Screening and Production of Xylanase by Thermophilic Microorganisms Isolated from Compost Using Sorghum Straw as Substrate Under Solid and Submerged Fermentation

[1]*Kazeem, M. O., [1]Fagbolagun, I. A. and [2]Agbaje, A. B.
[1] Department of Microbiology, Faculty of Life Sciences, University of Ilorin, Kwara State, Nigeria
[2]Department of Biological Sciences, Faculty of Natural and Applied Science, Al-Hikmah University, Kwara State, Nigeria
***Correspondence E-mails -** *kazeem.mo@unilorin.edu.ng*
Mobile: +2348130273407

Abstract
The development of renewable energy from lignocelluloic biomass is an economical and eco-friendly approach to the global energy crisis. The enzymatic hydrolysis of the biomass leads to xylose, as one of the fermenting sugars for bioethanol production. However, selection of a suitable microorganism for enzyme (xylanase) production plays a significant role in biomass conversion. This, however, requires selection of efficient xylanolytic organisms. The current study focuses on producing xylanase from selected bacteria (*Bacillus pumilus*, *Bacillus subtilis*, and *Micrococcus* sp.) and fungi species (*Aspergillus flavus*, *Aspergillus niger* and *Trichoderma viridae*) and enhancing their production capacities on sorghum straw substrate through solid state and submerge fermentation. Ability to produce xylanase was established by hydrolysis zone produced on xylan-agar plate. Among the fungi isolates, hydrolytic capacity reached the highest (2.0 mm) for *Aspergillus niger*, while *Bacillus subtilis* produced the highest (1.6 mm) among the bacteria species. *Aspergillus niger* showed the highest xylanase production (62.42 ± 0.78 U/mL) after 96 h. As for the bacteria isolates, the maximum xylanase activity (29.84±0.06 U/mL) was obtained at 48 h by *Bacillus subtilis.* Xylanase activity was further enhanced by mixed culture of bacteria species. Mixed culture of *Bacillus subtilis* and *Bacillus pumilus* improved xylanase activity to 33.58 U/mL. Xylanase production by the mixed culture grown on sorghum straw biomass resulted in a 1.7 – fold increase compared to commercial xylan. Findings suggest that sorghum straw has more economic advantage over xylan as a substrate for xylanase production by the test isolates.

1. Introduction

Lignocellulose are major source of organic material comprising of cellulose, hemicellulose and lignin. Forest waste, paper waste, agro-waste, wood waste among others are the major sources of lignocellulosic biomass. Biofuels, enzymes and biochemical products are some of the value added products obtained from lignocelluloses [1]. Some of the benefits provided by the use of lignocellulosic biomass include (i) reducing air pollution from burning and rotting in fields (ii) biomass is renewable and sustainable, (iii) carbon dioxide fixation (iv) creating job opportunities and (v) providing energy security [2]. Hemicellulose is the second largest polysaccharide in nature after cellulose consisting of β-1, 4 linked D-xylopyranosyl units linked with branches of O-acetyl, α-L-arabinofuranosyl and α-D-glucuronyl residues [3]. Xylanase together with other hemicellulase enzymes work synergistically for the complete degradation of hemicellulose. Therefore xylanase plays a vital role in lignocellulose degradation and has tremendous advantages especially in production fermentable sugars for bioethanol production [4] and the removal of hemicellulose /lignin linkages for bio-bleaching in the pulp and paper industry [5].

Diverse genera and species of microorganisms such as actinomycetes, bacteria, yeasts and molds have been recognized to be the rich sources of several xylanase. However, the recalcitrant nature of lignocellulosic biomass and enzyme efficiency are the major problems in lignocellulose bioconversion. These has led to continuous discovery of novel thermostable enzymes and efficient thermophilic microbes. Thermostable enzymes process tremendous advantages in lignocellulose deconstruction: (i) high specific activity (ii) high thermostability (iii) extends hydrolysis time (iv) reduces risk of contamination (v) reduces cost of cooling (vi) reduce viscosity. These benefits are of significant impotence in

reducing the overhead cost of biofuel production to which enzymes accounts for its one-half [1]. Thermophilic microorganisms are the main source of thermostable enzymes. The isolation of thermophilic microorganism from a thermophilic ecosystem is one of the quickest way in obtaining novel enzymes. For example *Geobacillus sp* MT-1, from deep sea produces a xylanase with a temperature optimum of 90 °C that is also active across the pH range 5.5–10 [6].

