

Research Article

XYLANASE from *STREPTOMYCES HYGROSCOPICUS* under Solid State Fermentation

***Prashant P. Dixit and Arvind M. Deshmukh**

Department of Microbiology,
Dr. Babasaheb Ambedkar Marathwada University,
Sub Campus, Osmanabad. (MS) India. 413 501
Corresponding author e-mail: prashantdixit74@rediffmail.com Phone
No. +91 02472 202045

[Received-06/01/2016, Accepted-12/01/2016, Published-17/01/2016]

ABSTRACT

The objectives of the present study were isolation, identification, and characterization of xylanase producing actinomycetes, optimization of medium composition and cultural conditions for xylanase production, production using cheaper sources and extraction of extra cellular xylanase from a potential strain.

Streptomyces hygroscopicus was selected and optimized for xylanase production in solid state fermentation using cheaper sources like wheat bran, rice bran, sugarcane molasses and cellulose paper powder. Maximum enzyme activity was observed in wheat bran (24.66 U/mL). Optimum pH and temperature for xylanase activity were found to be 6.0 and 50 °C. Xylanase was remarkably stable in the pH range (4-10), with more than 60 % activity and it was also stable at 50 °C and retained around 98 % residual enzyme activity up to two hours.

The use of wheat bran as a major carbon source is particularly valuable because oat spelt xylan is more expensive. Thus the present study proved that *Streptomyces hygroscopicus* used is highly potential and useful for industrial production of alkalistable thermostable xylanase using low cost agricultural residues under solid state fermentation.

Key words: Xylanases, solid state fermentation, alkalistable, thermostable, *Streptomyces hygroscopicus*

[I] INTRODUCTION

Xylan is the most abundant hemicelluloses in plant material and comprises up to one third of the total dry weight of higher plants. It is an important constituent of renewable resource. Microbial depolymerization of xylan is carried out by at least two enzymes; endo- β -xylanase (EC 3.2.1.8) and β -xylosidase (EC 3.2.1.37). Most microbial hemicellulolytic systems contain β -xylanase [6]. Actinomycetes play a considerable role in recycling nutrient and are

involved in the primary degradation of organic matter in compost and related materials [8]. They are considered as an important source of enzymes involved in lignocellulose degradation and activity against xylan. Information on actinomycetes xylanase activity is largely derived from studies on *Streptomyces* sp. [4] and *Thermommonospora* sp. [38]. Appropriate isolation and screening of alkalotolerant and thermotolerant actinomycetes will lead to the

production of the enzyme for biobleaching process and other applications. [16]. Stable enzymes active at high temperatures are favourable for increasing reaction rates and possibly for lessening contamination problems. The interests in xylan degrading enzyme and their application in the pulp and paper industries have advanced significantly over the past few years [2,9,10,12 AND 37] and therefore large scale production of xylanase at economical cost becomes more important. Solid state fermentation in this regard is a better choice for xylanase production.

Solid-state fermentation (SSF) is the growth of microorganisms on moist substrates in the absence of free-flowing water. The advantage of SSF processes over liquid batch fermentation include smaller volumes of liquid required for product recovery, cheap substrate, low cultivation cost for fermentation, and lower risk of contamination. The use of abundantly available and cost-effective agricultural residues, such as wheat bran, corn cobs, rice bran, rice husk, and other similar substrates, to achieve higher xylanase yields using SSF allows reduction of the overall manufacturing cost of biobleached paper. This has facilitated the use of this environment friendly technology in the paper industry.

The use of purified xylan as a substrate for enhanced xylanase production is uneconomical. Wheat bran, an important byproduct of cereal industry and is produced worldwide in enormous quantities, is an excellent cost-effective substrate for large scale production of xylanase in solid state fermentation (SSF) and therefore wheat bran was used as a substrate for xylanase production.

