# MICROBIAL XYLANASE: A REVIEW

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#### Abstract

Xylan is the major constituent of hemicellulose, the second most abundant natural polymer on earth. There are four main categories of xylans: arabinoxylans, glucuronoxylans, glucurono arabinoxylans and galacto glucurono arabinoxylans. The side chains of each xylan are being responsible for the solubility, physical conformation and reactivity of the xylan molecule with other components of the hemicellulose and therefore, influencing the mode and extent of enzymatic cleavage. Xylanases, as glycoside hydrolase members, are able to catalyse the hydrolysis of xylan, by breaking the  $\beta$ -1,4-glycoside linkages, in order to produce simpler compounds such as xylose. Because of the heterogeneity and complex chemical nature of xylan, the complete breakdown requires the action of several hydrolytic enzymes that are different considering their structure, the substrate specificities, their mode of action or biochemical properties. Many degrading microorganisms produce xylanases such as fungi (Aspergillus spp., Trichoderma spp.), bacteria (Bacillus spp., Streptomyces spp.), yeast (Cryptococcus spp.), marine algae etc. Depending on the source, the market of xylanases has expanded rapidly because of its potential in industrial use, especially in the biotechnological applications. In this review, are presented the significant aspects concerning the complete hydrolysis of xylan, and therefore of hemicellulose.

Key words: xylan, xylanases, bacteria, fungi, applications.

### INTRODUCTION

Lignocellulose is the most abundant renewable biomass on earth. The main constituents of lignocellulosic materials are cellulose, hemicellulose and lignin, along with smaller amounts of pectin, protein etc. The variation of the proportions of these components depends on: plant species, age, stage of growth or other conditions (Kumar et al., 2009)

The term "hemicellulose" was introduced by Schulze in 1891, describing the fractions isolated or extracted from plant materials with dilute alkali (Beg et al., 2001). Hemicellulose is not a well-defined compound (Polizeli et al., 2005), but a class of polymers, found in plant cell wall, based on pentose and hexose sugars, with xylan (the polymer of xylose) as the most abundant constituent (Uday et al., 2016). The names of the classes of the hemicellulose match the main sugar unit. Therefore, when a polymer is hydrolysed and produce xylose, it is a xylan (Polizeli et al., 2005).

Xylans or the hemicelluloses are located in plants between the lignin and the cellulose fibers underneath, being interspersed and covalently linked at various points with lignin, while covering cellulose, via hydrogen bonding (Beg et al., 2001). The term xylans is used to describe а group of non-cellulose polysaccharides, based on monosaccharides units such as D-xylose, D-mannose, D-glucose, L-arabinose, D-galactose, D-glucuronic acid and D-galacturonic acid (Polizeli et al., 2005; Shallom and Shoham, 2003). The xylose residues are linked by  $\beta$ -1,4-glycosidic bonds. Depending on their source and extraction method, these polysaccharides have different structure and composition. (Harmsen et al., 2010).

Xylanases represents a class of enzymes, which are responsible for the complete hydrolysis of the linear polysaccharide  $\beta$ -1,4-xylan into simpler compounds which are mainly consisted of xylose, thus breaking down hemicellulose.

#### XYLAN STRUCTURE

Based on the nature of its substituents, four main categories of xylans (Motta et al., 2013) can be considered:

- Arabinoxylans, comprising only side chains of single terminal units of  $\alpha$ -Larabinofuranosyl; - Glucuronoxylans, based only on  $\alpha$ -D-glucuronic acid and its 4-O-methyl ether derivative;

- Glucurono arabinoxylans, in which  $\alpha$ -D-glucuronic (and 4-O-methyl-  $\alpha$ -D-glucuronic) acid and  $\alpha$ -L-arabinose are both present;

- Galacto glucurono arabinoxylans, characterized by the presence of terminal  $\beta$ -Dgalactopyranosyl residues on complex oligosaccharide side chains of xylans. cells. It is found in large quantities in hardwoods (15-30% of the cell wall content) and softwoods (7-10%), as well in annual plants (<30%). In hardwoods, xylan exists as O-acetyl-4-O-methylglucuronoxylan and in softwoods. 28 arabino-4-Omethylglucuronoxylan, while in grasses and annual plants, it is as arabinoxylans (Beg et al., 2001; Kulkarni et al., 1999). Linear unsubstituted xylan has also been reported in esparto grass, tobacco and certain marine algae, with the latter containing xylopyranosyl residues linked by both  $\beta$ -1,3 and  $\beta$ -1,4 linkages (Motta et al., 2013).



