Int. J. of Food Ferment. Technol. 4(1): 1-11, June, 2014
DOI Number: 10.5958/2277-9396.2014.00001.4

Review Paper

Effects of Medium Formulation and Culture Conditions on Microbial Xylanase Production: A Review

Hooi Ling Ho

Faculty of Applied Sciences, UCSI University, 1, Jalan Menara Gading, UCSI Heights, Cheras, 56000 Kuala Lumpur, Malaysia.

Corresponding author: Hooi Ling Ho; hohooiling@gmail.com

Paper no. : 74 **Acceptance :** 25th May, 2014 **Received in revised form:** 27 August, 2014 **Accepted:** 27 August, 2014

Abstract

Xylanase is the enzyme that degrades xylan, causes decreasing in degree of polymerization, hence releasing oligomers such as xylobiose and xylose. Compared to the plants and animals, fungi are the most preferable microorganisms for this purpose due to their growth conditions and structural sturdiness. Xylanase is produced by several microorganisms such as *Aspergillus*, *Penicillium*, *Trichoderma*, *Streptomyces* and *Bacillus spp.* Nonetheless, *Aspergillus* is identified as the major producer of xylanase. Xylan is commonly used as a substrate for determining the xylanase activity. Due to the potential applications of xylanase in the industries and environment, it is essential to minimize the cost of production by optimising medium formulation and culture conditions of microorganisms in submerged fermentation. Its high demand worldwide, requires optimization of microbial xylanase production by specific carbon and nitrogen sources with optimum growth conditions, both are apparently managed to reduce production cost besides providing adequate amounts of nutrients for biomass and xylanase production, respectively. Xylanase has become valuable and attractive enzyme due to its vast applications in pulp and paper, food and beverage, detergent and textiles industry. All these aspects have been reviewed in this article.

Keywords: Xylanase production, medium formulation, cultural conditions, submerged fermentation

In the present era, various strains of filamentous fungi such as *Aspergillus niger*, *A. oryzae* and *Trichoderma spp.* have been reported to be the potent producers of xylanase. However, *A. niger* have received greater attention when it is utilised in the industrial production of xylanase (Abdeshahian *et al.,* 2009). Filamentous fungi are industrially important producers of xylanase due to the fact that they secrete xylanase into the medium thus, cell disruption is not required. Moreover, the xylanase yield from fungal culture is apparently higher than that of yeast and bacterial cultures. In addition, the

production of microbial xylanase is also preferred to that of plants and animals because of their availability, structural stability and genetic manipulation (Ahmad *et al.,* 2012). Xylanases are known to be inducible enzymes (Cai *et al.,* 2003) and are genetically single chain glycoproteins, ranging from 6 to 80 kDa and active between pH 4.5-6.5 at 40-60^{o}C. Xylanase can be divided into two types: plant and microbial xylanases. Plant xylanases are known to play major roles in seed germination, fertilization and programmed cell death while microbial xylanases are recognized due to their potential roles in biotechnological industry. There has been much concern and immense importance on xylanase due to its potential industrial applications. One of the major applications of xylanase is involved in pulp and paper industry where its application has increased considerably during the past few years (Thakur *et al.,* 2012). Kraft is made after the removal of residual lignin from paper pulp during bleaching process, whereby it requires the usage of large amounts of chlorine and chloride. These chemicals tend to generate toxic pollutants in the surrounding environment. Therefore, extensive research has been carried out to focus on the utilization of biological alternatives that can prevent the usage of these toxic chemicals. As a result, xylanase is used in the bleaching process to replace toxic chlorine-based bleach chemicals. Consequently, it produces better quality and properties of pulp and paper. Thus, xylanase plays an effective role in pulp and paper industry.