[III] MATERIALS AND METHODS

2.1 Isolation and screening

Soil and water samples were collected from laundry wastes from Sholapur city (Maharashtra), India. After serially diluting the

samples, a loopful of each sample was streaked on to separate sterile Glycerol Asparagine agar (GAA) plate and incubated at 50 °C for 5 days for isolation of actinomycetes. The isolated actinomycete strains were then spot inoculated on to sterile Reese and Mendel agar plates containing 1% (w/v) xylan and incubated at 50 °C for 2 days. The pH of the medium was adjusted to 8.0 by adding 1% (w/v) sodium carbonate. After incubation xylanase producing strains were selected by flooding the plates with 0.1% (w/v) Congo red for 15 min followed by repeated washing with 1 mol/L NaCl. All colonies showing a clear zone on agar plates were further screened by growing them in liquid medium and assaying the enzyme activity from the cell-free culture supernatant fluid. One strain showing maximum zone of xylan hydrolysis (8.4 cm), identified as *Streptomyces hygroscopicus* was selected for further studies.

2.2 Xylanase production in solid-state fermentation

Reese and Mendel's basal media was used as a moistening agent in a ratio of 1:3 (w/v). Erlenmeyer flasks (250 mL) containing 10 g of wheat bran were added with the Reses and Mandels basal medium (30 mL) just to wet the wheat bran. The flasks were inoculated with 2 mL of spore suspension prepared from a seven days old GAA slant of the culture grown at 50 °C. Sterile distilled water containing 0.1% (v/v) Tween 80 was used to prepare actinomycete spore suspension. Inoculated flasks were incubated at 50°C under static conditions for 6 days. The enzyme from one flask each day was extracted using 100 mL of 0.05M-phosphate buffer (pH 8.0) and filtered through a wet muslin cloth by squeezing. The extract was centrifuged at 5000 rpm for 20 min.

2.3 Estimation of xylanase activity

Xylanase activity was determined by incubating 1 mL of assay mixture containing 0.5 mL of 1 % (w/v) xylan and 0.5 mL of suitably diluted

enzyme in 50 mM phosphate buffer (pH 8.0) for 30 min at 50 °C. Enzyme and reagent blanks were also simultaneously incubated with the test samples. The reducing sugar formed was estimated by dinitrosalicylic acid method [25]. One international unit (IU) of enzyme activity for xylanase was defined as the amount of enzyme releasing 1 μ mol reducing sugar from xylan per minute using xylose as standard. The substrate xylan (1 %) was prepared by suspending two grams of xylan powder in 100 mL of distilled water and stirring the mixture for 16 h at 28 °C [13]. The insoluble fraction was separated by centrifugation at 10,000 rpm for 10 min at 4 °C and the soluble fraction was used for the estimation of xylanase activity.

2.4 Determination of protein concentration

Protein concentration in supernatant was determined according to the method of Bradford [7], using bovine serum albumin as standard.

2.5 Optimization of medium for enhanced production of xylanase

Optimization of medium for enhanced production of xylanase was carried out by use of variety of alternative carbon sources such as wheat bran, corncobs, cellulose paper powder, sugarcane molasses etc. separately. Different organic and inorganic nitrogenous compounds yeast extract, meat extract, peptone, tryptone, ammonium sulfate, diammonium hydrogen phosphate, ammonium chloride and sodium nitrate were used as nitrogen sources separately.

2.6 Effect of pH and temperature on the activity and stability of the crude enzyme

Estimation of xylanase activity at different pH (4-10) and temperature (29-80 °C) values were carried out under standard assay conditions to determine optimum pH and temperature for enzyme activity. The pH stability of the enzyme was measured by incubating 5 U of enzyme for one hour, at 50 °C in buffers of desired pH and then estimating the residual activity under standard conditions. The temperature stability

was determined by incubating 5 U of enzyme at various temperatures. Aliquots were removed at different time intervals and their residual activity was estimated under standard assay conditions.

[III] RESULTS

3.1 Time course of xylanase production in solid-state fermentation method

Maximum xylanase production (24.66 U/mL) was attained after incubation at 50 °C for 4 days (96 h) (Fig. 1).

