Journal of Biotechnology Research
ISSN(e): 2413-3256, ISSN(p): 2413-8878
Vol. 3, No. 3, pp: 15-24, 2017
URL: http://arpgweb.com/?ic=journal&journal=16&info=aims

Optimization of Xylanase Production from Fermentation of Water Hyacinth (*Eichhornia crassipes*) using *Trichoderma species*

Chinyere Blessing Udeh* Department of Microbiology, Ahmadu Bello University Zaria Nigeria

Joseph Baba Ameh Department of Microbiology, Ahmadu Bello University Zaria Nigeria

Saleh Alhaji Ado Department of Microbiology, Ahmadu Bello University Zaria Nigeria

Stanley Irobekhian Reuben Okoduwa Directorate of Research and Development, Nigerian Institute of Leather and Science Technology, Zaria Nigeria

Abstract: Background: In the present study, optimized cultural conditions for enhanced production of xylanase from local soil isolate of Trichoderma species, using water hyacinth as a substrate in submerged culture fermentation is presented. Method: The Megazyme assay method was used for endo 1, 4-β-xylanase using Azo-xylan (Birchwood). Results: A continuous increase in xylanase production was observed with increasing level of substrate concentration in the medium and highest production was obtained with water hyacinth at 6% w/v level. Maximum xylanase production was achieved with a pH 5.0, incubation temperature of 30ºC and agitation rate of 150 rpm. The highest production was achieved on day five of fermentation at optimum parameters under study. Result: The study showed that production of xylanase can be cost effective using water hyacinth and can be implored on large scale for industrial applications.

Keywords: Xylanase; Extracellular enzymes; Microorganism; Environment.

1. Introduction

Xylanases are extracellular enzymes produced by microorganisms like bacteria, fungi and a few yeasts. They are involved in the catalysis of β1,4-xylans present in lignocellulosic substances [1, 2]. The industrial application of xylanases in bioconversion processes has been limited as a result of the high cost implication associated with the enzymes production. This requires the production of these enzymes at low cost by establishing the optimal environmental conditions for its production as well as the use of agro-industrial wastes and plants rich in lignocellulose as substrates [3, 4]. It was reported that the global market for enzymes would reached 8.0 billion by 2015 [5]. In a recent publication it was reported that the global enzyme market was USD 8.18billion in 2015 and it is expected to witness a significant growth over the next eight years on account of its increasing application in detergents, pharmaceuticals and food & beverages [6].

Xylanases are used mainly in pulp and paper, feed, and baking industries. Xylanases are employed in the pre-bleaching of Kraft soft tissue to decrease the use of harsh compounds in the succeeding chemical bleaching phases [3, 6, 7]. The use of enzymes in treatments increases the chemical release of lignin by catalyzing residual xylan. This decreases the necessity for chlorine-based bleaching compounds, which is advantageous for the environment [7, 8]. [9] isolated indigenous white rot fungal strain which exhibited variable xylanase activity for possible use in the bio-bleaching of pulp. The combination of xylanases, glucanases, proteinases and amylases in feed preparations, decreases viscosity of the feed and enhances the up-take of nutrients [8]. Enzymes act in releasing nutrients either by catalyzing fibers that are non-degradable or by releasing nutrients obstructed by these fibers. Xylanases are used in food productions to enhance the dough properties and baking quality of bread and other baked goods by breaking down the polysaccharides in the dough [8]. Enzymatic treatment has advantageous effects on dough handling, bread size, consistency and stability [10]. Xylanases can also be employed in the conversion of agricultural waste and also the production of fuel ethanol [8, 11].

Filamentous fungi are producers of xylanases industrially due to the high production level and extra cellular secretion of enzymes. [12] isolated and screened potential cellulase-free xylanase producing fungi from various sources, all fungi isolated produced varying amount of xylanase. Xylanase activity levels from fungal cultures are usually much higher when compared to those obtained from yeasts or bacteria [13]. The production of xylanases by

*Corresponding Author

Trichoderma species using various lignocellulose materials has been reported [8, 12, 14-17]. There is however paucity of published literature on its optimizations using this approach for industrial utilization.

Water hyacinth (*Eichhornia crassipes)* is a free-floating water plant that emanated from the Amazon River basin in South America and has spread to more than 50 countries on five continents. Water hyacinth can stand extremes in water level fluctuations, seasonal disparities in flow velocity, nutrient accessibility, pH, temperature and toxic substances [18]. It can even grow at salinity levels up to 0.24% as was shown in Indonesia [19]. Water hyacinth is a detrimental aquatic weed creating hazard in many tropical and sub-tropical freshwater environments due to its faster growth rate than any other vascular macrophytes. This irritating aquatic weed adversely affects commerce by way of blocking irrigating channels, vitiating commercial fishing and seriously interfering with recreation. The coverage of waterways by water hyacinth has generated various difficulties such as the devastation of ecosystems, irrigation difficulties and an increase in mosquito populations. These adverse effects have made the plant one of the world's worst weeds and stirred the search for control methods [20]. Efforts to control the weed have caused high costs and labour requirements, leading to nothing but short-term elimination of the water hyacinths.

The approach of using refined xylan as inducer for improved xylanase production is uneconomical. One of the suitable approaches for this purpose is to use ligno-cellulose rich plant residue, which not only serve as cost-effective substrate but also offer environmental benefits. The approach of utilizing water hyacinth for xylanase production is expected to serve twin objectives of employing otherwise irritating weed as well as production of hydrolytic enzymes at reduced cost which in turn will decrease conversion cost of hemicellulose to xylose that can be used for producing bioethanol and variety of value added chemicals and other products. It is on this basis that this research aimed at optimizing the production of xylanase under submerged fermentation of water hyacinth (*Eichhornia crassipes*) as substrate using *Trichoderma* species.

2. Materials and Methods

2.1. Source of *Trichoderma* Species

Soil samples were collected from the Botanical Garden, Ahmadu Bello University Samaru Zaria-Nigeria. The samples were taken from 5cm depth after removing 5cm from the earth surface of plant remains.

2.2. Collection of Water Hyacinth

Water hyacinth plants were collected from ponds, lakes and River Kaduna in Kaduna state, North-West Nigeria and then taken to the herbarium Unit in the Department of Biological Sciences, Ahmadu Bello University, Zaria-Nigeria for authentication.

