A CASE STUDY:

Fungal xylanases : Theie application and future propspects

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Microbial xylanases are a group of industrial enzymes with applications in the paper and pulp industries, food and feed industry; animal feed industry and textile industries. This application of the xylanase is ecofriendly and the enzyme applications within the pulp and paper industry seem to be nearly endless. Fungal xylanases can be produced using two main methods, solid-state cultivation systems and submerged liquid cultivation systems. Most research has used submerged culture, which allows control of the degree of aeration, pH and temperature of the medium and the control of other environmental factors required for the optimum growth of microorganisms. In this review, the source of different fungi and their properties of hydrolyzing the cheaper agricultural residues are disscused.

The biodegradation and bioconversion of lignocelluloses into useful products and biological alleviation of pollution from lignocelluloses wastes is an environmental challenge (Panagiotou *et al.*,2003). Lignocellulosic materials from forest, agriculture, set aside lands, industry or urban solid wastes, mainly made up of lignin; cellulose and hemicelluloses are potential feedstocks for chemical utilization. LCM are heterogenous and hemicelluloses polymers are xylans, made up of biopolymers for practical applications, accounting for 25-35% of the dry biomass of woody tissues of dicots and lignified tissues of monocots and occur up to 50% in some tissues of cereal grains. The most common xylans are made up of a main backbone of xylose linked by β-1-4 bonds, where structural units are often substituted at positions C_2 or C_3 with arabinofuranosyl,4-0-methylglucuronic acid acetyl or phenolic substituents.

The xylan backbone is hydrolysed by endo-1,4-β-D-xylan xylanohydrolase;EC.3.2.1.8 degrade the xylan polymer into small oligomeres.Many of the species of fungal genera are known, which produce xylanase like *Aspergillus,Disporotrichum,Penicillium, Neurospora, Fusarium, Neocallimastix, Trichoderma* and *Coniothyrium.*

These species were able to utilize the agricultural residues as substrates. About 30-40% of the production cost of many industrial enzymes is accounted by the cost of growth substrate. The use of low cost substrate for the production of industrial enzymes is one of the ways to greatly reduce production costs. Fungal enzymes are commonly used in industries due to various technical reasons, including the feasibility of obtaining enzymes (Mitchell and Lonsane,1992).

A few fungal strains produce alkalitolerant,cellulase-free xylanase when grown under alkaline conditions pH 8-10 (Bansod *et al.*,1993).Tolerance to high pH and temperature are the desirable properties of xylanase improved production of fungal xylanase in submerged culture (Haltrich and Steiner,1994).The role of enzymes in many processes has been known for a long time. Fungal xylanases have attracted considerable research interest because of their potential industrial applications in food and bread making, fruit juice clarification,beverage,animal feed, fibre seperation, paper and pulp industries (Beg *et al.*,2000; Bajpai ,1997; Kenealy and Jefries, 2003).

Enzymatic action on xylan in the agricultural residues:

Abundance of xylan on earth:

Xylan is the most abundant non-cellulosic polysaccharide present in both hard woods and annual plants and accounts for 20-35% of the total dry weight in tropical plant biomass. In temperate soft woods, xylans are less abundant and comprise about 8% of the total dry weight. Xylan is found mainly in the secondary cell wall and is considered to be forming an interphase between lignin and other polysaccharide.Xylans are linear homopolymers that contains D-xylose monomers linked through β-1,4-glycosyl bonds

(Srinivasan and Rele,1999).

Structure feature of xylan:

The structure of xylan found in cell walls of plants can differ greatly depending on their origin but they always contain a β-1,4-linked D-xylose back bone (Ebringerova and Heinze,2000).

Arabinose is connected to the backbone of xylan via on ∝-1,2 or 1,3 linkage either as single residue or as short side chains.Glucuronic acid and its 4-0-methyl ether are attached to the xylan backbone via an ∝-1,2-linkage whereas aromatic feruloyl and P-coumaroyl residues have so far been found attached only to 0-5 of terminal arabinose residues (Saulnier *et al.*,1995).As a consequence of all these feature, the xylans form a very heterogenous groups of polysaccharide (Huisman *et al.*,2000).

