Chapter 19
Recent Advances and Industrial Applications of Microbial Xylanases: A Review

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Abstract Xylanase being a hydrolytic enzyme catalyses the hydrolytic breakdown of 1,4-β-D-xylosidic linkages in xylan which is an important constituent of hemicellulose. Xylanases are hemicellulases required for depolymerization of xylans which are the second most bountiful polysaccharide occurring in nature after cellulose having plant origin. A broad range of organisms have been reported to produce xylanases that include several fungi, bacteria, protozoans, crustaceans, marine algae, insects, snails, gastropods, arthropods, several seeds and plants. Filamentous fungi have been documented to be the useful producers of xylanase because of ease of cultivation, extracellular secretion of enzymes, higher yield and industrial aspect. Fungal xylanases from *Aspergillus* species and *Trichoderma* species have been widely studied and characterized and are commercially utilized in bakery and food processing industries. Microbial xylanases have been reported to be single-chain glycoproteins having molecular masses usually 8–145 kDa and exhibit maximum activity in temperature range 40–60 °C. Thermostable xylanases are ideally suited for use in industrial applications because of numerous advantages over thermolabile xylanase such as ability to work in broad temperature range, better substrate utilization and ability to tolerate high temperature in processes as well as better shelf life. Xylanases have widespread utilization in diverse industries such as food industry, textile industry and in pulp and paper industry. Xylanases have emerged to be extremely beneficial in terms of enhancing the production of numerous fruitful products. Over the years the advancements in molecular tools and techniques have enabled the better understanding of regulatory mechanisms heading xylanase production, underlying mechanism of action of xylanases as well as more precise knowledge of xylanase gene. Such advancements have paved the way for better utilization of enzymes in a much broader sense in commercial sector. Xylanases have tremendous industrial applications in commercial sector either on their own or by associating with different enzymes in numerous processes like processing of pulp and fibres; saccharification of agricultural, industrial and municipal wastes;

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flour improvement for bakery products; pretreatment of forage crops and lignocellulosic biomass; as well as an alternate to treating the textile-cellulosic waste with sulphuric acid.

Keywords Xylanase · Xylan · Hemicellulases · Saccharification · Glycoproteins · Immobilization

19.1 Introduction

Xylanase (E.C 3.2.1.8) is an enzyme belonging to glucanase family and has a quite an expanding group of enzymes which can hydrolyse the 1,4-β-D-xylosidic linkages in xylan. Being a complex molecule, the depolymerization of xylan necessitates a cooperative action of different enzymes for its thorough disintegration. β-1,4-Endoxylanase, β-xylosidase, α-L-arabinofuranosidase, α-glucoronidase, acetyl xylan esterase and phenolic acid esterase occupy a significant place amongst the xylanases that have been reported extensively. According to Sharma and Kumar (2013), xylanase is a significant industrial enzyme that causes the random disintegration of xylan by its endo-1,4-xylanase activity and produces xylose, xylooligosaccharides and xylobiose. Amongst these xylanases, endo-1,4- xylanases (1,4-β-D-xylan xylanohydrolase, E.C.3.2.1.8) catalyse xylan depolymerization by randomly hydrolysing xylan backbone. Whistler and Masek (1955) reported xylanase for the first time. According to Bastawde (1992), the importance of xylanases is discovered over 100 years ago by Hoppe-Seyler. Xylanases were primarily termed pentosanases and were recognized by International Union of Biochemistry and Molecular Biology (IUBMB) for the first time in 1961. The systematic name of xylanases is endo-β-1,4-xylanase, but more widely accepted and universally used synonyms of the enzyme include xylanase, endo-1,4-β-D-xylanase, endoxylanase, β-1,4-D-xylan xylanohydrolase, β-xylanase and β-1,4-xylanase, respectively. A number of key factors govern the yield of xylanases in fermentation that include accessibility of substrate, rate and extent of disentangling of the xylooligosaccharides, etc. Omar et al. (2008) reported that xylanase holds significant importance due to its proficiency of degrading the plant cell wall constituents. Xylanase has attained paramount industrial importance credited to their multidimensional and multifunctional role in fermentation processes and numerous other industries.