2.3. Isolation and Identification of *Trichoderma* species

Ten (10g) of the soil sample was homogenised in 90ml sterile distilled water. Serial dilution of the soil samples were carried out in sterilized distilled water. Aliquot of 0.1ml of 10^5 dilution was spread onto xylan agar medium (XAM) containing (% w/v): birchwood xylan, 0.5; Peptone, 0.09; Potassium chloride,0.01;Diammonium hydrogen phosphate, 0.04; Magnesium sulphate, 0.01%; agar, 1; pH 7.0 and incubated at 37^{o}C for 48-72 h. The colonies on the plates were transferred onto fresh XAM and incubated at 37^{o}C for 48 h in order to obtain pure culture.

Fungi isolated were cultured on *Trichoderma* Selective medium (TSM). Isolates of *Trichoderma* sp producing yellow zone in the red background were selected and preserved on Potato Dextrose Agar (PDA) for further studies. The *Trichoderma* strains were characterized by macro- morphological and microscopic studies.

2.4. Screening of Trichoderma sp Isolates

(*Qualitative xylanase assay)* Remazol brilliant Blue (RBB) plate screen method was used. 0.2% RBB-xylan (w/v) was added to potato dextrose agar and autoclaved. The medium was poured into petri dishes, swirled and allowed to solidify. Five (5ul) of overnight culture was inoculated onto the plates and incubated for 2 days. The plates were then observed for clear zones around xylanase producing cultures.

2.5. Quantitative Xylanase Assay

Trichoderma sp hydrolyzing xylan was analyzed for quantitative production of xylanase in the xylanase production medium (XPM). The isolates were grown in XPM at 37^{o}C for 48-72 h under shake flask culture in a shaker incubator (150 rpm). The culture was centrifuged at 10,000 rpm for 10 min and the xylanase activity in the supernatant was determined. Finally one fungal isolate with highest xylanase activity was selected for further studies [21].

2.6. Media Preparation for Fermentation

The birch wood xylan in the XPM was replaced with water hyacinth as carbon source at 1.0 % (w/v) concentration supplemented with (Trisodium citrate -5g, KH_2PO_4 –5g, NH_4NO_3 – 2g, $(NH_4)_2SO_4$ – 4g, $MgSO_4$ – 0.2g, peptone – 1g, yeast extract – 2g,glucose – 2g and distilled water 1000mL) with sodium phosphate buffer pH of 6.5.To this medium, 1 ml of *Trichoderma* sp (10^7-10^8 spores /ml) was inoculated and the mixture was incubated at 37^{o}C in water bath with shaking (150 rpm) for 5days. Liquid state cultures were harvested at 24 h interval and

centrifuged at 10,000 rpm for 20 minutes at 4°C and the resulting supernatant was called crude enzyme preparation and xylanase activity in the culture supernatant was monitored [22].

2.7. Inoculum Preparation

The inoculum was prepared by growing the isolates on malt extract agar at 37°C until sporulation. The spores were harvested using 0.1% (v/v) Tween 80 [23]. The number of spores (10^{7}-10^{8} spores /ml) was estimated by direct microscopic counting using haemocytometer.

2.8. Enzyme Extraction and Dilution

Using a positive displacement dispenser, 1ml of liquid enzyme preparation was transferred to 49ml of buffer and mixed thoroughly. This was termed the original extract. The solution was diluted 10-fold by transferring 1ml of diluted enzyme to 9ml of buffer until a dilution of enzyme suitable for assay was obtained [24].

2.10 Xylanase Assay: An aliquot of 0.5ml of buffered enzyme preparation (pre-equilibrated to 40°C) was added to 0.5ml of pre-equilibrated substrate solution (1% w/v Azo-Xylan birchwood) with thorough mixing on a vortex stirrer. The test tubes containing the mixture were immediately returned to water bath and incubated at 40°C for exactly 10 minutes from time of addition of the enzyme solution. The reaction was terminated by adding 2.5ml of ethanol (95% v/v) with vigorous stirring on a vortex mixer. This precipitated high-molecular weight, non-hydrolyzed substrate. The tubes were stored at room temperature for 5minutes and stirred again after wards they were centrifuged at 3,000 rpm for 10minutes. The supernatant solution was poured directly into a spectrophotometer cuvette and absorbance measured at 590nm against distilled water. A reaction blank was prepared by adding 2.5ml of ethanol to 0.5ml of the substrate solution with vigorous stirring.

One unit of enzyme activity is defined as the amount of enzyme required to release one µmole of D-xylose reducing sugar equivalents from Azo-xylan, at pH 6.0 per minute at 40°C [24].

2.9. Optimization of Culture Conditions for Xylanase Production

The effect of different substrate concentrations on xylanase production were studied by carrying out fermentation at concentrations of 2,3,4,5 and 6% w/v at 37°C for 5days and xylanase activity determined for each concentration.

The effect of agitation rate was studied by carrying out fermentation in at 150, 200, 250 and 300 rpm.

The effect of different initial pH on xylanase production were studied by carrying out fermentation at pH 5,6,7 and 8 at 37°C for 5days and xylanase activity determined for each concentration. The pH of the medium was adjusted by using 1N HCl or 1N NaOH.

In order to determine the effective temperature for xylanase production, *Trichoderma* sp fermentation was carried out in the temperature ranges of 30, 35, 40 and 45°C, and their effect on xylanase production was investigated by determining xylanase activity in the culture supernatant.

The effect of incubation period on xylanase production was determined by fermentation for 10 days at optimum culture parameters substrate concentration, pH, and temperature and agitation rate. Samples were withdrawn at 24h intervals and xylanase activity in the culture supernatant was monitored [24].

3. Results

3.1. Characterization and Screening of the Isolated *Trichoderma sp* for Xylanase Production

Two *Trichoderma* species were isolated from the soil samples obtained from botanical garden. The fungi grew in the xylan agar medium giving an initial whitish colouration which eventually turned greenish due to the formation of the mycelia (Plate I). The *Trichoderma* sp was characterized by the presence of septate mycelia (Plate II). The conidiophores were branched consisting of a group of 3 or 4 philiades which were swollen in the middle and narrow at the ends. The philiades bear conidia which were ellipsoidal in shape. The isolate was reconfirmed and placed under the genus of *Trichoderma* sp based on the colony and structural morphologies observed under light microscope (Table 1).

