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Xylanases and Industrial Applications: A Review

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***Abstract*—** *Hemicelluloses are a large family of heteropolysaccharides that are non-cellulosic and complicated. They are categorized based on the main monosaccharides that are found within their structure. The hydrolysis of the xylan polymers is carried out by an enzymatic complex, however two are the primary enzymes; one is beta-D-xylosidase and the other enzyme is endo-1,4-beta-xylanase. Fungal source, bacteria, algae, protozoans, insects, snails, plant beads, and other organisms all produce these enzymes, but filamentous fungi serve as the main commercial source. The most prevalent hemicellulose in lignocellulosic biomass is xylan. Purification of xylanases and their characterization is being carried out using several relevant procedures for biochemical, molecular investigations as well as for effective use in businesses. To address specific industrial and economic needs, xylanase is produced at a commercial scale through genetic engineering and cloning. Microbial xylanases are utilized in an array of industrial biotechnology applications, including the production of biofuels, operations in paper industry, the baking sector and brewing industry, various human food and animal feed production industries, pharmaceutical sector and the deinking process for recycling wastepaper.*

***Key Words*—** *Xylanases, β-D-xylosidase, Endo-1,4-β-xylanase, Xylan, Hemicellulose, Applications*

I. INTRODUCTION

Hemicelluloses are a broad category of heteropolysaccharides that are complex, noncellulosic, and are categorized based on the main monosaccharides they include, like arabinoxylan and galactomannan.[1] According to reports, it constitutes over 30% of the dry mass of terrestrial plants cell wall only and comes in a wide variety of connections and branching kinds. Their dissolution, consistency, and other physical properties are due to the branch points. By crosslinking these complex structures, ionic and covalent bonds form a physical barrier that resists bacterial and mechanical forces. An enzyme consortium comprising of enzymes that target distal and/or internal glycosidic connections is needed to enzymatically breakdown xylan. Enzymes that can dissolve the backbone and different side-branch points are essential in the context of xylan degradation for a thorough breakdown of the composition of xylan in plant cell walls. This provides more sites for enzymatic hydrolysis to take place.

They may also make it easier to introduce certain changes to the polysaccharide framework, such adjustments to the polymer's orientation, solubility, and linkages to lignin and phenolic compounds.[2] Joseleau et al. claim that careful understanding of the chemical and physical and chemical nature and configuration of the xylan molecule, either in the separated state or within the surroundings of the lignocellulosic molecule that makes up the plant cell wall, is necessary for the controlled use of xylan-degrading enzymes.

Hemicelluloses, or xylans, are the second most common type of natural polysaccharide. Both the middle lamella and the outer rigid cell wall of plantae have these polysaccharides. Hemicellulose describes a number of noncellulosic sugar heteropolymers comprised of varying ratios of the monomers (L-arabinose, D-glucuronic acid and galactronic acid, D-glucose, a deoxy sugar rhamnose, D-mannose, D-xylose and D-galactose). Depending on its primary sugar unit, hemicellulose is classified into various groups. So when a polymer hydrolyzes to form xylose, it becomes a xylan; similarly, cellulose fibers contain mannans, arabinans, glucans and galactans.[3]

In nature, wood hemicelluloses very never contain more than one type of sugar. The most common polymers found in them are arabinogalactans, glucuronoxylans, glucomannans, arabinoglucuronoxylans and galactoglucomannans. They frequently have complicated frameworks made up from multiple polymers. Each component's concentration varies between species and even between individual trees.[4] Hemicellulose, then, is a category of polymer components of plant fibers, each with unique features, rather than a clearly defined chemical substance.

The primary component of hemicelluloses is xylan, which is broken down by xylanolytic enzymes. Fungal xylanases have a vast array of industrial uses, encompassing the production of xylitol, animal feed, bread, juice, and wine. In the Netherlands' Wageningen International Agricultural Centre, a conference on xylans and xylanases was organized in 1991. During this conference, it was emphasized that a deeper understanding of the structure of xylan and the enzymes involved in its breakdown was essential to understanding the factors that regulate xylan molecules susceptibility to enzyme activity. Hemicellulase and hemicellulose were the topic of a colloquium at the 1992 Federation of European Biochemistry Societies meeting held at Trinity College, Dublin. Topics like the chemical composition and structures of hemicellulose, the biochemistry and molecular biology of microbial hemicellulases, and the applications of these enzymes in biotechnology were discussed during the colloquium.

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Given that xylan is the most prevalent hemicellulose, a wide range of xylans and xylan catalyzing enzymes were prioritized. Numerous scientists have worked hard to understand the workings of xylan catalyzing enzymes and how this breakdown affects the architecture of cell walls of plantae.

II. The Substrate Xylan

The natural world's second-most prevalent polysaccharide and the substrate for the activity of xylanases, xylan, contributes significantly to the structural integrity of plant cells and makes up around 1/3 of the world's renewable organic carbon.[5] Rye, rice, barley, wheat, sorghum and oat, in addition to certain added plants, comprising rye grass, pangola grass, and bamboo shoots, have been found to contain arabinoxylans. Although these polysaccharides make only a small portion of whole cereal grains, they are vital constituent of plantae cell wall. The secondary wall is home site to majority of glucuronoxylans and glucuronoarabinoxylans, which acting as binding agents, form chemical linkages (covalent and non-covalent) with lignin, cellulose and few additional polymers that are crucial for the stability of cell wall's structure. Gymnosperms include 7–12% xylans, which are less common than xylans, which make up 15–30% of the dry weight of angiosperms, the main class of hemicelluloses.[6]

The linear structured polymer glucuronoxylans are made up of beta-D-xylopyranosyl units which are connected by the (1-4) glycosidic linkages (xylose). This polymeric sugar backbone is composed of 4-O-methyl-alpha-D-glucuronopyranosyl units, that are methylated at the position number 4, and beta-D-xylopyranosyl units connected to positions 2 or 3. A high rate of acetyl group substitution (between 70 and 80 percent) occurs in angiosperm (hardwood) glucuronoxylans at positions number 2 or 3, or both, of the beta-D-xylopyranosyl, giving it a slightly miscible property in aqueous media.[7]