19.2 Substrates for Xylanase

Hemicelluloses are the major substrates for xylanases. The word hemicelluloses points to polysaccharides occurring in plant cell wall that are confederated with cellulose and glucans. Hemicelluloses are the second most bountiful constituents in

cell wall of plants behind cellulose (Nakamura 2003). Hemicelluloses consist of complex of polymeric carbohydrates that include xylan, glucomannan, xyloglucan, galactoglucomannan and arabinogalactan (Shallom and Shoham 2003). Classification of such polymeric carbohydrates relies upon the type of sugar entities present in their structure. Xylan is a major hemicellulose composed of xylose units interlinked through β-1,4-glycosidic linkage. Xylan has been reported to be the second most abundant polysaccharide behind cellulose that is loaded with a huge potential of being converted into a majority of products having paramount importance. Saha (2003) reported that xylan holds for nearly one third of entire replenishable organic carbon present on our planet. Xylan has a complex structure and heterogenic nature. Thus on account of this, xylanase plays a prodigious role in the combination of hydrolytic enzymes obligatory for the thorough disintegration of xylan (Takahashi et al. 2013). Xylan is a heterogenic polysaccharide comprising of xylose units interlinked through β-1,4-glycosidic bonds. Whistler and Richards (1970) reported that xylan major chain is built up of β-xylopyranose units. Xylan predominantly occurs in the secondary cell wall, and it constitutes the most part of the polymeric fraction of plant cell wall in association with lignin and cellulose. Xylan provides integrity to the cell wall by virtue of its association between lignin and cellulose through covalent and noncovalent bonds (Motta et al. 2013).

19.2.1 Structural Framework and Distribution of Xylan

Xylan being a convoluted heteropolysaccharide has an eminently branched structure that differs remarkably amidst distinctive plant species. On the grounds of common substituents occurring on the xylan backbone, xylans have been categorized into linear homoxylan, arabinoxylan, glucuronoxylan and glucuronoarabinoxylan. However as a matter of fact, there occurs microheterogeneity in each category of the xylan in relevance to the extent and characteristic of branching. The occurrence of side chains in the substituted forms of xylan determines several aspects that include physical configuration, degree of solubility, mode of the enzyme action, etc. (Motta et al. 2013). On account of its intricate structure and heterogenic nature, the thorough depolymerization of xylan necessitates a cooperative action of several enzymes (Subramaniyan and Prema 2002). Xylan has been reported to be distributed in a vast variety of tissues and cells and occurs in the major fraction of plant species. Singh et al. (2003) reported that xylan is known to exist up to significant levels in hardwoods of angiosperms (15–30%) and softwoods of gymnosperms (7–10%) and also in annual plants (<30%). Wood xylan predominantly occurs as O-acetyl-4-O-methylglucuronoxylan in hardwoods and in softwoods as arabino-4-O-methylglucuronoxylan. However xylans occurring in grasses and annual plants are generally arabinoxylans (Kulkarni et al. 1999). Xylan has a similarity to other polysaccharides having plant origin with respect to vast polydiversity and

polymolecularity (Sunna and Antranikian 1997). The extent of polymerization in xylans also has significant variability, for instance, hardwood xylans generally consist of 150–200 β-xylopyranose units, whereas softwood xylans generally consist of 70–130 β-xylopyranose units (Kulkarni et al. 1999). D-Xylans hold for 20–35% of entire dry weight in hardwood and annual plants and predominantly exist as the most prevalent non-cellulosic polysaccharides (Velkova et al. 2007).

19.2.2 Enzymatic Disintegration of Xylan

On account of its complex structure and heterogenic nature, thorough disintegration of plant xylan necessitates the cooperative involvement of a multienzyme hydrolytic complex having broad spectrum of activities and diverse approaches of action. The main enzymes participating in multienzyme hydrolysis of xylan include β-xylosidase, endoxylanase, acetyl xylan esterase, arabinofuranosidase, glucuronidase, galactosidase and feruloyl esterase. These enzymes act in a collegial fashion to depolymerize xylan into its monomeric units (Belancic et al. 1995). Endoxylanases hold foremost place amongst all xylanases on account of their direct participation in the cleavage of glycosidic bonds as well as in liberating short xylooligosaccharides (Verma and Satyanarayana 2012).

Xylanase randomly hydrolyses xylans into xylooligosaccharides, whereas β-xylosidase dislodges xylose units from the non-reducing ends of xylooligosaccharides. Despite this, thorough disintegration needs the activity of acetyl esterase to dislodge the acetyl substituents from the D-xylose backbone (β-1,4-linked) of xylan (Coughlan and Hazlewood 1993).

