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Use of hemicellulases in industries: An overview

Swati Tyagi, Kishor Sureshbhai Patil and Madhuri Gupta

Abstract
Hemicellulases from microbial cells have become biotechnologically important since they hydrolyze complex polysaccharides of plant tissues into simple molecules. In paper and pulp industry, role of hemicellulases is well established and recently its applicability in the food and feed technology, coffee extraction, oil drilling and detergent industry also have been found. Hemicellulases are enzymes produced mainly from microorganisms but its production from plants and animals have also been reported. Bacterial hemicellulases are mostly extracellular enzymes that can act in a wide pH and temperature range, though acidic and neutral hemicellulases are more common. In this review we will focus on complex hemicellulases structure and the microbial enzyme complex involved in its complete breakdown, hemicellulases sources, its production conditions and their applications in the commercial sector. However, the major emphasis of the review is on the microbial hemicellulases but reference to plant and animal hemicellulases also has been made to complete the overview.

Keywords: Hemicellulases, polysaccharides, and hydrolysis

Introduction
India's economy is based on agriculture that produce a huge amount of lignocellulosic waste (agricultural and forestry wastes, grasses, and woody materials) every year (Pandey *et al*., 2015; Binod *et. al.*, 2013) [10]. To eliminate these alarming levels of waste from environment or transform it into some value added products (such as animal feed, single-cell protein, bioethanol, organic acids etc.) a number of physical, chemical or biological methods are being employed by researchers (Tyagi *et al*., 2019) [76]. However, constant depletion of nonrenewable resources of energy and generation of secondary wastes forced the world to find to find an eco-friendly alternative sustainable sources to fulfil the product demand without polluting environment (Pandey *et al*., 2015; Binod *et. al.*, 2013) [10]. Enzymes are being used in many environmental friendly industrial purposes, as they are efficient enough, selective, can accelerate and speed up reaction by forming transition state complexes with their substrates which reduce the activation energy of the reaction (Kumar *et al*., 2017) [39]. Demand of enzymes for industrial and household catalysis is increased in past several years. Enzymes are amazing agents to catalyze the reaction quickly which would take several years in natural conditions (Kumar *et al*., 2017) [39]. However, ''White Biotechnology'' is facing a challenge since new biocatalysts have to compete economically with the well-established commercially available enzymes that have been optimized for years (Otten and Quax, 2005) [52]. Most of the enzymes secreted by microorganisms may display the desired activity, but are generally not suited for industrial use (Marrs *et. al.* 1999) [46]. Although many complicated chemical reactions can be efficiently performed by biocatalysts but industrial conditions are usually different from those in nature with respect to substrate concentrations, sheering forces, temperature, moisture and organic solvents (Pandey *et al*., 2015). With the advancement in the field of biotechnology, especially in the area of genetics and protein engineering have opened a new era of enzyme application in many industrial processes. This era is experiencing major R & D initiatives, resulting not only in the development of a number of new products but improvement in the process and performance of several existing processes also (Kumar *et al*., 2017; Binod *et. al.*, 2013) [39, 10].

Hemicellulose
After cellulose, hemicelluloses are the second most abundant natural polysaccharide present in plant cells and makes up 25–30% of total wood dry weight (Collins *et al.*, 2005) [12].

"Hemicellulose'' covers a wide range of non-cellulose polysaccharides composed in various proportions of monosaccharide units such as D-xylose, D-mannose, D-glucose, L-arabinose, D-galactose, D-glucuronic acid and D-galacturonic acid (Polizeli *et. al*., 2005) [57]. Naming of different classes of hemicellulose is done according to the presence of main sugar unit, for example when a polymer is hydrolyzed and yields xylose, it is called as xylan; similarly, hemicelluloses include mannans, glucans, arabinans and galactans (Ebringerova and Heinze 2000; Polizeli *et al.*, 2005) [19, 57]. In nature, hemicelluloses mostly consist of more than one type of complex sugar structures. The most common xyloses are glucuronoxylans, arabinoglucuronoxylans, glucomannans, arabinogalactans and galactoglucomannans and the amount of each component present varies from species to species and even from tree to tree (Subramaniyan and Prema 2002) [70]. Therefore, hemicellulose is not a well-defined chemical compound, but a class of polymer components of plant fibres, with properties peculiar to each one. Structures of hemicelluloses are described by many researchers. The main difference with cellulose is that hemicellulose has branches with short lateral chains consisting of different sugars. In contrast to cellulose, they are easily hydrolyzable polymers. They do not form aggregates, even when they are co-crystallized with cellulose chains.