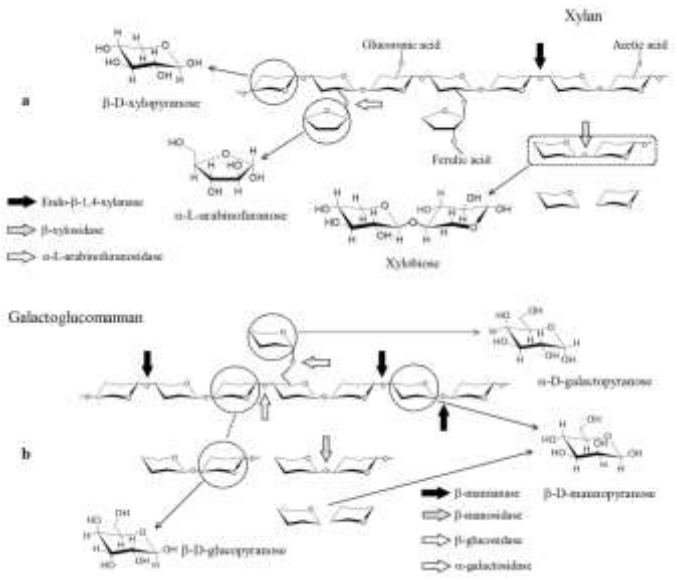

Fig. 1. The mode of action of certain hemicellulolytic enzymes on hemicellulosic material is schematically represented as follows: (a) Xylan—endoxylanase breaks down the xylan backbone, releasing shorter xylooligosaccharides. These are further hydrolyzed by accessory enzymes. Beta-xylosidases release xylose monomers from xylobiose, and arabinofuranosidase activity releases L-arabinose from the xylan chain. (b) Galactoglucomannan—beta-mannanase cleaves β-1,4 linkages between mannose and glucose or mannose and mannose sugars in the backbone chain. Alpha-galactosidase releases galactose residues attached to hydroxyl groups of main chain mannose or glucose residues. Betaglucosidase enzymes cleave glucose residues from the non-reducing ends of oligosaccharides produced by the action of β-mannanase enzymes.[9]

Glucuronoarabinoxylans, which are detected in softwood frequently, have exactly same xylan backbone, but each one has ten alpha-L-arabinofuranosyl units rather than beta-D-xylopyranosyl units. As compared to hardwood, more alpha-4-O-methyl-D-glucuronopyranosyl units are found in softwood. Non-acetylated furanoside structure of these xylans makes the arabinose portion-groups susceptible to acid degradation. We shall refer to glucuronoxylans and glucuronoarabinoxylans collectively as "xylans" in the following.[8]

III. Microbial Production of Xylanases

Many microorganisms produce xylanase enzymes and a variety of other enzymes possessing xylanolytic properties. To increase the efficacy and scope of hydrolysis, as well as variety and complexity of the enzymes, these may have a variety of physicochemical qualities, structures, particular activities, and yields. Utilization of xylanase preparations with an ideal pH of 5.5—always produced by fungi—has grown. Optimal performance for fungal xylanases typically occurs within a specific pH window (3.5 to 6.5) and a temperature range suitable for mesophilic organisms (40 to 60°C).[10] Most fungi sourced xylanases work best at a pH of around 5, while they are typically stable over pH ranges of 3 to 8. The majority of fungal organisms produce this enzyme, which can withstand thermal point lower than 50° Celsius. With very few exceptions, the initial cultivation pH of fungi that are known to be generating xylanases is often lower than 7. However, it differs when it comes to bacteria. Bacterial xylanases exhibit higher activity at a more neutral to alkaline pH range (5.0-8.0) and temperatures between 50 and 80°C compared to the fungal ones.[11] Similarly, most fungal xylosidases thrive in acidic conditions (pH 4.0-5.0) with optimal temperatures ranging from 40 to 80°C.[11] Among hemicellulases, production often occurs alongside pectinases by various microorganisms. Filamentous fungi excel in this area, secreting these enzymes in significant quantities into the surrounding environment. Key examples of such fungi include Aspergillus niger, Trichoderma spp., and Humicola spp..[12], [13]

Aspergillus niger, which secretes 15 external xylanases, and Trichoderma viride, that releases thirteen, are two typical examples of microbes that manufacture xylanase isoenzymes. There have been reports of instances of divergent post-translational processing, but this diversity may be the product of genetic superfluity.[14] The genome may contain numerous copies of the isoenzyme genes that are polycistronic or non-polycistronic, and in certain situations, multiple xylanases are expressed as separate gene products.

For instance, Caldocellum saccharolyticum (Caldocellulosiruptor saccharolyticus) has polycistronic xylanase, beta-xylosidase, and acetyl esterase genes, while Fibrobacter succinogenes S85's XynC gene has been shown to encode two different xylanase catalytic domains. In addition,

many xylanases exhibit a variety of auxiliary domains in addition to their numerous catalytic domains.[15] Examples include domains that bind to xylan or cellulose, dockerin domains, thermostabilizing domains, and domains whose functions are still unknown. These domains are generally divided by brief junction segments that are rich in hydroxyl amino acids and have the potential to fold and function independently.[16]

As entry of this substrate into the cell is prevented due to its huge size, an enormous quantity of the enzyme is secreted into the surrounding external environment. In reality, it is now considered as the end results of what they do are what triggers the formation of xylanase. Small amounts of constitutively produced enzymes are expected to release xylo-oligomers, which can then be transported into the cell and further broken down by beta-xylosidases or even intracellular xylanases, where they also act as an inducer of the production of more xylanases.[17]

The ability of bacteria to produce alkaline thermostable xylanase attracted researchers, much like it does in the case of many industrial enzymes. Bacillus spp. are noteworthy members that exhibit significant levels of xylanase function at alkaline pH as well as elevated temperature.[18] Under ideal nitrogen conditions, Bacillus SSP-34 exhibited higher levels of cellulase-deficient xylanase activity. At the chosen nitrogen source of the extracted yeast and peptone combination, this bacterium also generated a negligible amount of protease activity. In the ideal medium, Bacillus SSP-34 generated a xylanase function of 506 IU/ml.

TABLE I. Microbial sources and characteristics of some xylanases.

