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Industrial Applications of Bacterial Xylanases: A Review

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Abstract: Xylanase is an industrially important enzyme that hydrolyzes xylan and produces xylooligosaccharides, xylobiose and xylose. Wide range of bacterial genera is capable of producing xylanase enzymes. Bacterial xylanases are widely used in paper and pulp industry, ethanol production, animal feedstock preparation, defense molecule against phytopathogens, cloning to produce xylanase in large scale and protein engineering. Current studies on bacterial xylanases and xylanolytic systems are focused on improvement of enzyme production under diverse environmental conditions, purification, media optimization, cloning and gene expression and usage of enzyme for multiple industrial applications. This review paper mainly discusses the distribution of xylanase, assay methods, optimization of culture growing media and the diverse industrial biotechnological applications of bacterial xylanases.

Key words: Bacterial xylanases · Biotechnological application · Enzyme · Feed · Mutation · Pulp

INTRODUCTION

Industrial production and application of enzymes in large scale is associated mainly with the substrate of the enzyme. The usage of agricultural wastes as low-cost substrates for the production of industrial enzymes is a significant way to reduce production cost. Xylanase enzymes are of higher industrial importance that can be used in paper manufacturing industry to bleach paper pulp, increasing the brightness of pulp and improving the digestibility of animal feed and for clarification of fruit juices. Applications of xylanase to degrade the polymer xylan prevent the usage of chemicals that are very expensive and cause substantial pollution to the environment [1]. Microorganisms are the rich sources of xylanases, produced by diverse genera and species of bacteria and other microbes.

Xylanases: The demand for the industrial enzymes is estimated to be very high and food enzymes and general technical enzymes are given high priority for the usage in modern industries [44]. Enzyme xylanase converts xylan, which is the second most abundant natural polysaccharide, into xylose sugars. It removes precipitated xylan there by enhancing the access of chemicals to pulp fibers [56]. All xylanases are endo acting. Wide ranges of bacterial and fungal species are capable of producing a mixture of xylanase, α-xylosidase and accessory side-group cleaving enzymes in order to utilize xylan. Xylan found in nature consists of an α-1, 4-linked xylopyranose backbone substituted with acetyl, arabinosyl and glucuronosyl side chains [24]. Enzymatic hydrolysis of xylan to xylose is catalyzed by endo-1, 4-α-xylanase and α-xylosidase, the former randomly hydrolyzing xylan to xylooligomers and the latter producing xylose from xylooligomers. The enzyme cleaves the internal glycosidic bonds within the xylan backbone. The side chain groups are liberated by α-L-arabinofuranosidase, α-D-glucuronidase, α-galactosidase and acetyl xylan esterase [61]. Some xylanases can hydrolyze xylooligomers to xylose, especially in the cross-linked enzyme crystal form [17]. Arabinofuranosidase hydrolyzes arabinose side-chains, α-glucuronidase removes glucuronic acid side-chains from the xylosyl units; xylan esterase releases acetate group and finally â-xylosidase hydrolyzes xylobiose to xylose [69]. Many xylanases do not cleave glycosidic bonds between xylosyl units if substituted. Therefore, side chains must be cleaved before the xylan backbone can be completely hydrolyzed [38]. There are a lot of accessory enzymes that will only remove side chains from xylo-oligosaccharides. These enzymes require xylanases to partially hydrolyze the plant structural polysaccharides, before side-chains can be cleaved.

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Endoxylanases are differentiated from one another by the substrates upon which they act. Most of the enzymes hydrolyse unsubstituted xylooligomers with a degree of polymerization of more than two. The affinity increases with regions only to yield a mixture of unsubstituted xylo-oligosaccharides and short and long-chain substituted oligosaccharides. Some purified xylanases have been shown to liberate α-L-arabino-furanosyl residues from arabinoxylans or arabinoxylooligosaccharides [14]. On the basis of these observations xylanases have been classified in to two basic groups: arabinose liberating and non-arabinose liberating (Reilley, 1981). In some important applications the enzymes as in the bleaching of kraft pulp, there is the need for cellulose- free xylanase. Such enzymes have been reported to be produced by various fungi [23] and by a *Bacillus* sp [11].