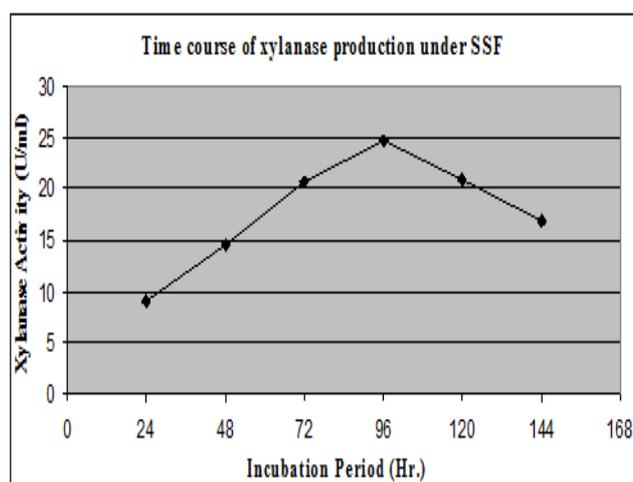


Fig.1: Time course of xylanase production under solid-state fermentation

3.2 Medium optimization for xylanase production

An assortment of substrates, mainly agricultural residues was evaluated for xylanase production by *Streptomyces hygroscopicus* under solid state fermentation. (Table 1). Wheat bran at a concentration of 10 % (w/v) was found optimal for maximum xylanase production (24.66 U/mL). Carbon sources such as rice bran (10% (w/v)) (13.22U/mL), sugarcane molasses (10% (w/v)) and wheat bran (10% (w/v)) with Cellulose Paper Powder (4 % (w/v)) (19.66U/mL) produced moderate amounts of xylanase while wheat bran (10% (w/v)) with soyabean meal (4 % (w/v)) produced negligible

amount of xylanase. The production of comparable amounts of xylanase using corncob (22.66 U/mL) is significant, as it is a cheap agricultural waste. However availability of wheat bran in large amounts at negligible costs promoted use of wheat bran as carbon source in the present study.

Table 1: Effect of different carbon sources on xylanase production

Sr. No.	Carbon source (w/v)	Xylanase Activity (U/mL)
1.	Wheat bran (10%)	24.66
2.	Rice bran (10%)	13.22
3.	Sugarcane molasses (10%)	19.66
4.	Wheat bran (10%) + Cellulose paper powder (4%)	19.66
5.	Wheat bran (10%) + Soyabean meal (4%)	06.66
6.	Corn cobs (10%)	22.66

Using 10 % (w/v) Wheat Bran as carbon source, different organic and inorganic nitrogen sources were evaluated for xylanase production (Table 2). Meat extract (17.66U/mL) was found to be the most suitable organic nitrogen source for the production of xylanase. Comparable results were obtained on using peptone and tryptone. When inorganic nitrogen sources were used, then a maximum xylanase production of 24.66 U/mL was obtained with 1 % (w/v) diammonium hydrogen phosphate.

An increase in the concentration of organic nitrogen did not result in enhanced xylanase production. This signifies the use of inorganic nitrogen source for obtaining high yields of xylanase.

Table 2: Xylanase production in the presence of different nitrogen sources

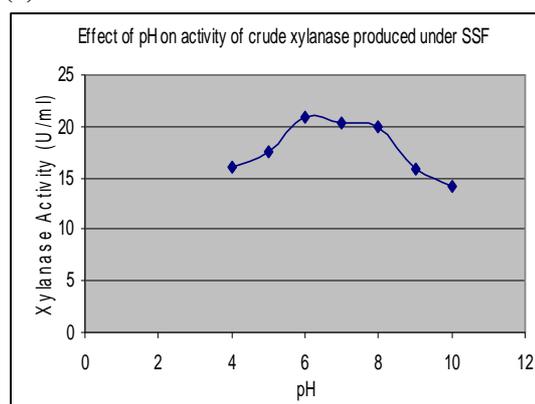
Sr. No.	Nitrogen source (1 % (w/v))	Xylanase activity (U/mL)
1.	Tryptone	17.33
2.	Peptone	17.00
3.	Yeast extract	15.33
4.	Meat extract	17.66
5.	Ammonium chloride	11.66
6.	Ammonium	20.33

	Sulphate	
7.	Di Ammonium hydrogen phosphate	24.66
8.	Sodium Nitrate	12.33

3.3 Effect of pH on activity and stability of crude xylanase produced in SSF

The activity of crude xylanase was determined in buffers of different pH (4-10) and results are given in Fig. 2 (a) and (b). The crude enzyme was active in a broad pH range (4-10) with maximum activity at pH 6. It yielded 97.59 and 75.99 % of its maximum activity at pH 8 and 9, respectively. The pH stability of crude xylanase was also determined in buffers of different pH (pH 4-10) and Xylanase was remarkably stable in the pH range (4-10), with up to more than 60 % activity. It had 66 and 62 % residual activity at pH 9 and 10, respectively.

