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# β 1,4-Xylosidase Production Potential of a *Fusarium sp.* on Wood Shavings

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Author FCA wrote the first draft of the manuscript, managed the literature searches managed the analyses of the study and performed the statistical analyses. Author AAO designed the work and wrote the protocol. All authors read and approved the final manuscript.*

***Research Article***

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## ABSTRACT

**Aims:** This study was aimed at screening fungal isolates from degrading wood samples for β 1,4-xylosidase production, selecting the best isolate based on the screening test to produce the enzyme through solid state fermentation of some wood shavings and determining optimum production conditions for the fungus on best substrate.
**Place and Duration of Study:** Microbial physiology laboratory, Department of Microbiology, University of Ibadan, Ibadan and Multi Discplinary Central laboratory University of Ibadan, Oyo State Nigeria. Between September 2010 and October 2011.
**Methodology:** Isolates obtained from degrading wood samples were identified and screened on agar plates of para–nitrophenyl β-xyloside as carbon source. Selected isolates were used to produce β 1,4-xylosidase using shavings of *Anogeissus leiocarpus, Gmelina arborea* and *Terminalia superba* moistened with a chemically defined medium as substrates. Assay was done every 3 days for 15 days. Production dependent parameters such as pH, temperature were varied on the best wood substrate for β 1,4-xylosidase production by selected fungus.
**Results:** Selected isolate was used for enzyme production on shavings of *Anogeissus leiocarpus, Gmelina arborea* and *Terminalia superba* as substrates, each was moistened with chemically defined medium. Assay was done every 3 days for 15 days. Production

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dependent parameters such as pH, temperature were varied on the best wood substrate for β 1,4-xylosidase production by fungus. Maximum β 1,4-xylosidase activity of fungus on each substrate was; *Anogeissus leiocarpus*, 31.620Ug$^{-1}$ on 12$^{th}$ day, *G. arborea,* 8.935U g$^{-1}$ on day 9 and *T. superba,* 6.053Ug$^{-1}$ on 6th day. Optimum β 1,4-xylosidase production was obtained on *A. leiocarpus* at 35ºC and pH 6 with 40% and 60% aeration. 50% moisture content of substrate supplemented with soy meal as nitrogen source supported the best β 1,4-xylosidase production by fungus.
**Conclusion:** Enzyme production was highly enhanced at optimum conditions.

*Keywords: β 1,4 xylosidase; wood substrates; screening; Anogeissus leiocarpus; optimisation.*

## 1. INTRODUCTION

Wood shavings and chips indiscriminately litter our environment in Nigeria and the common method of its disposal (burning) is environmentally hazardous. However, this waste could serve as a cheap substrate for production of industrially useful enzymes such as β 1,4-xylosidase (EC 3.2.1.37) by solid state fermentation (SSF). Xylan is one of the major hemicellulose components of lignocellulosic biomass, second to cellulose [1]. In synergistic action with β-xylanases (EC 3.2.1.8) and debranching enzymes, like α-glucuronidases, and glycosidases, β 1,4-xylosidase can potentially convert xylan into fermentable xylose for ethanol and methane production [2]. It is an industrially important biocatalyst: in the food industry, it is employed for hydrolysis of bitter compounds from grape fruit during juice extraction and liberation of aroma from grapes during wine making [3]. β 1,4-xylosidase releases xylose which act as inducer of hemicellulases from oligomeric saccharides [4,5]. It is also used in the production of biosurfactants from xylose or its polymers and alcohols of different sizes [6,7]. Its surface-active properties make it useful in pharmaceutical and cosmetic industries [8]. β 1,4-xylosidase from different sources find applications in biobleaching of pulp in the paper and pulp industry [5]. SSF offers advantages over liquid fermentation; especially for fungal cultures and it has considerable economic potential in agricultural, food, feed, pharmaceutical and pulp and paper industries (9). Research has proved that β-xylosidase level is generally higher in fungi than in other microorganisms [10]. Reducing the costs of enzyme production by optimizing the fermentation medium and the process is the goal of basic research for industrial applications [11].Therefore, this work is aimed at using a selected fungus obtained from degrading wood to produce β 1,4-xylosidase in solid state fermentation of some selected wood shavings as substrates and to determine the optimum production conditions β 1,4-xylosidase production by this fungus on the best substrate.

