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Xylanases current and future perspectives: a review

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ABSTRACT

Xylanase (EC 3.2.1.8, endo-(1-4)-β-xylan 4-xylanohydrolase, endo-1,4-xylanase, endo-1,4- β-xylanase, β-1,4-xylanase, endo-1,4-β-D-xylanase, 1,4- β-xylan xylanohydrolase, β-xylanase, β-1,4-xylan xylanohydrolase, β-D-xylanase) is the name given to a class of enzymes which degrade the linear polysaccharide beta-1,4-xylan into xylose, thus breaking down hemicellulose, one of the major components of plant cell walls. It plays a major role in micro-organisms thriving on plant sources for the degradation of plant matter into usable nutrients. Xylanases are produced by fungi, bacteria, yeast, marine algae, protozoans, snails, crustaceans, insect, seeds, etc. (mammals do not produce xylanases). Commercial applications for xylanase include the chlorine-free bleaching of wood pulp prior to the papermaking process, and the increased digestibility of silage (in this aspect, it is also used for fermentative composting). Apart from its use in the pulp and paper industry, xylanases are also used as food additives to poultry, in wheat flour for improving dough handling and quality of baked products, for the extraction of coffee, plant oils, and starch, in the improvement of nutritional properties of agricultural silage and grain feed, and in combination with pectinase and cellulase for clarification of fruit juices and degumming of plant fiber sources such as flax, hemp, jute, and ramie.

Key words: xylanase, mode of action, fruit juice clarification, biobleaching.

INTRODUCTION

Xylanases, a group of hydrolytic enzymes, catalyze the hydrolysis of xylan which are genetically single chain glycoproteins, 6–80 kDa, active between 40 to 60˚C (Butt et al. 2008). The complete enzymatic hydrolysis of xylan into its constituent monosaccharides requires the synergistic action of a consortium of xylanolytic enzymes. This is due to the fact that xylans from different sources exhibit a significant variation in composition and structure (Latif et al. 2006).

Xylanases are considered to be able to effectively hydrolyze xylan, the principal type of hemicellulose containing a linear polymer of β-D-xylopyranosyl units linked by (1-4) glycoside bonds. Microbial enzymes act cooperatively to convert xylan to its constituent simple sugars. These enzymes include β-1,4-endoxylanases (xylanases EC 3.2.1.8), cleave internal glycosidic bonds within the xylan backbone; arabinofuranosidase (EC 3.2.1.55), hydrolyzes arabinose side chains; R-glucuronidase (EC 3.2.1.131), removes glucuronic acid side chains from xylose units; xylan esterases (EC 3.1.1.6), release acetate groups and finally xylosidase (EC 3.2.1.37), hydrolyzes xylobiose to xylose. There is a considerable degree of synergy among these

enzymes. Many xylanases do not cleave glycosidic bonds between xylose units that are substituted. Thus, side chains must be cleaved before the xylan backbone is completely hydrolyzed. Conversely, several accessory enzymes will only remove side chains from xylooligosaccharides and therefore require xylanases to partially hydrolyze the plant structural polysaccharide, before side chains can be cleaved. These enzymes are potentially usefull in the biodegradation of lignocellulosic biomass to fuels and chemicals, in improving rumen digestion and for use in the prebleaching of kraft pulp, mainly because of a desire to move away the use of chlorine as a bleaching agent (Al-Bari et al. 2007). Xylans do not form tightly packed structures and are easily accessible to hydrolytic enzymes. Consequently, the specific activity of xylanase is two to three times greater than the hydrolases of other polymers like crystalline cellulose. In the pulping process, the resultant pulp has a characteristic brown colour owing to the presence of residual lignin and its derivatives. The intensity of pulp colour is a function of the amount and chemical state of remaining lignin. In order to obtain white and bright pulp suitable for manufacturing good quality papers, it is necessary to bleach the pulp to remove the constituents such as lignin and its degradation products. Biobleaching of pulp is reported to be more effective with xylanases than with lignin degrading enzymes. This is because lignin is crosslinked mostly to the hemicellulose which is more readily depolymerised than lignin.

