Journal of Advances in Microbiology

5(1): 1-12, 2017; Article no.JAMB.33936
ISSN: 2456-7116

Aspergillus Xylanases

Hooi Ling Ho[1*]

[1]Faculty of Applied Sciences, UCSI University, No. 1, Jalan Menara Gading, UCSI Heights, Cheras, 56000 Kuala Lumpur, Malaysia.

Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

Article Information

DOI: 10.9734/JAMB/2017/33936
Editor(s):
(1) Niranjala Perera, Department of Food Science & Technology, Wayamba University of Sri Lanka, Sri Lanka.
Reviewers:
(1) Ali Mohamed Elshafei, National Research Centre, Egypt.
(2) Rafael Lopes e Oliveira, Amazon State University – UEA, Brazil.
(3) Amit Kumar, Maharishi Markandeshwar University, India.
Complete Peer review History: http://www.sciencedomain.org/review-history/20487

Review Article

Received 4th May 2017
Accepted 25th July 2017
Published 12th August 2017

ABSTRACT

There has been tremendous growth and development in the use of xylanase in the biotechnology industry. The market trends reveal that xylanase takes major position of share up to 20% of the world enzymes market along with cellulases and pectinases. In fact, xylanase has become one of the major industrial enzymes in the pulp and paper industry as bio-bleaching agent. Besides that, xylanase is also used in the production of detergents and beverages. In the feed industry, enzyme preparation containing xylanases is used to improve the digestibility of animal feed. Furthermore, xylanase is added to swine and poultry cereal-based diets to improve absorption of nutrients. Additionally, xylanase is used in the modifications of flour in bakery products as well as in the saccharification of agricultural, municipal and industrial waste materials. Most commercial enzymes including xylanase are produced in submerged and solid state fermentation. Submerged fermentation is performed by culturing microorganisms in a liquid medium containing required nutrients with specific composition, volume and concentration. In contrast, solid state fermentation is defined as growth of microorganisms on a layer of moist solid substrate without presence of any free flowing water yet with enough moisture for growth and metabolism of microorganisms. *Aspergillus* spp*,* indeed, *A. brasiliensis* is a filamentous ascomycete fungus that has been widely used in the biotechnology field for xylanase production. The production of xylanase from fungal cultures is relatively high compared to the ones from bacteria and yeasts. As a result, the most desirable xylanases are produced by filamentous fungi known as fungal xylanases particularly *Aspergillus* xylanases where they have been involved in many industries for decades.

Keywords: Aspergillus brasiliensis; fungi; submerged fermentation; solid state fermentation; xylanase.

**Corresponding author: E-mail: hohooiling@gmail.com;*

1. INTRODUCTION: *Aspergillus*

Aspergillus is a group of approximately 200 species of molds. *Aspergillus* species are found throughout the world and are the most common type of fungi in our environment. Among the 200 species of molds, 16 species of *Aspergillus* molds are pathogenic. The species which trigger infection in humans include *Aspergillus brasiliensis, Aspergillus clavatus, Aspergillus fumigates, Aspergillus flavus, Aspergillus glaucus, Aspergillus nidulans, Aspergillus oryzae, Aspergillus ustus* and *Aspergillus terreus. Aspergillus* molds grow vigorously in oxygen-riched environments and survive well on carbon-riched materials. There are also *Aspergillus* molds which are known to be xerophilic. Xerophilic *Aspergillus* molds survive in conditions with low humidity and moisture. The most common species for the genus of *Aspergillus* is *A. brasiliensis. A. brasiliensis* is a haploid filamentous fungus which is essential in the biotechnology field. *A. brasiliensis* is originally isolated from the plant *Welwitschia mirabilis*, a plant estimated to be about 3000 years old in Namibia and Angola. On the other hand, in laboratory, *A. brasiliensis* is produced from chemostat cultures. This particular fungal species is widely used in the production of extracellular enzymes and citric acid. Moreover, it is also used for waste management and biotransformations. Nevertheless, *A. brasiliensis* infects humans the third most often as it causes growth of fungal balls in the lungs which eventually trigger severe lung infection. *A. brasiliensis* is generally found on food and waste materials including dead leaves and decaying compounds [1]. In fact, this fungus also causes spoilage of food. The ideal temperature for the growth of *A. brasiliensis* is 30℃. Thus, the difficulty of germination in the human body is anticipated as body temperature is approximately 37℃. Besides that, due to the acidophilic nature of the fungus, the optimum pH of the growth of this fungus is detected to be around pH 4.5 to 4.8. This fungus is usually grown on potato dextrose agar. Potato dextrose agar is a non-selective growth media used to cultivate fungi and yeasts. It is made from the infusion of potato and dextrose. On the agar, the colonial morphology of *A. brasiliensis* is described as wool-like and white in colour. Nonetheless, the colour of the colony turns black after the conidial production. In addition, the microscopic morphology of the fungus is seen as hyphae with conidial heads that split into columns.

