



Xylanase production by *Aspergillus niger* AD-81 using *Saccharum munja* as substrate under solid state fermentation

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Abstract

Xylanase are the industrially important class of the hydrolysing enzymes which hydrolyse xylan. Xylan is the most abundant of the hemicellulose which are linear backbone of beta-1,4 linked D-xylopyranose. Xylanase production can be performed on a variety of lignocellulosic material, such as wheat bran, wheat straw, rice husk, rice bran, rice straw, corncob, corn stalk, sorghum straw, apple pomace and sugarcane bagasse have been found to be most suitable. Microbial xylanase which is produced from fungus is most stable. Fungal species of *Aspergillus*, *Trichoderma* and *Penicillium* are mainly used for the production of xylanase at industrial scale. Xylanase have wide application in industry like food, feed, and pulp or paper industry. In the present study, *Aspergillus niger* AD-81 was used for the production of xylanase which was isolated from soil sample by serial dilution method. Whole fermentation process was carried out in 250 mL Erlenmeyer flask. *Saccharum munja* was used as substrate for xylanase production by *Aspergillus niger* AD-81 under submerged fermentation. Xylanase production is influenced by substrate as well as physiochemical conditions of the medium. Parameters that can affect activities and productivities of xylanase attained in fermentation process are pH, temperature, incubation period and rpm. The maximum xylanase production of 104.80 U/mL by *Aspergillus niger* AD-81 was obtained at initial medium pH of 6.0, 30°C and 180 rpm on 4th day.

Key words – xylan , hydrolyse, hemicellulose .

Introduction: -

Xylanase are a group of enzymes which degrade the linear polysaccharides beta 1-4 xylan to xylose. Due to structural heterogeneity of the xylan, xylan degrading enzyme system include several hydrolytic enzymes. The xylanolytic enzymes system includes beta -1-4-endoxylanase, beta –xylosidase, alpha –glucuronidases, alpha-L-arabinofuranosidase, acetyl xylan estrases (Motta et al,2013) and phenolic acid (ferulic and p-coumaric acid) esterase {Beg.et al,2001; Dhiman et-al,2008}. Among all of xylanase endoxylanase and beta-xylosidase are most important in depolymerizing xylan molecules into monomeric pentose units. Endoxylanases are involved in cleaving the glycosidic bonds and in liberating short xylooligosaccharides, while beta –xylosidases releases xylose residues from the nonreducing ends of xylooligosacchrides. Acetyl–esterase, ferulic esterase, glucoronidase and arabinosidase are required for the release of different side chain from the xylan backbone (Dhimananet et –al 2008) endo-1-4-beta xylanase are reported to be produced mainly by microorganism. Exo-beta-1-4-D-Xylosidases removes successive end by catalyzing the hydrolysis of beta-1-4-D xylo-oligosaccharides beta–



xylosidases can easily hydrolysis xylobiose that is not affected by the endoxylanases that release xylose during the hydrolysis of xylan.

The best known of these are endo-beta-1-4-xylanase which attack the main chain of xylans and beta –xylosidases which hydrolyze xylooligosaccharides to D –xylose. Hemicellulose is the second most abundant plant fraction available in nature after cellulose. Xylan is the most abundant of hemicellulose which has a linear backbone of beta-1-4-linked D-xylopyranose residues which is further substituted depending on plant sources to a varying degree with glucuronopyransosyl 4-0 methyl –D-gluconopyranosoyl, alpha –L-arabinofuransoyl, acetyl, as well as linked to ferulayl and component of lignin. Xylanase can be produced either in solid state fermentation (SSF) or in submerged fermentation (SmF). The enzyme productivity in SSF being much higher than in SMF.

Source of xylanase: -

Many study have reported the production of xylanase from fungi, bacteria, yeast, marine algae, seeds crustaceans, snails but the main source of these enzymes are fungi and bacteria. According to source xylanases have different characteristics which make them useful for an application or another.

Fungal xylanases: -

Fungi (*Aspergillus* sp. and *Penicillium* sp.) are important producer of xylanases due to high yields and extracellular releases of enzymes (Nair and Shashidhar 2008). Fungal xylanases have higher activity with compared with bacteria or yeast. Xylanase derived from fungal sources have characteristics that make them unavailable for some industrial application (Mandal, 2015). Most of these xylanases are efficient at temp. below 50 degrees and a pH range 4-6

(Beg et al., 2000) for eg. fungal xylanases is cannot be used in paper and pulp industry that need an alkaline pH and temp. more than 60° C (Mandal 2015). Another problem with fungal xylanases is presence of a cellulose.

