

INDUSTRIAL APPLICATIONS AND FUTURE PROSPECTS OF MICROBIAL XYLANASES: A REVIEW

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Microbial enzymes such as xylanases enable new technologies for industrial processes. Xylanases (xylanolytic enzyme) hydrolyze complex polysaccharides like xylan. Research during the past few decades has been dedicated to enhanced production, purification, and characterization of microbial xylanase. But for commercial applications detailed knowledge of regulatory mechanisms governing enzyme production and functioning should be required. Since application of xylanase in the commercial sector is widening, an understanding of its nature and properties for efficient and effective usage becomes crucial. Study of synergistic action of multiple forms and mechanism of action of xylanase makes it possible to use it for bio-bleaching of kraft pulp and for desizing and bio-scouring of fabrics. Results revealed that enzymatic treatment leads to the enhancement in various physical properties of the fabric and paper. This review will be helpful in determining the factors affecting xylanase production and its potential industrial applications in textile, paper, pulp, and other industries.

Keywords: Clarification; Desizing; Hemicellulose; Pulp; Paper; Xylitol; Xylanase

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INTRODUCTION

Microbial enzymes have shown tremendous potential for different applications. Over the years due to their remarkable features enzymes have occupied the centre stage of all the biochemical and industrial processes. Enzymes can carry out their myriads of biochemical reactions under ambient conditions, which makes their use eco-friendly and often the best alternative to polluting chemical technologies. Enzymatic treatment provides the same level of output as is achieved through conventional methods that use harsh chemicals. The twentieth century saw an unprecedented expansion in the field of enzyme kinetics because new fields like microbiology and biotechnology have rapidly begun to gain ground. Therefore, usage of enzymes at various industrial levels has also gained momentum.

Xylan is the most abundant renewable non-cellulosic polysaccharide present on earth. It is a major constituent of plant cell walls and constitutes around 20-30 % of the dry weight of tropical hardwood and annual plants. Studies reveal that xylan forms an interphase between lignin and other polysaccharides. It is mainly present in the secondary cell wall and covalently linked with lignin phenolic residues and other polysaccharides such as pectins and glucans.

Xylanases are the enzymes that can catabolize the xylan residues. Over the years usage of xylanase at the industrial level has increased significantly (Techapun et al. 2003; Haki and Rakshit 2003). An increasing number of reports and articles mentioning the

isolation of newer microbial species for xylanase production reveal an ever increasing interest by the scientific community in this field. Enzyme extracted from newer isolates has been used for pulp bleaching, fabric bio-processing, and for waste paper treatment sequences. A large quantity of raw material is processed in pulp and paper, textile, and in feed industries, and thus the volume of effluents released is very high. In order to deal with industrial waste and waste water, research and development departments have worked toward the establishment of strategies that are totally free from the use of hazardous chemicals and provide the same results as are achieved through conventional methods. Treatment with xylanase does not pose any environmental threat and therefore provides a glimmer of hope to environmentalists.

In this review article, an overview is presented over the current status and future prospects of the use of xylanase enzyme as an effective bio-agent at various industrial levels of processing and finishing of raw material.

XYLAN: STRUCTURE AND DISTRIBUTION

The most abundant hemicellulose present on the earth surface is xylan. Hemicellulosic material constitutes around 30-35 % of hardwood, 15-30 % of graminaceous plants, and 7-12 % of gymnosperms (Wong et al. 1988; Georis et al. 2000; Whistler and Richard 1980). Credit goes to Schulze (1981), who was the first ever to use the term “*hemicellulose*” for the fractions that he collected from plant material isolated with diluted alkali. Plant biomass, in terms of dry weight, comprises of an average of 23% lignin, 40% cellulose, and 33% hemicellulose. The main heteropolymers of the hemicellulosic component are xylan, mannan, galactans, and arabinans. D-xylose, D-mannose, D-galactose, and L-arabinose are examples of sugar moieties that are commonly attached with the heteropolymers and on the basis of which these heteropolymers are classified. Xylan molecules are mainly constituted by D-xylose as the monomeric unit, and traces of L-arabinose are also present (Bastawde 1992). Further, some substituents viz. acetyl, arabinosyl, and glucuronosyl are found on the backbone of xylan (Whistler and Richard 1980). Xylan occupies the central position in between the sheath of lignin residues and also covalently linked and intertwined with this sheath at several points. Covalent linkage of xylan with lignin sheath and intertwinedness through inter H-bonding gives an appearance of a “coat” around the cellulosic monomers (Biely 1985; Joseleau et al. 1992). Intra-chain H-bonding occurs through the 0 – 3 position, giving unsubstituted xylan a helical twist. These cellulose monomers will act as a barrier against the hydrolyzing action of cellulase enzyme (Uffen 1997).

Hardwood’s xylan molecules are made up of O-acetyl-4-O-methylglucuronoxylan and are attached through β -1,4-glycosidic bonds. Softwood xylan is made up of arabino-4-O-methylglucuroxylans. High rates of acetylation at C – 2 and C – 3 atoms of hardwood xylan make it partially soluble in water. These rates also are responsible for the easy exit of acetyl groups through alkali treatment (Sunna and Antranikian 1997). Unlike hardwood xylans, softwood xylans are not acetylated and are freely soluble in water. Further, they have α -L-arabinofuranose units linked through α -1,3-glycosidic bonds at the C – 3 position (Puls and Schuseil 1996), are shorter in length as compared to the hardwood xylan, and

also possess a lower degree of branching. Contrary to this, homoxylans are restricted to esparto grass (Chanda et al. 1950) and tobacco stalks (Eda et al. 1976) and only consist of xylosyl residues.

Degradability of native xylan is minimal; it is present as a formidable substrate, whereas alkaline extraction of the native form de-esterifies the substrate and is responsible for the removal of acetyl groups and breakdown of cross linkages, hence increasing the enzymatic degradability of xylan. Due to all these properties of commercial xylan, it is preferred over the native form for assay methods and screening procedures. Extraction of highly purified xylan is not achievable, as it contains some inseparable substituents. Therefore, several aspects of xylan structure remain unclear.