A. brasiliensis has been utilised for the production of enzymes such as xylanase, hemicellulases and cellulases. Besides that, the fungus has also been studied for the production of pectinases and invertases [2]. Enzyme is an essential class of globular proteins of biological origin that function as biochemical catalyst. The most distinct characteristics of an enzyme are its specificity and selectivity whereby each enzyme catalyses only a particular reaction involving a specific substrate. The sensitivity of enzymes towards the condition and environment in which they operate or function is one major property. Enzymes are generally needed in small quantities. They are able to work in within a specific range of pH, temperature and presence of cofactors and inhibitors. Most of the enzymes are specific where there is a single enzymatic reaction. Some enzymes have absolute specificity for one substrate and some would react with substrates with similar functional groups or side chains. Microbial enzyme production is becoming high in demand in biotechnology industry especially xylanase. Microbial enzymes are the most desired enzymes compared to that of animals and plants because of their low production cost and high predictability and reliability of their contents. The global market of industrial enzymes reached a billion dollars in 1990 and crossed $2.0 billion dollars in 2005 [3]. In the year of 2010, the market was estimated to be $3.3 billion dollars and expected to increase to $4.4 billion by the year of 2015. Nevertheless, the production costs and yields of enzymes are considered as the major problems. Therefore, investigations of the ability of using fungi to utilize inexpensive substrates have been done [4]. In fact, stronger efforts are constantly being made to elevate the production of hydrolytic enzymes using fermentation. Fermentation process provides a promising yield of enzymes. It is a very economical production method due to the ease accessibility of waste materials which eventually function as enzyme substrates. Indeed, one of the most desired enzymes used in the fermentation for industrial production is xylanase.

2. XYLANASE

Xylanase is an extracellular enzyme which hydrolyses β-1,4-D-xylosidic linkages of highly polymerized β-1,4-D-xylobiose, xylotriose and glucuronosyl residues. This particular enzyme is potential for the degradation of plant cells [5]. Xylanase is produced by a large group of different fungi, bacteria and yeasts. This enzyme

is also found in protozoa, insects, seaweeds and plants seeds [1]. Filamentous fungi are particularly well known producers of xylanase because they excrete the enzyme into the culture medium. Moreover, their enzyme activities are much higher compared to that of bacteria and yeasts [6]. Besides that, filamentous fungi are non-pathogenic [7]. Fungal xylanases are usually associated with the minor production of cellulase which occurs at the same time during the production of xylanase. Xylanases from different sources differ in their requirements in medium pH and temperature for optimum activity and productivity. According to Ho [8], xylanases are genetically single chain glycoproteins with molecular mass ranging between 6 kDa to 80 kDa. They are normally active between pH 4.5 to 6.5. There are several types of xylanases and these enzymes act under different conditions in terms of pH and temperature. The heterogeneity and sophistication of xylan result in abundance of a variety of xylanase with different specificities, primary sequences and structures. Therefore, xylanases have been grouped into two categories based on the basis of their physiochemical properties. The physiochemical properties include having a high molecular mass which is approximately more than 30 kDa with an acidic pH and otherwise having a low molecular mass with a basic pH. Although xylanases have been grouped, many xylanases especially fungal xylanases are not classified by this system as there is a complete classification system that also classifies all glycosidases. Thus, this system has been used as a standard classification for the enzymes that fall under the category of glycosidases.

According to Min-Jen et al. [9], xylanases are classified into endo-xylanases and exo-xylanases. Normally, exo-xylanases (β-D-xylopyranosidases) are referred as extracellular xylanases where they are being secreted into the culture medium. In 1991, xylanases were grouped into Family F and G based on hydrophobic cluster analysis and sequence homology. The Family F and G correspond to Family 10 and 11 in the numerical classification of glycosyl hydrolases [10,11]. According to Dijkstra and Krengel [12], the Family F is larger where it possesses a molecular mass of 35 kDa whereas Family G has a molecular mass of 20 kDa. Xylanases have been classified into Family 10 and 11 based on their molecular mass and isoelectric point. Subramamaniyian and Prema [13] pointed out that high molecular mass endoxylanases with low pI values belong to glycanase Family 10 whereas low molecular mass endoxylanases with high pI values belong to Family 11. According to Biely et al. [14], there has been addition of 123 poteins into the Family 11 and this Family is divided into three groups namely I, II and III, which are mainly consisted of fungal enzymes. Enzymes in group I and II are produced by *Ascomyceta* and *Basidiomyceta* while group III is produced by anaerobic fungi.

2.1 Xylan

Xylan is constructed from homopolymeric backbone chain of β-1,4-D-xylopyranose units which includes short chains of O-acetyl, α-L-arabinofuranosyl and D-glucuronyl or O-methyl-D-glucuronyl residues. There are four main categories of hemicelluloses namely xylans, mannan, B-glucans and xyloglucans. Among the four categories of hemicelluloses, xylan is the major component and approximately makes up to 35% of dried plant cells weight [15]. The hydrolysis of xylan relies on two classes of enzymes which are endoxylanases and xylosidases. The endoxylanases hydrolyse the xylan backbone into smaller components such as oligosaccharides which eventually degrade into xylose. Xylan consists of heteropolymers containing β-1,4-D-xylopyranose. There are four types of xylans and one of them is known as homoxylan. Homoxylan is a homopolymer which is found in seaweeds. In addition, another type of xylan is glucoronoxylan which is usually segregated from hardwoods. In fact, xylan is supposed to be O-acylated but due to the alkaline extraction, the acetyl groups are splited. The reduced ends of xylan are reportedly to be connected to rhamnose and galacturonic acid in order to form alkaline resistant end groups of xylan chain. Xylan is capable of producing intra-chain hydrogen bond which supports two β-1,4-cellulose helix folds. There is only one hydrogen bond between adjacent xylosyl residues in contrast with two hydrogen bonds between adjacent glycosyl residues of cellulose. The absence of primary alcohol functional group which is external to the pyranoside ring as in cellulose and mannan has a massive effect on the intra- and inter-chain of hydrogen bonding interactions. Intra-chain of hydrogen bonding which occurred in an O-3 position results in the helical twist to the structure. Lignin is bound to xylans by an ester linkage to 4-O-methyl-D-glucoronic acid residues. Glucoronoxylans are partially or fully insoluble in water. On the other hand, arabinoxylans are the main components of wheat, rice, corn and dietary fibres. Xylan in

general is found in most agricultural extracts such as soya bean hull and palm kernel. Indeed, most of the fruits especially the skins also contain xylan.