Biodegradation of xylan:

Biodegradation of xylan has been reviewed by Saha and Bothast,(1999).The total biodegradation of xylan requires endo β-1,4 xylanase, β-xylosidase and several accessory enzymes, such as α-arabino furanosidase, α-glucuronidase, acetyl xylan esterase, ferulic acid esterase and p-coumaric acid esterase, which are necessary for hydrolyzing various substituted xylans. The enzymes involved in the degradation of xylan are:

Acetyl xylan esterase – hydrolyses acetyl ester bonds in acetyl xylans.

Ferulic acid esterase: Hydrolyses feruloyl ester bonds in xylans

ρ-coumaric acid esterase: hydrolyses p-coumaryl ester bonds in xylans

Although the presence of β,1-3 hydrolyzing enzymes have been observed, the name xylanase refers to the β,1-4 hydrolyzing enzymes. When the action proceeds from one end to another they are of 'exo' type and when it occurs randomly at any point of the polymer, they are said to be of 'endo' type. The nomenclature has its origin in cellulases. The oligosaccharide hydrolyzing enzymes are called xylosidases.

Xylanase:

Xylanase (endo, β-1-4 xylanase) are glycoside hydrolases that catalyze the hydrolysis of internal b-1,four bonds of xylan,the major hemicellulose component of the plant cell.Xylanases belong to glucanase enzyme family and it contains 196 aminoacids.They show a remarkable potential for practical utilization in many fields,including food additives,pharmaceuticals,feed formulations and agricultural applications.

Occurrence of xylanases:

Xylanases are widely distributed in bacteria (saprophytic and phytopathogenous), mycrorrhizic fungi and some yeasts. The enzyme is also found in protozoa, insects, crustaceans, seaweed and also seeds and plants during the germination phase in the soil (Maria and Tronisielo,2005).

Xylanase production:

The basic factors for efficient production of xylanolytic enzymes are the choice of an appropriate inducing substrate and an optimum medium composition. The importance of cellulase – free xylanase systems in the paper and pulp industry has initiated research into the correlation between the production of xylanases and cellulases by microorganisms. Filamentous fungi are particularly interesting producers of xylanases (Haltrich *et al.*,1996). Since they excrete the enzymes into the medium and their enzymes levels are much higher than those of yeast and bacteria (Kulkarni *et al.*,1999).

Classification of xylanases:

The xylanases have been broadly classified into two major families of glycosyl hydrolases namely, Family F or 10 and G or 11 (Davis *et al.*,1999).

– Family G (11) xylanases have low molecular weight and broad pH activity, which usually extends into the alkaline range; seem to have a specific advantage for their application in pulp industry for biobleaching.

– Family F(10) xylanases have relatively high molecular weight, more complex and produce smaller oligosaccharides. F10 enzymes act mainly on xylan.

Induction of xylanase by cheaper hemicellulosic agrowastes:

Natural xylan sources such as agricultural and forestry wastes, paper industry wastes and various fruit wastes are potential raw materials for xylanase production. Cheaper agro-industrial residues are generally considered the best substrate for the production of xylanase enzyme.Although a *Pleothora* of xylanase producing strains have been described, their use for commercial production at present is restricted mainly to *Trichoderma* sp. and *Aspergillus* sp. Cheaper hemicellulosic substrates namely, cotton fibre, corncob, wheat bran, paddy straw, paddy husk, sugarcane bagasse, cornstalk, tamarind seed, saw dust and wheat straw were used as substrates for xylanase production. Corncobs supported maximum enzyme production in *Aspergillus foetidus* (Christov *et al.*,1999) and *Rhizopus oryzae* (Bakir *et al.*,2001).