Xylan as a substrate

Xylan or wood gum is a heterogeneous polysaccharide found in the cell walls of many plant species (Wong *et al.,* 1988). Xylan is consisted of complex structures of hemi-celluloses that have backbone composed of β-1,4-linked xylopyranose subunits. Hemi-celluloses of xylan are accounted for approximately 15 to 30% of the total dry weight in wood component of angiosperms (Gupta *et al.,* 2009). Besides cellulose, xylans are the most abundant polysaccharides found in nature (Rifaat *et al.,* 2005). Xylan found in cell walls of plants differed grately depending on their origins and molecules that are attached to the xylan backbone. The degradation of xylan into its monosaccharides constituent is achieved after several actions of hydrolytic enzymes that take place due to its heterogenous structure. In this case, β-1,4-xylanase cleaves the polysaccharide backbone of xylan while β-xylosidases hydrolyzes xylo-oligosaccharides to xylose (Haltrich *et al.,* 1997). In the past, the conversion of xylan into xylose was conventionally carried out using acid hydrolysis process. Nowadays, many studies considered xylan as one of the new substrates in the production of biofuels, pharmaceuticals and solvents.

Xylanase activity

Since xylanase is one of the most valuable enzymes in various biotechnological applications, there are a number of studies carried out regarding xylanase activity. Xylanase is useful in the production of xylose from xylan if the cellulolytic activities do not affect the cellulosic fibers. Xylanase activity depends on the xylan as the substrate, whereby there is a linear relationship between the time of hydrolysis and the amount of the reducing sugars produced. Khan *et al.* (1986) reported that the xylan substrate influences the xylanase activity assay, thus producing significant measurable amount and concentration of reducing sugars. Moreover, different concentrations of reducing sugars in xylanase activity are obtained from different laboratories using different substrates (Haltrich *et al.,* 1997). Furthermore, Bailey *et al*. (1992) also concluded that xylanase activity is depended on the types of substrates. Besides that, xylanase activity is also influenced by the incubation temperature of xylanase activity which is in a range of 45 to 60^{o}C (Coral *et al*., 2002). There are a number of xylanase activity assays used for the determination of reducing sugars, each with their own definition of xylanase activity unit. These assays however, differed in their procedure such as temperature, duration of incubation and substrate used. However, the principle is to quantify xylanase activity from the detection of reducing sugars released from the respective substrate. For example, one unit of xylanase activity is defined as the amount of xylanase required to release one micromole of xylose per mL of enzyme extract under the assay condition.

The most common method used for the quantification of xylanase activity assay is 3, 5-dinitrosalicyclic acid (DNS) assay (Miller, 1959). This method is relatively fast, less

hazardous and convenient. Another advantage of using DNS method is the response of non-stoichiometry of the colour. Besides DNS method, Somogyi-Nelson (SN) method is also commonly applied (Nelson, 1944; Somogyi, 1952; Goyal *et al.,* 2008). Nevertheless, DNS method was abundantly reported in most of the studies of xylanase activity assays (Yang *et al.,* 2005; Muthezhilan *et al.,* 2007; Kavya and Padmavathi, 2009; Singh *et al.,* 2009; Kaur *et al.,* 2011; Murugan *et al.,* 2011).