Classification of xylanases

The heterogeneity and complexity of xylan has resulted in abundance of diverse xylanases with varying specificities, primary sequences and folds, hence led to limitations with classification of these enzymes by substrate specificity alone. Wong et al. (1988) classified xylanases into two groups on the basis of their physicochemical properties: (*i*) having low molecular mass (<30 kDa) and basic pI, and (*ii*) having high molecular mass (>30 kDa) and acidic pI. However, many xylanases, in particular fungal xylanases, cannot be classified by this system. A more complete classification system has been introduced which allows the classification of not only xylanases, but also of glycosidases in general. This system has now become the standard means for the classification of these enzymes and is based on primary structure comparison of the catalytic domains only and classifies the enzymes in families of related sequences (Henrissat and Coutinho 2001). The initial classification grouped cellulases and xylanases into 6 families (A–F), which was updated to 77 families in 1999 (1–77) and still continues to grow as new glycosidase sequences are being identified. Enzymes within a particular family have similar three-dimensional structure and molecular mechanism and it has also been suggested that they may have a similar specificity of action on small, soluble, synthetic substrates (Henrissat and Coutinho 2001). Furthermore, divergent evolution has resulted in some of the families having related three-dimensional structures and thus the grouping of families into higher hierarchical levels, known as clans, has been introduced (Collins et al. 2005). Presently, 14 different clans have been proposed (GH-A to GH-N), most clans are composed of two to three families, apart from clan GH-A which currently encompasses 17 families. Within this classification system, xylanases are normally reported as being confined to families 10 and 11 which were formerly known as class F and G respetively (Collins et al. 2005).

The xylan degrading system include endo-1, 4-xylanases (1,4-β-xylan xylanohydrolase; EC 3.2.1.8), which release long and short xylooligosaccharides, or those that only attack longer chains and β-D-xylosidase (1, 4-β-xylan xylohydrolase; EC 3.2.1.3.7), which remove D-xylose residues from short xylo-oligosaccharides (Saha 2003). Enzymes such as β-arabinosidase, β-glucuronidase, ferulic acid esterase and acetyl xylan esterase are very important for the removal of side chain groups from xylan, especially when the fragments of the cleaved heteroxylans are present in high proportions of branched substituents (Aro et al. 2005).

Virtually all xylanases are "endo" acting, as readily determined by chromatography but the more detailed determination of kinetic properties, measuring the relative reaction rates on various substrates and determining the kinetics of intermediate product formation is much less common. Classifications based on molecular weight and pI are necessarily related to those based on sequence and sequence analysis can reliably predict crystal structure, but few studies have been performed that relate sequence or structural family to action patterns and substrate specificity. Even fewer studies have explored the specificity of hemicellulases with respect to branching patterns or substitution. Family 10 xylanases occasionally exhibit endocellulase activity; they generally have a higher molecular weight, and they occasionally will possess a cellulose binding domain. Even though all xylanases are endo acting, they can show variations in their product profiles. Some enzymes form predominantly xylose and xylobiose and others predominantly (or exclusively) form xylotriose and other higher oligosaccharide products. This difference appears to result from the number of substrate-binding subsites on the enzyme surface. The number of pyranose rings that the enzyme will bind effectively determines the nature of the oligoproducts. The family 10 catalytic domain is a cylindrical [α]-barrel resembling a salad bowl with the catalytic site at the narrower end, near the C-terminus of the [β]-barrel. There

are five xylopyranose binding sites. Catalytic domains of these enzymes belong to a "super family" that includes Family A cellulases, [β]-glucosidase, [β]-galactosidase, [β]-(1-3)-glucanases and [β]-(1-3, 1-4)-glucanases. Family 10 xylanases have relatively high molecular weights, and they tend to form oligosaccharides with a low degree of polymerization. Family 11 xylanases are considered true cellulase-free xylanases, which consistently exhibit a low molecular weight, and have either a high or low pI. They are produced by both bacteria and fungi. The positions of many amino acids are essentially identical in the family 11 xylanases from bacteria (*Bacillus circulans*).