The potential application of xylanases with or without concomitant use of cellulose including the bioconversion lignocellulose to sugar ethanol and other useful substance, clarification of juice and wine and nutritional value, improvement of silage and green feed.

The use of purified xylan as an inducer increases the cost of enzyme production. For this region different lignocellulosic residue, including wheat bran, wheat straw, corncob and sugarcane bagasse have been used as growth substrate in culture to produce xylanases. Lignocelluloses are mainly secondary plant cell wall material which consists of lignin cellulose and hemicelluloses. D-xylan is the major hemicellulose found in wood.

Large quantities of lignocellulosic wastes are generated through forestry, agricultural practices and industrial processes. Particularly through from agro-allied industries such as breweries pulp and paper, textile and timber industries. These wastes generally accumulated in environment therefor causing pollution problem.

In addition, cellulose free xylanases can be used for selective hydrolysis of the hemicellulose component in paper and pulp. D-xylan are the most abundant of cellulosic polysaccharides in hardwood and annual plants. It is a strong polymer in seeds, being also a structural component of a cell wall in plants. Where they account for 25-35% of total wt. in soft woods they are found



in lesser quantities, comprising approximately 8% of dry wt.

Biodegradation of hemicellulose require the action of several enzymes among which one is xylanase 1-4-beta-D-xylanohydrolase. Hemicellulose consists of a group of mixture of hexose, pentosanes and polyuronides and their monomers are useful in the production of different antibiotics, alcohols, animal's feeds and fuels. Xylanase hydrolyzes xylan fibers to shorter sugar residue which has markedly increased due to their potential applications in food and beverages industries.

Material and Method

Micro-organism: - The fungus *A. Niger* AD-81 was isolated from soil sample by serial dilution method. This fungus was selected for xylanase production in present study because of its higher xylanase activity among all isolates.

- 1) **Biomass:** - The pod of *Saccharum munja* were collected and dried in sun. The dried pods were grinded to size of _ to 1mm size and stored in plastic bag for further use.
- 2) **Submerged fermentation:** - Submerged fermentation was carried out in 250ml Erlang monger flask containing 50ml mendel and reese medium Nutrient medium containing 1% substrate (*Saccharum munja*) was sterilized at 121°C for 20minute, then cooled and inoculated with 1ml spore suspension (1×10^7 spore/ml). The flask was inoculated at 30°C at 170/180 rpm in incubator shaker. 1ml culture filtrate was isolated periodically and analysed for enzyme assay.
- 3) **Enzyme Assay:** - Xylanase was assayed by the addition of 0.1ml of appropriately diluted enzyme to 0.1ml of 1% (w/v) starch that was solubilized in 0.9M acetic buffer (pH 5.6). The reaction mixture was incubated for 10 min at 50°C and the liberated reducing sugars were measured using 3,5-dinitrosalicylic acid method. For this, 2.0ml of DNS is added to liberated reducing sugar and boiled for 5min. A separate blank was made for each sample to eliminate the non-enzymatic release of sugar. Sugar were estimated according to method of Miller (Miller 1959).

Result and Discussion: -

Isolation of fungal strains was done from soil enriched for xylanase producing microorganisms using serial dilution agar plate method. In the present investigation, we optimize and characterize a novel amylase from soil isolated fungal strain of *Aspergillus niger* AD-81.

The maximum xylanase production of 104.80 U/mL by *Aspergillus niger* AD-81 was obtained at initial medium pH of 6.0, 30°C and 180 rpm on 4th day.

References: -

- Eda S, Ohnishi A, Kato K (1976) Xylan isolated from the stalk of *Nicotiana tabacum*. Agric Biol Chem 40:359-364
- Gamez, S., Gonzalez-Cabriales, J.J., Ramirez, J.A. & Garrote, G. 2006. Study of the hydrolysis of sugar cane bagasse using phosphoric acid. Journal of Food Engineering 74: 78-88.
- Ganga, M. A., Piñaga, F., Vallés, S., Ramón, D. and Querol, A. (1999). Aroma improving in microvinification processes by the use of a recombinant wine yeast strain expressing the *Aspergillus nidulans*, xlnA gene. International Journal of Food Microbiology, 47, pp.171–178.
- Gawande PV, and Kamat MY, (1999). Production of *Aspergillus* xylanase by lignocellulosic waste fermentation and its application. JApplMicrobiol. 87(4), 511-519.
- Ghosh G, and Sudha ML, (2012). A review on polyols: News frontiers for health-based bakery products. Int J Food Sci Nutr. 63(3),372-379.



