CHAPTER 1

INTRODUCTION AND LITERATURE REVIEWS

1.1 Introduction

Xylanases are drawing increased attention, because of their usefulness in biotechnological applications. They might also be used in the bioconversion of lignocellulosic materials to fuels and chemicals. (Grag *et al.*,1996; Grag *et al.*,1998; and Jeffries,1996). *Streptomyces* Ab106.3, isolated from teak plantation area in Chiangmai University in order to use in waste utilization project, is able to produce xylanases. Study on production, purification, and characterization of xylanases produced by this microorganism may lead to further applications of the enzyme.

1.2 Xylan and Xylanases

1.2.1 Xylan

Wood is made up largely of lignocellulosic materials, cellulose hemicellulose and lignin, in various proportions (Erikkson *et al.*, 1990) as shown in Table 1.1

In general, plants contain 20-30 % of hemicellulosic materials (Kulkarni *et al.,* 1999). Hemicelluloses are non-cellulosic polysaccharides those are found in plant tissues as shown in figure 1.1, A) represent cell wall layers of tracheids; ML is middle lamella; P is primary cell wall; S_1.S_2, S_3 are layers of the secondary cell wall B) represent ultra structural of lignin, cellulose, and hemicellulose components. Figure 1.2. shows the arrangement of cellulose, hemicellulose, and lignin in plant cell wall a) transverse view, b) longitudinal view.

Xylan is the major constituent of hemicellulose and is second most abundant renewable resource with high potential for degradation to useful end products.

Eventually, xylan, in combination with cellulose, will supply most of the global demand for raw materials.

Table 1.1 Proportions of sugars and lignin in wood

Wood	Percent			
	Lignin[a]	Glucose[b]	Xylose[b]	Mannose[b]
Angiosperms				
Acacia koa	27.3	45.6	18.6	1.7
Acer saccharum	24.4	46.2	17.2	2.4
Alnus rubra	24.6	47.0	17.6	0.4
Betula papyrifera	19.0	44.9	24.3	2.1
B. verrucosa	21.7	35.1	20.7	0.9
Populus tremuloides	22.0	46.2	18.9	1.6
Quercus rubra	24.5	41.6	23.5	3.0
Tilia americana	21.5	43.9	16.1	0.3
Ulmus americana	23.6	55.8	16.0	3.1
Gymnosperms				
Abies balsamea	29.1	46.6	5.6	11.7
Picea abies	27.1	41.6	5.2	13.6
P. mariana	26.6	49.0	7.3	13.8
Pinus banksiana	29.9	44.6	8.4	10.0
P. resinosa	27.9	44.9	8.4	12.3
P. strobus	28.1	48.2	6.0	15.6
P. sylvestris	30.0	38.3	6.5	11.1
Tsuga canadensis	32.3	47.9	4.2	13.8

[a] Sulfuric acid lignin by the method of Effland (1977).
[b] HPLC analysis using method of Pettersen et al. (1985).

Source: Erikkson *et al.*: 1990

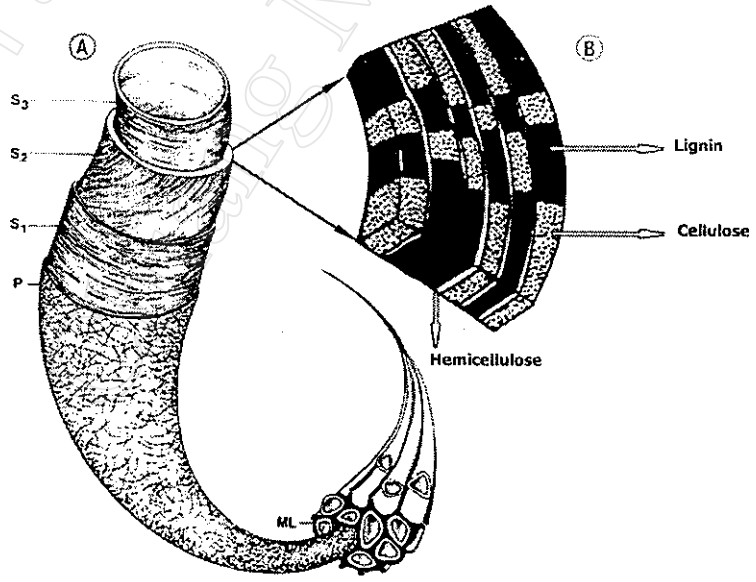


Figure 1.1 Cell wall layer of tracheids and ultrasturctural arrangement of lignocellulosic compounds.

Source: Erikkson *et al.*: 1990

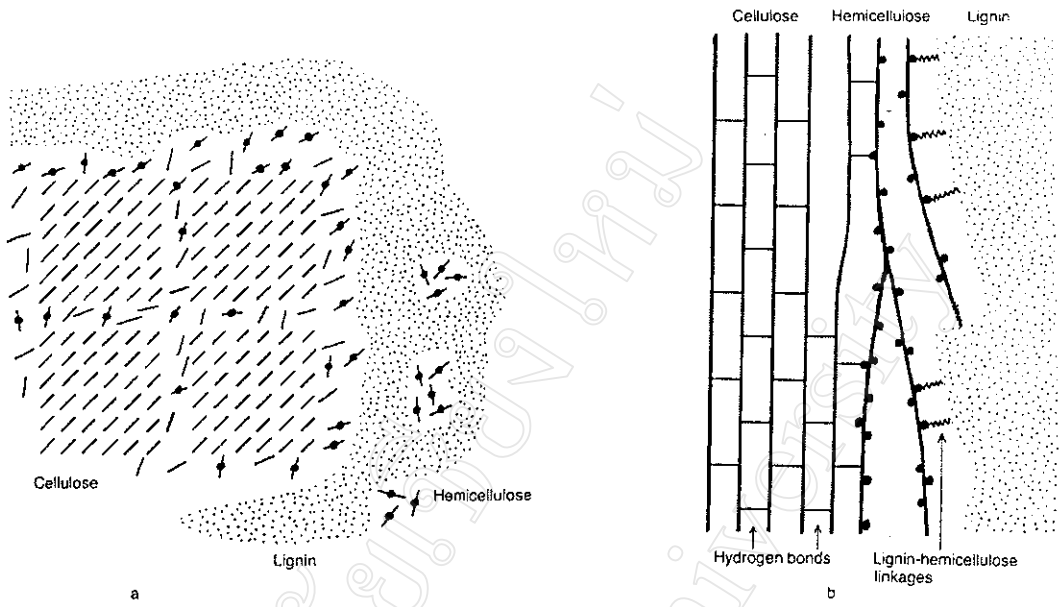


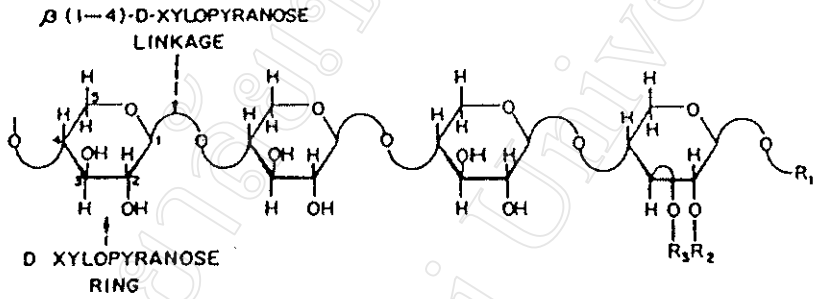
Figure 1.2 Model of cellulose, hemicellulose, and lignin
Source: Eaton and Hale : 1993

Xylan is the most common hemicellulosic polysaccharide in the cell wall of land plants, representing more than 30% of dry weight. In general, xylan is the major hemicellulosic material in wood from angiosperms, but is less abundant in wood from gymnosperms, which accounts for approximately 15-30 % and 7-12 % of total dry weight, respectively. (Sunna and Antranikian, 1997, Wong *et al.*,1998, Kulkarni *et al.*, 1999)

Xylan consists of 1,4-linked-β-D-xylopyranosyl. The β-1,4-xylans are heterogeneous polysaccharides found in the cell walls of all land plants. These polysaccharides are mainly found in secondary cell walls, the major component of mature cell walls in wood tissue (Erikkson *et al.*, 1990, and Wong *et al.*, 1998).. Most xylans occur as heteropolysaccharides, containing different substituent groups in the backbone chain and in the side chain. The common substituent groups found on the xylan backbone are acetyl, arabinosyl, and glucuronosyl residues. These groups are the limiting factor in complete degradation of xylan; they mainly comprise steric hindrance to enzyme substrate complex formation. (Erikkson *et al.*, 1990, Sunna and Antranikian, 1997 , Wong *et al.*, 1998).

1.2.2 Structure and Chemical Properties of Xylan

Xylan is a xylose-based hemicellulose in both softwood and hardwood. The xylose units are linked together by β-D-1,4 bond, which are similar to the linkage of glucose units in cellulose. Generally, They are categorized as linear homoxylan, arabinoxylan, glucuronoxylan and glucuronoarabinoxylan (Erikkson *et al.*, 1990, Kulkarni *et al.*, 1999). The basic backbone and the possible substituent groups are shown in Figure 1.3



R_1:
- p Coumaric acid
- α-D-Glucuronic acid
- α-D-Arabinofuranose

R_2:
- Acetyl group
- α-D-Glucuronopyranose [C_α Lignin]
- 4-O-Methyl-α-D-Glucuronopyranose [C_α Lignin]
- α-L-Arabinfuranose [C_α and C_β Lignin, Ferulic acid, Acetyl group, p Coumaric acid]
- C_α Lignin

R_3:
- Acetyl group
- α-L-Arabinofuranose [C_α and C_β Lignin, Ferulic acid, acetyl group, p Coumaric acid]
- β-D-Galactopyranosyl (1—5) α-L-Arabinofuranose
- β-D-Xylopyranosyl (1—2) α-L-Arabinofuranose
- α-L-Arabinofuranosyl(1—2,1—3,1-2,3 Arabinofuranose)n
- β-D-Galactopyranosyl (1—4) D-Xylopyranosyl(—2)-α-L-Arabinofuranose

Figure 1.3 The basic backbone of xylan and the substituent groups
Source: Kulkarni *et al.* : 1999

In various plants, xylans are usually present partly acetylated form. The presence of acetyl groups may responsible for the partial solubility of xylan in water.

