International Journal of Life Sciences Vol. 4. No. 3. 2015. Pp.178-187

Review Paper

Review on Microbial Xylanases and their Applications

Asish Mandal
PG Department of Botany, Ramananda College, Bishnupur, Bankura, West Bengal, India

Abstract
Xylanases are a group of enzymes that are responsible for hydrolysis of xylan, the main hemicellulose of plant cell wall. Xylanases are produced by different kinds of microorganisms like bacteria, fungi. But the level of xylanase in fungal culture is typically much higher than those from yeasts or bacteria. Xylanases have much importance in various industries like paper and pulp industries. Nowadays the enzymes are commercially produced and the genes responsible for its biosynthesis are cloned. The enzyme biosynthesis is regulated genetically and the scale up of its production can be regulated by different ways in optimized conditions in solid state fermentation and submerged fermentation.

Key Words: *Hemicelluloses, Xylanases, Microbial enzymes, pulp, paper*

Introduction

The enormous varieties of biochemical reactions that comprise life are nearly all mediated by biological catalysts i.e. enzymes. They have high degree of specificity for their substrates and they function in aqueous solutions under relatively mild conditions.

The demand for industrially important enzymes particularly of microbial origin is increasing owing to their low production cost. Since the diversity of microbial enzymes is great, they present a wide spectrum of characteristics that make them utilizable for quite specific applications. A number of microbial enzymes like protease, amylase, xylanase, cellulase, tannase, lipase etc. are being used in the field of food, agriculture, pharmaceuticals, cosmetics, and other biotech industries in our country and also in abroad.

Several works on different industrially important enzymes have already been reported but still research is going on for the hope of the best. Among the industrially important enzymes, xylanase (β-1, 4-D xylan-xylanohydrolase; EC - 3.2.1.8) responsible for the breakdown of xylan (a major component of plant hemicelluloses) has gained importance in different areas. This enzyme is mainly produced by microorganisms such as fungi and bacteria, whose activities are important for the maintenance of carbon flow in the carbon cycle.

For production of microbial enzymes the selection of suitable microorganism is very much important. The microorganisms are isolated generally from the soil. As xylan is one of major components of soil organic matters (Subramanian and Prema, 2002) one can easily gets xylanolytic enzyme producing microbes from soil. The physical and chemical environment of the isolated microorganism must be optimized for large-scale enzyme synthesis. In this context, an extracellular enzyme is preferred as it is cost effective and is available in a relatively pure form in the culture liquor. Submerged fermentation (SmF) technique is generally adopted in industries for rapid production of enzymes where the organisms are grown within large vessels containing liquid media with aeration device. Nowadays solid state fermentation has gained interest from researchers and has often been employed for the production of xylanases because of economic and engineering advantages. Solid state fermentation is becoming an attractive procedure in xylanolytic enzyme production because of its higher productivity and lower production cost. Hence, an approach to reduce the cost of xylanase production is the use of lignocellulosic materials as substrates rather than opting for the expensive pure xylans (Senthilkumar *et al*, 2005). Varieties of microbes are isolated for the xylanases production, varieties the xylanse enzymes are being purified and the genes encoding xylanases are being cloned and characterized (Roy and Habib, 2009)

In the present reviw, different aspects xylanase producing bacterial strain are described. With the increasing demands of xylanolytic enzymes in different industrial sector the knowledge on xylan and xylanses are increasing. In this context this review tries to focus the production, utilization and industrial application of the enzymes.

Xylan

Xylans are highly branched hetero-polymers. The backbone of xylan (Figure 1) is composed of β-1, 4-linked D-xylopyranosyl residues. The degree of polymerization of xylopyranosyl residues in birch wood xylan is 101 to 122 units (Jacobs and Dahlman, 2001). The common substituents found to be attached with the backbone are acetyl, arabinosyl and glucuronosyl residues (Bastawde, 1992). The frequency and composition of the branches are dependent on the source of xylan (Aspinall, 1980). In hardwood xylan, the substituent branches are linked with the backbone by β -(1, 2)-glycosidic bonds with 4- methylglucuronic acid group. In addition, O-acetyl groups sometime replace the OH groups in position C-2 and C-3 (Figure 1). The presence of acetyl groups is responsible for the partial solubility of xylan in water. In softwood xylan, the acetyl groups are fewer in the backbone chain. However, softwood xylan has additional branches consisting of arabinofuranose units linked by β -(1, 3)-glycosidic bonds to the backbone (Puls and Schuseil, 1993). Xylans in grasses are generally arabinoxylans (Wilkie, 1979; Aspinall, 1980), and some xylans have branches containing various combinations of arabinosyl, galactosyl, glucuronosyl, and xylosyl residues (Aspinall, 1980; McNeil et al., 1984). Both hardwood and softwood xylans have a reducing end group consisting

of rhamnosyl, galacturonosyl, and xylosyl residues (Andersson et al., 1983). Homoxylans, which consist of xylosyl residues exclusively, have been isolated from esparto grass (Chanda et al., 1950) and tobacco (Eda et al., 1976).

Xylans from different sources, such as grasses, cereals, softwood and hardwood differ in composition. Birch wood xylan contains 89.3% xylose, 1% arbinose, 1.4% glucose and 8.3% anhydrouronic acid (Kormelink et al., 1991). Wheat arabinoxylan contains 65.8% xylose, 33.5% arabinose, 0.1% mannose, 0.1% galactose and 0.3% glucose (Gruppen et al., 1992). Corn fibre hemicellulose contains 48-54% xylose, 33-35% arabinose, 5-11% galactose, and 3-6% glucuronic acid (Doner and Hicks, 1997; Hespell, 1998).

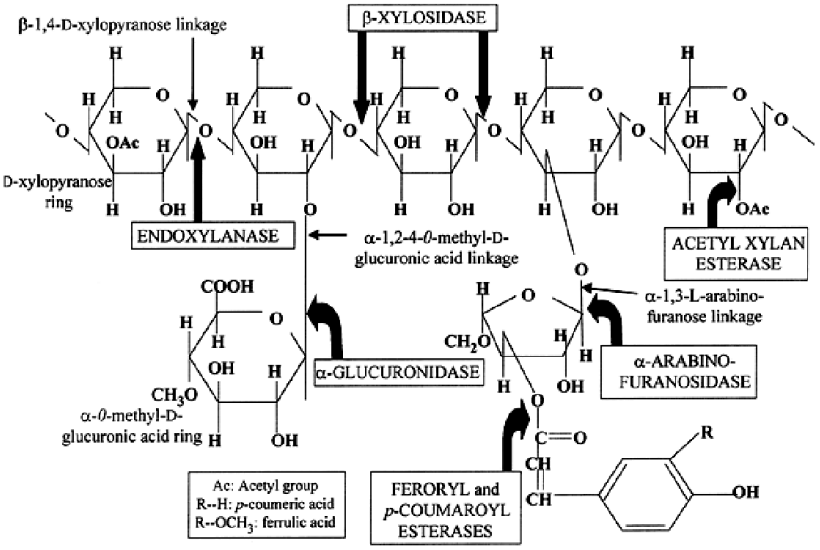

Fig 1. Structure plant xylan showing different substituent groups with sites of attack by microbial xylanases

Occurrence of xylan

Lignocellulosic biomass, the major reservoir of fixed carbon in nature is one of the most abundant materials in the world. Principal sources of these materials are trees and agricultural residues. The main components of lignocelluloses are divided into four major groups like cellulose, hemicellulose, lignin and extractives (Puls and Schseil, 1993; Dahlman and Sjöberg, 2002). A report on distribution of lignocellulosic components is given in the table 1.