2.2 Xylanase Activity

Comparison of xylanase activity produced by different types of fungi and some of the higher producing strains has been carried out by many researchers. The definition of enzyme activity according to the International Union of Biochemistry states that one unit of enzyme activity corresponds to the amount of enzyme that catalyses the hydrolysis of 1 micro mole of substrate per minute under reaction conditions. In the case of xylanase activity, 1 U corresponds to the amount of enzyme needed to release 1 micromole of reducing sugar of xylose per minute under reaction conditions [8]. The principle of the xylanase assay works in a way where xylanase catalyses the enzymatic hydrolysis of xylan by releasing xylose. The reaction is hindered by the addition of 3, 5-dinitro salicylic acid (DNS) and a red coloured complex is formed with the presence of xylose. Hence, the amount of xylose released is generally measured at 575 nanometers using a spectrophotometer after the detection using DNS.

3. XYLANASE PRODUCTION AND PURIFICATION

Fermentation is a process that utilises microorganisms to convert solid or liquid substrates into various products. In the earlier days, fermentation was applied to produce wine, beer and cheese unlike today where fermentation is also used to produce hydrogen gas and industrial alcohol such as biofuel. In the 1850s and 1860s, Louis Pasteur was the first scientist to study and demonstrate the fermentation process. Commercially useful fermentations are grouped into two categories namely submerged and solid state fermentation. In solid state fermentation, microorganisms grow on a moist solid with little liquid. On the other hand, in submerged fermentation, microorganisms and medium are mixed in a fermenter or culture flask with constant supplied of oxygen. Submerged fermentation is a method of fermentation employed to cultivate microorganisms in a liquid nutrient medium. According to Esser and Bennett [16], the most prominent feature of submerged fermentation is the fungal morphology as the germinating spores form stubby, forked and bulbous hyphae aggregation. In fact, the enzymes productivity is affected by fungal morphology [17]. Some studies have shown that the correlation between the fungal morphology and enzymes productivity is due to its effect on the viscosity of the medium [18]. The advantage of this process is the minimal monitor of various parameters. Moreover, fungal spores are evenly distributed in the medium [19]. In most cases, the productivity of enzymes in solid state fermentation would be much higher than that of submerged fermentation especially fungal xylanases. Okafor et al. [20] investigated the potential of agro-wastes for xylanase production by *A. brasiliensis* using submerged fermentation whereby wheat bran aroused as the optimum substrate by producing 6.47 U/mL of xylanase activity. Since the contents in submerged fermentation are in liquid medium, the transfer of heat and mass are more efficient and effective. The process can also be disadvantageous where a higher risk of contamination is likely to occur. Furthermore, higher production costs are tend to incur especially in larger scale of production where larger bioreactors are employed.

Submerged fermentation began in the 1930s, apparently, it is still the main method used until today. The advantage of the submerged fermentation is the parameters that can be controlled and monitored by the means of adding reagents or nutrients. Submerged fermentation is subdivided into aerobic process which requires oxygen while anaerobic process which is carried out in the absence of oxygen. One example of aerobic submerged fermentation process is xylanase production by *A. brasiliensis.* Apparently, fermentation process is influenced by a number of factors such as pH, temperature, medium composition, dissolved oxygen, dissolved carbon dioxide, types of fermentation operation mode and shear rate of the fermenter. The operation mode of fermentation could either be batch, fed-batch, or continuous. The difference in these factors may affect the rate of fermentation together with the product yield, texture and smell. Shake flask fermentation is the type of fermentation carried out in shake flasks, especially in Erlenmeyer flasks. Generally, 250 mL or 500 mL Erlenmeyer flasks are used to accommodate the culture medium. In most cases, baffles have been used in shake flask fermentation in order to assist in oxygen transfer and to prevent vortex formation. One disadvantage of using baffles in shake flask fermentation is in fact suitable for lower volume and shorter term of fermentation due to excessive splashing of the medium onto the

cotton wool which could eventually prevent free flow of oxygen. There are a variety of plugs used for the shake flask fermentation such as cotton wool, glass wool and gauze. The volume of medium in a shake flask culture plays an important role. A lower volume of medium in a shaker flask culture would increase the rate of oxygen transfer. Low volumes of medium are usually used in fermentation processes which are carried out in a short period of time. Medium with high volume would trigger evaporation of the nutrients, thus, causing the medium to be concentrated and viscous. Shaker incubators are used in shake flask culture. The shaker provides stable temperature and conditions where the cells are growing. The shaker incubator contains a tray which moves in a circular motion provides orbital agitation to the culture medium. Agitation affects the rate of aeration and the mixing of the cells culture. Large scale fermentation is carried out either in a batch, fed-batch or continuous fermentation mode. Batch and fed-batch operations are quite common while continuous fermentation is rare. In most cases, batch fermentation runs for 4 to 5 days or maybe even up to 9 days in a bioreactor. There are seven major parts of the bioreactor namely peristaltic pumps, drive system, operational amplifiers, base units, control panel, reagent bottle holder, vessel and support frame. Each part holds specific functions which allows the bioreactor to work efficiently. The peristaltic pumps connect the inlet side of the pumps to the reagent bottles and the outlet to the two way inlet fitting on the vessel top plate. The drive system is the essential part of the bioreactor as it is connected to the impeller. The impeller provides the shear force for culture medium. The motor is located within the base unit. The connection to the vessel is simply lifted for removal and replaced for operation. The operational amplifiers function as sensors for temperature, pH and air pressure which connect the antifoam probes to the fixed cables. The reagent bottle holder holds the scott bottles on the side of the bioreactor. Meanwhile, the base units create space for the rotameter. The rotameter controls the gas flow rate. The vessel is the container which carries the culture medium while the support frame holds the vessel in place. The parameter keys such as pH, temperature, air pressure, antifoams and pumps are displayed on the control panel for operation.