Xylan is the most abundant and principal class of hemicellulose that accounts up to one-third of the polysaccharide component of plant biomass (contributing 15–30% of the total dry weight in angiosperms and 7–12% in gymnosperms) (xxxxx; Haltrich *et al*. 1996) [32]. Structurally, xylan is a hetero-polysaccharide with a 1, 4-linked xylose monomers backbone that may be branched with arabinose, glucuronic acid, and acetate substituents at the 2- and 3-position of xylose. Xylan can be used as a sole carbon source by various bacteria and fungi (Otten and Quax, 2005) [52].

Xylanase and its distribution

Xylanases are complex enzyme systems and shows variability from organism to organism (Heidorne *et al.*, 2006) [34]. Several xylanolytic enzymes for example, endo-1, 4-β-xylanase, β-D-xylosidase, α-L-arabinofuranosidases, α-glucoronidases, and acetyl esterases act synergistically to hydrolyze xylan completely (Kar et. al, 2008; Colllins *et al*. 2005; Pandey *et al*., 2015) [36].

Xylanases are ubiquitous, diverse by nature and can be obtained by different sources including bacteria (Battan *et al*. 2006) [6], fungi, actinomycetes, and yeast (Kuhad *et al.*, 1998; Liu *et al.*, 1998; Niehaus *et al.*, 1999) [38, 42, 51]. A number of bacteria and fungi belonginh to the genus *Bacillus, Streptomyces. Thermomonospora, Chaetomium, Nonomuraea, Arthrobacter, Clostridium, Melanocarpus Dictyoglomus Thermotogales, Thermoactinomyces, Fusarium, Aspergillus and other brown and white rot fungi* are reported to produce xylanases (Maheshwari *et al.*, 2000; Kohilu *et al.*, 2001) [45, 37].

Because of their outstanding application in breakdown or convert lignocellulosic biomass into value added products, xylanases are considered as commercially important class of enzymes (Polizeli *et al*., 2005a,b) [57, 58]. Xylanolytic enzymes breakdown arabinoxylans or ther xylans into simple sugars making them available for fermenting process, improve digestibility of animal feed (Twomey *et al*., 2003) [75]. In paper industry during paper recycling, pretreatment of pulp with xylanase is the major application (Twomey *et al*., 2003) [75].

Microbial xylanases are favored over animal and plant xylanases because they are available easily, more stable, and can be easily modified (Bocchini *et al*., 2005). There are several reports when lignocellulosic waste biomass such as sugarcane bagasse, wheat bran, rice straw and waste food material have been used as primary carbon sources for xylanase production (Tyagi *et al*., 2019) [76]. Xylanases production via genetic engineering has received great attention in the past years due to its large scale application in several industries. This review discusses the potential applications of xylanases in various industries.

Xylanase Structure

Complexity in the structure and composition of xylans has led to the evolution of hundreds of xylanases. Initially, xylanases were classified on the basis of molecular weight and isoelectric point (PI), where one class of xylanase had low molecular weight proteins (< 30 kDa) with basic pI, whereas the other class had high molecular weight proteins (> 30 kDa) with acidic pI (Arabi *et al*., 2011) [3]. But this system was inadequate as several exceptions have been reported (Collins *et al.*, 2005) [12]. Therefore, a more complete system, based on the primary structure of catalytic domains and the enzymes sharing sequence similarities was developed. Mainly, xylanases have been classified in Glycoside hydrolase families 10 (GH10) and 11 (GH11). However, the xylanolytic activities have also been found in other classes, of which some are bifunctional enzymes (Bajpai *et al*., 2012) [5].