Source of enzyme	*Code*	Mw (kDa)	pl	pH opt	T_{opt} (°C)	Substrate	Reaction product[1]	Reference
Fungi								
Aspergillus niger		20.8*	6.7	5.0	55°	Larchwood	X_2, X_3, X_4	[19]
Aspergillus niger	*I*	13.0*	8.6	6.0	45°	Larchwood	X_3, X_5, (X_2, X_4, X_6)	[20]
	II	13.0*	9.0	5.5	45°	Larchwood	X_3, X_5, (X_2, X_4, X_6)	
Aspergillus niger		28.0	3.65	5.0	40°-50°	Larchwood	X_3, X_5, (X_2, X_4, X_6)	[21]
Aspergillus niger		14.0*	4.5	4.9	45°	Larchwood	X_2, X_3, X_5	[22]
Aspergillus niger van Tieghem	*I*			5.5		Cotton shell, Rice straw	X, X_2	[23], [24]
Aspergillus ochraceus		48.0 50.0*		6.0	50°	Larchwood xylan	X_2, X_3	[25]
Aspergillus oryzae VTT-D-85248	*I*	28.0*	7.0	5.0		Beachwood glucuronoxylan	X_n	[26]
	II	26.0*	4.9	5.0			X_n	
Aureobasidium pullalans CBS 58475		24.0	8.0	4.25	60°	Larchwood	X, X_2, X_3, X_4	[27]
Cephalosporium sacchari	*HC-IV*	9.55*	6.0			Hemicellulose B	X, X_2, X_3, AX_3, AX_4	[28]
Ceratocytis paradoxa	*HC-II*		4.5	5.1	80°	Hemicellulose B	X, X_2, X_3, AX_2, AX_3	[29]
Chaetomium thermophile var. coprophile 1-110	*I*	26.0 25.0*		5.4-6.0	70°	Larchwood	X_2, X_3	[30]
	II	7.0 6.8*		4.8-6.4	60°	Larchwood	X_2	
Dichomitus squalens	*EXI*	39.0	4.6	5.0	60°	Larchwood, Wheat straw	X_2, X_3	[31]
	EXII	36.0	4.5	5.0	60°		X_2, X_3	
Gliocladium virens IFO 8349		7.0*		6.0	45°	Rice straw arabinoxylan	X, X_2, X_3	[32]
Humicola lanuginosa		21.5 20.0*	4.1	6.0	65°	Xylan	(X), X_2, X_3	[33]
Lentinula edodes		41.0	3.6	4.5-5.0	65°	Aspen glucuronoxylan	X, X_2, X_3	[34]
Malbranchea pulchella var. sulfurea No. 48			8.6	6.0-6.5	70°	Hardwood xylan	X, X_2, X_3	[35], [36]
Mesophilic fungus strain Y-94	*A*	51.0 44.0*	5.05	4.9	80°	Larchwood	X, X_2	[37]
	B	48.0	4.40	4.9	80°	Larchwood	X, X_2	
	C	35.0	4.10	4.9	80°	Larchwood	X, X_2	
Paecilomyces varioti IMD RK 032	-	20.0	5.2	4.0	50°	-	-	[38]
Polyporus tulipiferae (Irpex lacteus)	*I*	38.0		6.0	60°	Larchwood	X, X_2, X_3	[39]
	III	62.0		6.0	70°	Larchwood	X_2, X_3	
Polyporus tulipiferae		38.0	7.6-8.0	4.6-5.2	60°	Larchwood glucuronoxylan	X, X_2, X_3	[40]
Robillarda sp. Y-20	*I*	17.6 18.0*	9.7	4.5-6	50°	Larchwood	X, X_2, X_3	[41]
	II	59.0 56.5*	3.5	4.5-6	50°	Larchwood	X, X_2, X_3	
Schizophyllum commune strain # 13 Dellmar (ATCC 38548)	*A*	21.0	4.5	5.0	50°	Larchwood xylan	X, X_2	[42], [43]
Schizophyllum radiatum CMI 90347		25.7		4.9	55°	Steamed wheat straw	X_2, X_3, X_4, X_5	[44]
Sporotrichum dimorphosporum	*II*	32.0*	3.9	4.5-5	65°-70°	Birchwood xylan	X, X_2, X_3, X_4, $GlcAX_3$	[45]