The xylanase producing *Trichoderma* isolate (TS2) produced the largest zone of clearance on Remalzol brilliant blue xylan agar plate and also showed the highest extracellular xylanolytic activity (366 U/ml) under submerged fermentation, (Table 2) thus it was selected for further studies.

Table-1. Cultural and Microscopic Characteristics of *Trichoderma* Isolates

Isolates	Cultural characteristics/microscopic characteristics	Inference
TS 1	White fluff covers agar in a few and then becomes more compact and woolly. Green patches were eventually produced due to formation of conidia (initially at the center of the colony and spread to the margin). Hyphae are septate with short conidiophores which are branched at wide angles; phialides are flask shaped and conidia round and clustered.	*Trichoderma* sp
TS2	At the early stage whitish to greenish mycelia appeared. Next a deep green colour developed in central part and gradually extended to the periphery. Finally, it appeared a whitish green colour. Mostly globose conidia developed on phialides produced in the opposite direction in each point	*Trichoderma* sp

TS: *Trichoderma* species

Plate-I. Colonial morphology of *Trichoderma* sp isolate on Potato Dextrose Agar (PDA)

Plate-II. The Microscopic Characteristic of *Trichoderma* sp Isolates

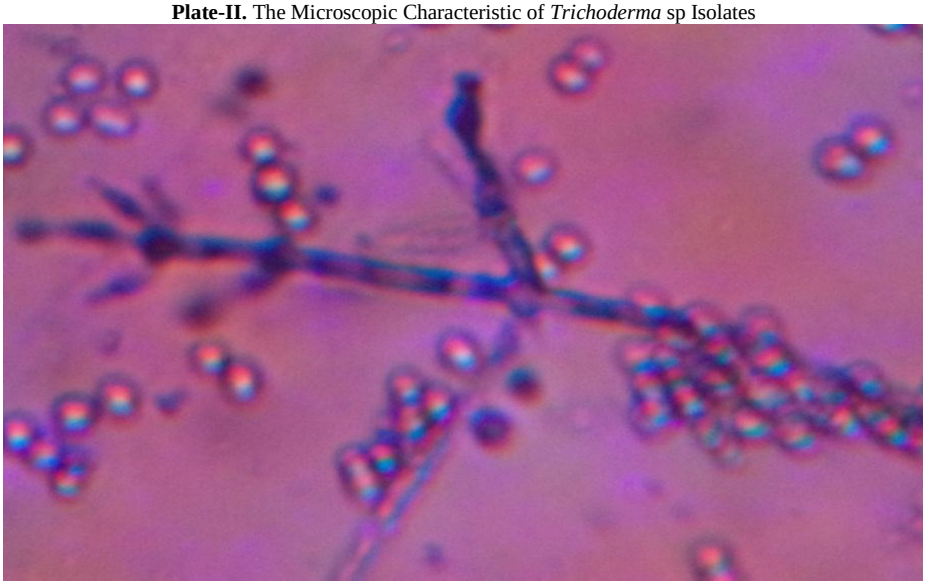


Table-2. Isolates and their Xylanase Production in Xylanase Production Media

Isolates	Xylanase activity (U/ml)
TS1	284
TS2	366*

TS : *Trichoderma* sp (values are mean of three replicates). *1U xylanase activity= 1µmol xylose min-1 * higher xylanase activity

3.2. Effect of Substrate Concentration on Xylanase Production

With a view to further improve the xylanase production without increasing the cost of production, the substrate concentrations was varied from 1 to 6% w/v. With increasing concentrations of lignocellulosic in the production medium, substantial increase in enzyme production was recorded. The result from this finding showed that xylanase production is concentration dependent as seen in Fig 1. In this case the best induction for xylanase was at 6% concentration (3170 U/ml).

3.3. Effect of Agitation Rate on Xylanase Production

Fig 2 shows the effects of agitation speed (from 100 rpm to 300 rpm) on xylanase production by *Trichoderma* sp in submerged fermentation system using shake flasks. The results revealed that the optimal agitation speed was 150 rpm which produced a maximal xylanase activity of 3650 U/ml. Agitation speed of 100rpm, 200rpm and 250rpm had maximum enzyme activity of 2160 U/ml, 1220 U/ml and 730 U/ml respectively.

3.4. Effect of pH on Xylanase Production

The effect of different initial pH on the production of xylanase is presented in Fig 3. The optimum pH for xylanase activity was found at acidic value of pH 5 (3630 U/ml). This was followed by pH 6, 7 and 8 with 3170 U/ml, 2960 U/ml, and 2080 U/ml respectively. The enzyme activity gradually decreases with increasing pH above the optimum.

3.5. Effect of Temperature on Xylanase Production

The effect of temperature on xylanase production was determined by incubating the flask at a range of temperature of 30°C, 35°C, 40°C, and 45°C. The results of the test showed that the optimal temperature for maxima xylanase production (3780 U/ml) was 30°C (Fig 4).

3.6. Effect of incubation period on xylanase production

For determining the time for maximum xylanase production, the various parameters for highest activity were taken into consideration. This resulted in an increase in xylanase activity of 2550 U/ml at 24 h of growth and maximum of 3910 U/ml at 120h (day 5) of incubation. The activity then declined slowly to 1410 U/ml at 240 hours (day 10) (Fig 5).