- Ghotora SK, Chada BS, Badhan AK, Saini HS, and Bhat MK, (2006). Identification and characterization of diverse xylanases from thermophilic and thermotolerant fungi. *BioResources* 1(1), 18-33.
- Gilbert, H. J. and Hazlewood, G. P. (1993). Bacterial cellulases and xylanases. *Journal of General Microbiology*, 139, pp. 187-194.
- Gomez, L. D., Steele-King, C. G. and McQueen-Mason, S. J. (2008). Sustainable liquid biofuels from biomass: the writing's on the walls. *New Phytologists*, 178(3), pp. 473-485.
- 47, pp.171-178.
- Gawande PV, and Kamat MY, (1999). Production of *Aspergillus* xylanase by lignocellulosic waste fermentation and its application. *J Appl Microbiol.* 87(4), 511-519.
- Ghosh G, and Sudha ML, (2012). A review on polyols: News frontiers for health-based bakery products. *Int J Food Sci Nutr.* 63(3),372-379.
- Ghotora SK, Chada BS, Badhan AK, Saini HS, and Bhat MK, (2006). Identification and characterization of diverse xylanases from thermophilic and thermotolerant fungi. *BioResources* 1(1), 18-33.
- Gupta U, and Kar R, (2009). Xylanase production by a thermo-tolerant *Bacillus* species under solid-state and submerged fermentation. *Braz Arch Biol Technol.* 52(6), 1363-1371.
- Harbak, L. and Thygesen, H. V. (2002) Safety evaluation of a xylanase expressed in *Bacillus subtilis*. *Food Chem Toxicol*, 40, pp. 1-8.
- Harris AD and Ramalingam C, (2010). Xylanases and its application in food industry: A review. *J Exper Sci.* 1(7), 1-11.
- Heck, J. X., Flores, S. H., Hertz, P. F. and Ayub, M. A. Z. (2006). Statistical optimization of thermotolerant xylanase activity from Amazon isolated *Bacillus circulans* on solid-state cultivation. *Bioresource Technology*, 97, pp. 1902-1906.
- HinzSWA, Pouvreau L, Joosten R, Bartels J, Jonathan MC, Wery J and Schols H, (2009). Hemicellulase production in *Chrysosporium lucknowense* C1. *J Cereal Sci.* 50(3), 318-323.
- Juturu, V. and Wu, J. C. (2014). Microbial exo-xylanases: a mini review. *Applied Biochemistry and Biotechnology*, 174, pp. 81-92.
- Kansoh, A. L. and Gammal, A. (2001). Xylanolytic activities of *Streptomyces* sp. 1, taxonomy production, partial purification and utilization of agricultural wastes. *Acta Microbiologica ET Immunologica Hungarica*, 48, pp. 39-52.
- Keskar, S. S., Rao, M. B. and Deshpande, V. V. (1992). Characterization and sequencing of an active-site cysteine-containing peptide from the xylanase of a thermotolerant *Streptomyces*. *Journal of Biochemistry*, 281, pp. 601-605.
- Khandeparker R and Numan MT, (2008). Bifunctional xylanases and their potential use in biotechnology. *J Ind Microbiol Biotechnol.* 35(7), 635-644.
- Ghosh G, and Sudha ML, (2012). A review on polyols: News frontiers for health-based bakery products. *Int J Food Sci Nutr.* 63(3),372-379.
- Ghotora SK, Chada BS, Badhan AK, Saini HS, and Bhat MK, (2006). Identification and characterization of diverse xylanases from thermophilic and thermotolerant fungi. *BioResources* 1(1), 18-33.
- Gouda, M. K. (2000). Purification and partial characterization of cellulose free xylanase produced in solid state and submerged fermentation by *Aspergillus tamaritii*. *Advances in Food Sciences*, 22(1/2), pp. 31-37.
- Guo S, Liu D, Zhao Z, Li C, and Guo Y, (2014). Xylanase supplementation of a wheat-based diet improved nutrient digestion and mRNA expression of intestinal nutrient transporters in broiler chickens infected with *Clostridium perfringens*. *Poult Sci.* 93(1),94-103.
- Gupta U, and Kar R, (2009). Xylanase production by a thermo-tolerant *Bacillus* species under solid-state and submerged fermentation. *Braz Arch Biol Technol.* 52(6), 1363-1371.
- Harris AD and Ramalingam C, (2010). Xylanases and its application in food industry: A review. *J Exper Sci.* 1(7), 1-11.
- He J, Yu B, Zhang K, Ding X, and Chen D, (2009). Expression of *endo*-1,4-beta xylanase from *Trichoderma reesei* in *Pichia pastoris* and functional characterization of the produced enzyme. *BMC Biotechnol.* 9, article 56.
- Heck, J. X., Flores, S. H., Hertz, P. F. and Ayub, M. A. Z. (2006). Statistical optimization of thermotolerant xylanase activity from Amazon isolated *Bacillus circulans* on solid-state cultivation. *Bioresource Technology*, 97, pp. 1902-1906.
- Hessing JG, Rotterdam CV, Verbake JM, Roza M, Maat J, Gorcom RFV, and Hondel CAVD, (1994). Isolation and characterization of a 1,4-b-endoxylanase gene of *A. awamori*. *Curr Genet.* 26(3), 228-232.
- HinzSWA, Pouvreau L, Joosten R, Bartels J, Jonathan MC, Wery J and Schols H, (2009). Hemicellulase production in *Chrysosporium lucknowense* C1. *J Cereal Sci.* 50(3), 318-323.
- Juturu, V. and Wu, J. C. (2014). Microbial exo-xylanases: a mini review. *Applied Biochemistry and Biotechnology*, 174, pp. 81-92.
- Juturu, V. and Wu, J. C. (2011). Microbial xylanases: Engineering, production and industrial applications. *Biotechnol*