Source of enzyme	*Code*	Mw (kDa)	pl	pH opt	T_{opt} (°C)	Substrate	Reaction product[1]	Reference
		32.0*	4.0	4.5-5	65°-70°		X, X_2, X_3, X_4, $GlcAX_3$	
		32.0*	4.4	4.5-5	65°-70°		X, X_2, X_3, X_4, $GlcAX_3$	
		32.0*	4.7	4.5-5	65°-70°		X, X_2, X_3, X_4, $GlcAX_3$	
		26.0*	5.5	4.5-5	50°		X, X_2, X_3, X_4, $GlcAX_3$	
Talaromyces byssochlamydoides YH-50	*XbI*	54.0	3.8	4.5	70°	Xylan	X, X_2	[46]
	XbII	45.0	4.0	5.0	70°		X, X_2	[47]
Thermoascus aurantiacus C436		32.0	7.1	5.1	80°	Oat spelt	X_2	[48]
Thermoascus aurantiacus		31.8		5.2	63°	Larchwood	X, X_2, GlcAX	[49], [50]
Trichoderma harzianum E58		20.0	9.4	5.0	50°	Xylan (Aspen, Larch, Oat Spelt)	X, X_2, X_3, X_4	[51], [52]
		29.0	9.5	5.0	60°-65°		X, X_2, X_3, X_4	
Trichoderma koningii IMI 73022	*2*	17.7	7.3	4.9-5.5	50°	Oat straw	(X), X_2, X_3, AX_4	[53]
Trichoderma lignorum	*A*	21.0	5.1	3.5	45°	Oat spelt arabinoxylan	X, X_2, X_3, AX_4, AX_5	[54]
	B	20.0	8.7	6.5	45°		X, X_2, X_3, X_4, AX_4, AX_5	
Trichoderma longibrachiatum Rifai	*A*	21.5	9.45	5.0	55°	Larchwood	X_2, X_3	[55]
	B	33.0	9.25	5.0	60°	Larchwood	X, X_2	
Trichoderma reesei VIT-D-80133	*I*	32.0	4.1-4.2	4-5		Birchwood	X, X_2	[56]
	II	23.0	6.4-6.5	4-5		Birchwood	X, X_2	
Bacteria (Next Section)								
Aureomonas sp. No. 212 (ATCC 31085)	*L*	145.0		7-8	50°	Xylan	X_2, X_3, X_4	[57]
	M	37.0		6-8	50°	Xylan	X_2, X_3, X_4	
	S	23.0		5-7	60°	Xylan	X_2, X_3, X_4	
Bacillus circulans WL-12	*A*	85.0*	4.45	5.5-7		Xylan	X, X_2, X_3, X_4	[58]
	B	15.0*	9.10	5.5-7		Xylan	X_2, X_3, X_4	
Bacillus coagulans str.26 (ATCC 8038)		22.0*	10.0	6.0	37°	Xylan	X, X_2	[59]
Bacillus no. C-59-2			6.3	6-8	60°	Xylan (Oryza sativa)	X_2, X_3, X_4	[60]
Bacillus pumilus IPO		24.0		6.5	40°	Larchwood xylan	X_2, X_3, X_4, X_5	[61], [62]
Bacillus sp. 11-1S		56.0		4.0	80°	Larchwood xylan	X, X_2, X_3	[63]
Bacillus sp. No. C-125	*A*	43.0		6-10	70°	Xylan	X_2, X_3, X_4	[64]
	N	16.0		6.0-7.0	70°		X_2, X_3, X_4, X_5	
Bacillus sp. WI	*I*	21.5	8.3-8.5	6.0	65°	Larchwood xylan	X_2, X_3, X_4, X_5	[65]
	II	49.5	3.5-3.7	7.0-9.0	70°		X, X_2, X_3, X_5	[66]
		48.5*						
Bacillus sp. WII	*I*	22.5	8.3-8.5	6.0	65°	Larchwood xylan	X_2, X_3, X_4, X_5	
	II	50.0	3.5-3.7	7.0-9.0	70°		X, X_2, X_3, X_5	
		51.0*						
Bacillus stearothermophilus str. 21		39.5	4.83	7.0	60°	Oat spelt xylan	X_2, X_3	[67]
Bacillus subtilis	*A*	27.0		4.5-6.0				[68], [69]
	B	27.0		4.5-6.0				
	C	45.0		6.5-7.0		Maize xylan	$GlcAX_6$	
	D	45.0		6.5-7.0				
	E	45.0		6.5-7.0				
Bacillus subtilis PAP 115		32.0		5.0	50°	Soluble larchwood xylan`	(X), X_2, (X_3)	[70]
Cellvibrio gilvus		40.0	5.0	6.5	55°	Larchwood	X, X_2, X_3	[71]
Clostridium acetobutylicum ATCC 824	*A*	65.0	4.45	5.0	50°	Larchwood	X_2, X_3, X_4, X_5, X_6	[72]
		63.0*						
	B	29.0	8.50	5.5-6.0	60°	Larchwood	X_2, X_3	
		29.5*						
Clostridium stercorarium	*A*	44.0	4.53	5.5-7	75°	Larchwood xylan	(X), X_2, X_3	[73]
	B	72.0	4.43	5.5-7	75°		(X), X_2, X_3	
	C	62.0	4.39	5.5-7	75°		(X), X_2, X_3	
Clostridium stercorarium str. HX-1	*D*	53.0	4.5	6.5	75°	Oat spelt xylan	X_2, X_3	[74]
Clostridium thermolacticum str. TC21	*I*	39.0	4.9	6.5	80°	Larchwood glucuronoxylan	X_2, X_3	[75]
Nocardiopsis dassonvillei subsp. Alba OPC-18	*XI*	23.0	4.9	7.0	60°	Oat spelt xylan	X_2, X_3	[76]
	XII	23.0	5.3	7.0	60°	Oat spelt xylan	X_2, X_3	
	XIII	37.0	4.1	7.0	50°	Oat spelt xylan	X_2, X_3	
Streptomyces lividans 1326		43.0	5.2	6.0	60°	Oat spelt xylan	(X), X_2	[77]
Streptomyces sp. Strain 3137	*X-I*	50.0	7.10	5.5-6.5	60°-65°	Hardwood xylan	X, X_2	[78]

Source of enzyme	*Code*	Mw (kDa)	pl	pH opt	T_{opt} (°C)	Substrate	Reaction product[1]	Reference
	X-IIA	25.0	10.06	5.0-6.0	60°-65°		X, X_2	[79]
	X-IIB	25.0	10.26	5.0-6.0	60°-65°		X, X_2	
Streptomyces sp. Strain E-86		40.5	7.3	5.5-6.2	55°-60°	Hardwood xylan	(A), X, X_2	[80], [81]
Streptomyces sp. Strain KT-23		44.0 42.0*	6.9	5.5	55°	Rice straw xylan	X, X_2	[82]
Streptomyces T7		21.88 20.5*	7.8	4.5-5.5	60°	Larchwood	(X), X_2, X_3, X_4, X_5, X_6	[83]
Yeasts (Next Section)								
Cryptococcus albidus CCY 17-4-4		48.0	5.7 5.3			Oat spelt xylan	X, X_2	[84]
Cryptococcus albidus CCY 17-4-1	*I*	26.0*				Hornbeamwood xylan	X_2, X_3	[85]
Cryptococcus albidus CCY 17-4-1		48.0 26-28*	5.0	5.4		Arabinoxylan, glucuronoxylan, linear xylan	X, X_2, X_3	[86]
Cryptococcus flavus IFO 0407		25.0 23.0*	10.0	4.5	55°	Xylan	X, X_2, X_3, X_4	[87]
***** Determined by gel permeation chromatography								
[1] X, xylose; A, arabinose; Gl, glucopyranosyl uronic acid								

In general, xylans are degraded by endo-xylanases mainly to xylose (X), xylobiose (X2), xylotriose (X3), and a mixture of xylo-oligosaccharides. Some microbial sources of the enzymes and their characteristics are presented in the table given above. Below are given the crystal structures of xylanase from Trichoderma longibrachiatum obtained by X-ray diffraction studies.[88]