These groups are readily removed when xylan is treated with alkali extraction (Sunna and Antranikian, 1997).

Acetylating occurs more usually at the C-2 and C-3 positions of xylose units. These acetyl groups block the access of xylanolytic enzymes to xylan backbone, probably by steric hindrance. The small amounts of feruric acid, *p*-coumeric acids, and hydroxy cinnamic acids also present as substituent groups. Arabinose and ester linkage of glucuronic acid crosslink to lignin, feruryl reisdues may also crosslink xylan and lignin. (Sunna and Antranikian, 1997, and Kukani *et al.,* 1999).

The xylan in hardwood is *O*-acetyl-4-*O*-methylglucuronoxlan. This polysaccharide consists at least of ten xylose units, average degree of polymerization (DP) is between 150 and 200. Figure 1.4 shows the structure of *O*-acetyl-4-*O*-methylglucuronoxylan. Every tenth xylose unit usually carries a 4-*O*-methylglucuronic acid attached to the C-2 of xylose. Hardwood xylans are highly acetylated, in birch wood; acetyl group content is more than 1 mol per 2 moles of xylose. (Erikkson *et al.,*1990, Sunna and Antranikian, 1997)

Softwood xylans are composed of arabino-4-*O*-methylglucuronoxylan as shown in figure 1.5. They contain a higher 4-*O*-methylglucuronic acid than those of hardwood xylans. The 4-*O*-methylglucuronicacid residues are attached to the C-2 position. Softwood xylans are not acetylated, and instead of acetyl groups they have α-L-arabinofuranose units linked by α-1,3-glycosidic bonds to the C-3 position of the xylose units.

The arabinose substituents occur on almost 12% of the xylosyl residues. The proportion of β-D-xylopranose, 4-*O*-methyl- α-D-glucuronic acid and L-arabinofuranose is 100 : 20 : 13. In general, softwood xylans are shorter than hardwood xylans, with DP between 70 and 130, they are also less branched (Sunna and Antranikian, 1997, Wong *et al.,* 1998)

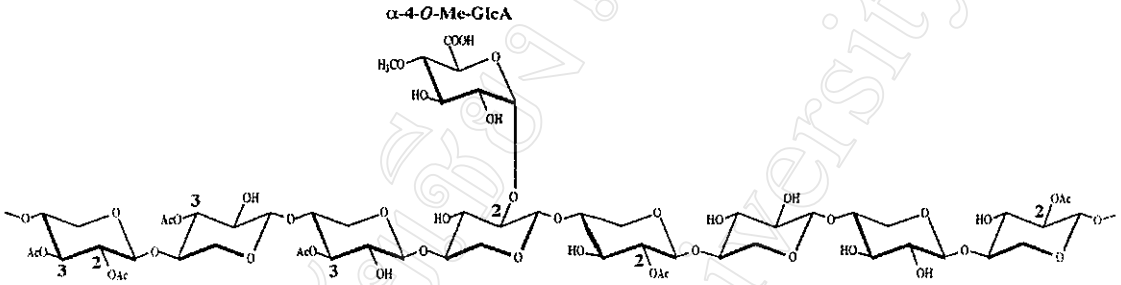


Figure 1.4 Compositions of *O*-acetyl-4-*O*-methylglucuronoxylan

Source: Sunna and Antranikian: 1997

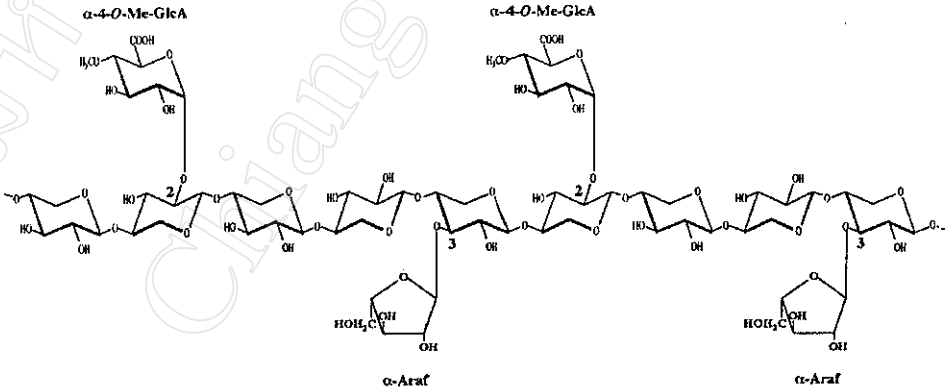


Figure 1.5 Compositions of arabino-4-*O*-methylglucuronoxylan

Source: Sunna and Antranikian: 1997

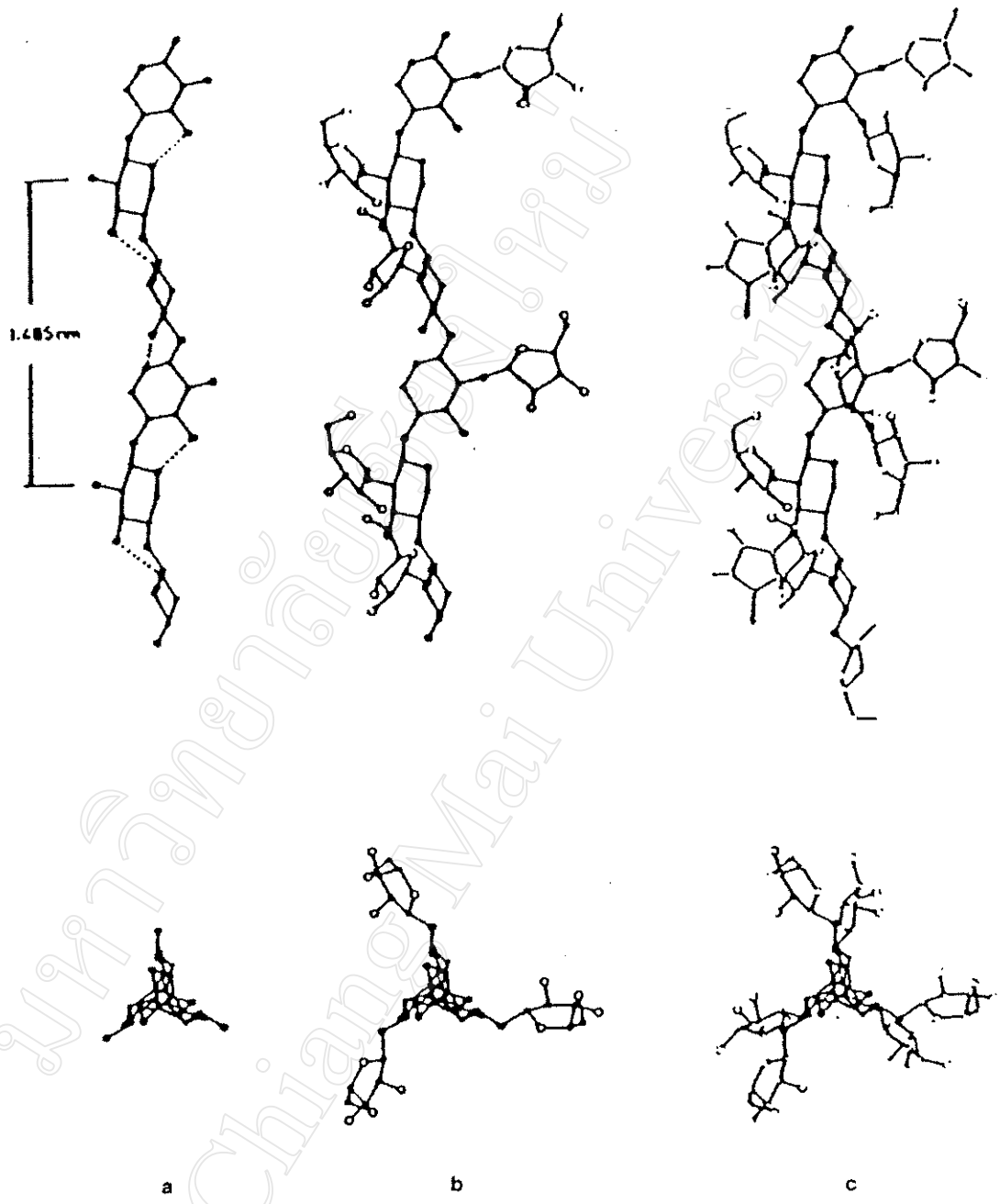


Figure 1.6 Three-dimensional structure of xylan

Source: Adapted from Kulkarni *et al.*: 1999

The three-dimensional structure of the xylan molecule has been described in 1992 by Atkins. The xylan backbone shows a three fold left-handed conformation under crystallized conditions; the side chains do not affect the geometry of the glycosidic linkage as show in figure 1.6, a) xylan backbone; b) xylan backbone and one L-

arabinose; c) xylan backbone and two L-arabinose. Electron diffraction patterns also confirm the three-fold conformation and show that the chains are organized in a trigonal lattice with hexagonal morphology. (Kulkarni *et al.*: 1999)

1.2.3 Xylanases

Since, the structure of xylan is variable, involving not only both linear backbones, but also branched with substituent groups. Then, the assemble of enzymes is required to archive the complete degradation of xylan.