19.3 Xylanase Sources

Xylanase sources range from microorganisms like bacteria, fungi, protozoans, actinomycetes, crustaceans, marine algae, insects, snails, gastropods, arthropods, several seeds and plants that tears the glycosidic linkages in xylans thus resulting in hemi acetyls and glycans (Motta et al. 2013). Certain invertebrate organisms such as earthworms also possess a broad spectrum of fibrolytic microbes in their gut. Thus it is quite possible that a few amongst these organisms might be the producers of novel endoxylanases (Park et al. 2007). Rumen is one of the most fascinating sources of xylanases wherein the effective hydrolysis of plant polysaccharides commences. Filamentous fungi have been documented to be the useful xylanase producers because of ease of cultivation, extracellular secretion of enzymes, higher yield and industrial aspect. Fungal xylanases from *Aspergillus* species and *Trichoderma* species have been widely and thoroughly studied and characterized and are commercially utilized in bakery and food processing industries. Certain microorganisms

such as *Aspergillus* and *Penicillium* have been documented to efficiently synthesize xylanases in the presence of cheaper hemicellulosic substrates such as rice bran, bagasse corn stalk, corn cob, wheat bran and rice straw. White rot fungus is extensively utilized in manufacturing pharmaceutical products, in food industries and in the manufacturing of cosmetics (Qinnhe et al. 2004). White rot basidiomycetes conducts its depolymerization action on lignocellulosic materials through the enzymatic action of several xylanase enzymes. Several bacterial strains including *Bacillus* sp. have also been reported to be the efficient producers of thermostable xylanases having good stability at varying range of temperature, pH, presence of metal ions, etc. (Battan et al. 2007).

19.3.1 Fungal Xylanases

Fungal xylanases from genera *Aspergillus*, *Trichoderma*, *Pichia* and *Fusarium* species have been considered as the efficient and producers of novel xylanases (Absul et al. 2005). Other efficient fungal species producing xylanases include *Streptomyces* (Kansoh et al. 2001) *Aspergillus kawachii* (Ito et al. 2000) and *Cunninghamella subvermispora* (Ferraz et al. 2004). Haq et al. (2002) documented *Aspergillus niger* as the most potent xylanase producer.

19.3.2 Bacterial Xylanases

Bacterial species producing high activity xylanases at alkaline pH and higher temperature are Bacillus sp. (Subramaniyan et al. 2001). Main bacterial genera that are efficient producers of novel xylanases are *Bacillus halodurans* (Ebrahimi 2010), *Saccharopolyspora pathunthaniensis* (Verma and Satyanarayana 2012), *Thermotoga* sp. (Yoon et al. 2004), *Bacillus circulans* D1(Bocchini et al. 2005), *Pseudomonas* sp. XPB-6 (Sharma and Chand 2012), *Stenotrophomonas maltophilia* (Raj et al. 2013) and *Bacillus* genus (Kaur et al. 2015). Certain filamentous bacterial strains documented to be the efficient producers of endoxylanases, xylanase and polygalacturonase are *Streptomyce*s sp., *S. roseiscleroticus* and *S. cuspidosporus* (Maheswari and Chandra 2000).

19.3.3 Thermophilic Xylanases

A good proportion of xylanase-producing thermophilic and hyperthermophilic microorganisms have been reported and successfully explored from a vast variety of sources including thermal springs, self-heating decaying organic debris, hot pools

and terrestrial and marine solfataric fields (Sunna and Bergquist 2003). Family 10 xylanases have been successfully explored from a diverse range of thermophilic and hyperthermophilic organisms, including *Thermoascus aurantiacus* (Khasin et al. 1993) and *Nonomuraea flexuosa* (Hakulinen et al. 2003). Xylanases from *Dictyoglomus thermophilum* and *Nonomuraea flexuosa* have been reported amongst the most stable xylanases and possess temperature optima of 80 °C and 85 °C, respectively. Certain hyperthermophilic archaea have also been reported to be the efficient producers of novel xylanases, e.g. *Pyrodictium abyssi* (Andrade et al. 1999), *Thermofilum* strains (Andrade et al. 1999), *Pyrococcus furiosus* (Cady et al. 2001), *Thermococcus zilligii* (Cady et al. 2001), *Sulfolobus solfataricus* (Cannio et al. 2004) and *T. leycettanus* (Wang et al. 2016) (Tables 19.1, 19.2, and 19.3).