Xylanases can be produced by submerged fermentation and solid state fermentation. In recent years, solid state fermentation has gained relatively much interest in biotechnology industry especially in the production of many enzymes. This is due to the fact that minimised operation costs are incurred with higher product concentration and productivity with lower risk of microbial contamination as compared to submerged fermentation [21]. In comparison to submerged fermentation, solid state fermentation involves heterogeneous interactions of microbial biomass with moist solid substrate. The lower moisture content in solid state fermentation promises a more efficient way of downstream processing. The solid state technique involves cultivation of microorganisms on solid substrate with a high content of nutrients. According to Rengasayee et al. [22], the content of moisture in substrate highly lowers the threat of bacterial infections. The unique morphological characteristics of filamentous fungi allow them to colonise and penetrate solid substrate in search for nutrients, as a result, fungi are excellently suitable for solid state fermentation [23]. Several studies have reported higher xylanase production in solid state fermentation as compared to submerged fermentation. Malarvizhi et al. [24] observed 30-fold increment of xylanase production in solid state fermentation than in liquid culture fermentation when wheat bran was used as the substrate for the culture of *Ganoderma lucidum*. Nikhil et al. [25] obtained higher xylanase production of 710.4 U/gds/min in solid state fermentation over submerged fermentation on a study of xylanase production by *Aspergillus flavus* FPDN1. The major downside of this technique is not possible to feed ingredients to the medium during cultivation. Likewise, the changes in pH and carbohydrate concentration can not be controlled during the solid state fermentation process.

There are two important factors in xylanase fermentation. These two factors include strain selection and growth cycle of xylanase producers. In xylanase production, a relatively active producer strain should be considered to produce high activity of enzyme. On the other hand, it is essential to recognize the stage of the growth cycle where the enzyme activity is at its optimum peak. As in many cases, enzymes rapidly disappear as soon as they reach their optimal activity. Microbial growth in a newly inoculated fermenter shows four stages or patterns. The stages of microbial growth proceed from lag phase, exponential phase, stationary phase and eventually to death phase. Therefore, during the lag phase, the cells concentration does not increase much. The length of the lag phase depends on the growth history of the

inoculum and the composition of the medium. The lag phase is also known as the adaptation phase of the microorganism in a new environment. Cells weight increases drastically during the exponential phase. Consequently, the nutrients become exhausted while the inhibitory products of metabolism build up in the culture causing the microorganisms to enter the stationary phase. The death phase begins after the stationary phase, whereby cell lyses begins and biomass concentration declines. The mass and morphology of the pellet change as the growth of the fungi proceeds. It is indicated that in a submerged culture, the mass of the microorganisms increase at a slower rate compared to the exponential rate in a condition where there is a reasonable approximation of the data. The rate of increase in the colony length is constant whereas the radius of the fungal colony elevates at a constant rate on culture surface. The pellet size and morphology of the fungi are determined by the medium composition, concentration of the pellets and agitation rate. The growth of fungal mycelia happens through chain elongation and branching which proceeds from the tip of the mycelium as apical growth forming septa between the cells. Furthemore, when the mycelia grow on a surface, it becomes intertwined and thick mats are formed. In submerged culture, the mycelia may exist as diffuse mycelia or form pellets with the diameter ranges from 0.1 to 10 mm. In a study on xylanase production by Laxmi et al. [26], they revealed that the xylanase production by an isolated *Aspergillus* spp RSP-6 was depended on the microbial growth and fermentation medium. Moreover, the biochemical and physical properties of the enzyme produced vary from one microorganism to another depending on the genetic strain. Human beings are unable to produce this enzyme but naturally occurring bacteria in the human intestine are able to produce xylanase. As such the enzyme is beneficial not only to animals, but also to human health. Some of its benefits to human health are better digestion of plant-based foods, assist in reducing intestinal discomfort from eating foods like beans, cereals and fibrous vegetables and also increase of xylan-based prebiotics to support healthy intestinal bacteria.

Xylanase production is influenced by fermentation conditions such as temperature, medium pH, shear force and dissolved oxygen. Fermentation temperature affects growth rate of the microorganisms and productivity of xylanase. Medium pH is one of the essential factors. Growing fungi at an unfavourable medium pH limits the growth rate which eventually results in reduced yields of xylanase. There were several findings which stated that lower accessibility of the hemocellulosic substrate due to unfavourable pH conditions result in lower induction of xylanase. Higher shear force in other words, higher agitation rate has several negative effects towards the microorganisms used in xylanase production especially filamentous fungi. The examples of the effects are leakage of intracellular material, disintegration of hyphae and changes of morphology of the fungi [27]. Dissloved oxygen does not really play an important role in the productivity of xylanase. Enzyme activity and production of extracellular proteins are not affected by oxygen levels of 20% or above but severely lowered at oxygen level of 10%. Other factors such as the addition of minimal amounts of purified xylan into the lignocellulosic substrate or carbon source have been reported to be beneficial to induce the activity of the xylanase. Nevertheless, the culture medium used in the prolonged fermentation process may contain undesirable metabolites produced by microorganisms that reduce the enzyme activity. In contrast, some distinctive compounds are added in the medium to enhance the production of xylanase by fungi include several mineral salts such as KH_2PO_4, $MgSO_4$, $CaCl_2$, and NH^{4+} or NO^{3+}. In fact, Fe^{2+}, Co^{2+} and Zn^{2+} are mostly added in the medium for xylanase production. Complex nitrogen sources are added in the medium as well to improve the production of xylanase [8]. Generally, higher xylanase activity could be obtained when peptone or yeast extract is added in the production medium. Cheaper complex nitrogen supplements such as cotton seed derived protein and corn steep powder have also been used. Additionally, other surfactants mainly Tween 80 or fatty acids are mostly added into the medium to improve the yields of xylanase. Over the years, many xylanase producing strains have been found. However, the commercial xylanase production is mostly restricted to *Trichoderma* spp and *Aspergillus* spp*,* some examples are *Trichoderma reesei* [28] and *Aspergillus nidulans* [29]. Table 1 illustrates the production of xylanase from various types of microorganisms reported in literatures and Table 2 summarizes the characteristics of xylanases including molecular weight, optimum pH and temperature, Km, Vmax and pI values from different microorganisms.