XYLANASE: XYLAN HYDROLYZING ENZYME

Over 100 years ago the role of enzymes in the breakdown of xylan was observed by Hopper-Seyler (Bastawde 1992). Xylanases, the xylan hydrolyzing enzymes, are ubiquitous and diverse by nature (Collins et al. 2005). A cocktail of enzymes with diverse specificity and mode of action can catabolize the xylan molecule, as it contains several substituted groups and side chains. Synergistic action of hydrolyzing enzymes may be responsible for the complete breakdown of the xylan molecule. Enzymes actively involved in the breakdown are β -1,4-endoxylanase, β -xylosidase, α -L-arabinofuranosidase (EC 3.2.1.55), α -glucuronidase, acetyl xylan esterase (EC 3.1.1.6), and phenolic acid esterase. Endoxylanases (β -1,4-D-Xylanohydrolase; EC 3.2.1.8) and β -xylosidase (EC 3.2.1.37) are a group of enzymes that catalyze the hydrolysis of the main backbone of xylan. Different enzymes, such as acetyl esterase (Linden et al. 1994), ferulic esterase (Faulds et al. 1995), glucuronosidase (Wood and Wilson 1995), and arabinosidase (Manin et al. 1994), are required for the release of different side chains from the xylan backbone, but the discussion of such enzymes is beyond the scope of this review. Cooperative action of all these enzymes converts the xylan molecule into constituent sugars.

XYLANASE: PRODUCTION UNDER SSF AND SmF

The main motivation for investigating the different parameters of xylanase production is the wide variety of biotechnological applications. A number of different sources, including bacteria (Sunna and Antranikian 1997; Gilbert and Hazlewood 1999; Battan et al. 2006), fungi (Sunna and Antranikian 1997; Kuhad et al. 1998), actinomycetes (Ball and McCarthy 1989), and yeast (Harmova et al. 1984; Liu et al. 1998), etc., have been reported for xylanase production under solid state fermentation (SSF) and submerged fermentation (SmF). Aerobic microbial transformation of solid materials or solid state fermentation can be defined in terms of the following properties:

1. A porous biodegradable or non-biodegradable solid substrate bed should provide a large surface area per unit volume ranging from 10^3 to 10^6 m^2/cm^3 and also allow easy exchange of gases for the growth of microorganisms (Raimbault 1998).

2. The solid substrate bed should have high affinity for water molecules and be able to hold more water than its own dry weight and also allow a high rate of biochemical processes (Raimbault 1998).

SSF provides a medium devoid of free-flowing water molecules, and the water is present in an absorbed or in complex form with the solid matrix and the substrate (Canel and Young 1980). Low capital investment, lower operating cost, and many other characteristic properties associated with SSF make it an attractive alternative for enzyme production (Chahal et al. 1996; Haltrich et al. 1996; Jecu 2000). SSF provides an environment that is very similar to the natural habitats of microorganisms; therefore it induces the microorganisms to produce more enzymes and metabolites that will not be produced or will be produced only in low yields in SmF (Jecu 2000). Several reports are available on xylanase production using fungal systems, but few reports are available for bacterial systems (Archana and Satyanarayana 1997; Gesses and Mamo 1997; Subramanian and Prema 1997; Sonia et al. 2005) under SSF and SmF. A few reports are also available on the high yield of xylanase (Battan et al. 2006; Sanghi et al. 2007) under SSF using bacterial sources. In SSF using wheat bran as the solid substrate, *Bacillus stearotheophilus* SDX produces high xylanase yield at a substrate-to-moisture ratio of 1:2.5 (Dhiman et al. 2008).

Under SmF conditions too, the reported microbes produce only small yields of xylanase. Among the bacterial systems, *Bacillus licheniformis* 77-2 produced only 41 IU/ml of xylanase (Damiano et al. 2003) and *Bacillus subtilis* produced only 1120 IU/ml of the recombinant xylanase (Qureshy et al. 2000). Various fungal and actinomycetes system are also reported to give low xylanase production, namely *Aspergillus nidulans* (Taneja et al. 2002), *Streptomyces cuspidosporium* (Maheshwari and Chandra 2000), and *Schizophyllum commune* under SmF (Haltrich et al. 1999).

Xylanase: Families and Catalytic Sites

Different types of xylanases come under the category of glycosyl hydrolases, and these can be further classified into two families (Collins et al. 2005). One family has the designation of Family 10 or (F) and the other is Family 11 or (G) (Davies and Henrissat 1995; Hazlewood et al. 1994). Relatedness and similarities of these families was further confirmed through pair-wise alignment and by performing BLAST (Basic Local Alignment Search Tool). BLAST is a tool through which one can compare the two different proteins (enzymes) at the amino acid level and can also check their local and global alignment. BLAST score confirmed the sequence similarity between these two families and exclusively identified the set of enzymes that were mutually similar. BLAST search also found some similarity between the Family 10 of xylanase and $\beta - (1 - 3)$ and $\beta - (1 - 4)$ of glucanases. By using BLAST, a list was prepared in which 77 different proteins belonging to (F) and another 88 proteins related to (G) were identified and compiled. In this list only one sequence has a set of bifunctional properties that match to catalytic domains of both families (F) and (G) (Jeffries 1996). The main substrates for Family 10 are *p* – nitrophenyl (PNP) – xylobiose and PNP – cellobiose. However PNP – xylobiose acts 50 times higher than the PNP – cellobiose. Family 10 or (F) members have low molecular weight and have the tendency to form oligosaccharides having less degree of polymerization. These

observations clearly indicated that Family 10 mainly acts over the xylan residues (Zhang et al. 1994).

The catalytic domain of Family (F) bears five xylopyranose binding sites and constitutes a bowl-shaped structure. Catalytic sites of Family (F) were on the narrower end near to the carboxyl terminus of a β – barrel (Derewenda et al. 1994). Catalytic domains of these enzymes belong to a superfamily that includes Family A cellulases, β – glucosidases, β – galactosidases, β – (1 – 3) – glucanases, and β (1 – 3, 1 – 4) – glucanases (Jenkins et al. 1995). Double layer structure constituted by β – pleated sheets surrounds the catalytic sites of Family (G) (Miao et al. 1994; Withers and Aebersold 1995). The positions of many amino acids in Family 11 are identical to those of bacterial species (*Bacillus circulans*) or fungal (*Trichoderma harzianum*) origin.