Bacterial enzymes have tremendous potential for different industrial applications. Over the years due to their characteristic features, bacterial enzymes have taken important role of all the biochemical and industrial processes. Enzymes can perform their myriads of biochemical reactions under ambient environmental and cultural conditions that make their use eco-friendly and often the best alternative to polluting bio-chemical technologies. Currently, the massive usage of chemical additives is being replaced by the microbial enzymes in diverse applications. The use of microbial enzymes has become an unavoidable need of the time, because they highly promote the effects that are similar to those of chemical additives with the advantage of being catogorized as safe natural harmless additives. Enzymatic application provides the same level of output as it is achieved through conventional methods with non eco friendly chemicals. Therefore, promotion of enzyme usage at various industrial levels has now been active. General organic natural wastes from forests and agricultural wastes always comprise cellulose, hemicelluloses and lignin [1]. Rising prices of crude oil due to increasing fuel demands has lead the need for alternative sources of bio-energy in the coming future. Among potential alternative bio-energy resources, Lignocelluloses have been considered as the principal source of bio-fuels. The primary components of lignocellulosic biomass have the potential to be a renewable source for energy. Large quantities of lignocellulosic wastes are generated through forestry, agriculture and industries that generally accumulate in the environment and thereby cause serious pollution problem in the long run. Since last decade, there has been increasing trend of research in the bio materials recovered from residual biomass and agro-wastes [18]. These residues can be effectively used as the raw material for the commercial production of diverse value added materials by bringing about a significant reduction in the cost of production and environmental pollution [25].

Xylanase Producing Organisms: Many species of bacteria and fungi are capable of producing xylanases even in extreme environments (hot, alkaline, etc.) and this feature makes them more suitable for industrial applications. Transgenic (recombinant) bacteria, fungi, or yeast transformed with genes from other microorganisms can produce xylanases. Transgenic *Brassica napus* (canola) has also been invented. The meal produced from this canola can be used as an animal feed supplement (Canola meal is the protein-rich residue left after the production of canola oil) [63]. Occurrence of xylanases is confirmed in the digestive juices of crustaceans, snails and herbivorous insects, protozoa, marine algae, actinomycetes group and in germinating seeds of terrestrial plants [33]. Xylanases have however not been found among the vertebrates [14]. Xylanases (endo-1-4-â-D-xylosidases) are distributed among bacteria inhabiting a wide range of environments. For the convenience of the molecular biologist, they are not found in *E.coli*, the most common host for molecular biology work. Many fungi (saprophytes, phytopathogens and mycorrhiza) are reported to be xylanase producers. Among the fungi *Trichoderma* spp. should be mentioned specifically, being extensively used as xylanase producers. Xylanases from only some species of yeasts have been reported. *Saccharomyces cerevisiae* is another commonly used organism for recombinant xylanase production in which the xylan degrading enzymes are absent [70].

Xylanases from Bacteria: The xylanase producing bacteria occur in several different environments. Both marine (*Rhodothermus marinus, Thermotoga* spp.) and terrestrial (*Clostridium* sp., *Streptomyces* spp.) environments are represented. They are reported from halophiles and alkalophiles [26]. They are of course also, produced by several rumen bacteria in the bacteroides group, in which they can be looked upon as catalysts for hydrolysis of polysaccharides in order to increase nutrient uptake.

Xylanases are produced in bacterial species growing in wide range of temperature. i.e. thermophiles, mesophiles and psychrophiles. Xylanases are produced by thermophilic eubacteria and archaea have considerably longer half-life (T1/2) at 80°C or higher temperatures than those from thermophilic fungi [57].

Table 1: Different bacterial species producing xylanase enzyme under different culture conditions.

Microorganisms	Xylanase (U mL^{-1})	Culture conditions	Media	Reference
Bacillus pumilus	327.53	pH 9.0; 30°C	Paddy husk	[34]
Bacillus subtilis	31.41	pH 9.0; 27°C	XFM	[35]
Bacillus circulans	400	pH 7.0, RT	XFM	[50]
Bacillus sp	120	pH 9.0, RT	XFM	[7]
Bacillus sp	11.5	pH 7.2, RT	XFM	[46]
Bacillus stearorhermophilus StrainT6	2.33	pH 7.0, RT	XFM	[40]
Bacillus circulans D1	8.41	pH 9.0; 45°C	Bagasse hydrolysates	[12]
*Streptomyces sp.*strain Ib 24D	1447.0	pH 7.5; 28°C	Tomato pomace	[51]
Micrococcus (DG10)	3.3	pH 7.0, RT	XFM	[53]
Cellulomonas flavlgena NIAB 441	16	pH 7.3, RT	XFM	[48]
Bacillus sp	24.4	pH 7.0, 25°C	XFM	[30]