Fig-1. Effect of Substrate Concentration on Xylanase Production

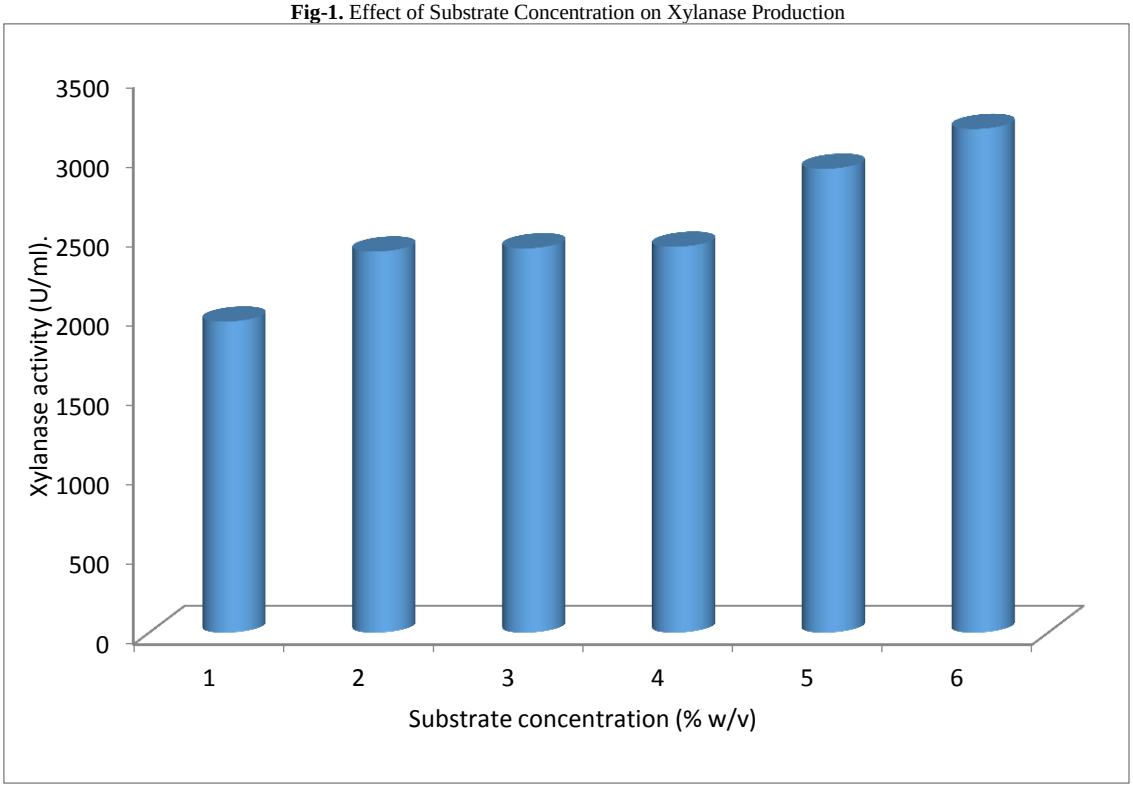


Fig-2. Effect of Agitation Rate on Xylanase Production

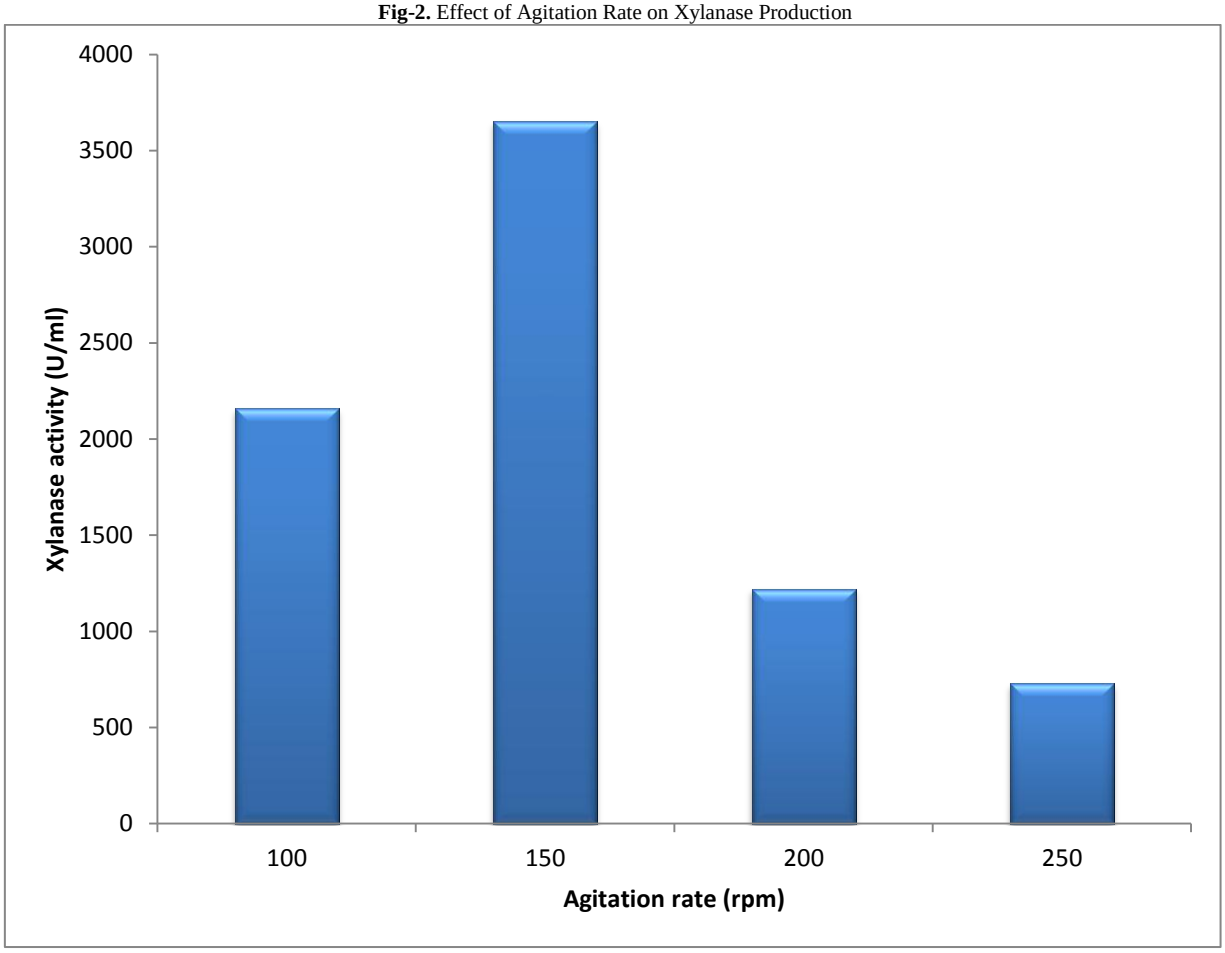


Fig-3. Effect of pH on Xylanase Production

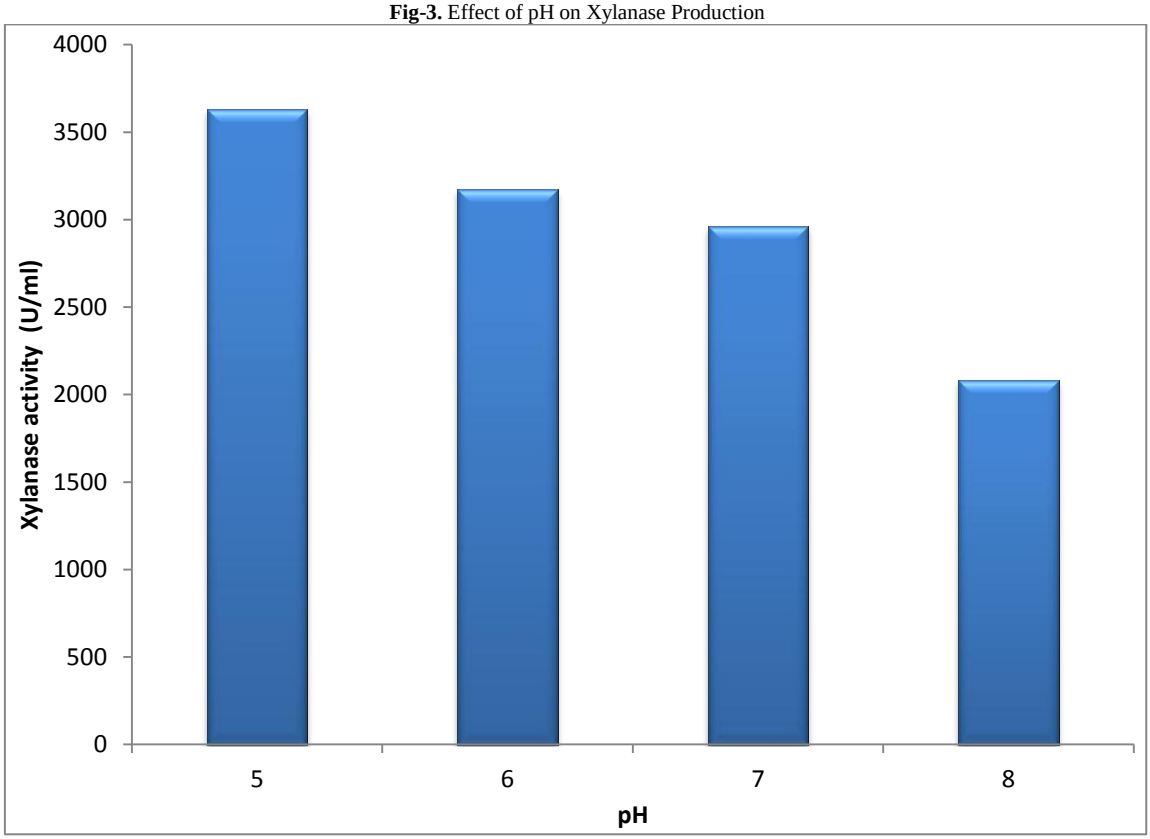


Fig-4. Effect of Temperature on Xylanase Production

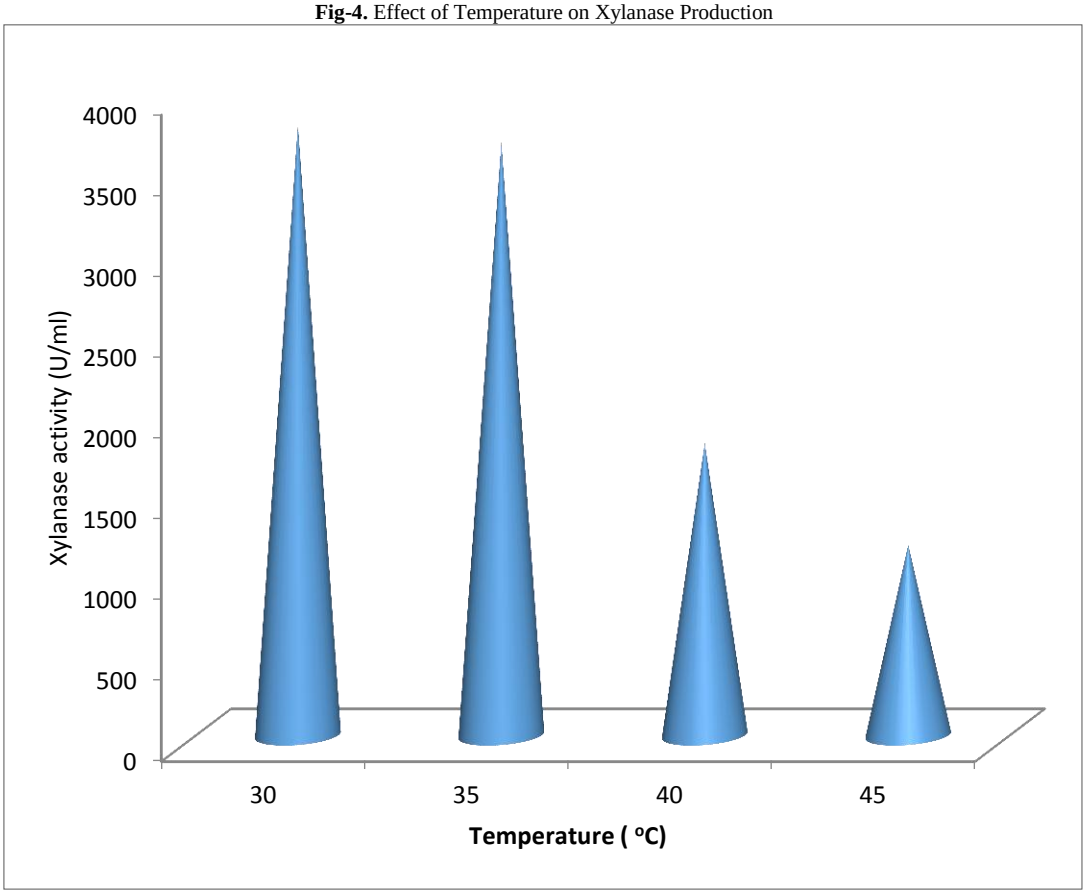
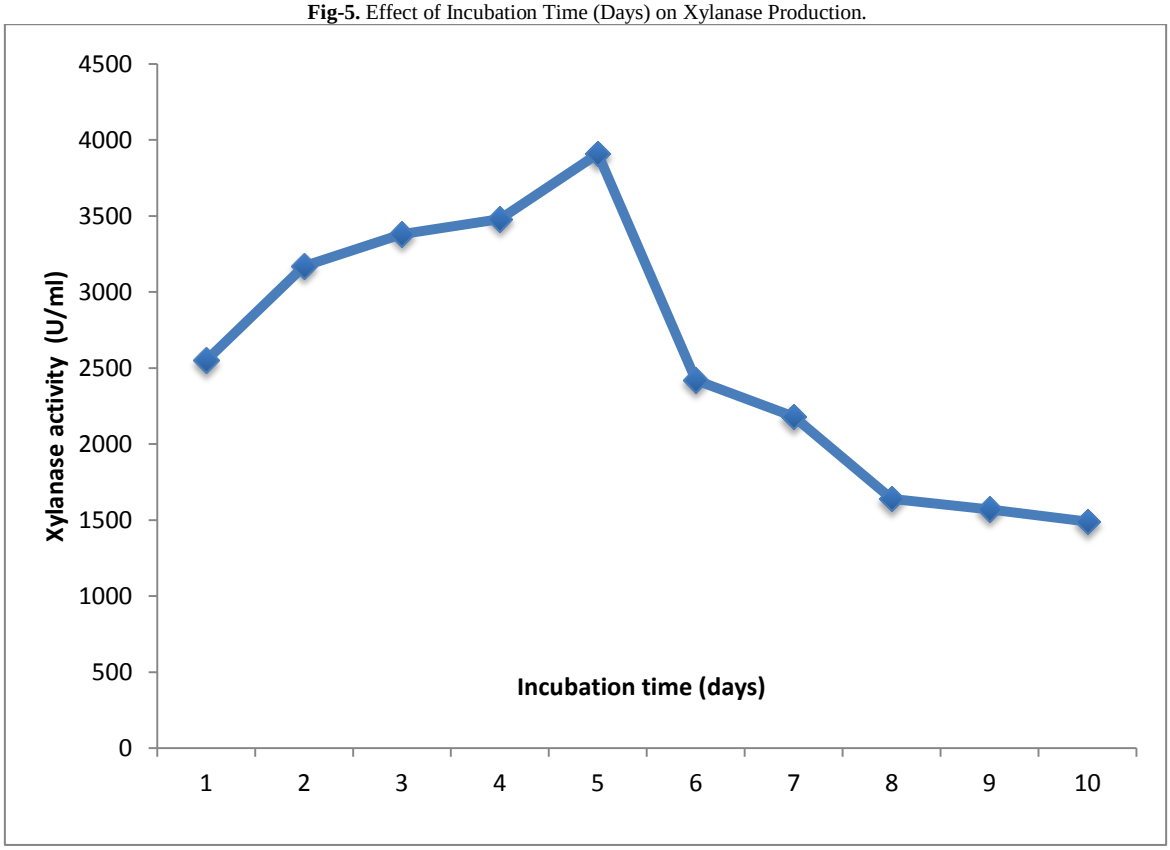


Fig-5. Effect of Incubation Time (Days) on Xylanase Production.



4. Discussion

The bioconversion of lignocellulosic rich plant materials to fermentable sugar has caught the attention of industrial and scientific researches as it represents a substitute feed stock for the production of biofuels, chemicals, cheap energy source for fermentation and enhanced animal feed. In this study, two *Trichoderma* strains from botanical garden were screened for xylanolytic activity in xylan agar medium. By using the Remalzol Brilliant Blue

(RBB) plate screen, a xylanase producing isolate (TS2) produced the largest zone of clearance and also showed the highest extracellular xylanolytic activity (366 U/ml) under submerged fermentation. The colony morphology and microscopic examination method was used to characterized the fungal isolate. At the early stage, whitish to greenish mycelia