- Adv. 30(6), 1218-1227.
- Kansoh, A. L. and Gammal, A. (2001). Xylanolytic activities of *Streptomyces* sp. 1, taxonomy production, partial purification and utilization of agricultural wastes. *Acta Microbiologica ET Immunologica Hungarica*, 48, pp. 39-52.
- Keskar, S. S., Rao, M. B. and Deshpande, V. V. (1992). Characterization and sequencing of an active-site cysteinecontaining peptide from the xylanase of a thermotolerant *Streptomyces*. *Journal of Biochemistry*, 281, pp. 601–605.
- Gawande PV, and Kamat MY, (1999). Production of *Aspergillus* xylanase by lignocellulosic waste fermentation and its application. *JApplMicrobiol.* 87(4), 511-519.
- Ghosh G, and Sudha ML, (2012). A review on polyols: News frontiers for health-based bakery products. *Int J Food Sci Nutr.* 63(3),372-379.
- Gomez, L. D., Steele-King, C. G. and McQueen-Mason, S. J. (2008). Sustainable liquid biofuels from biomass: the writing's on the walls. *New Phytologists*, 178(3), pp. 473–485.
- Gouda, M. K. (2000). Purification and partial characterization of cellulose free xylanase produced in solid state and submerged fermentation by *Aspergillus tamaritii*. *Advances in Food Sciences*, 22(1/2), pp. 31-37.
- Guo S, Liu D, Zhao Z, Li C, and Guo Y, (2014). Xylanase supplementation of a wheat-based diet improved nutrient digestion and mRNA expression of intestinal nutrient transporters in broiler chickens infected with *Clostridium perfringens*. *Poult Sci.* 93(1),94-103.
- Gupta U, and Kar R, (2009). Xylanase production by a thermo-tolerant *Bacillus* species under solid-state and submerged fermentation. *Braz Arch Biol Technol.* 52(6), 1363-1371.
- Harbak, L. and Thygesen, H. V. (2002) Safety evaluation of a xylanase expressed in *Bacillus subtilis*. *Food Chem Toxicol.* 40, pp. 1–8.
- Harris AD and Ramalingam C, (2010). Xylanases and its application in food industry: A review. *J Exper Sci.* 1(7), 1-11.
- He J, Yu B, Zhang K, Ding X, and Chen D,(2009).Expression of *endo-1,4-beta* xylanase from *Trichoderma reesei* in *Pichia pastoris* and functional characterization of the produced enzyme. *BMC Biotechnol.* 9, article 56.
- Heck, J. X., Flores, S. H., Hertz, P. F. and Ayub, M. A. Z. (2006). Statistical optimization of thermotolerant xylanase activity from Amazon isolated *Bacillus circulans* on solid-state cultivation. *Bioresource Technology*, 97, pp. 1902–1906.
- Herculano, P.N., Porto, T.S., Moreira, K.A., Pinto, G.A.S., Souza-Motta, C.M. & Porto, A.L. 2011. Cellulase Production by *Aspergillus japonicus* URM5620 Using Waste from Castor Bean (*Ricinus communis* L.). *Applied Biochemistry and Biotechnology* 165: 1057-1067.
- Hessing JG, Rotterdam CV, Verbake JM, Roza M, Maat J, Gorcom RFV, and Hondel CAVD,(1994). Isolation and characterization of a 1,4-b-endoxylanase gene of *A. awamori*. *Curr Genet.* 26(3), 228-232.
- HinzSWA, Pouvreau L, Joosten R, Bartels J, Jonathan MC, Wery J and Schols H, (2009). Hemicellulase production in *Chryso sporiumlucknowense* C1. *J Cereal Sci.* 50(3), 318-323.
- Jain A, Morlok CK, and Henson JM, (2013). Comparison of solid state and submerged state fermentation for the bioprocessing of switchgrass to ethanol and acetate by *Clostridium phytofermentans*. *ApplMicrobiolBiotechnol.* 97(2), 905-917.
- Juodeikiene G, Basinskiene L, Vidmantiene D, Makaravicius T, Bartkiene E.Benefits of β -xylanase for wheat biomass conversion to bioethanol. *J Sci FoodAgric.* 2012 Jan 15;92(1):84-91.
- Juturu, V. and Wu, J. C. (2014). Microbial exo-xylanases: a mini review. *Applied Biochemistry and Biotechnology*, 174, pp. 81–92.
- Juturu, V. and Wu, J. C. (2011). Microbial xylanases: Engineering, production and industrial applications. *Biotechnol Adv.* 30(6), 1218-1227.
- Kansoh, A. L. and Gammal, A. (2001). Xylanolytic activities of *Streptomyces* sp. 1, taxonomy production, partial purification and utilization of agricultural wastes. *Acta Microbiologica ET Immunologica Hungarica*, 48, pp. 39-52.
- Keskar, S. S., Rao, M. B. and Deshpande, V. V. (1992). Characterization and sequencing of an active-site cysteinecontaining peptide from the xylanase of a thermotolerant *Streptomyces*. *Journal of Biochemistry*, 281, pp. 601–605.
- KhandeparkerR and Numan MT, (2008).Bifunctional xylanases and theirpotential use in biotechnology. *J Ind MicrobiolBiotechnol.* 35(7), 635-644.
- Khonzue, P., Laothanachareon, T., Rattanaphan, N., Tinnasulanon, P., Apawasin, S., Paemanee, A., Ruanglek, V., Tanapongpipat, S., Champreda, V. and Eurwilaichitr, L. (2011) Optimization of xylanase production from *Aspergillus niger* for Eda S, Ohnishi A, Kato K (1976) Xylan isolated from the stalk of *Nicotiana tabacum*. *Agric Biol Chem* 40:359-364
- Fan G,Katrolia P, Jia H, Yang S, Yan Q, and Jiang Z, (2012). High-level expression of a xylanase gene from the thermophilic fungus *Paecilomycesthermophila* in *Pichia pastoris*. *Biotechnol Lett.* 34(11), 2043-2048.