Figure 1. Structure of xylan and the xylanolytic enzymes involved in its degradation. Ac: Acetyl group; α-Araf: α-arabinofuranose; α-4-O-Me-GlcA: α-4-O-methylglucuronic acid. Source: Sunna and Antranikian, 1997

In the Figure 1 are presented the enzymes involved in the hydrolysis of xylan.

Additionaly there is another category, named homoxylans, consisting exclusively of xylosyl residues, but this type of xylans are not widespread in nature, being isolated from limited sources (tobacco stalks, guar seed husks) (Sunna and Antranikian, 1997).

Among these categories, the complexity increases from linear to highly substituted xylans. The side chains are responsible for the solubility, physical conformation and reactivity of the xylan molecule with other hemicellulosic components and therefore, influence the mode and extent of enzymatic cleavage (Kulkarni et al., 1999).

Xylan is present in a variety of plant species, being distributed in several types of tissues and

#### ENZYMATIC HYDROLYSIS OF XYLAN

The role of enzymes in the breakdown of xylan was observed by Hopper-Seyler over 100 years ago (Bastawde, 1992). Xylan is a complex chemical compound with a heterogeneous nature. Therefore, its complete breakdown requires the action of several hydrolytic enzymes with diverse modes of action and specificities.

The xylanolytic enzyme system includes  $\beta$ -1,4endoxylanase,  $\beta$ -xylosidase,  $\alpha$ -glucuronidase,  $\alpha$ -L-arabinofuranosidase, acetyl xylan esterase

(Motta et al., 2013) and phenolic acid (ferulic and p-coumaric acid) esterase (Beg et al., 2001; Dhiman et al., 2008). The synergic action of all these enzymes convert xylan into its constituent sugars. Among all of xylanases, endoxylanases

and  $\beta$ -xylosidases are the most important in depolymerizing xylan molecule into monomeric pentose units. Endoxylanases are involved in cleaving the glycosidic bonds and in liberating short xylooligosaccharides, while  $\beta$ -xylosidase releases xylose residues from the nonreducing ends of xylooligosaccharides (Motta et al., 2013). Acetyl esterase, ferulic esterase, glucuronidase, and arabinosidase are required for the release of different side chains from the xylan backbone (Dhiman et al., 2008). Endo-1.4- $\beta$ -xylanases (E.C.3.2.1.8) are reported to be produced mainly bv microorganisms such as bacteria (Bacillus sp., Streptomyces sp.) and fungi (Aspergillus sp., Trichoderma sp.). However, there are reports that indicate several other sources, such as: Japanese pear fruit during the over-ripening period, the flour of European wheat (Triticum *aestivum*) water or fresh molluse (Subramaniyan and Prema, 2002).

Exo- $\beta$ -1,4-D-xylosidase (E.C.3.2.1.37) removes succesive D-xylose residues from the non-reducing end, by catalysing the hydrolysis of  $\beta$ -1,4-D-xylo-oligosaccharides.  $\beta$ -xylosidase can easily hydrolise xylobiose that isn't affected by the endoxylanases that release xylose during the hydrolysis of xylan. Among the producing microorganisms, there are reports that include *Bacillus* sp. and different fungi (Subramaniyan and Prema, 2002).

 $\alpha$ -L-arabinofuranosidases (E.C.3.2.1.55) hydrolyse the terminal, non-reducing  $\alpha$ -Larabinofuranosyl groups of arabinans, arabinoxylans and arabinogalactans. This type of enzyme is produced by fungi, actinomycetes and other bacteria (*Bacillus polymyxa*, *Rhodothermus marinus*).

