Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2016, 8(3):334-339

Review Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Xylanase: A promising enzyme

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ABSTRACT

Xylanase is a naturally occurring enzyme found in microbes and fungi. Xylanase belonging to pentosanases, a class of enzyme which breaks down cell wall matrix of plants breaks down xylan into xylose. Thus this property of xylanase makes it important at commercial level. Recently, xylanases are being produced by the help of genetically modified organisms (bacteria and fungus). Xylanases also find its application in various industries. These include textile and paper industry, beverage industry, bakery industry, pharmaceuticals industry, biofuel production. In this review we are tried to highlight the importance of xylanase at commercial level.

Keywords: Xylan, enzyme, microorganism, activity, applications

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INTRODUCTION

Xylan is the polysaccharide with major structures in plant cells and in nature the most abundant polysaccharide accounting for approximately one third of renewable organic carbon sources on the earth. The major component of hemi cellulose, these polysaccharide constitute a complex of carbonate hydrolase including xylan, xyloglycan, glucomannan, galactoglucomannan and arabinogalactan. Xylans are present in large quantity in hard wood from angiosperm (the cell content 15_30% of the cell wall content) and soft wood from gymnosperms (7-10% as well as in annual plans less than 30%) [1]. Being highly branched polysaccharide, xylan varies in structure between different plant species and the homopolymeric backbone chain of 1,4 linked beta -D xylopyranosyle units can be substituted with the number of other side chain groups. Due to its heterogeneity and complexity, complete hydrolysis of xylan require a large variety of cooperatively acting enzymes like endo-1,4-beta-D-xylanases (cleave the xylan backbone), beta-D xylosidases (cleave xylose monomers from non-reducing end of xylooligosaccharides and xylobiose), alpha-L-arabinofuranosidases, alpha-D--glucuronidases etc. for removal for different substituent side chain. Complete xylanolytic enzyme system including all of this activity is well documented in the microorganisms like fungi [2]. Some of the most important xylanolytic microbes include *Aspergilli, trichodermi, streptomycetes, Pheanerochaetas, Chyridiomycetes, Ruminococci, Fibrobacters, Clostridia, and Bacilli* etc. The ecological niches of these microorganisms are diverse and wide spread and typically include environments (for e.g. forests) where plant materials accumulate and deteriorate. Xylanolytic microorganisms have also been reported to produce extremophilic xylanases and have been isolated from variety of sources like thermal springs marine solfateric fields, antarctic environments, soda lakes, soda deserts etc. xylanases constitute the major portion of hemicelluloses and have wide range of application in industrial and biotechnology. Their commercial exploitation in the area of food, feed and paper and pulp industry are well documented. Recently xylanases are also being exploited

for biofuel production from agricultural residues.

Xylans and its enzymatic hydrolysis
The nature of its substituents is based on a broad distinction may be made therefore in xylans, in which their increment in complexity from linear to highly substituted xylans. Xylans can be categorized into four major groups: 1. Arabinoxylans, having only side chains of single terminal units of alpha-L-arabinofuranosyl substituents. Particulary in the case of cereals, arabinoxylans which varies in the degree of arabinosyl substitution, with either 2-O-and 3-O-monosubstituted or double (2-O-, 3-O-) substituted xylosyl residues. 2. Glucuronoxylans, in which α-D-glucoronic acid or its 4-O-methyl ether derivative represents the only substituent. 3. Glucuronoarabinoxylan, in which α-D-glucoronic (and 4-O-methyl-α-D—glucoronic) acid and α-l-arabinose are both present. 4. Galactoglucuronoarabinoxylans, which are characterized because of the presence of terminal β-D-galactopyranosyl residues on complex oligosaccharide side chains of xylans and are found typically in perennial plants. There is a category which exhibits micro-heterogeneity with respect to the degree and in branching nature. Basically the side chains determining the solubility, physically conformation, and reactivity of the xylan molecule with the other hemicellulosic components and hence greatly influencing the mode and extent the enzymatic cleavage. Endospermic arabinoxylans of annual plants, also called pentosans, are water soluble and alkaline solutions than xylans of lignocellulosic materials because of their branched structures.