- Gamez, S., Gonzalez-Cabriales, J.J., Ramirez, J.A. & Garrote, G. 2006. Study of the hydrolysis of sugar cane bagasse using phosphoric acid. *Journal of Food Engineering* 74: 78-88.
- Ganga, M. A., Piñaga, F., Vallés, S., Ramón, D. and Querol, A. (1999). Aroma improving in microvinification processes by the use of a recombinant wine yeast strain expressing the *Aspergillus nidulans*, xlnA gene. *International Journal of Food Microbiology*, 47, pp.171–178.
- Gawande PV, and Kamat MY, (1999). Production of *Aspergillus* xylanase by lignocellulosic waste fermentation and its application. *JApplMicrobiol.* 87(4), 511-519.
- Ghosh G, and Sudha ML, (2012). A review on polyols: News frontiers for health-based bakery products. *Int J Food Sci Nutr.* 63(3),372-379.
- Ghotor SK, Chada BS, Badhan AK, Saini HS, and Bhat MK, (2006). Identification and characterization of diverse xylanases from thermophilic and thermotolerant fungi. *BioResources* 1(1), 18-33.
- Gilbert, H. J. and Hazlewood, G. P. (1993). Bacterial cellulases and xylanases. *Journal of General Microbiology*, 139, pp. 187-194.
- Gomez, L. D., Steele-King, C. G. and McQueen-Mason, S. J. (2008). Sustainable liquid biofuels from biomass: the writing's on the walls. *New Phytologists*, 178(3), pp. 473–485.
- Gouda, M. K. (2000). Purification and partial characterization of cellulose free xylanase produced in solid state and submerged fermentation by *Aspergillus tamarii*. *Advances in Food Sciences*, 22(1/2), pp. 31-37.
- Guimaraes, N.C.A., Sorgatto, M., Peixoto-Nogueira, S.C., Betini, J.H.A., Zanoelo, F.F., Marques, M.R., Polizeli, M.L.T.M. & Giannesi, G.C. Bioprocess and biotechnology: effect of xylanase from *Aspergillus niger* and *Aspergillus flavus* on pulp bio-bleaching and enzyme production using agroindustrial residues as substrate. *SpringerPlus* 380: 1-7.
- Guo S, Liu D, Zhao Z, Li C, and Guo Y, (2014). Xylanase supplementation of a wheat-based diet improved nutrient digestion and mRNA expression of intestinal nutrient transporters in broiler chickens infected with *Clostridium perfringens*. *Poult Sci.* 93(1),94-103.
- Gupta U, and Kar R, (2009). Xylanase production by a thermo-tolerant *Bacillus* species under solid-state and submerged fermentation. *Braz Arch Biol Technol.* 52(6), 1363-1371.
- Harbak, L. and Thygesen, H. V. (2002) Safety evaluation of a xylanase expressed in *Bacillus subtilis*. *Food Chem Toxicol*, 40, pp. 1–8.
- Harris AD and Ramalingam C, (2010). Xylanases and its application in food industry: A review. *J Exper Sci.* 1(7), 1-11.
- He J, Yu B, Zhang K, Ding X, and Chen D, (2009). Expression of *endo*-1,4-beta xylanase from *Trichoderma reesei* in *Pichia pastoris* and functional characterization of the produced enzyme. *BMC Biotechnol.* 9, article 56.
- Heck, J. X., Flores, S. H., Hertz, P. F. and Ayub, M. A. Z. (2006). Statistical optimization of thermotolerant xylanase activity from Amazon isolated *Bacillus circulans* on solid-state cultivation. *Bioresource Technology*, 97, pp. 1902–1906.
- Herculano, P.N., Porto, T.S., Moreira, K.A., Pinto, G.A.S., Souza-Motta, C.M. & Porto, A.L. 2011. Cellulase Production by *Aspergillus japonicus* URM5620 Using Waste from Castor Bean (*Ricinus communis* L.). *Applied Biochemistry and Biotechnology* 165: 1057-1067.
- Hessing JG, Rotterdam CV, Verbake JM, Roza M, Maat J, Gorcom RFV, and Hondel CAVD, (1994). Isolation and characterization of a 1,4-b-endoxylanase gene of *A. awamori*. *Curr Genet.* 26(3), 228-232.
- HinzSWA, Pouvreau L, Joosten R, Bartels J, Jonathan MC, Wery J and Schols H, (2009). Hemicellulase production in *Chrysosporium lucknowense* C1. *J Cereal Sci.* 50(3), 318-323.
- Jain A, Morlok CK, and Henson JM, (2013). Comparison of solid state and submerged state fermentation for the bioprocessing of switchgrass to ethanol and acetate by *Clostridium phytofermentans*. *ApplMicrobiolBiotechnol.* 97(2), 905-917.
- Juodeikiene G, Basinskiene L, Vidmantiene D, Makaravicius T, Bartkiene E. Benefits of β -xylanase for wheat biomass conversion to bioethanol. *J Sci Food Agric.* 2012 Jan 15;92(1):84-91.
- Juturu, V. and Wu, J. C. (2014). Microbial exo-xylanases: a mini review. *Applied Biochemistry and Biotechnology*, 174, pp. 81–92.
- Juturu, V. and Wu, J. C. (2011). Microbial xylanases: Engineering, production and industrial applications. *Biotechnol Adv.* 30(6), 1218-1227.
- Kansoh, A. L. and Gammal, A. (2001). Xylanolytic activities of *Streptomyces* sp. 1, taxonomy production, partial purification and utilization of agricultural wastes. *Acta Microbiologica ET Immunologica Hungarica*, 48, pp. 39-52.
- Keskar, S. S., Rao, M. B. and Deshpande, V. V. (1992). Characterization and sequencing of an active-site cysteine-containing peptide from the xylanase of a thermotolerant *Streptomyces*. *Journal of Biochemistry*, 281, pp. 601–605.
- Khandeparker R and Numan MT, (2008). Bifunctional xylanases and their potential use in biotechnology. *J Ind MicrobiolBiotechnol.* 35(7), 635-644.