appeared. Next a deep green colour was developed in central part and gradually extended to the periphery. Finally, they appeared as whitish green colour. *Trichoderma* species is characterized by the presence of septate mycelia. This was observed in the present research. The conidiophores are branched consisting of a group of 3 or 4 philiades which is swollen in the middle and narrow at the ends. The philiades bear conidia which are ellipsoidal in shape. The isolate was reconfirmed and placed under the genus of *Trichoderma* based on the colony and structural morphologies observed under light microscope.

An important factor affecting the rate of reaction catalyzed by an enzyme is the substrates' concentration. A continuous increase in xylanase production was observed with increasing level of lignocellulosics in the medium and highest production was observed with water hyacinth at 6% level. [25] had reported an increasing xylanase production with higher levels of lignocellulosic (3-5%) substrate concentration. [Fortkamp and Knob [26]] found the best induction for xylanase production for *Trichoderma viride* using pineapple peel at 2%.w/v. This could be attributed to the distinct composition and accessibility of the substrate. However, Grigorevski-Lima, *et al.* [27], found high xylanase production by *Trichoderma atroviride* at 3% w/v using untreated sugarcane bagasse as substrate. The results obtained by Sanghvi, *et al.* [28] revealed that increase in substrate concentration led to a decrease in xylanase production by *Trichoderma harzianum*. In the present study, substrate concentration could not exceed 6% w/v due to increased viscosity which influenced medium component and oxygen transfer. In the course of enzyme substrate reaction, the initial velocity (V_0) progressively increases with increasing concentration of the substrate, till a point is reached beyond which the increase in V_0 will not be subject to the substrate concentration. Conversely beyond a particular substrate concentration, the velocity remains constant without any additional increase.

Agitation speed that was above 150 rpm resulted in reduced enzyme production and this condition could be due to the fungal cell instabilities caused by shear pressure or shear forces. This agrees with the findings of Darah et al., Darah, *et al.* [29]. However, lower agitation speed of less than 150 rpm resulted in low growth, which thus resulted in low xylanase production. This could be due to low amount of dissolved oxygen in the production medium. The similar conditions were reported by Darah, *et al.* [29] Bai, *et al.* [30].