 $\alpha$ -D-glucuronidases (E.C.3.2.1.1) catalyse the hydrolysis of the  $\alpha$ -1,2-glycosidic linkages between xylose and D-glucuronic acid or its 4-O-methyl ether linkage.

For the complete hydrolysis of natural glucuronoxylans is necessary to use esterases to remove the bound acetic and phenolic acids. Acetyl xylan esterase (E.C. 3.1.1.6) breaks the bonds of xylose to acetic acid, feruloyl esterase (E.C. 3.1.1.73) the arabinose side chain residues to ferulic acid and p-coumaroyl esterase the arabinose side chain residue to p-coumaric acid.

Usually, xylanases are inducible enzymes secreted in media containing pure xylan or xylan-rich residues (Balakrishnan, 1997). The immobilization of xylanases is necessary for practical purposes. Therefore, either the microorganism or the enzymes are immobilized on solid material, technique that offers several advantages such as repetead usage on enzyme, ease of product separation and improvement of enzyme stability (Beg et al., 2001).

## SOURCES OF XYLANASE

Many studies have reported the production of xylanase from fungi, bacteria, yeast, marine algae (Mandal, 2015), seeds, crustaceans, snails (Polizeli et al., 2005) but the main sources for these enzymes are fungi and bacteria. According to the source, xylanases have different characteristics which makes them useful for an application or another.

### Bacterial xylanases

Xylanases produced by bacteria and actinomycetes (*Bacillus* sp., *Pseudomonas* sp., *Streptomyces* sp.) are effective in a broader pH range of 5-9, with the optimum temperature for xylanase activity between 35°C to 60°C (Beg et al., 2001; Mandal, 2015; Motta et al., 2013). Bacterial strains studied for their xylanase

activity are shown in the Table 1 (Mandal, 2015; Amore et al., 2014; Dhiman et. al, 2008; Maheshwari and Chandra, 2000).

Table 1. Xylanase producing bacteria

Microorganism			
Bacillus pumilus			
Bacillus subtilis			
Bacillus amyloliquefaciens			
Bacillus cereus			
Bacillus circulans			
Bacillus megatorium			
Bacillus licheniformis			
Bacillus stearothermophilus			
Streptomyces sp.			
Streptomyces roseiscleroticus			
Streptomyces cuspidosporus			
Streptomyces actuosus			
Pseudonomas sp.			
Clostridium absonum			
Thermoactinomyces thalophilus			

Studies on *Bacillus* spp. showed higher xylanase activity at alkaline pH and high temperature. Therefore, bacterial xylanases are used in industrial application due to their alkali tolerance and thermostability (Mandal, 2015).

## Fungal xylanases

Fungi (Aspergillus spp., Fusarium spp., Penicillium spp.) are important producers of xylanase due to high yields and extracellular release of the enzymes (Nair and Shashidhar, 2008). Also, fungal xylanases have higher activity, compared with bacteria or yeast. However, xylanases derived from fungal sources have some characteristics that makes them unavailable for some industrial applications (Mandal, 2015). Most of these xylanases are efficient at temperature below 50°C and a pH range of 4-6 (Beg et al., 2000). For example, fungal xylanases can't be used in the pulp and paper industry, that needs an alkaline pH and temperature more than 60°C (Mandal, 2015). Another problem with fungal xylanases is the presence of a cellulase, few studies reporting fungal xylanase without cellulase activity (Subramaniyan and Prema, 2002).

Fungal strains studied for their xylanase activity are shown in the Table 2 (Mandal, 2015; Huitron et al., 2008; Ja'afaru, 2013; Taneja et al., 2002; Haltrich et al., 1993; Ghanen et.al, 2000; Haltrich et al., 1996).

Table 2	) Xylanase	producing	fungi
Table 2	2. Aylanase	producing	Tungi

Microorganism
Aspergillus niger
A. foetidus
A. brasiliensis
A. flavus
A. nidulans
A. terreus
Penicilium sp.
Trichoderma reesei
T. longibrachiatum
T. harzianum
T. viride
T. atroviride
Fusarium oxysporum
Thermomyces lanuginosus
Alternaria sp.
Talaromyces emersonii
Schizophyllum commune
Piromyces sp.