Table 1. Distribution of lignocellulosic components in softwoods, hardwoods and wheat straw.

	Weight, % of dry material		
	Softwoods	**Hardwoods**	**Wheat straw**
Cellulose	42 ± 2	45 ± 2	36 ± 5
Hemicellulose	27 ± 2	30 ± 5	27 ± 3
Lignin	28 ± 3	20 ± 4	11 ± 3
Extractives	3 ± 2	5 ± 3	26 ± 5

Among the lignocellulosic substances, hemicellulose remains as an intermediate cross-linking component that strengthens the structure of cell walls. Schulze (1891) first introduced the term 'hemicellulose' for the fractions isolated or extracted from plant materials with dilute alkali. These are partially soluble or swellable in water. Hemicelluloses include xylan, mannan, galactan, and arabinan, which are heteropolymers. These heteropolymers differ from each other according to the types of sugar moieties present within the backbone. Hardwood hemicelluloses contain mostly xylans, whereas softwood hemicelluloses contain mostly glucomannans (McMillans, 1993). The backbone of a hemicellulose can be a homopolymer or heteropolymer. The sugar components of hemicelluloses are D-xylose, D-mannose, D-galactose, and L-arabinose (Polizeli et al., 2005). Among them most abundant sugar is xylose.

Xylan appears to be a major component of hemicelluloses and interfaces between lignin and other carbohydrates in secondary walls of plant cell (Brownell, 1970; Axelsson et al., 2005). Linkages between xylan and pectic substances have also been suggested as xylan-glucan-protein complexes (Selvendran, 1985). Xylan tends to be adsorbed onto cellulose (McNeil et al., 1975; Linder et al., 2003) and to be aggregated with other hemicellulosic components (Kato, 1981) by hydrogen-bonding interactions.

The heteroxylans, which are probably highly cross-linked by diferulic bridges, constitute a network in which the cellulose microfibrils may be embedded (Saulnier et al., 1995).

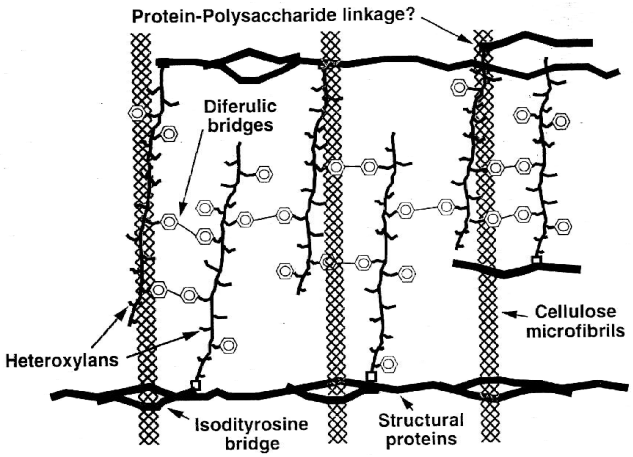

Fig 2. A hypothetical model of the maize bran cell wall (Saulnier and Thibault, 1999)

Xylanolytic enzymes:
The complex structure of xylan needs different enzymes for its complete hydrolysis. Endo-1, 4-β-xylanase (β-1, 4-D-xylan-xylanohydrolase, EC - 3.2.1.8) depolymerizes xylan by the random hydrolysis of the xylan backbone and β-1, 4- D-xylosidase (β-1, 4-D-xylan-xylohydrolase EC - 3.2.1.37) splits off small oligosaccharides into xylose. The side groups of xylan are hydrolyzed by α-L-arabinofuranosidase, α-D-glucuronidase, galactosidase, and acetyl xylan esterase (Figure 1). The endoxylanases, reported to release xylose during hydrolysis of xylan, do not have any activity against xylobiose, which could be easily hydrolysed by β-xylosidases.

Xylanase can be classified into the following types (Bastawde, 1992) according to the site of cleavage

i) Endo-β-(1, 4)-D-xylan xylanaohydrolase, (E.C. 3.2.1.8)

It initially, hydrolyzes xylan to β-D-xylopyranosyl oligomers, but at a later stage, small molecules such as mono, di and trisaccharides of β-D-xylopyranosyl may be produced.

ii) Exo-β -(1, 4)- xylosidase (E.C.-3.2.1.37)

It hydrolyzes small xylooligosaccharides and xylobiose, releasing β-D-xylopyranosyl residues from the non-reducing terminus.

Xylanases are again classified into families 10 (or F) and 11 (or G) glycosyl hydrolases by hydrolytic cluster analysis (Henrisssat and Bairoch, 1993). Family 10 xylanases are larger, more complex and require two unsubstituted xylopyranosyl residues to be attached with xylan, whereas, endoxylanases of family 11 require three unsubstituted xylopyranosyl residues (Biely, 1985) and it makes the enzyme to effect on more specific position.

Sources of xylanase
Xylanases are produced by fungi, yeast, bacteria and marine algae. Microorganisms are the potent producer of xylanase and a list of xylanase producing microorganisms are given in Table 2.

