990.00 ±17.90$^{a}$ | 1001.00 ±21.80$^{a}$ | 895.00 ±15.60$^{b}$ | 890.00 ±18.40$^{b}$ | 990.00 ±14.50$^{a}$ |

Values are Mean ± SD, Values with different superscript on same row are significantly different at p <0.05 using

Table 6: Effect of Nitrogen Sources on Laccase Enzyme Production from Corn Cobs

| Nitrogen source | Yeast | Peptone | Urea | Ammonium | Sodium | Potassium |
|---|---|---|---|---|---|---|
| *T. reesei* | 715.00±12.40$^{a}$ | 723.00±11.30$^{a}$ | 777.00±17.80$^{b}$ | 687.00±13.50$^{c}$ | 557.00±9.50$^{d}$ | 1001.00±24.90$^{e}$ |
| *Lenzites betulina* | 866.00±18.20$^{a}$ | 513.00±7.80$^{b}$ | 901.00±15.00$^{c}$ | 287.00±4.70$^{d}$ | 895.00±14.50$^{e}$ | 815.00±13.90$^{f}$ |
| *A. niger* | 650.00±9.60$^{a}$ | 450.00±5.70$^{b}$ | 755.00±17.40$^{c}$ | 501.00±11.30$^{d}$ | 812.00±14.80$^{e}$ | 856.00±16.00$^{f}$ |
| *Lach. flavidum* | 1550.00±25.00$^{a}$ | 834.00±19.60$^{b}$ | 1330.00±23.40$^{c}$ | 650.00±12.60$^{d}$ | 998.00±15.70$^{e}$ | 990.00±21.00$^{f}$ |
| *T. reesei & L. betulina* | 801.00±17.80$^{a}$ | 775.00±13.40$^{b}$ | 758.00±14.50$^{b}$ | 901.00±18.90$^{c}$ | 850.00±16.40$^{d}$ | 656.00±12.50$^{e}$ |
| *T. reesei & A. niger* | 555.00±8.90$^{a}$ | 888.00±11.50$^{b}$ | 701.00±12.10$^{c}$ | 945.00±18.40$^{d}$ | 695.00±11.90$^{e}$ | 794.00±9.80$^{f}$ |
| *T. reesei & L. flavidum* | 998.00±13.40$^{a}$ | 1302.00±21.60$^{b}$ | 1550.00±24.10$^{c}$ | 786.00±19.30$^{d}$ | 980.00±16.70$^{e}$ | 990.00±18.50$^{f}$ |
| *L. betulina & A. niger* | 666.00±10.30$^{a}$ | 756.00±12.80$^{b}$ | 954.00±17.20$^{c}$ | 958.00±13.20$^{c}$ | 799.00±14.30$^{d}$ | 901.00±20.10$^{e}$ |
| *L. betulina & L. flavidum* | 1601.00±27.80$^{a}$ | 1105.00±22.30$^{b}$ | 888.00±12.40$^{c}$ | 1450.00±23.00$^{d}$ | 1222.00±24.50$^{e}$ | 991.00±21.00$^{f}$ |
| *A. niger & L. flavidum* | 559.00±8.70$^{a}$ | 1200.00±19.70$^{b}$ | 860.00±16.50$^{c}$ | 569.00±10.30$^{a}$ | 990.00±20.00$^{d}$ | 1340.00±27.00$^{e}$ |

Values are Mean ± SD, Values with different superscript on same row are significantly different at p <0.05

Figure 1: Fiber Components from Fungal Fermentation of Corn Cobs (%)

| Fungi | Cellulose | Hemicellulose | Lignin |
|---|---|---|---|
| *Control* | 39.40±4.30$^{g}$ | 42.00±4.00$^{f}$ | 15.40±1.55$^{g}$ |
| *Unfermented* | 35.30±2.90$^{f}$ | 40.10±3.90$^{f}$ | 15.00±1.20$^{fg}$ |
| *Trichoderma reesei* | 12.60±0.84$^{ab}$ | 15.30±1.30$^{a}$ | 12.10±0.90$^{cd}$ |
| *Lenzites betulina* | 20.40±2.10$^{de}$ | 18.90±1.70$^{bc}$ | 11.80±1.60$^{cd}$ |
| *Aspergillus niger* | 15.70±1.60$^{bc}$ | 19.20±1.90$^{bc}$ | 9.90±0.70$^{b}$ |
| *Lachnocladium flavidum* | 18.20±1.80$^{cd}$ | 21.30±2.00$^{c}$ | 7.20±0.80$^{a}$ |
| *T. reesei & L. betulina* | 22.50±1.72$^{e}$ | 20.90±1.85$^{bc}$ | 13.00±1.30$^{e}$ |
| *T. reesei & A. niger* | 10.50±1.20$^{a}$ | 16.70±1.45$^{ab}$ | 10.30±1.40$^{bc}$ |
| *T. reesei & Lach. flavidum* | 11.90±1.50$^{ab}$ | 17.00±2.00$^{b}$ | 9.00±0.50$^{ab}$ |
| *L. betulina & A. niger* | 18.70±1.40$^{cd}$ | 29.20±3.10$^{e}$ | 12.60±1.45$^{d}$ |
| *L. betulina & Lach. flavidum* | 20.90±2.10$^{de}$ | 22.00±1.80$^{d}$ | 11.30±1.70$^{c}$ |
| *A. niger & Lach. flavidum* | 15.20±1.50$^{bc}$ | 17.90±1.60$^{b}$ | 10.30±1.02$^{bc}$ |

Values are Mean ± SD, Values with different superscript letter down the column are significantly different at p <0.05

## DISCUSSION

### Effect of fermentation conditions on laccase enzyme production

After 15days of incubation, highest laccase activities were observed between 7-9 days of fermentation, with *Lach. flavidum* showing highest activity at about 8days of incubation. Nearly similar results were found by Kumar *et al*. (2011) who reported that maximal laccase activity in *p. ostreatus* was on the 9th day (570 U/L). In addition to this Sivakumar *et al*., (2010) also reported optimum laccase production by *Ganoderma sp*. after 10th day of incubation.