Xylanase: Biosynthesis and Multiple Forms

The mechanism that regulates the synthesis of xylanase production is still not clear. One of the proposed mechanisms of xylanase regulation includes the movement of low molecular weight xylan fragments across the microbial cells and the inability of high molecular weight compounds to execute such movement. Low molecular weight, small xylan fragments are constitutively produced in the medium and regulate the xylanase synthesis (Bastawde 1992; Kulkarni et al. 1999). Xylanase production can be of an inducible type as well as constitutive by nature. Medium components are responsible for the inducible production of xylanase, whereas enhanced production is obtained only in those media that contain pure xylan and xylan-rich substrates (Balakrishnan et al. 1997). However most of the available reports describe the constitutive production of xylanase in the presence of production medium (Khanna and Gauri 1993; Khasin et al. 1993; Linder et al. 1994; Segura et al. 1998). A xylan-rich substrate mainly acts as the inducer for the xylanase synthesis (Levin and Forschiassin 1998) as in the case of *Aspergillus awamori* (Siedenberg et al. 1998) or *Streptomyces* sp QG – 11 – 3 (Beg et al. 2000a), as well as a weak inducer in other cases *Cellulomonas flavigena* (Avalos et al. 1996). However the extent of induction is species-specific. Several other compounds such as L-sorbose, xylo-oligosaccharides, and lignocellulosic residues induce xylanase production significantly. Many fungal (Xu et al. 1998; Liu et al. 1998; Sachslenhner et al. 1999) actinomycetes and bacterial (Lopez et al. 1998) species are positively induced by L-sorbose for enhanced xylanase production. But under the same reaction conditions, readily metabolizable sugars such as glucose and xylose act as repressors of xylanase yield (Fernandez-Epsinar et al. 1992; Ishihara et al. 1997; Siedenberg et al. 1998; Bataillon et al. 1998; Liu et al. 1999). Similarly, reports are also available on the positive regulation by lignocellulosic substrates (wheat bran, rice straw, rice bran etc) on the enhanced xylanase production (Beg et al. 2000a; Gupta et al. 2001; Kesker 1992; Kuhad et al. 1998; Puchart et al. 1999; Battan et al. 2006; Sanghi et al. 2007). Production media containing amino acids have also shown positive influence on regulation of xylanase production in *Bacillus* sp. (Ikura et al. 1987; Kulkarni et al. 1999), *Streptomyces* sp. (Beg et al. 2000b), and on *Staphylococcus* sp. (Gupta et al. 1999).

A synergistic effect involving multiple forms of xylanases having different specialities is required for the complete hydrolysis of xylan (Wong et al. 1988). Complete degradation of acetylated xylan is achieved only through the synergistic action between

acetyl xylan esterase and endoxylanases (Biely et al. 1986). This leads to the release of acetic acid, which in turn increases the affinity of endoxylanases for xylan backbone, accompanied by the release of shorter chains of acetylated polymers that are readily catalyzed by the esterase enzymes (Biely 1985; Biely et al. 1986). Several fungal species possess multienzyme systems of xylanase, producing endoxylanases, β -xylosidases, α -L-arabinofuranosidases, and acetyl esterases (Bachmann and McCarthy 1991).

XYLANASE: INDUSTRIAL ASPECTS

The potential of hemicellulose as a renewable raw material is immense. It is produced and wasted annually in huge amounts and has several likely uses if suitably applied. Xylanase has been categorized as one of the more industrially important enzymes (Collins et al. 2005; Haki and Rakshit 2003; Pandey et al. 1999), and the judicious use of xylanases in industries could result in cleaner reactions, higher yields, and lower consumption of energy.

Before using xylanase at industrial levels, several criteria have to be fulfilled. Pilot scale processes are generally carried out at high temperatures; therefore bacterial xylanase having broad ranges of pH and temperature stability are preferred in industry (Kulkarni and Rao 1996). Similarly, xylanases extracted from actinomycetes are also operational over a broad range of reaction parameters (Garg et al. 1998; Beg et al. 2000c), whereas fungal xylanases are stable under acidic pH conditions, varying from pH 4 to 6 only (Silva et al. 1994; Tenkanen et al. 1997; Maximo et al. 1998; Christov et al. 1999b). However, some fungal species produce xylanases that are active at highly alkaline pH, but their number is few and they are less efficient in comparison to the acidic fungal xylanases. In order to obtain the maximal effect of xylanase, some of the reaction parameters, such as enzyme dose, retention time, pH, and temperature, should be optimized.

Bio-Processing of Fabrics

Current research is focused on the replacement of harsh chemicals with commercial enzymes that can specifically target the non-cellulosic and hemicellulosic impurities, yet maintaining the standard production outcomes of textile industries (Li and Hardin 1998; Hartzell and Hsieh 1998; Eppers 1999; Traore and Buschle-Diller 1999; Buchert et al. 2000; Csiszar et al. 2001a; Yachmenev 2001; Lenting et al. 2002; Agrawal et al. 2004; Lenting et al. 2004; Dhiman et al. 2008). Enzymatic treatment can significantly increase the water absorbing properties of fiber by removing complex impurities situated in the primary cell wall. Pretreatment of low-quality jute fibre with pure and thermostable xylanase is attractive for selective removal of xylan without affecting the fibre strength during the spinning process (Saha 2000). A number of reports are available that cite the use of cellulases and pectinases for bioprocessing of fabric, but there are very few reports on xylanases for the purpose of desizing and scouring (Csiszar et al. 2001b; Losonczy et al. 2005).

Processing of the fabric includes desizing (removal of adhesive sizing material), scouring (improving absorbency and whiteness of the textile material), and bleaching (imparting fixed standard whiteness to the fabric) (Karmakar 1999; Rouette 2001). Sizing

materials (i.e. starch and waxes etc.) protect the fabric against abrasive forces during weaving. Desizing is carried out to remove the adhesive material in order to render the fabric more accessible to the subsequent stages of the processing. Conventionally it is carried out at higher temperature with strong oxidizing agents in alkaline solution. After desizing, the fabric needs to be scoured to remove inhibitory materials for its efficient finishing, wetting, and dyeing (Harris et al. 1998).

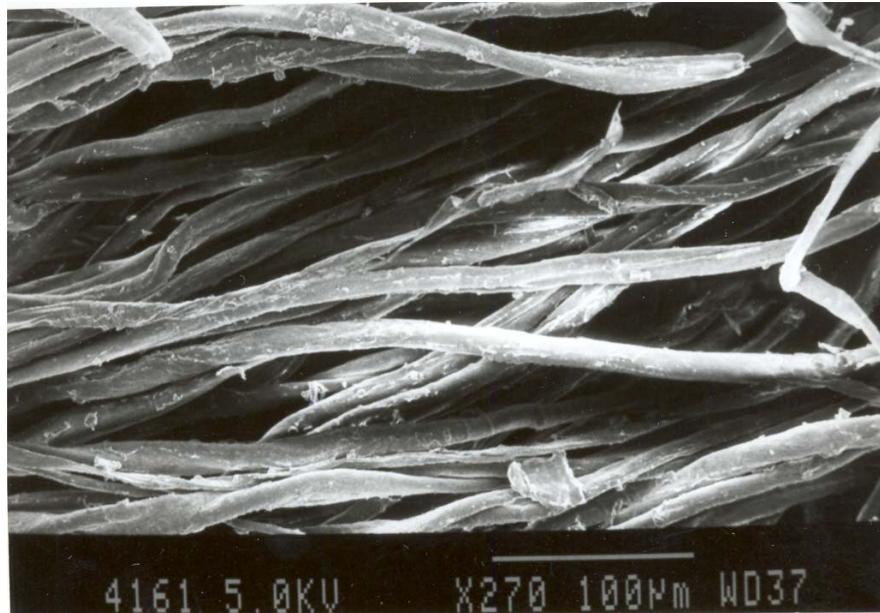


Fig. 1a. Scanning electron micrograph of control cotton (0701DR001) fabric at low magnification