(a)



(b)

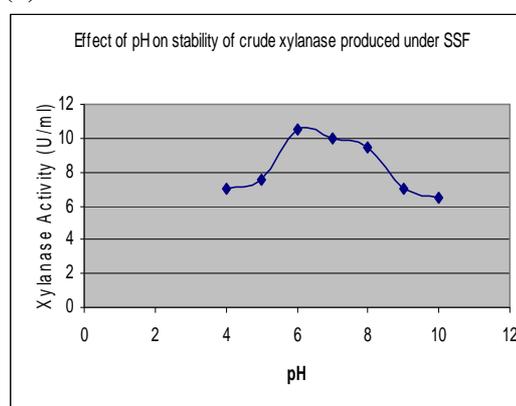
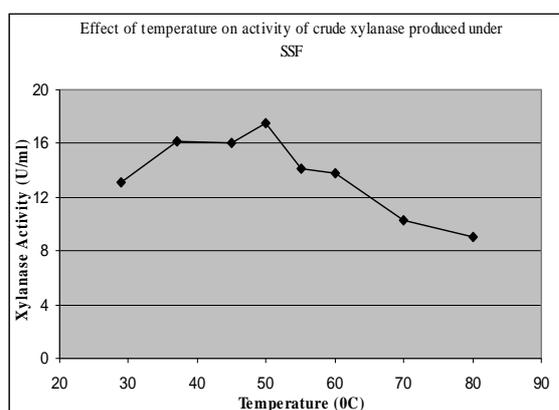


Fig. 2: Effect of pH on (a) activity and (b) stability of crude xylanase produced in SSF

3.4 Temperature activity profile and thermal stability of crude xylanase produced in SSF

Effect of temperature on activity and stability of crude xylanase produced by SSF are given in Fig.3. The crude xylanase had an optimum temperature of 50 °C. It showed more than 70 % residual enzyme activity in the temperature range 29- 60 °C. However, at 70 and 80 °C there was a steep decline in enzyme activity with 59 and 51 % residual enzyme activity, respectively. The xylanase was stable at 50 °C; it showed around 98 % residual enzyme activity up to two hours and retained 90% and 85 % residual enzyme activity at 45 °C and 60 °C respectively.

a.



b

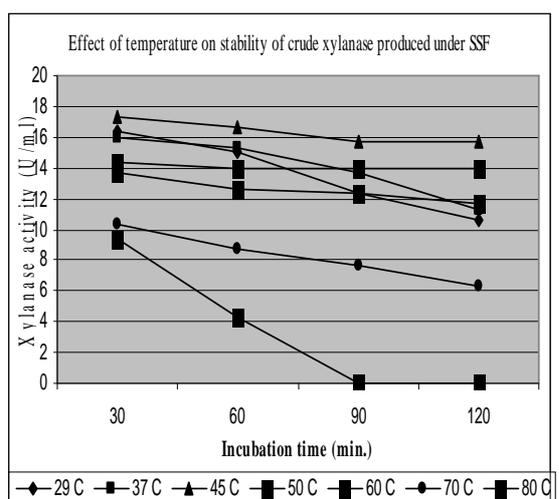


Fig. 3: Effect of temperature on (a) activity and (b) stability of crude xylanase produced in SSF

[IV] DISCUSSION

Xylanase production by *Streptomyces hygroscopicus* under solid state fermentation and using different agricultural waste was studied. Wheat bran was found to be the most suitable carbon source for maximum xylanase production. The use of purified xylan as a substrate for enhanced xylanase production is uneconomical. Using abundantly available and low cost agricultural byproducts as substrates for the production of xylanase is one of the ways to substantially reduce the enzyme production cost [19,37]. Wheat bran, an important byproduct of cereal industry and is produced worldwide in enormous quantities, is an excellent cost-effective substrate for large scale production of xylanase in solid state fermentation (SSF) and therefore wheat bran was used as a substrate for xylanase production. Xylanase production by *Streptomyces hygroscopicus* under solid state fermentation was obtained in 96 hr. Identical fermentation periods for xylanase production were reported by Tandon D. and Sharma N. [39] for xylanase from *Bacillus atrophaeus*, Kumar S. *et al.*, [14] for xylanase from *Bacillus tequilensis*, and Peter Albert C.F. *et al.*, [29] for xylanase from *Aspergillus niger*.