Cultural conditions for xylanase production:

Optimum pH for xylanase production:

pH of the culture media is an important factor that influences xylanase enzyme production. The optimum pH for xylanase production was pH 4-4.9 in *Pencillium jantinellum* (Tanaka *et al.*,2005) and in *Rhizopus oryzae* (Bakir *et al.*,2001), 5-5.5 in *Aspergillus japanicous* (Palma *et al.*,1996), 6.5 in *Acrophialophora nainiana* (Cardoso and Filho,2003) and pH 5.5 in *Penicillium purpurogenum* (Belanic *et al.*,1995).

Optimum temperature for xylanase production:

The optimum temperature for endo-xylanase production by bacterial and fungal sources varies between 40 and 60^{0}C (Kulkarni *et al.*,1999). In thermophilic fungi, maximum enzyme production occurred between 40 – 55^{0}C in *Thermomyces lanuginosus* at 40 – 50^{0}C (Hoq and Deckwer,1995) and in *Thermoactinomyces thalophilus* at 50^{0}C (Kolhi *et al.*,2001).

Carbon and nitrogen sources on xylanase production:

Enhancement of xylanase production by sugars, starch was studied by several workers.Glucose repressed xylanase production in *Melanocarpus albomyces* (Maheswari and Kamalam,1985).Xylose repressed xylanase production in *Aspergillus giganteus* (Coelho and Carmona,2003). Effect of organic and inorganic nitrogen sources in the culture medium enhanced the xylanase production.Organic nitrogen sources namely, yeast extract was used. Yeast extract supported more xylanase in *Aspergillus flavus* (Ruckmani and Rajendran,2001). The inorganic nitrogen source ammonium nitrate supported maximum xylanase production in *A.niger* (Ikram-ul-Haq *et al.*,2002) and ammonium phosphate in *Aspergillus flavus* (Ruckmani and Rajendran,2001).

Biochemical properties of xylanase:

Characteristics of xylanases from different microorganisms are listed in the (Table 1)**.** The optimum temperature for the activity of most xylanases is reported to be 50-60^{0}C.Some xylanases have been reported to exhibit higher thermo stability and optimal activity ranging from 80 to 100^{0}C.

Applications of xylanase:

Xylanases from different organisms have been evaluated for their interaction with various kinds of pulps. The use of xylanases leads to a reduction in organ chlorine pollutants such as dioxin from the paper making other chlorine free bleaching can achieve brighter results with the addition of xylanase. Xylanases from *Trichoderma longibrachiatum* has been used to treat kraft pulp of different fibre length.

Xylanase promotes bleaching by the hydrolysis of relocated reprecipitated xylan on the surface of the pulp fibres allowing for better chemical penetration and thus improving lignin extractability. Xylanases are important in food and feed technologies, since their target enzymes are often applied industrially. Indeed, endo xylanases are used in many food and feed applications to degrade or modify the arabinoxylan population in order to improve processing and or/ end use quality of cereal products. Xylanases also play a key role in maceration of vegetable matter, protoplastation of plant cells, clarification of juices liquefaction of coffee, recovery of oil from subterranean mines, extraction of flavours and pigments, plant oils and starch and to improve the efficiency of agricultural silage production. The xylanases find application in the bakery and the fodder industries due to the presence of substantial amounts of residual hemicellulose in the raw material. The use of xylanases together with the hemicellulases corrects the problems and also increases the nutritive value of the feed. These biotechnological potentials of xylanases have promoted the search for suitable enzymes and technologies for large-scale economic production.

Future prospects:

Several applications of xylanase are being developed for the food and paper industries, which are based on the partial hydrolysis of xylan. Xylans with the cellulases is not yet economically feasible. In order to make the application of xylanases realistic the improvement in enzyme yields is of utmost importance. The production of xylanolytic enzyme is higher with increasing substrate concentration. However, the high concentration of solid substrate gives rise to mass transfer limitations in batch cultivation. A fed-batch mode of cultivation where much higher substrate concentrations can be used is an attractive alternative process. Selecting a suitable strain for the production of xylanase is the greater attention needs to be focused on this aspect. There is an urgent need for identifying, developing the strain capable of producing a high specific activity of xylanase.