Fig. 2. Crystal structure of xylanase from different geometric angles, obtained from Protein Data Bank.[88]

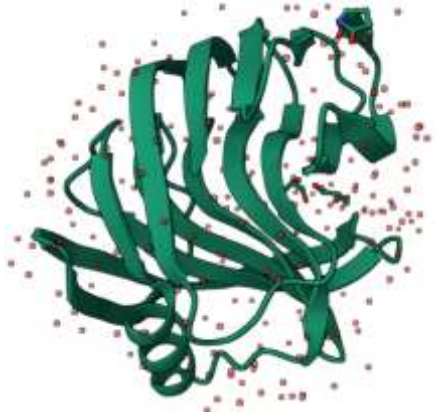

Fig. 3. Crystal structure of xylanase from different geometric angles, obtained from Protein Data Bank.[88]

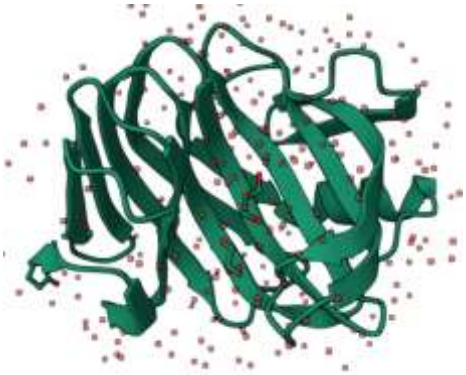

Fig. 4. Crystal structure of xylanase from different geometric angles, obtained from Protein Data Bank.[88]

Fig. 2, 3 and 4 are Crystal structures of xylanase presented from different geometric angles, obtained from Protein Data Bank.[88]

IV. Mechanism of Action

While acetyl or feruloyl esterases hydrolyze ester bonds of side groups of acetate or ferulic acid in the structure of plant cell walls, xylanases, glucuronidases, arabinofuranosidases, galactosidases, and mannanases are the primary microbial hemicellulases that act on glycosidic bonds.[9] The action of xylanase has been explained by a number of models. The enzymatic catalysis or more specifically, hydrolysis of xylan is caused by the activity of xylanases. The anomeric core of the reduced sugar forms of the carbohydrate can typically be retained or inverted as a result of hydrolysis. This implies that a few chemical state changes are involved. The anomeric conformation is either retained or inverted during glycosyl transfer, which typically causes the nucleophilic replacement at the rich carbon of the anomeric core. It is known that the majority of polysaccharide hydrolyzing enzymes, including cellulases and xylanases, hydrolyze their substrates while retaining the C1 anomeric structure. The anomeric preservation of the product involves a twofold displacement mechanism.[89]

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Following characteristics are included in the double displacement mechanism:

- a substrate-protonating acid catalyst
- a covalent glycosyl enzyme in-between with this carboxylate and an enzyme carboxyl group attached, with the sugar's anomeric conformation opposite that of the substrate.
- Through transition states involving oxo-carbonium ions, this covalent intermediate is obtained from both directions.
- bulk of the rate increase is provided by various noncovalent interactions.

Based on the crystallography analysis of the interaction between xylopentaose and Pseudomonas fluorescens Xylanase A, a scientist developed an enzyme mechanism that incorporates both the traditional ideas mentioned above and the findings of their investigation. In terms, xylanases recognize and attaches with xylan, while its residual component at position-1 is distorted and it is attracted towards catalytic component along with it, bond is formed (glycosidic bond) and ultimately substrate and enzyme complex is formed but due to its exposure with ionized water, hydrolases acts and product is released.[90]

The majority of the findings on the hydrolysis pattern of xylanases from Bacillus spp. release xylobiose, xylotriose, and xylotetraose, with the synthesis of xylose occurring only after a protracted incubation period. On various xylan substrates, xylanases A and B from Trichoderma reesei and two forms (B&C) from Trichoderma harzianum show synergistic interactions. Combinations of xylanases worked better than a single xylanase to hydrolyze pine holocellulose. The main products of xylan's hydrolysis by Bacillus circulans WL-12's xylanase B (pI 9.1) were xylobiose, xylotriose, and xylotetraose.[91] Since it was discovered that this enzyme needs at least four xylopyranoside leftovers to form a functional complex, xylotetraose emerged as the most effective substrate for saturating all of the enzyme's binding sites. However, the same source's Xylanase A quickly converted xylan to xylotetraose, and extended incubation led to the primary end products of xylose, xylobiose, and xylotriose.

V. Cellulose and Pulp

The whitening of cellulose pulp is the primary industrial use of xylanases. Since peroxidases were used to accelerate the lignin degradation process twenty years ago, enzymatic processes are now being employed in industry.[18], [92] Currently, the chemical treatment processes rather than hydrolytic treatment by enzymes is used to make paper in many nations, including Brazil. The standard procedure is called the Kraft process, which is a German name that means "force" or "strength."

As a source of raw materials, three kinds of eucalyptus are especially preferred. The process begins by pretreating wood shavings in a digestor with a mixture of NaOH and Na_2S at 165°C, under 8 kgf/cm^2. The cooking solution's two reagents work to speed up lignin removal along with recovering cellulosic fibers. The cellulose pulp is referred to as brown mass at this point and has a dark appearance due to the black liquor. It is likely that 90–95 percent of the lignin and hemicellulose that are involved in this process dissolve and partially breakdown. The accumulated lignin gives the pulp a dark color, and pre-bleaching is used after washing the brown mass carried out, which involved removing a few minor impurities and some of the lignin that was left over from the cooking process.

Oxygen is employed in this procedure to reduce the expenses of other reagents that are utilized in the process for brightening i.e., bleaching. Pulp before bleaching exhibits a light yellowish hue and can't yet be utilized to produce high-quality writing or printing paper. Remaining lignin on the fiber walls is what is causing this discoloration. The paste is then bleached to achieve a pure white color by removing light-absorbing compounds created as byproducts of the breakdown of lignin. There are three stages to the bleaching procedure. O_3 and Cl_2O are utilized in the first stage, and sodium hydroxide, oxygen, and hydrogen peroxide are employed in the second. An additional procedure with Cl_2O is applied in the final step.