Table 2: List of some xylanase producing microbes (● = poor producer, ●● = good producer, ●●● = best producers)

Organisms	Reference
Fungi	
●●● *Melanocarpus albomyces*	Gupta et. Al. (2013)
●●● *Aspergillus foetidus*	Shah, et al., (2005)
●●● *Aspergillus niger* SL-05	Liu et al., (2008)
● *Fusarium oxysporum* VTT-D-80134	Poutanen (1987)
● *Penicilium* sp. ZH-30	Li, et al., (2008)
● *Piromyces* sp. E 2	Tenuissen et al., (1992)
●●● *Schizophyllum commune*	Steiner et al., (1987)
●● *Talaromyces emersonii* CBS 814.70	Tuohy et al., (1990)
●●● *Thermomyces lanuginosus*	Singh (2000)
●● *Trichoderma longibrachiatum*	Azin, et al., (2007)
●● *Trichoderma reesei* SAF3	Kar et al., (2006)
Yeast	
●● *Aureobasidium pullulans* Y-12311-1	Li et al., (1993)
● *Cryptococcus albidus*	Morosoli et al., (1986)
Bacteria	
● *Bacillus cereus* BSA1	Mandal et al., (2008)
● *Bacillus circulans* AB 16	Dhillon et al., (2000)
● *Bacillus megatorium*	Sindhu et al., (2006)

●●● *Bacillus pumilus*.	Batton et al., (2006)
●●● *Bacillus* sp. NCL 87-6-10	Balakrishnan et al., (2000)
●●● *Clostridium absonum* CFR – 702	Rani, (1996)
● *Pseudonomas* sp. WLUN 024	Xu et al., (2005)
●● *Streptomyces actuosus* A-151	Wang et al., (2003)
● *Streptomyces cuspidosporus*	Maheswari et al., (2000)
● *Streptomyces roseiscleroticus* NRRL-B-11019	Grabski and Jeffries, (1991)
●● *Streptomyces* sp. QG-11-3	Beg et al., (2000)
● *Thermoactinomyces thalophilus* sub group C	Kohli et al., (2001)

Bacterial xylanases

Xylanases derived from bacteria (Kulkarni and Rao, 1996; Khanongnuch et al., 1999) and actinomycetes (Garg et al., 1998; Beg et al., 2000) are effective in over a broader pH range of 5–9. The optimum temperature for xylanase activity was found between 35°C to 60°C. In industries bacterial xylanases are more fascinating for their alkali tolerance and thermostability. Higher levels of xylanase activity at alkaline pH and high temperature are reported mainly from *Bacillus* spp. (Subramaniyan et al., 1997). Some characters of xylanase from bacterial sources are listed below (Table 3).

Table 3. Characteristics of some bacterial xylanases as described by Beg et al. (2001)

Bacteria	Molecular weight (kDa)	Optimum pH	Optimum temperature (ºC)	pH stability	Temperature (ºC) stability
Acidobacterium capsulatum	41	5.0	65	3.0-8.0	20-50
Bacillus sp. W-1	21.5	6.0	65	4.0-10.0	40
Bacillus circulans WL-2	15	5.5-7.0	-	-	-
Bacillus sterothermophillus T-6	43	6.5	55	6.5-10	70
Bacillus sp.strainBP-7	22-120	6.0	55	8.0-9.0	65
Bacillus sp. BP-23	32	5.5	50	9.5-11.0	55
Bacillus polymyxa CECT 153	61	6.5	50	-	-
Bacillus sp. BPK-1	23	5.5	60	5.0-12.0	50-60
Bacillus sp. NG-27	-	7, 8.4	70	6.0-11.0	40-90
Bacillus sp. SPS-0	-	6.0	75	6.0-9.0	85
Bacillus sp. AR-009	23, 48	9-10	60-75	8.0.0-9	60-65
Bacillus sp. NCIM 59	15.8, 35	6	50-60	7.0	50
Cellulomonas fimi	14-150	5-6.5	40-45	-	-
Cellulomonas sp. 2353	22, 33, 53	6.5	55	-	-
Micrococcus sp. AR-135	56	7.5-9	55	6.5-10	40
Staphylococcus sp. SG-13	60	7.5, 9.2	50	7.5-9.5	50
Thermoacetobacterium sp. JW/SL-YS 485	24-180	6.2	80	-	-
Termotoga maritiana MSB8	40, 120	5.4, 6.2	92-105	-	-

Fungal xylanases and associated problems

Xylanase obtained from fungal sources have some fundamental problems. Due to these problems bacterial xylanases are getting more interest in the industrial applications. The problems of using fungal xylanase are listed below.

i) Most of the fungal xylanases can tolerate temperature below 50ºC and the optimum pH for activity is within of 4.0 to 6.0 (Beg et al., 2000). Industrial applications, especially the paper and pulp industries require alkaline pH and elevated temperature (more than 60ºC) and therefore, fungal xylanases are less suitable.

ii) The presence of considerable amount of cellulase activity is another problem. Reports of fungal xylanase without cellulase activity are very rare (Subramaniyan and Prema, 2000).

iii) Moreover large-scale production is often difficult with the fungi because of the slow generation time and co-production of highly viscous polymer that lower the oxygen transfer (Bernier et al., 1983). Agitation is needed to maintain the medium homogeneity, but the shearing forces in a fermenter can disrupt the fragile fungal biomass leading to the low productivity (Subramaniyan and Prema, 2000). A higher rate of agitation speed leading to hyphal disruption may again decrease xylanase production.

Regulation of xylanase biosynthesis

Xylanase is an inducible enzyme and secreted in media containing pure xylan or xylan-rich residues (Balakrishnan et al., 1997; Segura et al., 1998; Beg et al., 2001). Induction is mostly initiated by xylan. Xylan is a comparatively large heteropolysaccharide, and cannot enter into the cell matrix. Hydrolysis of it is done outside the cell by the constitutively produced xylanase. The products of xylan hydrolysis are small molecular weight xylobiose, xylotriose, and other oligosaccharides. These hydrolyzed products can enter directly into the cell matrix where they are degraded by the intracellular β-xylosidase that releases xylose residues. The oligomers are also reported to be hydrolyzed to monomers during their transportation through the cell membrane into the cell matrix by the action of hydrolytic transporters having exo-β-1, 4-bond cleaving proteins like the β-xylosidases (Conrad and Nothen, 1984). The xylose and oligoxylose residues in tern induce the biosynthesis of the enzyme (Wang et al.,

1992). In *Bacillus* sp. BP-7 (Lopez et al., 1998) and *Trichosporon cutaneum* SL409 (Liu et al., 1998), xylanase is induced by xylose, but is repressed in the presence of glucose. In some cases, readily metabolizable sugars, such as glucose and/or xylose, are suppressors of xylanase synthesis (Beg et al., 2000, Mandal et al. 2012). A hypothetical model for the regulation is focused in Figure 3.