## 2. METHODOLOGY

### 2.1 Sample Collection

Wood samples were obtained from Bodija plank market and sawmills in Ibadan, Nigeria. Specific fractions of each of the wood samples were made to undergo spontaneous degradation for six months.

## 2.2 Isolation Procedure

Isolation of fungi from the degrading wood samples was done by the methods of Onilude, [12] first on two weeks basis for a period of three months and later at four weeks interval for another three months.

## 2.3 Identification of Isolates

Fungal isolates were identified by comparing their cultural and morphological characteristics with those described in Domsch *et al*., [13], and Kiffer Morelet, [14]. The selected fungus was maintained on Potato Dextrose Agar (PDA) slant at 4ºC.

## 2.4 Primary Screening of Isolates

Obtained isolates were screened on agar plates using a modified method of Downie *et al*., [15] with para – nitrophenyl -β-xyloside as the only carbon source incorporated into a chemically defined medium made up of the following constituents: ($gl^{-1}$): 0.05 $MgSO_4.7H_20$, 0.005 $CaCl_2$, 0.005 $NaNo_3$, 0.009 $FeS0_4.7H20$, 0.002 $ZnSO_4$, 0.012 $MnSO_4$, 0.23 KCl, 0.23 $KH_2PO_4$, 2 peptone, 19 Agar (LAB M).

## 2.5 Preparations of Fungal Inoculum

Inoculums for the solid state fermentation of the wood substrates were prepared by introducing 20ml of 0.1% Tween 80 solution into Potato Dextrose Agar slants containing sporulating 5 day old cultures of the organism. The suspension was kept under agitation with a magnetic stirrer. 0.5ml of the spore suspension was introduced in a Neubauer chamber using a Pasteur pipette and counted until the amount in ml of fungal suspension that will give $10^6$ spore $ml^{-1}$ was obtained.

## 2.6 Pretreatment of Wood Samples and Inoculation of Substrates

Wood samples of *Anogeissus leiocarpus, Gmelina arborea* and *Terminalia superba* were differently used as sole carbon source for the fungus in SSF. Each wood sample was made into shavings of size 2mmx 2mm [16] using saw milling machine and subjected to thermal treatment at 121ºC for one hour [17]. The moistening medium was prepared according to Giselle *et al.,* (18) with the following composition in $gl^{-1}$ of 50mM citrate buffer: $K_2HPO_40.23$, $MgSO_4 \cdot 7H_2O$ 0.05, $CaCl_20.005$, $NaNO_3$ 0.05, $FeSO_4 \cdot 7H_2O$ 0.009, $ZnSO_4$ 0.002 ,$MnSO_4$ 0.012 using 7g of peptone as the organic nitrogen source and the pH adjusted to 5.6. 10g of each of the wood substrates was measured into 250ml Erlenmeyer flask and moistened up to 70% using the liquid moistening medium. The moistened wood chip substrates were autoclaved for 15 min at 121ºC and allowed to cool to ambient temperature. Cooled substrates were each inoculated with spore suspension of $10^6$ $sporesg^{-1}$ of dry matter. After thorough mixing, the flasks were incubated at 28ºC ± 2ºC for 15 days in static mode. Replicates of the flasks were also subjected to same treatment.

## 2.7 Separation of Enzyme from Fungal Mycelia

Enzyme extraction was carried out on the 3rd, 6th, 9th 12th and 15th day of incubation following a modified method of Giselle *et al.,* [18] by using 50mM citrate buffer (pH5.6) instead of 0.9% NaCl containing 0.1% tween 80. 30ml of the buffer was poured into the fermentation tube

and stirred at 120 rpm in a shaker for 5 minutes. Solids were filtered under vacuum through Whatman no. 1 filter paper and the filtrate was further centrifuged at 4ºC in a cold centrifuge machine (Model: IEC B-20A) at 15,000 rpm for fifteen minutes. The supernatant was used as the crude enzymatic extract and kept at 4ºC.