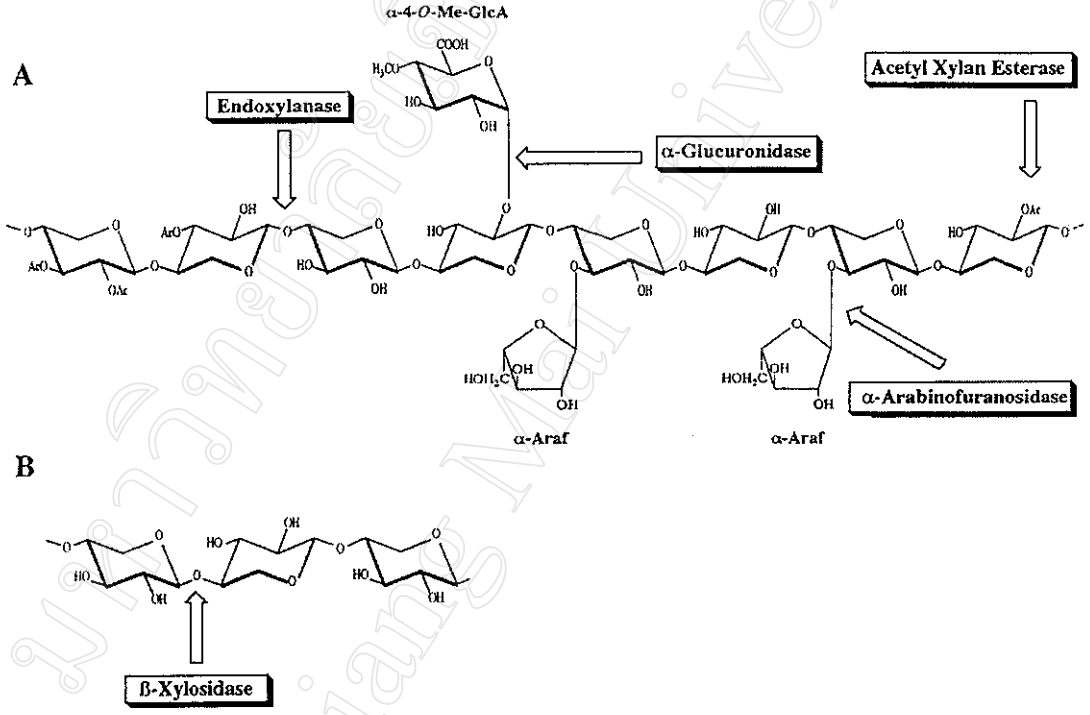


Figure 1.7 Mechanisms of xylanases

Source: Adapted from Sunna and Antranikian : 1997

Xylanolytic enzymes are a group of enzymes, which can degrade xylan. Generally, the members of them are endo-1,4-β-xylanase (EC 3.2.1.8), β-xylosidase (EC 3.2.1.37), α-glucuronidase (EC 3.2.1.-), α-L-arabinofuranosidase (EC 3.2.1.55), and acetylxylanesterase (EC 3.1.1.6). Among these enzymes, the endoxylanase is the well known and best characterized. Figure 1.7 shows the hydrolysis of xylan A) The

xylanolytic enzymes involved in the degradation of xylan. B) Hydrolysis of xylooligosaccharides by β-xylosidase

These enzymes are the major components of xylanolytic systems produced by bio-degradative microorganisms such as fungi and bacteria, whose activities are important from the maintenances of carbon flow in the carbon cycle and thus, biomass turnover in nature (Wong *et al*, 1998, Sunna and Antranikian, 1997).

Xylanolytic enzymes are widely spread in nature. They have been reported to produced in marine and terrestrial bacteria, rumen and ruminant bacteria, fungi, marine algae, protozoa, snails, crustaceans, insects and seeds of terrestrial plants. The functions of xylanolytic enzymes are biodegradation in order to provide a source of metabolized energy as found in the degradation of plant cell wall components in interaction with other polysaccharide degrading enzymes, degradation of xylan during germination of barley, and digestion of dietary fiber (Sunna and Antranikian, 1997)

1.2.4 Types of xylanases

1.2.4.1 Endo-β-1,4-xylanase

Both fungi, bacteria, produce this enzyme. However, xylanases from fungi have been more extensively studied because the amount of enzyme production is larger than the latter. (Erikkson, 1990).

Endo-β-1,4,-xylanase cleaves the internal glycosidic linkages of the branched xylan backbone, resulting in a decrease DP (degree of polymerization) of the substrate. The attack of the substrate is no random, and the bonds to be hydrolyzed depend on the nature of the substrate, length, and degree of branching of substrate or present of substituent groups (Li *et al.*, 2000). During the early course of hydrolysis of xylan, the main products formed are xylooligosaccharides. As hydrolysis proceeds, these oligosaccharides will be further hydrolyzed to xylotriose, xylobiose, and xylose.

Endo-β-1,4-xylanase could be grouped into those that are basic proteins (High pI) with MW below 30,000 and those that are acidic (Low pI) with MW>30,000

However, there are several exceptions to this general pattern, Endoxylanase that has low pH and low MW value has been reported, and vice versa. Most characterize Endoxylanase is optimally active at temperature range between 45 and 75 ^{o}C (Sunna and Antranikian, 1997)

1.2.4.2 β-xylosidase

β-D-xylosidase (β-D-xyloside xylohydrolase; EC 3.2.1.37) is exoglycosidase that hydrolyzes short xylooligosaccharides and xylobiose from non-reducing end to liberate xylose. True β-xylosidase is able to cleave artificial substrates such as *p*-nitrophenyl β-D-xyloside which has been reported in bacteria and fungi (Erikkson, 1990,Clark, *et. al.*, 1996, Saxena *et al.*,1995, Kitamono *et al.*,1999). There are larger enzymes with molecular weights between 60-360 kDa, and they may be mono- or dimeric proteins and shown in Table 1.2

β-xylosidase appears to be mainly cell associated in bacteria and yeast. However, extracellular β-xylosidase activity has also been reported (Belfaquih and Penninckx, 2000). An important role of β-xylosidase seems to be relieving the end product inhibition of Endoxylanase. Most of the reported β-xylosidase shows the highest activity toward xylobiose and no activity toward xylan.(Erikkson, 1990, Sunna and Antranikian, 1997, and Wong *et al.*, 1998)

Table 1.2 Properties of Purified Fungal and Bacterial β-Xylosidase

Organism	MW (kDa)	Subunit (kDa)	Form	pI
Fungi				
Aspergillus awamori	110.0		Monomeric	4.2
A. niger	78.0		Monomeric	N.D.[a]
A. oryzae	168.0	82.0	Dimeric	4.1
A. pulverulentus	180.0	65.0	Trimeric	4.7
	190.0	100.0	Dimeric	3.5
Aureobasidium pullulans	240.0	121.0	Dimeric	<3.0
Chaetomium trilaterale	240.0	118.0	Dimeric	4.8
Emericella nidulans	240.0	116.0	Dimeric	3.2
Neurospora crassa	83.0		Monomeric	4.3
Penicillium wortmanni	100.0		Monomeric	5.0
Pichia stipitis	34.0		Monomeric	N.D.[a]
Talaromyces emersonii	181.0	97.5	Dimeric	8.9
Trichoderma reesei	100.0		Monomeric	4.7
T. viride	101.0		Monomeric	4.4
Bacteria				
Bacillus pumilus	130.0	70.0	Dimeric	4.4
B. stearothermophilus	150.0	75.0	Dimeric	4.2
Clostridium acetobutylicum	224.0	85.0 63.0	Dimeric	5.8
Thermoanaerobacter ethanolicus	165.0	85.0	Dimeric	4.6
Thermotoga sp. FjSS3-B.1	174.0	92.0	Dimeric	4.1

[a] N.D.: not determined.

Source: Sunna and Antranikian : 1997

1.2.4.3 α-L arabinofuranosidase

L-arabinose is one of the substituent groups on xylan. Although the amount of this sugar is relatively small, restrict the hydrolysis of hemicellulose by xylanases. The definition of α-L-arabinofuranosidase is that it hydrolyzes non-reducing α-L-arabinofuranosyl residues of α-L-arabinofruanosides, arabinans, arabinoxylans, and arabinogalactans (Erickkson, 1990).

α-L arabinofuranosidases are produced by fungi and bacteria (Debeche *et al.*, 2000, Gilead and Shoham, 1995, Matsuno *et al.*,2000, Nogawa *et al.*,

1999, Spagnuolo *et al.* 1999). There are two types of arabinases, the exo-acting α-L-arabinofuranosidase (EC 3.2.1.55), which is active against *p*-nitrophenyl-α-L-arabinofuranosidase and on branched arabinans, and the endo-1,5-α-L-arabinase (EC 3.2.1.99), which is active only toward linear arabinans. The size of native arabinofuranosidases may reach up to 495 kDa and are found in mono-, di-, tetra, hexa-, and octameric forms as shown in table 1.3 (Sunna and Antranikina, 1997).

α-L-arabinofuranosidases are capable of hydrolyzing both 1,3- and 1,5-α-L-arabinofuranosyl linkages in arabinoxylan, which have been reported in *A. niger, B. subtilis.* The *A. niger* enzyme first attacks the α-L-1,3-linked arabinofuranosyl residue in arabinan to extent of 30% and then proceeds with the slow attack of the α-L-1,5-arabinan, which will then be completely converted to arabinose. (Clark *et al.*, 1996, Erikkson, 1990, Sunna and Antranikian, 1997)

Multiple forms of α-L-arabinouranosidase in *Streptomyces* have been reported. (Matsuno *et al.*, 2000 ,Sunna and Antranikian, 1997).

Synergism between α-L-arabinofurnosidase and xylanases have been reported. An increase in xylose, xylobiose and arabinose production was observed when both enzymes were used simultaneously (Gilead and Shoham, 1995, and Sunna and Antranikian, 1997).

1.2.4.4 α-Glucuronidase

The 4-O-methylglucuronic acid side chains in xylan are a steric hindrance for the hydrolysis of the xylan backbone by endo-β1,4-xylanase in the vicinity of the branching points. The 4-O-methyl-D-glucuronic acid residues are linked α-glycosidically to the O-2 position. (Erikkson *et al.*,1990).

Table 1.3 Occurance and Properties of α-Arabinofuranosidases

Organism	MW (kDa)	Subunit (kDa)	Form	pI
Fungi				
Aspergillus niger	53.0		Monomeric	3.6
A. niger 5-16	67.0		Monomeric	3.5
Phanerochaete chrysosporium	55.0		Monomeric	7.3
Talaromyces emersonii	210.0	105.0	Dimeric	3.5
Trichoderma reesei	53.0		Monomeric	7.5
Bacteria				
Bacillus polymyxa	166.0	65.0 33.0	Dimeric	4.7
B. subtilis	65.0		Monomeric	5.3
B. stearothermophilus	256.0	64.0	Tetrameric	6.5
Bacteroides xylanolyticus	364.0	61.0	Hexameric	N.D.[a]
Butyrivibrio fibrisolvens	240.0	31.0	Octameric	6.0
Clostridium acetobutylicum	94.0		Monomeric	8.2
Ruminococcus albus	310.0	75.0	Tetrameric	3.8
Streptomyces sp. 17-1	92.0		Monomeric	4.4
Streptomyces diastaticus	38.0 60.0		Monomeric Monomeric	8.8 8.3
S. purpurascens	495.0	62.0	Octameric	3.9
Thermomonospora fusca	92.0	46.0	Dimeric	N.D.[a]

[a] N.D.: not determined.