The catalytic mechanism of classes 10 and 11 involves hydrolysis of xylans with the retention of the anomeric center of the reducing sugar monomer of the carbohydrate. This is a a double-displacement reaction where a covalent glycosyl enzyme is formed as an intermediate and then hydrolyzed via oxocarbenium ion-like transition state (Pandey *et al*., 2015). Two glutamic acid residues, which are appropriately located at the active site, are involved in the formation of a transition state. One acts as a general acid catalyst by protonating the substrate, whereas the other attacks nucleophilically, which results in a release of a leaving group to form the α-glycosyl enzyme intermediate (β to α conversion). In the second step, the first carboxylate group functions as a general base to extract a proton from a water molecule that attacks the anomeric carbon (Biely *et al*., 1993, 1997) [9]. This leads to a cleavage of a glycosidic bond and an inversion of the configuration of the anomeric carbon that is, α to β conversion (Tyagi *et al*., 2019, Biely *et al*., 1993, 1997) [76, 9]. Glycoside hydrolase family 10 comprises endo-1,4-βxylanases and endo-1,3-β-xylanases. Members of this family typically have a high molecular mass, a low pI, and display an (α/β)-barrel fold, can hydrolyze the aryl β-glucosides of xylobiose and xylotriose at the aglyconic bond. Kinetic, crystal structure and end product analysis of these xylanses on xylooligosaccharides of various sizes have revealed that this family typically has four to five substrate-binding sites (Teplitsky *et al*., 2000; Meng *et al*., 2015) [73, 49].

Family 11 is composed only of xylanases (EC3.2.1.8), and considered as "true xylanases," as they are exclusively active on D-xylose-containing substrates. Family 11 members have a small size, low molecular weight, a high pI, high catalytic efficiency, high substrate selectivity and a variety of optimum pH and temperature values that make them suitable for several industrial applications (Paës *et al.*, 2012) [53]. The catalytic mechanism is similar to that of GH10 xylanases. They possess β-jelly roll fold structure. GH11 xylanases exhibit high substrate selectivity and a high catalytic

efficiency. The products of GH11 xylanase reactions can be further degraded by family 10 enzymes. GH10 xylanases are catalytically more versatile and have low substrate specificity as compared with that of GH11 xylanases. Xylanases from family 11 preferentially cleave the unsubstituted regions of the arabinoxylans backbone, whereas GH10 enzymes cleave the regions with greater substitution (Collins *et al*., 2005) [12].

Xylanase production

The main incentive towards investigation of different parameters for xylanase production is the broad area of biotechnological approaches. A number of microorganisms including bacteria, fungi, actinomycetes yeast etc have been reported for xylanse production under solid state fermentation (SSF) or submerged fermentation (SmF) (Dhiman *et al*., 2008) [18].

First and major step towards efficient xylanase production are the choice of an appropriate inducing substrate (inducer) and an optimum medium composition. β-D Xylopyranosyl residues and β-methyl xyloside, a non-metabolizable structural analogue of xylobiose (can be made at low cost) can act as an inducer of the xylanolytic complex (Tyagi *et al*., 2019, Rizzatti *et al*. 2001) [76, 63], however in case of some microorganisms this can give rise to inequitable control, leading to catabolite repression of endoxylanases (Flores *et al*. 1996; Mach *et al*. 1996) [21, 43]. Synthetic compounds, such as 3-O-β-D-xylopyranosyl D-xylose (Xylβ1-3Xyl), 2-O-β-D-glucopyranosil D-xylose (Glcβ1-2Xyl), and 2-O-β-D-xylopyranosyl D-xylose (Xylβ1- 2Xyl), has also been reported (Hrmová *et al*. 1991) [35].