Low levels of inoculum concentration may not initiate adequate growth while higher levels can create competition among fermenting organisms (Sabu *et al*. 2005). In this study, higher laccase activities were observed with single cultures of *Lach. flavidum* at lower inoculum concentrations, while the mixed cultures showed higher activities at higher concentrations. Similar pattern of production was observed with the production of laccase by an indigenous strain of *Pleurotus ostreatus* HP-1 studied on solid state fermentation (Patel *et al.,* 2009). The activities were however increasing with increasing inoculum concentrations in most of the fermenting cultures before decreasing. Laccase production was decreased because of fast depletion of the nutrients, resulting in a and adaptation of fungi, hence affecting the enzyme production. The maximum activity of 1% carbon sources on laccase production obtained from supplementation with sucrose, fructose and CMC from *Lach. flavidum* and co-cultures *Lach. flavidum /L betulina* and *A. niger/ Lach. flavidum* are in agreement Elsayed *et al*., (2012) who reported fructose to be the best carbon source for laccase production. Fructose instead of glucose was reported to have resulted in a 100-fold increase in activity of laccase in Basidiomycetes (Mansur *et al*., 1997), while Tavares *et al.* (2006), showed that initial glucose concentration was the factor most important for laccase production in *Trametes versicolor.* decrease in metabolic activity. (Wang *et al*., 2019)

Effect of pH on laccase production showed that laccase activities were significantly higher at lower pH (4-6) among the mixed culture fermentations. The mono cultures had higher activities within a higher pH range (6-8). The results obtained in the present study are in agreement with Nadeem *et al.,* (2014) who reported that most of the fungal cultures preferred a slightly acidic pH of medium for growth and enzyme production. Sivakami *et al*. (2012) also showed that in *Schizophyllum commune* and P. *ostreatus* the optimal pH for fungi mycelia biomass yield and laccase activities was 5.5.

Moisture ratio/level is a key factor in SSF that influences the laccase production. Among the various moisture levels tested, mixed cultures of *Lach. flavidum* with *T. reesei* and single culture of *Lach. flavidum* showed highest laccase activities at 65 – 70% moisture contents. Niladevi *et al*., (2007) and Patel *et al*., (2009) reported optimum moisture level for fungal laccase production to be 65% and 60% respectively**.** Patel and Gupte (2010) also observed higher laccase production at 80% moisture content by utilizing *Tricholoma giganteum* on wheat straw as substrate. Selection of an appropriate carbon source is important in growth, metabolism

The effect of 1% nitrogen source showed yeast, urea and potassium nitrate were seen to have induced the most significant effect on laccase activities and production. Fermentation with *Lach. flavidum* had the highest cellulase activity along with co-culture of *Lach. flavidum / A. niger* Ligninolytic enzymes have been seen to be regulated by the usable concentration of nitrogen in a medium. Lignolytic enzyme production can be stimulated by low nitrogen levels while repression can be caused by high levels (Kirk and Chang 1990). The current result is in line with the reports of Bakkiyaraj *et al.,* (2013) which reported organic nitrogen sources being more suitable than most inorganic sources

for most fungi. Some strains require excess nitrogen to protect the enzyme, while others are only induced by nitrogen starvation (Jing 2010). From some studies, we can also see that organic nitrogen is more conducive to the production of laccase than inorganic nitrogen (Karp ,2015). The carbon/nitrogen ratio is one of the key parameters for laccase production in lignocellulosic wastes and it varies due to differences in lignocellulosic resources and fungal strains (Economou, 2017).

Fiber component analysis showed that, cellulose, hemicellulose and lignin were seen to 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, *T. reesei* had the most significant reduction in hemicellulose. Lignin was most significantly reduced by *Lach. flavidum* and its mixture with *A. niger*. Considerable variations occur in Polymer degradation selectivity of fungi and their delignification potentials (Dong *et al.*, 2013). Growth conditions as well as genetic variation are largely responsible for lignolytic enzyme production and breakdown of fibre. 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).

## CONCLUSION

In conclusion, all exploited fungi in this present study following the optimization of fermentation conditions have showed solid state fermentation of single and mixed cultures of four fungal species (*Aspergillus niger, Trichoderma reesei, Lachnocladium flavidum* and *Lenzites betulina)* can lead to improved degradation maize cobs. All fungi showed ability to produce laccase and degrade lignin. Mixed fermentation of *L. flavidum* and *T. reesei* proved to be the most effective lignin degraders with optimal lignolytic activities. Optimal conditions were supported at pH 6-7, moisture at 65-70%, incubation period of 8-9 days, culture conc. of 8 x $10^3$ spores/ml, with sucrose and yeast as best supporting carbon and nitrogen sources respectively. Results of fiber component analysis showed that, cellulose, hemicellulose and lignin were seen to 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, *T. reesei* had the most significant reduction in hemicellulose. Lignin was most significantly reduced by mixed culture of *Lach. flavidum/ A. niger*. Improved lignin degradation has been seen to greatly enhance the nutritive value and utilization of maize cob.

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