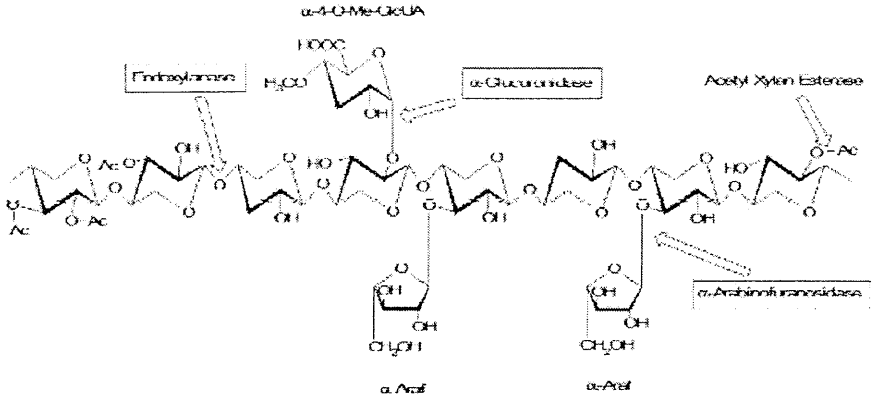

Fig. 1: Structure of xylan and the xylanolytic enzymes involved in its degradation. Source: [62]

A diverse spectrum of xylanase producing organisms, have been isolated from various bacteria (McCarthy, 1987). Although the xylanase activities from bacteria generally are low, other characteristics like specificity, thermostability and extreme pH-tolerance are important for a number of industrial applications such as in pulp processing [27, 64]. Thermophilic alkaline xylanase was produced by *Bacillus pumilus* isolated from open xylan agar medium [31].

Bacteria enzymes fascinated the scientists for alkaline thermostable endo-1, 4-â-xylanase production. *Bacillus* SSP-34 showed higher levels of endo-1, 4-â-xylanase activity under optimum nitrogen condition. *Bacillus* SSP-34 produced an endo-1, 4-â-xylanase with activity of 506 IU/ml in the optimized fermentation medium (Subramanian and Prema, 2002). Earlier, Endo-1, 4-â-xylanase with an activity of 400 IU/ml from *Bacillus circulans* [50]. It had optimum activity at pH 7 and 40% of the activity was retained at pH 9.2. However, the culture supernatant also showed low levels of cellulolytic activities with 1.38 IU/ml of endoglucanase and 0.05 U/ml of cellobiohydrolases. *Bacillus stearothermophilus* strain T6 has been reported to produce cellulase free endo-1, 4-â-xylanase. It actually had slight cellulolytic activity of 0.021 IU/ml [74]). Table 1 highlights the diverse bacterial species that produce xylanase enzyme under different environmental and cultural conditions.

Xylanase Assay: The xylanase activity is often assayed based on measurement of reducing sugar released during the course of hydrolysis of xylan, by DNS or Nelson-Somogyi methods [31]. Due to absent of standardization [5] compared the measurement of xylanase activity by twenty different laboratories. According to the author, the major source of variation between apparent xylanase activities was probably the substrate chosen, although small differences in protocols were also significant. After standardization of substrate 260 Sustainable Degradation of Lignocellulosic Biomass - Techniques, Applications and Commercialization and method, the inter-laboratory

standard variation of the results decreased from 108% to 17% from the mean. Other researchers use the 4-o-methylglucuronoxylan covalently dyed with Remazol Brilliant Blue (RBB xylan) as substrate and the xylanase is assayed based on the release of the dyed fragments [6]. Xylanase activity can also be measured in terms of reducing sugar produced by its action on xylan by Dinitro salicylic acid (DNSA) method [41]. There are also available some commercial methods for xylanase assays, as the fluorescence-based method EnzChek® Ultra Xylanase Assay Kit (Invitrogen, Carlsbad, CA) or the Xylazyme tablet (Megazyme, Bray, Ireland), which employs azurine-cross linked arabinoxylan (AZCLArabinoxylan) as substrate and its hydrolysis by xylanase produces water soluble dyed fragments. The figure 1 demonstrates the typical xylan structure and the sites of action of diverse xylanolytic enzymes.