Mode of action of xylanases

Several models have been proposed to explain the mechanism of xylanase action. Xylanase activity leads to the hydrolysis of xylan that may result either in the retention or inversion of the anomeric centre of the reducing sugar monomer of the carbohydrate and suggests the involvement of one or two chemical transition states (Subramaniyan and Prema 2002). Glycosyl transfer usually results in nucleophilic substitution at the saturated carbon of the anomeric centre and takes place with either retention or inversion of the anomeric configuration. Most of the polysaccharide hydrolyzing enzymes like cellulases and xylanases are known to hydrolyse their substrates with the retention of the C1 anomeric configuration. There is the involvement of double displacement mechanism for the anomeric retention of product (Rye and Withers 2000; Collins et al. 2005). Based on the crystallographic study of xylopentaose binding to *Pseudomonas fluorescens,* Leggio et al. (2000) have proposed a most suitable enzyme mechanism which combine the classical concepts listed above and facts derived from their study. According to them xylanase recognises xylan as a left-handed three fold helix, the xylosyl residue at subsite-1 is distorted and pulled toward the catalytic residues, and the glycosidic bond is strained and split to form the enzyme-substrate covalent intermediate, which is attacked by an activated water molecule, retaining glycosyl hydrolase mechanism and release the product (Subramaniyan and Prema 2002). There are several reports regarding the hydrolytic pattern of xylanases from *Bacillus* sp. and most of them are mainly releasing xylobiose, xylotriose and xylotetrose while formation of xylose occurred only during prolonged incubation. Xylanases A and B from *Trichoderma reesei* and C and D from *Trichoderma harzianum* under different combinations showed synergistic interactions on different xylan substrates. Xylanase were more effective in combinations than single xylanase for hydrolysing pine holocellulose (Wong and Maringer, 1999). Xylanase II of *Bacillus circulans* WL-12 (pI 9.1) hydrolysed xylan principally to xylobiose, xylotriose and xylotetrose. This enzyme was shown to be requiring a minimum of four xylopyranoside residues to form the productive complex, thus xylotetrose out of other substrates tried was the most preferred substrate to saturate all binding sites of the enzyme. But the Xylanase I from the same source degraded xylan rapidly to xylotetraose and prolonged incubation resulted in xylose, xylobiose and xylotriose as the main end products (Subramaniyan and Prema 2002). Sugars like xylose, xylobiose and xylo-oligomers can be prepared by the enzymatic hydrolysis of xylan. Bioconversion of lignocelluloses to fermentable sugars has a great economic prospect. The depolymerization action of endo-1, 4-xylanase (EC 3.2.1.8) results in the conversion of the polymeric substance into xylo-oligosaccharides and xylosidases. Lignin is bound to xylans by an ester linkage (Himmel et al. 1999).

Sources of xylanases

Xylanases are produced by diverse genera and species of bacteria, actinomycetes and fungi. While several *Bacillus* species secrete high levels of extracellular xylanase, filamentous fungi secrete high amounts of extracellular proteins where xylanase secretion often accompanies cellulolytic enzymes for example as in species of *Trichoderma*, *Penicillium*, and *Aspergillus* (Polizeli et al. 2005). To use xylanase enzymes for pulp treatment, it is preferable not to have any accompanying cellulolytic activity, since the cellulase may adversely affect the quality of the paper pulp.

Microbial xylanase production has been extensively reviewed by several groups as these enzymes are produced by a diverse variety of microorganisms (Shah et al. 1999; Knob and Carmona 2008; Yang et al. 1995; Velkova et al. 2007; Sharma and Chand 2012a). Many of the bacteria and fungi are reported to produce xylanases. Several microorganisms, including fungi and bacteria have been reported to readily hydrolyze xylans by synthesizing 1,4-β-D-endoxylanases and β-xylosidases.

Since the introduction of xylanases in the paper-pulp and food industries, there have been many reports on xylanases from bacterial and fungal microflora. Characterization of xylanolytic enzymes is important for its biotechnological application. The cost of enzyme is one of the main factors determining the economics and viability of a process which can be partially achieved by optimizing fermentation medium. Several industrial processes are carried out using whole cells as the source of enzymes but the efficiency can be improved by using isolated and purified enzymes. Criteria for selection of particular method for isolation and purification depend on its end use. A high state purity is generally not required in food processing, detergent as well as paper pulp

industry; however it may be necessary to exclude certain contaminating enzymes. A number of xylanase producing microorganisms have been isolated and characterized.

Xylanolytic Bacteria

Yang et al. (1995) reported that an alkaliphilic *Bacillus* sp. isolated from kraft pulp produced 49.0 IU/ml of xylanase when cultivated in alkaline medium with 2.0% birchwood xylan and 1.0% corn steep liquor at pH 9.0. Kapoor et al. (2008) optimized the cultural conditions (pH 9.0, agitation 200 rpm, inoculum size 1.25% and inoculum age 2 h) for maximum xylanase production by an alkaliphilic *Bacillus pumilus* strain MK001. The bacterium secretes high levels of xylanase on agricultural residues (wheat bran 1220 IU/ml; wheat straw 900 IU/ml) as well as on synthetic xylans (birch wood xylan 1190 IU/ml; oat spelt xylan 1150 IU/ml). Xylanases have expanded their use in many processing industries, such as pulp and paper, food and textile (Knob and Carmona 2008). Xylanases, widely detected in bacteria have been well characterized in terms of their properties and mode of action towards xylan. Balakrishnan et al. (1993) have reported an alkalophilic *Bacillus* strain (NCL 87-6-10) which produced cellulase free xylanases in a commercially viable medium containing wheat bran and organic nitrogen.