Table 19.1 List of xylanase-producing microorganisms

Microorganisms	References
Fungi	
Trichoderma harzianum	Sanghvi et al. (2010)
Aspergillus niger	Subbulakshmi and Priya (2014)
Trichoderma reesei SAF3	Kar et al. (2006)
Marasmius sp.	Ratanachomsri et al. (2006)
Aspergillus terreus UL 4209	Chidi et al. (2008)
Fusarium solani F7	Gupta et al. (2009)
Aspergillus awamori	Teixeira et al. (2010)
Penicillium citrinum	Ghoshal et al. (2011)
Aspergillus usamii	Zhou et al. (2011)
Trichoderma sp.	Norazlina et al. (2013)
Cladosporium sp.	Patel and Prajapati (2014)
Penicillium crustosum	Mushimiyimana and Padmavathi (2015)
Aspergillus sp.	Thomas et al. (2016)
Aspergillus nidulans	Gabriela et al. (2016)
Rhizopus oryzae SN5	Pandey et al. (2016)
Aureobasidium pullulans NRRL Y-2311-1	Yegin (2016)
Bacteria	
Sclerotinia sclerotiorum S2	Ellouze et al. (2008)
Bacillus cereus	Roy and Habib (2009)
Bacillus pumilus	Monisha et al. (2009)
Bacillus sp. *YJ6*	Yin et al. (2010)
Bacillus sp.	Azeri et al. (2010) and Bahri et al. (2011)
Streptomyces sp. *P12–137*	Coman and Bahrim (2011)
Pseudomonas sp. *XPB-6*	Sharma and Chand (2012)
Colletotrichum graminicola	Zimbardi et al. (2013)
Bacillus genus	Kaur et al. (2015)

Table 19.2 Thermophilic organisms for family 10 xylanase

S. No.	Name of the organism	Temperature	Reference
1.	*Clostridium thermocellum*	70 °C	Herbers et al. (1995)
2.	*Rhodothermus marinus*	65 °C	Karlsson et al. (2004)
3.	*Thermotoga* sp.	105 °C	Shi et al. (2013)
4.	*Thermoascus aurantiacus* RCKK	45 °C	Jain et al. (2014)
5.	*Geobacillus stearothermophilus* KIBGE-IB29	60 °C	Bibi et al. (2014)
6.	*Caldicellulosiruptor* sp.	75 °C	Meng et al. (2015)

Table 19.3 Thermophilic organisms for family 11 xylanase

S. No.	Name of the organism	Temperature	Reference
1.	*Dictyoglomus thermophilum*	70 °C	McCarthy et al. (2000)
2.	*Paecilomyces variotii*	60 °C	Kumar et al. (2000)
3.	*Thermomyces lanuginosus*	60 °C	Singh et al. (2003)
4.	*Chaetomium thermophilum*	70 °C	Ahmed et al. (2012)
5.	*Anoxybacillus flavithermus* TWXYL3	65 °C	Ellis and Magnuson (2012)
6.	*Humicola insolens* Y1	50 °C	Shi et al. (2015)

19.4 Classification of Xylanases

Xylanases can be categorized into two separate groups: (1) one possessing low molecular mass (<30 kDa) and basic pI and (2) another one possessing high molecular mass (>30 kDa) and acidic pI (Wong et al. 1998). Also on the basis of primary sequence homology, xylanases have been categorized into two distinct families (family F or 10 and family G or 11). Xylanase under family F has lower pI values as compared to family G. Earlier in a study, family 10 xylanases have been reported to be more complex and diverse having high molecular mass (>30 kDa) (Ducros et al. 2000). In contrast family 11 xylanases are much simpler, have consistency in their structure, have greater specificity for xylan and possess low molecular mass (>20 kDa) in comparison to family 10 xylanases. Woodward (1984) reported that three distinct types of xylanases are known for their involvement in xylan degradation.

19.4.1 Endo-1,4-β-xylanase (1,4-β-D-Xylan Xylanohydrolase)

Endo-1,4-β-xylanase (1,4-β-D-xylan xylanohydrolase; EC 3.2.1.8) disunites the glycosidic linkages in the xylan backbone thus leading to reduced substrate polymerization. Xylan is not degraded randomly. As a matter of fact, the xylan hydrolysis relies upon the attributes of the substrate molecule, i.e. upon the chain length, presence of substituents and extent of branching (Li et al. 2000). Xylanase categorizes themselves into two distinct types on the grounds of type of end products of the

reaction: (a) non-debranching or non-arabinose liberating and (b) branching or arabinose liberating.

(a) Non-debranching endoxylanases: Non-debranching or non-arabinose liberating endoxylanases are those endoxylanases that can be compartmentalized into two distinct variants, one giving off xylose and xylobiose as the end products and the other one giving off xylooligosaccharides as the end product.
(b) Branching endoxylanases: Branching or arabinose liberating endoxylanases can be compartmentalized into two discrete groups: group 1 that possess the capability to hydrolyse branching points hereby giving off xylooligosaccharides and arabinose as the end products and group 2 that cleaves off the xylan and branching points thereby giving off principally xylobiose, xylose and arabinose, respectively.