Use of wheat bran as an effective substrate for xylanase production has been reported by many users and for variety of microorganisms viz. *Trichoderma harzianum* [35], *Rhizopus stolonifer* [14], *Trichoderma sp.* FETL C³-2 [28], *Humicola grisea var. Thermoidea* [33], *Aureobasidium pullulans* [27], *Bacillus pumilus* [5], *Bacillus subtilis* [32], *Bacillus subtilis* CO1 [24] and *Aspergillus foetidus* MTCC 4898 [34]. Many researchers also insisted usefulness of wheat bran as an efficient substrate for xylanase production. Gupta *et al.*, [15] for xylanase production by *Staphylococcus sp.* SG-13, Xu *et al.*, [40] for xylanase production by *Pseudomonas sp.* WLI- 11 (wild type) and *Pseudomonas sp.* WLUN 024 (mutant) ,

Balakrishnan *et al.*, [3] for xylanase from *Bacillus* sp. NCL 87-6-10, Ahmad Z, *et al.*, [1] for xylanase from *Aspergillus niger* and Kamble R.D. and Jadhav A.R [20] for xylanase from a newly isolated *Bacillus* sp.. Hooi Ling Ho and Ke Li Heng [18] studied xylanase production by *Bacillus subtilis* using different agricultural residues such as rice bran, palm kernel cake, corn cobs, barley husk and wheat bran and found wheat bran as the best carbon source.

Wheat bran is supposed to be the best substrate for xylanase production because it is able to remain in loose condition, provides large surface area, contains around 40% xylansubstrates for xylanase, also contains around 28% proteins, low lignin and silica content, over all it is completely nutritive for variety of microorganisms. [5, 32].

An increase in the concentration of organic nitrogen did not result in enhanced xylanase production. This signifies the use of inorganic nitrogen source for obtaining high yields of xylanase. Diammonium hydrogen phosphate was found to be the most suitable nitrogen source for maximum xylanase production by *Streptomyces hygroscopicus*. Gupta *et al.*, [15] also reported use of wheat bran as carbon source and diammonium hydrogen phosphate as source of nitrogen for xylanase production by *Staphylococcus* sp. SG-13. However, Dey *et al.*, [11] reported yeast extract as the most suited nitrogen source and wheat bran as the carbon source for enhanced xylanase production by alkalophilic thermophilic *Bacillus* sp. and George *et al.*, [13] reported enhanced production of xylanase using corncobs as a source of carbon and yeast extract as the most suited nitrogen source by *Thermomonospora* sp. Lemes J.L. *et al.*, [23] reported use of yeast extract as a supplement to the nitrogen sources like sodium nitrate and ammonium sulphate resulted in considerable improvement in production of xylanase. Rehmani *et al.*, [31]

also showed use of urea as nitrogen source for xylanase production in submerged fermentation but it resulted in low activity.

Xylanase from *Streptomyces hygroscopicus* has a pH optimum at pH 6.0. Similar results were reported for *Rhizopus stolonifer* [14] whereas *Penicillium oxalicum* showed pH optima at 8.0 [26]. However the enzyme from *Streptomyces hygroscopicus* retained considerable activity at elevated pH. Enzyme also showed stability in a broad pH range of pH (pH 4-10). Xylanase from terrestrial *Streptomyces* sp. C24 also was stable in the range of pH 6.0 to 10.0 [30] whereas xylanase from *Paenibacillus* sp. XJ18 was active in a broad pH range of 4.5 to 9.0 [41], Singh S. *et al.*, [36] also reported that xylanase from *Thermomyces lanuginosus* SSBP was active and stable in a pH range of 5.5 to 10.0.