Shah et al. (1999) isolated an alkalophilic strain of *Bacillus* Sam-3, producing 131U/ml of cellulase free xylanase at pH and temperature 8.0 and 60 °C respectively. *Bacillus circulans* AB-16 was reported to produce cellulase free xylanase, when grown on untreated wheat straw. The pH optimum of the crude enzyme was 6.0-7.0 with a temperature optimum of 80 °C. The enzyme showed high pH and thermal stability retaining 100% residual activity at 60 °C, pH 8.0 and 9.0 even after 2.5 h of incubation (Dhillon et al. 2000). Bataillon et al. (2000) have reported xylanase from a strain of *Bacillus* sp. strain SPS-0, isolated from a hot spring in Portugal. The enzyme was purified to homogeneity and the optimum temperature and pH for activity were 75 °C and 6.0 respectively. Xylanase was stable up to 70 °C for 4 h at pH 6.0. *Bacillus megaterium* B6 ATCC 51946, a potent producer of β-amylase, produced extracellular xylanase when grown in the presence of xylan as sole carbon source. The strain showed maximum xylanolytic activity after 12 hour of incubation and enzyme was stable in the pH range 7.0-9.0 for 7 min at 85 °C and for 20 min at 50 °C (Ray et al. 1997).

An alkalophilic *Bacillus* SSP-34 was reported to produce more than 600 IU/ml of xylanase with a specific activity of 200 IU/ml (Subramaniyam 2000). Rani and Nand (2000) while studying the cellulase free thermostable xylanase production from *Clostridium absonum* CFR-702 in a defined medium (anaerobic cultivation, 65 ºC) reported the optimum activity at 72-96 h, pH 8.5 and cultivation temperature of 75-80 ºC. Aerobic bacteria and fungi isolated from *Ziziphus mauritiana*, *Sclerocarya birrea* fruits and cattle compost from Zimbabwe, released maximum endo-xylanase at pH 8.0 (stable over a pH range of 6.0-9.0) and a temperature 60°C (Chivero et al. 2001).

Xylanolytic fungi

Melanocarpus albomyces, a thermophilic fungus isolated from compost by enrichment culture in a liquid medium containing sugarcane bagasse, produced cellulase free xylanases which released xylobiose, xylotriose and higher oligomers from different sources (Prabhu et al. 1999). *Trichoderma reesei* LM-UC4E1, *Aspergillus niger* ATCC 10864, *A. phoenicis* QM 329 have also been cultured in solid substrate fermentation on sugarcane bagasse for xylanase production (Gutierrez et al. 1998). Xylanolytic fungal strain *Aspergillus niger*, *A. flavipes* (Mukhopadhyay et al. 1997) and several others have been reported to produce quite high levels of xylanases but they also co-secrete cellulases. Although filamentous fungi such as *Trichoderma, Penicillium* and *Aspergillus* are known to produce high levels of xylanases, they normally also co-secrete cellulases. For better application in pulp biotechnology, it is preferable that the xylanase preparations are free of cellulase. Pioneering research work in the area of cellulase free xylanases in India has been carried out at National Chemical Laboratory (NCL), Pune and thereafter lot of work in this direction has been done at Central Pulp and Paper Research Institute (CPPRI), Saharanpur and Thapar Institute, Patiala.

Muthezhilan et al. (2007) have reported xylanase production from *Penicillium oxalicum* and optimized production in solid state fermentation using cheaper sources like wheat bran, rice bran, rice straw, sesame oil cake and wood husk. Maximum enzyme activity was observed in wheat bran representing 3.89 U/ml with pH optima of 8.0 and temperature 45 °C. To produce an enzyme preparation useful for industrial applications requiring high temperature, the region encoding the CBM was deleted from the *N. flexuosa xyn11A* gene and the truncated gene was expressed in *Trichoderma reesei* (Paloheimo et al. 2007). A feasibility study of the enzyme treatment on different types of pulps using a commercial cellulase free xylanase enzyme carried out at ABC paper mills in collaboration with Department of Biotechnology, H.P. University showed that enzyme pretreatment resulted in approximately 20% and 15% reduction in terms of chlorine and alkaline consumption respectively under optimized reaction conditions in subsequent steps of bleaching (Chatterjee et al. 2002).