Generally, xylanases production could be performed in large scale in Submerged Liquid Culture (SLC) or on a solid substrate (SSF). SSF provide a medium that is devoid of free floating of water molecules and water present is absorbed with substrates and solid matrix. The submerged culture processes have already been well-established in the bioprocess industry as it is more amenable to control and scale-up (Rodriguez *et al*., 2014). In SSF processes several parameters such as growth medium, pH, temperature, agitation, aeration, and time of cultivation is optimized by researchers to get remarkable enhancement in the xylanase production yield (Ghoshal *et al*., 2015a; Gupta *et al*., 2013; Rodriguez *et al*., 2014; Thomas *et al*., 2013) [27, 30, 74]. It is reported that about 80–90% of all xylanases are produced in submerged culture using various substrates (Gomes *et al*. 1994; Liu *et al*. 1999; Rani and Nand 2000) [28, 43, 62]. SSF provide an environment that is similer to the natural environment of the organisms. Solid-state fermentation is also popular because of high yield, simplicity of the process, and low risk of contamination (Agnihotri *et al*., 2010) [1]. In case of solid substrate fermentation (SSF), wheat bran and rice are regarded as inducers and some alternative substrates such as sugarcane bagasse, rice husks, corn cob and wood pulp also have been used (Anthony *et al*. 2003; Medeiros *et al*. 2000; Damaso *et al*. 2000) [2, 48, 16].

Applications of xylanses in industry

Biotechnological use of xylanases has grown extraordinarily in recent years. The important applications of xylanases are in the paper industries, feed industries, food industries, pharmaceutical industries, and bio-fuel production. (Aristidou and Bhat 2000; Beg *et al*. 2001ab; Subramaniyan and Prema 2002ab; Techapun *et al*. 2003) [4, 7, 70, 72]. The end-products of xylan degradation have very much importance in commercial applications (Parajó *et al*. 1998) [55].

Agro waste treatment

By enzymatic hydrolysis with Xylanase, Xylan rich agro waste can be treated and converted from xylan to xylose. As a pleasant surprise, xylanase can itself be generated from agro waste. Fang *et al.*, 2010 reported production of a low molecular weight xylanase by *Aspergillus carneus* M34 by solid-state fermentation using agricultural waste as the substrate. Development of an efficient enzymatic hydrolysis process offers new prospects for treating hemicellulosic wastes and application in biogas production unit (Stalin *et al*., 2012; Goswami and Pathak, 2013) [68, 29].

Paper and pulp industries

From past several years, use of enzymes in paper and pulp industry has caught the attention of researchers all over the world as it have been proven a cost-effective means for mills to take advantage of a variety of applications (Bajpai, 2012) [5]. Bleaching is the process of removal of lignin from chemical pulps to produce bright or completely white finished pulp (Beg *et al.*, 2001) [7]. Xylanase in the pulp and paper industries is used for hydrolysis of xylan, which facilitates the release of lignin from paper pulp and, consequently, reduces the usage of chlorine as the bleaching agent (Subramaniyan and Prema, 2002; Motta *et al*., 2013) [70, 50]. Process of bleaching is necessary due to the presence of residual lignin and its derivatives during pulping process, which causes pulp to gain a characteristic brown color (Subramaniyan and Prema, 2002 ; Motta *et al.*, 2013) [70, 50]. Bleaching of pulp usually requires large amounts of chlorine-based chemicals and sodium hydrosulfite, which cause several effluent-based problems like generation of chlorinated organic substances, some of which are toxic, mutagenic, persistent, and highly resistant to biodegradation (Beg *et al*., 2001; Subramaniyan and Prema, 2002; Motta *et al.*, 2013) [7, 70, 50].

The xylanases act by cleaving the remaining bridges between the lignin and xylan, opening the structure of the cellulose pulp and leading to the fragmentation of xylan and subsequent extractions of the fragments (Paice *et al*. 1992) [54]. Treatment with xylanase renders the pulp more permeable to subsequent chemical extraction of the residual brown lignin and lignincarbohydrate from the fibres. Madlala *et al*., 1998 [44] used different preparations of commercial Xylanase P and crude xylanase from *Thermomyces lanuginosus* to evaluate the bleaching process of paper pulp and found that the use of enzymes could increase brightness 5 times over the control the pulp. With xylanases bleaching of pulp is more effective then lignin-degrading enzymes cause removal of small hemicellulose portion could be sufficient to open b006E up the polymer, which facilitates removal of the residual lignin by mild oxidants (Motta *et al*., 2013) [50]. The efficiency of various microbial xylanase in the bleaching process has been studied for *Streptomyces thermoviolaceus, Streptomyces roseiscleroticus, Streptomyces sp., Bacillus sp. Bacillus pumilus, Bacillus circulans, Aspergillus kawachii, Aspergillus oryzae, Aspergillus niger, Chaetomium cellulolyticum, Thermomyces lanuginosus, Trichoderma reesei.*