- Khonzue, P., Laothanachareon, T., Rattanaphan, N., Tinnasulanon, P., Apawasin, S., Paemane, A., Ruanglek, V., Tanapongpipat, S., Champreda, V. and Eurwilaichitr, L. (2011) Optimization of xylanase production from *Aspergillus niger* for Eda S, Ohnishi A, Kato K (1976) Xylan isolated from the stalk of *Nicotiana tabacum*. *Agric Biol Chem* 40:359-364
- Fan G, Katrolia P, Jia H, Yang S, Yan Q, and Jiang Z, (2012). High-level expression of a xylanase gene from the thermophilic fungus *Paecilomyces thermophila* in *Pichia pastoris*. *Biotechnol Lett.* 34(11), 2043-2048.
- Gamez, S., Gonzalez-Cabriales, J.J., Ramirez, J.A. & Garrote, G. 2006. Study of the hydrolysis of sugar cane bagasse using phosphoric acid. *Journal of Food Engineering* 74: 78-88.
- Ganga, M. A., Piñaga, F., Vallés, S., Ramón, D. and Querol, A. (1999). Aroma improving in microvinification processes by the use of a recombinant wine yeast strain expressing the *Aspergillus nidulans*, xlnA gene. *International Journal of Food Microbiology*, 47, pp.171–178.
- Gawande PV, and Kamat MY, (1999). Production of *Aspergillus* xylanase by lignocellulosic waste fermentation and its application. *JApplMicrobiol.* 87(4), 511-519.
- Ghosh G, and Sudha ML, (2012). A review on polyols: News frontiers for health-based bakery products. *Int J Food Sci Nutr.* 63(3), 372-379.
- Gilbert, H. J. and Hazlewood, G. P. (1993). Bacterial cellulases and xylanases. *Journal of General Microbiology*, 139, pp. 187-194.
- Gomez, L. D., Steele-King, C. G. and McQueen-Mason, S. J. (2008). Sustainable liquid biofuels from biomass: the writing's on the walls. *New Phytologists*, 178(3), pp. 473–485.
- Gupta U, and Kar R, (2009). Xylanase production by a thermo-tolerant *Bacillus* species under solid-state and submerged fermentation. *Braz Arch Biol Technol.* 52(6), 1363-1371.
- Harbak, L. and Thygesen, H. V. (2002) Safety evaluation of a xylanase expressed in *Bacillus subtilis*. *Food Chem Toxicol*, 40, pp. 1–8.
- Harris AD and Ramalingam C, (2010). Xylanases and its application in food industry: A review. *J Exper Sci.* 1(7), 1-11.
- He J, Yu B, Zhang K, Ding X, and Chen D, (2009). Expression of *endo*-1,4-beta xylanase from *Trichoderma reesei* in *Pichia pastoris* and functional characterization of the produced enzyme. *BMC Biotechnol.* 9, article 56.
- Heck, J. X., Flores, S. H., Hertz, P. F. and Ayub, M. A. Z. (2006). Statistical optimization of thermotolerant xylanase activity from Amazon isolated *Bacillus circulans* on solid-state cultivation. *Bioresource Technology*, 97, pp. 1902–1906.