Due to the heterogeneity and complex chemical nature of plant xylan, its complete breakdown requires action of complex several hydrolytic enzymes with diverse specificities and mode of action. Thus, it is not surprising for xylan-degrading cells to produce and arsenal of enzymes. The xylanolytic enzyme system that carries hydrolysis of xylan is normally composed of a repertoire of hydrolytic enzymes, including endoxylanase (endo-1,4-β-xylanase, E.C.3.2.1.8), β-xylosidase (xylan -1,4-β-xylosidase, E.C. 3.2.1.37), α-glucuronidase (α-glucosiduronase, E.C.3.2.1.139), α-arabinofuranosidase (α-Larabinofuranosidase, E.C. 3.2.1.55) and acetylxylan esterase (E.C.3.1.1.72). All of these enzymes act cooreratively to convert xylan into its constituent sugars. Among all xylanases, endoxylanases are the most important due to their directly involving in cleaving the glycosidic bonds and in liberating short xylooligosaccharides. Xylan, being a molecularly highly mass polymer, cannot penetrate the cell wall. The low mole ular mass fragments of xylan play a key role in the regulating the xylanase biosynthesis. These include fragments like xylose, xylobiose, xylooligosaccharides, heterodisaccharide of xylose and glucose and their positional isomers. These molecules are liberated from xylan through the action of small amounts of constituently produced enzymes. Xylanase catalyzes the random hydrolysis of xylan to xylooligosaccharides, while β-xylosidase releases xylose residues from the non-reducing ends of oligosaccharides. However, a complete degradation requiers the synergistic action of acetyl esterase to remove the acetyl substituent's from the β-1,4-linked D-xylose backbone of xylan.

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Based on hydrophobic cluster analysis of the catalytic domains and similarities in the amino acid sequences, Xylanases have been primarily classified as GH 10 and 11 [3]. Several models have been proposed to explain the mechanism of xylanase action. Generally, the hydrolysis of xylans by GH 10 and 11 results in the retention of the anomeric centre of the reducing sugar monomer of the carbohydrate. Families 10 and 11 contain enzymes that catalyze the hydrolysis with the retention of the anomeric configuration, with residues of two glutamates being implicated in the catalytic mechanism. This indicates a double-displacement mechanism, in which a covalent glycosyl enzyme is formed intermediating subsequently hydrolyzed, and two carboxylic acid residues, suitably located in the active site, are involving in the formation of the intermediate; one acts as a general acid catalyst by protonating the substrate, while the nucleophilic attack a second performer, which results in the leaving group departure and form the α-glycosyl enzyme intermediate (β to α inversion). In the second step, the first carboxylate group instead behave as a general base functions, abstracting a proton from a nucleophilic water molecule, which attacks the anomeric carbon. This a second substitution leads to, in which the anomeric carbon again passes via a transition state and the product is rising with the β configuration (α to β inversion). Glycoside hydrolase family 10 is composed of endo-1, 4-β-xylanases, and endo-1, 3-β-xylanases (EC 3.2.1.32) (34). Members of this family can also hydrolyze the aryl β-glucosides of xylobiose and xylotriose at the aglyconic bond. Furthermore, these enzymes are highly active on short xylo oligosaccharides, thus it indicates small substrate-binding sites. Crystal structure analyses, kinetic analyses of the activity on xylo oligosaccharides of various sizes and end product analyses have indicated that family 10 xylanases typically have four to five substrate-binding sites. Members of this family also

typically have a high molecular mass, a low pI and display an $(\alpha/\beta)_8$-barrel fold [4]. Compared to other xylanases, GH11 members display several interesting properties, such as high substrate selectivity and high catalytic efficiency, a small size, and a variety of optimum pH applications. Family 11 is composed only of xylanases (EC3.2.1.8), leading to their consideration as "true xylanases," as they are exclusively active on D-xylose-containing substrates. GH11 enzymes are generally characterized by a high pI, a low molecular weight, a double-displacement catalytic mechanism, two glutamates that act as the catalytic residues and a β-jelly roll fold structure [5]. Additionally, the products of their action can be further hydrolyzed by the family 10 enzymes. Similar to family 10 xylanases, these enzymes can hydrolyze the aryl β-glycosides of xylobiose and xylotriose at the aglyconic bond, but they are inactive on aryl cellobiosides. Furthermore, in contrast to the family 10 xylanases, but similar to the family 8 cold-adapted xylanases, these enzymes are most active on long-chain xylooligosaccharides, and it has been found that they have larger substrate-bindind clefts, containing at least seven subsites. Xylanases belonging to GH10 exhibit greater catalytic versatility and lower substrate specificity than those belonging to GH11. According to Sabini et. al. [5], the binding sites for xylose residues in xylanases are termed subsites, with bond cleavage occuring between the sugar residues at the -1 (non-reducing) and the +1 (reducing) ends if the polysaccharides substrate. As observed in assays using arabinoxylan as the substrate, GH10 products have arabinose residues substituted at the +2 subsite. These results suggest that GH 10 enzymes are able to hydrolyze xylose linkages closer to the side. Therefore, xylanases from family 11 preferentially cleave the unsubstituted regions of the arabinoxylan backbone, whereas GH10 enzymes cleave the decorated regions, being less hampered by the presence of substituents along the xylan backbone. The xylan side-chain decorations are recognized by xylanases, and the degree of substitution in xylan will influence the hydrolytic products; this difference insubstrate specificity has important implications in the deconstruction of xylan.