Compost microbes represent a potential source of commercially important bioactive compounds and their degradation capability is also remarkable. The development of compost is characterized by hostile environment such as high temperature. Therefore, exploring the compost niche for potential xylanase producing microbes is imperative. Among the many microbial sources, filamentous fungi are especially interesting as they secrete these enzymes into the medium and their xylanase levels are very much higher than those found in yeasts and bacteria [7]. However, improvement in xylanase production from bacteria might be achieved by mix culture. The use of mix culture for the improvement of enzyme production from bacteria have been reported. Mix culture of *Bacillus licheniformis* and *B. paralicheniformis* improved cellulase production by 2.28 folds over the mono culture [8].

Several agro-waste product such as rice straw, corncobs, wheat bran, sugar cane bagasse and rice husk have been implicated as substrate for xylanase production [3]. However, our knowledge on surghum straw as potential substrate for xylanase production have been limited due to reliance on the common agro-waste biomass. Sorghum straw is a renewable and cheap agro-waste residue generated from *Sorghum bicolor*, cultivated in Nigeria. The straw consists of stalks and leaves which contain mainly cellulose, hemicellulose, and lignin. The hemicellulose content of sorghum straw is between 19.18 and 28.07 % [9], which makes it a potential as a substrate for exploration of xylanse production. Traditionally, industrially important enzymes have been obtained from submerge fermentation due to greater control of environmental parameters such as temperature and pH. However, solid state fermentation improves the yield of enzymes [10]. Fungi are the most commonly used microorganisms that thrives in solid state fermentation due to their ability to grow at low moisture content on a solid. On the whole efficient xylanase production is influenced the source of microorganism, its strain, type of culture and the mode of fermentation. Moreover, successful industrial applications requiring cost effective bioprocess which can be achieved through the selection of suitable cheaper and efficient substrate.

As a result, in the current investigation, thermophilic microbes isolated from compost were tested for xylanase production and their capability was assessed using mix culture and growing of sorghum straw under immerse and solid state fermentation.

2. Materials and Method

2.1 Preparation of Sorghum Straw

Sorghum straw was collected from an agricultural farm, of the University of Ilorin, Kwara state, Nigeria. It was washed, dried and then milled into smaller particles using local industrial grinder. A fine powder was obtained after grinding and it was used as substrate for xylanase production.

2.2 Microorganisms

Three fungal and three bacterial isolates which had been previously isolated from the compost mixture of chicken manure and sorghum straw were obtained from the culture collection of Enzyme and Bioprocess Laboratory, Department of Microbiology, University of Ilorin. They were resuscitated on Nutrient Agar and Potato Dextrose Agar for 24 h at 50 °C.

2.3 Primary Screening of the Isolates for Xylanolytic Activity

The bacteria and fungi isolates were screened for xylanolytic activity using modified xylan agar. Bacterial isolates were allowed to grow on media containing (g/L): 0.1 g Yeast extract, 0.08 g K_2HPO_4, 0.02 g KH_2PO_4, 0.02 g NaCl,0.001 g $CaCO_3$, 1.0 g Xylan, 17.5 g Agar powder, 0.02 g $MgSO_4.7H_2O$ [11]. The fungal isolates were screened using a media composition containing (g/L): 5.0 g Peptone, 5.0 g Yeast extract, 1.0 g K_2HPO_4, 0.2 g $MgSO_4.7H_2O$, 20.0 g Agar, 0.1g Xylan [12]. After sterilization, 10µL of bacteria suspension was spot inoculated on the center of the xylan-agar plate and incubated at 50 °C for 24 h. As for the fungi, a 5 mm agar plug from 3-day-old fungi was inoculated in the middle of the xylan agar plate and incubated at 35°C for 72 h. After

incubation, the plates were flooded with 1% Congo red solution for 15 min and then counterstained with 1 M NaCl. The hydrolysis zones were measured and the relative xylanolytic activity was determined using the ratio of the diameter of the clearance zone (CZ) to the diameter of colony size (CS). The isolates were then used for quantitative xylanase production on sorghum straw substrate.