Xylanase producing microorganisms

In recent years, considerable attention has been focused on the use of microorganisms in industrial fermentation processes, especially xylanase production. Usage of xylanase in industry has increased significantly over the years (Techapun *et al.,* 2003). Xylanase as the major enzyme is involved in the degradation of the hemicelluloses backbone of xylose has been isolated extensively from various microorganisms. The enzyme from different sources work differently at different temperatures and pH values. Several studies have been reported on xylanase producing microorganisms in nature which include yeast, bacteria and fungi (Simoes *et al.,* 2009). Filamentous fungi, such as *Aspergillus* spp (Haq *et al.,* 2004), *Penicillium spp* (Fadel and Fouda, 1993), *Streptomyces* spp (Kansoh and Gammel, 2001), *Bacillus* spp (Rashid, 1999) and *Trichoderma* spp (Azin *et al.,* 2007; Seyis and Aksoz, 2005) have been most extensively studied and manipulated in the production of xylanase. In addition, production of xylanase from fungi has higher enzyme activity compared to those of bacterial strains (Subramaniyan and Prema, 2002). As a result, fungi have been proved as the organisms with the capability for biosynthesis of xylanase. However, *Aspergillus niger* remains the fungi of choice for xylanase biosynthesis (Wong *et al.,* 1998). *A. niger,* a filamentous fungi, is one of the most common species of the genus *Aspergillus* which consists of over 185 species. This strain of fungi is commonly mesophilic and is one of the most established microorganisms used for the industrial production of xylanase. *A. niger* is also commonly used for the production of extracellular enzymes such as lipases, amylases and proteases as well as production of citric acid, oxalic acid and gluconic acid (Takahashi *et al.,* 1991). Table 1 illustrates some examples of xylanase production from different types of microorganisms.

Table 1. Various microorganisms that are involved in production of xylanase

Microorganism	Source
Aspergillus niger	Abdel-Naby *et al.,* 1992
Aspergillus niger	Prasertsan *et al.,* 1997
Aspergillus niger	Ahmad *et al.,* 2012
Aspergillus flavus	Bhushan *et al.,* 2012
Aspergillus terreus	Shahriarinour *et al.,* 2005
Bacillus pumilus	Rashid, 1999
Coprinopsis cinerea	Kaur *et al.,* 2011
Coprinellus disseminatus	Singh *et al.,* 2009
Penicillium spp.	Fadel and Fouda, 1993; Li *et al.*, 2011
Paecilomyces themophila	Yang *et al.,* 2005
Streptomyces spp.	Kansoh and Gammel, 2001
Trichoderma harzianum	Seyis and Aksoz, 2005
Trichoderma longibrachiatum	Azin *et al.,* 2007

Production of xylanase under submerged fermentation

The enzyme is produced both in submerged and solid state fermentation, many studies on the production of xylanases have been performed in submerged liquid culture (Murugan *et al.,* 2011; Simoes *et al.,* 2009). Currently, 80-90% of commercial xylanase are produced in submerged culture because it has a higher degree of intensification, better level of automation and greater flexibility of scaling up. The use of submerged fermentation for production of xylanase is rising due to the better understanding of fungal metabolism and their positive responses. Using this technique, microorganisms are cultured in a liquid medium containing required concentrations of nutrients for the optimisation of medium formulation. Similarly, growth conditions of incubation temperature and pH medium are easily maintained in this fermentation. Hakim (2006) reported the advantages of using submerged fermentation over the solid state fermentation. In submerged fermentation, it produces higher enzymatic yield and productivity, involves lesser man

power, requires lower maintenance cost, exposes lesser risks of contamination with better temperature, pH, dissolved oxygen, agitation and aeration control during fermentation process especially in a bioreactor system. Using submerged fermentation, the study on the optimisation of various parameters is very convenient whereby bioreactor is easily monitored, periodic sampling of broth can be regularly conducted. If necessary, the addition of further nutrients can be regulated under continuous fermentation process.