The ability to recover the chemical compounds from the black liquor is the Kraft process' principal benefit. The sodium hydroxide and other organic compounds found in the black liquor are not, however, recovered by all companies. The process's low yield (40–50%), unpleasant gas emissions, expensive beginning expenditures, and high bleaching costs are its drawbacks, on the other hand. It is clear that the Kraft process makes extensive use of chemicals that cause pollution. The formation of highly hazardous and carcinogenic organochlorines from the breakdown products of lignin caused by the use of chlorine necessitates the treatment of the papermaking facility's effluents. Environmental law restrictions have prohibited the usage of Chlorine based chemicals in bleaching procedures carried out in paper and cellulose related sectors mainly in the Western Europe and North America. It has been investigated how effective microbial xylanase is in the bleaching procedure. e.g. Streptomyces spp.[93]

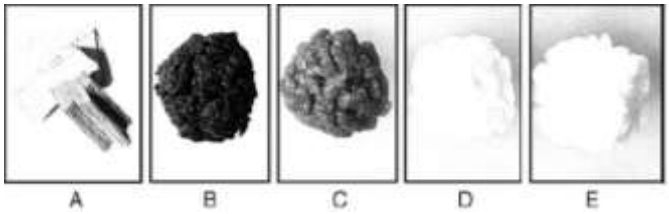

Fig. 5. Cellulose pulp bleaching stages. Shavings of wood **(A)**, post Kraft processing pulp **(B)**, pre-bleaching stage via oxygen use **(C)**, bleached with ozone and chlorine dioxide **(D)**, stage using chlorine dioxide only, after treatment with sodium hydroxide, hydrogen peroxide and oxygen **(E)**. [11]

There are two theories about how the xylanases work in the bleaching process used for cellulose pulp. In the initial step, the lignin-precipitated xylan would be subject to action by xylanases. This xylan precipitates as a result of a pH decrease at the conclusion of the cooking process.[94] The lignin would be more exposed to the chemicals used in the whitening of cellulose pulp if it were removed by the activity of xylanases. The 2nd theory is supported by the fact that lignin can form compounds with polysaccharides like xylan and that some of the linkages may not have been dissolved during the Kraft process because they are alkali-resistant. The residual lignin-

xylan bridges are broken by the xylanases, opening the cellulose pulp's framework, and causing xylan to shatter. The broken particles are then removed.[95] After being treated by xylanases, pulp becomes much amenable to chemical elimination of any remaining brown colored lignin and lignin carbohydrates from the fibers.

VI. Baking and Brewing

Due to their potential utility in the production of bread, the use of xylanolytic enzymes has grown during the past few decades. Hydrolyzing enzymes for starch and non-starch carbohydrates are frequently utilized as bread enhancers in the bread-making industry. The rheological qualities of dough, bread's specific volume, and the firmness of the crumb are all improved by the enzyme-mediated hydrolysis of non-starch polysaccharides.[96] The xylanases, along with the other hemicellulases, help to redistribute water and make dough that is softer and simpler to knead by catalyzing the hemicellulose in wheat flour. They delay crumb's development during bread baking, allowing expansion of dough. Bread quantities have increased, water absorption has increased, and fermentation resistance has improved because to the usage of xylanases.[97] Additionally, bread with more arabinoxyl-oligosaccharides would be better for your health.

Xylanase transforms the hemicellulose that is insoluble in water into water soluble form that keeps H_2O in dough, reduces stiffness, gives volumetric increase, and creates finer, uniformed crumbs. Xylanases and other enzymes that hydrolyze the complex cell walls are employed to enhance the quality of bread, increase lifetime by lowering the rate of stalling and to improve dough handling capabilities. They seem to be particularly successful in straight dough processes. With better dough pliability, productivity, stability, loaf volume, and crumb structure, xylanases also improve bread quality. Numerous enzymes, including cellulases, xylanase, and proteases, strengthen the structure of gluten and consequently enhance the quality of bakery goods. The ideal level of enzymatic hydrolysis of pentosans by hemicellulases or pentosanases enhances the qualities of the dough, leading to more uniformity in quality parameters.[98] The dough is more adaptable to varying flour characteristics and processing procedure variations thanks to xylanases. Tempered pan bread volume is increased greatly, dough is softened, and less sheeting effort is needed by utilizing xylanases.

In comparison to breads without any enzyme added, xylanase, protease, amylase and lipase are greatly more effective at producing bread with increased specific volume when heated in a microwave oven. Xylanases significantly altered the feel of profile analysis and decreased the stiffness of bread crumb. The reorganization of water from the pentosan cycle to the gluten phase by xylanase results in an increase in bread volume. Decent oven spring will be the result of the extensibility of the gluten portion increasing with volume.[99] Due to the activity of the enzyme in lowering the gelling starch's viscosity and allowing greater and longer expansion in the oven prior to the inhibition of enzymes and denaturation of proteins, the improving effect caused by pentosanases on bread volume could possibly be linked to a better gas retention during proofing.

When bran is hydrated with a combination of cellulase and xylanase, the amount of soluble sugar increases and the amount of insoluble fiber decreases. Starch gelatinization is either delayed or inhibited, and water absorption for dough development is reduced. Bread made with bran hydrated with a low dose of xylanase or a combination of cellulase and xylanase has a higher loaf volume.[100] The quality of whole wheat bread was found to be improved by enzymes such as amylase, cellulase, glucose oxidase, and xylanase. The highest improvement in loaf volume was achieved with the highest dose of xylanase, and there was also a trend of decreasing crumb hardness and slowing the rate of crumb firming.[101]

Xylanase is advised when preparing biscuits to make cream crackers more lighter, to enhance the texture and palatability and for giving uniformity to wafers.[102] By liquefying fruit and vegetables, stabilizing fruit pulp, recovering more scents, important oils, nutrients, and minerals, palatable dyes, pigments, and other compounds, reducing viscosity, and hydrolyzing compounds that obstruct the structural or chemical eliminating of the juice or that may result in cloudiness in the concentrate, xylanases, in combination with cellulases, amylases, and pectinases, improve juice yield. Together with endoglucanase, xylanase isolates and separates the starch and gluten during the breaking down of arabinoxylan and starch in wheat flour. The mucilage made from coffee beans also uses this enzyme. Higher stability and optimal activity at an acidic pH are xylanases' major desirable traits for application in the food sector. Other applications of xylanases are being found as molecular biology techniques advance. A wine with a stronger scent than usual was produced recently using a recombinant yeast that contained the xylanase gene from Aspergillus nidulans, xlnA.[103]