2.4 Selection of Xylanase Producing Strains

Isolates showing golden-yellow zone were selected as xylanase positive strain. The hydrolysis zones were measured and the relative xylanolytic activity was determined using the ratio of the diameter of the clearance zone (CZ) to the diameter of colony size (CS). The isolates (*Bacillus subtilis, Bacillus pumilus, Micrococcus, Apergillus flavus, Apergillus niger, Trichoderma viridae)* were used for quantitative xylanase production on sorghum straw substrate.

2.5 Inoculum Preparation

Each bacterial strain was inoculated aseptically into nutrient broth and incubated for 24 h at 50°C, after which 5 % inoculum corresponding to OD_{600}= 1.0 was used for the inoculation process. The fungal inoculum was prepared by growing the isolate on malt extract agar at 35ºC until sporulation. Three mycelia discs were plugged and used as inoculum.

2.6 Xyalanase Production on Sorghum Straw Substrate

A 5% inoculum of bacteria suspension was cultured in 250 mL Erlenmeyer flasks containing 100 mL basal medium containing: 0.5 g/l NH_4Cl; 0.5 g/l KH_2PO_4; 0.75 g/l KCl; 2.5 g/l K_2HPO_4; 7.0 g/l $MgSO_4.7H_2O$; 10 g/L sorghum straw (Carbon source); 3.0 g/l NaCl. The flasks were incubated at 50 °C under shaking at 200 rpm for 72 h. After incubation, the culture filtrate was centrifuged at 10,000rpm for 15 min at 4°C. The supernatant was stored at 4 °C till further use. Modified method of Mandels and Weber [13] was used for the xylan production medium. The medium consist (g/L); 1.40 g $(NH)_2SO4$, 2.0 g KH_2PO4, 0.63 g Urea, 0.75 g Peptone, 2.0 mL Tween 80, 0.30 g $CaCl_2$, 0.3 g $MgSO_4.7H_2O$, 2.0 g Sorghum straw (carbon source), 1.0 mL of Trace element. The trace elements consisted of (g/L) $FeSO_4$ (5×10^{-3}); $MnSO_4 .H_2O$ (1.6×10^{-3}); $ZnSO_4.7H_2O$ (1.4×10^{-3}) and $CoCl_2$ (2.6×10^{-3}). Fermentations were performed in 250 mL Erlenmeyer flasks containing 2 g of Sorghum straw moistened with 5 mL mineral salt. The flask was covered with cotton wool and aluminum foil and was autoclaved at 121 ºC for 15 min. After cooling, three mycelia discs of fungi were inoculated aseptically a cork borer (7mm in diameter) and incubated at 37 ºC for 7 days. The samples were withdrawn every 24 hours. The enzyme was extracted by adding 30 mL of sodium citrate buffer of pH 5.0 and shake at 50 °C for 30 min. The whole content of the flasks was sieved using a sterile muslin cloth. The filtrate was centrifuged at 3000 rpm for 10 min. The cell free supernatant was used for a crude enzyme assay.

2.7 Protein Determination

Total protein was determined by modified Bradford reagent and bovine serum albumin (BSA) as standard [14]. For this, the sample appropriately diluted was mixed with the Bradford reagent. The samples were then incubated at room temperature for 5 min. After that, the absorbance was determined using a spectrophotometer at 595 nm.

2.8 Xylanase Production by Mixed Culture

Basal medium was inoculated with a cocktail of the three bacteria species in order to determine its xylanase production potential. Using Birchwood xylan and sorghum straw as carbon sources, the difference in the xylanase activity using the cocktail organisms was also carried out using basal salt medium.

