

SOLID STATE CULTIVATION AND APPLICATION OF XYLANASEKianoush Khosarvi-Darani¹ and Dina Karamad²

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ABSTRACT

Research for xylanase biosynthesis is an interesting area due to its important industrial application. This review paper serves as an overview of xylanase bioproduction and application as well as its producing microorganisms, substrates and process variables, to consider the future prospects of xylanases in biotechnological applications. Several approaches should be applied to overcome main limitations which inhibit widespread commercial and industrial application of this enzyme; low production yield and the high total cost.

Keywords: xylanase, producing microorganisms, process variables, substrates, solid state fermentation.

INTRODUCTION

Plant biomass is a huge substrate on the earth in which consists of cellulose, hemicellulose and lignin, which should be hydrolyzed by acids or enzymes to lower molecular weight carbohydrates and finally to monomeric sugars (Yoonan and Kongkiattikajorn, 2004). Xylan, the polymer of xylose is the main component of hemicellulose. This heteropolysaccharide can be used as substrates for microbial growth and production.

Hydrolysis of xylan to xylose is possible by acid or enzymatic methods. Hydrolysis by enzymes has main advantages of higher purity and lower chemical pollution problem. Xylose can be used for the production of useful biometabolites e.g. alcohols (ethanol, butanol, and xylitol) and single cell proteins. Production of purified xylanase and cellulose enzymes are reported on rice straw and rice husk (Dutta *et al.*, 2014). In this research, the amount of produced xylose and reducing sugars are estimated.

Xylanase chemistry

Xylanase as a heteropolysaccharide is a major component of cell walls of plant hemicelluloses. Endoxylanase randomly hydrolyses the main chain of xylan to form xylooligosaccharides, which are then degraded by xylanolytic enzymes such as xylosidase and arabinofuranosidases. Accessory enzymes, are able to cleave side chain groups of heteroxylan. The final hydrolysis products of xylan are xylose and oligosaccharides, which have potential industrial application in the foods, paper, agricultural industries, as well as pharmaceuticals, and renewable fuel (Sriyapai *et al.*, 2011; Heck *et al.*, 2006; He *et al.*, 2010).

Based on the physicochemical properties and amino acid sequence similarities of their catalytic domains by hydrophilic cluster analysis, xylanases are classified into two glycoside hydrolase groups: family 10 (formerly family F, a high molecular mass >30 kDa and low isoelectric point) and family 11 (formerly family G, with low molecular mass <30 kDa and high isoelectric point) (Coughlan *et al.*, 1993). Many xylanases belonging to family 11 are obtained from *Actinomyces* (Sriyapai *et al.*, 2011; Callins *et al.*, 2005).

The high optimum temperature of xylanase and its alkaline optimal pH leads to its tremendous potential for application of enzyme for special benefits e.g. bleaching of kraft pulps and other biotechnological processes (Mohana *et al.*, 2008; Lakshmi *et al.*, 2009).

Xylanase producing microorganism

The xylanase producing microorganisms are isolated from soil collected from decaying agricultural waste. Screening of xylanase producing bacteria should be carried out on xylan-containing medium (He *et al.*, 2010). Many isolates are reported as good producer of xylanase in solid state fermentation (Yang *et al.*, 2008; Yang *et al.*, 2006). Table 1 shows a list of xylanase producing fungi were grown on agricultural waste. Also in Table 2, different xylanase producers on several substrates in different conditions are listed.