Source: Sunna and Antranikian : 1997

Table 1.4 Occurrence of Microbial α-Glucuronidases

Organisms
Fungi
Agaricus bisporus
Aspergillus niger
Pleurotus ostreatus
Schizophyllum commune
Thermoascus aurantiacus
Trichoderma reesei
Bacteria
Fibrobacter succinogenes
Streptomyces flavogriseus
S. olivochromogenes
Thermoanaerobacterium sp. JW/SL-YS485

Source: Sunna and Antranikian : 1997

α-D-glucuronidase (3.2.1.-) hydrolyzes the α-1,2 linkages between glucuronic acid and xylose residues in glucuronoxylan. (Sunna and Antranikian, 1997, Tenkanen and Siika-aho, 2000). The early studies of this enzyme is in fungi. The occurrence of α-D-glucuronidase in some fungi and bacteria are listed in table 1.4

1.2.4.5 Acetyl xylan esterase

Acetyl groups have long been known to be present as substituents in various plant polysaccharides, hemicellulose and pectin. In glucuronoxylans, 60%-70% of the xylose residues are esterified. Alkaline treatment removes acetyl residues from wood hemicellulose. Acetyl xylan esterase was first reported in fungal culture (Erikkson *et al.*, 1990).

Acetyl xylan esterase (EC 3.1.1.6) removes the O-acetyl substituents at the C2 and C3 positions of xylose residues in acetylxylan. First report of acetyl

xylan esterase production was 1985. Acetyl groups present on the xylan backbone inhibit the action of xylanases by steric hindrance. Acetyl xylan esterase releases acetic acid from xylan, thereby forming new unsubstituted sites on the xylan backbone. This, therefore, increases the susceptibility of the polymeric xylan to endoxylanase. (Sunna and Antranikian, 1997)

1.2.4.6 Feruric and *p*-coumeric acid esterases

Ferulic and *p*-coumeric acid are substituent groups on xylan backbone, which inhibit the action of xylanases by steric hindrance. They are linked to xylan by ester bonds. Feruric acid esterase (EC 3.1.1.-) cleaves the ester linkages between arabinose and *p*-coumeric acid. These enzymes were first described by MacKenzies *et al.* (Sunna and Antranikian, 1990).

1.2.5 Xylanase Production

Fungal and bacterial xylanases are generally associated with cellulases (Haapala, *et al.,* 1996. Kluepfel, *et. al,* 1986, MacKenzie *et al.*, 1987, Van Zyl, 1985, Vlave *et. al.*, 1997). The mechanisms those govern the formation of extracellular enzymes with reference to carbon sources present in the medium are influenced by the availability of precursors for protein synthesis. Therefore, in some fungi, growing the cells on xylan not contaminated by cellulose under a lower nitrogen/carbon ration in the medium may by one of the strategies for producing xylanolytic systems free of cellulases. However, cellulosic substrates were also found to be essential in the medium for maximum xylanase production by *Clostridium sterecoraium, Thermonospora curvata* and *Neurospora crassa* (Kulkarni, *et. al.*, 1999). Cheaper hemicellulosic substrates such as orange peel (Ismail, 1996) corn cob (Christov *et al.,*1999, Rani and Nand 2000, Singh, *et al.*, 2000b), wheat bran (Archana and Satyanarayana, 1997,Beg *et al.*, 2000, Liu *et. al.*, 1998), rice bran, rice straw, corn stalk, and baggase have also been found to be most suitable for the production of xylanases, in the case of certain microorganisms such as *Aspergllus awamori* (Sidenberg *et al.,*1997), *Penicillium purpurogenum* and alkaliphilic thermophilic

Bacillus sp. NCIM 59 (Kulkarni *et al.*, 1999), *Streptomyces albogriseolus, Streptomyces nitrosporous, Micromonospora melanospora* (Van Zyl, 1985), *Streptomyces* HM-15 (Patel and Ray, 1994). The xylanases activity is found to be higher in fungi than in bacteria (Haltrich, *et al.*, 1997). Actinomycetes and bacteria exhibit near-neutral pH optima for growth and enzyme production (Van Zyl, 1985, Patel and Ray, 1994), in contrast to the general acidic pH requirements of fungi (Kuhad *et al.*, 1998, Vlaev *et al.*, 1997). However, certain alkaliphilic bacilli are known having pH optima for growth and enzyme production at alkaline pH (Erikkson, 1990, Kulkarni *et al.*, 1999,Shah *et al.*, 1999).

1.2.6 Factors Affecting Xylanase Yield

When xylanase production is carried out on complex heterogeneous substrates, various factors have a combined effect on the level of xylanase expression (Haltrich *et al.*, 1997). They include substrate accessibility, rate and amount of release of the xylooigosaccharides and their chemical nature and quantity of xylose released-which acts as the carbon source and as an inhibitor of xylanase synthesis in most of the cases. The slow release of the inducer molecules and the possibility of the culture filtrate converting the inducer to its non-metabolizable derivative are believed to boost the level of xylanase activity.

The xylanases bind tightly to the substrate. Ionic interaction appeared to plays an important role in the binding of xylanase to xylan (Tenkanen *et al.*, 1995). At 4 °C, the absorption of xylanase from *Bacillus* sp. on straw, lignin, and insoluble xylan was irreversible (Zillox and Debeire, 1998). Due to a binding property, some amount of xylanases produced during the fermentation is often lost and discarded, as bound enzyme, along with the insoluble substrate such like baggase and corncob, for example. The metabolic enzymes of the xylanase producer such as protease and transglycosidases also affect the actual yield of the enzyme (Kulkarni, *et al.*,1999). These enzymes are optimally expressed at the end of the exponential phase; and the harvesting time of the xylanases must be correlated to the production of these enzymes on the medium under consideration. Other bioprocess parameters those can affect the activity and productivity of xylanase attained in a

fermentation process including the composition of culture medium, pH, temperature, and agitation (Kulkarni, *et al.,* 1999).

Addition of Tryptone and media modification enhanced more than two and a half folds of xylanase yield obtained from *Bacillus circulan* AB16 (Dhillon *et al.,* 2000). Xylanase production by *Fusarium oxysporum* was increased when peptone and wheat bran were added to the culture medium (Kuhad *et al.,* 1998). Tween 80 has been reported to be a stimulator for xylanase production in *Trichosposon cutaneum* SL409. (Liu *et al.*, 1998), *Fusarium oxysporum,* (Kuhad *et al.*, 1998).

In xylanase production by *Aspergillus awamori,* using wheat bran as a carbon source, when agitation rate increased growth and xylanase production decreased. It was suggested that higher agitation rate reduced the intimate contact between pellets and the wheat bran. Substrate particle size also affected to xylanase production. When ground wheat bran was applied, the growth and substrate uptake rate increased, but the xylanase production decreased due to less contact between the fungus and the wheat bran (Siedenberg *et al.,* 1997).

1.2.7 Regulation of Xylanase Synthesis

Xylanase productions by various bacteria and fungi have been shown to be inducible. In general, the xylanase induction is a complex phenomenon and the level of response to an individual inducer varies with organisms. The substrate derivatives and the enzymatic end products may often play a key positive role in the induction of xylanases (Samain *et al.,* 1997); they can also act as the end product inhibitors, possibly at much higher concentrations. (Kulkarni *et al.,* 1999)

1.2.7.1 Induction of xylanase

Xylan, being a high molecular mass polymer, cannot penetrate the cell wall. The low molecular mass fragments of xylan play a key role in the regulation of xylanase biosynthesis. These fragments include xylose, xylobiose,

xylooligosaccharides, heterodisaccharides of xylose, glucose and their positional isomers. These molecules are liberated from xylan by the action of small amounts of constitutively produced enzyme. In general, xylan is the potential inducer for xylanase production (Bahkali, 1996, Christov *et al.,* 1999, Rani and Nand, 2000, Tuncer *et al,* 1999). Cellulose has also been shown to act as an inducer of the xylanase in a few cases (Bahkali, 1996, MacKenzie *et al.,* 1987). In *Streptomyces* sp. xylanase activity appears to increase with the crystallinity of the cellulosic substrate (Morosoli *et al.*, 1986). Sugarcane baggase is found to be the best inducer of xylanase and β-xylosidase in *Cellulomonas flavigena* (Kulkarni *et al.,* 1999), *Streptomyces* HM-15 (Patel and Ray, 1994), *Streptomyces albogriseolus, S. nitrosporus* , and *Micromonospora melanospora* (Van Zyl, 1985).

A synergistic effect on the synthesis of cellulases and hemicellulases was observed when cellulose and hemicellulose were used together as the carbon source. In the presence of xylose, higher enzyme yields were obtained from *Bacillus pumilus* , *Streptomyces lividans 66* , and *Aureobasidium pullulans* (Kulkarni *et al.*, 1999)

The level of inducers and/or the required enzymes in the culture filtrate also affect the xylanase synthesis. The possible factors affecting xylanase induction have been diagrammatically represented in figure 1.8 (Kulkarni *et al.*, 1999).