2.9 Xylanase Assay

A 0.5 mL of 1% xylan in 0.05M phosphate buffer pH 7.0 was added to 0.5 mL of diluted crude enzyme and incubated for 30 min at 50˚C. After incubation, the reactions were terminated by adding 3 mL of DNS reagent and allow boiling in water bath for 15 min. After cooling, the absorbance was read at 575 nm against the blank in the spectrophotometer [15]. One unit of xylanase activity was determined as the amount of enzyme that liberates 1µmole of xylose per minute under the specified assay condition. The same procedure was repeated for fungal xylanase assay except that the phosphate buffer was replaced by citrate buffer pH 4.8.

3. Results and Discussion

In this research, a total of three bacteria (*Bacillus subtilis*, *Bacillus pumilus* and *Micrococcus* sp.) and three fungi (*Apergillus flavus*, *Apergillus niger* and *Trichoderma viridae*) were shown to be xylanolytic on xylan-agar plate as shown in Table 1. *Aspergillus niger* and *Bacillus subtilis* emerged as best isolate for xylanase production among the fungi and bacteria species respectively. *Aspergillus niger* produced a clear zone at 2.0 ± 0.2, while *Bacillus subtilis* hydrolytic capacity reached 1.6 ± 0.5. It is worth noting that there was no difference between hydrolytic capacity produced by *Bacillus subtilis* and *Trichoderma viridae*. Notably, all the isolates produced hydrolytic capacity above 50 % with the least (68 %) occurring *Micrococcus* sp.

Since SSF provides an environment closer to the natural habitat of fungi, the method was adopted in ways to stimulate more xylanase secretion. The time course of enzyme production in solid-state fermentation from *Apergillus flavus*, *Apergillus niger* and *Trichoderma viridae* were analyzed using sorghum straw as a carbon source (Figure 1). It was observed that a lower level of xylanase appeared in the early stages of incubation and the enzyme levels steadily reached a maximum level of 62.42 U/ mL, 55.78 U/ml and 53.36 U/ mL in *A. niger*, *T. viridae* and *A. flavus* respectively, after 96 h of fermentation. A prolonged incubation time beyond 96 h results in a sharp decline in xylanase production. This decrease was constant till the end of the incubation period at 168 h. The production of xylanases by *A. niger* is a trend to be observed being the highest producer of xylanase out of the three fungal species studied. Similarly, Kulkarni and Gupta [16] also proved through their studies that *A. niger* is highly potential and useful for xylanase production. *Aspergillus niger* is more active in xylanase production compared to *Trichoderma viridae* grown on barley bran [17]. *A. niger* also produced higher xylanase when cultivated on wheat straw under solid state fermentation [18]. When comparing the xylanase production by A. *flavus*, it was almost 15% lower than that of *A. niger* at 96 h. Thus, A. *flavus* presents a very lower xylanase production rate than *A. niger* and *T. viridae.* A report by Oyedeji *et al*., [19] also aligns in that regard.

Bacillus species are industrially significant bacteria for the degradation of xylan due to their capacity to secrete important extracellular enzymes [20, 21]. As shown in Figure 3, maximum xylanase production of 29.84 U/mL was attained by *Bacillus subtilis* at 48 h followed by *Bacillu pumilus* at 36.06 U/ mL. In contrast, Gandarillas *et al*., [22] reported 10.0 U/mL xylanase productions when barley bran was used as substrate. Torkashvand *et al*. [23], also noticed higher xylanase production from *Bacillus subtilis* T4b grown on canola meal and tomato pomace. Further increase in the fermentation period beyond this resulted in decline in enzyme production which might be due to the production of toxic metabolites during microbial growth which inhibits the enzyme synthesis. Likewise the release of a small amount of protease from the aging cells gradually entering into autolysis could hinder enzyme activities. In the case of xylanase production by *Micrococcus* sp., the activity was found to be maximal (24.53 U/mL) at 48 h. This is in contrast to the findings of Mmango-Kaseke *et al*., [24] where the xylanase activity was found to be maximal at 84 h.