Induction of Bacterial Xylanase Production by Mutation: The potential of a microorganism to mutate is an important property conferred by DNA, since it creates new variations in the gene pool. The challenge is to isolate those strains which are true mutants that carry beneficial mutations (Parekh *et al.*, 2000). UV rays are important inducers of strain mutations. The pyrimidines are especially sensitive to modifications by UV rays. This may result in the production of thymine dimers that distort the DNA helix and block future replications [52]. Sometimes UV treatment might have caused inhibition of xylanase production and disturbed the basic metabolic activities of the bacterial strain. This may be the reason for the lower production of xylanase in most number of mutants. The mutant strains showing more enzymatic activity were those labeled GS1-S059 and GS1-S067. Endo-xylanase by the mutant of *Humicola lanuginose* TH1 was 1.6-fold more than that produced by the parental organism in solid-state fermentation of rice bran at 45°C [47]. A newly isolated *Pseudomonas* strain was improved to produce lipase by mutation [29]. *Pseudomonas* sp. ATCC 31461 was improved by UV-mutation for polysaccharide production [67]. Strain by exposing the wild-type strain to UV-irradiation, heat shock and EMB chemical treatment. When *Bacillus pumilus* was subjected to UV-irradiation for 3 cycles, xylanase production was increased by 1.22 times in the first two cycles and there was no increase in the third cycle. But when this mutant was further subjected to heat shock and chemical mutation by EMS, there was no significant improvement in the xylanase production [32]. UV-mutation was applied to *Bacillus* sp. to improve the protease production [29].

Organic Wastes as Support for Bacterial Xylanase Production: Various lignocellulosic materials and microbial cultures have been used successfully in solid-state fermentation for xylanase production [58]. Paddy husk was used as a support for the production of different cellulases [36, 10] α-amylase [3] glucoamylase [3] and protease [4]. Paddy husk, a major agricultural waste in Sri Lanka, was examined as a solid support for the production of xylanase by *Bacillus pumilus*. Highest xylanase production by *Bacillus* sp AR-009 ($600Ug^{-1}DM$) was obtained when wheat bran was used as substrate [22]. When *Bacillus amyloliquifaciens* MIR 32 was grown in birchwood xylan-containing medium, the highest xylanase activity was obtained at 48h, with agitation [11]. Substrates that have been used included sugar cane bagasse, wheat bran, rice bran, corncobs, coconut coir pith, maize bran, gram bran, wheat straw, rice straw, paddy husk, soyhull, sago hampas, grapevine trimmings dust, saw dust, banana waste, tea waste, cassava waste, palm oil mill waste, aspen pulp, sugar beet pulp, sweet sorghum pulp, apple pomace, peanut meal, rapeseed cake, coconut oil cake, mustard oil cake, cassava flour, wheat flour, corn flour, steamed rice, steam pre-treated willow, starch, etc [16, 10, 54]. The suitability of a substrate depends on the availability of the nutritional content. In SSF, free movement of air and better oxygen transfer can be achieved by incorporating fibrous substrate or porous coarsely granulated substrate and mainly on the microorganism used [39].

Bacillus pumilus was able to produce xylanase by both submerged and SSF fermentation systems. In submerged fermentation, *Bacillus pumilus* produced highest xylanase activity of $29.35UmL^{-1}$ at 42 hours [34]. Time for higher xylanase production in SSF system is higher (6 days) than that of submerged fermentation system (42 hours). The yield of xylanase production per one gram of xylan was $1465UmL^{-1}$ in SMF and $8598UmL^{-1}$ in SSF. Xylanase production by SSF process is more than 5.87 times as compared to that of submerged culture process [33].