In general, nutrients supplements in submerged fermentation are supplied in the medium in the form of cheaper and readily available complex waste materials including rice bran and rice straw (Wang *et al.,* 2003), corn cobs (Ahmad *et al.,* 2012), sugarcane bagasse, oat straw and wheat bran (Khandeparkar and Bhosle, 2007), which are among the sources of carbon and energy for the growth of microorganisms and xylanase biosynthesis. Thus, it is critical to minimize the cost of production by optimising the medium formulation and growth conditions of microorganisms in submerged fermentation. Submerged fermentation is the culture of microorganisms in liquefied medium as compared to solid state fermentation which involves the growth of microorganisms in the absence or near-absence of free moving water. Generally, medium is used to culture xylanase producers at a specified temperature and pH with constant aeration and agitation until the sufficient concentration of xylanase is achieved. Low xylanase activity is apparently obtained in non-agitated flasks, most probably due to oxygen or mass transfer limitations, while on proper agitation speed, higher xylanase activity is produced, probably due to satisfactory oxygen supply. In addition, the volume in the submerged fermentation has a great impact on oxygen and nutrients supply that affects the growth of microorganism and production of enzymes (Mimura and Shinichi, 1999; Ivanova *et al*., 2001). Therefore, in submerged fermentation, aeration and agitation are very important to ensure availability of nutrients, transfer of oxygen and absorption of other essential substances to the growing cells. In submerged fermentation, it is possible to use wide range of substrates and adequate standard inoculum size to minimise the amount of time needed for fermentation compared to solid state fermentation. There are two methods that can be used as the standard inoculum for submerged fermentation, either by direct inoculation of spores to the sterile liquid medium or using vegetative cells.

However, submerged fermentation using fungi such as *A. niger* for xylanase production was commonly initiated by direct inoculation of spores to the sterile liquid medium. Apparently, higher inoculums size in the submerged fermentation encourages better xylanase production in shorter lag phase followed by faster log phase of cell growth. Table 2 summarizes details of production of xylanase by different microorganisms in submerged fermentation.

Table 2. Production of xylanase by different microorganisms through sporulation in submerged fermentation

Microorganism	Standard inoculum (spores/mL)	Xylanase activity (U/mL)	Source
Aspergillus niger	1×10^4	44.10	Loera and Cordova, 2003
Aspergillus niger	1×10^7	125.14	Cao *et al.,* 2008
Aspergillus niger	1×10^5	293.82	Bakri *et al.,* 2008
Aspergillus carneus	1×10^6	22.20	Fang *et al.,* 2007
Aspergillus flavus	1×10^6	31.00	Nair *et al.,* 2008
Bacillus subtilis	1.85×10^6	128.00	Annamalai *et al.,* 2009
Streptomyces spp.	1.4×10^8	70.00	Nascimento *et al.,* 2002
Trichoderma viride	1×10^6	2.45	Goyal *et al.,* 2008

Medium formulation for xylanase production

Xylanase activity is generally influenced by the medium composition. Thus, medium optimisation has been advocated as one of the approaches for the improvement of microbial xylanase activity. The effect of different types and concentrations of carbon and nitrogen sources on the performance of xylanase production from various studies are summarized here.

Effects of carbon source

The production of microbial xylanase on submerged fermentation is strongly affected by the types of carbon source used. A fairly large number of carbon containing substances have been evalued. Different carbon sources for optimising medium formulation for xylanase production by various microorganisms have been employed such as starch, sucrose, maltose, glucose, lactose, fructose, mannose, galactose, xylose, sorbitol and glycerol. When Seyis and Aksoz, (2005) studied the effect of different carbon sources on xylanase activity by *Trichoderma harzianum,* sucrose and glucose were found to produce xylanase and out of these, sucrose showed higher xylanase biosynthesis compared to glucose.

Table 3: Effect of carbon sources on xylanase activity by various fungi

Fungi	Carbon source	Xylanase activity (U/mL)	Source
Aspergillus niger	Maltose	17.80	Simoes *et al.,* 2009
Aspergillus niger	Oat spelt	13.88	Tallapragada and Venkatesh, 2011
Aspergillus niger	Corn cobs	60.03	Ahmad *et al.,* 2012
Fusarium solani	Wheat straw	52.81	Gupta *et al.,* 2009
Penicillium implicatum	Lactose	19.50	Simoes *et al.,* 2009
Streptomyces chartreusis	Corn cobs	334.34	Li *et al.,* 2011
Tetracheatum elegans	Sucrose	90.00	Sati and Bisht, 2006
Tetracladium marchalianum	Glucose	100.00	Sati and Bisht, 2006
Tetracladium marchalianum	Sucrose	107.00	Sati and Bisht, 2006
Trichoderma viride	Sorbitol	169.00	Simoes *et al.,* 2009