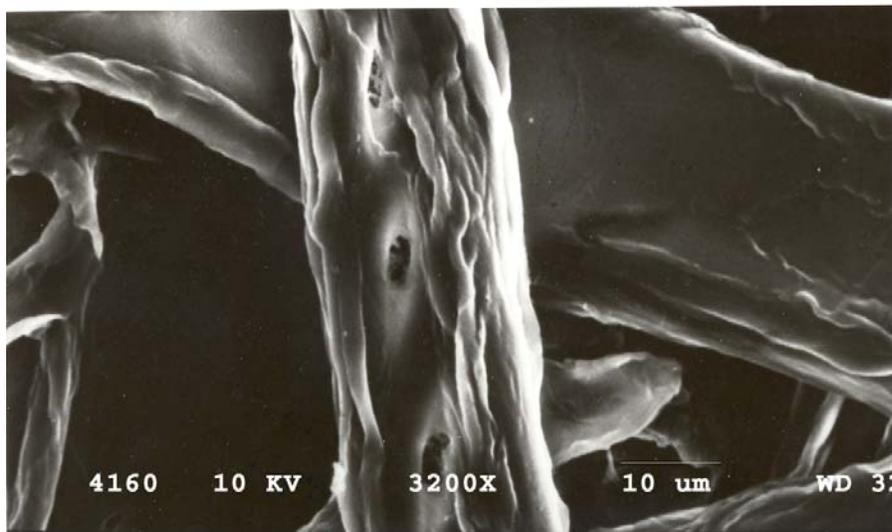


Fig. 1b. SEM of enzymatically desized cotton (0701DR001) fabric at high magnification

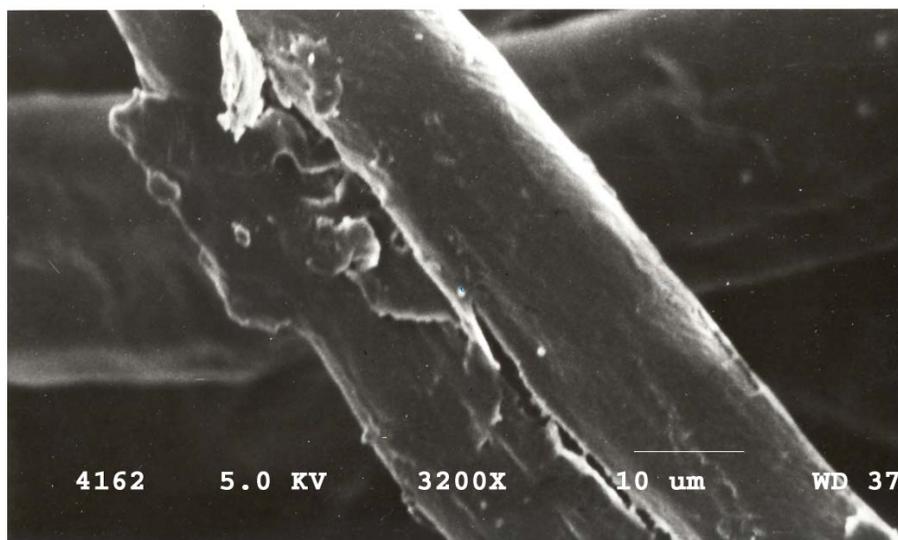


Fig. 1c. SEM of bioscoured cotton (0701DR001) fabric using xylanase, at high magnification

Conventional scouring is a chemical-intensive, non-optimal process, and concentrated sodium hydroxide (scouring) and hydrogen peroxide (H_2O_2)/sodium hypochlorite (bleaching) solutions are used to eliminate the inhibitory non-cellulosic impurities of wax (0.4–1.2%), pectic substances (0.4–1.2%), ashes (0.7–1.6%), and lignin-containing proteins (1.0–1.9%). Conventional scouring solutions attack non-specifically on cellulosic material of fiber, which in turn causes strength loss. The advantage associated with enzymatic pretreatment is the highly specific action of the enzyme. Xylanase specifically acted over the hemicellulosic impurities and efficiently caused their removal. Enzymatic treatment did not cause any strength loss of the fiber (Dhiman et al. 2008). Figure 1 clearly shows the specific action of xylanase on the impurities. Fiber became more soft and smooth after desizing (Fig. 1b) and bioscouring process (Fig. 1c) as compared to the control (Fig. 1c) untreated fiber. The greatest hurdle in the commercialization of enzymatic method is offered by the presence of seed coat fragments attached with the fibres and linters (Verschraege 1989). Enzymatic pretreatment with xylanase leads to the partial hydrolysis of these seed coat fragments, thereby making them more accessible to the chemicals during the later stages of bleaching and finishing (AATCC Technical Manual 1980).

Biobleaching of Pulp

A few decades ago researchers proposed a hypothesis based on the use of microbial enzymes in paper processing, noting that paper is composed of natural polymers, such as cellulose, hemicellulose, and lignin materials. The last decade has witnessed the conversion of that hypothesis into reality, as lignin-degrading enzymes and hemicellulases, etc., have been successfully used in paper processing, viz. bleaching with xylanases, pitch removal with lipases, and increasing the freeness of pulp fibers (Li and Hardin 1998; Techapun et al. 2003). These enzymes are being used in the treatment of cellulosic pulps to remove residual xylans in the production of dissolving pulps. Xylanases, mannanases, and their accessory enzymes are able to degrade the hemicellulose portion in the pulp selectively without affecting the cellulose.

Xylan-degrading enzymes aid in enzymatic debarking and pulp refining to reduce energy demands in mechanical pulping processes. They also can facilitate enzymatic deinking of recycled fibres in combination with cellulases. In an enzymatic beating process, the enzymes are added to bleached pulp fibres to increase external fibrillation (Kuhad et al. 1997). Apart from improving pulp fibrillation, xylanase application can increase water retention, reduce beating times in virgin pulps, restore bonding and increase freeness in recycled fibres, and selectively remove xylan from dissolving pulps (Srinivasan and Rele 1999).

In the process of paper production, chemical pulping is the first step, in which fibers are broken apart and most of the lignin is removed (Hong et al. 1989). The chemical pulping process that is most frequently used around the world is the sulphate (kraft) process (Damiano et al. 2003; Duarte et al. 2003; Oakley et al. 2003). The residual lignin is then removed by a multistep bleaching process, since the kraft process in the absence of bleaching imparts a brownish colour to the paper (Bajpai 1999; Damiano et al. 2003). As we gain more experience in enzymatic processing of paper, one of the biggest challenges of papermaking, i.e. bleaching of pulp, may be solved with the use of xylanase enzyme. These enzymatic processes also have potential to reduce organic pollutant loads on the environment (Onysko 1993; Kulkarni and Rao 1996; Dhillon et al. 2000). Some of the achievements in the field of enzymatic bleaching are given in Table 1.