## 2.8 Determination of β 1,4-xylosidase Activity

This was done according to the method Baldrian and Gabriel, [19] using 50mM citrate buffer (pH5.6). β 1,4-xylosidase activity of the crude enzyme extract was assayed at 3 days intervals in a reaction mixture (1 ml) containing 0.5ml of 2 milli mol $l^{-1}$ *p*-nitrophenyl-β- -xyloside (*p*NPβX) in 50 mM citrate buffer(pH 5.6) and 0.5ml of appropriately diluted enzyme solution. After incubation at 50ºC for 30 min, the reaction was stopped by adding 1ml of ice-cold 0.5 M $Na_2CO_3$ and the color that developed as a result of *p*-nitrophenyl (*p*NP) liberation was measured at 410 nm. A standard curve was plotted using optical density values obtained at different concentrations of *p*-nitrophenyl (*p*NP) to determine the sugar (*p*NP) concentration within each assayed reaction mixture. One unit (U) of endo-β 1,4-xylosidase activity was defined as the amount of enzyme that liberated 1 µmol *p*NP per minute in the reaction mixture under these assay conditions. Data was presented as averages and standard errors of three replicate experiments.

## 2.9 Optimization of Production Conditions for *β* 1,4-xylosidase by *Fusarium compactum* through Solid State Fermentation of *A. leiocarpus*

The substrate that supported the best β 1,4-xylosidase production by the fungus was utilised for these tests.

### 2.9.1 Effect of different initial aeration condition on β 1,4-xylosidase production by *F. compactum*

The moistened substrate with initial pH of 5.6 was subjected to different initial aeration of 30, 40, 50, 60, 70 (%) using different initial concentrations of thioglycolate to give the required initial aeration followed by inoculation with the fungus. The flasks were incubated at 28ºC ± 2ºC, for 12 days, after which the enzyme activity was determined.

### 2.9.2 Effect of different inoculum sizes on β 1,4-xylosidase production

Spore counts of $10^3$,$10^6$, $10^9$, $10^{12}$ and $10^{15}$ of five day old cultures of the fungus were used as inoculums in Erlenmeyer flasks containing initial production substrate. The flasks were incubated at 28ºC ± 2ºC for 12 days and β-xylosidase activity was determined afterwards.

### 2.9.3 Effect of different initial moisture content on β 1,4-xylosidase production by the fungus

The substrate in 250 ml Erlenmeyer flasks were moistened at different initial moisture levels of 30%, 40%, 50%, 60% and 70% separately using the moistening solution in 50mM citrate buffer with pH adjusted to 5.6. Flasks were afterwards inoculated with appropriate spore suspension of fungus and incubated at 28ºC ± 2ºC for 12 days after which β-xylosidase activity was determined as previously described.

### 2.9.4 Effect of various nitrogen sources on β 1,4-xylosidase production by the fungus

The moistening medium used for this test was the initial one described previously but the nitrogen source was differently replaced by yeast extract, peptone, soy meal, corn steep liquor and urea using the same proportion as the one used for the nitrogen source employed at initial production of enzyme and maintaining the same pH. The different flasks containing the substrate was there after moistened with the media differently, sterilized, cooled, inoculated accordingly and incubated at 28ºC ± 2ºC for 12 days. β 1,4-xylosidase activity of the media was determined thereafter.

### 2.9.5 Effect of various initial pH of substrate on β 1,4-xylosidase production by the fungus

The pH of the moistening medium was adjusted with 50mM sodium citrate or citric acid to various pH values ranging from 4,5,6,7 to 8 and applied separately to the substrate in different flasks and sterilized. The sterilized flasks containing the medium was inoculated with the appropriate fungal inoculums and incubated at 28ºC ± 2ºC for 12 days. β 1,4-xylosidase activity of the medium in each flask was determined thereafter.

### 2.9.6 Effect of varied incubation temperature on β 1,4-xylosidase production by the fungus

The fermenting medium was prepared according to the method of Giselle *et al.,* [18] using shavings of *A. leiocarpus* and incubated for twelve days. The temperatures of incubation tested were 30ºC, 35ºC, 40ºC, 45ºC and 50ºC.