Complex undefined agricultural extracts have also been reported by several studies as the essential carbon sources for xylanase activity. During biosynthesis of xylanase from *A. niger*, wheat bran delivered the optimal activity of xylanase when compared with other alternative substrates (Park *et al.,* 2002; Haq *et al.,* 2002). Besides that, xylanase activity by *A. niger* was also examined using various carbon sources such as maltose, oat spelt and corn cob (Simoes *et al.,* 2009; Tallapragada and Venkatesh, (2011) and Ahmad *et al.,* 2012). Based on their results, growth medium containing corn cob as carbon source produced higher xylanase activity of 60.03 U/mL compared to 17.80 and 13.88 U/mL from maltose and oat spelt, respectively. Additionally, Li *et al.,* (2011) produced the highest xylanase activity of 334.34 U/mL by *Streptomyces chartreusis* using corn cobs as carbon source. In other study, Haq *et al.,* (2002) investigated the production of xylanase by mutant strain of *A. niger,* GCBMX-45 using different concentrations of carbon and nitrogen sources. From their results, the mutated fungi produced the highest xylanase activity of 2350 U/g using 2% starch and contributed towards 2480 U/g by using 0.2% ammonium sulphate. Table 3 summarizes the xylanase production by various fungi using different carbon sources.

Effects of nitrogen source

The effect of nitrogen source on xylanase production has been extensively studied on submerged fermentation. Ammonium chloride, ammonium sulphate, ammonium nitrate, peptone and yeast extract were among the suitable nitrogen sources for the production of xylanase (Haltrich *et al.,* 1997; Hoq *et al.,* 1994). Hence, nitrogen source helped to enhance the efficient growth of fungi and improved activity of xylanase also (Oshoma *et al.,* 2010). Yeast extract was commonly used in many studies as nitrogen source. Xylanase activity by *A. niger* was investigated using yeast extract (Tallapragada and Venkatesh, 2011) and $(NH_4)_2SO_4$ (Abdel-Naby *et al.,* (1992). Based on their results, yeast extract provided higher xylanase activity of 14.37 U/mL compared to 13.90 U/mL using $(NH_4)_2SO_4$, respectively. In addition, the production of xylanase by *A. flavus* was also optimised using yeast extract and peptone and it was concluded that yeast extract and peptone were the most

recommended nitrogen source compared with other organic and inorganic nitrogen sources (Bhushan *et al.,* 2012). Similarly, Pal and Kaushik, (2012) elucidated the effect of various nitrogen sources on the growth of *Rhizoctonia solani* and it was concluded that the highest xylanase activity was obtained from yeast extract and peptone. Table 4 summarizes the xylanase production by various fungi using different nitrogen sources.

Table 4: Effect of nitrogen sources on xylanase activity by various fungi

Fungi	Nitrogen source	Xylanase activity (U/mL)	Source
Aspergillus niger	Yeast extract	14.37	Tallapragada and Venkatesh, 2011
Aspergillus niger	$(NH_4)_2SO_4$	13.90	Abdel-Naby *et al.,* 1992
Cladosporium macrocarpum	$NaNO_3$	32.00	Fattah *et al.,* 2011
Streptomyces chartreusis	Yeast extract	42.54	Li *et al.,* 2011

Cultural conditions for xylanase production

Fungal fermentation is widely recognized as a complex process due to diverse fungal morphology. In order to achieve high production of xylanase, it is essential to study the influence of cultural conditions such as incubation temperature and pH of medium on fungal growth. Hence, the effects of different incubation temperatures and pH of medium on the performance of xylanase production from various studies are illustrated here.