- Herculano, P.N., Porto, T.S., Moreira, K.A., Pinto, G.A.S., Souza-Motta, C.M. & Porto, A.L. 2011. Cellulase Production by *Aspergillus japonicus* URM5620 Using Waste from Castor Bean (*Ricinus communis* L.). *Applied Biochemistry and Biotechnology* 165: 1057-1067.
- Hessing JG, Rotterdam CV, Verbake JM, Roza M, Maat J, Gorcom RFV, and Hondel CAVD, (1994). Isolation and characterization of a 1,4-b-endoxylanase gene of *A. awamori*. *Curr Genet.* 26(3), 228-232.
- HinzSWA, Pouvreau L, Joosten R, Bartels J, Jonathan MC, Wery J and Schols H, (2009). Hemicellulase production in *Chryso sporium lucknowense* C1. *J Cereal Sci.* 50(3), 318-323.
- Jain A, Morlok CK, and Henson JM, (2013). Comparison of solid state and submerged state fermentation for the bioprocessing of switchgrass to ethanol and acetate by *Clostridium phytofermentans*. *ApplMicrobiolBiotechnol.* 97(2), 905-917.
- Juodeikiene G, Basinskiene L, Vidmantiene D, Makaravicius T, Bartkiene E. Benefits of β -xylanase for wheat biomass conversion to bioethanol. *J Sci FoodAgric.* 2012 Jan 15;92(1):84-91.
- Juturu, V. and Wu, J. C. (2014). Microbial exo-xylanases: a mini review. *Applied Biochemistry and Biotechnology*, 174, pp. 81–92.
- Juturu, V. and Wu, J. C. (2011). Microbial xylanases: Engineering, production and industrial applications. *Biotechnol Adv.* 30(6), 1218-1227.
- Kansoh, A. L. and Gammal, A. (2001). Xylanolytic activities of *Streptomyces* sp. 1, taxonomy production, partial purification and utilization of agricultural wastes. *Acta Microbiologica ET Immunologica Hungarica*, 48, pp. 39-52.
- Keskar, S. S., Rao, M. B. and Deshpande, V. V. (1992). Characterization and sequencing of an active-site cysteine-containing peptide from the xylanase of a thermotolerant *Streptomyces*. *Journal of Biochemistry*, 281, pp. 601–605.
- Khandeparker R and Numan MT, (2008). Bifunctional xylanases and their potential use in biotechnology. *J Ind MicrobiolBiotechnol.* 35(7), 635-644.
- Khonzue, P., Laothanachareon, T., Rattanaphan, N., Tinnasulanon, P., Apawasin, S., Paemane, A., Ruanglek, V., Tanapongpipat, S., Champreda, V. and Eurwilaichitr, L. (2011) Optimization of xylanase production from *Aspergillus niger* for
- Nair, S. G., Sindhu, R. and Shashidhar, S. (2008). Fungal xylanase production under solid state and submerged fermentation conditions. *African Journal of Microbiology Research*, 2, pp. 82–86.