Bioconversion of lignocellulose in biofuels

Xylanase synergistically with laccase, glucanase, mannanase xylosidase, glucosidase etc., used for the generation of biofuels, such as ethanol and xylital from lignocelluloic biomass (Stalin *et al.*, 2012) [68]. Currently, second-generation biofuels are the primary products of the bioconversion of lignocellulosic materials. Ethanol is the most important renewable fuel in terms of volume and market value.

Bioconversion of lignocellulses into ethanol requires de-lignification of lignocelluloses to generate small sugars followed by fermentation of mixed pentose & hexose to produce ethanol (Lee 1997) [40]. However, enzymatic hydrolysis is still a major cost factor in the conversion of lignocellulosic raw materials to ethanol (Viikari *et al.*, 2012) [78].

The pharmaceutical, food and feed industries

Xylanase play a central role in baking technology. Xylanase, with amylase, pectinase, and carboxymethylcellulase can be used for clearing up of juices. It helps in increasing juice yield from fruits or vegetables and also in the maceration process. Besides, it reduces the viscosity of the fruit juice improving its filterability (Biely *et al*., 1985). Xylanase may also improve the extraction of coffee, plant oils, and starch (Wong and Saddler) as turbidity observed in it is due to presence of both pectic materials suspended in a stable colloidal system (Sreenath and Santhanam, 1992) [67]. In bakery industry, thermophilic enzymes are suitable as they are generally optimally active at the temperatures required (at or below 35 °C) for dough preparation (Collins *et al.*, 2006) [13]. Enzymatic hydrolysis of non-starch polysaccharides leads to improvement of rheological properties of dough and crumb firmness (Martinez-Anaya and Jimenez, 1997) [47].

Xylanase improves dough machinability, dough stability, oven spring, loaf volume, crumb structure, and shelf life (Hamer, 1995; Poutanen, 1997) [33, 59] when optimum dose is used. Pariza and, Johnson, 2001 reported that endo-1, 4-β-xylanases from bacterial/fungal sources can be use in baking industries and also improve crumb softness after storage.

The use of enzymes in the production of feed is an important sector of agribusiness. Xylanases with glucanases, pectinases, cellulases, proteases, amylases, phytase, galactosidases and lipases.are used in animal feed as it improve the nutritional properties of agricultural silage and grain feed. (Twomey *et al*., 2003) [75]. Hydrolysis of xylan may result in oligomers called as xylooligosaccharides (XOs), which have have prebiotic effects as they are neither be hydrolyzed nor absorbed in the upper gastrointestinal tract, may be used in pharmaceutical, agriculture and feed products.

These XOs thus affect the host by selectively stimulating the growth or activity of one or a number of bacteria in the colon, thus improving health (George *et al.*, 2001; Hakulinen *et al.*, 2003; Virupakshi and Kyu, 2005; Ghatora *et al*, 2006; Garg *et al.*, 2009; Garg *et al.*, 2010) [24, 31, 25, 22]. For XOs production, the enzyme complex must have low exoxylanase or β-xylosidase activity, to prevent the production of high amounts of xylose, as it have inhibitory effects on XO production (Ghatora *et al.*, 2006; Garg *et al.*, 2009) [26, 23].

Xylanases, sometimes in combination with a complex of enzymes (hemicellulases, proteases and others) used as a dietary supplement or to treat poor digestion, but few medicinal products can be found with this formulation. It is a non-carcinogenic sweetener, suitable for diabetic and corpulent individuals and recommended for the prevention of osteoporosis and respiratory infections, lipid metabolism disorder, kidney and parenteral lesions. A variety of commercial products containing xylitol, such as chewing gum, can be found on the market.

The development of a more appropriate technology for xylitol production has generated great hope of its wider use in the food, pharmaceutical and odontological industries.