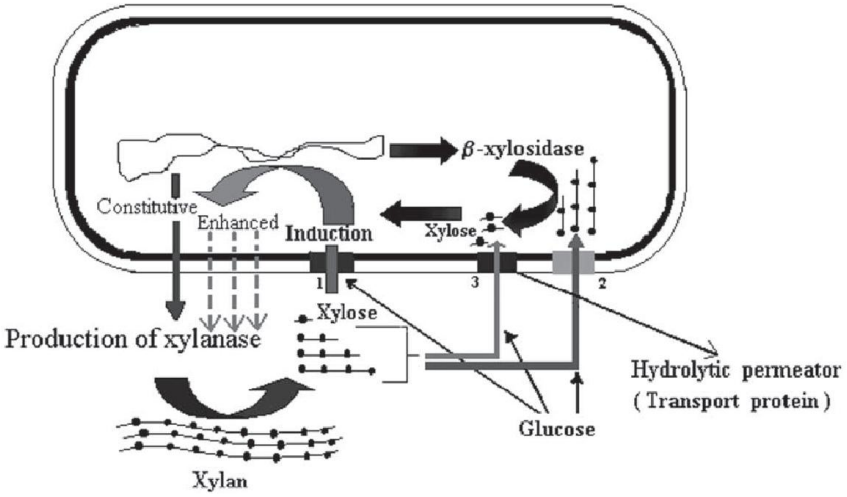

Fig 3. Hypothetical model for xylanase gene regulation in bacteria (1) xylose monomers can be easily transported through the cell membrane, which induce the enhanced xylanase synthesis, (2) the action of constitutively produced xylanases results in xylooligosaccharides, for example, xylotriose, the transportation of which into the cell later cause the enhanced synthesis, (3) the hydrolytic permeator can result in the transportation-coupled hydrolysis of xylooligomers from the constitutive xylanase action. All the cases could be affected by the presence of glucose. (Wang et al., 1992; Polizeli et al., 2005)

Applications of xylanase

Xylanases has paramount importance in different industries due to its specific catalytic ability. The major uses are-

1. Biobleaching of paper pulp

The use of xylanase leads to a reduction in organochlorine pollutants such as dioxin from the paper making process. Xylanase can reduce the requirement for oxidizing chemicals by up to 20%–40% (Garg et al., 1998; Vicuna et al., 1997). Xylanase does not harm cellulose; the strength of the paper product is not adversely affected.

The most common pulping process is the Kraft process or sulfate process where cooking of wood chips is carried out at about 170ºC for 2h in a solution of Na_2S/NaOH for degradation and solubilization of lignin. The resulting pulp has a characteristic brown color, which is primarily due to the presence of residual lignin and lignin derivatives. The bleaching of the pulp can be regarded as a clearing process involving the destruction, alteration or solubilization of the lignin, colored organic matters, and other undesirable residues (Madlala et al., 2001). Conventionally, chlorine is used for bleaching and, in fact, chlorine selectively degrades lignin in the unbleached pulp. The residual lignin is converted to water or alkali soluble products. The effluents of the bleaching process are the major contributors to wastewater pollution from the pulp and paper industries (Subramaniyan and Prema,2000). A considerable amount of xylan remains present in the fibers after the pulping by this process. Enzymatic hydrolysis of the reprecipitated and relocated xylans from the surface of fibers apparently renders the structure of the fiber more permeable. The increased permeability allows the leaching of lignin or lignin-carbohydrate molecules in higher amounts. Ligninase and xylanase were reported as the most efficient biobleaching agents. The use of xylanase was first demonstrated by Viikari et al., (1986) that resulted in the reduction of chlorine consumption. Xylanase also releases chromophores associated with carbohydrates (Patel et al., 1993). Lundgren et al. (1994) demonstrated a mill trial of TCF (total chlorine free) technology for bleaching of pulp with xylanase from *Bacillus stearothermophilus* strain T6, which has optimum activity at pH 6.5. Axelsson and Lindström, (2004) also studied the condition of TCF biobleaching during kraft pulping.

2. Improvement of animal feed

Addition of xylanase to animal feed increases digestibility, which also improves the health of animals. For example, chicken feeds contain wheat, rye and many other grains which are incompletely digested due to their hard seed coat. These grains tend to be too viscous in the intestine of chickens for complete digestion. Xylanase partially digests the grains and thereby improves digestibility (Twomey et al., 2003). It also converts hemicelluloses to small sugars so that nutrients formerly trapped on the cell walls are released (Bedford and Classen, 1992). The chicken gets sufficient energy from less feed and results in improvement of both weight and feed conversion efficiency. In a sense, the addition of xylanase to animal feed causes a kind of pre-digestion of the feed (Wu and Rabindran, 2004). Partial xylan hydrolysis in animal feed may improve cellulose accessibility to ruminal digestion and thus improve nutritional value of the feed. However, complete removal of xylan from animal feed does not appear desirable because hemicelluloses are important components of "dietary fiber" and their removal may increase bowel diseases (Dekker, 1985).

3. Bread making

Xylanases may be employed in bread-making, together with α-amylase, malting amylase, glucose oxidase and proteases. The xylanase breaks down the hemicellulose in wheat-flour, helping in the leavening the dough and make it softer. During the bread-

baking process, they delay crumb formation, allowing the dough to grow. Application of xylanase helps in increase in bread volumes, greater absorption of water and improved resistance to fermentation (Maat et al., 1992; Harbak and Thygesen, 2002). Also, a larger amount of arabinoxylo-oligosaccharides in bread would be beneficial to health. In biscuit-making, xylanase is recommended for making cream crackers lighter and improving the texture, palatability.

4. Application in solid waste treatment

Large amount of xylan is present in wastes of agricultural and food industries, therefore, xylanase can be used in treatment of these wastes for conversion of xylan into xylose. The development of an efficient process of enzymatic hydrolysis offers new prospects for treating hemicellulosic wastes (Biely, 1985; Rani and Nand, 1996).

5. Preparation of juice from fruits or vegetables

The juice industries make up a good part of the enzyme market. The production of fruit and vegetable juices requires methods of extraction, clearing and stabilization. In the 1930s, when the manufacture of citrus fruit juices began, the yields were low and problems were encountered in the filtration of the juice, owing to its turbidity. The increase in knowledge of the chemical constituents of fruits and the use of microbial enzymes helped to solve these problems. Nowadays, xylanases, in conjunction with celluloses, amylases and pectinases, lead to an improved yield of juice by means of liquefaction of fruit and vegetables; stabilization of the fruit pulp; increased recovery of aromas, essential oils, vitamins, mineral salts, edible dyes, pigments etc., reduction of viscosity, hydrolysis of substances that hinder the physical or chemical clearing of the juice, or that may cause cloudiness in the concentrate (Polizeli et al., 2005). Xylanase, in combination with endoglucanase, takes part in the hydrolysis of arabinoxylan and starch, separating and isolating the gluten from the starch in the wheat flour. This enzyme is also used in the preparation of coffee-bean mucilage (Wong et al., 1988; Wong and Saddler, 1993). The main desirable properties for xylanases for use in the food industry are high stability and optimum activity at an acid pH.