19.4.2 Exo-1, 4-β-xylanase (β-1,4-D-Xylan Xylohydrolase)

These enzymes discharge the single xylose units from the non-reducing end of the xylan chain.

19.4.3 β-D-Xylosidase (1,4-β-D-Xylan Xylohydrolase)

β-D-Xylosidases (1,4-β-D-xylan xylohydrolase; EC 3.2.1.37) are exoglycosidase that actively depolymerizes short xylooligosaccharides to give off xylose. β-D-Xylosidases can be classified on the grounds of their relative fondness towards xylobiose and larger xylooligosaccharides, respectively. Octavio et al. (2006) documented that a huge fraction of bacteria and fungi efficiently produce such xylanases. They may show their existence in the culture broth surrounding the cell, in alliance with the cell, or they may also exist in both. β-D-Xylosidases play a significant role in lowering the end product inhibition of endoxylanases which is a rate-limiting factor in xylan depolymerization (Andrade et al. 2004).

19.5 Mechanism of Xylanase Action

Numerous stereotypes have been put forward to elucidate the action mechanism of xylanases. Subramaniyan and Prema (2002) reported that xylanase action eventually results in the hydrolysis of xylan that may cause retention or inversion of the anomeric centre of the reducing sugar monomer of the xylan thus giving an intimation of one or two chemical transition states being involved. Transfer of glycosyl

eventually causes nucleophilic substitution at the saturated carbon of the anomeric centre and commences with either retention or inversion of the anomeric configuration. A great majority of hydrolytic enzymes like xylanases and cellulases that are well recognized for hydrolysing polysaccharides eventually result in the hydrolysis of their corresponding substrates with the retention of the C1 anomeric configuration. Double displacement mechanism has been reported to be directly indulged in the anomeric retention of product (Clarke et al. 1993).

19.6 Xylanase Production

The main driving force behind search for novel xylanases is the broader range of its tremendous industrial applications. Both solid-state fermentation (SSF) system and submerged fermentation (SMF) system can be successfully utilized. Xylanase production can be efficiently carried out using solid-state cultivation systems and submerged cultures methods. Submerged fermentation (SMF) technique has been the method of choice by most of researchers because of easier regulation of various process parameters such as pH, temperature of medium, degree of aeration as well as several environmental factors indispensable for the optimal growth of microorganisms. However as a matter of fact, solid-state fermentation has procured significant attention and acceptance from the researchers worldwide over the years and has been successfully and widely utilized for xylanase synthesis (Haltrich et al. 1996). This is credited to numerous economic and engineering advantages. Submerged fermentation (SMF) system has been preferred over solid-state fermentation (SSF) system for products involving large-scale production because the yield of enzyme is higher (about 90%) and also more cost-effective as compared to solid-state fermentation (Gouda 2000). SMF has the advantage over solid-state fermentation system in extracting a large fraction of purified enzymes. Wheat bran came out to be the best carbon source in the studies conducted on xylanase production by *Stenotrophomonas maltophilia* after using commercial xylans and different agro-industrial residues (Raj et al. 2013). *Bacillus arseniciselenatis* DSM-15340 resulted in a thermoalkalophilic cellulose-free xylanase in significant level, while it was grown in solid-state conditions by utilizing economically accessible agro-residual substrate wheat bran. Thus it could be efficiently utilized for production of xylanase on large scale by utilizing such agro-residual substrates (Kamble and Anandrao 2012). SSF conditions are exclusively favourable for the fungal growth since these organisms possess the ability to grow at rather low water activities in contrast to most of the bacteria and yeast that do not grow and proliferate efficiently in such culture environment. Mushimiyimana et al. (2015) reported that xylanolytic enzymes are efficiently produced by fungi under submerged conditions. Microbes such as *Trichoderma*, *Aspergillus*, *Phanerochaete*, *Streptomyces*, *Clostridia*, *Ruminococcus*, *Chytridiomycetes*, *Bacillus* and *Fibrobacteres* are loaded with huge potential to efficiently produce xylanase enzymes (Qinnhe et al. 2004).