It was observed from the current study that xylanase production from the fungi species was much higher than that of the bacteria species. Therefore, an attempt was made to improve production of xylanase by the bacteria species via cocktail culture. The cocktail cultures include: *Bacilus pumilus*, and *Bacillus subtilis* (BpBs), *Bacillus subtilis* and *Micrococcus* sp. (BsM), *Bacilus pumilus* and *Micrococcus* sp. (BpM), *Bacilus pumilus*, *Bacillus subtilis* and *Micrococcus* sp. (BpBsM). The culture cocktail BpBs produced the highest (33.58 U/ mL) xylanase activity among the 4 cocktails tested. This was followed by BpBsM, with maximal activity at 31.22 U/ mL. This is attributed to the ability of the organisms to work synergistically to enhance xylanase activity. Furthermore, xylanase activity by BpM and BsM followed a similar trend from the early stages of fermentation till the optimal 48 h with maximal activity at 26.64 U/ mL. Both BpM and BsM produced the least xylanase activity. The least activity observed could be due to antagonistic effects of the bacteria species. It is worth noting that xylanase production by *Micrococcus* sp. cannot be improved singly by either *B. subtils* or *B. pumilus*. Thus, the synergistic activity of both *Bacillus* species is essential towards improving xylanase production by the *Micrococcus* sp.

This suggests that cocktails produced by mixed culture BpBs enhanced xylanase production and can therefore be used for efficient hemicellulose degradation. A cocktail of microrganisms brings about higher product yield. The growth rate becomes higher as one microorganism may produce essential growth factors such as carbon, amino acids and vitamins that is beneficial to the microorganisms thereby improving the activity the enzymes.

Different lignocellulosic wastes such as *Delonix regia* pods [25] wheat bran [26], rice straw [27], corn cob [28], wheat straw [29], has been used as carbon source for xylanase production. However little attention have been focused on the use of sorghum straw as substrate for the production of commercial xylanase. Sorghum straw used in the current study resulted in xylanase production comparable to previous studies. However, it is essential to compare it with commercial xylan popularly known and used as substrate for xylanase production. Therefore, xylanase activity of cocktail BpBs on sorghum straw was also studied against activity on beechwood xylan. The result showed an increased production at 33.58 ±0.03 U/mL after 48 h as compared to its production at 23.044 ± 0.06 U/ml on xylan (Figure 5). In contrast to our study, all the seven agro-waste carbon sources tested failed to significantly improve xylanase production above xylan in *Bacillus subtilis* T4b [23]. In their study xylanase production on rice straw reached 65.89U/ mL while on Birchwood xylan, production was 64.72U/ mL. The higher yield on the lignocellulosic waste is as a result of the substrate serving as a carbon source and also an inducer for enzyme production. In any industrial production process, the cost of the substrate plays a crucial role which makes low-value lignocellulosic waste preferred.

Conclusion

According to the findings, *Bacillus subtilis* produces the most xylanase activity in submerged fermentation, but *Aspergillus niger* produces the most xylanase activity in solid state fermentation when sorghum straw is employed as the sole carbon source. Cocktail culture BpBs produced more xylanase than monoculture. When compared to commercial xylan, xylanase production by the cocktail culture grown on sorghum straw increased by 1.7-fold. Sorghum straw can be utilized as an alternative, less expensive substrate for xylanase production, thereby lowering the cost of industrial xylanase for a variety of applications. The utilization of sorghum straw will also aid in the resolution of the environmental waste disposal issue.

Table 1: Qualitative Screening for Xylanolytic Isolates

Isolates	Hydrolytic capacity (HC)	Relative HC (%)
Fungi		
Aspergillus flavus	1.5 ± 0.6	75.0
Aspergillus niger	2.0 ± 0.2	100
Trichodermaviridae	1.6 ± 0.2	80.0
Bacteria		
Bacillus subtilis	1.6 ± 0.5	100
Bacillus pumilus	1.4 ± 1.7	87.5
Micrococcus sp	1.1 ± 0.6	68.8

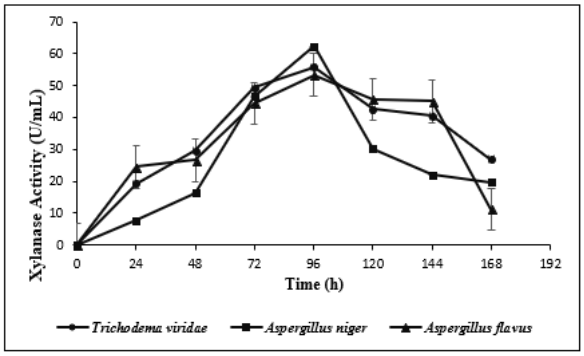

Figure 1: Xylanase production profile of xylanolytic fungal grown on sorghum straw in solid state fermentation.