Xylanases 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 (Nair and Shashidhar, 2008).

Additionaly, large scale production of fungal xylanase is difficult due to the slow generation time and coproduction of highly viscous polymer that lowers the oxygen transfer (Mandal, 2015).

### APPLICATIONS OF MICROBIAL XYLANASE

The market of xylanase has increased significantly worldwide, over the past few years (Techapun et al., 2003; Haki and Rakshit, 2003; Dhiman et al., 2008). Microbial xylanases have attracted a great deal of attention, because of their biotechnological potential in various industrial processes such as food, feed and pulp and paper industry.

Also, they have shown an immense potential for increasing the production of several useful products in a most economical way. The main possibilities are the production of SCPs, enzymes, liquid or gaseous fuels and solvents and sugar syrups, which can be used as such or as feed stock for other microbiological processes (Kuhad and Singh, 1993). Therefore, xylanases are considered as "one of the more industrially important enzymes" (Dhiman et al., 2008)

## Pulp and paper industry

Chemical bleaching in the pulp and paper industry is used to increase the paper brightness. Unfortunately, this causes serious damages of the cellulose components and reduce the yield and viscosity of the pulp. The level of viscosity is related to the degree of cellulose polymerization and to the paper strength (Cheng et al., 2013), so decreasing viscosity is not desirable.

The role of xylanase in the biobleaching of pulp was first reported by Viikari et al. in 1986. Since then, many studies were conducted, toward newer microbial isolates (Sunna and Antranikian, 1997; Beg et al., 2000; Gilbert and Hazlewood, 1993; Liu et al., 1998), as well as bleaching experiments (Cheng et al., 2013; Manimaran et al., 2009; Garg et al., 2011; Khandeparkar and Bhosle, 2007; Li et al., 2005). The researchers, focused on using xylanase mainly for reducing the chemical consumption, few studies being centered on the effects of this enzyme on the yield and viscosity of the pulp (Cheng et al., 2013)

With this technique, the pulp is usually treated with xylanase before chemical bleaching (Martin-Sampedro al.. et 2012). The reprecipitated xylan is hydrolyzed, in the presence of xylanase, this facilitating pulp and bleaching lowering the chemical consumption. By this means, this technique reduces the toxic compounds discharged into the environment (Cheng et al., 2013).

## Animal feed

Including xylanases into a rye-based diet of broiler chickens results in reduced intestinal viscosity, thus improving both the weight gain of chicks and their feed conversion efficiency (Bedford and Classen, 1992)

Xylanases, used as pretreatment of forage crops, improve the nutritional properties of agricultural silage and grain feed (Subramaniyan and Prema, 2002; Kuhad and Singh, 1993; Bedford and Classen, 1992), thus improving the digestibility of ruminant feeds and facilitating composting (Gilbert and Hazlewood, 1993).

However, the complete removal of xylan is not wanted, because hemicelluloses are important components of diet and their removal may increase bowel diseases (Mandal, 2015)

## Food industry

Xylanases improve the quality of bread, by increasing the specific bread volume. This is further enhanced when amylase is used in combination with xylanase (Maat et al., 1992). Also, they are applied in rye baking, where the addition of xylanase makes the doughs soft and slack (Subramaniyan and Prema, 2002). During the bread-baking process, they delay crumb formation, allowing the dough to grow (Mandal, 2015).

Another use of xylanases is as dough strengthners, because they provide excellent tolerance to the dough towards variations in processing parameters and in flour quality (Subramaniyan and Prema, 2002). Also, a larger amount of arabinoxylo-oligosaccharides in bread would be beneficial to health (Mandal, 2015). In biscuit-making, xylanase is recommended for making cream crackers lighter and improving the texture and tastiness (Mandal, 2015).

Along with cellulase and pectinase, xylanase can be used for the preparation of dextrans, used as food thickeners (Mandal, 2015).