19.7 Characterization and Purification

Xylanases from distinct sources vary significantly in their temperature and pH optima for the maximal activity. Concentrated and pure enzymes exhibit enhanced activity and reduced risk of inhibitory substances thus putting forward themselves as ideal materials having tremendous potential for use in industrial applications. Standard column chromatography, size exclusion chromatography and ion exchange chromatography are principally utilized techniques for purification of xylanases. Dean et al. (1991) reported that low molecular weight of xylanases has rendered their successful segregation from other proteins utilizing ultra filtration technique. Widjaja et al. (2009) purified cellulase-free xylanase from *Aspergillus niger* and *Trichoderma reesei* using ion exchange chromatography. The effective utilization of xylanase for the treatment of pulp fibres demands cellulase free xylanase. Goulart et al. (2005) successfully produced cellulase-free xylanase by utilizing *Rhizopus stolonifer* cultured on wheat bran. This cellulase-free xylanase exhibited optimum activity at pH 6.0 and temperature 45 °C, respectively. For maximal xylanase activity, pH 5.5 and temperature 60 °C were documented as most appropriate conditions by Huang and Penner (1991). Coelho and Carmona (2003) documented that xylanases are significantly thermostable within the pH range 4.5–10.5. Camacho and Aguilar (2003) documented a molecular weight of 22 kDa for xylanase from *Aspergillus* sp. Sardar et al. (2000) reported a molecular weight of 24 kDa for purified xylanase upon SDS-PAGE. Yasinok et al. (2010) reported the 186-fold purification of xylanase from *Bacillus pumilus* SB-M13A by hydrophobic interaction.

19.8 Xylanase Immobilization

Pioneer immobilization reaction was executed for introduction of reactive groups onto inert glass surface so as to increase the accessible surface area for immobilization. Therefore activation of glass beads was undertaken. Free xylanase exhibited optimum activity at pH 5.0 and 35 °C temperature. Crude enzyme was immobilized onto glass beads by physical adsorption binding. Immobilized enzyme can be reused two to three times under assay conditions. The free and immobilized xylanase activity was assayed at different pH of buffer (0.1 M) ranging from 4.0 to 8.0 and at various temperatures (35–65 °C) to determine the optimum activity under reaction conditions. The immobilized xylanase was tested for its reusability using 1 g of immobilized support repeatedly up to four times and percent relative activity determined (Kumar et al. 2014).

19.9 Industrial Aspects and Applications of Xylanases

Xylans and xylanases have witnessed significant rise in their biotechnological values and have shown remarkable rise in their use. The end products of xylan degradation, furfural and xylitol have gained remarkable utility in industrial applications (Parajo et al. 1998). The industrial utilization of xylanase commenced in the 1980s. Initially xylanases were used in animal feed preparation. In the successive years, they were significantly utilized in the food, textile and paper industries, respectively. Xylanases are predominantly employed in food industry to quicken the baking process of cookies, crackers, cakes as well as various other foods since they depolymerize the polysaccharides in the dough into corresponding monomeric units.

19.9.1 Bioprocessing of Fibres

Modern research centres on substituting the hazardous chemicals with the commercial enzymes that can precisely act upon the non-cellulosic and hemicellulosic impurities are still sustaining the quality as well as upholding the production yields of textile industries (Dhiman et al. 2008). Treatment with enzyme can apparently strengthen the water soaking characteristics of fibres by eradicating the complex impurities present in the primary cell wall. (Saha 2000) reported that utilization of pure and thermostable xylanase for pretreatment of low quality jute fibers for selectively removing xylan is entrancing. Plant fibres such as linen can be effectively processed by utlizing the xylanolytic enzyme complex. This method has an advantage that the step involving usage of strong bleaching step is bypassed as lignin won't face oxidation which would have darkened the fibres (Csiszar et al. 2001). Xylanase precisely acts upon the hemicellulosic impurities and effectively eradicates them. Such enzymatic treatment do not create any harm to the fibre in terms of loss in fibre strength (Dhiman et al. 2008).

