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Optimization of Culture Conditions for Xylanase Production by Mixed Fungal Fermentation: Effects on Pretreated Maize Cobs

[1]Olagunju, A., [1]Onyike, E., [1]Ameh, D. A., [2]Atawodi, S. E. and [1]Muhammad A.

[1]Department of Biochemistry Ahmadu Bello University Zaria
[2]Department of Biochemistry Federal University Lokoja

Abstract

The use of microorganisms for xylanase production plays important role in the bioconversion of lignocelluloses and also required in huge amount for industrial level application. This necessitates the need to select potent microorganisms for xylanase production, followed by optimization of media components for enhanced production. The effects of altering cultural fermentation conditions on the xylanase production ability in maize cobs were investigated. A consortium of four fungi; *Lenzites betulina, Trichoderma reesei, Lachnocladium specie* and *Aspergillus niger* were used to carry out single and mixed solid-state fermentation on NaOH pretreated maize cobs. Optimization of fermentation factors were carried out from ten groups of individual and co-fermented fungal combinations. *L. flavidum* was found to be the most effective xylanase producer with optimal conditions at pH of 5.5, moisture 75%, inoculum concentration at 5-6 x 10^3 spores/ml, incubation period of 7-9 days and 1% peptone as the best nitrogen media supplement. Variation to different degrees in the degradation of the maize cobs were observed. A 10% decrease in cellulose was observed with co- cultures of *T. reesei* and *A. niger* and a 15% decrease in the hemicellulose fraction. The biotechnological potential of corn cobs has been enhanced by the screening and optimizing the culture conditions.

Keywords: Xylanase, hemicellulose, maize cobs, fungi, fermentation.
Corresponding authors email: nutribas@gmail.com +234-8038534580

Introduction

The interest in biotechnological applications of xylanase has increased tremendously, such as bioconversion of lignocellulosic material and agro-wastes to fermentable products, improving the digestibility of animal feed stocks, pre-bleaching of pulp, and mixtures, modification of cereal-based stuffs. (Sunkar et al., 2020). Xylose is the second most abundant component in the cell wall of plants, and results from the breakdown of the hemicellulolytic component of lignocellulose by action of xylanases. Thus, the continuous search for xylanase producing microorganisms is ever increasing (Bhardwaj et al., 2019). Estimated 40-50% of corn is corn cobs and contains the highest xylan content among several other lignocellulosic residues. It consists of about 39% total fiber content and up to 40% of xylan (Terrone et al., 2018). The use of fermentation biotechnology for the increased production of value-added products thus necessitates efficient manipulation of corn cobs residue. Improved utilization of the fibre components through structural modification of maize cobs can be achieved through fermentation (Xu et al., 2023). Alkaline pretreatment of agroresidues has proved to be one of the most effective process due to high degree of polymerization and crystallinity reduction and the lesser operational cost implication (Shukla et al.,

2023). Solid State Fermentation (SSF) is a technique where both physical support as well as nutrient source for the growth of organisms is provided by the solid matrix in the presence of little or no free moisture (Sathendra et al., 2022). Significant role and impact are played by several cultural factors on the final yield and productivity which necessitates their optimization for improved product formation. These include inoculum size/ concentration, amount of moisture content, pH, temperature, and the nature of nutrient source (Bhardwaj et al., 2019). Several potential organisms which have abilities to degrade hemicellulose and cellulose need to be identified for production of wide range of digestive enzymes.

Materials and Methods

Collection and Preparation of Sample

Samples of corn cobs were collected in clean containers from Samaru and Giwa markets in Zaria, Kaduna state, they were dried in the open to a constant weight and milled dried to constant weight at 50^0C and milled to a particle size of 2mm using domestic blender 9 model (MX-391N Matsuhita Electric) at the department of Biochemistry Ahmadu Bello University Zaria. Alkaline pretreatment was carried out with 4.5L of NaOH solution on one (1) kilogram of milled cobs at ambient temperature for 1hour. This was terminated with HCL. Samples were ready for fermentation after washing with distilled water and dried at ambient temperature

Test organisms and growth of inoculums

Four fungal degrading organisms; *Trichoderma reesei, Aspergillus niger*, *Lenzites betulina* and *Lachnocladium flavidum* previously stored under refrigerated condition were grown in culture medium containing (g/L): 0.3g glucose, distilled water, 0.1g potassium dehydrogenate sulphate, 1g corn steep liquor and finally 0.2g sodium nitrate. After 5 days at room temperature, the cultures were w washed with sterile distilled water and served as the inoculums.