Optimization of Culture Conditions for Higher Xylanase Production by Bacteria: The fact that bacteria grow and produce maximum xylanase activity at lower water ratio offers significant advantage in reducing the risk of contamination, since most bacterial species are unable to grow at reduced moisture level [28]. Bacteria prefer media with higher moisture content for maximum enzyme production [49]. Water causes the swelling of the substrate and facilitates good utilization of substrates by

the microorganisms. Increasing moisture level is believed to have reduced the porosity of substrate, thus limiting the oxygen transfer into the substrate [33]. Likewise, a lower moisture ratio leads to reduced solubility of the nutrients of the solid substrate, lower degree of swelling and a higher water tension [28]. The organisms which grow at and above pH 7.0 are said to be "alkaliphilic". *Bacillus pumilus* is also an alkaliphilic organism. The highest xylanase activity (160.85Ug^{-1}DM) by *Bacillus pumilus* on solid media was produced on the 6th day of inoculation when the initial pH of the medium was kept at 9.0. When the initial pH of the medium was optimized at 9.0, xylanase activity was increased by 1.2 times, than the non optimized condition [33]. An alkaline xylanase producing alkaliphilic *Bacillus* sp AR-009 was active at pH 9.0 and temperature 60-65°C. The optimum growth temperature varies with the organism. The optimum temperature for the growth of *Bacillus laterosporus* was 42°C [30].

Considering that the market of corn-based products is intensive worldwide and that a high amount of xylan is found in corn cob, the utilization of this lignocellulosic residue is a very good choice for xylanase production. Corn cobs available in market have 45% of cellulose, 35% of hemicellulose and 15% of lignin [27]. The raw corn cobs contained 34.8% (w/w) xylan and 35.0% (w/w) cellulose [71]. The amount of xylan in corn cob goes up to 40% [27]. When xylan was extracted from corncob, the yield obtained was 6.33%. Corn cob has shown to be the most important carbon source for xylanase production while using [13].

Sugars have two possible effects on xylanase production. One is induction and the other one is repression. If sugars are added as sole carbon source, they will induce the xylanase production. If they are added to a medium containing Birchwood xylan they will affect the enzymatic synthesis negatively, probably acting as end product repressors [19]. All regulation of enzyme synthesis takes place by means of repression. Repression will take place at the level of transcription of information between DNA and mRNA, by interfering in the synthesis of mRNA at the DNA template. Therefore repression leads to a failure in the synthesis of mRNA as well as protein. Higher amount of xylanase will be produced only when grown in media containing lower sugar concentrations. Sugars like glucose, xylose strongly repressed xylanase production by *Bacillus* sp [22].

Several oil seed cakes, because of their abundant availability and low price, are used as cattle feed, fertilizer and in rare cases after proper processing, food for human. Therefore the local nitrogen sources such as ground nut powder, sesamum seed cake powder, coconut seed cake powder and soymeal powder containing media are used. The total nitrogen content of local nitrogen sources is not the only factor which has direct influence on xylanase production. Among the local nitrogen sources soymeal powder containing medium gave highest xylanase activity than other local nitrogen sources and the commercially available nitrogen sources [31]. Several reports also indicated that soybean meal was the best nitrogen source for xylanase production by *Botryodiplodia theovromae* [43], *Bacillus sp.* SN-1 [15] and *Bacillus sp.* AS-1 [59].

Variations in xylanase production could be due to the variation in the amino acids contents of proteins of these nitrogen sources. Generally xylanase production is reduced in the media added with ground nut powder, sesamum seed cake powder and coconut seed cake powder and the reason could be due to the fat contents of these sources and variations in the compositions of the fats present in these powders could have inhibited xylanase production [31]. In soymeal most of the fats were already removed during the preparation of soymeal from soy bean [68]. Therefore fat may be inhibiting xylanase production or some fats present in soymeal could be essential for xylanase production.