19.9.2 Biobleaching of Pulp and Paper

Treatment of the paper pulp with xylanase effectively hydrolyses the hemicellulosic chain amidst cellulose and lignin thereby eliminating the loosely held lignin from the desired cellulose. Xylanases have turned out to be a valuable as well as cost-effective asset for mills to have an edge over a vast number of bleaching benefits (Bajpai 2012). In this context, it lessens the discharge of organochlorine pollutants, for instance, dioxin, ultimately resulting in chlorineless bleaching without posing any detrimental harm to the paper's strength (Li and Hardin 1998). The major utilization of xylanases in commercial sector is in cellulose pulp bleaching. The usage of enzymes commenced in this context ever since peroxidases were employed for

degrading the lignin (Sandrim et al. 2005). Dhillon and Khanna (2000) reported chemical process is preferred over enzymatic hydrolysis method for paper production in several countries, including Brazil. The routinely used method is popularly called as the kraft process. The paper production process involves chemical pulping as the pioneer step which is characterized by the breakdown of fibres and removal of majority of lignin fraction (Hong et al. 1989). Pulp bleaching can be represented as a purification process which involves the attributes such as destruction as well as the alteration or solubilization of the coloured organic matters, lignin and other inadmissible leftovers on the fibres (Madlala et al. 2001). The efficacy as well as efficiency of microbial xylanase in context of bleaching process has been well studied for *Streptomyces galbus* (Kansoh and Nagieb (2004), *Bacillus pumilus* (Duarte et al. 2003), etc. The optimum pH of bacterial xylanases in common is somewhat uplifted compared to optimum pH of fungal xylanases (Khasin et al. 1993), which is a desirable asset in majority of paper and pulp industries.

19.9.3 Significant Role in Improving the Animal Feed

Xylanase is well recognized to play a significant role in uplifting the quality of animal feed. Xylanase treatment lessens the viscosity of the fodder thereby rendering the fodder readily digestible by the animal gut. It significantly elevates propagation of the pancreatic enzymes into the food thereby boosting the overall absorption of the nutrients. Gilbert and Hazlewood (1993) documented utility of xylanase in enhancing the digestibility of ruminant feeds and also in speeding up the composting process. Xylanases have been well documented for their utilization in animal feed in association with cellulases, amylases, galactosidases, glucanases, lipases, pectinases, proteases and phytases. Twomey et al. (2003) reported that these enzymes depolymerize arabinoxylans present in the constituents of the feed thereby rendering the raw material less viscous. Young fowl and swine synthesize endogenous enzymes in comparatively lesser amount as compared to adults, so that food supplements loaded with exogenous enzymes should uplift their performance as livestock. Furthermore, such diet has been found to cut down the undesirable leftovers in the excreta (nitrogen, zinc, copper and phosphorus), an effect that might play a productive role in scaling down of the environmental contamination (Polizeli et al. 2005). Addition of xylanase to feed comprising of low viscosity foods like maize and sorghum may enhance the digestion and absorption of nutrients in the foremost part of the digestive tract eventually resulting in an improved use of energy (Van Paridon et al. 1992).

19.9.4 Pharmaceutical and Chemical Applications of Xylanase

The end product of xylan hydrolysis, xylitol a polyalcohol, is as an artificial sweetener that is significantly used in candies, chewing gums and several other food items (Parajo et al. 1998). Xylanases are sometimes added as a cocktail (mixture) of enzymes comprising of proteases, hemicellulases and various other enzymes as a dietary supplement or as a measure to cure weak digestion. Xylitol being a noncarcinogenic sweetener is highly suited for individuals suffering with diabetes and obesity. Xylitol is also recommended in cases such as lipid metabolism disorder and respiratory infections, for the prevention of osteoporosis as well as for persons suffering with kidney and parental lesions. A vast variety of commercially available products such as candies, chewing gums and several other food products are known to have xylitol as the artificial sweetener. The advancements in xylitol production technology have facilitated a way for its utilization in a broad sense in the food, odontological and pharmaceutical (Nigam and Singh 1995). The production of ecofriendly biological fuels like bioethanol is witnessing a significant hike as the other available energy sources are depleting continuously. Moreover most of the fuels currently in use generate high levels of toxic aerosols and other polutants that pose several health hazards. The products of xylan hydrolysis can be efficiently transformed into important biological fuels such as ethanol (Sun and Cheng 2002).

19.9.5 Applications in Recycling of Waste Paper

Xylanases have also proved their utility in recycling of waste paper. The recycling is principally accomplished via two-staged processes, i.e. pulping and beating. Stage one essentially consists of the sundering of fibre or fibre dissemination. The entire exercise is called as hydrating process. The foremost step in enzymatic treatment primarily consists of prefatory soaking of paper followed by enzyme incubation. However stage two essentially consists of mechanical shearing of pulp, subsequent heating of pulp with a purpose of disaggregation of fibres and deactivating the enzyme. Xylanase treatment accounts for dislodging numerous reducing sugars from waste paper pulp. The release of reducing sugars is directly correlated to temperature probably on grounds that elevated temperature hydrolyses the majority of xylans held amidst the pulp fibres. Enzymatic treatment eventually promotes the swelling of pulp fibres, which aids in further processing of the pulp material as well as significantly upgrades its physical properties (Kenealy and Jeffries 2003).