Solid-state Fermentation

The method of Ali et al. (1991) was used for solid state fermentation. A mineral salt medium containing (g/L): $CoC1_2.6H_20$ 0.0067; KH_2PO_4,10.0; $(NH_4)_2SO_4$,10.5; $MnSG_4$. H_2O, 0.004; $MgSO_4.7H_2O$, 0.33; $CaCl_2$ 0.5; $FeS0_47H_2O$, 0.013; $ZnS0_4.7H_20$; 0.004; yeast- extract, 0.5 and 70-l00g of the untreated and treated maize cobs. Media was autoclaved at 121^0C for 15minutes and initial pH adjusted to 5.0.

Five milliliters (5ml) of spore suspension were added to the medium after sterilization and incubated at ambient temperature. Same conditions were applied to another 50g of corn cobs without addition of inoculums to serve as the control. Fermentation was carried out for a period of 15 days.

Optimizing fermentation conditions

Spore suspension with concentration between 2 - 8 x 10^3 spores/ml was used to determine the effect of inoculum concentration, effect of moisture was determined by varying the moisture content between 60-80% using a citrate phosphate buffer. The effect of nitrogen supplementation was determined using 1% organic and inorganic nitrogen sources added to different fermentation set-ups, enzyme activity at interval between three to fifteen days were used to assess determine the effect of incubation time. The effect of moisture content on xylanase production was determined by increasing the moisture from 60% to a maximum of 80% using a citrate phosphate buffer. Effect of pH on the fermentation medium was obtained by carrying out fermentation at varying pH between 4.0 - 8.5 using 1M sodium hydroxide /1M hydrochloric acid.

Preparation of Crude Extract for Enzyme Analysis

Ten (10) ml of distilled water was added to 5g each of the fermented samples and mixed thoroughly. The supernatant obtained after centrifugation at 4000 rpm for 10 minutes were used for the biochemical analysis.

Determination of Xylanase Activity

The method of Bailey et al. (1992) was used to determine the activity of xylanase enzyme following the dinitrosalicylic acid method of reducing sugars from birch wood xylan (Miller 1958). The amount of enzyme required to release 1 μmol of xylose from birch wood xylan in 1 min under the assay conditions was defined as one unit of xylanase.

Data Analysis

Experiments were repeated three times to address variability and stability of fermented samples and values expressed as Mean ± standard deviation. Differences between the groups were analyzed by one-way analysis of variance (ANOVA) with the aid of Statistical Package for Social Sciences (SPSS) (2021) software. Values are considered significantly

different at p <0.05 using Duncan Multiple range test (DMRT).

Results and discussion

Results

Effect of incubation period on xylanase production using mono and co-cultures of different fungal strains for SSF of corn cobs is shown in table 1 Fermentation over the course of 15days showed higher xylanase activities after seven (7) days of incubation, with a mono-culture of *Lach. flavidum* showing highest activity (234.00±14.20 U/l) at about 7days of incubation. Single and co-culture fermentation did not show statistical difference in xylanase activity in terms of the period of incubation. However single culture of *A. niger* and co-culture of *T. reesei and Lach. flavidum* also showed higher activities.

The effect of inoculum size on xylanase production using mono and co-cultures of different fungal strains for SSF of corn cobs is shown in table 2. Higher xylanase activities (210.00±10.70 U/L) were experienced with mixed cultures at lower inoculum concentrations (4×10^3 to 5.5×10^3spores /ml), this was observed with the mixed cultures of *T. reesei/ A. niger* and also single cultures of *T. reesei*.

Effect of pH on xylanase production using mono and co-cultures of different fungal strains for SSF of corn cobs is shown in table 3. Xylanase activity was significantly high following fermentation at pH (5-7) with *Lach. flavidum.* Among the co- culture fermentations *A. niger/ Lach. flavidum* had highest xylanase activity 236.00±12.70 U/l.

Table 4 shows the effect of moisture content on xylanase production using mono and co-cultures of different fungal strains for SSF of corn cobs. Most cultures exhibited higher activities at lower moisture contents; *A. niger/T. reesei* had the highest activity (231±17.40) at 65% moisture content.