Textiles Industry

Little research has been done on the enzymatic preparation for treating textile fibres, and thus appears to be a hopeful market demanding the development of new techniques. In textile industry, xylanolytic complex (free of cellulolytic enzymes) can be used for progression of plant fibres, such as hessian or linen. After using incubated dried ramee (China grass) stems with xylanase to liberate the long cellulose fibres intact, no need to use the strong bleaching step, since the lignin does not undergo oxidation, which would lead to darkening of the fibres (Prade 1995; Brühlmann *et al*. 2000; Csiszár *et al*. 2001) [60, 11, 15].

Conclusion

Xylanase is an industrially important enzyme which degrades xylan randomly and produces xylooligosaccharides, xylobiose and xylose. It is mainly present in microbes and plants but not in animals and has been studied and commercially produced extensively from fungal and bacterial sources. Considering the world market in enzymes, Great hopes are placed on technological advances, but there is also a search for new microorganisms, within the great biodiversity of this planet, that may possess better physiological characteristics in relation to temperature, pH of the medium and adaptability to low-cost substrates, which have been until now hardly exploited, such as sugarcane bagasse of which massive amounts are produced in Brazil and India. (Randomski, *et al.*, 1997; Polizeli *et al.*, 2005; Sharma and Kumar, 2013; Motta *et al.*, 2013) [61, 57, 50].

Several applications of xylanases are being developed for different industries which are based on the partial hydrolysis of xylan. Conversion of renewable biomass into liquid fuels is long term application of xylanases where it play a crucial role in conjunction with the cellulases, is not yet economically feasible. In order to make the application of xylanases realistic the improvement in enzyme yields is of paramount importance. Considerable progress has been made in the last few years in identifying the process parameters which are important for obtaining high xylanase yields and productivities, and thus influencing the economics of xylanase production. The production of xylanolytic enzymes is higher with increasing substrate concentration. More research efforts are necessary to obtain constitutive mutants which will also eliminate the hindrance of using insoluble carbon sources for the production of xylanase in fermentors.

Although the enzyme recovery methods are outside the scope of the present review article, their importance in the economics of enzyme production is beyond doubt and greater attention needs to be focused on this aspect. The complete bioconversion of xylans to the sugar monomers has so far not been achieved. This is the main hurdle in the commercial success of bioconversion processes from the technical as well as the economic point of view. The enzymatic cleavage of xylan to smaller oligosaccharides is itself a reversible reaction and most of the enzymes show transglycosylating activity and synthesis of higher oligosaccharides under certain reaction conditions. Hence a special process for the enzymatic cleavage of xylan needs to be designed such as the two-phase reactor in which the product can be continuously separated from the reactants.

Xylanases have potential to be use in various industries has been discussed which is directly related to reduction in environmental pollution. It has role in bio-bleaching paper pulp and increasing pulp brightness. Besides, it can be exploited for ethanol production and as an additive in animal

feedstock to improve its nutritional value. Endo-1,4-β-xylanase can also be exploited in baking and fruit juice industries. Here, we reviewed its distribution, structural aspects, industrial / biotechnological applications (Kumar *et al*., 2017; Sharma and Kumar, 2013; Morosoli *et al.*, 1993; Deberie-Gosselin *et al.*, 1992) [39, 17].

However, specific activity of the xylanase preparations is much lower than that of commercial preparations such as amylases, proteases and glucose isomerases therefore this is an urgent need for identifying, otherwise developing, the strain capable of producing a high specific activity xylanase, but the task may not be simple, mainly because of the heterogeneous nature of the substrate xylan.

In addition to the chemical heterogeneity, the substrate is also divided into soluble and insoluble physical states which make the actual catalysis an extremely complex phenomenon.This catalytic complexity may have resulted in multiplicity amongst the xylanases. Hence the xylanase multiplicity must be analyzed taking into account the microheterogeneity of the substrate. Thus it may be possible to design a mixture of xylanases that has a better speci¢c activity than the individual components. Cleaner biobleaching technology for the paper and pulp industry is currently concentrated in the developed countries, whereas renewable energy generation from agricultural waste has more relevance for the developing nations. A lot of work needs to be done to bring these research ideas to reality.

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