Table 1. Production of xylanase from different types of microorganisms

Microorganism	Reference
Aspergillus niger	Pang and Omar [30]
Aspergillus niger	Ahmad et al. [31]
Aspergillus niger	Kavya and Padmavathi [32]
Aspergillus carneus	Fang et al. [33]
Aspergillus foetidus	Shah and Madamwar [34]
Bacillus licheniformis	Archana and Satyanarayan [35]
Paecilomyces themophila	Yang et al. [36]
Penicillium chrysogenum	Jayant et al. [37]
Streptomyces spp	Saurav and Kannabiran [38]
Streptomyces cyaneus	Ninawe and Kuhad [39]
Tricoderma viride	Juwaied et al. [40]

Although there are many producer strains that yield high activity of xylanase, *A. brasiliensis* is considered as one of the optimum xylanase producers because it carries more advantages compared to other strains. The application of *A. brasiliensis* as the optimum strain not only improves the performance of the microorganism, in fact it also produces the desired enzyme of xylanase more progressively. As a result, *A. brasiliensis* has been investigated and elucidated enormously for xylanase production. Apparently, many have reported *A. brasiliensis* produced higher xylanase activity and productivity. According to Ho [56], xylanase activity by *A. brasiliensis* was found to be 2.175 ± 0.103 U/mL after using wheat bran as the prime carbon source in shake flask culture. Indeed, much higher production of 7.074 ± 0.089 U/mL was observed in batch bioreactor system. In the study, agricultural residue such as wheat bran has been introduced as the substitute carbon source to reduce the production cost of xylanase. In the further study, the *Aspergillus* xylanase was subsequently purified with DEAE Sepharose and Sephadex G-75 column chromatography. At the end of purification, xylanase was purified up to 3.6-fold with its recovery yield of 1.68% and specific activity of 116.64 U/mg. Additionally, the purified xylanase was detected to be a low molecular weight protein. Indeed, a molecular weight of 36 kDa of the purified xylanase was visualized and detected on SDS-PAGE [57].

4. XYLANASE APPLICATIONS

Xylanase possesses multiple industrial applications. Conventionally, the application of xylanase has been majorly considered for the bioconversion of lignocellulosic materials, especially agricultural and forestry wastes and residues in order to produce high-value products such as biofuel. Moreover, xylanase has been used in many hydrolysis processes such as production of oligosaccharides mainly xylobiose and xylotiose from isolated xylans [58]. These oligosaccharides are subsequently used as functional additives and sweeteners. Additionally, xylanase is also being utilised in clarification of final products of wine and fruit juice. This enzyme increases fruit pulp production while releasing aroma precursors. Xylans, pectins and hemicelluloses available in fruits are broken down into simpler molecules such as xylose and glucose with the presence of xylanase. Hemicelluloses are polysaccharides found in plant cell walls that are not cellulose (polymer of β-1,4-D-glucose) and pectins (polymer of galacturonic acid). The breakdown of the cells wall yields the extraction of the juice from the fruits.

Apart from this, xylanase is also used to reduce beers haze and viscosity as well as to increase wort filterability in brewing. Besides that, xylanase is widely used in food industry. Among the different types of food industry, much importance attention has been given to bread production. Bread is highly consumed around the globe. Commonly, enzymes such as fungal α-amylases are being used in bread making process. Throughout the years, enzymes have gained much importance and popularity in the bread making industry as they improve the quality and characteristic of the dough. Other enzymes including proteases, amylases and cellulases are also involved in conjuction with xylanase to strengthen the gluten network of the bread. In the baking process, xylanase degrades the glycosidic linkages in arabinoxylans. This action improves the handling properties of the dough and volume of the bread. Furthermore, attention on xylanase has grown recently mainly due to the fact that this enzyme is applied in pulp and paper industry. Xylanase improves chemical bleaching of pulps resulting in more economical and environmental advantages over the chemical method of using chlorine [59]. Xylanase strengthens cellulose fibers in bleached kraft pulp and improves fibrillation water retention of pulp. In fact, microscopic analysis reveals xylanase possesses the ability to open up fibre surfaces which exhibit detached material on xylanase treated pulps. This enzyme enhances pulp bleaching up to 25% in savings campared to chlorine based chemicals. Enzymatic pre-treatment of the pulps increases their final brightness value. In order to conduct this