Industrial Application of Bacterial Xylanases: Xylanases have numerous applications and biotechnological potential for various industries including chemicals, fuel, food, brewery and wine, animal feed, textile and laundry, pulp and paper and agriculture [62]. Even though there are several applications of xylanases in industry [37, 61] the major applications of xylanases are in pulp and paper, feed, food and baking industries currently [60]. In combination with pectinases and other enzymes, xylanases have also been used in other processes such as clarification of juices, extraction of coffee and extraction of plant oils and starch. Other potential applications include the conversion of agricultural waste into fuel ethanol [13]. Xylanase has useful applications in many ways:

Biobleaching paper pulp - Paper producers need to retain cellulose while removing the lignin from paper pulp. The ideal way to perform this operation is to add chlorine-based bleaches to the pulp. Xylanase breaks the hemicellulose chains that are responsible for the close adherence of lignin to the cellulose network. There is thus a reduced need for bleach to remove the loosened lignin. When the bleach used is chlorine-based, the use of xylanase leads to a reduction in organo-chlorine

pollutants. In addition, chlorine-free bleaching can achieve brighter results with the addition of xylanase. Because xylanase does not harm cellulose, the strength of the paper product is not adversely affected [34]. Xylanases are used in the prebleaching of kraft pulp to reduce the use of harsh chemicals in the subsequent chemical bleaching stages. Bleaching in the kraft paper making process consists in decolorizing and removing the highly colored residual lignin from washed pulp.

Realistic cost estimates and improvement in process economics are the key factors in the commercial success of any technology and therefore it must be clearly understood that no enzyme-based process for bleaching can be as inexpensive as using chlorine or even organic chlorine compounds. Thus, the added expenses incurred by the use of enzymes must be viewed in terms of their accrued indirect benefits like prevention of environmental derangement and reduced health hazards to mankind [60]. The enzymatic treatments improve the chemical liberation of lignin by hydrolyzing residual xylan. This reduces the need for chlorine-based bleaching chemicals, which is beneficial for the environment [8, 64, 65]. General concern over the accumulation of pollutants from paper and pulp industries that use chlorine as an agent for bleaching involves in the development and usage of biotechnology based techniques for biobleaching process. However the occurrence of cellulase is a major issue in the usage of xylanases as biobleaching tool. The cellulases easily result in the hydrolysis of cellulose that is the main recovered product in paper and pulp industry. However, the enzyme preparations from microorganisms producing higher levels of xylanases with no cellulase activity would be the best choice in the paper industry because the enzyme cellulase may adversely affect the quality of the paper pulp produced.

Improving animal feed - Adding xylanase stimulates growth rates by improving digestibility, which also improves the quality of the animal litter. Chicken feed based on wheat, rye and many other grains is incompletely digested without added enzymes. These grains tend to be too viscous in the chicken's intestine for complete digestion. Xylanase thins out the gut contents and allows increased nutrient absorption and increased diffusion of pancreatic enzymes in the digesta. It also changes hemicellulose to sugars so that nutrients formerly trapped within the cell walls are released. The chickens get sufficient energy from less feed. The barn is cleaner because the feed is more thoroughly digested so the chicken waste is drier and less sticky. The addition of xylanase to animal feed pre-digests that feed [42].

Making bread fluffier and keeping it fresh longer - In the food industry, xylanases are used to improve the dough properties and baking quality of bread and other baked goods by breaking down the polysaccharides in the dough. The enzyme treatment has favourable effects on dough handling, bread volume, texture and stability [31]. In the food industry, xylanases are used to improve the dough properties and baking quality of bread and other baked goods by breaking down the polysaccharides in the dough. The enzyme treatment has favourable effects on dough handling, bread volume, texture and stability [42].

Fuel-alcohol/Biological ethanol production. Xylanase decreases the viscosity of the mash and prevents fouling problems in distilling equipment. The ethanol production process from lignocellulosic biomass includes de-lignification of plant biomass and hydrolysis of cellulose and hemicellulose to monosaccharides [9]. This hydrolysis process can be performed by treatment with mineral acids or by enzyme action. The acidic hydrolysis requires higher energy consumption and the process is very expensive with the production of many by-products. Therefore the enzymatic hydrolysis is preferred. Bi-functionality of endo-1, 4-â-xylanase could result in more efficient and cheaper saccharification process of the agricultural residues and industrial wastes used for production of bio-ethanol [20]. Hexoses and pentoses sugars present in lignocelluloses were fermented to produce into methanol [55].

Extracting more fermentable sugar from barley for making beer. Xylanase has the ability to break hemicellulose down into sugars. In addition, added xylanase can reduce the viscosity of the brewing liquid, improving its filterability [63]. In beer production, the cellwall of the barley is hydrolyzed and long chains of arabinoxylans are released. These arabinoxylans increase the viscosity of the beer and this will lead to a muddy appearance of the beer. Xylanases are used to hydrolyze the arabinoxylans to oligosaccharides. By this, viscosity of the beer will be kept low and the formation of ugly looking muddy appearance of the beer will also be eliminated [55].