Effects of incubation temperature

The incubation temperature is one of the important parameters to determine the performance of fermentation. Most filamentous fungi are mesophilic which require the optimal growth temperatures between 25 and 35°C, although some species thrive best at 50°C (Reid, 1998; Suresh and Chandrasekaran, 1999). *A. niger* was reported to grow best at 25 to 30°C. At temperatures higher than the optimum, the heat transfer between molecules is high, which leads to enzymes denaturation process. Heat also destroys the properties of the nutrients of the raw substrate materials of xylanase and so decreases the xylanase activity of the fungi. Conversely, the lower temperatures lead to lower fungal metabolic and enzymatic activity (Adinarayana *et al*., 2003). For the production of xylanase, many *Aspergillus* species were grown at the optimum temperature of 30°C and the highest xylanase activity of 276 U/mL has been reported by Bailey *et al.,* (1989), when *A. foetidas* was incubated at 30°C. Similarly, *A. niger* gave a xylanase activity of 69.88 U/mL when incubated at 30°C (Gupta *et al.,* 2009). Table 5 shows the studies on xylanase production by various fungi at different temperatures.

Table 5. Effects of incubation temperature on xylanase activity by various fungi

Fungi	Incubation temperature (°C)	Xylanase activity (U/mL)	Source
Aspergillus foetidas	30	276.00	Bailey *et al.,* 1989
Aspergillus niger	25	23.20	Simoes *et al.,* 2009
Aspergillus niger	28	8.98	Tallapragada and Venkatesh, 2011
Aspergillus terreus	35	35.00	Chidi *et al.,* 2008
Fusarium solani	30	69.88	Gupta *et al.,* 2009
Fusarium solani	28	-	Chirstakopoullos *et al.,* 1999

Effects of initial pH of medium

The pH of medium has a great influence on the performance of xylanase activity. It was reported that most filamentous fungi are observed to grow well under slightly acidic conditions, ranging from 3 to 6, but some fungi are able to growth at a pH below 2 (Fawole and Odunfa, 2003). Several studies investigated the effects of various initial pH of medium for xylanase production by different strains of

fungi. Ahmad *et al.,* (2012) reported that the initial pH of medium of 5.5 produced the optimum xylanase activity of 60.03 U/mL by *A. niger.* Conversely, when the initial pH of medium was adjusted to 8.0, Tallapragada and Venkatesh (2011) obtained the optimum xylanase activity of only 5.51 U/mL. Table 6 shows a few reports on xylanase production by various fungi at different initial pH values of medium.

Table 6. Effects of initial pH of medium on xylanase activity by various fungi

Fungi	pH of medium	Xylanase activity (U/mL)	Source
Aspergillus niger	8.0	5.51	Tallapragada and Venkatesh, 2011
Aspergillus niger	5.5	60.03	Ahmad *et al.,* 2012
Aspergillus terreus	6.0	28.60	Chidi *et al.,* 2008
Fusarium solani	5.5	58.80	Gupta *et al.,* 2009
Penicillium implicatum	5.0	27.40	Simoes *et al.,* 2009

Industrial applications of xylanase

Xylanase plays a major role in the breakdown of xylan into xylose in industry. (Table 7) There are various important applications of xylanase. One of the major functions is involved in the pulp and paper industry. The process of bleaching of kraft pulp is involved with xylanase, that is one of the major chemical replacement constituents (Coral *et al*., 2002). Alkaline xylanase that is soluble in alkaline solutions is one of the most important xylanase applications due to their eco-friendly competence in pulp and paper industry (Horikoshi, 1996). The role of xylanase enzyme in pulp and paper industry is to replace the use of harsh chemicals in the bleaching of kraft pulp before the paper is manufactured (Beg *et al.,* 2001). The xylanase enzyme is able to remove the colour from the kraft paper during the bio-bleaching process. Throughout the bio-bleaching process at high temperature and pH, xylanase is able to reduce 20% of kappa amount from the pulp. Consequently, it reduces the usage of chlorine, improves pulp quality and viscosity, and thus, increases the brightness of colour of paper (Khandeparkar and Bhosle, 2007). Xylanase in cellulose pulp treatment is also able to reduce the utilization of chlorine compound.