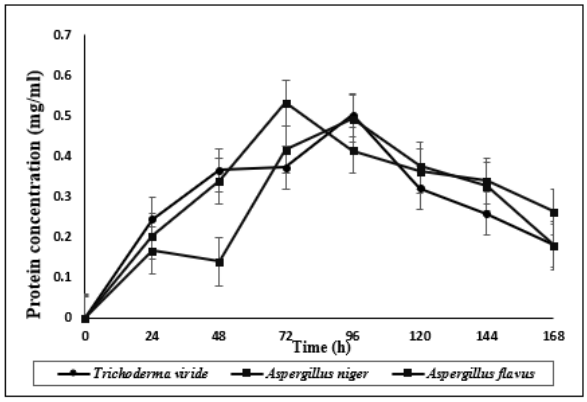

Figure 2: Protein production profile of xylanolytic fungal grown on sorghum straw in solid state fermentation.

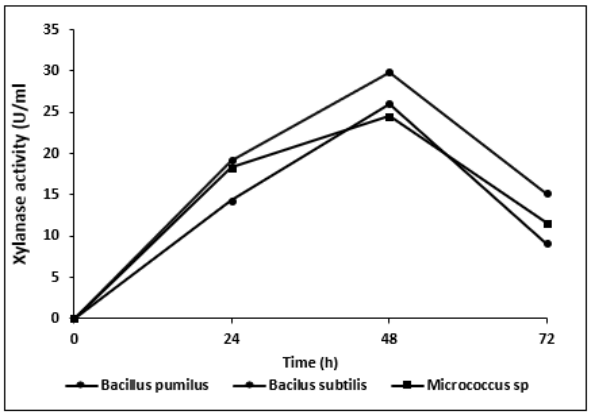

Figure 3: Xylanase production profile of xylanolytic bacteria grown on sorghum straw in submerged fermentation.

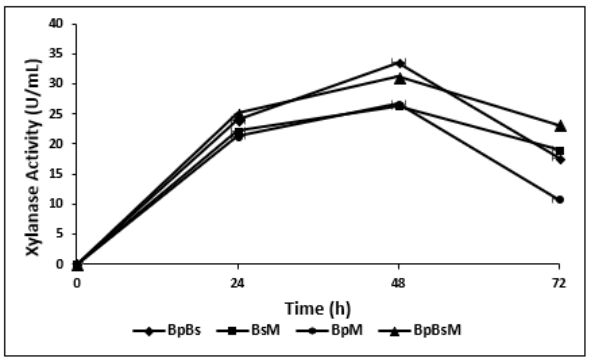

Figure 4: Xylanase production profile of xylanolytic bacteria cocktail in submerged fermentation.

BpBs= *Baciluspumilus*. and *Bacillus subtilis*
BsM= *Bacillus subtilis* and *Micrococcus* spp.
BpM= *Baciluspumilus* and *Micrococcus* spp.
BpBsM= *Baciluspumilus*,*Bacillus subtilis* and *Micrococcus* spp.

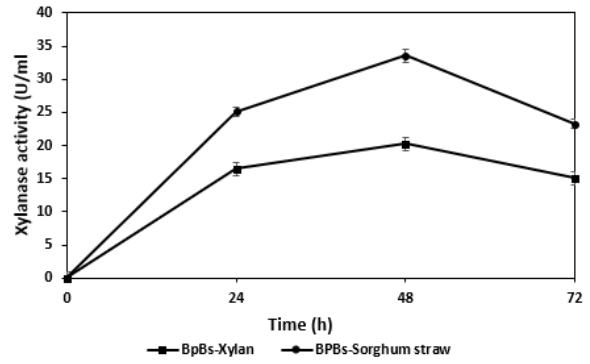

Figure 5: Comparison of xylanase production profile of BpBs grown on xylanand sorghum straw under submerged fermentation.