Beer is created by hydrolyzing the barley cell membrane, which results in arabinoxylans long chains which grants the beverage its "muddy" presentation by raising viscosity. As a result, xylanases are utilized to break down arabinoxylans to lower oligosaccharides, which lessens the viscosity of the beer and as a result, eliminates its muddy appearance. For aromatizing musts, fruit juices and wines, alpha-L-Arabinofuranosidase and beta-D-glucopyranosidase are used in the food processing industry. [104]

VII. Animal Feed

Forage crops are pretreated with xylanase to increase ruminant feed digestibility and to make composting easier. Along with glucanases, pectinases, cellulases, proteases, amylases, phytase, galactosidases, and lipases, xylanases are used in animal feed. The viscosity of the raw material is decreased by these enzymes as they catalyze arabinoxylans present in the constituents of feed.[105] In feed comprised of low viscosity cereals like maize and sorghum, addition of xylanase may improve nutrient breakdown in the first portion of the Gastrointestinal Tract, that results in more effective usage of energy. Endogenous enzymes are not produced as much in fledgling birds and pigs as is produced in adults, so exogenous enzyme food supplements should help them function better as

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livestock. Additionally, it has been demonstrated that diet of this type decreases unwelcome residues in excreta, a finding that may contribute to a decrease in environmental contamination.[106] M. B. Café fed the chicken birds nutrient-dense diets with or without the adding to it 0.1% Avizyme-1500. As compared to the control groups, birds fed diets that were supplemented with Avizyme showed noticeably greater body weights, lower death rates, and higher amounts of energy derived from net from their diet. By adding xylanase in poultry feed, Babalola [107] noted better perceived nitrogen and fiber uptake as well as the feed time for transit. Additionally, it was determined that the addition of these enzymes to boiled castor seed food was acceptable and had no negative effects on growth efficiency or blood constituents.

VIII. Xylanases in Chemical Production

Waste made of lignocellulose underwent a preliminary oxidation process using H_2O_2. The pretreated material was combined with xylanases and cellulases for the saccharification process to produce C_5 and C_6 sugars, and then Lactobacillus was used to ferment the sugars that reduced to lactic acid.[108] Two stages were required to convert xylose into xylitol. Using isolated endo-xylanase and beta-xylosidase at 40 °C for 15 h, the lignocelluloses' aqueous phase was chemically pretreated to produce xylose, which was then hydrogenated to produce xylitol. The aqueous phase's contaminants did not prevent the enzyme from working.[109] Chemically, xylooligomers were saccharified to xylose at elevated thermal point (40–80 °Celsius) and pH extreme of 4–9. At lower temperatures and a pH range of 4.5-7, the Selenomonas ruminantium GA192 beta-D-xylosidase, however, was capable to decompose the xylooligomers.[110]

During the pretreatment of lignocellulose, xylooligomers are generated that prevent the hydrolysis of cellulose during saccharification. By assisting in the hydrolysis of xylooligomers, the system was improved by the xylanases addition such as endo-xylanase or beta-D-xylosidase. With the use of sodium hydroxide and Irgazyme 10A-X4, a commercial enzyme complex made up from both endo-xylanase and beta-D-xylosidase, xylan was isolated from aspen pulp. In a mild environment (pH 4.5–5, 45 °C) for 22–45 hours, the xylan hydrolysis produced a xylose yield of 91% maximum, based on the dry solids that were used.[111] [112]

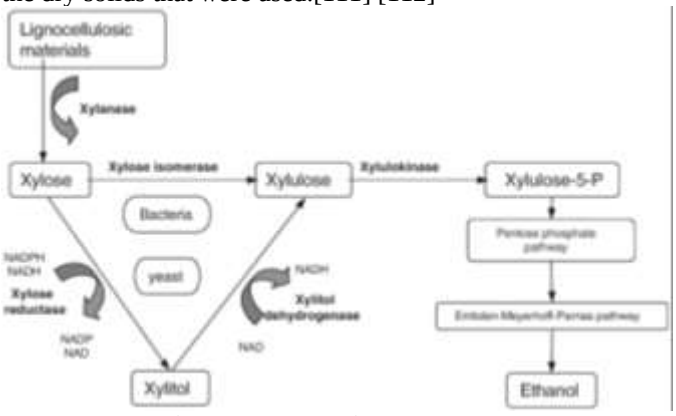

Fig. 6. Production of ethanol and xylitol from lignocellulosic materials. [11]

IX. Biofuel

Lignocellulosic biomass being biologically converted results in bioethanol production. Biopolymers including pectin, cellulose, xylan, lignin, and peptides can all be present in varying concentrations in lignocellulosic biomass. From lignocellulosic biomass, biofuels like xylitol and ethanol can be produced by xylanases, working in synergy with other enzymes like ligninase, glucanase, xylosidase, glucosidase and mannanase etc.[113] In production of bio-ethanol, lignocellulosic containing materials as biomasses can be delignified in order to release hemicellulose and cellulose, subsequently catalyzed to create free carbohydrates and lastly brewing to generate C_2H_5OH from mixed C_5 sugar and C_6 sugar. Prior to the synthesis of bioethanol, the lignocellulosic biomass must undergo the necessary saccharification procedure. The sugar production during saccharification is improved by enzymatic hydrolysis before the agroresidue pretreatment.[114]

By employing enzyme cocktail pretreatment on lignocellulosic biomasses, Bala and Singh demonstrated a novel, improved technique for the production of bioethanol. Pennacchio noted a significant succinic acid yield from the basfia succiniciproducens BPP7-fermented sugars [115]. According to research by Bala and Singh from 2017, an enzyme cocktail can be utilized to produce xylo-oligosaccharides for monomers and prebiotics for producing biofuel.[116] In another study, expression vector pace2-hph-PRIM was created for overexpressing gene encoding Angiotensin Converting Enzyme-2 in a fungal specie Trichoderma reesei. This recombinant fungal strain was applied in synthesis of xylitol from tree bark and was used to increase xylanse production.[117] Xylitol can be used for middle ear infection prevention, better dental health promotion, and inclusion in oral care goods.