Table 2. Characteristics of xylanases from different microorganisms

Species	Molecular weight (kDa)	Optimum		K_m (mg/ml)	V_{max}	pI	References
		pH	Temperature (℃)				
Aspergillus niger	13.5-14.0	5.5	45	-	-	9	Frederick et al. [41]
Aspergillus kawachii IFO 4308	26-35	2-5.5	50-60	-	-	3.5-6.7	Ito et al. [42]
Aspergillus nidulans	22-34	5.4	55	-	-	-	Fernandez-Epsinar et al. [43]
Aspergillus sydowii MG 49	30	5.5	60	-	-	-	Ghosh and Nanda [44]
Aspergillus fischeri Fxn1	31	6	60	4.88	5.88 µM/min/mg	-	Raj and Chandra [45]
Aspergillus sojae	32.7	5.0	60	-	-	3.50	Kimura et al. [46]
	35.5	5.5	50	-	-	3.75	
Aspergillus caespitosus	26.3	6.5	50	2.5	1679 µ/mg	-	Sandrim et al. [47]
	27.0	7.0	55	3.9	113 µ/mg	-	
Aspergillus ficuum AF-98	35	5	45	3.267	18.38 M/min/mg (beechwood xylan)	-	Lu et al. [48]
	35	5	45	3.747	11.1 M/min/mg (birchwood xylan)	-	Lu et al. [48]
Aspergillus niger BCC14405	21	5	55	8.9	11,100 U/mg	-	Asano Krisana et al. [49]
Trichoderma harzianum	20	5	50	0.58	0.106 µM/min/mg	-	Tan et al. [50]
Trichoderma reesei	20	5-5.5	45	3.0-6.8	-	9.0	Tenkanen et al. [51]
	19	4-4.5	40	14.8-22.3	-	5.5	
Thermomyces lanuginosus-SSBP	23.6	6.5	70-75	3.26	6300 µM/min/mg	3.8	Lin et al. [52]
Thermomyces lanuginosus DSM 5826	25.5	7	60-70	7.3	-	4.1	Cesar and Mrsa [53]
Penicillium purpurogenum	33	7.0	60	-	-	8.6	Belancic et al. [54]
	23	3.5	50	-	-	5.9	
Fusarium oxysporum	20.8	6	60	9.50	0.41 µM/min/mg	-	Christakopolous et al. [55]
	23.5	6	55	8.45	0.37 µM/min/mg	-	

application, xylanase is prepared with the absence of cellulase to avoid the damage of the kraft pulp. The outcome of this application has opened up doors of using xylanase in other applications of paper manufacturing. One example is deinking of newsprint. In most cases, xylanase is blended with cellulase in a desired ratio for deinking process. At the optimum ratio of 50:50, much higher brightness of deinked pulp is obtained. The busting and tearing index as well as breaking length of enzymatically deinked pulp are higher compared to that of chemically-treated pulp. On the other hand, in the feed industry, xylanase is used to improve digestibility of animal feed, thus, increasing feed viability. Xylanase is added into poultry feed materials such as wheat bran and rice bran to enhance absorption of nutrients especially for the birds. This would eventually reduce the amount and concentration of nutrients in bird droppings. Furthermore, xylanase is also applied in forage digestions whereby it is sprayed on forages along with other fibrolytic enzymes. The forages are then fed to cows to enhance their milk production.

5. SUMMARY

Fungal xylanase particularly from *Aspergillus* spp gains potential growing demand as one of the important industrial enzyme due to its diverse applications in many industries ranging from fruit pulp production to pulp and paper manufacturing. In a nutshell, xylanase attracts many research interests as a result of its huge revenues generated in the global market of industrial enzymes.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Wang CH, Hangling Y, Haiwan H, Honghai G. Xylanase production and its application in degradation of hemicellulose materials. International Congress on Biotechnology in the Pulp and Paper Industry. 1998(7):65-67.
2. Montiel-González AM, Viniegra-González G, José Fernández F, Loera O. Effect of water activity on invertase production in solid state fermentation by improved diploid strains of *Aspergillus niger.* Process Biochemistry. 2004(39):2085-2090.
3. Krishna C. Solid-state fermentation system: An overview. Critical Reviews in Biotechnology. 2005(25):1-30.
4. Kang SW, Park YS, Lee JS, Hong SI, Kim SW. Production of cellulases and hemicellulases by *Aspergillus niger* KK2 from lignocellulosic biomass. Journal of Bioresource Technology. 2004;91:153-156.
5. Omar AW, Khataibeh MH, Abu-Alruz K. The use of xylanases from different microbial origin in bread making and their effects on bread quality. Journal Applied Science. 2008;8(4):672-676.
6. Haltrich D, Nidetzky B, Kulke KD, Steiner W, Zupanie S. Production of fungal xylanases. Bioresource Technology. 1997; 58:137 -161.
7. Kar S, Mandal A, das Mohapatra PK, Mondal KC, Pati BR. Production of cellulase-free xylanase by *Trichoderma reesei* SAF3. Brazilian Journal of Microbiology. 2006;37:462-464.
8. Ho HL. Effects of Medium formulation and culture conditions on microbial xylanase production using agricultural extracts in submerged fermentation (SmF) and solid state fermentation (SsF): A Review. Journal of Biodiversity, Bioprospecting and Development. 2014;1:130.
   DOI: 10.4172/2376-0214.1000130
9. Min-Jen T, Mee-Nagan Y, Khanok R, Khin LK, Shui-Tein C. Purfication and characterization of two cellulose free xylanases fron an alkaliphilic *Bacillus firmus*. Enzyme and Microbial Technology. 2002;30:590-595.
10. Henrissat B. A classification of glycosyl hydrolases based on amino acid sequence similarities. Biochemical Journal. 1991; 280:309-316.
11. Henrissat B, Bairoch A. New families in the classification of glycosyl hydrolases based on amino acid sequence similarities. Biochemical Journal. 1993;293:781-788.
12. Dijkstra U, Krengel BW. Three-dimensional structure of endo-1,4-β-xylanase I from *Aspergillus niger*: Molecular basis for its low pH optimum. Journal of Molecular Biology. 1996;263:70-78.
13. Subramaniyan S, Prema P. Biotechnology of microbial xylanase: Enzymology, molecular biology and application. Critical Reviews in Biotechnology. 2002;22:33-64.
14. Biely P, Markovik O, Mislovicova D. Sensitive detection of endo-1,4-glucanases

& endo-1,4-xylanases in gels. Analytical Biochemistry. 1985;144:147-151.