BpBs= *Baciluspumilus* and *Bacillus subtilis*

References

[1] Yeoman, CJ, Han, Y, Dodd, D, Schroeder, CM, Mackie, RI, Cann, IKO, Thermostable enzymes as biocatalysts in the biofuel industry, *Advance Appllied Microbiology*, 70, 2010, 1-55.

[2] Balan, V, Current challenges in commercially producing biofuels from lignocellulosic biomass, *International Scholarly Research Notices*, 2014, 1-31

[3] Irdawati, I, Sofiyyana, A, Advinda, L, Fiffendy, M, Salvia, S, Syamsuardi, S, et al. Optimization of Agricultural Waste Substrate as an Alternative Medium for Xylan in Producing Xylanase Enzymes by Thermophilic Bacteria. In: *Journal of Physics*: Conference Series, IOP Publishing, 2021, 1940, p. 012052.

[4] Roy, S, Dutta, T, Sarkar, TS, Ghosh, S, Novel xylanases from *Simplicillium obclavatum* MTCC 9604: comparative analysis of production, purification and characterization of enzyme from submerged and solid state fermentation, *SpringerPlus*, 2, 2013, 1-10. https://doi.org/10.1590/S1517-83822011000300036

[5] Sridevi, A, Ramanjaneyulu, G, Suvarnalatha Devi, P, Biobleaching of paper pulp with xylanase produced by *Trichoderma asperellum*, *3 Biotech*, 7, 2017, 266.

[6] Wu, S, Liu, B, Zhang, X, Characterization of a recombinant thermostable xylanase from deep-sea thermophilic *Geobacillus* sp. MT-1 in East Pacific, *Applied Microbiology and Biotechnology*, 72, 2006, 1210-1216.

[7] Cunha, L, Martarello, R, Souza, PMd, Freitas, MMd, Barros, KVG, Ferreira Filho, EX, et al., Optimization of xylanase production from *Aspergillus foetidus* in soybean residue, *Enzyme research*, 2018, 1-7. https://doi. org/10.1155/2018/6597017

[8] Kazeem, M, Ajijolakewu, K, Rahman, NA, Cellulase production by co-culture of *Bacillus licheniformis* and *B. paralicheniformis* over monocultures on microcrystalline cellulose and chicken manure-supplemented rice bran media, *BioResources*, 16, 2021, 6850-6869.

[9] Dong, M, Wang, S, Xu, F, Wang, J, Yang, N, Li, Q, et al., Pretreatment of sweet sorghum straw and its enzymatic digestion: Insight into the structural changes and visualization of hydrolysis process, *Biotechnology for Biofuels*, 12, 2019, 276.

[10] Mrudula, S, Murugammal, R, Production of cellulase by *Aspergillus niger* under submerged and solid state fermentation using coir waste as a substrate, *Brazilian Journal of Microbiology*, 42, 2011, 1119-1127.

[11] Mahilrajan, S, Balakumar, S, Arasaratnam, V, Screening and identification of a thermophilic and alkalophilic bacterium producing xylanase, *Advances in Applied Science Research*, 3, 2012, 242-50. https://doi.org/10.1007/s13205-013-0160-2

[12] Adesina, FC, Onilude, AA, Isolation, identification and screening of xylanase and glucanase-producing microfungi from degrading wood in Nigeria, *African Journal of Agricultural Research*, 8, 2013, 4414-4421. https://doi.org/10.5897/ajar2013.6993

[13] Mandels, M, Weber, J. The production of cellulases. 1969, 95, 231-413 http://dx.doi.org/10.1021/ba-1969-0095.ch023

[14] Bradford, MM, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, *Analytical Biochememistry*, 72, 1976, 248-54.

[15] Miller, GL, Use of dinitrosalicylic acid reagent for determination of reducing sugar, *Analytical Chemistry*, 31, 1959, 426-428. doi:10.1021/ac60147a030

[16] Kulkarni, P, Gupta, N, Screening and evaluation of soil fungal isolates for xylanase production, *Recent Research in Science and Technology*, 5, 2013, (2), 33-36

[17] Soliman, HM, Sherief, A, EL-Tanash, AB, Production of xylanase by *Aspergillus niger* and Trichoderma viride using some agriculture residues, *International Journal of Agricultural Research*, 7, 2012, 46-57.