Reported Malbranchea pulchella thermostable xylanase expressed in Aspergillus nidulans states that the significant amount of sugar that ferments produced from processed sugarcane bagasse allows xylanase to function on substrate for a long period of time and displays many biotechnological applications including second-generation ethanol production.[118] Amaya-delgado et al. identified Cellulomonas flavigena's Cfl Xyn11A xylanase, that was used to enhance the yield of fermentable sugars to make bioethanol (microbial C_2H_5OH) by pre-treatment of lignocellulosic biomass.[119]

X. Deinking The Paper

The deinking treatment, which eliminates ink specks from the paper, is an important step in the recycling of wastepaper. Disposal of hazardous wastewater occurs as a result of the extensive usage of chlorine-based derivatives in this process, including $NaSiO_2$, Na_2CO_3, chelating agents, and hypochlorites.[120] Compared to chemical treatment, by using a crude enzyme solution that included T. harzianum cellulase and xylanase, photocopier wastepaper deinking was improved, leading to better toner removal and stronger paper. Longer fiber lengths, which are indicative of the preservation of fiber integrity, and the promotion of fibrillation are responsible for this improvement. A 94% deinking efficiency could be attained

with an enzyme dosage of 0.8 IU/g, a pulp consistency of 10%, and a reaction duration of 60 minutes. When enzyme therapy was administered "During-Hydra Pulping", it produced a higher deinking efficiency than when it was administered "Pre-Hydra Pulping" or "Post-Hydra Pulping." Treatments with high enzyme doses and lengthy reaction times degraded the qualities of paper strength. The brightness of the pulp improved when xylanase was included in the crude enzyme. Enzymatic activity on fines showed improvement in pulp freeness.[121]

Cellulase-free xylanase preparations from six fungal isolates were evaluated, with an emphasis on how they affected deinking pulp. It was shown that individual xylanases might improve pulp brightness in comparison to untreated and chemically deinked pulp. Results of DX-23, DX-41, DX-K and DX-36 enzymes were nearly same. Remarkably, the deinked pulp treated with DX-23 xylanase showed a 22% improvement in ISO brightness, measuring 34.5%, compared to untreated pulp. This specific xylanase also produced the lowest yellowness (11.7±0.3) and the maximum whiteness (31.7±1.2). The results demonstrate improvements in brightness, whiteness, and yellowness that might be useful in the paper sector, highlighting DX-23 xylanase as a good choice for efficient deinking procedures. The high level of unwanted yellowness that was achieved when pulp was deinked chemically was caused by the pulp's alkaline state at the time of deinking. DX-23's xylanase was thus chosen for additional analysis. Based on its physical and cultural traits, the isolate DX-23 was determined to be Aspergillus niger. DX-23's 600 bps ITS sequencing revealed 99% identity with A. niger. [122]

Nowadays, wastepaper is deinked using enzyme combinations like laccase and xylanase, which significantly improves the paper's brightness, luminosity, rip factor, rupture factor, etc. According to Chutani and Sharma (2016), who used the xylanase and cellulase enzyme combination from T.longibrachiatum MDU-6, deinked newspaper has a brightness of 52%, which is 9.6% more than compared to the control sample.[123]

XI. Pharmaceutical Applications

There are not many applications of xylan or xylanase in the pharmaceutical sector. A mixture of enzymes may occasionally have xylanases added to it as a nutritional supplement or as a treatment for impaired digestion, but few pharmaceutical products use this composition. One of the hydrolytic byproducts of xylan, beta-D-xylopyranosyl residues, can be converted into solvents, flammable fluids (ethanol) and low-calorie artificial sweets. The 1st stage is delignification of xylan-rich hemicellulose material, which is then hydrolyzed by hemicellulases and xylanases to produce sugars like beta-D-xylopyranosyl units.[124] The products are then fermented, primarily by yeasts, to create ethanol or xylitol. A polyalcohol, xylitol has a sweetening effect similar to sucrose. It is a non-cariogenic sweetener that can be used by diabetic people and those who are obese. It is recommended to prevent developing osteoporosis, lipid metabolism abnormalities, renal harm, parenteral damages and respiratory tract infections. There are many commercial products that have xylitol in them, including chewing gum.[125]

Although obtaining beta-D-xylopyranosyl units from the hydrolysis process of xylan is an ensuring method, Chemical catalytic processes are now used to produce industrial xylitol on a huge stage. The fact that the xylose must firstly be purified in a variety of processes is the fundamental reason why this procedure is seen as being expensive. In addition, hazardous byproducts of the chemical processes often impact fermentation. For instance, during the hydrolysis of lignocellulosic material, compounds that result from the degradation of xylose, lignin and glucose may be generated additionally to the release of sugars. Acetic acid, extracted materials such as terpenes and their derivatives, tropolones, and phenolic compounds like flavonoids, stilbenes, quinones, lignans, and tannins, as well as metals (chromium, nickel, copper and iron) can all be effective microbial activity blockers. There is considerable hope that xylitol will be used more widely in the food industry, pharmaceutical industry, and odontological industries because of the development of more appropriate processes and techniques for its manufacturing.[11]

XII. Conclusion

Further research is needed to understand the role of xylan-degrading enzymes in the degradation of lignocellulose, particularly the function of xylanases and attachment enzymes in achieving and enhancing the full disintegration of lignocellulose. It may be helpful to comprehend the microfeatures of cellulose recalcitrance and its ability to resist enzymatic hydrolysis by conducting a thorough research of the microscale and nanoscale characteristics of the plant cell wall and the cellulose bonding hydrogen network. Microbial xylanases offer a lot of potential and are highly advantageous for industrial use. In the food processing sector, xylanase enzyme must be promoted to take the place of chemical emulsifiers and additives. Combining the xylanase enzyme with other enzymes can improve outcomes.

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