6. Improve retting of Flax fibers

Retting is the decomposition of the outer bark from the stem of the plants like flax, jute etc. This process is necessary before the fibers are processed into linen. Addition of xylanase enhances the retting process. A judicious application of the xylanolytic enzyme in conjunction with the pectinolytic enzyme results in the degumming of bast fibres of plants such as flax, hemp, jute and ramie (Sharma, 1987; Puchart et al., 1999) etc. A xylanase-pectinase combination is also used in the debarking process, which is the first step in wood processing (Wong and Saddler, 1992; Bajpai, 1999). The fibre liberation from plants is affected by retting, i.e., the removal of binding material present in plant tissues using enzymes produced *in situ* by microorganisms. Pectinases are believed to play a major role in this process, but xylanases must be involved (Sharma, 1987). Replacement of slow natural process of retting by treatment with artificial mixtures of enzymes could become a new technology of procurement of plant fibers in the near future (Bajpai, 1999).

7. Production of biofuels

Xylanase can be used for the generation of biolfuels, like ethanol and xylitol from lignocellulosic biomass (Dominguez, 1998; Eriksson et al., 2002; Sorensen et al., 2003). The biological process of ethanol production requires delignification of lignocellulose to liberate cellulose and hemicellulose from their complex with lignin. A mixture of enzyme like xylanase, mannanase, ligninase, xylosidase, glucanase, glucosidase, etc. can depolymerize the carbohydrate polymers (cellulose and hemicellulose) produces free sugars and finally fermentation of mixed pentose and hexose sugars produces ethanol.

8. Other uses

Along with that of cellulase and pectinase, xylanase can be used for the preparation of dextrans for use as food thickeners (Thompson, 1983) and the production of fluids and juices from plant materials (Biely, et al., 1985; Wood, 1985,). Xylanase may also be used to prepare rayon, cellophane, and cellulose esters, cellulose ethers (carboxy methyl cellulose, methyl and ethyl cellulose). Selected xylanases may be suitable for the production of branched/unbranched, short/long, or labeled xylooligosaccharides, model compounds for studying mechanisms of xylanase action. Similarly, some xylanases may be used to improve cell wall maceration for the production of plant protoplasts. Purified alkalophilic xylanase can improve the cleaning ability of detergents that are especially effective in cleaning stains of fruit, vegetable, soil and grass (Kumar et al., 2004).

Commercial xylanases

As the utilization of xylanase is increasing gradually, many commercial firms have come out for research and marketing of this highly potent enzyme which are available under different trade names (Table 4).

Future prospects

Since lignocellulose is an abundant and renewable resource with high potential, the biodegradation of lignocellulose has interested researchers and technologists in many fields. The xylanolytic systems of microorganisms involved in lignocellulose biodegradation, have received substantial attention in the past decade.

Production of xylanolytic systems or specific xylanases may be improved when there is more known about the induction and secretion of these enzymes. Alternatively, these goals may be achieved by mutagenesis or genetic engineering. Genetic engineering further offers the opportunity to produce single xylanolytic enzymes selected for intended applications or researches. Some of the examples of gene cloning are listed in the Table 5 The hydrolysis efficiency, hydrolysis specificity, or stability of these enzymes may also be improved by using protein engineering. These goals would be facilitated by a better understanding of the relationship between the structure and function of xylanases.

Table 4: List of some commercial xylanase suppliers

Supplier	Product trade name	Application
Alko Rajamaki, Finland	Ecopulp	Pulp bleaching
Sandoz, Charlotte, N.C. and Basle, Switzerland	Cartazyme	Pulp bleaching
Clarient, UK	Cartazyme HS 10, Cartazyme HT, Cartazyme SR 10 Cartazyme PS10, Cartazyme 9407 E,	Pulp bleaching
Genercor, Finland; Ciba Giegy, Switzerland	Irgazyme 40-4X/Albazyme 40-4X, Irgazyme-10A, Albazyme-10A	Pulp bleaching
Voest Alpine, Austria	VAI Xylanase	Pulp bleaching
Novo Nordisk, Denmark	Pulpzyme HA, Pulpzyme HB, Pulpzyme HC	Pulp bleaching
Bicon India, Bangalore	Bleachzyme F	Pulp bleaching
Rohn Enzyme 0Y; Primalco, Finland	Ecopulp X-100, Ecopulp X-200, Ecopulp X-200/4, Ecopulp TX-100, Ecopulp TX-200,	Pulp bleaching
Meito Sankyo, Japan	Nogazyme	Food
Rohm, Germany	Rholase 7118	Food
Solvay Interox, USA	Optipulp L-8000	Pulp bleaching
Thomas Swan, UK	Ecozyme	Pulp bleaching
Iogen, Canada	GS-35, HS70	Pulp bleaching
Sankyo, Japan	Sanzyme PX, Alpelase F	Feed, Food
Enzyme Development, USA	Enzeko xylanase	Baking, food, feed

Table 5: Examples of some cloned xylanase genes.

Organisms	Name of the cloned genes	Authors
Streptomyces sp. S9	*xynAS9*	Li N, Meng K, Wang Y, Shi P, Luo H (2008)
Paenibacillus sp. strain W-61	*xyn1*	Watanabe S, Viet DN, Kaneko J, Kamio Y, Yoshida S (2008)
Bacillus subtilis	xynA	Bai Y, Yang P, Yao B (2007)
Bacillus sp.	Xyl	Lu P, Feng, MG, Li WF, Hu CX (2006)
Bipolaris sorghicola	*xyn11A* *xyn11B*	Emami & Hack, (2002)
Cochliobolus sativus	*xyl2*	Emami & Hack, (2002)
Cochliobolus- heterotrophus	*xyn11A*	Emami & Hack (2002).
Fusarium oxysporumf	*xyl4* *xyl5*	Gómez E, Roncero MIG, Di Pietro A, Hera C (2001)
Claviceps purpurea	*cpxyl1, cpxyl2*	Giesbert S, Lepping HB, Tenberge KB, Tudzynski P (1998)
Magnaporthe grisea	*xyl1*	Wu SC, Kauffmann S, Darvill AG Albersheim P (1995)
Cochliobolus-carbonum	*Xyl1*	Apel PC, Pannacione DG, Holden FR, Walton JD (1993)

References

Andersson SI, Samuelson M, Ishihara 0, Shimizu K (1983) Structure of the reducing end-groups in spruce xylan. Carbohydr Res 111:83-288

Apel PC, Pannacione DG, Holden FR, Walton JD (1993) Cloning and targeted disruption of *XYL*1, a β-1,4-xylanase gene from the maize pathogen *Cochliobolus carbonum*. Mol Plant Microbe Interact 6:467-473

Aspinall GO (1980) Chemistry of cell wall polysaccharides. In Preiss J (ed) The biochemistry of plants (a comprehensive treatise), vol 3, Carbohydrates: structure and function. Academic Press, Inc., New York, pp 473-500