[18] Azzouz, Z, Bettache, A, Boucherba, N, Prieto, A, Martinez, MJ, Benallaoua, S, et al., Optimization of β-1, 4-Endoxylanase Production by an *Aspergillus niger* Strain Growing on Wheat Straw and Application in Xylooligosaccharides Production, *Molecules*, 26, 2021, 2527. https://doi.org/10.3390/molecules26092527

[19] Oyedeji, O, Iluyomade, A, Egbewumi, I, Odufuwa, A, Isolation and screening of xylanolytic fungi from soil of botanical garden: xylanase production from *Aspergillus flavus* and *Trichoderma viride*, *J Microbiol Res*, 8, 2018, 9-18. https://doi.org/10.5923/j.microbiology.20180801.02

[20] Kumar, L, Kumar, D, Nagar, S, Gupta, R, Garg, N, Kuhad, RC, et al., Modulation of xylanase production from alkaliphilic *Bacillus pumilus* VLK-1 through process optimization and temperature shift operation, *3 Biotech*, 4, 2014, 345-56.

[21] Mittal, A, Nagar, S, Gupta, VK, Production and purification of high levels of cellulase-free bacterial xylanase by *Bacillus* sp. SV-34S using agro-residue, *Annals of Microbiology*, 63, 2013, 1157-1167. https://doi.org/10.1007/s13213-012-0574-

[22] Gandarillas, C, Soto, R, Vargas, VA, Xylanase production using barley straw by Bacillus sp. LB-4 isolated from laguna Blanca, Potosi-Bolivia, *Revista Boliviana de Química*, 29, 2012, 63-70.

[23] Torkashvand, N, Soltan Dalal, MM, Mousivand, M, Hashemi, M, Canola meal and tomato pomace as novel substrates for production of thermostable *Bacillus subtilis* T4b xylanase with unique properties, *Biomass Conversion and Biorefinery*, 2020, 1-13.

[24] Mmango-Kaseke, Z, Okaiyeto, K, Nwodo, UU, Mabinya, LV, Okoh, AI, Optimization of Cellulase and Xylanase Production by *Micrococcus* Species under Submerged Fermentation, *Sustainability*, 8, 2016, 11, 1-15 https://doi.org/10.3390/su8111168

[25] Kazeem, MO, Mhii, U, Arekemase, MO, Tiamiyu, AO, Maiangwa, J, Response surface optimization of xylanase diminutive cellulase by a thermo-alkaline tolerant *Trichoderma harzanium* FC50 under solid state cultivation on *Delonix regia* pods, *Journal of Taibah University for Science*, 17, 2023, (1) 2221824. https://www.tandfonline.com/doi/full/10.1080/16583655.2023.2221824

[26] Cui, F, Zhao, L, Optimization of xylanase production from *Penicillium* sp. WX-Z1 by a two-step statistical strategy: Plackett-Burman and Box-Behnken experimental design, *International journal of molecular sciences*, 13, 2012, 10630-1046.

[27] Bangyeekhun, E, Singhajutha, S, Romruen, U, Xylanase production by *Aspergillus fumigatus* UR01 by solid state fermentation of rice straw, *Asian Journal of Agriculture and Biology*, 8, 2020, (4), 422-427. https://doi.org/10.35495/ajab.2019.06.273

[28] Richhariya, J, Sharma, TK, Dassani, S, Production and optimization of enzyme xylanase by *Aspergillus flavus* using agricultural waste residues, *Journal of Applied Biology and Biotechnology*, 8, 2020, 82-9. https://doi.org/10.7324/JABB.2020.80413

[29] Matrawy, AA, Khalil, AI, Marey, HS, Embaby, AM, Use of wheat straw for value-added product xylanase by *Penicillium chrysogenum* strain A3 DSM105774, *Journal of Fungi*, 7, 2021, 696. https://doi.org/10.3390/jof7090696