Xylanase from *Streptomyces hygroscopicus* showed temperature optima at 50 °C. Xylanase from *Penicillium rolfii* c3-2(1) IBRL [22], *Fusarium heterosporum* [17], newly isolated *Bacillus* sp. [20] also showed temperature optima at 50 °C. However, *Rhizopus stolonifer* [14], *Fusarium heterosporum* [17] and *Penicillium oxalicum* [26] showed temperature optima at 45 °C whereas *Aspergillus foetidus* MTCC 4898 xylanase reported by Shah and Madamwar [34] was stable at 50 °C and had a half-life of 57.53 min at 50 °C. Enzyme from *Streptomyces hygroscopicus* showed high degree of stability at 50 °C and did not show any decrease in the activity even after 2 hours. Enzyme retained more than 60% activity after 2 h at 70 °C where as it had half-life of 60 min at 80 °C. However, these results are not in accordance with xylanase from *Thermomyces lanuginosus* SSBP which had half-life of two days at 65°C [36].

Thus, *Streptomyces hygroscopicus* produced alkalistable thermostable xylanase using wheatbran as the source of carbon and diammonium hydrogen phosphate as the source

of nitrogen using solid state fermentation technique.

[V]CONCLUSION

The use of purified xylan as a substrate for enhanced xylanase production is uneconomical. Using abundantly available and low cost agricultural byproducts as substrates for the production of xylanase is one of the ways to substantially reduce the enzyme production cost. Wheat bran, an important byproduct of cereal industry and produced worldwide in enormous quantities, is an excellent cost-effective substrate for large-scale production of xylanase in solid-state fermentation (SSF).

Streptomyces hygroscopicus produced 24.66 U/ml of xylanase when grown in Solid-state fermentation with wheat bran as a substrate at pH 8 and 50 °C for 48 h. The crude xylanase was active in a broad range of pH (4-10) and temperature (29-80°C). The optimum pH and temperature were 6 and 50 °C respectively. The crude enzyme was stable in the pH range 5-8 and was found to be thermostable with half-lives of two hour at 60 and 70 °C respectively, but only 60 min at 80 °C. Enzyme also had pH optima of 6.0 and temperature optima of 50 °C.

REFERENCES:

- Ahmad, Z., Butt, M.S. and Riaz, M. (2013), Partial purification and characterization of xylanase produced from *Aspergillus niger* using wheat bran, Pakistan Journal of Agri. Sci. Vol. 50, Issue 3, pg 433-437
- Bajpai, P., Bhardwaj, N.K., Bajpai, P.K., Jauhari, M.B. (1994), The impact of xylanases on bleaching of eucalyptus kraft pulp. *Journal of Biotechnology* vol.38, pg 1-6
- Balakrishnan, H., Kamal Kumar, B., Dutta-Choudhury, M., Rele, M.V. (2002), Characterization of alkaline thermoactive cellulase-free xylanases from alkaliphilic *Bacillus* sp. (NCL 87-6-10), *Journal of Biochemistry, Molecular Biology and Biophysics*, Vol. 6, Issue 5, pg 325 - 334.
- Ball, A.S., McCarthy, A.J. (1989), Production and purification of xylanase from actinomycetes. *J Applied Bacteriology* Vol. 66, pg 439-444
- Battan, B., Sharma, J., Kuhad, R.C. (2006) , High-level xylanase production by alkaliphilic *Bacillus pumilus* ASH under solid-state fermentation, *World Journal of Microbiology and Biotechnology* Vol. 22, pg 1281-1287
- Biely, P. (1985), Microbial xylanolytic systems. *Trends Biotechnology* Vol. 3, pg 286-290
- Bradford, M.M. (1976), A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* Vol. 72, pg 248-254
- Burman, N.P. (1973), The occurrence and significance of actinomycetes in water supply. *In: Actinomycetes: Characteristics and practical Importance* Sykes G. and F.A. Skinner, (eds), Academic Press, London and New York pg. 219-230.
- Christov, L.P., Myburgh, J., O'Neill, F.H., Tonder, A.V. and Prior, B.A. (1999) , a. Modification of carbohydrate composition of sulfite pulp by purified and characterized β -xylanase and β -xylosidase of *Aureobasidium pullulans*. *Biotechnol Prog* Vol. 15, pg 196-200
- Christov, L.P., Myburgh J., O'Neill, F.H., Tonder, A.V., Prior, B.A. (1999) , b. Production, partial characterization and use of fungal cellulase-free xylanases in pulp bleaching. *Process Biochemistry* Vol. 34, pg. 511-517
- Dey, D., Hinge, J., Shendye, A., Rao, M. (1992), Purification and properties of extracellular endo-xylanases from alkalophilic thermophilic *Bacillus* sp. *Canadian Journal of Microbiology* Vol. 38, pg 436-442
- Garg, A.P., Roberts, J.C., McCarthy, A.J. (1998), Bleach boosting effect of cellulase-free xylanase of *Streptomyces thermoviolaceus* and its comparison with two commercial enzyme preparations on birchwood kraft pulp. *Enzyme Microbial Technology* Vol. 22, pg 594-598