Xylanolytic Microorganisms

Xylanases are produced by diverse organisms like fungi, bacteria, algae, protozoa, gastropods and arthropods. Bacterial genera like *Bacillus, Cellulomonas, Micrococcus, Staphylococcus, Thermotoga, Paenibcillus, Arthrobacter, Microbacterium, Pseudoxanthomonas, Rhodococcus* have been reported to produce xylanases. Among the *actinomycetes group, Streptomyces, Thermomonospora, Thermoactinomycetes* etc. are commonly reported for xylanase production fungal genera like *Aspergillus, Fusarium, Penicillium, Geotrichum, Paecilomyces, Cephalosporium, Trichoderma* are reported for xylanase production. Bacteria have an advantage over fungi for xylanase production as large-scale cultivation of fungi is often difficult due to slow generation time, co-production of highly viscous polymers and low oxygen penetration. Moreover low pH optima for fungal growth may necessitate additional downstream processing for fungal xylanases. Among the bacterial genera, Bacillus is the most predominant genus as xylanase producer. Members of genus Bacillus like *Bacillus circulans*, *B. Stearothermophilus, B.polymyxa, B subtilis, B. Amyloliquifaciens* have been reported to produce considerable amount of xylanases. Many of the extremophilic xylanases are produced by the thermophilic Bacillus like *B. Acidocaldarius, B. Thermoalkalophilus* have been reported to produce considerable amount of xylanases. Many of the extremophilic xylanases are produced by the thermophilic bacillus like *B. Acidocaldarius, B. Thermoalkalophilus* have been reported to produce xylanases which are active at temperature ranging from 50-80℃. Thermotoga are another group of bacteria which produce extremely thermostable xylanases active in the temperature ranging from 80-110℃. Many of the thermophilic actinomycetes also produce thermostable xylanases showing activity at temperature ranging from 60-70° C. Recently endoxylanases from thermophilic actinomycetes *Microtetraspora flexuosa* SIIX have been reported to show optimum activity at 80℃ [6]. Due to low pH requirement, fungi are very rarely reported to produce alkophilic xylanases. However fungal species like *Aspergillus Kawachii, Cryptococcus* sp. H-2 and *Penicillium* Sp. 40 are reported to produce most acidophilic xylanases stable at pH 1-2 [7]. Many thermophilic fungi like *Gloephyllum trabeum, Talaromyces Byssochlamydoides, Thermoascus aurantiacus, Thermomyces Lanugimosus* are reported to produce thermostable xylanases activity in the range of 50-80℃ [8,9].

Factors affecting xylanase yield and activity

Commercial production of xylanase involves industrial fermentation processes using suitable bacteria or fungi. The yield of xylanases in fermentation process are generally governed by a number of factors as production of xylanases in microorganisms is dependent on the availability of appropriate substrate, the carbon sources present in the media are the most important factor for xylanase production a number of substrate like corn cap, wheat bran, rice bran, ponds stalk, bagasse, etc have been found to be very suitable substrate on production of xylanases. In addition to the nature of substrate, substrate accessibility, rate and amount of release of the xylooligosaccharide, their chemical in nature, quantity of xylose release also influence the production of xylanase during fermentation process [10] have reported sugarcane bagasse has a base inducer of xylanase and beta-xylosidase in *Cellulomonas flavigena*.