Xylanase of *Acinetobacter junii* has been lyophilized to enhance practical applicability and storage stability (Lo *et al.*, 2010). *Kluyvera*

species strain OM3 isolated from spent mushroom substrate could produce a high level of cellulose – free xylanase (5.12 u/ml) with maximum activities at 70°C and pH 8. In this study, 100% and 71% activity has retained after incubation at 60°C and 70°C and maintain stability over a pH range of 5 to 9 (Xin *et al.*, 2013). *Kluyvera* species is a good anaerobic bacterium which is capable of producing effective cellulase and xylanase and has high temperature and pH stability (Xin *et al.*, 2013). Production of 41 kDa xylanase from *Paenibacillus campinensis* is reported under various pH, temperature as well as alternative carbon and nitrogen sources. The results showed that the highest specific activity of xylanase in crude extract was obtained at 24 h, 37°C, pH 8. Xylanase activities of 56.8 % and 51.9 % were founded after 4 h incubation in pH 7 and 9 at 65°C, respectively (Ko *et al.*, 2010).

Thermomyces lanuginosus reported as producer of thermostable GF11 endo-xylanase encoded by XynA gene. *Escherichia coli* is also one of the most extensively used prokaryotic organism for the industrial production of enzyme because of its well-characterized genetics, and its ability to grow rapidly and at high density on inexpensive substrates (Le *et al.*, 2014).

There are very few reports showing the ability of the fungus to produce industrially important enzymes under nonsterile condition. Anyway, *Promicronospora* sp is capable of producing xylanase from rice straw in nonsterile fermentation (Kumar *et al.*, 2011). *Trichoderma* sp. can secrete large amounts of efficient xylanase for industrial production. (He *et al.*, 2010; Wong *et al.*, 1992). Xylanase production synthesized by *Pleurotus eryngii*. Xylanase activity was checked by using oat-spelt xylan as a substrate and the reducing group was detected through dinitrosalicylic assay method (Altaf *et al.*, 2010).

The selective production of xylooligosaccharides is conducted by partially purified xylanase from *Aspergillus foetidus* MTCC 4895 (Chapla *et al.*, 2012).

In recent years, many cellulolytic bacteria have been recognized for their ability to hydrolyze lignocellulosic materials for bioenergy production. Those cellulolytic bacteria include the genera of *Bacillus*, *Ruminococcus*, *Streptomyces*, *Bacteroides* and *Cellulomonas* (Lo *et al.*, 2010; Gessesse *et al.*, 1999; Rapp *et al.*, 1986).

Solid state fermentation (SSF) in xylanase production: SSF is the growth of organisms on moist substrates in the absence of free-flowing

water. The use of SSF for the production of enzymes and other products has many advantages over submerged fermentation (Gessesse *et al.*, 1999). SSF do not need for complex machinery and sophisticated control system with less volume of liquid for product recovery, which leads to reduced cost of downstream processing and subsequent waste treatment. Also, other advantages of this system are usability of simple and cheap media for the fermentation and lower energy demand, (often a high product yield) and lower risk of contamination due to the inability of most contamination to grow in the absence of free flowing water (Gessesse *et al.*, 1999).

A large number of fungal species are known to grow well on moist substrates in the absence of free-flowing water whereas many bacteria are unable to grow under this condition. As a result, most studies involving SSF have been conducted by using fungi.

SSF has interest for production of xylanase similar to many other enzymes due to lower operation costs and energy requirements, as well as simple plant and equipment projects in compared to submerged fermentation (Heck *et al.*, 2006; Heck *et al.*, 2005; Khosravi Darani *et al.*, 2008). Xylanase production by *P. thermophile J18* was carried out in SSF using wheat straw as substrate (Yang *et al.*, 2008). Also, xylanase production by a newly isolated *Aspergillus terreus* MTCC866 has been optimized using palm fiber in SSF (Table 1) (Lakshmi *et al.*, 2009; Yang *et al.*, 2008).