## Hemicellulosic wastes

Xylan is present in large amounts in hemicellulosic wastes. There is a massive accumulation of agricultural, forestry and municipal solid waste residues, therefore the development of an efficient process of enzymatic hydrolysis offers new prospects for treating wastes (Subramaniyan and Prema, 2002; Rani and Nand, 1996).

## Biofuels

Production of biofuels is gaining great importance as the energy resources are shrinking. The combined action of xylanase with several enzymes such as mannanase, ligninase, xylosidase, glucanase, glucosidase etc. can be applied for the generation of biofuels (ethanol and xvlitol). from lignocellulosic biomass (Dominguez, 1998). The production of bioethanol requires the delignification of lignocellulose to liberate cellulose and hemicellulose. The next steps depolymerization include the of the carbohydrate polymers to produce free sugars and the fermentation of mixed pentose and hexose to produce bioethanol (Lee, 1997).

## Fabric bio-processing

Xylanase treatment can significantly remove hemicellulosic impurities, thus increasing the water absorbing properties of fiber, without affecting the fibre strength during the spinning process. In the end, fiber becomes more soft and smooth after desizing (Dhiman et al., 2008).

## Treatment of plant cells

Treatment of tobacco suspension cells (*Nicotiana tabacum* CV.KY 14) with a purified endoxylanase from *Trichoderma viride* increased the levels of acylated sterol glycosides and induces the synthesis of

phytoalexins (Moreau et al., 1994). Additionaly, a truncated bacterial xylanase gene from *Clostridium thermocellum* has been demonstrated in rhizosecretion in transgenic tobacco plants. (Borisjuk et al., 1999). Some xylanases improve cell wall maceration for the production of plant protoplasts (Beg et al., 2001).

## Beverage and juices industry

Xylanases used in combination with cellulase and pectinase helps clarifying must and juices, liquefying fruits and vegetables (Beg et al., 2001), stabilization of the fruit pulp, reduction of viscosity, hydrolysing the substances that hinder the physical or chemical clearing of the juice,or that may cause cloudiness in the concentrate (Polizeli et al., 2005; Mandal, 2015).

Particularly,  $\alpha$ -L-arabinofuranosidase and  $\beta$ -Dglucopyranosidase are used for aromatizing musts, wines and fruit juices (Spagna et al., 1998). Also, the xylanolytics enzymes are employed for extracting coffee, plant oils and starches (Subramaniyan and Prema, 2002).

In the fermentation industries, such as beer brewing, xylanases, used as a pre-treatment of the arabinoxylans containing substrates (barley, wheat) reduce the viscosity, thus increasing process efficiency (Subramaniyan and Prema, 2002).

## Surfactants

Alkyl glycosides are surfactants widely used in industrial applications, being produced commercially from monomeric sugars. Using polysaccharide is more feasible for their industrial production, because several steps in the process can be omitted (Matsumara et al., 1999). Therefore, xylanase presents a challenging opportunity.

Other application of xylanase is in the detergent industry, as it improves the cleaning ability of detergents that are more efficient in cleaning fruit, vegetable, soils and grass stains (Kumar et al, 2004; Dhiman et al., 2008).

## **Retting of Flax fibers**

A combined xylanase-pectinase system is used in the debarking process, which is the first step in wood processing, the addition of xylanases enhancing the retting process. Other applications of this combined system are used in the degumming of bast fibers such as flax, hemp, jute and ramie or the fiber liberation from plant instead of retting (Beg et al., 2001).

## CONCLUSIONS

For the breakdown of xylan, the main component of hemicellulose, is requierd the combined action of xylanolytic enzymes such as:  $\beta$ -1,4-endoxylanase,  $\beta$ -xylosidase,  $\alpha$ glucuronidase,  $\alpha$ -L-arabinofuranosidase, acetyl xylan esterase and phenolic acid esterase.

Depending on the source, mainly bacteria or fungi, xylanases have different characteristics which makes them useful for an application or another.

Xylanases present immense potential in various industrial areas or research fields such as: pulp and paper, animal feed, food industry, hemicellulosic wastes, biofuels, fabric bioprocessing, treatment of plant cells, surfactants, retting of flax fibers, beverages and food industry.

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