Sugumaran *et al.* [11] reported production of thermoalkilic tolerant xylanase from *B. Subtilis* in submerged fermentation using cassava bagasse reported sugarcane bagasse as a best inducer for xylanase production from bacillus sp. Rice bran and soybean residues served as excellent substrate for xylanase production by Bacillus sp using soil state fermentation [12] yeast extract, beef extract, peptone, soy bean meal have been reported to serve as good nitrogen soure for yeild of xylanase from different bacteria [13-15] reported trypton as a most efficient source for xylanase production from *Streptomyces* sp. 7b. When different agriculture waste substrate, like wheat bran, sugarcane, bagasse, rice bran and saw dust are subjected to solid state fermentation by streptomyces sp. 7b, maximum xylanase contain observed in wheat bran containing medium. Using yeast extract, peptone and beef extract as organic nitrogen source, enhanced xylanase level from *Streptomyces cyaneus* SN32, *Bacillus* sp., *Streptomyces* sp., *Streptomyces* sp. CA24 has been reported [16]. Yeast extract, peptone and beef extract has an important role in xylanase production by bacillus ap., probably because of some important elements contained in these organic nitrogen sources that are necessary for this bacterium metabolism are a complex nitrogen source was essential for bacterial growth and high enzyme activity.

Besides the nutritional components, other bioprocess parameters like pH of the medium, temperature for incubation, agitation, whistle size, initial load of inoculums etc. can also influence the xylanase production during fermentation process.

Application of xylanases

Major applications of xylanases are in paper and pulp industry, animal feed processing, clarification of beverages and recent application involves biofuel production from agroresidues.

Paper Industry

Chlorinated phenolic compounds as well as polychlorinated biphenyls, produced during conventional pulp bleaching being toxic and highly resistant to biodegradation, from one of the major sources of environmental pollution.

Removal of residual lignin from kraft pulp is physically and chemically restricted by hemicelluloses. Lignin has been reported to link with hemicelluloses. The most common pulping process is the kraft process where cooking of wood chips is carried out in a solution of Na_2S/NaOH at about 170°C for two hours resulting in the degradation and solubilisation of lignin. To obtain pulp of very high brightness and brightness stability, all the lignin must be remove from the pulp. For that, chemical pulping is more effective than mechanical pulping. However, there is the formation of residual lignin which has to be removed by bleaching process. The residual lignin in chemical pulp is dark in colour because it has been extensively oxidized and modified in the cooking process. This residual lignin is difficult to be removed due to its covalent bonding to the hemicellulose and perhaps to cellulose fibers. The bleaching of the pulp can be regarded as a purification process involving the destruction, alteration, or solubilization of the lignin, coloured organic matters and other undesirable residues on the fibres.

Conventionally chlorine is used for bleaching of pulp. Chlorination of pulp does not show any decolorizing effect, and in fact, the colour of the pulp may increase with chlorination and it is the oxidative mechanism which aids the pulp bleaching. The dominant role of chlorine in bleaching is to convert the residual lignin in the pulp to water or alkali solute products. The effluent that are produced during the bleaching process, especially those following the chlorination and the first extraction stages are the major contributors to waste water pollution from the pulp paper industry. During the Kraft process part, of the xylan is relocated on the fibre surfaces. Conserable amount of xylan is present in the fibres afer pulping process. Enzymatic hydrolysis of the reprecipitateted and the relocateted xylans on the surface of the fibres apparently renders the structures of the fibres more permeable. Increased permeability allows the passage of lignin carbohydrate molecules in higher amoun t and of high molecular masses in the subsequent chemical reactions. Ligninases and hemicellulases (xylanases) were tested bio-bleaching. Use of hemicellulases was first demonstrated [17] which resulted in the reduction of chlorine consumption. Lund gren et al [18] even tried a mill trail on TCF (total chlorine free) technology for bleaching of pulp with xylanase from *bacillus stearothermophilus* strain T6 which is having optimum activity at pH 6.5. Even though there are many reports on microbial xylanases only a limited number of them are having characteristics applicable in paper and pulp industry. Two types of phenomenon are involved in enzymatic pretreatment. The major effect is due to hydrolysis of reprecipitated and reabsorbed xylan or xylan lignin complexes that are seperated during the cooking process. As a result of the enzymatic treatment, the pulp becomes more accessable to the oxidation by the bleaching chemicals. A minor effect is due to the enzymatic hydrolysis of the residual non disolved hemicellulose by endo xylanases. Residual lignin is unbleached pulp (Kraft pulp) is linked to hemicelluloses and that cleavage of this linkage will

allow the lignin to be released.