The effect of 1% nitrogen source (yeast, peptone, urea, sodium nitrate, ammonium nitrate and potassium nitrate) on xylanase enzyme production from mono and co-culture solid state fermentation of corn cobs is shown in table 6. Yeast, peptone, and urea were seen to have induced the most significant effect on xylanase activities and production. Fermentation with co-culture of *T. reesei/A. niger* had the highest xylanase activity (189.00±14.00 U/L).

Results of fiber component analysis from fungal mono and Co-culture solid state fermentation of corn cobs (%) is shown in table 6. Following mono and co-culture fermentations, cellulose and hemicellulose 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 (10.50±1.20%), *T. reesei* had the most significant reduction in hemicellulose (15.30±1.30%).

Table 1: Effect of Incubation Period on Xylanase Enzyme Production from Solid State Fermentation of Corn Cobs (U/L)

Fermenting fungi	**3 days**	**5 days**	**7 days**	**9 days**	**11 days**	**15 days**
T. reesei	196.00±15.90^{d}	205.00±13.60^{e}	215.00±14.70^{c}	220.00±11.50^{g}	180.00±10.20^{c}	178.00±11.60^{c}
Lenzites betulina	135.00±11.60^{bc}	142.00±12.30^{bc}	157.00±10.50^{b}	150.00±13.40^{b}	133.00±14.00^{b}	122.00±9.60^{b}
A. niger	210.00±11.40^{d}	213.00±10.75^{e}	224.00±12.50^{c}	205.00±15.00^{f}	199.00±11.50^{d}	201.00±10.10^{d}
Lach. flavidum	195.00±12.30^{d}	217.00±13.40^{e}	234.00±14.20^{c}	239.00±11.80^{h}	215.00±13.50^{e}	206.00±11.20^{d}
T. reesei & L. betulina	125.00±9.50^{b}	128.00±11.40^{b}	130.00±10.30^{a}	168.00±15.60^{c}	177.00±12.90^{c}	180.00±17.80^{c}
T. reesei & A. niger	145.00±13.70^{bc}	155.00±12.50^{c}	168.00±8.90^{b}	196.00±11.80^{e}	210.00±14.30^{e}	213.00±10.90^{d}
T. reesei & L. flavidum	196.00±12.00^{d}	210.00±16.40^{e}	221.00±14.50^{c}	230.00±14.30^{h}	217.00±11.60^{e}	211.00±10.10^{d}

L. betulina & A. niger	79.00±5.20[a]	84.00±3.90[a]	126.00±10.40[a]	129.00±10.70[ab]	96.00±6.80[a]	90.00±7.00[a]
L. betulina & L. flavidum	95.00±8.50[a]	104.00±11.30[a]	125.00±10.50[a]	122.00±13.40[a]	119.00±11.50[b]	110.00±9.00[b]
A. niger & L. flavidum	155.00±12.50[c]	165.00±14.70[d]	177.00±16.10[b]	180.00±15.00[d]	172.00±13.60[c]	166.00±12.80[c]

Values are Mean ± SD, Values with different superscript letters down the column are significantly different at $p < 0.05$, using Duncan Multiple range test.

Table 2: Effect of Inoculum Concentration on Xylanase Enzyme Production from Solid State Fermentation of Corn Cobs. (U/L)

Fermenting fungi	2×10^3	4×10^3	5.5×10^3	6.5×10^3	8×10^3
T. reesei	145.00±10.50^{c}	152.00±12.40^{c}	184.00±15.60^{e}	180.00±15.70^{d}	150.00±12.40^{c}
Lenzites betulina	65.00±7.80^{a}	77.00±6.90^{a}	84.00±7.50^{a}	89.00±8.10^{a}	79.00±5.50^{a}
A. niger	122.00±14.20^{bc}	136.00±13.50^{bc}	147.00±13.80^{c}	135.00±13.60^{bc}	133.00±12.70^{bc}
Lach. flavidum	138.00±11.60^{c}	149.00±11.10^{c}	159.00±15.30^{d}	152.00±11.80^{c}	131.00±12.70^{bc}
T. reesei & L. betulina	121.00±11.20^{bc}	132.00±13.20^{bc}	159.00±14.10^{d}	119.00±10.20^{b}	92.00±8.90^{a}
T. reesei & A. niger	173.00±16.70^{d}	196.00±15.90^{d}	210.00±10.70^{f}	201.00±13.40^{d}	179.00±12.60^{e}
T. reesei & L. flavidum	128.00±11.20^{bc}	132.00±12.30^{bc}	144.00±11.60^{c}	131.00±13.50^{bc}	122.00±10.80^{b}
L. betulina & A. niger	105.00±11.90^{b}	119.00±17.20^{b}	121.00±13.60^{b}	134.00±12.40^{bc}	125.00±13.70^{b}
L. betulina & L. flavidum	130.00±14.20^{c}	139.00±15.10^{bc}	127.00±15.70^{bc}	125.00±13.90^{b}	119.00±11.90^{b}
A. niger & L. flavidum	169.00±14.50^{d}	180.00±18.50^{d}	184.00±16.40^{e}	190.00±20.10^{d}	159.00±14.60^{d}