Table 7. Major applications of xylanase in ndustry

Industry	Applications	Source
Pulp and paper	Kraft pulp bio-bleaching	Wong *et al.,* 1988; Subramaniyan and Prema, 2002; Li *et al.,* 2005; Khandeparkar and Bhosle, 2007.
Food and beverage	Clarification of wine and juices; alternative sweeteners and food additives	Beg *et al.,* 2001; Subramaniyan and Prema, 2002
Feedstock processing	Improve digestibility of animal feed and increase feed efficiency	Pucci *et al.,* 2003
Agriculture	Bioconversion of agricultural wastes to produce higher value products such as biofuel and other chemicals	Eriksson, 1990; Damaso *et al.,* 2003; Dhiman *et al.,* 2008
Baking	Modifying baking products, improving bread volume, texture and stability and enhancing the recovery of starch from wheat flours	Li *et al.,* 2000; Bhat *et al.,* 2001; Tallapragada and Venkatesh, 2011

The importance of xylanase is not only restricted to the pulp and paper industry, but is also involved in other industries with equal values of importance. Most commonly xylanase is used in baking, feedstock processing, food and beverage, detergent and textile industries. Xylanase exhibits excellent performance during dough handling process by improving elasticity and strength of the dough, subsequently, improving the desirable texture, loaf volume and overall quality of bread. Xylanase also reduces viscosity of animals feed and subsequently, improves the digestion and absorption of nutrients into the animal body. As a result, it increases the body weight of farm animals including cattle and sheep. On the other hand, xylanase also aids in the clarification and taste consistency of juice and wine in food and beverage

industry (Table 7). Additionally, other potential applications of xylanase are involved in the bioremediation and bioconversion of agricultural, municipal and food wastes for the production of fermentable bioproducts and renewable biofuel such as bioethanol.

Conclusion

Growth of microorganisms is affected by many factors including xylanase activity when the complex undefined substrates are utilized in the submerged fermentation system. In some cultural media, the growth of microorganisms that generated higher yields of biomass concentration gave higher xylanase activity. The production of xylanase has been always directly proportional to the biomass concentration. which indicates that particular medium composition and culture conditions that produce the optimum xylanase activity are essential to produce higher biomass concentration. Moreover, the fermentation parameters such as the optimum incubation temperature and pH of medium on xylanase activity significantly affect the biomass production at various levels. Xylanase plays the role as one of the primary metabolites that is needed for the growth of microorganisms. This enzyme operates through hydrolysis of complex substrates to reducing sugars that are eventually absorbed into the cells. In conclusion, in order to increase the xylanase biosynthesis, complex undefined carbon sources are generally supplied after the addition of the defined sugar as carbon source in order to stimulate the initial growth of microorganisms in submerged fermentation.

References

Abdel-Naby, Mohamed, A. and Kwon, D. Y. 1992. Production of xylanase and xylosidase by *Aspergillus niger* NRC 107. *Korean Journal of Applied Microbial Biotechnology,* **20**(5): 543-550.

Abdeshahian, P., Samat, N. and Wan Yusoff, W. M. 2009. Xylanase production by *Aspergillus niger* FTCC 5003 using palm kernel cake in fermentative bioprocess. *Pakistan Journal of Biological Sciences,* **12**(15): 1049-1055.

Adinarayana, K., Prabhakar. T., Srinivasulu, V., Anitha, R.M., Jhansi, L.P. and Ellaiah, P. 2003. Optimization of process parameters for cephalosporin C production under solid state fermentation from *Acremonium chrysogenum*. *Process biochemistry*, **39**: 171-177.