Thermophilic xylanases

Xylan, which is the dominating component of hemicelluloses, is one of the most abundant organic substances on earth and has a great application in the pulp and paper industry. The wood used for the production of the pulp is treated at high temperature and basic pH, which implies that the enzymatic procedures require proteins exhibiting a high thermostability and activity in a broad pH range (Jacques et al. 2000). Treatment with xylanase at elevated temperatures disrupts the cell wall structure. This, as a result, facilitates lignin removal in the various stages of bleaching.

Xylanases for such a purpose (1) must lack cellulolytic activity to avoid hydrolysis of the cellulose fibres, (2) need to be of low molecular mass to facilitate their diffusion in the pulp fibres, and (3) most importantly, high yields of enzyme must be obtained at a very low cost (Niehaus et al. 1999). According to these authors all commercially available xylanases can only partially fulfill these requirements of the optimum temperature for the activity of most xylanases is reported to be 50–60 ˚C with a half-life of about 1 h at 55˚C (Jacques et al. 2000). However, some xylanases have been reported to exhibit higher thermal stability and optimal activity ranging from 80 to 100 ˚C (Morris et al. 1998).

Production, purification and characterization of xylanases

The choice of an appropriate substrate and nutritive media composition is of great importance for the successful production of xylanases. The substrate not only serves as carbon and energy source, but also provides the necessary inducing compounds for the enzyme production (Velkova et al. 2007). The use of purified xylan as a substrate to induce xylanase synthesis increases the cost of enzyme production. Therefore for commercial applications, there have been attempts to develop a bioprocess to produce xylanase in high quantities from simple and inexpensive substrates. Among all agro industrial material hydrolyzing enzymes, xylanases gained importance due to their role in production of xylose based products of industrial importance (Laxmi et al. 2008; Sharma and Chand 2012b).

The extracellular microbial enzymes often contain some level of impurities in their native form. Therefore, purification of enzymes is of great importance for their molecular characterization and applications. The purity of enzymes is one of the most important aspects for their specific application.

Xylanase purification schemes have generally used standard column chromatographic techniques. Ion exchange and size exclusion are the generally utilized schemes for xylanase purification, but there are also reports of purification with hydrophobic interaction column chromatography. The low molecular weight of certain xylanases has also enabled their separation from other proteins using ultra filtration. There are several reports regarding the purification of xylanases to electrophoretic homogeneity, however, the yield and purification fold varies in different cases (Sharma and Chand, 2012a). In all cases the crude enzyme was initially concentrated using precipitation or ultra-filtration techniques. A moderately thermostable xylanase was purified from *Bacillus* sp. strain SPS-0 using ion-exchange, gel and affinity chromatography (Bataillon et al. 2000). Purification of two proteins from culture supernatant of *Bacillus circulans* AB 16 through ion-exchange and gel filtration yielded 40% enzyme recovery while purification in other *Bacillus* sp. yielded percentage recovery of 14% and 28% (Dhillon and Khanna 2000). For potential applications on pulp fibres, the purification of xylanases is considered to be incomplete as long as cellulase activity is totally eliminated. .

Sanghvi et al. (2010) partially purified xylanases from *Trichoderma harzianum,* concentrated from 30 to 80% ammonium sulfate saturation with 62% and 67% yield. The fraction was subjected to SDS–PAGE and native PAGE analysis. It was found that xylanase has a homogenous nature in band pattern (mw 29 kDa). Kulkarni et al. (1999) reported that microbial xylanase are single subunit proteins within a range of 8–145 kDa. Gupta et al. (2009) reported a xylanase from *Fusarium solani* F7 of 89 kDa. Anthony et al. (2005) used *A. niger* BRFM 281 in shake flask fermentation for hyper production of XynB and reported a yield of 900 mg/l. The recombinant enzyme was purified to 1.5-fold by immobilized metal affinity chromatography with enzyme recovery of 71%. During characterization of the enzyme, it was found that it had molecular weight 23 kDa, optimum pH 5.5 and optimum temperature 50 °C. The enzyme showed stability over a pH range of 4.0 to 7.0 and temperature up to 50 °C.