Table 1: Chronological Order of Use of Enzyme in Paper and Pulp Industry (Kirk and Jeffries 1996)

| Year | Development | Investigator (Reference) |
|------|---|--|
| 1959 | Pulp fibrillation by cellulases | Bolaski et al. 1959 |
| 1984 | Enzymatic beating with xylanases Hemicellulose removal from dissolving pulp by xylanases | Comtat et al. 1984 Paice and Jurasek 1984 |
| 1986 | Prebleaching with xylanases | Viikari et al. 1986 |
| 1988 | Enhanced drainage with cellulases | Fuentes and Roberts 1988 |
| 1988 | Decreased vessel picking with cellulases | Uchimoto et al. 1988 |
| 1989 | Depitching pulp with lipases | Irie et al. 1989 |
| 1991 | Deinking with cellulases and xylanases | Kim et al. 1991 |
| 1993 | Pulp delignification with laccase | Call 1993 |
| 1996 | Bleaching with manganese peroxidase | Harazono et al. 1996 |

Nowadays the demand of cellulase-free xylanase has been increasing for the production of rayon grade paper pulps or superior quality dissolving pulps (Jurasek and Paice 1986; Techapun et al. 2003). Any cellulase activity hampers the pulp properties, leading to degradation in pulp quality and increased effluent treatment cost (Haki and Rakshit 2003).

The application of xylanases from *Bacillus pumilus* ASH (Battan et al. 2007), *Streptomyces* sp. QG-11-3 (Beg et al. 2000c), *Streptomyces cuspidosporus* (Maheshwari and Chandra 2000), *Bacillus licheniformis* 77-2 (Damiano et al. 2003), *Chaetomium cellulolyticum* (Baraznenok et al. 1999), and *Streptomyces* sp. strain S38 (Georis et al. 2000) for improvement in pulp bleaching has been reported by several workers. A chlorine savings in the range of 30–35 % and 12–40 % using cellulase-free xylanase from *S. thermoviolaceus* (Garg et al. 1998) and commercial xylanase (Ecopulp, Cartazyme NS-10

and Pulpzyme HC) (Vicuna et al. 1997), respectively, have been reported earlier. Beg et al. (2000c) reported a reduction of 8 % chlorine by using xylanase from *Streptomyces* sp. QG-11-3.

Pretreatment of pulp with xylanase prior to treatment with a CDED₁D₂ bleaching procedure enhances various physical properties of the pulp viz. viscosity, tensile strength, breaking length, burst factor, burstness, tear factor, and tearness. Enzymatic prebleaching facilitates an increase in pulp fibrillation and water retention, restoration of bonding, increased freeness in fibers, and selective removal of xylan from dissolving pulps, thus rendering the fibers more accessible to chemical bleaching (Techapun et al. 2003). Effects of xylanase treatment can be clearly observed from the scanning electron micrographs of handsheets, prepared from the pretreated pulp sample. Figure 2b clearly reveals that enzymatic action caused more dispersion of the fiber as compared to the control sample (Fig. 2a), which leads to an increase in water retention properties of the paper. Further biobleaching caused softening of the fiber, which was due to the highly specific action of xylanase over the impurities.

This reduces the load of chlorine consumption and requirements of other chemical agents in the subsequent chemical bleaching stages. Beg et al. (2000c) have reported improvement in the tensile strength and burst factor by up to 63% and 8%, respectively, by xylanase produced from *Streptomyces* sp. QG-11-3. This enzymatic bleaching process efficiently reduces the COD value of the effluent, which is highly significant, as it decreases the environmental pollution caused by the release of waste effluents from paper industry into the surroundings.

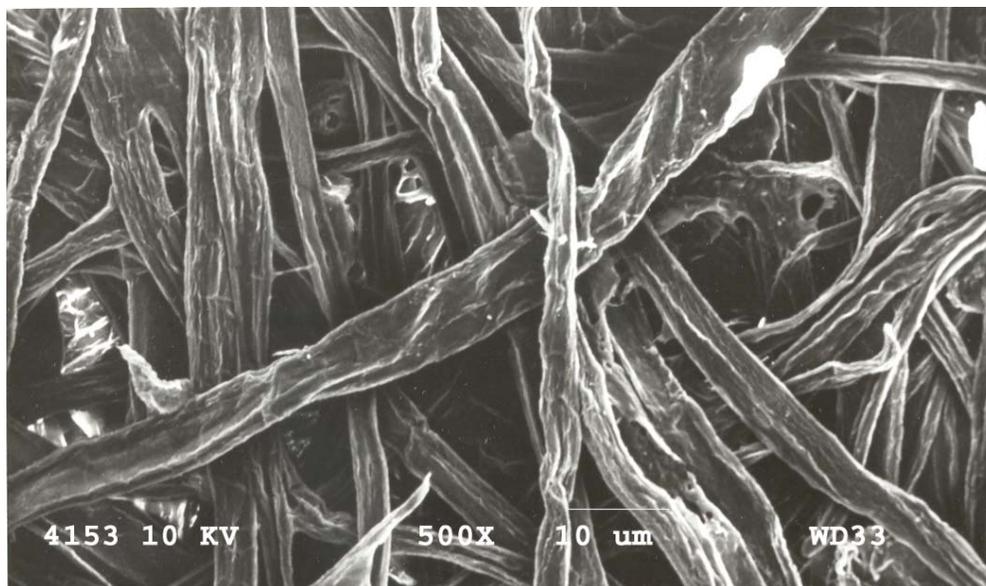


Fig. 2a. Scanning electron micrograph of unbleached (control) eucalyptus kraft pulp (500X)

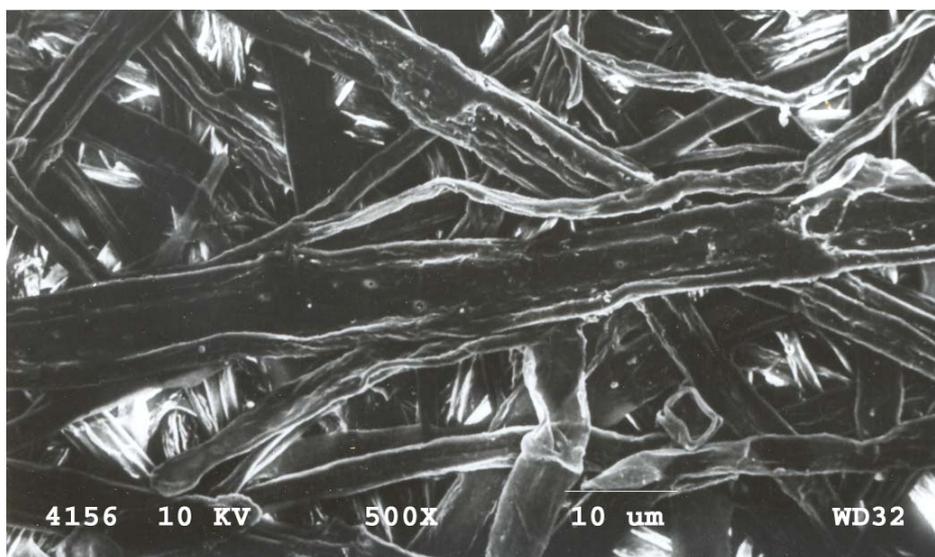


Fig. 2b. Scanning electron micrograph of eucalyptus kraft pulp after enzymatic treatment (300X)