13. George, S.P., Ahmad, A., Rao, M.B. (2001), A novel thermostable xylanase from *Thermomonospora* sp.: Influence of additives on thermostability. *Journal of Bioresource Technology* Vol. 78, pg 221-224.
14. Goulart Antonio José, Carmona E. C., Monti, R. (2005) , Partial purification and properties of cellulase-free alkaline xylanase produced by *Rhizopus stolonifer* in solid-state fermentation, *Brazilian Archives of Biology and Technology*, Vol. 48, Issue 3, pg 327-333
15. Gupta, S., Kuhad, R.C., Bharat Bhushan, Hoondal, G.S. (2001), Improved xylanase production from a haloalkalophilic *Staphylococcus* sp. SG-13 using inexpensive agricultural residues, *World Journal of Microbiology and Biotechnology* Vol. 17 pg 5-8
16. Haltrich, D., Nidetzky, B., Kulbe, K.D., Steiner, W. and Zupancic, S. (1996) , Production of fungal xylanases. *Bioresour. Technol.* Vol. 58, pg 137-161
17. Heinen, P.R., Henn, C., Peratta, R.M., Bracht, A., Garcia, R., Luis de Conceicao, J., Lourdes, M., Polizeli, T.M., Kadowaki, M. K. (2014), Xylanase from *Fusarium heterosporum*: Properties and influence of thiol compounds on xylanase activity, *African Journal of Biotechnology* Vol. 13, Issue 9, pg 1047-1055
18. Ling, Ho. H. and Heng, K.L. (2015), Xylanase production by *Bacillus subtilis* in cost effective medium using soybean hull as part of production medium composition under submerged fermentation and solid state fermentation, *J. Biodivers. Biopros. Dev.* Vol. 2, Issue 1 pg1-13 (1000143)
19. Jain, A. (1995), Production of xylanase by thermophilic *Melanocarpus albomyces* IIS 68. *Process Biochem* Vol. 30, pg 705–709
20. Kamble, R.D. and Jadhav, A.R. (2012), Isolation, purification and characterization of xylanase produced by a newly isolated *Bacillus* in SSF, *Hindawi Publishing Corporation International Journal of Microbiology* Vol. 2012 Article ID 683193
21. Kumar, S., Sharma, N., and Pathania, S. (2014),. Purification, characterization and gene encoding of xylanase produced from *Bacillus tequilensis* SHO isolated from compost using low cost wheat bran as substrate, *Global Journal of Biology, Agriculture and Health Sciences*, Vol. 3, Issue 3, pg 1-15
22. Lee, K.C., Takamitsu, A., Ibrahim, D., Prawitwong, P., Lan, D., Murata, Y., Mori, Y., and Kosugi, A. (2015), Purification and characterization of a xylanase from the newly isolated *Penicillium rolfsii* c3-2(1) IBRL, *Bioresources* Vol. 10, Issue 1, pg 1627-1643
23. Lemos, J.L., Fontes, M.C., and Pereira, N. Jr. (2001), Xylanase production by *Aspergillus awamori* in solid-state fermentation and influence of different nitrogen sources, *Appl. Biochem. Biotechnol.* Spring; Vol. 91 Issue 3, pg 681-9
24. Manimaran, A. , Vatsala, T.M. (2007), Biobleaching of banana fiber pulp using *Bacillus subtilis* C O1 xylanase produced from wheat bran under solid-state cultivation, *Journal of Industrial Microbiology and Biotechnology* Vol. 34, pg 745–749
25. Miller, G.L. (1959), Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry* Vol. 31, pg 426-428.
26. Muthezhilan, R., Ashok, R., Jayalakshmi, S. (2007), Production and optimization of thermostable alkaline xylanase by *Penicillium oxalicum* in solid state fermentation, *African Journal of Microbiology Research* pg 020-028
27. Nasr, S., Soudi, M.R., Salmanian, A.H. and Ghadam, P. (2013) , Partial optimization of Endo 1,4 –B- xylanase production by *Aureobasidium pullulans* using agro industrial residues, *Journal of Basic Medical Sciences* Vol. 16, pg 1245-1253
28. Pang, P.K., Darah, I., Poppe, L., Szakacs, G., Ibrahim, C.O. (2006), Xylanase Production by a local isolate, *Trichoderma* sp. FETL C³⁻² via solid state fermentation using agricultural wastes as substrates, *Malaysian Journal of Microbiology*, Vol. 2, Issue 1, pg 7-14
29. Peter Albert, C.F., Ajayi, A. A, and Awosika, F.A.(2015) , Studies on xylanase production by *Aspergillus niger* on Tomato Pomace medium, *African Journal of Applied Research* Vol. 1