Xylanase applications

Research for xylanase biosynthesis is an interesting area due to its important industrial application e.g. improving the digestibility of animal feed, bleaching of kraft pulp, bioconversion of lignocellulosic waste into their constituent sugars, clarification of juices, (Mohana *et al.*, 2008) as well as extraction of plant oils, extracellular polymeric substances, improving nutritional value of silage, green feed, coffee, starch and as bleaching agents in pulp and paper industry (Lakshmi *et al.*, 2009). However, low production yield and the high total cost inhibit widespread commercial and industrial application of this enzyme (Lo *et al.*, 2010; Rapp *et al.*, 1986). As it was mentioned before, xylanase is able to hydrolyze the water soluble arabino-xylanase. This reaction leads to the release of lower molecular weight fraction with improved impact on specific volume expansion capacity and firmness of bread (Primo-Martin *et al.*, 2005). According to this 0.01% (w/w), xylanase led to

approximately 19.6% decline of the total phenolic content (Yang *et al.*, 2014).

The potential application of xylanases also includes reducing sugars by hydrolysis of lingo-cellulosic biomass. These sugars are further fermented for the biofuel production (e.g., ethanol, butanol). (Xin *et al.*, 2013). In compared to aerobic fungi and bacteria, few investigations are reported on hydrolytic enzymes by anaerobic bacteria (Bajpai 1996).

Browning is a problem of wheat products (e.g. wheat dough, chapatti's, pasta, and fresh oriental noodles) (Demekke *et al.*, 2001) during storage, transport and marketing (Baik *et al.*, 1995). This phenomenon is due to the activity of polyphenol oxides (PPO) and peroxidase (PO) which catalyze the oxidation of free, reduced phenolic compounds to pigment, forming elements (Kruger *et al.*, 1992) (with an exception to the color resulting from carotenoids (Francis, 2000). Reported approaches to overcome browning focus on inactivating the PPO, the PO or eliminating the substrates of these enzymes. Glucose oxidase (GOX) was reported as a dough bleaching enzyme because of its β -carotene degradation capacity (Gélinas *et al.*, 1998). Apart from GOX, xylanase was also commonly used for improving the properties of whole wheat dough (Bonet *et al.*, 2006; Primo Martin *et al.*, 2005; Steffolani *et al.*, 2012).

One of the exciting application of xylanases is the production of xylo- oligosaccharides (XOS) from many agrowastes such as concorb (also known as maize cores) (Anand *et al.*, 2013). These XOS exhibit prebiotic effect when consumed as a part of the diet (Driss *et al.*, 2014).

Results

Xylanases can be applied for waste management and production of many useful products. Production of oligosaccharides can be further considered as functional food sweeteners and additives. To meet the needs of industry, more attention of research should be focused on the increasing ability of to hydrolyze soluble or insoluble xylans as well as improved enzyme stability in different temperature pH, and inhibitors. Genetic engineering and recombinant DNA technology may have an important role in the large-scale expression of xylanases. No individual enzyme may meet all of the requirements of the feed and food industries. Moreover, as industrial applications require cheaper enzymes, the elevation of expression

levels seems crucial to ensure the sustainability of the process.

Conclusion

Agricultural wastes possess large quantities of hemicellulose (e.g. 25% in rice straw). The process for bioconversion of them to value-added products e.g. biofuels and chemicals are receiving increased attention. Such renewable resources are required for reduction of petroleum consumption. This is the best way for hydrolysis of agro-industrial wastes in an enzymatic solution.

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Table-1: Comparison of xylanase production from other fungi strains grown on agricultural waste

Microorganisms	Substrate	Xylanase activity	References
<i>Aspergillus terreus</i> (MTCC8661)	Palm oil fiber	115269u/g	Lakshmi <i>et al.</i> , 2009
<i>Thermomyces lanuginosus</i>	Sorghum straw	48000u/g	Bakri <i>et al.</i> , 2003
<i>Trichoderma longibrachiatum</i>	Wheat bran and wheat straw	592.7 u/g	Azin <i>et al.</i> , 2007
<i>Aspergillus niger</i> BO3	1.5% wheat bran+ 2.4% corn cobs+0.6% malt sprout	996 u/ml	Dobrev <i>et al.</i> , 2006
<i>Fusarium oxysporum</i>	2% corn cobs	245 u/ml	Kekos <i>et al.</i> , 1996
<i>Pseudomonas</i> sp.WLUNO24	7% wheat bran	450 u/ml	Xu <i>et al.</i> , 2005
<i>Aspergillus terreus</i> mutated strain	1% xylan, 0.5% peptone, 0.5% yeast extract, 0.1% KH ₂ PO ₄ , 0.05% MgSO ₄	42.2 u/ml	Geweely <i>et al.</i> , 2006