Biobleaching and bioprocessing of pulps using a combination of xylanase and laccase further broadens the horizon of enzymes in the pulp and paper industry. Once modified, hemicellulose(s) is removed by xylanase; the lignin layer is easily available for the penetration and degradative action of laccase (Viikari et al. 1994; Bajpai 2004). The exposed lignin moiety thus requires less chlorine for its removal (Kuhad et al. 1997; Bajpai 2004). Kapoor et al. (2007) describe differential and synergistic effects of a xylanase and laccase mediator system in bleaching of soda and waste pulps for ecofriendly production of paper. Most of the biobleaching studies are confined to kraft and sulfite pulps (Damiano et al., 2003; Duarte et al. 2003; Oakley et al. 2003), but a few studies have reported the use of non-woody pulp, for which a slight increase in brightness was achieved. Xylanase-pretreated pulp samples of banana, silk cotton, and cotton showed an increased brightness of 19.6, 11.6, and 7.9%, respectively, in the case of xylanase produced by *Bacillus subtilis* C01, using agriresidues (Ayyachamy and Vatsala 2007).

Presently a significant number of European, North American, South American, and Japanese mills are using xylanase in the bleaching process. Pulpzyme HA, introduced by Novo Nordisk A/S, was the first commercially available xylanase for use in biobleaching of wood pulps. It was extracted from a strain of *Trichoderma reesei* and was used in the first bleaching stage to reduce the dosage of active chlorine. Several multinational biotech companies are marketing various xylanase preparations, such as Irgazyme (Genencor International), Cartazyme (Sandoz), Ecopulp (Alko), and VAI xylanase (Voest Alpine). Canada, which is the largest producer of pulp, is producing more than 10% of its bleached pulp by using xylanase.

Waste Paper Recycling

Xylanase has been used successfully also in waste paper treatment. Treatment is mainly carried out through two stages of pulping and beating. The first stage involves the fiber separation or fiber dispersing, which is responsible for the moistening of loose fibers, and the process is known as hydrating process. The first step of enzymatic treatment

involves initial soaking of paper and enzyme incubation, whereas the second stage includes mechanical shearing (gentle refining) of pulp, followed by heating of pulp for the separation of fibers and enzyme deactivation. Xylanase treatment is responsible for the liberation of more reducing sugars from the waste paper pulp, and their release is directly related to temperature. It may be due to the fact that at higher temperature most of the xylan situated in between the pulp fibers gets hydrolyzed.

Enzymatic treatment caused swelling and separation of fibers of waste paper pulp. Fibers of pretreated pulp (Fig. 3b) sample appeared more dispersed and smooth as compared to the untreated pulp (Fig. 3a) due to hydrolysis and detachment of xylan residues attached to the surfaces of fibers.

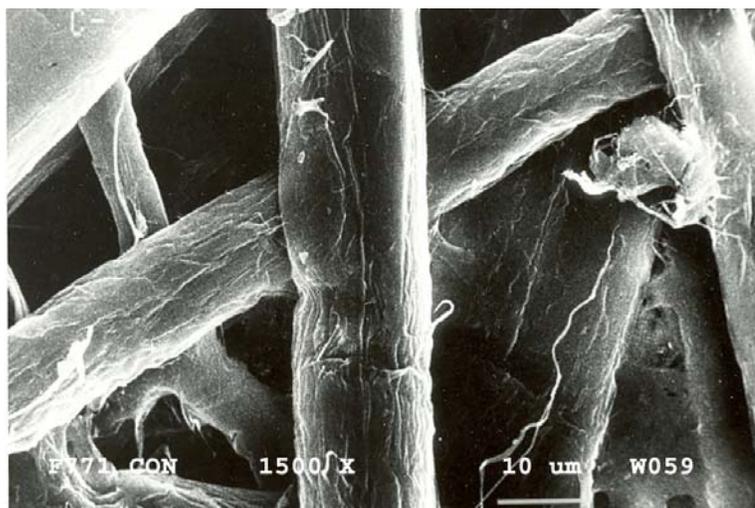


Fig. 3a. SEM micrograph (1500 X) of the untreated pulp, after refining; the fiber surfaces were observed to be smooth.

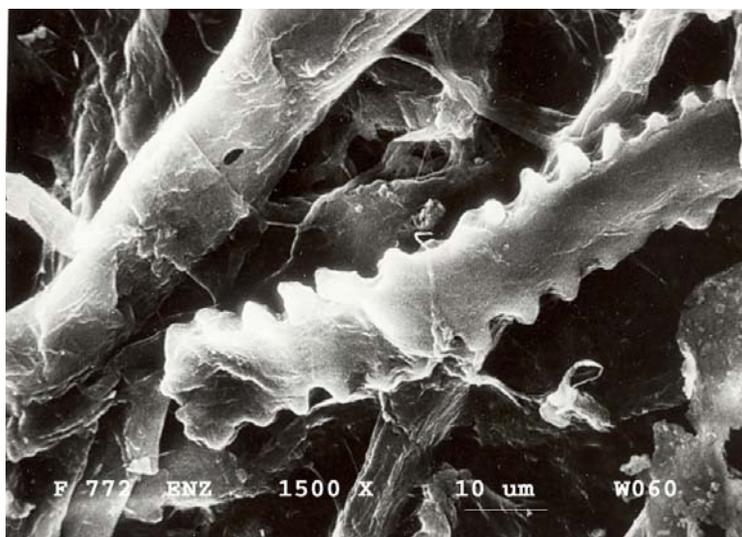


Fig. 3b. SEM micrograph (1500 X) of enzyme-treated pulp; the fiber surfaces were observed to be rough, with wrinkles and peeling of microfibrils.

Poorna and Prema recorded similar findings after treatment of carton and office paper waste with endoxylanase (Poorna and Prema 2007). Enzymatic treatment facilitates the swelling of the pulp fiber, which proves to be beneficial in the further processing of the pulp material and also enhances the physical properties (Kenealy and Jeffries 2003).