Values are Mean ± SD, Values with different superscript letters down the column are significantly different at $p < 0.05$, using Duncan Multiple range test.

Table 3: Effect of pH on Xylanase Enzyme Production from Solid State Fermentation of Corn Cobs (U/L)

Fermenting fungi	**pH 4**	**pH 5**	**pH 6**	**pH 7**	**pH 8**
T. reesei	172.00±13.10^{d}	177.00±11.90^{d}	193.00±13.20^{c}	210.00±14.20^{d}	221.00±15.10^{e}
Lenzites betulina	146.00±10.60^{c}	150.00±13.50^{c}	155.00±12.60^{b}	159.00±14.50^{c}	160.00±9.40^{c}
A. niger	101.00±7.20^{b}	105.00±8.60^{b}	134.00±11.70^{ab}	130.00±12.60^{ab}	129.00±13.20^{ab}
Lach. flavidum	104.00±10.20^{b}	123.00±13.10^{b}	155.00±12.00^{b}	154.00±11.50^{c}	150.00±12.60^{b}
T. reesei & L. betulina	145.00±11.30^{c}	155.00±13.40^{c}	158.00±14.50^{b}	166.00±10.80^{c}	160.00±14.00^{c}
T. reesei & A. niger	134.00±12.60^{c}	145.00±11.50^{c}	156.00±13.70^{b}	167.00±15.30^{c}	180.00±16.80^{d}
T. reesei & L. flavidum	199.00±11.40^{e}	207.00±12.20^{e}	219.00±13.40^{d}	231.00±11.90^{d}	225.00±16.50^{e}
L. betulina & A. niger	78.00±4.30^{a}	84.00±5.60^{a}	124.00±9.60^{a}	122.00±10.50^{a}	120.00±11.30^{a}
L. betulina & L. flavidum	139.00±13.10^{c}	144.00±11.20^{c}	156.00±14.20^{b}	146.00±12.90^{b}	122.00±10.20^{a}
A. niger & L. flavidum	225.00±15.40^{f}	236.00±12.70^{f}	217.00±12.30^{d}	211.00±11.50^{d}	205.00±14.30^{e}

Values are Mean ± SD, Values with different superscript letters down the column are significantly different at $p < 0.05$, using Duncan Multiple range test.

Table 4: Effect of Various Moisture Content on Xylanase Enzyme Production from Solid State Fermentation of Corn Cobs. (U/L)

Fermenting fungi	**60%**	**65%**	**70%**	**75%**	**80%**	**85%**
T. reesei	175.00±9.20^{cd}	190.00±13.40^{c}	110.00±12.50^{b}	150.00±11.90^{c}	97.00±7.80^{a}	175.00±15.10^{b}
Lenzites betulina	110.00±12.30^{ab}	111.00±10.60^{a}	73.00±7.20^{a}	69.00±6.80^{a}	93.00±9.70^{a}	88.00±5.50^{a}
A. niger	201.00±17.80^{e}	230.00±18.20^{d}	180.00±10.70^{d}	210.00±15.60^{de}	167.00±15.40^{c}	199.00±13.80^{cd}
Lach. flavidum	195.00±14.60^{d}	211.00±12.50^{cd}	202.00±13.40^{e}	195.00±14.50^{d}	180.00±12.40^{cd}	205.00±12.50^{cd}
T. reesei & L. betulina	155.00±10.40^{c}	167.00±13.50^{b}	149.00±10.30^{c}	111.00±11.50^{b}	126.00±13.60^{b}	189.00±15.80^{bc}
T. reesei & A. niger	210.00±16.20^{ef}	231.00±17.40^{d}	219.00±13.90^{e}	221.00±12.40^{e}	209.00±11.50^{e}	210.00±10.70^{cd}
T. reesei & L. flavidum	216.00±12.20^{ef}	215.00±11.70^{d}	211.00±13.50^{e}	219.00±12.40^{e}	210.00±13.90^{e}	215.00±10.60^{d}
L. betulina & A. niger	94.00±6.70^{a}	97.00±9.80^{a}	86.00±6.90^{a}	75.00±7.50^{a}	88.00±6.80^{a}	105.00±8.65^{a}
L. betulina & L. flavidum	118.00±11.60^{b}	120.00±12.30^{a}	116.00±11.80^{b}	99.00±9.10^{b}	98.00±10.30^{a}	108.00±10.80^{a}
A. niger & L. flavidum	221.00±15.20^{f}	229.00±10.40^{d}	201.00±14.30^{e}	215.00±12.60^{de}	199.00±13.50^{d}	210.00±12.00^{cd}