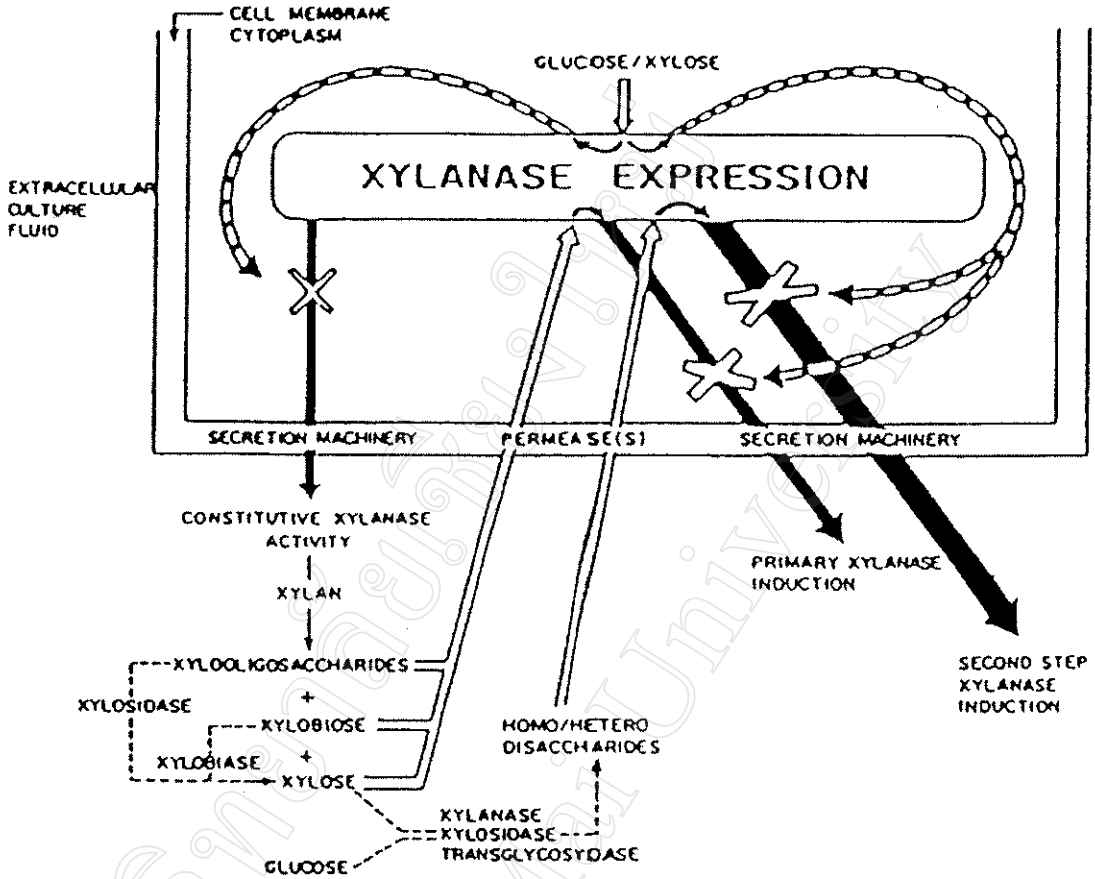


Figure 1.8 Xylanases regulation and expression

Source: Kulkarni *et al.* : 1999

1.2.7.2 Regulation at the molecular level

A separate regulation for the formation of xylanase and cellulase has been reported in a few microorganisms. Esteben *et al.* (1999) have reported that xylanase was undetected in glucose grown culture of *Bacillus circulans* WL-12 but xylose, mannose and cellobiose supported xylanase production. The xylanase and xylosidase in *Butyrivibrio fibrisolvens* GS 113 were shown to be under coordinate control. They were induced by xylan and repressed by glucose. An analysis of DNA fragments containing β-xylanase genes from *B. pumilus* indicated that the xylanase and xylosidase genes were closely

associated and were linked in a 14.4 kb DNA fragment. However, they did not appear to be controlled by the same operon (Kulkarni *et al.*, 1999).

1.2.7.3 Catabolite repression

Catabolite repression by glucose is a common phenomenon observed in xylanase biosynthesis. Catabolite repression of xylanase gene appeared to be controlled at two levels, directly by repression of gene transcription and indirectly by repression of transcriptional activator. The same pattern of regulation was observed in *A. niger* and *A. nindulans*. (Kulkarni *et al.*, 1999), but there were some exceptions. The xylanase biosynthesis by *Thermonospora curvata*, and mutant of *Cellulomonas biazotea* were not repressed by glucose (Busch and Stutzenberger, 1997, Rajoka *et al.*, 1997). Glycerol was reported to be a repressor of xylanase production in *Streptomyces* sp. EC10 (Belfaquih and Penninckx., 2000).

It was found that enzyme synthesis was repressed when easily metabolizable carbon sources were present in the growth medium, suggesting that transition state regulators and catabolite repression control the synthesis of the enzyme. The biosynthesis of xylanase occurs several hours after the depletion of the inducer added to the medium, in contrast to the synthesis of β-xylosidase, which has very short induction periods. (Kulkarni *et al.*, 1999)

1.2.8 Biochemical properties of xylanases

The available information about the properties of xylanase stems mostly come from studies on bacterial and fungal enzymes. Microbial xylanases are single sub unit proteins with molecular masses in the range of 8-145 kDa (Sunna and Antranikian, 1997). In general, the optimum temperature for endoxylanase from bacterial and fungal sources varies in the range of 40-80 °C (Breccia, 1998, Cesar and Mrsa, 1996, Gessesse, 1998, Ismail, 1996, Kuhad *et al.*, 1998, Liu, *et. al*, 1998, Shah *et al.*, 1999). Xylanases from different organisms are usually stable over a wide pH range 3-10 (Baraznenok *et al.*,1999, Christakopoulos, 1996, Gessesse,

1998, Jain *et al.,* 1998) and show optimum pH in the range of 4-8 (Cesar and Mrsa, 1996, Gessesse, 1998, Ismail, 1996, Shah *et al.,* 1999). The amino acid compositions of xylanases, reported from various sources, indicate predominantly aspartic acid glutamic acid, Glycine, serine and threonine. (Kulkarni *et al.,* 1999)

1.2.8.1 Carbohydrate content (Kulkarni *et al.,* 1999)

The occurrence of glycosylated enzymes are common phenomena among many eukaryotic xylanases. The xylanases from prokaryotic sources, such as *Clostridium stercorarium Streptomyces* sp. and alkaliphilic, thermophilic *Bacillus* sp., were found to be glycoproteins. Carbohydrate groups are covalently liked with protein or are present as dissociable complexes with xylanases. Gycosylaton has been implicated in the stabilization of glycanases against extreme environments. It has been suggested that both differential glycosylation and proteolysis may contribute to the multiplicity of xylanases. (Breccia *et al.,* 1998, Kulkarni *et al.,* 1999)

1.2.8.2 Substrate specificity

Different xylanases have different activities against various xylan structures. The key factors that influence the rates of xylan hydrolysis are chain length and degree of substitution. (Li *et al.,* 2000).

An endoxylanase from *Streptomyces roseiscleorticus* has also been shown to be a debranching type of enzyme. It is commonly observed that substituents in the highly branched polysaccharides interfere with xylanase activity. The xylanases also vary in their activities against various cellulosic substrates. The relaxed specificity of some xylanases and the more restricted specificity of others could be due to differences between residues involved in the catalytic groups. Generally, xylanases appear to be specific toward the intersugar linkage. (Kulkarni *et al.,* 1999)

1.2.8.3 Thermophilic xylanases

Despite the occurring of xylan-degrading enzymes in actinomycetes, comparatively little information about xylanases from the thermophilic actinomycetes is available, other microbial xylanases were shown in the table 1.5

1.2.8.4 Mechanism of action of the xylanases

It has frequently been suggested that the catalytic mechanism of glycosidases resemble that of lysozyme. The hydrolysis reaction, catalyzed by xylanases as well as cellulases, proceeds through an acid-base mechanism involving two residues. The first residue acts as a general catalyst and protonates the oxygen of the osidic bond. The second residue acts as a nucleophile which, in the case of retaining enzymes, interacts with the oxocarbonium intermediate or promotes the formation of an OH^- ion from a water molecule, as observed for inverting enzymes. Reaction with retention of configuration involves a two-step mechanism in which proton transfer occurs to and from an oxygen atom in an equatorial position at the anomeric center (Kulkarni *et. al.,* 1999)

Table 1.5 Xylanases from Thermophilic bacteria

	Source	Xylanase	Optimum conditions			
			Growth		Activity	
			pH	Temp. (°C)	pH	Temp. (°C)
	Thermophilic bacteria					
1	*Bacillus acidocaldarius*		3.5–4.0	65	4.0	80
2	*Bacillus licheniformis* A 99		7.0	60	7.0	50
3	*Bacillus stearothermophilus* T-6	T-6	7.0–7.3	60	9.0	65
4	*Bacillus stearothermophilus* No. 21	A	7.0	55	7.0	60
5	*Bacillus thermoalkalophilus*		90	60	6.0–7.0	60, 70
6	*Clostridium acetobutylicum* ATCC 824	A	6.0	37	5.0	50
		B		37	5.5–6.0	60
7	*Clostridium stercorarium* HX-1	D	6.0–7.0	60	6.5	75
8	*Clostridium stercorarium* F-9	A	6.0–7.0	65	6.5	75
9	*Clostridium thermolacticum* (TC 21)		6.0–7.0	65	6.5	80
10	*Dictyoglonus thermophilum* strain B1		7.0	68	7.0	80
11	*Microtetraspora flexuosa* S II X		6.0	80	9.0	52
12	Thermophilic *Bacillus* Strain XE		7.0	55	6.0	75
13	Thermophilic *Bacillus* sp		7.0	65	6.5–7.0	78
14	Thermophilic bacteria ITI 283, ITI 236		7.5	65	8.0	80
15	*Thermoanaerobacterium* sp. JW/SL-YS485		6.0	60	6,2	80
16	*Thermotoga* sp. (Fjss3-B.1)	A	6.8–7.0	80	5.3	105–110
17	*Thermotoga maritima* (MSB 8)	A	7.0	80	6.2	92
		B		80	5.4	105
18	*Thermotoga thermarum*	1	6.0–7.0	77	6.0	80
		2		77	7.0	90–100
19	*Thermomonospora curvata*	1	6.0–7.0	55	7.8	75
		2			7.2	75
		3			6.8	75
20	*Thermomonospora chromogena* MT814		8.0	50	5.0–8.0	75
21	*Thermomonospora fusca* BD 21		8.0	50	6.0–8.0	65
23	*Thermomonospora fusca* YX		8.0	50	6.0–8.0	70
24	*Streptomyces thermoviolaceus* OPC-520	I	7.0	50	7.0	70
		II		50	7.0	60
	Thermophilic fungi					
1	*Gloephyllum trabeum*			–	4.0	80
2	*Talaromyces byssochlamydoides* YH-50		6.2	50	5.0	70
3	*Thermoascus aurantiacus*		6.0	45	5.0	75
4	*Thermomyces lanuginosus* DSM5826		6.5	50	6.5	50
5	*Talaromyces emersonii* CBS 814.7	II	4.5	45	4.2	78
		III	4.5	45	3.5	67