Best production conditions were determined based on the evaluated parameters.

## 3. RESULTS AND DISCUSSION

A total of 220 fungal isolates were obtained from the degrading wood samples, isolates that did not show any β 1,4-xylosidase activity in the screening were not identified while those that showed activity were selected and identified. Identification of the fungal isolates obtained and their frequencies of occurrence during isolation respectively were *Emericella nidulans*(11)*, Aspergillus flavus*(8), *Aspergillus niger(45*), *Fusarium chlamydosporium* (17), *Fusarium compactum*(15), *Fusarium oxysporium*(14), *Penicillium purpurogenum*(5), *Rhizopus spp.*(33), *Trichoderma harzianum*(37), *Trichoderma reesei*(21). (Table 1) shows that *Fusarium compactum* had the highest average relative β 1,4-xylosidase activity 13.3%±0.01 among the screened isolates. This informs why the isolate was chosen for this work.

Solid-state fermentation (SSF) has many advantages over submerged fermentation. These include higher productivity, simpler technology, and lower capital investment, lower energy demand, less wastewater output, better and economical product recovery and lower foam build-up [20,21].

**Table 1. Relative β 1,4-xylosidase activities of fungal isolates from degrading wood**

| Isolates and Codes | Average relative xylosidase activities (%) |
|---|---|
| *Emericellanidulans*ENTS4 | 12.1 ± 0.00 |
| *Aspergillus flavus*AFAL5 | 12.3 ± 0.00 |
| *Aspergillus niger* ANGA1 | 12.6 ± 0.02 |
| *Fusarium chlamydosporium*FCGA7 | 12.0 ± 0.01 |
| *Fusarium compactum* FCAR4 | 13.3 ± 0.01 |
| *Fusarium oxysporium*FOSM1 | 11.7 ± 0.01 |
| *Penicilliumpurpurogenum*PPMA3 | 11.4 ± 0.02 |
| *Rhizopus spp.*RSMA3 | 10.2 ± 0.00 |
| *Trichoderma harzianum*THMA1 | 12.4 ± 0.01 |
| *Trichoderma reesei* TRTS4 | 12.3 ± 0.01 |

*Each value is a mean of triplicate determinations + mean error*

β 1,4-xylosidase activity of the fungus increased on wood substrates as compared to what was obtained from the plate screening test which may be an indication that plate screening method may only be used as a preliminary test. From the result on (Figure 1), all the substrates supported the production of β 1,4-xylosidase by the fungus but *A.leiocarpus* supported the highest β 1,4-xylosidase production by this fungus. The endo-β 1,4-xylosidase activity of the fungus on *A.leiocarpus* was highest on the 12$^{th}$ day with 31.620Ug$^{-1}$of dry weight (dw) of substrate while on *G. arborea*, it was highest on the 9$^{th}$ day with a value of 9.315Ug$^{-1}$ dw and on *T. superba* the highest activity obtained was on the 6$^{th}$ day which was 6.063Ug$^{-1}$ dw. The fact that *A.leiocarpus* supported the highest β 1,4-xylosidase activity of the fungus among the substrates on all the days assay was done may be because this substrate may have higher available hemicellulose content than others or that its xylan component serves as a preferred carbon source for the fungus. It is also possible that the substrate is more easily biodegraded by the fungus than the other substrates as a result of easier access of the fungus to the hemicellulose components of this substrate.

Enzyme production however reduced swiftly on the 15$^{th}$ day of incubation on this substrate, this could be as a result of exhaustion of available nutrient by the fungus or the accumulation of secondary metabolites which are capable of impairing β 1,4-xylosidase production by the isolate. From the value of β 1,4-xylosidase activity on 15$^{th}$ day, it is possible the enzyme had started degrading probably because of proteases released by the fungus.