Table - 2: Comparison of different xylanase producers on several substrates in different condition

No	Microorganism	Substrate	Enzyme activity	Production rate	Productivity	Heat Resistance (C)	Optimal pH	Culture method	References
1	<i>Streptomyces thermocarboxidus</i> subsp <i>MW8</i>	1%(w/v) soytone+ 1% (w/v) NaCl, and 0.5%(w/v) xylan	35714 u/g	96 hours	372.02 u/g/h	50	7	Solid-state fermentation	Chi <i>et al.</i> , 2013
2	<i>Bulkholderia sp. DMAX</i>	Distillery spent wash	5200-5600 u/g	15 hours	346.66-373.33 u/g/h	50	8.6	Solid-state fermentation	Mohana <i>et al.</i> , 2008
3	<i>Bacillus stearothermophilus</i> ATCC12980 (Rockville Co.)	Xylan, 10g/ polypepton, 20g/ yeast extract, 2.5g/ Ammonium nitrate, 2g/ phosphate mono-potasic, 2g/ MgSO ₄ .H ₂ O, 1g/ MnSO ₄ , 0.05g	8700 u/g	48 hours	181.25 u/g/h	60	7	Solid-state fermentation	
4	<i>Paecilomyces thermophile</i> J18	Wheat straw	18580 u/g	168 hours	110.59 u/g/h	50		Solid-state fermentation	Yang <i>et al.</i> , 2006
5	<i>Aspergillus niger</i> P 602	Corn cob Wheat Straw	6320u/g	64 hours	98.75 u/g/h	55	5	Solid-state fermentation	Gawande <i>et al.</i> , 1999
6	<i>Streptomyces albus</i> & <i>Streptomyces chromofuseus</i>	Rice straw pulp	4301 u/g	48 hours	89.60 u/g/h	28	7.2	Solid-state fermentation	Rifaat <i>et al.</i> , 2005
8	<i>Paenibacillus Campinasensis</i> BL11	Kraft pulp mill	2939 u/g	24-48 hours	61.22-122.45 u/g/h	37	8	Solid-state fermentation	Ko <i>et al.</i> , 2010
9	<i>Aspergillus niger</i>	Cottonseed oil	1761 u/g	36 hours	48.91 u/g/h	40	4.6	Solid state fermentation	Wang <i>et al.</i> , 2006
10	<i>Bacillus Stearothrmophilus</i> SDX	Wheat bran	3446 U/g	72 hours	47.86 u/g/h	37	7	Solid state fermentation	Dhimanet <i>et al.</i> , 2008
11	<i>Aspergillus niger</i> KK2	Straw rice	5071u/g	120hours	42.25 u/g/h	50	4.8	Solid state fermentation	Kalogeris <i>et al.</i> , 1999
12	<i>Aspergillus awamori</i>	Sugarcane bagasse	2500 IU/g	60 hours	41.66 u/g/h	30		Solid state fermentation	Lemos <i>et al.</i> , 2002
13	<i>Aspergillus niger</i> N218	Corn cob Wheat Straw	2989 u/g	72 hours	41.51 u/g/h	55	5	Solid state fermentation	Gawande <i>et al.</i> , 1999
14	<i>Thermoascuc aurantiacus</i>	Straw wheat	5465u/g	168 hours	32.52 u/g/h	50	5	Solid-State Fermentation	Topakaset <i>et al.</i> , 2003
15	<i>Aspergillus foetidus</i>	Corn cob	3065u/g	96hours	31.92 u/g/h	50	5.3	Solid-State Fermentation	Wu <i>et al.</i> , 2005
16	<i>Bacillus circulans</i> BL53	Fibrous soybean residue	3700 u / g	120 hours	30.83 u/g/h	60	7	Strong inhibitors: Hg, SDS Slight: Na, Cu, Fe, Zn, g, Ca, PHMB General :Ions react with sulphhydryl group e.g. Hg ⁺	Heck <i>et al.</i> , 2005
17	<i>Aspergillus niger</i> CCUG33991	wheat Straw & bran	1465u/g	50 hours	29.30u/g /h	40	5	Solid state fermentation	Shahi <i>et al.</i> , 2011
18	<i>Aspergillus niger</i> LPB 326	Sugarcane bagasse +soybean meal	1937 IU/g	96 hours	20.17 u/g/h	30		Solid state fermentation	Macielet <i>et al.</i> , 2008