Animal feed processing
Arabinoxylans are partly water soluble polysaccharides present in considerable amount in cereals like barley and result in a highly viscous aqueous solution. This high viscosity of cereal parameter for the use of cereal grains in animal feeding. Application of xylanases for pre-treatment of arabinoxylan containing substrates has been proved to be a good solution for this. Babalola *et al*. [19] observed improved apparent nitrogen and fibre absorption as well as feed transit time by the application of xylanase in poultry feed. Cafe et al. [20] gave nutritionally rich diets, with or without the addition of 0.1%m Avizyme 1500 (xylanase, protease, and amylase) to the poultry birds. Birds fed on the diets supplemented with Avizyme exhibited significantly higher body weights, less mortality and greater amount of net energy from their diets as compared to the control group.

Beverage industry
The xylanolytic enzymes are also employed for clarifying juices and wines. For extracting coffee, plant oils and starches for improving the nutritional properties of agricultural silage and grain feed, Xylanases have also been reported to be very effective. Xylanases, in conjunction with cellulases, amylases and pectinases, lead to an improved ill of juice by means of liquifaction of fruit and vegetables; stabilization of the food pulp; increased recovery of aromas, essentials oils, vitamins, mineral salts, edible dyes, pigments etc reduction of viscoscity, hydrolysis of substances that hinder the physical and chemical clearing of the juice, or that may cloudiness in the concentrate [21].

Bakery Industry
Xylanases are used as dough strengtheners since they provide excellent tolerance to the dough towards variation and processing parameters and in flour quality. They also significantly increased volume of the baked bread. With the use of xylanases, there has been an increased bread volumes, greater absorption of water and improved resistance fermentation [22-24] Also, a larger amount of arabinoxylooligosaccharide in bread would be beneficial to health [21]. Xylanase transform water insoluble hemicellulose into soluble form, which binds water in the dough, therefore decreasing the dough furnace, increasing volume in creating finer and more in uniform crumps [25].

Pharmaceutical industry
Hydrolytic products of xylan known as xylooligosacharides (Xos), which may be used in pharmaceutical industry. Xos have prebiotic effect, as they are neither hydrolyzed nor absorbed in the upper gastrointestinal tract, and they effect the host by selectively instumulating the growth or activity of one or a number of bacteria in a column, thus improving health. Among their key physiological advantages re the reduction of cholesterol, maintainance of gastrointestinal health, and improvement of the biological availability of calcium, they also inhibit starch retrogradation, improving the nutritional and sensibly properties of food. For the production of XOs, the enzyme complex must have low exoxylanase are β-xylosidase activity, to prevent the production of high amounts of xylose which has inhibitory effects on XO production.

Biofuel Production
Production of environmently friendly fuel is gaining great importance as the energy sources are depleting in a faster rate. There are reports regarding production of ethanol from the agro wastes by incorporating xylanase treatment. Xylanase, together with other hydrolytic enzymes, can be used for the generation of biological fuels, such as ethanol, from lignocellulosic biomass. However, enzymatic hydrolysis is still a major cost factor in the conversion of lignocellulosic raw materials to ethanol [18]. In the bioethanol fuel production, the first step is the delignification of lignocellulose, to liberate cellulose and hemicellulose from their complex with lignin. The second step is a depolymerization of the carbohydrate polymers to produce free sugars followed by the fermentation of mixed pentose and hexose sugar to produce ethanol [26]. Simultaneous saccharification and fermentation is an alternative process, in which both hydrolytic enzyme and fermentative microorganisms are present in the reaction.

CONCLUSION

Several applications of xylanases are being developed for the food and paper industries which are based on the partial hydrolysis of xylan. The long term applications of xylanases such as conversion of renewable biomass into liquid fuels, where xylanases play a crucial role in the conjunction with the celluloses, is not yet economically feasible. However, stringent environmental regulations and awareness to reduce the emission of greenhouse gases

have added and incentive for future research developments in the study of xylanases. In order to make the application of xylanases realistic the improvement in enzyme yields is od almost importance. Considerable progress has been made in the last few years in identifying the process parameters which are important for obtaining high xylanases yields and productivities and thus influencing the economics of xylanase production. The production of xylanolytic enzymes is higher with increasing substrate concentrations. However, the high concentration of solid substrate give rise to mass transfer limitations in batch cultivations. A fed batch mode of cultivation, where much higher substrate concentrations can be used, is an attractive alternative process. Hence it is necessary to identify the potent xylanase producer by screening for potential xylanolytic micro-organism.

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