Applications of xylanases

Xylanases have made a remarkable impact in the world of biotechnology because of their applications in the pulp or paper, feed, food and fermentation industries. The most promising application of xylanases is pre-bleaching of Kraft pulp (Beg et al. 2001). Other applications include extraction of coffee, plant oils, and starch, improvement of nutritional properties of agricultural silage and grain feed and in combination with pectinase and cellulase, clarification of fruit juices, wine and recovery of fermentable sugars from hemicelluloses (Lama et al. 2004). Moreover, xylanases show immense potential for increasing the production of several valuable products like xylitol and ethanol in a most economical way (Beg et al. 2001). Production of xylanase from a single microorganism renders its industrial application more feasible and

economical. Filamentous fungi have been widely used to produce hydrolytic enzymes for industrial application, including xylanases, whose levels in fungi are generally much higher than those in yeast and bacteria. *Aspergillus awamori* has been used for the production of enzymes such as glucoamylase, protease and xylanase. The important advantage of the application of *Aspergillus awamori* as suitable strain is that the organism has a long history of safe use for the manufacture of food products destined for human consumption and is regarded as non toxigenic and non-pathogenic. The cost of an enzyme is one of the main factors determining the economy of a process. Reducing the costs of enzyme production by optimization of the fermentation medium and process is the goal of basic research for industrial application. With the increasing concern about the environmental hazards caused by the chemicals discharged in pulp and paper industry effluents, the potential of microbial enzymes in reducing the pollution load of the environment is being realized. Xylan degrading enzymes have wide industrial application, either alone or with association of other enzymes. Xylanases show great potential mainly for the bioconversion of lignocelluloses to sugar, ethanol and other useful substances, clarification of juices and wines, improving the nutritional quality of silage and green feed and the de-inking processes of waste papers (Kheng and Omar 2005).

Xylanases in paper and pulp industry

Conversion of wood into paper pulp via the Kraft process accounts for the vast majority of the paper production worldwide. Paper pulp produced by this process contains residual lignin and lignin derivatives entrapped in the pulp matrix by xylan. Bleaching is required in the kraft process to remove the residual lignin and brighten the pulp. Present day bleaching of kraft pulp uses large amounts of chlorine and chloride chemicals (Angayarkanni et al. 2006). Due to environmental pressure and market forces demanding low adsorbable organic halide (AOX) pulp, a pulp mill is investigating options to reduce chlorine and chlorine dioxide consumption in its bleach plant. One option now being used is the use of xylanase enzyme as a pre-bleaching chemical. Work has shown that enzymes can be used in the treatment of unbleached pulp for delignification and brightening. Biotechnology has the potential to increase the quality and supply of feed stocks for pulp and paper, reduce manufacturing costs and create novel high-value products. Novel enzyme technologies can reduce environmental problems and alter fibre properties. Because the pulp and paper industry is capital-intensive with facilities specific to the tasks, new technology must either reduce expenses or fit easily into the existing process design. Pulp and paper companies are reluctant to build or expand plants when the overall industry has enough capacity to satisfy market demands. Although xylanases have been widely used, their application in the paper, pulp and feed industries is limited somewhat by certain enzymatic characteristics such as poor resistance to high temperature and narrow optimal pH range. Therefore, it is essential to improve the properties of xylanases for broader applications (Yang et al. 2007). The idea of enzyme bleaching first emerged in 1984 when xylanase was used as a trial enzyme. The pulp and paper industry is applying new, ecologically sound technology in its manufacturing processes. Many interesting enzymatic applications have been proposed in the literature. Implemented technologies tend to change the existing industrial process as little as possible. Commercial applications include xylanases in prebleaching kraft pulps and various enzymes in recycling paper (Angayarkanni et al. 2006). In the future, value-added products could be built around enzyme processes.

Xylanases have saved chemical costs for the industry without interfering with the existing process. This technology has increased the bleaching speed in both TCF (Total chlorine free) and ECF (Elemental chlorine free) processes (in the case of chlorine dioxide bleaching, has actually increased the throughput of the plant due to debottlenecking at the chlorine dioxide generator). The use of xylanases for prebleaching kraft pulp has been one of the greatest success stories of enzymes in the pulp and paper industry. Enzyme use helps to solve some of the environmental concerns associated with the use of chlorine in bleaching. The xylanase attacks hemicellulose and alters the interface between the cellulose and lignin, thereby facilitating the removal of lignin-associated hemicellulose fraction with minimal damage to the pulp. This process is less expensive, less drastic and especially less toxic than conventional chemical treatment. Xylanase treatment can improve lignin extraction, alter carbohydrate and lignin associations, or cleave redeposited xylan (Beg et al. 2001). It also reduces the cost of this application as installation of expensive oxygen delignification facilities may be avoided. Besides bleaching through lignin removal, the use of xylanases helps increase pulp fibrillation, reduce beating times in the original pulp and increase the freeness in recycled fibres.