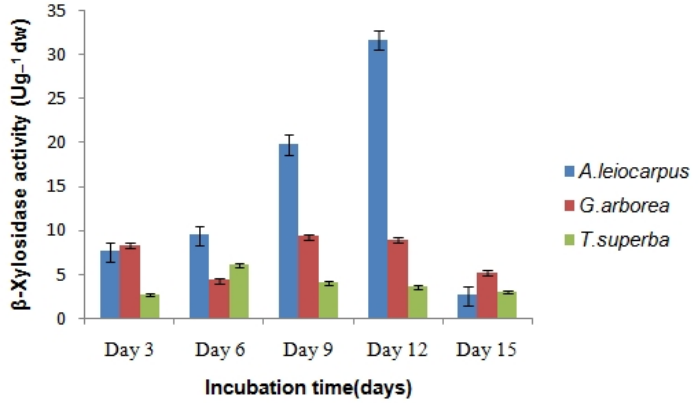


**Figure 1. β 1,4-xylosidase activity(Ug$^{-1}$dw) of *Fusarium compactum* FCAR4 on different wood shavings at different days of incubation**

All enzyme-based biotechnological processes require optimization to increase their activities in the culture supernatant by selecting the best carbon and nitrogen sources and optimizing other process variables [22]. For the optimisation of production condition test, (Figure 2a) showed that soy meal supported the best β 1,4 xylosidase production by the fungus (35.754$Ug^{-1}$dw) among all the nitrogen sources employed in this work. Although peptone served as a very good nitrogen source for the production of β 1,4-xylosidase by the fungus supporting an activity of 32.394 $Ug^{-1}$dw (Figure 2a), corn steep liquor could also serve as an equally good nitrogen source for production of β 1,4-xylosidase by the fungus as seen in (Figure 2a) (31.747 $Ug^{-1}$dw). The low cost of corn steep liquor precludes the use of peptone for large-scale fermentation and this will help reduce production cost of the enzyme especially in developing countries like Nigeria. Saha [23] also reported a good support of β 1,4-xylosidase production when soy meal was used as nitrogen source.

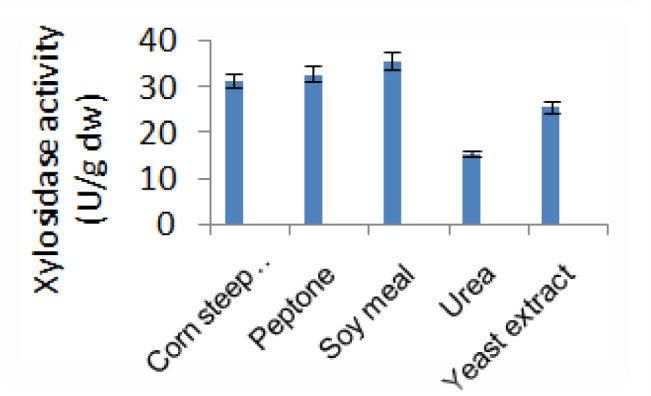


**Figure 2a. Effect of Different Nitrogen Sources on production of β 1,4-xylosidase by *F.compactum*FCAR on *A. leiocarpus* as substrate**

The critical importance of moisture level in SSF media and its influence on the biosynthesis and secretion of enzymes can be attributed to the interference of moisture with the physical properties of the solid particles [24]. Results shown on (Figure 2b) revealed that the fungus produced β 1,4-xylosidase optimally at 50% moisture content giving a β 1,4-xylosidase activity of 33.020$Ug^{-1}$dw and enzyme production was observed to increase with moisture content from 30% - 50% until there was drop at 60% and 70% moisture contents progressively. An increase in moisture level has been reported to wet through the substrate (leaving free water); besides, increase in moisture content is also believed to reduce the porosity of the substrate, thus limiting oxygen transfer [9]. This explains why there was a drop of β 1,4-xylosidase activity at 60% and 70% after a peak of activity at 50% moisture content. The least β 1,4-xylosidase activity was at 30% moisture content and 40% moisture content also supported low β 1,4-xylosidase activity. Ellaiah *et al.,* [25] reported that low moisture content cause reduction in the solubility of nutrients within the substrate and low degree of swelling, thus the low β 1,4-xylosidase activity at 30% and 40% moisture content. The critical importance of moisture level in SSF media and its influence on the biosynthesis and secretion of the enzyme can be attributed to the interference of moisture with the physical properties of the so lid particles. High substrate moisture results in decreased substrate porosity, which in turn prevents oxygen penetration. At the same time, low moisture level leads to poor microbial growth and poor accessibility to nutrients [26]. Different microorganisms show better performance at different moisture levels.