Bioconversion

Bioconversion of xylan to useful, higher value products normally requires multi-step processes that include: (i) pretreatment (mechanical, chemical or biological), (ii) hydrolysis of the polymers to produce readily metabolizable molecules (e.g. hexose or pentose sugars), (iii) bio-utilization of these molecules to support microbial growth or to produce chemical products, and (iv) separation and purification (Howard et al. 2003).

Dilute acid pretreatment at a relatively low temperature to minimize the formation of inhibitory compounds, followed by enzymatic saccharification, is an excellent workable process for generating fermentable sugars from hemicellulosic biomass. It is essential that a xylose-tolerant β -xylosidase, along with other xylan-degrading enzymes, is developed in order to make enzymatic saccharification of any xylan substrate a commercial success (Roberto et al. 2003).

Some bacteria, such as *E. coli*, *Klebsiella*, *Erwinia*, *Lactobacillus*, *Bacillus*, and *Clostridia*, can utilize mixed sugars but produce no, or only a limited quantity of ethanol. Several microorganisms have been genetically engineered to overproduce ethanol from mixed sugar substrates (Sun and Cheng 2002). Various recombinant strains, e.g. *E. coli* K011, *E. coli* SL40, *E. coli* FBR3, *Zymomonas* CP4 (Pzb4), and *Saccharomyces* 1400 (Plnh32) have been evaluated for the fermentation of mixed sugar substrates.

Xylitol, a five-carbon sugar alcohol, used as a natural food sweetener (Nigam and Singh 1995) has odontological applications such as teeth hardening, remineralisation, and as an antimicrobial agent; is used in toothpaste formulations (Roberto et al. 2003; Parajo et al. 1998) and is currently produced by chemical reduction under alkaline conditions of the xylose derived mainly from wood hydrolyzate. Xylitol is used as a natural food sweetener, and its demand in the food and pharmaceutical industries has created a strong market for the development of low cost xylitol production processes. The recovery of xylitol from the xylan fraction is about 50-60%, or 8-15% based on the raw material employed. The bulk of the xylitol produced is consumed in various food products, such as chewing gum, candy, soft drinks, and ice cream. It gives a pleasant cool and fresh sensation due to its high negative heat of solution.

A product of hemicellulosic hydrolyzate, 2,3-butanediol, is a valuable chemical feedstock because of its application as a solvent, liquid fuel, and as a precursor of many synthetic polymers and resins (Fig. 4).

Dehydration of 2,3-butanediol yields the industrial solvent methyl ethyl ketone, which is much more suited as a fuel because of its much lower boiling point. Further dehydration of 2,3-butanediol yields 1,3-butanediene, which is the starting material for synthetic rubber and is also an important monomer in the polymer industry (Howard et al. 2003).

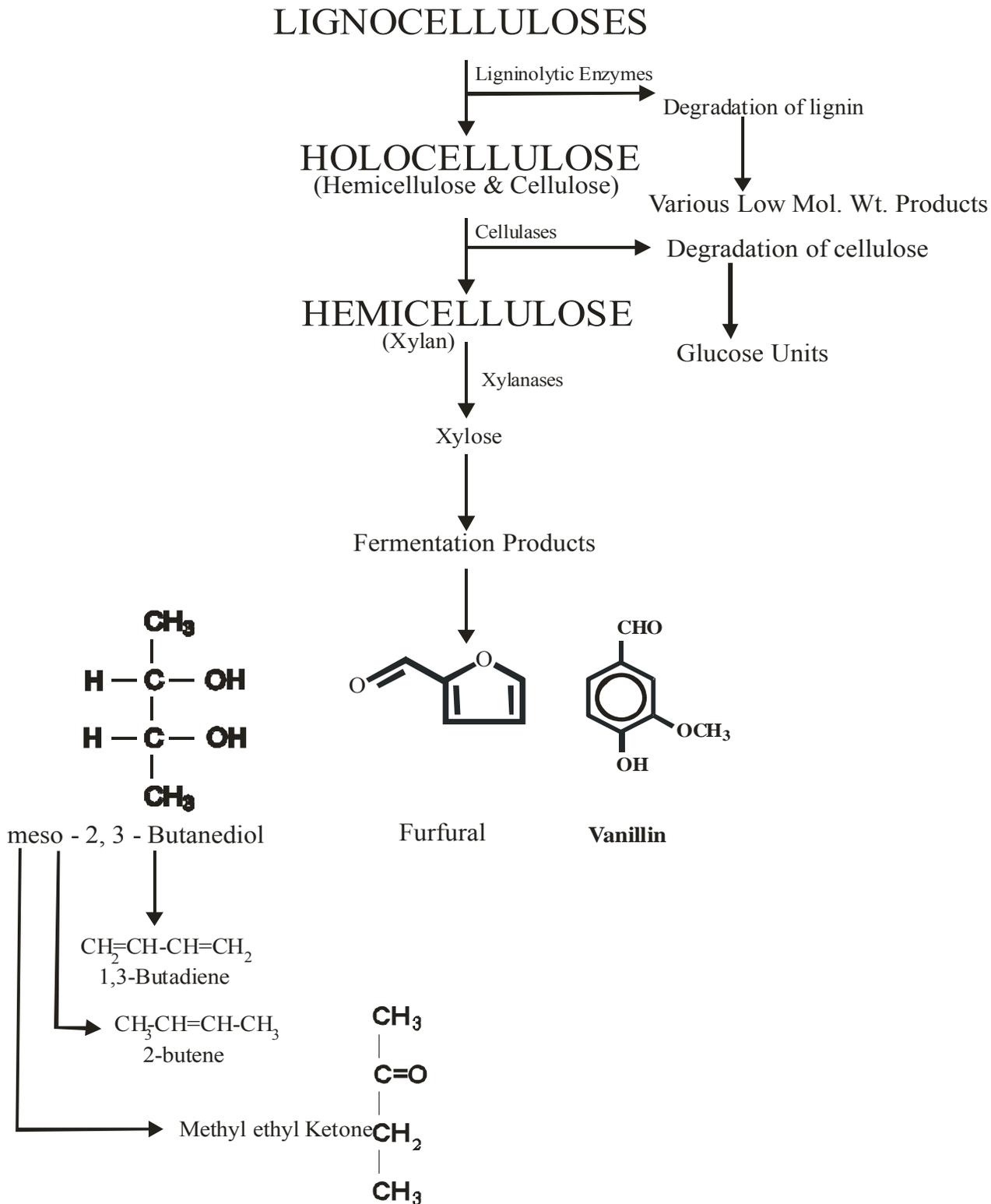


Fig. 4. Various fermentation products of xyloses

Another value-added product from hemicellulose hydrolyzates is lactic acid, which is used in the food, pharmaceutical, and cosmetic industries. It is a component of biodegradable plastic polylactate, the market for which is expected to grow significantly. Furfural is used in the manufacture of furfural-phenol plastics, varnishes, and pesticides (Zeitch 2000; Montane et al. 2002). Ferulic acid is the major source of cinnamic acid, which is found in a variety of plant cell walls. Ferulic acid esterase from *A. niger* was first used to convert ferulic acid to vanillic acid, which was then reduced to vanillin (Priefert et al. 2001).