19.9.6 Applications of Xylanases in Food, Bread and Drinks Production

The industrial applications of xylanases have witnessed a significant rise in last few decades credited to their potent efficacy in bread making process (Butt et al. 2008). The usage of starch- as well as non-starch-hydrolysing enzymes is a common practice in bread making industry for uplifting the quality and texture of bread (Javier et al. 2007). Like other hemicellulases, xylanases act in a similar manner thereby depolymerizing the hemicellulose existent in wheat flour thus assisting in uniform circulation of water thereby rendering the dough more softer as well as easy to knead. Xylanases assist in delaying the crumbing process during bread baking process thereby letting the dough to grow (Polizeli et al. 2005). Xylanase utilization in baking industry has significantly aided in a significant rise in bread volumes, better absorption of water as well as enhanced resistance to fermentation (Camacho and Aguilar 2003). Butt et al. (2008) reported that xylanases effectively transform the hemicelluloses that are water insoluble into a soluble form that actively binds to the water in dough thereby reducing the firmness in dough, enhances the volume and generates finer crumbs with increased uniformity. Various enzymes like xylanases, cellulases and proteases enhance the firmness of the gluten network thereby uplifting the worthiness of bakery products (Gray and Bemiller 2003). Xylanases are highly commended for use in biscuit industry with a purpose to make cream crackers lighter as well as to uplift the texture, uniformity and palatability of the wafers. The major desirable aspects of xylanases in food industry are endurance and ability to show optimum activity in acidic pH range. The advancements in molecular tools and techniques have paved the way for more and more uses of xylanases. Xylanases play a significant role in beer production process. They effectively depolymerize arabinoxylans to lower xylooligosaccharides thereby rendering the beer less viscous thus eliminating its muddy appearance to significant levels (Dervilly et al. 2002).

19.9.7 Other Important Applications of Xylanases

Xylanases along with other hydrolases can be efficiently employed for the synthesis of important biofuels like ethanol by utilizing lignocellulosic biomass (Ahring et al. 1999). Xylanase in association with pectinase, amylase and carboxymethylcellulase can be efficiently utilized for clarification of juices. Xylanases may also be employed to enhance the extraction of coffee, plant oils and starches. Xylanases may also be successfully capitalized for boosting the nutritional aspects of agricultural silage and grain feed (Malathi and Devegowda 2001). Xylanases also have significant use in rye baking wherein the addition of xylanase prompts the dough to become more soft and sloppy (Harbak and Thygesen 2002).

19.10 Future Prospects

The industrial importance of xylanase is well established. Amongst different hydrolytic enzymes, xylanase has attained widespread commercial importance credited to its enormous potential applications in food, in feed and in pharmaceutical industries. The surplus availability of hemicellulosic biomass especially xylan becomes a major factor in xylanase production by various microorganisms. Fungal xylanases from *Aspergillus* species and *Trichoderma* species have been widely studied and characterized and are commercially utilized in bakery and food processing industries. Xylanase production economics is governed by several key factors that include accessibility of substrate and rate and extent of disentangling of the xylooligosaccharides besides several other decisive factors including inoculum size, pH, temperature, inducers, medium additives, aeration, activators and inhibitors. Submerged fermentation (SMF) system is the most promising technique used worldwide for xylanase production. This is credited to ease of control over various key process parameters, to the higher yield of enzyme (about 90%) and because of being cost-effective as compared to solid-state fermentation. The present review focuses on the various microbial sources for novel xylanase production, the range of available substrates that can be successfully utilized to meet the industrial demands of xylanases and the widespread industrial applications of microbial xylanases.

The prospects of xylan hydrolysis by xylanase from fungal species such as *Aspergillus* and *Trichoderma* and bacterial species like *Bacillus* sp. look quite promising. Thus future studies to increase the xylan hydrolysis rate as well as to assure enhanced process control for increased yield of xylanase would be envisaged.

In conclusion, xylanase is an industrially important enzyme that is loaded with huge potentials for use in commercial sector in various processes such as processing of pulp and fibres; saccharification of agricultural, industrial and municipal wastes; flour improvement for bakery products; manufacturing of several food products; and enhanced bleaching of cellulose pulps that is mainly used in food and pharmaceutical industry. Since the range of applications of this enzyme is very broad, so there is always a scope for novel xylanase with better and improved characteristics, which may be utilized for various industrial applications.

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