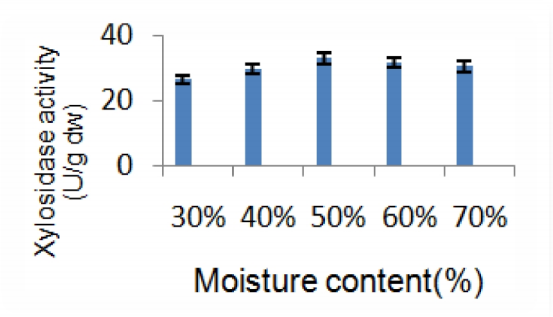


**Figure 2b. Effect of Varied Moisture Content on production of β 1,4-xylosidase by *F.compactum*FCAR on *A. leiocarpus* as substrate**

Inoculum size affected xylosidase production as shown on (Figure 2c) β 1,4-xylosidase production by *F. compactum* increased with inoculum size from $10^3$ to $10^{12}$ and declined at $10^{15}$. Lowest production of the enzyme was at$10^3$ followed by $10^{15}$ with an activity of 25.895$Ug^{-1}$dw and 28.948$Ug^{-1}$dw respectively. High inoculum size has been reported to cause dense fungal ramification and rapid utilisation and exhaustion of available nutrient in SSF [24]. Reduction in available nutrient will reduce enzyme activity this may be why low β 1,4-xylosidase activity was recorded at high inoculum size($10^{15}$). The peak enzyme production was obtained at $10^{12}$ inoculum size which gave a β 1,4-xylosidase activity of 34.895$Ug^{-1}$dw.

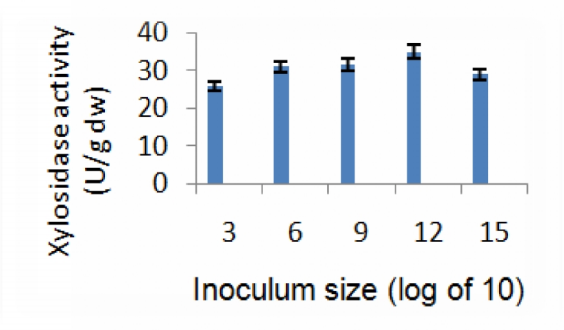


**Figure 2c. Effect of Different Inoculum Sizes on production of β 1,4-xylosidase by *F. compactum* FCAR on *A. leiocarpus* as substrate**

When aeration of the fermentation medium was varied the enzyme production was at its peak at 40% aeration while the production of the enzyme was lowest at 70% aeration as shown on (Figure 2d).

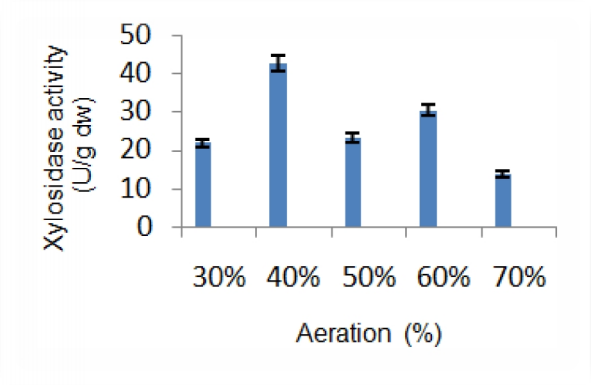


**Figure 2d. Effect of Varied Aeration of Fermentation Medium on production of β 1,4-xylosidase by *F. compactum* FCAR on *A. Leiocarpus***

Temperature is the most important variable in the SSF process [27].

(Figure 2e) shows that the best enzyme activity was obtained at 35ºC however the production of β 1,4-xylosidase by the fungus was still relatively higher from 40ºC to 50ºC than what was obtained at 30ºC making it a good candidate for industrial processes and large scale production of the enzyme.