Cleaner biobleaching technology for the paper and pulp industry is currently concentrated in the developed countries, where as renewable energy generation from agricultural has more relevance for the developing nation. Wasting the agricultural wastes in the road side as creates the environmental problems instead of this we can make use of them in an ecofriendly manner. Research efforts should be focused on the improvement of such strain as

Table 1 : Characteristics of xylanases from different microorganisms

Fungi	Optimum		Stability		Activity		Molecular weight (kDa)	Reference
	pH	Temperature (°C)	pH	Temperature (°C)	pH	Temperature (^{0}C)		
Acrophialophora nainiana	6	50	5	50	-	-	17	Ximenes *et al.*,1999
Acrophialophora nainiana	7	55	-	55	-	-	22.6, 22.1	Salles *et al.*,2000
Aspergillus fischeri	6	60	5 – 9.5	55	-	-	31	Raj and Chandra,1996
A. niger	-	-	5.0-6.0	60	5.5&6.0	45	-	Frederick *et al.*,2004
A niger	-	28	-	-	-	-	-	Yuan *et al.*,2005
A flavus	5.5-6.0	-	-	-	-	-	-	Desouza *et al.*,1999
A tamarii	6.0-6.5	45	-	-	-	-	-	Gouda and Naby,2002
A fumigatus	-	30-42	-	-	-	-	45.7,39.8,18.2	Lenartovicz *et al.*,2003
A versicolor	6.5	-	-	-	6-7	55	28	Carmona *et al.*,2005
A versicolor	6.5	30	-	-	6.0	55	-	Carmona *et al.*,2007
A tamarii	8	60	6.0-9	-	-	-	-	Chivero *et al.*,2001
A flavipes	-	-	-	-	-	45-50	-	Mukhopadhyay *et al.*, 1997
A orchraceus	6.0	45	-	-	-	-	-	Biswas *et al.*,2004
A caespitosus	6.5-7.5	50-55	-	-	-	-	-	Sandrim *et al.*,2005
A giganteus	-	-	-	40,50,60	6.0	50	-	Coelho and Carmona,2003
A niger	5.0	50	3.0-8.0	-	-	-	-	Liu *et al.*,2005
Fusarium oxysporum	6	60, 55	7 – 10	30	-	-	20.8, 23.5	Christapolous *et al.*,1996
F. oxysporum	-	30	-	-	-	-	-	Singh *et al.*,1995
F.verticillioides	5.5	50 30	4 – 9.5	50	-	-	24	Saha ,2001
Penicillium purpurogenum	7	60	6 – 7.5	40	-	-	33	
Talaromyces	3.5	50	4.5 – 7.5				23	Belanic *et al.*,1995
P.janthellum	-	-	-	-	5.5	30	-	Palma *et al.*,1996
Thermonospora sp	5-10	80		-	-	-	38	George *et al.*,2001
Thermomyces lanuginosus	7	60 – 70	5 – 9	60	-	-	25.5	Cesar and Mrsa,1996
T.lanuginosus	6-7	40	-	-	6-6.5	70	24.7	Singh *et al.*,2000
T.lanuginosus	6.5	50-70	6.5	2-3	6.5-8	-	24.5	Kaur *et al.*,2004
Melanocarpus albomyces	5.6	65	-	50	6.6	-	-	Prabhu and Maheswari,1999
Myceliopthora sp			9.2	60	6	75	53	Chadha *et al.*,2004
Sporotrichum thermophile	-	-	5	60-70	5	70	25	Katapodis *et al.*,2003
Schizophyllum commune	-	-	5.7	40	5.5	50	30	Kolenova *et al.*,2005

for the efficient utilization of biomass. Xylan being an abundant agricultural residue can be used as a suitable for xylanase production.

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