Values are Mean ± SD, Values with different superscript letters down the column are significantly different at $p < 0.05$, using Duncan Multiple range test.

Table 5: Effect of Various Nitrogen Sources on Xylanase Enzyme Production from Solid State Fermentation of Corn Cobs. (U/L)

Fermenting fungi	**Yeast**	**Peptone**	**Urea**	**Ammonium**	**Sodium**	**Potassium**
T. reesei	170.00±3.40^{ef}	185.00±6.50^{f}	150.00±5.60^{d}	149.00±7.10^{de}	95.00±2.90^{bc}	115.00±6.70^{d}
Lenzites betulina	86.00±8.50^{a}	72.00±5.40^{a}	94.00±6.80^{a}	105.00±8.10^{ab}	93.00±4.60^{b}	99.00±7.40^{b}
A. niger	125.00±6.20^{b}	131.00±4.00^{bc}	119.00±7.60^{bc}	106.00±5.90^{ab}	96.00±3.20^{bc}	102.00±4.50^{c}
Lach. flavidum	145.00±10.50^{cd}	153.00±9.60^{de}	133.00±11.20^{cd}	129.00±9.30^{c}	115.00±7.90^{d}	105.00±10.30^{c}
T. reesei & L. betulina	153.00±9.80^{de}	144.00±12.40^{d}	130.00±9.90^{c}	118.00±10.00^{b}	125.00±8.70^{de}	109.00±7.90^{cd}
T. reesei & A. niger	181.00±13.50^{f}	189.00±14.00^{f}	173.00±11.60^{e}	160.00±9.50^{e}	165.00±15.00^{f}	122.00±12.00^{e}
T. reesei & L. flavidum	121.00±11.20^{b}	134.00±13.10^{c}	122.00±10.70^{c}	130.00±9.20^{c}	118.00±10.60^{d}	120.00±8.10^{de}
L. betulina & A. niger	111.00±12.40^{b}	115.00±11.90^{b}	103.00±9.50^{ab}	98.00±7.60^{a}	76.00±5.90^{a}	82.00±8.30^{a}
L. betulina & L. flavidum	129.00±14.20^{bc}	120.00±13.10^{b}	127.00±12.70^{c}	109.00±9.80^{ab}	110.00±10.30^{c}	95.00±7.60^{ab}
A. niger & L. flavidum	173.00±15.30^{f}	157.00±11.40^{e}	148.00±10.50^{d}	145.00±12.60^{d}	128.00±10.90^{e}	131.00±13.20^{f}

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

Table 6: Hemicellulose and Cellulose Fractions from fungal Mono and Co-culture Solid State Fermentation of Corn Cobs (%)

Fungi	Cellulose	Hemicellulose
Control	39.40±4.30^{g}	42.00±4.00^{f}
Unfermented	35.30±2.90^{f}	40.10±3.90^{f}
Trichoderma reesei	12.60±0.84^{ab}	15.30±1.30^{a}
Lenzites betulina	20.40±2.10^{de}	18.90±1.70^{bc}
Aspergillus niger	15.70±1.60^{bc}	19.20±1.90^{bc}