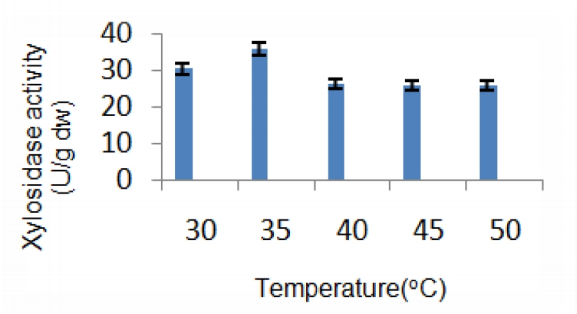


**Figure 2e. Effect of Different Temperature of Incubation on production of β 1,4-xylosidase by *F. compactum* FCAR on *A. leiocarpus* as substrate**

β 1,4-xylosidase activity was observed to increase progressively from pH 4 to pH 6 where the peak was recorded and started to drop from pH 7 to pH 8 where the lowest activity was recorded. The result shown on (Figure 2f) for the influence of pH on the enzyme production of the fungus is in agreement with that obtained by Macielli, [24] whose optimum pH fell within the range of 6 and 6.5. Other fungal species were reported to show higher β 1,4-xylosidase yields when the initial pH was kept below 6.0 [5].

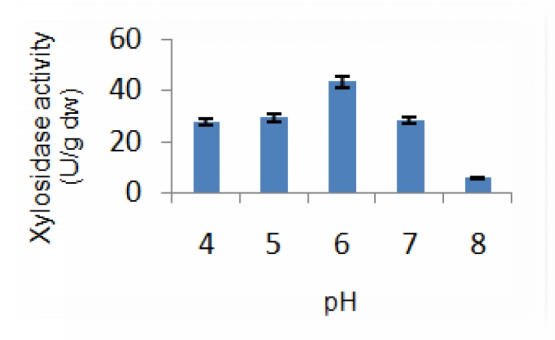


**Figure 2f. Effect of initial pH variation on β 1,4-xylosidase production by *Fusarium compactum*FCAR on *A .leiocarpus* as substrate**

## 4. CONCLUSION

This work has shown that *Fusarium compactum* strain obtained from degrading wood in this work is capable of producing beta 1,4-xylosidase considerably at favourable moisture content, temperature, pH, inoculums size, initial aeration and using appropriate organic nitrogen source as obtained in this work. This is a pointer to a better way of utilising wood wastes in generating income in Nigeria. This will also make the environment safer to thrive in.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Jiguang ZQ, Cong Q, Yan NK, Du X. Characterisation of a thermostable xylanase from *Chaetomium* sp. and its application in Chinese steamed bread. Food Chem. 2010;120:457-62.
2. Basaran P, Ozcan M. Characterization of beta-xylosidase enzyme from *Pichiastipis*. Bioresource Technol. 2008;99:38-43.
3. Romanowska I, Polak J, Bielecki S. Isolation and properties of *Aspergillus niger* IBT-90 xylanase for bakery. Applied Microbiol. Biotechnol. 2006;69:665-71.
4. Afzal AJ, Latif F, Rajoka MI, Siddiqui KS. Innovative kinetic and thermodynamic studies of a purified superactive xylanase from *Scopulariopsis* sp. Appl. Biochem Biotechnol. 2005;120:51-7.
5. Sonia KG, Chadha BS, Saini HS. Sorghum straw for xylanase hyper-production by *Thermomyces lanuginosus* (D2W3) under solid-state fermentation. BioresourTechnol. 2005;96:1561-69.
6. Kurakake M, Osada S, Komaki T. Transxylosylation of β-xylosidase from *Aspergillusawamori* K4. Biosci Biotechnol Biochem.1997;61:2010-14.