30. Porsuk, I., Ozakin, S., Bali, B., and Yilmaz, E. I. (2013), A cellulose free, thermoactive and alkaline xylanase production by terrestrial *Streptomyces* sp. CA24, Turk. J. Biol. Vol. 37, pg 370-375
31. Rehmani, N., Jabbar, N.U., Suparto, I. H. And Yopi (2014), Optimization of production of xylanase from marine bacterium *Bacillus safensis* P20 on sugarcane baggase by submerged fermentation, International Journal on Advanced Science, Enginnering and Information Technology Vol. 4 Issue 6, pg 31-34
32. Sanghi, A., Garg, N., Sharma J., Kuhar K., Kuhad, R. C., Gupta, V. K. (2008), Optimization of xylanase production using inexpensive agro-residues by alkalophilic *Bacillus subtilis* ASH in solid-state fermentation, World Journal of Microbiology and Biotechnology Vol. 24, pg 633-640
33. Severino, de Albuquerque Lucena-Neto, Filho, E.X.F. (2004), Purification and Characterization of A New Xylanase From *Humicola grisea* var. *Thermoidea*, Brazilian Journal of Microbiology Vol. 35, pg 86-91
34. Shah, A. R., Madamwar D. (2005), Xylanase production under solid-state fermentation and its characterization by an isolated strain of *Aspergillus foetidus* in India, World Journal of Microbiology and Biotechnology Vol. 21, pg 233-243
35. Silveira, F.Q. de Paula, de Sousa, M.V., Ricart, C.A.O., Milagres, A.M.F., de Medeiros, C.L., Filho, E.X.F. (1999), A new xylanase from a *Trichoderma harzianum* strain, Journal of Industrial Microbiology and Biotechnology Vol. 23, pg. 682-685
36. Singh, S., Pillay, B. and Prior, B.A. (2000), Thermal stability of *B*-xylanases produced by different *Thermomyces lanuginosus* strains, Enzyme Microbial Technology Vol. 26 Issue 7, pg. 502-508
37. Srinivasan, M.C., Rele, M.V. (1999), Microbial xylanases for paper industry. Current Science Vol. 77, pg. 137-142
38. Stutzenberger, F.J., Bodine, A.B. (1992), Xylanase production by *Thermomonospora curvata*. Journal of Applied Biotechnology Vol. 72, pg. 509-511
39. Tandon, D., and Sharma, N. (2014), Isolation of potential cellulolytic and xylanolytic bacteria and optimization of their cultural conditions for enhanced production of cellulose and xylanase, Journal of Agroalimentary processes and Technologies Vol. 20, Issue 3, pg. 231-240
40. Xu, Z.H., Yun-Ling Bai, Xia Xu, Jing-Song Shi, Wen-Yi Tao (2005), Production of alkali-tolerant cellulase-free xylanase by *Pseudomonas* sp. WLUN024 with wheat bran as the main substrate, World Journal of Microbiology and Biotechnology 21, pg. 575-581
41. Yun, K., Yopi and Meryandini, A., Characterization of xylanase activity produced by *Paenibacillus* sp XJ18 from TNBD Jambi, Indonesia, HAYATI Journal of Biosciences, Vol. 22, Issue 1, pg. 20-26