Source: Kulkarni *et al.* : 1999

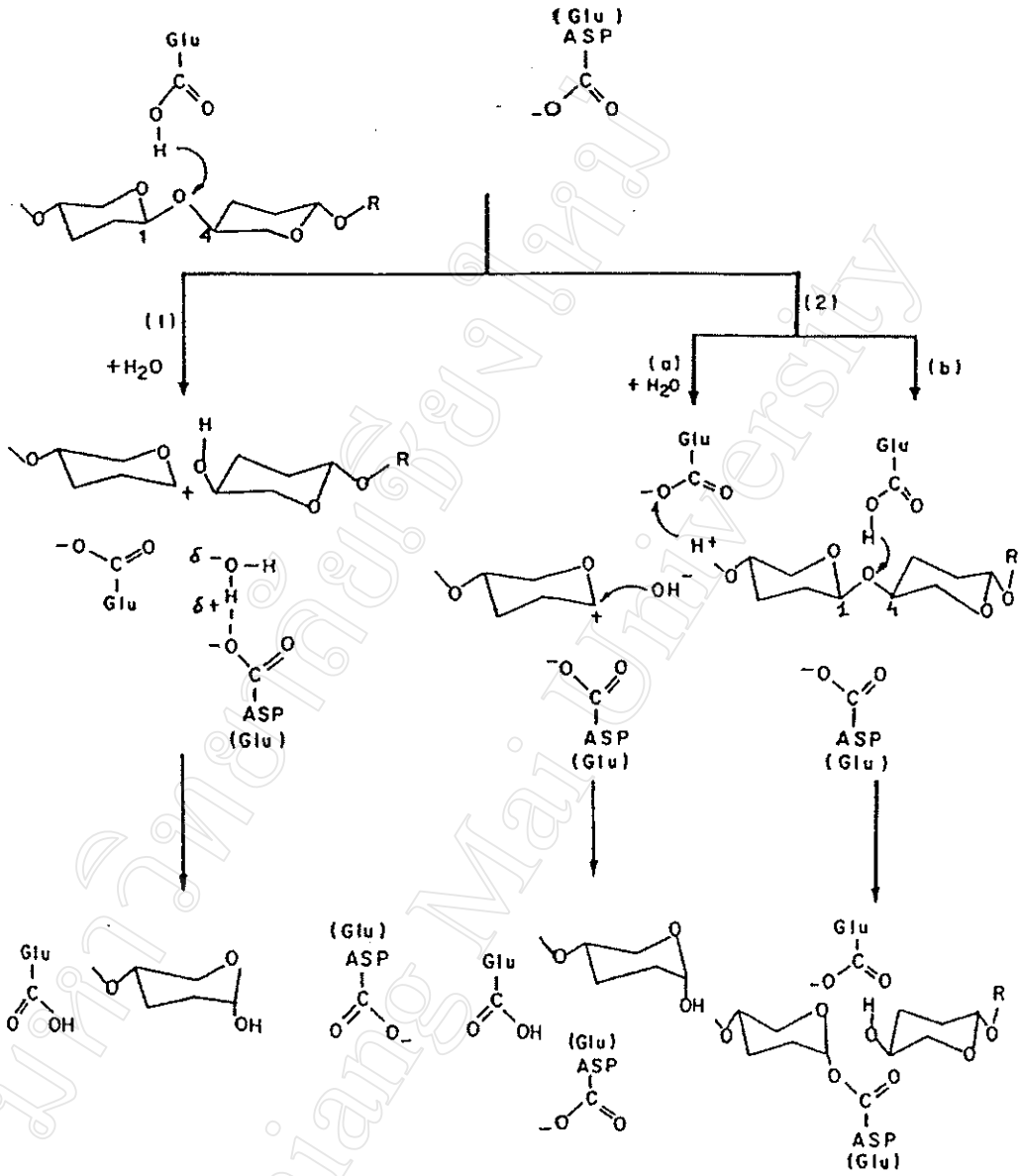


Figure 1.9 Mechanisms of xylanases

Source: Kulkarni *et al.*, 1999

From figure 1.9, the reaction mechanisms of the xylanases could have 2 pathways, a single displacement reaction and a double displacement reaction. The single displacement reaction is shown as (1) in the left side of the tree diagram. The important elements involve in this reaction are a general acid (Glu), a general base (Asp or Glu) and a nucleophilic attacked by a water molecule. The double displacement reaction is shown as (2) in the right side of tree diagram. It consists of 2 possible reactions, which are (a) stabilization of an

oxocarbonium ion by electrostatic interaction with the carboxylate of an Asp (or Glu) at the active site or (b) the formation of a covalent intermediate by nucleophilic attack of the Asp (or Glu) on C-1 of the incipient sugar (Kulkarni *et. al.*, 1999)

Another example of xylanase catalytic has been reported (Torronen and Rouvinen, 1997). The catalytic mechanism of XYNII, xylanase from *Trichoderma reesei*, is shown in figure 1.10

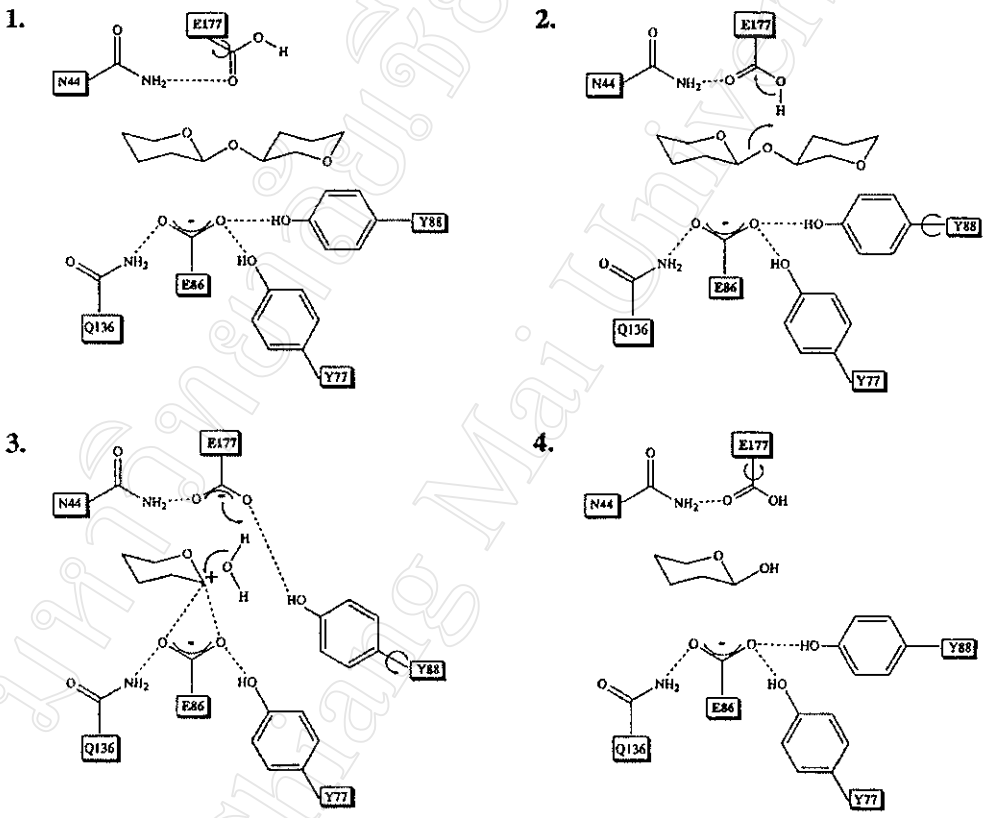


Figure 1.10 Catalytic Mechanisms of XYNII from *Treichoderma reesei*.
Source: Torronen and Rouvinen : 1997

From Figure 1.10, the catalytic mechanism proceeds as follows:

1. The substrate binding triggers conformational change in E177 (from down to up position) and increase its pKa;

2. E177 acts as an acid and breaks the glycosidic bond between the xylose units of the substrate. Y88 also changes its conformation

3. Y88 is hydrogen bonded to E177 and water molecule attacks to the carboniumion intermediate

4. Y88 forms a hydrogen bond with E86, and E177 changes its conformation from up to down position. (Torronen and Rouvinen, 1997)

1.2.9 Biotechnological Applications of xylanases

Xylanases are drawing increased attention, because of their usefulness in facilitation the bleaching of kraft pulp. They increase the extractability of lignin and release chromophores from pulp. They might also be used in the bioconversion of lignocellulosic materials to fuels and chemicals. (Grag *et al.*,1996; Grag *et al.*,1998; and Jeffries,1996)

The major role of xylanase is in pulp and paper industry. There are many reports about the attempt to add xylanase in bleaching process of pulp from different sources such as eukalypt sulfite pulp (Baraznenok *et al,* 1999, Christov *et al.*,2000), baggase pulp (Kulkarni *et al.*,1996, MacCabe *et al.,* 1996, Shah *et al.,* 1999). Using of xylanase results in chemical reduction in bleaching process. It is environmental friendly (Grag *et al.*,1996; and Grag *et al.*,1998). It was reported that xylanase enhanced deinking efficiency of magnetic and flotation deinking of xerographic and laser printed papers (Gubitz *et al.,* 1998)

In European community, over 90 % of the poultry diets contain wheat and/or barley. Using of xylanase reduces feed viscosity in gastrointestinal trace of poultry caused by non-starch polysaccharides (NSP), which impair digestion. Addition of xylanases can increase the digestibility of feed resulting in improving of animal performance. (Beauchemin *et al.*,1999, Hew *et al.,* 1998, Marquardt *et al.*, 1996, Schingoethe *et. al.*, 1999, Yang *et al.*,1999). The feasibility of using xylanase and others polysaccharide-degrading enzymes as feed additives for ruminant animal were also reported (Hristov *et. al.*,1998)

In large bread making operation, quality of wheat flour varies. In order to produce consistent products for the consumers and to make operation more efficient, the mixture of xylanases, α-amylase, proteases, glucoseoxidase, and lipases are used as supplements in bread making process. These supplements are

enable better handling of dough, and control of characteristics in the finish bread such as taste, loaf volume, crumb texture, and anti-stalling properties. (ECS, 2001b)

Mixture of exo- and endo- xylanases were used to produce a hemicellulose hydrolysate , which *Propionibacterium* acidipropioniei used as carbon source for producing propionic acid (Ramsy *et al.* ,1998)

Yeast cellulase-free xylanase was used for treatment of different agricultural wastes, corncob powder, raw jute fibers and sugar cane baggase pulp. Xylose is the major product of enzymatic hydrolysis (Gokhale *et al.,*1998)

Recombinant wine yeast strain, expressing xylanase gene obtained from *Aspergillus nidulans*, is able to secrete xylanase into culture medium. It was found that the concentration of some ester, higher alcohols and terpenes were increased in wine produced from this recombinant yeast strain by microvinification. (Ganga *et al.,* 1999)

1.3 Actinomycetes

Actinomycetes are especially common in soil. Taxonomically, the actinomycetes are bacteria. They are usually Gram-positive. Actinomycetes are filamentous bacteria, their morphology resembles that of the filamentous fungi; However, the filaments of actinomycetes consist of prokaryotic cells with diameters much smaller than those of molds.(Totora *et al.*1995). Many actinomycetes also have aerial mycelia those extend above the substratum and form conidia, asexual spore with thin-wall. The spores can vary greatly in shape. (Prescott *et al.*,1993).