Improving silage or enhanced fermentative composting. Treatment of forages with xylanase results in better quality silage and improves the subsequent rate of plant cell wall digestion by ruminants. In addition to converting hemicellulose to nutritive sugar that the cow or other ruminant can digest, xylanase also produces compounds that may be a nutritive source for the ruminal microflora. Endo-1, 4-â-xylanase and cellulase treatment of

forages produces a better quality silage that improves the subsequent rate of plant cell wall digestion by ruminants. There is a significant amount of sugar sequestered in the xylan of plant weight. As a result of endo-1, 4-â-xylanase treatment, there is an increase in sugar and that is useful for digestion in cow and other ruminants.Endo-1, 4-â-xylanase also produces compounds that are nutritive source for a variety of ruminal microflora [21].

Improve degradability of plant waste material (agricultural wastes) for bioconversion process that converts lignocellulose to fermentative products various polymeric sugars are utilized to a higher level. Xylanolytic systems are required to perform maximum hydrolysis of complex substrates to yield monomeric end products. In most of the bioconversion processes, complete hydrolysis of substrate may not be required at the hydrolysis phase. Fermentative organisms *Klebsiella pneumonae* can utilize disaccharides such as xylobiose. Hydrolysis limitations associated with product inhibition can be relieved by the usage of sequential co-culture [72] or simultaneous saccharification and fermentation [73]. Finally, the release of end products or residues of the hydrolysis may interfere with the fermentation process.

Protein Engineering: There are efforts going on to get more thermostable xylanases by using protein engineering. Mining of new genes from nature and rational engineering of known genes are required to get tailor made xylanases for diverse industrial applications. A disulfide bridge Q1C-Q24C was introduced in the N-terminal region of xylanase TLX isolated from *Thermomyces lanuginosus* GH11. The engineered enzyme exhibits higher temperature optimum. The resistance to thermal inactivation was increased by about 10°C. The N-terminal disulfide bridge equally increased both the kinetic and thermodynamic stability [66]. Engineered thermos table xylonite XT6 from *Geobacillus stearothermophilus* having 13 amino acids substitutions was analyzed and showed that engineered enzyme had half life of inactivation 52 fold of that of the wild type [74]. Therefore, the mutant enzyme is of potential interest for industrial applications.

Increasing juice yield from fruits or vegetables. Xylanase aids in the maceration (chewing up) process. In addition, added xylanase can reduce the viscosity of the juice, improving its filterability [74].

Improve retting of flax fibres. Retting is the decomposition of the outer stem of the flax plant necessary before the fibers are processed into linen. After comparing micrographs of soft wood sulphate pulp with that of the same pulp after xylanase pre-bleaching and alkali extraction, it was found that there is no considerable change in the shape and quality of fiber after xylanase pre-bleaching. However, flattening of the fiber arises little bit after alkaline extraction, confirming that the lignin extraction from the cell wall results in its collapse [45].

Aiding in separation of wheat or other cereal gluten from starch - Other potential applications include the conversion of agricultural waste and the production of fuel ethanol [47].

Suggestion for the Future Researchers: The bacterial strains that produce higher amount of xylanase should be identified clearly by molecular level. Diverse optimization techniques should be tried to increase the bacterial xylanase production. Genetic manipulations by mutation techniques and by the use of DNA Recombinant technology could be used to increase the amount of microbial proteins. The usage of cheap local carbon and nitrogen sources should be studied in detail for the economical enzyme production. Techniques that increase the stability of enzyme should be identified. Large scale studies should be done to identify the bacteria and to efficiently use their xylanases in the industrial application.

CONCLUSION

Xylanase is an industrially important enzyme which degrades xylan and produces xylooligosaccharides, xylobiose and xylose. Wide range of bacterial genera is producing xylanase enzymes. Bacterial xylanases are widely used in paper and pulp industry, ethanol production, animal feedstock preparation, defense molecule against phytopathogens, cloning to produce xylanase in large scale and protein engineering. Research in xylanase should be explored more in the wide range of industrial applications to meet the demand of future.

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