Axelsson P, Berggren R, Berthold F, Lindström ME (2005) Molecular mass distributions of lignin and lignin-carbohydrate complexes in birch Kraft pulps - changes caused by the conditions in the cook and their relation to unbleached pulp brightness and bleachability. J Pulp Paper Sci 31:1-20

Axelsson P, Lindström ME (2004) Influence of the conditions during birch kraft cooking on unbleached brightness, and on ECF - and TCF-bleachability. Nordic Pulp Paper Res J 19: 309-317

Azin M, Moravej R, Zareh D (2007) Production of xylanase by *Trichoderma longibrachiatum* on a mixture of wheat bran and wheat straw: optimization of culture condition by Taguchi method. Enzyme Micrb Technol 40:801-805

Bai Y, Yang P, Yao B (2007) Cloning, characterization, and expression of xylanase, a gene from *Paenibacillus* sp. DG-22 in *Escherichia coli*. J Microbiol Biotechnol 17:29-36

Bajpai P (1999) Application of enzymes in the pulp and paper industry. Biotechnol Prog 15:147-157

Balakrishnan H, Srinivasan MC, Choudhury K, Rele MV, Chsndwadkar AJ (2000) Effect of syntheticzeoites on xylanase production from an alkalophilic *Bacillus* sp. Current Sci 79:95-97

Balakrishnan H, Srinivasan MC Rele MV (1997) Extracellular protease activities in relation to xylanase secretion in an alkalophilic *Bacillus* sp. Biotechnol Lett 18:599–601

Bastawde KB (1992) Xylan structure, microbial xylanase and their mode of action. World J Microbiol Biotechnol 8:353-368

Battan B, Sharma J, Kuhad RC (2006) High level xylanase production by alkaliphilic *Bacillus pumilus* ASH under solid state fermentation. World J Microbiol Biotechnol 22: 1281-1287

Bedford MR Classen HL (1992) The influence of dietary xylanase on intestinal viscosity and molecular weight distribution of carbohydrates in rye-fed broiler chick. In: Visser J, Beldman G, vanSomeren MAK, Voragen AGJ (eds) Xylans and xylanases. Elsevier, Amsterdam, pp 361–370
Beg QK, Kappor M, Mahajan L, Hoondal GS, (2001) Microbial xylanases and their industrial application: a review. Appl Microbiol Biotechnol 56:326-338
Beg QK, Bhushan B, Kapoor M, Hoondal GS (2000) Production and characterization of thermostable xylanase and pectinase from a *Streptomyces* sp. QG-11-3. J Ind Microbiol Biotechnol 24:396–402
Bernier RJ, Michel D, Lubomir J, Michael GP (1983) Isolation and characterization of a xylanase from *Bacillus subtilis*. Appl Environ Microbiol 46: 511-514
Biely P (1985) Microbial xylanolytic systems. Trends Biotechnol 3:286-290
Brownell HH (1970) Spruce milled wood lignin and lignin-carbohydrate complex: molecular size and shape and frequency of lignin-carbohydrate linkages. Tappi 53:1278-1281
Chanda SK, Hirst EL, Jones JKN, Percival EGV (1950) The constitution of xylan from esparto grass (*Stipa tenacissima*, L.). J Chem Soc 1289-1297
Conrad D, Nothen W (1984) Hydrolysis of xylodextrins (DP 2-6) by xylanolytic enzymes from *Aspergillus niger*. In: Proceedings of the 3rd Europian Congrress of Biotechnology. Vol 2, pp169
Dahlman O, Sjöberg J (2002) In: Proceedings of the 7th European Workshop on Lignocellulosics and Pulp. Åbo, Finland, pp 111-114.
Dekker RFH (1985) Biodegradation of the hemicelluloses, In: Higuchi T (ed) Biosynthesis and biodegradation of wood components. Academic Press, Inc., Orlando, Fla. pp505-533
Dhillon A, Khanna S (2000) Production of a thermostable alkali-tolerant xylanase from *Bacillus circulans* AB 16 on wheat straw. World J Microbiol Biotechnol 16:325-327
Dominguez JM (1998) Xylitol production by free and immobilized *Debaryomyces hansenii*. Biotechnol Lett 20:53-56
Doner LW, Hicks KB (1997) Isolation of hemicellulose from corn fibre by alkaline hydrogen peroxide extraction. Cereal Chem 74:176-181
Eda S, Ohnishi A, Kato K (1976) Xylan isolated from the stalk of *Nicotiana tabacum*. Agric Biol Chem 40:359-364
Emami K, Hack E (2002) Conservation of *XYN11A* and *XYN11B* xylanase genes in *Bipolaris sorghicola*, *Cochliobolus sativus*, *Cochliobolus heterotrophus* and *Cochliobolus spicifer*. Current Microbiol 45:303-306
Eriksson O, Goring DAI, Lindgren BO (1980) Structural studies on the chemical bonds between lignin and carbohydrates in spruce wood. Wood Sci Technol 14:267- 279
Garg AP, Roberts JC, McCarthy AJ (1998) Bleach boosting effect of cellulase-free xylanase of *Streptomyces thermoviolaceus* and its comparison with two commercial enzyme preparations on birch wood Kraft pulp. Enzyme Microbial Technol 22:594– 598
Giesbert S, Lepping HB, Tenberge KB, Tudzynski P (1998) The xylanolytic system of *Claviceps purpurea*: cytological evidence for secretion of xylanases in infected rye tissue and molecular characterization of two xylanase genes. Phytopathology 88:1020-1030
Gómez-Gómez E. Roncero MIG, Di Pietro A, Hera C (2001) Molecular characterisation of a novel endo-1, 4-β-xylanase gene from the vascular wilt fungus *Fusarium oxysporum*. Current Genetics 40:268-275
Grabski AC, Jeffries TW (1991) Production, purification and characterization of β-(1-4)-endoxylanase of *Streptomyces roseiscleroticus*. Appl Environ Microbiol 57:987-992
Gruppen H, Hamer RJ, Voragen AGJ (1992) Water unextractable cell wall material from wheat flour. 2. Fractionation of alkali extracted polymers and comparison with water extractable arabinoxylans. J Cereal Sci 16:53-67
Gupta G, Sahani V, Gupta RK (2013) Optimization of xylanase production from Melanocarpus albomyces using wheat straw extract and its scale up in stirred tank bioreactor.. Indian J of Chem Technol 20: 282-289.
Harbak L, Thygesen HV (2002) Safety evaluation of a xylanase expressed in *Bacillus subtilis*. Food Chem Toxicol 40:1–8
Henrissat B, Bairoch A (1993) New families in the classification of glycosyl hydrolases based on amino acid sequence similarities. Biochem J 293:781-788
Hespell RB (1998) Extraction and characterization of hemicellulose from corn fiber produced by corn wet-milling process. J Agric Food Chem 46:2615-2619
Jacobs A, Dahlman O (2001) Characterization of the molar masses of hemicelluloses from wood and pulps employing size exclusion chromatography and matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Biomacromolecules 2:894-905
Kar S, Mandal A, Das Mohapatra PK, Mondal KC, Pati BR (2006) Production of cellulase free xylanase by *Trichoderma reesei* SAF3. Brazilian J Microbiol 37:462-464
Kato K (1981) Ultrastructure of the plant cell wall: biochemical viewpoint. Encyclopedia of Plant Physiology. New Ser pp 29-46
Khanongnuch C, Lumyong S, Ooi T, Kinoshita S (1999) A noncellulase producing strain of *Bacillus subtilis* and its potential use in pulp biobleaching. Biotechnol Lett 21: 61–63.
Kohli U. Nigam P, Singh D, Chaudhary K (2001) Thermostable, alkalophilic and cellulase free xylanase production by *Thermoactinomyces thalophilus* subgroup C. Enzyme Microbial Technol 28:606–610.
Kormelink FJM, Searle-Van Leewan MJF, Wood TM, Voragen AGJ (1991) Purification and characterization of a (1, 4)-β-arabinoxylan arabinofuranohydrolase from *Aspergillus awamori*. Appl Micribial Biotechnol 35:753-758
Kulkarni N, Shendye A, Rao M (1999) Molecular and biotechnological aspects of xylanases. FEMS Microbiol Rev 23:411–456
Kumar BK, Balakrishnan H, Rele MV (2004) Compatibility of alkaline xylanases from an alkaliphilic *Bacillus* NCL (87-6-10) with commercial detergents and proteases. J Ind Microbiol Biotecnol 31:83-87
Li N, Meng K, Wang Y, Shi P, Luo, H (2008) Cloning, expression, and characterization of a new xylanase with broad temperature adaptability from *Streptomyces* sp. S9. Appl Microbiol Biotechnol online:3 June.