- Narasimha G, Sridevi A, Buddolla V, Chandra MS, and Reddy BR, (2006). Nutrient effects on production of cellulolytic enzymes by *Aspergillus niger*. Afr J Biotechnol. 5(5), 472-476.
- Ninawe, S., Kapoor, M. and Kuhad, R. C. (2008). Purification and characterization of extracellular xylanase from *Streptomyces cyaneus* SN32. Bioresource Technology, 99, pp. 1252–1258.
- Okafor, U. A., Okochi, V. I., Onyegeme-okerenta, B. M. and Nwodo- Chinedu, S. (2007). Xylanase production by *Aspergillus niger* ANL 301 using agro–wastes. African Journal of Biotechnology, 6(14), pp. 1710-1714.
- OmogbenigunFO, Nyachoti CM, and Slominski BA, (2004). Dietary supplementation with multienzyme preparations improves nutrient utilization and growth performance in weaned pigs. J Anim Sci. 82(4), 1053-1061.
- OsipovDO, Rzhkova AM, Matys VY, Koshelev AV, Okuney ON, Rubtsova EA, Pravil’nikov AG, Zorov IN, Sinitsyna OA, Oveshnikov IN, Davidov ER, and Sinitsyn AP, (2011). Production of biocatalysts on the basis of recombinant heterologous xylanase producer strains in the *Penicillium vericulosum* fungus: Their application in the hydrolysis of timber and wood processing industry wastes. Catalysis Ind. 3(1), 34-40.
- OtteLG, and Quax WJ, (2005). Direct evolution: Selecting today’s biocatalysts. Biomol Eng. 22(1-3), 1-9.
- Pandey A, Soccol CR, and Mitchell D,(2000). New developments in solid state fermentation I: Bioprocess and products. Process Biochem. 35(10), 153-169.
- Pang, P. K., Darah, I., Poppe, L., Szakacs, G. and Ibrahim C. O. (2006). Xylanase production by a local isolate, *Trichoderma* spp. FETL c3-2 via solid state fermentation using agricultural wastes as substrates. Malaysian Journal of Microbiology, 2(1), pp. 7-14.
- Parameswaran, B. 2009. Sugarcane Bagasse. In: P.S.N. Nigam, P., A. Pandey (ed.). Biotechnology for agro-industrial residues utilisation - Utilisation of agro-residues. Springer, pp. 239-252.
- Polizeli, M.L.T.M., Rizzatti, A.C.S., Monti, R., Terenzi, H.F., Jorge, J.A. & Amorim, D.S. 2005. Xylanases from fungi: properties and industrial applications. Applied Microbiology Biotechnology 67: 577-591.
- Puls J, Schuseil J (1993) Chemistry of hemicelluloses: relationship between hemicellulose structure and enzyme required for hydrolysis. In: Coughlan MP and Hazlewood GP (eds) Hemicellulose and hemicellulases. Portland Press, London, pp 1 -28
- Raghukumar C, Muraleedharan U, Gaud VR, Mishra R. Xylanases of marine fungi of potential use for biobleaching of paper pulp. J Ind Microbiol Biotechnology. 2004;31:433–441.
- Rizzatti, A.C., Jorge, J.A., Terenzi, H.F., Rechia, C.G. &Polizeli, M.L. 2001. Purification and properties of a thermostable extracellular β -D-xylosidase produced by a thermotolerant *Aspergillus phoenicis*. Journal of Industrial Microbiology & Biotechnology 26: 156-160.
- Rodrigues, R.C.L.B., Felipe, M.D.G.A., Silva, J.B. A. &Vitolo, M. 2003. Response surface