Azeri et al. (2010) tested crude xylanase dose of three strains (Ag12, Ag20 and Ag32) of *Bacillus* sp. for biobleaching of kraft pulp at 60ºC at pH 9.0, pulp consistency of 5.0% and observed optimum enzyme dose 10.0 IU/g OD.

The treated and untreated pulps applied (prior to EDTA and peroxide treatment) by Ag12, Ag20 and Ag32 reduced the kappa number by 7.04 (27.4%), 3.72 (61.7%) and 2.39 (75.3%), increased the brightness by 80 (1.0%), 80.5 (1.5%) and 82 (3.0%) ISO units, respectively.

Damiano et al. (2003) used xylanase from alkaliphilic *Bacillus licheniformis* 77-2 at a dose of 14.0 IU/g dry of eucalyptus kraft pulp at pH 6.0 and 60°C for 4 h, reduced the Kappa number and resulted in an increase in brightness of the paper. Thus Kappa number and brightness, respectively 28.5 and 30% less ClO_2 was required in comparison to the enzymatically untreated samples. Zheng et al. (2000) have reported that cellulase-free xylanase from an alkaliphilic *Bacillus* sp. was maximally active at pH 10.0 and 60°C on biobleaching of ramie fibers and they have reported a brightness increment of 5.2% and boosted the effect of H_2O_2 bleaching. Angayarkanni et al. (2006) reported that xylanase enzymes of three fungi, *Aspergillus indicus, A. flavus* and *A. niveus*, increased in brightness of 42.0 – 45.0 ISO units from 19.83 ISO units and reduced kappa number of 5.0-6.8 from 18.60 after the enzyme pretreated pulp when subjected to alkali extraction process. Thus, increase of brightness and Kappa number reduction observed in this study with xylanases treated of kraft pulp are consistent with other studies.

Xylanases in fruit juice clarification

The production of fruit and vegetable juices is important both from the human health and commercial standpoints. The availability of nutritious components from fruits and vegetables to a wide range of consumers is thus facilitated throughout the year by the marketing of their juices. The production of fruit juice (Sharma and Chand 2012c) and vegetable juices requires methods for extraction, clarification and stabilization. During the early 1930s, when fruit industries began to produce juice, the yields were low and many difficulties were encountered in filtering the juice to an acceptable clarity (Uhlig 1998). Extracellular xylanase activity was produced in a submerged culture by *Sclerotinia sclerotiorum* S2 fungus, on wheat bran as an inducing substrate. The enzyme was partially purified and biochemically characterized. The novel xylanase activity was useful as an aid in orange juice clarification. The clarification was observed with concomitant production of reducing sugars after 24 h of incubation of juice with the xylanase. Xylanase aided juice clarification, which resulted in a 27% decrease in insoluble materials. Dhiman et al. (2010) produced xylanase from *Bacillus stearothermophilus* and used for juice clarification including independent variables viz. temperature, incubation time and enzyme dose to study the dependent variables such as yield, acidic neutrality and filterability etc. On the basis of the contour plots the optimum enzyme dose was 12.5IU/g of xylanase. Enzymatic treatment has resulted in the improvement of two fold in the release of reducing sugars and 52.97% in juice yield, whereas 35.34% reduction in turbidity was observed. The extracellular *Pseudomonas* sp. XPB-6 xylanse was used for apple, mosambi and orange juice clarification by sharma and Chand (2012c).

Xylanases in baking

Bread is the most common and traditional food around the world whose preparation is closely linked with enzymes. For years, enzymes such as malt and fungal α -amylases have been used in bread making. Due to the changes in baking industry and the ever-increasing demand for more natural products, enzymes have gained real importance in bread making, where they improve dough and bread quality leading to improved dough flexibility, stability, loaf volume and crumb structure (Guy and Sarabjit 2003).