There exists a strong effect of initial pH of the medium on enzyme production. To assess the effects of pH value in the substrate on xylanase synthesis, the pH values were adjusted by the addition of HCl or NaOH to 5.0, 6.0, 7.0 and 8.0. The results showed that the production of xylanase by *Trichoderma* sp was found to be in the range of 5 and 7. The optimum pH for xylanase activity was found at acidic pH value of 5 (3630 U/ml). The enzyme activity progressively decreased when increasing the pH above the optimum. Effect of pH on xylanase production by these fungi supports the findings of Silveira, *et al.* [15], who reported that xylanase activity exhibit a pH optimum of 5.0 for *Trichoderma harzianum*. Fortkamp and Knob Fortkamp and Knob [26] observed high production levels of xylanase at pH range of 7.0 -7.5 by *Trichoderma viride* using pineapple peel as substrate. The results obtained in this study agrees fairly well with that obtained by Mohan, *et al.* [31] with a record pH for *Trichoderma species* at 5.5 - 5.7. The optimal pH medium for xylanase production not only depends on the strain of the fungal species employed but also on the nature of the carbon source used [31].

The present study showed that enzyme activity decreased with increasing temperature. Findings have been reported for different temperatures for maximum xylanase production either in flask or in fermentor studies using *Trichoderma species* suggesting that the optimal temperature for xylanase production also depends on the strain variation of the microorganism [32]. The optimum temperature for enzyme production is similar to the optimum temperature for growth of *Trichoderma* sp in its natural habitat. Similar observation was also reported by Goyal, *et al.* [25] in *Trichoderma viride*; Sudgen and Bhat Sudgen and Bhat [33] in *Sporotrichum thermophile* and by Biswas, *et al.* [34] in *Aspergillus orchraceus*. Fortkamp and Knob [26] recorded a temperature of 28^0C for *Trichoderma viride*.

The progressive decrease in enzyme production with an increase in the incubation period might be due to the exhaustion of nutrients in the medium which stressed the fungal physiology resulting in the inactivation of secretary machinery of the enzymes [35]. However, Sanghvi, *et al.* [28] observed that xylanase production by *Trichoderma harzianum* with wheat straw increased up to 12 days of fermentation. Norazlina et al., [36] recorded an increase in xylanase production to the sixth day by *Trichoderma* sp in the fermentation process. The production level of xylanase in this study was significantly higher than many reported in the literature using different agro-industrial wastes. *T. atroviride* grown on sugarcane bagasse had a xylanase titre level of 61.3 U/ml [27]. Fortkamp and Knob [26] reported a titre level of 73.09 U/ml. Usually, additional increase in the incubation time resulted in the decrease of xylanase production.

5. Conclusions

It can be concluded from the present study that the production of xylanase can be made cost effective by using water hyacinth as carbon source by submerged fermentation. Xylanase activity of 3170 U/ml was recorded at 6 % w/v substrate concentration. Agitation speed of 150 rpm had the best induction of xylanase production. Also an optimum pH of 5, 30^oC temperature and a maximum incubation period at day 5 was recorded. At optimum parameters xylanase activity increased to 3910 U/ml.

Acknowledgments

The authors expressed their sincere gratitude to the technical staff of Microbiology Department, Ahmadu Bello University Zaria for their assistance during the bench work Thanks to the management of SIRONigeria Global Limited, Abuja Nigeria for the critical suggestions in the preparation of the manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

References

[1] Kheng, P. P. and Omar, I. C., 2005. "Xylanase production by a local fungal isolate, Aspergillus niger USM AI 1 via solid state fermentation using palm kernel cake (PKC) as substrate." *Songklanakarin J. Sci. Technol*, vol. 27, pp. 325-336.

[2] Silva, L. A. O., Terrasan, C. R. F., and Carmona, E. C., 2015. "Purification and characterization of xylanases from trichoderma inhamatum." *e-J. Biotechnol*, vol. 18, pp. 307-313.

[3] Ali, S. B. R., Muthuvelayudham, R., and Viruthagiri, T., 2012. "Statistical optimization of nutrients for production of cellulase and hemicellulase from rice straw." *Asia J. Biochem. Pharm. Res.*, vol. 2, pp. 154-174.

[4] Buthelezi, S. P., Olaniran, A. O., and Pillay, B., 2011. "Sawdust and digestive bran as cheap alternative substrates for xylanase production." *Afr. J. Microbiol. Res.*, vol. 5, pp. 742-752.

[5] S., L., Yang, X., Yang, S., Zhu, M., and Wang, X., 2012. "Technology prospecting on enzymes: application, marketing and engineering." *Comput. Struct. Biotechnol. J.*, vol. 2, pp. 1-11.

[6] GVR (Grand View Research), 2016. "Enzyme market by type. Forecast to 2024. ISBN Code 978-1-68038-022-4, 2016. Retrived from http://grandviewresearch.com on Sunday 3/7/16."

[7] Beg, Q. K., Kapoor, M., Mahajan, L., and Hoondal, G. S., 2001. "Microbial xylanases and their industrial applications: A review." *Appl. Microbiol. Biotechnol*, vol. 56, pp. 326-338.

[8] Albert, S., Chauhan, D., Pandya, B., and Padhiar, A., 2011. "Screening of Trichoderma species as a potential fungal partner in co-culturing with white rot fungi for efficient bio-pulping." *Global J. Biotechnol. Biochem.*, vol. 6, pp. 95-101.

[9] Singh, S., Dutt, D., and Tyagi, C. H., 2013. "Screening of xylanases from indigenously isolated white rot fungal strains for possible application." *Pulp Biobleaching*, vol. 2, p. 602.

[10] Bhat, M. K. and Hazlewood, G. P., 2001. *Enzymes in Farm Animal Nutrition, In: Bedford MR and Partridge CC (eds.)*. CAB International: Wallingford, UK.

[11] Damaso, M. C. T., Almeida, M. S., Kurtenbach, E., Martin, O. B., Pereira, N. J., Andrade, C. M. M. C., and Albano, R. M., 2003. "Optimized expression of a thermostable xylanase from Thermomyces lanuginosus in pichia pastoris." *Appl. Environ. Microbiol*, vol. 69, pp. 6064-6072.