7. Lin Y, Lee Y, Tseng M, Lee W. Xylooligosaccharides as By-products of Fuel Ethanol from Cellulosic Biomass. Innovative Bio-production of Fuels and Chemicals from Renewable Resources (i-BioS) Singapore, 21st-22nd January. 2013;26.
8. Levasseur A, Asther M, Record E. Overproduction and characterization of xylanase B from *Aspergillus niger*. Canadian J Microbiol. 2005;51(2):177-83.
9. Pandey A, Soccol CR, Nigam P, Soccol VT, Vandenberghe LPS, Mohan R. Biotechnological potential of agro industrial residues. II: Cassava bagasse, Bioresour Technol. 2000;74:81-7.
10. Brink J, Vries RP. Fungal enzyme sets for plant polysaccharide degradation. Appl Microbiol Biotechnol. 2011;91(6):1477-92.
11. Park Y, Kang S, Lee J, Hong S, Kim S. Xylanase production in solid state fermentation by *Aspergillus niger* mutant using statistical experimental designs, Appl. Microbiol. Biotechnol. 2002;58:761-6.
12. Onilude AA. Effect of cassava cultivar, age and pretreatment processes of cellulase and xylanase production from cassava waste by *Trichoderma harzianum.* J Basic Microbiology.1996;36:421–31.
13. Domsch KI, Gam W, Anderson TH. Compendium of soil fungi. Vol.1. Academic Press, London: U.K; 1980.
14. Kiffer E, Morelet M. The Deuteromycetes. Microscopic Fungi Classification and Generic Keys. Science Publishers Inc, U.S.A; 2000.
15. Downie B, Hilhorst WM, Bewley JD. A new assay for quantifying endo-beta-mannanase activity using congo red dye. Phytochemistry. 1994;36:829-35.
16. Kirk TJ, Jeffries TW. Roles of microbial enzymes in pulp and paper processing. ACS Symposium Series.1996;655:2-14.
17. Goyal A, Kalra KL, Sareen VK, Soni G. Xylanase production with xylan rich lignocelluloses wastes by a local soil isolate of *Trichoderma viride*. Braz. J. Microbiol. 2008;39(3):1-10.
18. Giselle MM, Luciana P, Vandenberghel C, WindsonIsidoro H, Ricardo CF, Eli Della B, Tahiana Q, Brandalize IAP. Carlos RS. Xylanase Production by *Aspergillus niger* LPB326 in Solid-State Fermentation Using Statistical Experimental Designs ISSN 1330-9862 (FTB-2050); 2007.
19. Baldrian P, Gabriel J. Lignocellulose degradation by *Pleurotus ostreatus* in the presence of cadmium. FEMS Microbiology Letters. 2003;220:235-40.
20. Hölker F, Höfer M, Lenz J. Biotechnological advantages of laboratory-scale solid-state fermentation with fungi. Appl. Microbiol. Biotechnol. 2004;64:175-86.
21. Wang CL, Li DF, Lu WQ, Wang YH, Lai CH. Influence of cultivating conditions on the α-galactosidase biosynthesis from a novel strain of *Penicillium* sp. in solid-state fermentation. Lett Appld Microbiol. 2004;39:369-75.
22. Santos E, Piovan T, Roberto IC, Milagres AMF. Kinetics of the solid state fermentation of sugarcane bagasse by *Thermoascus aurantiacus* for the production of xylanase. Biotechnol Letter. 2003;25:13-6.
23. Saha BC. Purification and properties of an extracellular β-xylosidase from a newly isolated *F. proliferatum*. Bioresource Technol. 2003;90(1):33-8.
24. Macielli GM. Xylanase Production by *A. niger* in SSF, Food Technol Biotechnol. 2008;46(2):183–9.
25. Ellaiah P, Adinarayana K, Bhavani Y, Padmaja P, Srinivasulu A. Optimization of process parameters for glucoamylase production under solid state fermentation by a newly isolated *Aspergillus* species, Process Biochem. 2002;38:615-20.
26. Bhatti HN, Rashid MH, Nawaz R, Asgher M, Perveen R, Jabbar A. Optimization of Media for Enhanced Glucoamylase Production in Solid-State Fermentation by *Fusarium solani* Food Technol Biotechnol. 2007;45(1):51-6.

27. Bokhari IAS, Latif F, Akhtar WM, Rajoka IM. Characterization of a β – xylosidase produced by a mutant derivative of *Humicola lanuginosa* in solid state fermentation. Annals of Microbiology. 2010;10:1007/S 13213-010-0026-3.



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