The applications of xylanolytic enzymes have increased for the last few decades owing to their potential effectiveness in bread making. Starch and non-starch carbohydrate- hydrolyzing enzymes are commonly used in the bread making industry as bread improvers (Javier et al. 2007). Over recent years, the role of xylanases in bread making has been investigated intensively (De Schyver et al. 2008). Enzymatic hydrolysis of non-starch polysaccharides leads to the improvement of rheological properties of dough, bread specific volume, and crumb firmness. Xylanase transforms water-insoluble hemicellulose into soluble form, which binds water in the dough, therefore decreasing dough firmness, increasing volume and creating finer and more uniform crumbs. It significantly improves manufacturing conditions: dough is made more 'machine-friendly' as it does not stick to the machinery parts (Rouax 1993). Xylanase can directly or indirectly improve the strength of the gluten network and so improve the quality of the finished bread. Although the complete mechanism of hemicellulase, pentosanase or xylanase in breadmaking has not been clearly demonstrated, it is well known that the addition of certain types of pentosanase or xylanase at the correct dosage can improve dough machinability, yielding more flexible, easier-to-handle dough. Consequently, the dough is more elastic, stable and gives better ovenspring during baking resulting in a larger volume and improved crumb texture. Xylanases degrade the pentosans (arabinoxylans) in such a way that the thereby modified pentosans improve dough elasticity, stability and development. The addition of endoxylanases during dough processing is expected to increase the concentration of arabino xylo-oligosaccharides in bread, which has beneficial effects on human health (Poutanen 1997).

In wheat flour, xylans are mainly present as arabinoxylans which are the cell wall components. Arabinoxylans can be in both water-soluble and water-insoluble forms. Water-insoluble pentosans will hold water about 10 times of their weight in water (Mannie 2000). In order to increase the amount of water-insoluble pentosans, xylanases can

break glycosidic linkages in arabinoxylans, leading to smaller fragments of carbohydrates and therefore water is released in the dough (Al-Widyan et al. 2008).

Xylanases in animal feed

Xylanases have attracted considerable research interest because of its potential industrial applications. In animal feed, it increases the body weight gains (Medel et al. 2002). Xylanase breakdown the cell walls of cereal to improve nutrient digestibility. Xylanases increase cereal by-product usage in feed formulation to reduce feed cost. Enzymes supplementation on diets improves production efficiency of poultry by increasing the digestion of low quality products and reducing nutrient loss through excreta, allowing the reduction of diets nutritional levels with likely economic advantages. Enzymes are added to animal ration with the goal of increasing its digestibility, removing antinutritional factors, improving nutrient availability, as well as for environmental issues (Costa et al. 2008). Angelovieova et al. (2005) verified that exogenous enzymes utilization (xylanases and proteases) improved, in average, 8.41% and 2.51% the efficiency of feed utilization of a wheat-based diet in the first and second assays, respectively.

Xylanases in bioconversion of hemicellulosic wastes to ethanol

In this context, effective utilization of abundantly available agro industrial wastes for biotechnological production of value added products of different nature such as biofuels, organic solvents, microbial enzymes and other metabolites using microbial strains or biocatalysts edge over conventional chemical methodologies. All photosynthetic materials are made of simple carbohydrates, however present in the form of polymers of cellulose, hemicellulose and lignin and are entangled in complex manner. Hence, their digestion to produce simple monomeric forms requires strong alkali or acidic environment. This chemical hydrolysis method generates the effluents of xenobiotic nature and cause environmental pollution. Use of specific hydrolyzing enzymes provides a better solution. Among different hemicellulosic material hydrolyzing enzymes, xylanases are gaining importance because of their wide application in various industrial sectors especially in bioconversion of hemicellulosic waste materials to ethanol, xylitol and arabitol in addition to their application in animal feed and different biotechnological applications (Subramaniyan & Prema 2002).

Xylanases in textile industry

The use of enzymes in textile industry is one of the most rapidly growing fields in industrial enzymology. The enzymes used in the textile field are xylanases, amylases, catalases and lactases which are used to remove the starch, degrade excess hydrogen peroxide, bleach textiles and degrade lignin. As enzymes become more readily available, their application is rapidly developing in various areas of textile processing. The textile industry could greatly benefit from the expanded use of these enzymes as they are non-toxic and environmental friendly. The removal of noncellulosic compounds of bast fibers, such as lignin and hemicelluloses, can be approached using xylanases in blends with cellulases. After using this method, there is no need to use the strong bleaching step, which would lead to darkening of the fibres (Polizeli et al. 2005).

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