[12] Sridevi, B., Charya, C., and Singara, M. A., 2011. "Isolation, identification and screening of potential cellulase-free xylanase producing fungi." *Afr J. Biotechnol.*, vol. 10, pp. 4624-4630.

[13] Paloheimo, M., Mäntylä, A., Kallio, J., and Suominen, P., 2003. "High yield productionof a bacterial xylanase in the filamentous fungus Trichoderma reesi requires a carrier polypeptide wwith an intact domain structure." *Appl. Environ. Microbiol*, vol. 69,

[14] Gomes, I., Gomes, J., Steines, W., and Esterbaner, H., 1992. "Production of cellulase and xylanase by a wild strain of Trichoderma viride." *Appl. Microbiol. Biotechnol*, vol. 36,

[15] Silveira, F. Q. P., Ximenes, F. A., Cacais, A. O. G., Milagres, A. M. F., Mederros, C. L., Puls, J. E., and Filno, E. X. F., 1991. "Hydrolysis of xylans by enzyme system from solid cultures of Trichoderma harzianum strains." *Braz. J. Med. Biol. Res.*, vol. 32, pp. 947-952.

[16] Seyis, I. and Aksoz, N., 2005a. "Xylanase production from Trichoderma viride 1073 D3 with alternative carbon and nitrogen sources." *Food Technol. Biotechnol*, vol. 43, pp. 37-40.

[17] Seyis, I. and Aksoz, N., 2005b. "Investigations of factors affecting xylanase activity from Trichoderma herzianum 1073 D31." *Braz. Arah Biol. Technol*, vol. 148, pp. 187-93.

[18] Gopal, B., 1987. *Water hyacinth*. New York: Elsevier Science Publishers Armsterdam.

[19] Kikuchi, T., Takagi, M., Tokuhisa, E., Suzuki, T., Panjaitan, W., and Yasuno, M., 1997. "Water hyacinth (Eichhornia crassipes) as an indicator to show the absence of Anopheles suncaicus larvae." *Med. Entomol. and Zool*, vol. 48, pp. 11-18.

[20] Gunnarson, C. C. and Peterson, M. C., 2007. "Water hyacinths as a resource in agriculture and energy production: A literature review." *Waste Manage*, vol. 27, pp. 117-129.

[21] Teather, R. M. and Wood, P. J., 1982. "Use of Congo red-polysaccharide interactions in enumeration and characterization of cellolytic bacteria from the bovine rumen." *Appl. Environmental Microbiol.*, vol. 43, pp. 777-780.

[22] Ghosh, M., Das, A., Mishra, A. K., and Nanda, G., 1993. "Aspergillus sydowii MG 49 is a strong producer of thermostable xylanolytic enzymes." *Enzyme Microbiol. Technol.*, vol. 15, pp. 703-709.

[23] Smith, P. J., Rinzema, A., Tramper, J., Schlosser, E. E., and Knol, W., 1996. "Accurate determination of process variables in a solid state fermentation system." *Proc. Biochem*, vol. 31, pp. 669-678.

[24] Megazyme International Ireland, 2007. "Assay of endo- 1,4-β-Xylanase using Azo-Xylan(Birchwood), S-AXBP."

[25] Goyal, M., Kalra, V. K., Sareen, S., and Soni, G., 2008. "Xylanase production with xylan rich lignocellulose wastes by a local soil isolate of Trichoderma viride." *Braz. J. Microbiol.*, vol. 39, pp. 535-541.

[26] Fortkamp, D. and Knob, A., 2014. "High xylanase production by Trichoderma viride using pineapple peel as a substrate and its application in pulp bio bleaching." *Afr. J. Biotechnol*, vol. 13, pp. 2248-2259.

[27] Grigorevski-Lima, A. L., Oliveira, M. M., Nascimento, R. P., Bon, E. P., and Coelho, R. R., 2013. "Production and partial characterization of cellulase and xylanases from Trichoderma atroviride 676 using lignocellulosic residual biomass." *Appl. Biochem. Biotechnol*, vol. 169, pp. 1373-1385.

[28] Sanghvi, G. V., Koyani, R. D., and Rajput, K. S., 2010. "Thermostable xylanase production and partial purification by solid -state fermentation using agricultural waste wheat straw." *Mycol.*, vol. 1, pp. 106-112.

[29] Darah, I., Erman, E., and Ibrahim, C. O., 1997. "Proceedings on the first colloquim on potential utilization of Starch and Lignocellulosic material for value added application." pp. 4-77.

[30] Bai, D. M., Jia, M. Z., Zhao, X. M., Ban, R., Shen, F., Li, X. G., and Xu, X. M. L., 2003. "Lactic acid production by pellet from Rhizopus oryzae R10121 in a stirred tank fermentor." *Chem. Eng. Sci.*, vol. 58, pp. 785-791.

[31] Mohan, G., Guhankumarand, P., and Balakrishnan, V., 2011. "Isolation of xylan degrading enzyme from Trichoderma species." *Res. Plant Biol.*, vol. 1, pp. 15-20.

[32] Lu, W., Li, D., and Wu, Y., 2003. "Influence of water activity and temperature on xylanase activity biosynthesis in pilot-scale solid state fermentation by aspergillus sulphureus." *Enzyme Microbiol. Technol*, vol. 32, pp. 305-311.

[33] Sudgen, C. and Bhat, M. K., 1994. "Cereal straw and pure cellulose as carbon sources for growth and production of plant cell wall degradation enzymes by Sporotrichum thermophile." *World J. Microbiol. Biotechnol.*, vol. 10, pp. 444-251.

[34] Biswas, S. R., Jana, S. C., Mishra, A. K., and Nanda, G., 1990. "Production, purification and characterization of xylanase from a hyperxylanolytic mutant of Aspergillus ochraceus." *Biotechnol. Bioeng.*, vol. 35, pp. 244-251.

[35] Nochur, S. V., Roberts, M. F., and Demain, A. L., 1993. "True cellulose production by Cloistridium thermocellum grown no different carbon sources." *Biotechnol. Lett.*, vol. 15, pp. 641-646.

[36] Norazlina, I., Meenalosani, N., and Ku-Halim, K. H., 2013. "Production of xylanase by Trichoderma species via solid- state culture using sugarcane bagasse." *Int. J. Environ. Sci.*, vol. 3, pp. 99-105.