Li XL, Zhang, ZQ, Dean JFD, Eriksson KEL, Ljungdahl LG (1993) Purification and characterization of a new xylanase (APX-II) from the fungus *Aureobasidium pullulans* Y-2311–1. Appl Environ Microbiol 59:3213–3218
Linder AP, Bergman R, Bodin A, Gatenholm P (2003) Mechanism of assembly of xylan onto cellulose surfaces. Langmuir 19:5072-5077
Liu C, Sun ZT, Du JH, Wang J (2008) Response surface optimization of fermentation conditions for producing xylanase by *Aspergillus niger* SL-05. J Ind Microbiol Biotechnol DOI 10.1007/s 10297-008-0330-0
Liu W, Zhu W, Lu Y, Kong Y and Ma G (1998) Production, partial purification and characterization of xylanase from *Trichosporon cutaneum* SL409. Process Biochem 33:331–326
Lopez C, Blanco A, Pastor FIJ (1998) Xylanase production by a new alkali-tolerant isolate of *Bacillus*. Biotechnol Lett 20:243–246
Lu P, Feng MG, Li WF Hu CX (2006) Construction and characterization of a bifunctional fusion enzyme of Bacillus-sourced beta-glucanase and xylanase expressed in *Escherichia coli*. FEMS Microbiol Lett 261:224-30
Lundgren KR, Bergkvist L, Hogman S, Joves H, Eriksson G, Bartfai T, Laan JVD, Rosenberg E, Shoham Y (1994) TCF Mill Trial on softwood pulp with Korsnas thermostable and alkaline stable xylanase T6. FEMS Microbiol Rev 13:365-368
Maat J, Roza M, Verbakel J, Stam H, DaSilra MJS, Egmond MR, Hagemans MLD, VanGarcom RFM, Hessing JGM, VanDerhondel CAMJJ, van-Rotterdam C (1992) Xylanases and their application in bakery. In: Visser J, Beldman G, VanSomeren MAK, Voragen AGJ (eds) Xylans and xylanases. Elsevier, Amsterdam, pp 349-360
Madlala AM, Bissoon S, Singh S, Christove L (2001) Xylanase induced reduction of chlorine dioxide consumption during elemental chlorine-free bleaching of different pulp types. Biotechnol Lett 23:345 351
Maheswari U, Chandra TS (2000) Production and potential applications of a xylanase from a new strain of *Streptomyces cuspidosporus*. World J Microbiol Biotechnol 16:257-263
Mandal A, Kar S, Das Mohapatra PK, Maity C, Pati BR, Mondal KC (2012) Regulation of xylanase Biosynthesis in *Bacillus cereus* BSA1. Appl Biochem Biotechnol 167 (5): 1052-1060
Mandal A, Kar S, Das Mohapatra, PK, Maity C, Mondal KC, Pati BR (2008) Xylanase production under submerged fermentation by newly isolated *Bacillus cereus* BSA1: parametric optimization of cultural conditions. J Pure Appl Microbiol 2:55-160
McMillan JD (1993) Pertreatment of lignocellulosic biomass. In: Himell ME, Baker JO, Overend, RP (eds) Enzymatic conservation of biomass for fuel production, American Chemical Society, Washington DC, pp 292-323
McNeil M, Darvill AG, Fry SC, Albersheiin P (1984) Structure and function of the primary cell walls of plants. Annu Rev Biochem 53:625-663
Morosoli R, Roy C, Yaguchi M (1986) Isolation and partial primary sequence of a xylanase from the *Cryptococcus albidus*. Biochem Biophys Acta 870:473–478
Patel AN, Grabski AC, Jeffries TW (1993) Chromophore release from Kraft pulp by purified *Streptomyces roseiscleroticus* xylanase. Appl Microbiol Biotechnol 39:405–412
Polizeli MLTM, Rizzatti ACS, Monti R, Ternzi HF, Jorge A, Amorim (2005) Xylanase from fungi: properties and industrial applications. Appl Micrbiol Biotechnol 67:577-591
Poutanen K, Ratto M, Puls J, Viikari L, (1987) Evaluation of different microbial xylanolytic systems. J Biotechnol 6:49-60
Puchart V, Katapodis P, Biely P, Kremnicky L, Christakopoulos P., Vrsanska M, Kekos D, Marcis BJ, Bhat MK (1999) Production of xylanases, mannanases, and pectinases by the thermophilic fungus *Thermomyces lanuginosus*. Enzyme Microbial Technol 24:355-361
Puls J, Schuseil J (1993) Chemistry of hemicelluloses: relationship between hemicellulose structure and enzyme required for hydrolysis. In: Coughlan MP and Hazlewood GP (eds) Hemicellulose and hemicellulases. Portland Press, London, pp 1-28
Rani S, Nand K (1996) Development of cellulase free xylanase producing anaerobic consortia for the use of lignocellulosic wastes. Enzyme Microbial Technol 18:23-28.
Roy N and Rowshanul Habib M. (2009), Isolation and Characterization of Xylanase Producing strain of *Bacillus cereus* from soil. Iranian J of Microbiology, 2: 49-53
Saulnier L, Thibault JF (1999) Ferulic acid and diferullic acid as component of sugar-beet pectins and maize bran heteroxylan. J Sci Food Agric 79:396-402
Saulnier L, Marot C, Chanliaud E, Thibault JF (1995) Cell wall polysaccharide interactions in maize bran. Carbohydr Polymers 26:279–287
Schulze E (1891) Information regarding chemical composition of plant cell membrane. Ber Dtsch Chem Ges 24:2277-2287
Segura BGD, Durand R, Fevre M (1998) Multiplicity and expression of xylanases in the rumen fungus *Neocallimastix frontalis*. FEMS Microbiol Lett 164:47–53
Selvendran RR (1985) Developments in the chemistry and biochemistry of pectic and hemicellulosic polymers. J Cell Sci Suppl 2:51-88
Senthilkumar SR, B. Ashokkumar, K. Chandra Raj and P. Gunasekaran (2005).Optimization of medium composition for alkali-stable xylanase production by AspergillusfischeriFxn 1 in solid-state fermentation using central composite rotary design.Bioresour. Technol. 96: 1380-1386.
Shah AR, Madamwar D (2005) Xylanase production under solid state fermentation and its characterization by an isolated strain of *Aspergillus foetidus* in India. W J Microbiol Biotechnol 21:235-243
Sharma HSS (1987) Enzymatic degradation of residual non-cellulosic polysaccharides present on dew-retted flax fibers. Appl Microbiol Biotechnol 26:358-362
Sindhu I, Chibber S, Capalash N, Sharma P (2006) Production of cellulose free xylanase from *Bacillus megatorium* by solid state fermentation for biobleaching of pulp. Current Microbiol 53:167-172
Singh S, Reddy P, Haarhoff J, Biely P, Janse B, Pillay D, Prior BA (2000) Relatedness of *Thermomyces lanuginosus* strain producing a thermostable xylanase. J Biotechnol 81:119-128