When growing on a solid medium, the hyphae of actinomycetes grow both on the surface and substratum of the medium. The individual filaments of hyphae are subdivided into units in which there is an inward growth of the cell wall at regular intervals along the filament, a process called septation. It's usually divided hyphae into long cells, 20 μm and longer. Each septated unit in the filament contains at least one DNA molecule.

Actinomycete spores develop by septation at filament tips, usually in the stage of nutrient deprivation. The spores reproduced by most species are not heat resistant

as are the endospores of the genera *Bacillus* and *Clostriudium*, but do with strand desiccation well. (Boyd,1998 and Prescot, 1993, Totora *et. al,*1995)

Actinomycetes make up approximately 20% to 60% of the total microbial population of the soil. The so-called "earth smell" of soil is due to the production of perpenoids (geosmins) and extracelluar enzymes produced by actinomycetes. They produce a multitude of extracellular enzymes those are capable of degrading plant and animal organic matter. (Boyd, 1998, and Prescott *et al.*,1993, and Totora *et al.*,1995,).

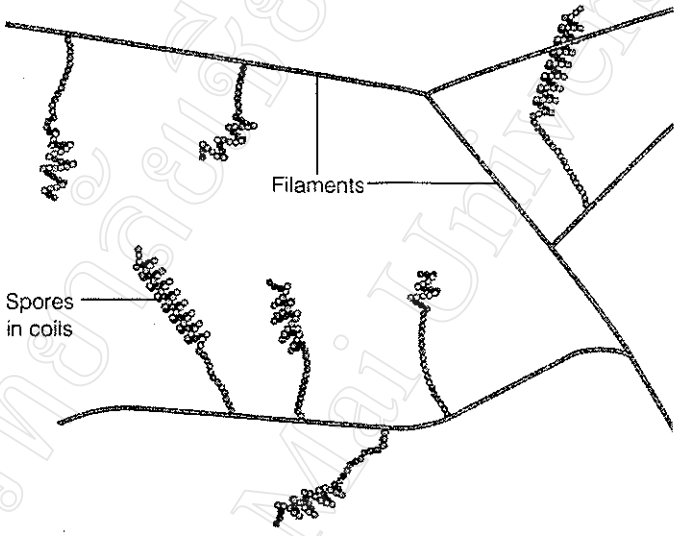


Figure 1.11 A typical drawing of *Streptomyces* showing filamentous, branching growth with asexual reproductive spores at filament tips.

Source: Totora: 1995

The best-known genus of actinomycetes is *Streptomyces,* which is one of the bacteria most commonly isolated from soil. *Streptomyces* derived from Greek *streptos* means bent or twists and *myces* means fungus (Totora *et al.*, 1995). The *Streptomyces* is actinomycetes that has arial hyphae devide in a single plane to form chains of 5 to 50 or more nonmotile conidiospores with surface texture ranging from smooth to spiny and warty. The substrate mycelium, when present, does not undergo fragmentation. The reproductive asexual spores of *Streptomyces,* called conidiospores, are formed at the ends of aerial filaments. If each conidiospore lands on a suitable substrate, it is capable of germination into a new colony. These organisms are strictly aerobes. They often produce extracellular enzymes those are enable them to utilize proteins,

polysaccharides such as starch or cellulose, and many other organic materials found in soil. (Boyd, 1998,Prescott *et al.*,1993, and Totora *et al.*,1995,).

Streptomyces is an enormous genus, 142 species are described in Bergey's Manual. Members of the genus are strictly aerobes, form chains of nonmotile spores within a thin fibrous sheath. The three to many conidia in each chain are often pigmented and can be smooth, hairy, or spiny in texture. The natural habitat of most Streptomycetes is the soil. They are very flexible nutritionally and can aerobically degrade resistant substances such as pectin, lignin, chitin, keratin, latex and aromatic compounds. (Boyd, 1998, Prescott *et al.*,1993, and Totora *et al.*,1995).

1.4 Related works on Xylanase Production and Purification from *Streptomyces*

1.4.1 Xylanase Production

Van Zyl (1985) studied cellulase production by three mesophilic actinomycetes grown on baggase as a carbon source. *Streptomyces albogriseolus, S. nitrosporeus* and *Micromonospora melanospora* were grown on untreated ball-milled baggase. It was found that these mesophilic actinomycetes produced xylanase concomitantly with cellulase.

Kluepfel *et al.* (1986) studied cellulase and xylanase productions from *Streptomyces lividans* 1326 in shake flask culture. When grown on a defined salt medium containing 1% (w/v) xylan as a carbon source, *S. lividans* produced optimal levels of extracellular cellulase of 1 U/ml after 48 h or 72 h of cultivation at 37 °C and 34 °C, respectively. Large amounts of xylanase of 50 U/ml was obtained after 72 h of cultivation at 40 °C. After approching the optimum level of enzyme production, enzyme production decreased concomitantly with fermentation time. A better production of both enzymes was observed when xylan was used instead of cellulose as a substrate.

MacKenzie *et al.* (1987) studied the induction of cellulolytic and xylanolytic enzyme system in *Streptomyces* spp. It was found that *Streptomyces flavogriseus* and *Streptomyces olivochromogenes* produced only cellulase when grown on

cellulose, but produced both cellulase and xylanase when grown on xylan-containing materials. Growth on xylan and wheat bran resulted in higher CMCase levels than did growth on cellulose. Growth conditions that gave high endoxylanases levels also resulted in the production of enzymes involved in the hydrolysis of non-xylose components of xylan: α-L-arabinofuranosidase, α-L-O-methylglucuronidase and feruric esterase. *S. flavogriseus* produced two major endoxylanases, and *S. olivochromogenes* produced several such activities.

Adhi *et al.* (1989) studied xylanase production by *Streptomyces viridosporus* T7A and *S. badius* 252. The fermentation was carried out in 2-L fermentor, working volume of 1.3 l using 1% (w/v) 100/200 mesh dried corn lignocellulose as a carbon source. The fermentation parameters, agitation rate, aeration rate, pH, and temperature were 200 rpm, 0.5 l/min, 8.5, and 37 °C, respectively. Xylanase production rapidly increased and reached the maximum activity of 0.03 U/ml in the first 2 days of fermentation then declined and remained at a relatively constant level along with the fermentation time. To compare xylanase production of *S. viridosporus* T7A to *S. badius* 252, corn lignocellulose of 2% (w/v) was used as a carbon source. The fermentation parameters, agitation rate, aeration rate, pH and temperature were 300 rpm, 1 l/min, 8.5, and 37 °C, respectively. Xylanase production by *S. viridosporus* T7A and *S. badius* reached the maximum activity of about 0.09 U/ml at 4 days and 0.06 U/ml at 3 days, respectively. There seemed to have a 24 h lag phase for xylanase production by *S. viridosporus* T7A, but no lag phase for *S. badius*.

Patel and Ray (1994) studied xylanase production by *Streptomyces* HM-15 on agricultural waste. Sugarcane baggase and corn stalk were used as carbon sources for shaking flask culture. Sugarcane baggase was the best un-treated source, but after alkaline treated, corn stalk gave maximum xylanase production. After 36 h of cultivation at 30 ^{o}C, maximum xylanase production of 53 U/ml and 84 U/ml were obtained from untreated baggase medium and treated corn stalk medium, respectively.

Avalos *et al* .(1996) studied xylanase production of *Cellulomonas flavigena* in 750-ml bench fermentor containing 500 ml of mineral medium supplemented with 1% of sugarcane baggase, Avicel, Solka folk, or xylan, at 37 °C, pH 7.2 , and aeration rate of 0.5 vvm. It was found that sugarcane baggase was the best xylanase inducer. The levels of xylanase induction by cellulose-based substrates like Solka folk and Avicel were higher than xylan did. Xylanase production in baggase supplemented medium started after 24 h of cultivation and reached 18 U/mg·protein at 40 h of cultivation. The enzyme production remained constant through 45 h, then decreased along with the fermentation time. Xylanase activity was found only in the supernatant. These results indicated that xylanase produced by this microorganism could be induced by either cellulose or hemicellulose, with a synergistic effect when both polysaccharides were presented.

Palma *et al.* (1996) studied xylanase production by *Penicillium janthinellum* on sugarcane baggase hemicellulosic hydrolysate. The maximum xylanase activity obtained in shake flask was 98 U/ml at agitation rate of 60 min^{-1}, pH 5.5, 30 ^{o}C. To determine the effect of aeration and agitation rate on xylanase production, the fungi was cultivated in 4-L fermentor. Three agitation rates of 100, 300, and 400 min^{-1} and three aeration rates of 0.2, 0.4, and 0.6 vvm were used. They suggested that xylanase synthesis was growth associated and a function of K_La. The highest enzyme activity of 8.9 U/ml obtained at agitation rate of 100 min^{-1}, aeration rate of 0.2 vvm, and a K_La of 1.24 h^{-1}. Increased in agitation speed lowered the enzyme production, suggested that excess mechanical shear cause hyphae disruption and reduced enzyme productivity. In fermentor, xylanase activity decreased with increase of oxygen concentration. This could be due to oxygen inhibiting or inactivation of enzyme.

Liu *et al.* (1997) found that addition of wheat bran and Tween 80 to the medium enhanced enzyme biosynthesis significantly. The highest xylanase activity obtained in liquid culture was 74 IU/ml.

Vlaev *et al.* (1997) studied cellulase and xylanase production by *Trichoderma* sp. Strain 414 cultivated in Tanaka media using corn fiber pulp from cornstarch production as a carbon source. The fermentation parameters agitation rate, aeration rate, pH, and temperature were 400 rpm, 0.5–1 vvm, pH 4.5, and 29 °C, respectively. The maximum cellulase production of 3.4 U/ml and xylanase production of 3.7 U/ml were obtained at 72 h and 96 h of cultivation, respectively.