Feed

Enzymes for ruminants feed have received considerable attention recently because of their potential to improve animal performance. Pretreatment of agricultural silage and grain feed by xylanases has been reported to improve its nutritional value. Incorporation of xylanase into a rye-based diet of broiler chickens results in reduced intestinal viscosity, thus improving both the weight gain of chicks and their feed conversion efficiency (Bedford and Classen 1992; VanParidon et al. 1992). Most of the low-quality feedstuff contains large amounts of incompletely digestible nutrients and energy values. The nutritional value of these low-quality feedstuffs can be increased significantly if they are effectively processed before feeding. Thus, xylanases are used in the pretreatment of forage crops to improve the digestibility of ruminant feeds and to facilitate composting (Gilbert and Hazlewood 1993).

Xylanases reduce viscosity and increase absorption by breaking down the non-starch polysaccharides (NSPs) in high fibre rye- and barley- based feeds. Most commercial xylanases are produced by *Trichoderma*, *Bacillus*, *Aspergillus*, *Penicillium*, *Aureo-basidium*, and *Talaromyces*. Lignocellulosic wastes from pulp and paper industries, as well as wastes from dairy and agricultural industries, are potential substrates for use in the production of single-cell protein (SCP) for feed and food purposes (Kuhad and Singh 1993).

Other Applications

Microbial xylanases have shown tremendous potential in different processing stages at various industrial levels. Xylanase has numerous applications in the food and feed industries (Ball and McCarthy 1988; Kuhad and Singh 1993) and can be applied for the production of several useful economical products such as SCPs (Single Cell Proteins), sugar syrups, and liquid and gaseous fuels (Wong and Saddler 1992; Kuhad and Singh 1993; Niehaus et al. 1999; Bajpai 1999). Apart from the above-mentioned promising applications, some of the other crucial uses of xylanase are listed below:

1. Recently xylanase in combination with amylase has been world-wide tested for its ability to enhance the quality and volume of bread (Maat et al. 1992).
2. Xylanase, along with other pectinolytic enzymes, is currently used for clarification of juices and for liquification of fruits (Biely 1985; Yusof and Ibrahim 1994).
3. Treatment of forage crops with xylanases lead to their digestibility by ruminants (Gilbert and Hazlewood 1993).
4. A potential application of the xylanolytic enzyme system is in conjunction with the pectinolytic enzymes in paper and pulp industry (Ahlawat et al. 2007a,b). A

- xylanase-pectinase combination is also used for degumming of bast fibers such as flax, hemp, jute, and ramie (Sharma 1987; Puchart et al. 1999).
5. A xylanase-pectinase combination also has been used in the debarking process, which is the first step in wood processing (Bajpai 1999; Wong and Saddler 1992).
 6. Other applications include the rhizosecretion in transgenic tobacco plant (Borisjuk et al. 1999), improvement in cell wall maceration (Wong et al. 1988), processing of aromatizing nuts, wines (Spanga et al. 1998), and induction in glycosylation and fatty acylation of phytosterols (Moreau et al. 1994).
 7. Acidic oligosaccharides obtained from birchwood xylan by treatment with family 11 xylanase from *Sporotrichum thermophile* have been tested as antimicrobial products against Gram positive bacteria and *Helicobacter pylori* (Vardakou et al. 2003).
 8. Xylanases also find applications in the detergent industry, as they improve the cleaning ability of detergents that are especially effective in cleaning fruit, vegetable, soils, and grass stains (Kuhad et al. 1997).
 8. Xylanases also help in fuel-alcohol production (Dominguez 1998; Kuhad and Singh 1993; Sun and Cheng 2002). They decrease the viscosity of the mash and prevent fouling problems in distilling equipment. Xylo-oligomers produced by enzymatic hydrolysis of xylan may be valuable for their rheological properties.

FUTURE PROSPECTS

In the future, emphasis will be placed on the proper and economical utilization of abundantly present xylan and lignin compounds. These compounds accumulate in the environment due to industrialization and urbanization. Paper industry and agricultural activities release significant quantity of xylan, which gets deposited in rivers and ponds. Therefore, in order to maintain the ecological balance and to fulfill the ever increasing demand of fuel and energy, attention must be paid to the proper conversion of these waste hemicellulosic compounds to renewable sources of energy and biofuels (Biely 1985; Niehaus et al. 1999; Monica et al. 2004; Inoue et al. 2008).

Deep knowledge of molecular aspects of xylanase and cloning in suitable expression vectors will be the second major target. This is so because new industrial uses of xylanases have been explored, and such kind of xylanases are required that are stable and active over a broad range of pH and temperature; therefore, genes encoding for the thermophilic, alkaline form of xylanases will be cloned for industrial purposes. Few reports are available on the molecular aspects and molecular cloning of xylanase for enhanced production and for the synthesis of xylanase with desired properties (Kulkarni et al. 1999; Liu et al. 2003; Huang et al. 2006). In order to constitute such expression vectors that can be used for the expression of desired type of xylanase, the latest techniques in the field of protein engineering will need to be used.

A principal hurdle in the commercialization of enzymatic processes is the bulk production of enzymes at a cost effective rate. In order to meet this goal, such strategies should be explored by which cost-efficient bulk production can be achieved. Therefore, the coming years will see advancement in production methods to exploit such microbial

species that can easily metabolize the available waste material by using the simplest techniques at affordable prices. Much progress has been made in understanding the basic mechanisms of xylanases and in engineering their properties. In order to bring such a revolution in production and industrial applications to fruition, biotechnologists, microbiologists, and biochemists should work together for the future.

CONCLUSIONS

1. The characteristics of xylanases are well suited for biobleaching of pulp, and cellulase-free enzymatic cocktails can be used as bleach boosting agents, thereby decreasing the bleaching chemical requirements when such technology is applied in the pulp and paper industries.
2. Use of alkalothermophilic xylanase enzyme in textile industry could lead to reactions that can reduce the negative environmental impact and also improve the physical properties of the fabric.
3. Other industries such as feed, jute processing, and fruit juice industry, etc., also are employing methods that are enzyme-based and eco-friendly in nature.
4. In the coming time new methods will be developed for an easy and cheap production of xylanase enzyme to fulfill the demands of various industries.

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