19	<i>Bacillus SY30A</i>	Wheat bran + (g/L):K ₂ HPO ₄ , 1; NaCl, 3; MgSO ₄ .7H ₂ O, 0.3)	1157 u/g	72 hours	16.06 u/g/h	55 (40-75)	7 (4-10)	Solid state fermentation	
20	<i>Aspergillus fumigates F-993</i>	White corn flour	720 u/g	48 hours	15 u/g/h	50 (50-65)	3.5 (3.5-6.5)	Solid state fermentation	Fadelet <i>et al.</i> , 2014
21	<i>Aspergillus fischeri Fxn 1</i>	Wheat Straw	1024 u/g	72 hours	14.22 u/g/h	50	6	Solid state fermentation	Weber <i>et al.</i> , 2002
22	<i>Thermoascus aurantiacus</i>	Bagasse	2700u/g	240 hours	11.25 u/g/h	50	5	Solid-state fermentation	Panagioto uet <i>et al.</i> , 2003
23	<i>Streptomyces sp.</i> (strain 1b 24D)	Tomato pomace	1447 u/ml	240 hours	6.02 u/ml/h **min**	60	6.5	Submerge state fermentation	Rawashde het <i>et al.</i> , 2005
24	<i>Bacillus subtilis NS7</i>	Nutrient broth suppl. With ylan, soybean meal, NaCL, and KH ₂ PO ₄	353 u/ml	72 hours	4.9 u/ml/h	37 (37-70)	6.5 (5-9)	Submerge state fermentation	Bansalet <i>et al.</i> , 2012
25	<i>Bacillus mojavensis</i>	Oat husk + yeast extract, 5g/ oat spelt xylan, 5g/ peptone, 5g/ K ₂ HPO ₄ , 1g/ MgSO ₄ .7H ₂ O, 1g	249.308 u/ml	48 hours	4.36 u/ml/h	55 (35-65)	9 (7-11)	Submerge state fermentation	Akhavan Sepahyet <i>et al.</i> , 2011
26	<i>Poliporus caliatus MRL7</i>	g/L (NH ₄) ₂ SO ₄ , 1.4/MgSO ₄ .7H ₂ O, 0.3/FeSO ₄ , 0.05/ZnSO ₄ .7H ₂ O, 0.014 / COCl ₂ , 0.02/ MnSO ₄ , 0.016)	292.8 u/ml	216 hours	1.35 u/ml/h	30 (25-37)	5	Submerge state fermentation	Saleemet <i>et al.</i> , 2014
27	<i>Lentinus pigrinus MRL6</i>	g/L:(NH ₄) ₂ SO ₄ , 1.4/ gSO ₄ .7H ₂ O, 0.3/FeSO ₄ , 0.05/ ZnSO ₄ .7H ₂ O, 0.014 / COCl ₂ , 0.02/MnSO ₄ , 0.016)	278.52 u/ml	216 hours	1.28 u/ml/h	30 (25-37)	5	Submerge state fermentation	Saleem <i>et al.</i> , 2014
28	<i>Phanerochaete sordid MRL3</i>	g/L :Proteous peptone, 0.5/ urea, 0.3/ KH ₂ PO ₄ , 0.2/ CaCL ₂ ,0.3/tween -80, 0.2)	272.7 u/ml	216 Hours	1.25 u/ml/h	30 (25-37)	5	Submerge state fermentation	Saleem <i>et al.</i> , 2014
29	<i>Aspergillusniger</i>	2.5% / 3%/ 3.5% concentration Sugarcane pagasse	39.07 u/ml	72 hours	0.54 u/ml/h	37 (25-32.5)	5.5 (5-6.5)	Submerge state fermentation	Robl <i>et al.</i> , 2015