Lachnocladium flavidum	18.20±1.80^{cd}	21.30±2.00^{c}
T. reesei & L. betulina	22.50±1.72^{e}	20.90±1.85^{bc}
T. reesei & A. niger	10.50±1.20^{a}	16.70±1.45^{ab}
T. reesei & Lach. Flavidum	11.90±1.50^{ab}	17.00±2.00^{b}
L. betulina & A. niger	18.70±1.40^{cd}	29.20±3.10^{e}
L. betulina & Lach. Flavidum	20.90±2.10^{de}	22.00±1.80^{d}
A. niger & Lach. Flavidum	15.20±1.50^{bc}	17.90±1.60^{b}

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

Discussion

As fermentation progresses, nutrient depletion usually occurs in the media and this could lead to reduction in growth and consequent enzyme production, this could be complemented by the accumulation of end products which are toxic in nature. Higher xylanase activities were recorded after seven (7) days of incubation with most fermenting fungi. *T. lanuginosus* has been reported to produce xylanase optimally after 7 days (Kumkum et al., 2013), while maximum of five days of incubation was required by *A. niger* when grown rice straw under solid state fermentation. (Kang et al., 2004). 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.

Adegboye et al., (2021) reported that most production of enzymes associated with growth of organisms is usually directly proportional to microbial biomass. At lower inoculum concentrations, higher xylanase activities were recorded with mixed cultures. Large inoculum size decreases enzyme production and growth of organism in the lag phase, whereas smaller inoculum size increases the lag phase. (Soni et al., 2023). *Aspergillus terreus* was reported to produce maximum xylanase using 1.2 x10^4 spores per 2.0 ml (Ghanem *et al.*, 2000). It has been generally suggested that in solid state fermentation, lower inoculum concentrations do not support mycelial expansion and product formation.

The effect of pH on microbial growth and the production of enzymes is considered to be an important (Akhavan et al., 2020). What happened in your own experiment? What was the effect of pH in your work? Effect of pH on xylanase production shows that xylanase activity was significantly high following fermentation at pH 5-7. Sherief et al*.,* (2012) reported maximum enzyme production by *A. fumigatus* and *A. terreus* at pH 6.0 and 7.0. The optimal pH for the activity and growth of organisms usually occurs within a range of pH and this influences the transport systems of enzymes across cell membranes (Lin et al*.,* 2013)

In solid state fermentation, microbial growth and activity is critically affected by an appropriate level of moisture (Almowallad**,** et al., 2022). In this research, most cultures exhibited better activities at medium moisture contents 65%. The results also indicate that xylanase activities decreased under lower (33-60%) and higher (83-88%) moisture contents. In SSF, most microbial viable cells require about 60-80% moisture content for new cell synthesis (Sun, 2022).

Nitrogen sources are basically involved in protein synthesis by serving as regulators for the precursors involved the production of extracellular

enzymes in majority of microbial fungi. Fermentation with mono-culture of *A. niger* had the highest xylanase activity. This result is similar to other results where *A. niger* and *A. fumigatus* showed maximum xylanase activity using peptone as nitrogen source (Betini, et al., 2009). In general, organic nitrogen sources being more complex and richer in nitrogen content, favor cell mass production more than inorganic nitrogen sources. (Devi et al., 2022).

The success in the utilization of most lignocellulosic and potentially renewable carbon sources depends on the success of economically friendly and feasible biotechnological approaches to hydrolytic enzyme production (Behera and Ray, 2016). *T. reesei* proved to be the most efficient hemicellulose degrader, with 15% reduction and the 10% reduction in hemicellulose was observed with the co-fermentation with *A. niger* and *T. reesei*.

Conclusion

The production of xylanase and the simultaneous breakdown of maize cobs following the single and mixed fermentations using the consortia of fungi was demonstrated in this study. The ability to produce xylanase to different degrees was demonstrated by all the fermenting fungi. *L. flavidum* was also found to be most effective xylanase producer with optimized conditions at pH of 5.5, moisture 75%, inoculum conc at 5-6 x 10^3 spores/ml, incubation period of 7-9 days, 1% peptone and glucose as media supplements.

All fermenting fungi also showed ability to reduce the cellulose and hemicellulose levels to different degrees. With co-cultures of *T. reesei and A. niger* standing out with 10% cellulose reduction and *T. reesei* standing out with 15% hemicellulose reduction. By screening and optimizing the culture conditions for xylanase production, better degradation of corn cobs for biotechnological use was has been enhanced.

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