15. Jiang Z, Cong Q, Yan Q, Kumar N, Du X. Characterisation of a thermostable xylanase from *Chaetomium* sp. and its application in chinese steamed bread. Food Chemistry. 2010;120:457-62.
16. Esser K, Bennett JW. The Mycota. Germany: Springer. 2002;224-225.
17. Wang L, Ridgway D, Gu T, Moo-Young M. Bioprocessing strategies to improve heterologous protein production in filamentous fungal fermentations. Biotechnology Advances. 2005;23:115-129.
18. Bhargaba S, Wenger K, Marten M. Pulsed addition of limiting-carbon during *Aspergillus oryzae* fermentation leads to improved productivity of a recombinant enzyme. Biotechnology and Bioengineering. 2003(82):111-117.
19. Rao M, Mishra C, Seeta R, Srinivasan MC, Deshpande VV. *Penicillium janthinellum* as a source of fungal biomass protein from lignocellulosic waste. Biotechnology Letters. 1983;5:301-304.
20. Okafor U, Okochi VI, Onyegeme-okerenta BM, Nwodo-Chinedu S. Xylanase production by *Aspergillus niger* ANL 301 using agro-wastes. African Journal of Biotechnology. 2007;6(14):1710-1714.
21. Ashorkkumar B, Gunasekaran P, Kayalvizhi N. Optimization of media for β-fructoranosidase production by *Aspergillus niger* in submerged and solid state fermentation. Process Biochemistry. 2001; 37:331-338.
22. Rengasayee V, Thomas JN, Siddarth B. Optimisation of production of xylanase enzyme production in *Aspergillus* spp. Thesis. Chennai: Anna University; 2005.
23. Maghsoodi V, Yaghmaei S. Comparison of solid substrate and submerged fermentation for chitosan production by *Aspergillus niger*. Transactions C: Chemistry and Chemical Engineering. 2010;17(2):153-157.
24. Malarvizhi K, Murugesan K, Kalaichelvan PT. Xylanase production by *Ganoderma lucidum* on liquid and solid state fermentation. Indian Journal of Experimental Biology. 2003;41:620-626.
25. Nikhil B, Dharmes A, Thakor P. Production of xylanase by *Aspergillus flavus* FPDN1 on pearl millet bran: Optimization of culture conditions and application in bioethanol production. International Journal of Research in Chemistry and Environment. 2012;2(3):204-210.
26. Laxmi GS, Sathish T, Rao CS, Brahmaiah P, Hymavathu M, Prakasham RS. Palm fiber as novel substrate for enhanced xylanase production by isolated *Aspergillus* sp. RSP-6. Trends in Biotechnology and Pharmacy. 2008; 2(2):447-455.
27. Shi M, Chen L, Wang XW, Zhang T, Zhao PB, Song XY, Sun CY, Chen XL, Zhou BC, Zhang YZ. Antimicrobial peptaibols from *Trichoderma pseudokoningii* induce programmed cell death in plant fungal pathogens. Microbiology. 2012;158:166-175.
28. Liu C, Qiang Y, Qing Z, Shi-yuan Y. Study on the selective production of xylanase by *Trichoderma reesei.* Linchan Huaxue Yu Gongye. 1999;19(2):8-12.
29. Pinaga F, Fernandez-Espinor MT, Valles S, Roman D. Xylanase production in *Aspergillus nidulans.* Induction and carbon catabolite repression. FEMS Microbiology Letters. 1994(115):319-324.
30. Pang PK, Omar IC. Xylanase production by a local fungal isolate, *Aspergillus niger* USM AI 1 via solid state fermentation using palm kernel cake (PKC) as substrate. Songklanakarin Journal of Science and Technology. 2005;27:325-336.
31. Ahmad Z, Butt MS, Anjum FM, Asgher M. Effect of wheat bran concentration on xylanase biosynthesis by *Aspergillus niger*. International Journal of Agriculture and Biology. 2009;11:571-576.
32. Kavya V, Padmavathi T. Optimization of growth conditions for xylanase production by *Aspergillus niger* in solid state fermentation. Polish Journal of Microbiology. 2009;58:125-130.
33. Fang HY, Chang SM, Hsieh MC, Fang TJ. Production, optimization growth conditions and properties of the xylanase from *Aspergillus carneus* M34. Journal of Molecular Catalysis B: Enzymatic. 2007; 49:36-42.
34. Shah AR, Madamwar D. Xylanase production under solid-state fermentation and its characterization by an isolated strain of *Aspergillus foetidus* in India. World Journal of Microbiology and Biotechnology. 2005;21:233-243.
35. Archana A, Satyanarayana T. Xylanase production by thermophilic *Bacillus*

licheniformis A99 in solid-state fermentation. Enzyme and Microbial Technology. 1997;21:12-17.