Kuhad *et al.* (1998) studied xylanase production by hyper-xylanolytic mutant strain (NTG-19) of *Fusarium oxysporum* in shake flask culture using agricultural residues as carbon sources. Among different agricultural residues, 4% (w/v) wheat bran produced highest amount of xylanase within 96 h of cultivation, pH 3-3.5 and 30 $^{\circ}$C. Addition of Tween-80 and olive oil to the medium improved the enzyme yield significantly. When this fungi was grown in a fermentor, the xylanase yield was enhanced by approximately of 50%.

Pham *et al.* (1998) studied the effect of aeration on xylanase production by *Bacillus* sp. I-1018 in 2-L fermentor, working volume of 1.6-L. The combination of agitation rate of 250 and 350 rev/min and aeration rate of and 0.5 and 1.5 generated K_La of 21.2, 36.7, 46.4, and 73.8, respectively. The maximum xylanase production, about 2700 nkat/ml, was obtained at 36 h of fermentation with fermentation parameters, aeration rate, agitation rate, K_La, and temperature were 1.5 vvm, 350 rev/min, 73.8 h^{-1}, and 50 °C, respectively. They suggested that the increase of aeration enhanced the production rate but not the final concentration of xylanase.

Christov *et al.* (1999) studied cellulase-free xylanase produced by five strains of fungi. Four different carbon sources; wheat bran, corncobs, oat spelts xylan and bleach plant effluent were used. When grown on corncobs, *Aspergillus foetidus* (ATCC 14916) produced significant levels of xylanase (547.4 U/ml) and 6.6 U/ml of cellulase activities.

Tuncer *et al.* (1999) studied the production of extracellular lignocellulose-degrading enzymes during the growth of *Thermomonospora fusca* BD25 in basal

salts-yeast extract medium containing different carbon sources. In shake flask culture; 50 ^{o}C, 150 rev min $^{-1}$, xylanase production increased rapidly during the growth phase. It reached the highest values of 2.68 U/ml at 72 h of cultivation, and then slightly decreased along with the fermentation time. The optimum temperature and pH of enzyme production were, 50 ^{o}C and 7.0-8.0, respectively. They suggested that xylanase production by this microorganism was growth associate.

Rani and Nand (2000) studied production of thermostable cellulase-free xylanase by *Clostridium absonum* CFR-702. The optimum fermentation parameters were 72-96 h, pH 8.5, 75-80 °C. Among different carbon sources tested, xylan and xylose induced xylanase activity whereas xylobiose had less induction effect. Cultivation of this microorganism in 1000 ml of Hungate bottles with 1% birchwood xylan at 75 ^{o}C and pH 8.5 exhibited the highest enzyme activity of 7,025 nkat/ml without filter paper degrading CMCase and β-glucosidase activities. They suggested that corncobs was a good carbon source for xylanase production by this microorganism.

Singh *et al.* (2000b) studied hemicellulase production by *Thermomyces lanuginosus* strain SSBP in shake flask culture and 15-L fermentor. After 7 days of cultivation in shake flask cultureing on coarse corn cobs medium at 50 °C, endo-β-1,4-xylanase of 56,500 nkat/ml was obtained, whereas other hemicellulases, β-xylosidase, β-glucosidase, and α-L-arabinofuranosidase were produced at levels less than 7 nkat/ml. Beech wood xylan medium was used to study xylanase production in fermentor instead of coarse corn cobs because of Rheological difficulties. The production was carried out in 15-L fermentor, working volume of 9-L, pH 6.5, 50°C, agitation rate of 400 rpm. A lag phase of xylanase production was observed during the first 6 h of cultivation. Xylanase increased concomitantly with growth. Xylanase production reached the maximum level of 1,060,000 nkat/ml at 72 h of cultivation.

Kohli *et al.* (2001) studied cellulase free xylanase productions by *Thermoactinomyces thalophilus* subgroup C on stationary submerge culture, shaking

submerge culture (150 rpm), and semi-solid culture. Culture media used in submerge culture was basal medium supplemented with various carbon sources: glucose, sucrose, and birchwood xylan at 0.5% (w/v), corn stover, wheat straw, sugarcane baggase, and wheat bran at 5% (w/v). Semi-solid culture was carried out in basal medium supplemented with >10% of sugarcane baggase. Cultivation was carried out at 50 °C. Xylanase production in submerge culture was higher that that of shaking submerge culture and semi-solid culture, suggested that this organism may sensitive to shear stress. Xylanase production on stationary submerge culture reached the maximum levels at 96 h of cultivation then decreased along with the fermentation time. The optimum temperature of xylanase was 65 °C. At pH 8.5, 65 °C the half-life of this enzyme was 125 min.

1.4.2 Xylanase Purification

Keskar *et al.* (1989) purified an extracellular xylanase obtained from *Streptomyces* T_7. Extracellular xylanase produced in submerged culture by thermotolerant *Streptomyces* T_7, growing at 37-50 °C was purified to homogeneity by DEAE-cellulose and gel filtration on Sephadex G-50. The purified enzyme had molecular weight of 20,643 and a pI of 7.8. The optimum pH and temperature for enzyme activity were 4.5-5.5 and 60 °C, respectively. The enzyme retained 100% activity of its activity on incubation at pH 5.0 for 6 days at 50 °C, and for 11 days at 37 °C. The xylanase had no cellulase activity.

Tsujibo *et al.* (1992). Purified two types of xylanases from a thermophilic actinomycetes, *Streptomyces thermoviolaceus* OPC-520. The purified enzymes (STX-I and STX-II) had molecular weight of 54,000 and 33,000, respectively. The pIs were 4.2 (STX-I) and 8.0 (STX-II). The optimum pH for enzyme activity of STX-I and STX-II was 7.0. The optimum temperature for the activity of STX-I was 70 °C, and that for the activity of STX-II was 60 °C. They were endo-xylanases.

Elegir *et. al* (1994) purified and characterized endoxylanase complex from *Streptomyces* sp. strain B-12-2. When grown on oat spelt xylan, *Streptomyces* sp.

strain B-12-2 produced five distinct xylanases in absence of significant cellulase activity. On the basis of their physiochemical characteristics, they could be divided into two groups: the first group (xyl 1a and xyl 1b) consisted of low-molecular-weight (26.4 and 23.8 kDa) and neutral- to high-pI (6.5 and 8.3) endoxylanases, respectively. The first group endoxylanases were unable to hydrolyze areyl-β-D-cellobioside, had low levels of activity against xylotetraose (X4) and limited activity against xylopentaose, produced little or no xylose, and formed products having a higher degree of polymerization with complex substrates. These enzymes apparently carried out trans-glycosylation. The second group (xyl2, xyl3, and xyl 4) consisted of high-molecular-weight (36.2, 36.2, and 40.5 kDa) and low-pI(5.4, 5.0, and 4.8) xylanases. The second group of endoxylanases were able to hydrolyze aryl-β-D-cellobioside, showed higher levels of activity against X4 and xylose.

Patel and Ray (1994) studied the production and some properties of xylanase obtained from *Streptomyces* HM-15. Alkali-treated corn stalk gave maximum xylanase production, and baggase was the best untreated source. Xylanase was stable for 24 h over pH 5.0-7.0. It had optimum temperature for enzyme activity between 50 and 60 $^{\circ}$C and a half-life of 5 h at 60 $^{\circ}$C. Xylanase production and activity were inhibited by xylose.

Ruiz-Arribas *et al.* (1997) studied 2 xylanases obtained from *Streptomyces halstedii* JM8. This organism was isolated from straw, produced and secreted at least two proteins with hydrolytic activity towarded xylan into the culture supernatant. Both proteins were cloned and overproduced in many strain of *Streptomyces*. From N-terminal sequence determination, immunoblot assay, and time course overproduction experiments showed that both xylanases were encoded by the same gene that the smallest form (35 kDa) originated from the large one (45 kDa) by proteolytic clevage on the C-terminus. The optimum pH and temperature in 7.5-min assays for both enzyme activities were 6.3 and 60 $^{\circ}$C, respectively. Both proteins were highly stable in a wide range of pHs (4 to 10) and temperature (4 to 50 $^{\circ}$C); Nevertheless, after 1 h incubation, both enzymes lost most of their activities at temperature over 55 to 60 $^{\circ}$C.

Kluepfel *et. al.* (1986) studied the production of cellulases and of xylanase by *Streptomyces lividans* 1326 under different growth conditions. When grown on a defined salt medium containing xylan as main carbon source at 40 ^{o}C, *Streptomyces lividans* 1326 exhibited 50 IU/ml of xylanase activity. The optimum pH and temperature for xylanase activity were 6.0-7.0 and 60 ^{o}C, respectively.

Morosoli *et al.* (1986) Studied an extracellular xylanase produced by a cellulase-negative mutant strain of *Streptomyces lividans* 1329. The purified xylanase had an apparent molecular weight of 43,000 kDa and pI of 5.2. The optimum pH and temperature for the enzyme activity were 6.0 and 60 ^{o}C, respectively. The values of K_m and V_{max}, determined with a soluble oat spelts xylan, were 0.78 mg/ml and 0.85 mmol/min per mg of enzyme. The xylanase showed no activity against CM-cellulose and *p*-nitrophenyl-β-D-xyloside. The enzyme degraded xylan, producing mainly xylobiose, a mixture of xylooligosaccharides and a small amount of xylose as end products. Its pattern of action on β-1,4-D-xylan indicated that it was a β-1,4-endoxylanase (EC 3.2.1.8)

Magnuson and Crawford (1997) isolated and purified an alkaline xylanase from culture filtrate of *Streptomyces viridosporous* T7A. The xylanase had a molecular weight of 59 kDa, determined by capillary electrophoresis. The purified xylanase had pI of 10.2-10.5, a pH optimum of 7.0-8.0, and a temperature optimum of 65-70 ^{o}C.

Kaneko *et al.* (2000) purified xylanase from the culture filtrate of *Streptomyces olivaceoviridis* E-86 by successive chromatography on DE-52, CM-Sepharose and Superose 12. The molecular mass of the xylanase was estimated to be 23 kDa, indicating that the enzyme consisted of a catalytic domain only. The optimum pH and temperature of enzyme activity were 6 and 60 ^{o}C, respectively. The pH stability ranged from 2-11 and the thermal stability was up to 40 ^{o}C.