Sorensen HR, Meyer AS, Pederson S (2003) Enzymatic hydrolysis of water-soluble wheat arabiloxylan. 1. Synergy between □-L-arabinofuranosidases, endo-1, 4-β-xylanases and β -xylosidase activities. Biotechnol Bioeng 81:726-731
Steiner W, Lafferty RM, Gomes I, Esterbauer H (1987) Studies on a wild strain of *Schizophyllum commune*: cellulase and xylanase production and formation of the extracellular polysaccharide. Biotechno Bioeng 30:169-178
Subramaniyan S, Prema P (2000) Cellulasefree xylanases from *Bacillus* and other microorganisms. FEMS Microbiol Lett 183:1-7
Subramaniyan S, Prema P (2002) Biotechnology of microbial xylanases: enzymology, molecular biology, and application. Critical Rev Biotechnol 22: 33–64
Subramaniyan S, Prema P, Ramakrishna, SV (1997) Isolation and screening for alkaline thermostable xylanases. J Basic Microbiol 37:431–437
Tenuissen M J, de Kort GVM, Op den Camp HJM, Huis in t Veld JHJ (1992) Production of cellulolytic and xylanolytic enzymes during growth of the anaerobic fungus *Piromyces* sp. on different substrates. J Gen Microbiol 138:1657-1664
Thompson NS (1983) Hemicellulose as a biomass resource, In: Soltes EJ (ed) Wood and agricultural residues. Research on use for feed, fuels, and chemicals.
Tuohy M, Coughlan TL, Coughlan MP (1990) Solid-state versus liquid cultivation of *Talaromyces emersonii* on straw and pulps: enzyme productivity, In: Coughlan MP, Amaral Collaco MT (Eds) Advances in biological treatment of lignocellulosic materials. Elsevier Applied Science, London, pp153
Twomey LN, Pluske JR, Rowe JB, Choct M, Brown W, McConnell MF, Pethick DW (2003) The effects of increasing levels of soluble non-starch polysaccharides and inclusion of feed enzymes in dog diets on faecal quality and digestibility. Animal Feed Sci Technol 108:71–82
Vicuna R, Escobar F, Osses M, Jara A (1997) Bleaching of eucalyptus Kraft pulp with commercial xylanase. Biotechnol Lett 19:575–578
Viikari L, Ranua M, Kantelinen A, Sundiquist J Linko M (1986) Bleaching with enzymes. In Proceedings of the 3rd International Conference of Biotechnology on Pulp and Paper Industry. Stockholm, 16-19th June, pp 67-69
Wang P, Ali S, Mason JC, Sims PFG, Broda P (1992) Xylanases from Streptomyces *cyaneus*. In Visser J, Kusters-van-Someran MA, Voragen AGJ (eds) Xylans and Xylanases., Elsevier Science Publishers, Amsterdam BV, pp225
Wang SL, Yen YH, Shih IL, Chang AC, Chang WT, Wu WC (2003) Production of xylanases from rice bran by *Streptomyces actuosus* A-151. Enzyme Microbiol Technol 33:917–925
Watanabe S, Viet DN, Kaneko J, Kamio Y, Yoshida S (2008) Cloning, expression, and transglycosylation reaction of *Paenibacillus* sp. strain W 61 xylanase 1. Biosci Biotechnol Biochem 72:951-958
Wilkie KCB (1979) The hemicelluloses of grasses and cereals. Adv Carbohydr Chem Biochem 36:215-264
Wong KKY, Saddler JN (1992) *Trichoderma* xylanases, their properties and purification. Critical Rev Biotechnol 12:413– 435
Wong KKY, Tan LUL, Saddler JN (1988) Multiplicity of β-1,4- xylanase in microorganisms, functions and applications. Microbiol 52:305–317
Wu SC, Kauffmann S, Darvill AG Albersheim P (1995) Purification, cloning and characterisation of two xylanases from *Magnaporthe grisea*, the rice blast fungus. Mol Plant-Microbe Interact 8:506-514
Wu YB, Rabindran V (2004) Influence of whole wheat inclusion and xylanase supplementation on the performance, digestive tract measurement of broiler chickens. Animal Feed Sci Technol 116:129-139
Xu ZH, Bai YL, Shi JS, Tao WY (2005) Production of alkali tolerant cellulose free xylanase by *Pseudomonas* sp. WLUN 024 with wheat bran as the main substrate. World J Microbiol Biotechnol 21:575-581

(Acceptance date: 18.05.2015; Online available from: 04.09.2015)