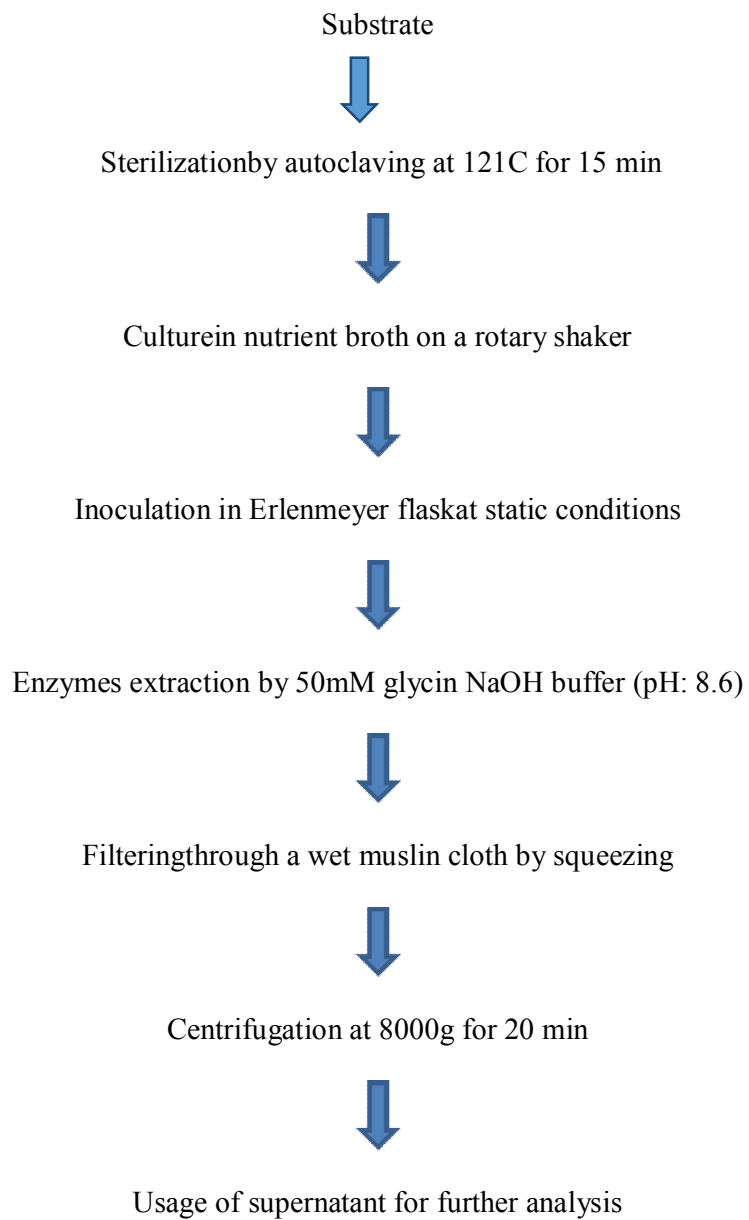


Figure - 1: Xylanase production Flowchart (modified from Shahi *et al.*, 2011)