36. Yang SQ, Yan QJ, Jiang ZQ, Li LT, Tian HM, et al. High-level of xylanase production by the thermophilic *Paecilomyces themophila* J18 on wheat straw in solid-state fermentation. Bioresource Technology. 2006;97:1794-1800.
37. Jayant M, Rashmi J, Shailendra M, Deepesh Y. Production of cellulase by different co-culture of *Aspergillus niger* and *Penicillium chrysogenum* from waste paper, cotton waste and baggase. Journal of Yeast and Fungal Research. 2011;2:24-27.
38. Saurav K, Kannabiran K. Diversity and optimization of process parameters for the growth of *Streptomyces* VITSVK9 spp isolated from Bay of Begal, India. Journal of Natural and Environmental Sciences. 2010;1:56-65.
39. Ninawe S, Kuhad RC. Use of xylan-rich cost effective agro-residues in the production of xylanase by *Streptomyces cyaneus* SN32. Journal of Applied Microbiology. 2005;99:1141-1148.
40. Juwaied AA, Al-amiery AAH, Abdumuniem Z, Anaam U. Optimization of cellulase production by *Aspergillus niger* and *Tricoderma viride* using sugar cane waste. Journal of Yeast and Fungal Research. 2011;2:19-23.
41. Frederick MM, Kiang C, Frederick JR, Reilly PJ. Purification and characterization of endo-xylanases from *Aspergillus niger*. I. Two isozymes active on xylan backbones near branch points. Biotechnology and Bioengineering. 1985; 27:525-532.
42. Ito K, Ogasawara H, Sugimoto T, Ishikawa T. Purification and properties of acid stable xylanases from *Aspergillus kawachii*. Bioscience, Biotechnology and Biochemistry. 1992;56:547-550.
43. Fernandez-Epsinar MT, Ramon D, Pinaga F, Valles S. Xylanase production by *Aspergillus nidulans.* FEMS Microbiology Letters. 1992;91:91-96.
44. Ghosh M, Nanda G. Purification and some properties of xylanase from *Aspergillus sydowii* MG 49. Applied and Environmental Microbiology. 1994;60:4620-4623.
45. Raj KC, Chandra TS. Purification and characterization of xylanase from alkalitolerant *Aspergillus fischeri* Fxn 1. FEMS Microbiology Letters. 1996;145:457-461.
46. Kimura I, Sasahar H, Tajima S. Purification and characterization of two xylanases and an arabinofuranosidase from *Aspergillus sojae*. Journal of Fermentation and Bioengineering. 1995;80:334-339.
47. Sandrim VC, Rizzatti ACS, Terenzi HF, Jorge JA, Milagres AMF, Polizeli MLTM. Purification and biochemical characterization of two xylanases produced by *Aspergillus caespitosus* and their potential for kraft pulp bleaching. Process Biochemistry. 2005;40(5):1823-1828.
48. Lu FX, Lu M, Lu Z, Bie XM, Zhao HZ, Wang Y. Purification and characterization of xylanase from *Aspergillus ficuum* AF-98. Bioresource Technology. 2008;99(13): 5938-5941.
49. Asano Krisana, Sriprang R, Gobsuk J, Eurwilaichitr L, Tanapongpipat S, Kirtikara K. Endo-1,4-β-xylanase B from *Aspergillus niger* BCC14405 isolated in Thailand: Purification, characterization and gene isolation. Journal of Biochemistry and Molecular Biology. 2005;38(1):17-23.
50. Tan LUL, Wong KKY, Yu EKC, Saddler JN. Purification and characterization of two D-xylanases from *Trichoderma harzianum*. Enzyme and Microbial Technology. 1985; 7:425-430.
51. Tenkanen H, Puls J, Poutanen K. Two major xylanases of *Trichoderma reesei*. Enzyme and Microbial Technology. 1992; 14:566-574.
52. Lin J, Ndlovu LM, Singh S, Pillay B. Purification and biochemical characteristics of β-D-xylanase from a thermophilic fungus, *Thermomyces lanuginosus*-SSBP. Biotechnology and Applied Biochemistry. 1999;30:73-79.
53. Cesar T, Mrsa V. Purification and properties of xylanase produced by *Thermomyces lanuginosus*. Enzyme and Microbial Technology. 1996;19:289-296.
54. Belancic A, Scarpa J, Peirano A, Diaz R, Steiner J, Eyzayuirre J. *Penicillium purpurogenum* produces several xylanases: Purification and properties of two of the enzymes. Journal of Biotechnology. 1995;41:71-79.
55. Christakopoulos P, Nerinckx W, Kekos D, Marcis B, Claeyssens M. Purification and characterization of two low molecular mass alkaline xylanases from *Fusarium*

oxysporum F3. Journal of Biotechnology. 1996;51:181-189.

56. Ho HL. Batch submerged fermentation in shake flask culture and bioreactor: Influence of different agricultural residuals as the substrate on the optimization of xylanase production by *Bacillus subtilis* and *Aspergillus brasiliensis*. Journal of Applied Biotechnology and Bioengineering. 2016;1(3):00016.
DOI: 10.15406/jabb.2016.01.00016
57. Ho HL, Soh LS, Ong SH. Production, purification and characterisation of a purified low molecular weight and thermo-alkaline tolerance xylanase by *Aspergillus brasiliensis* in submerged fermentation. British Microbiology Research Journal. 2016;11(2):1-25.
58. Pellerin P, Gosselin M, Lepoutre J, Samain E, Debeire P. Enzymatic production of oligosaccharides from corncob xylan. Enzyme and Microbial Technology. 1991;13:617-621.
59. Viikari L, Kantelinen A, Sundquist J, Linko M. Xylanases in bleaching: From an idea to the industry. FEMS Microbiology Reviews. 1994;13:335-350.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/20487