Ahmad, Z., Butt, M. S., Anjum, F. M., Awan, M. S., Rathore, H. A., Nadeem, M. T., Ahmad, A. and Khaliq, A. 2012. Effect of corn cobs concentration on xylanase biosynthesis by *Aspergillus niger. African Journal of Biotechnology*, **11**(7): 1674-1682.

Annamalai, N., Thavasi, R., Jayalakshmi, S. and Balasubramanian, T. 2009. Production and optimization of cellulase-free, alkali-stable xylanase by *Bacillus pumilus* SV-85S in submerged fermentation. *Indian Journal of Biotechnology,* **8**: 291-297

Azin, M., Moravej, R. and Zareh, D. 2007. Production of xylanase by *Trichoderma longibrachiatum* on a mixture of wheat bran and wheat straw: Optimization of culture condition by Taguchi method. *Enzyme and Microbiology Technology,* **40**: 801-805.

Bailey, M. J., Biely, P. and Poutanen, K. 1989. Production of xylanolytic enzymes by strains of *Aspergillus. Applied Microbiology and Biotechnology,* **30**: 5-10.

Bailey, M. J., Biely, P. and Poutanen, K., 1992. Interlaboratory testing of methods for assay of xylanase activity. *Journal of Biotechnology*, **23**: 257-270.

Bakri, Al-Jazairi, M. and Al-Kayat, G. 2008. Xylanase Production by a Newly Isolated *Aspergillus niger* SS7 in Submerged Culture. *Polish Journal of Microbiology,* **57**(3): 249-251.

Beg, Q. K., Kapoor, M., Mahajan, M. L. and Hoondal, G. S. 2001. Microbial xylanase and their applications: a review. *Applied Microbiology Biotechnology,* **56**: 326-338.

Bhat, M. K. and Hazlewood, G. P. 2001. Enzymology and other characterisitcs of cellulases and xylanases. *Enzymes in farm animal nutrition*, CAB International, Wallinford, UK. **38**: 11-60.

Bhushan, B., Pal, A. and Jain, V. 2012. Isolation, screening and optimized production of extracellular xylanase under submerged condition from *Aspergillus flavus* Mtcc 9390. *EEG, an open access journal,* **1**(1).

Cai, Q., Yue, T., Cheng, J. and Ma, Q. 2003. The screening of culture condition and properties of xylanase by white rot fungus *Pleorotus ostreatus*. *Process Biochemistry,* **33**: 2-6.

Cao, Y., Meng, D., Lu, J. and Long, J. 2008. Statistical optimization of xylanase production by *Aspergillus niger* AN-13 under submerged fermentation using response surface methodology. *African Journal of Biotechnology*, **7**(5): 631-638.

Chidi, S. B., Godana, B., Ncube. I., vanRensburg, E. J. and Cronshaw, A. 2008. Production, purification and characterization of cellulase-free xylanase from *Aspergillus terreus* UL 4209. *African Journal of Biotechnology*, **7**: 3939-3948.

Chirstakopoullos, P., Mamma, D., Nerinkxw, W., Kekos, D., Macris, B. and Claeyssens, M. 1999. Production and partial characterisation of xylanase from *Fusarium oxysporum*. *Bioresource Technology*, **58**: 115-119.

Coral, G., Arikan, B., Unaldi, M. N. and Guvenmez, H. K. 2002. Some properties of thermostable xylanase from an *Aspergillus niger* strain. *Microbiology,* **52**: 299-306.

Damaso, M. C. T., Almeida, M.S., Kurtenbach, E., Martins, O. B., Pereira, N., Andrade C. M. M. C. and Albano, R. M. 2003. Optimised expression of a thermostable xylanase from Thermomyces lanuginosus in *Pichia pastoris*. *Applied Environment Microbiology*, **69**: 6064-6072.

Dhiman, S. S., Sharma, J. and Battan, B. 2008. Industrial applications and future prospects of microbial xylanases: A review. *Bioresources*, **3**(4): 1377-1402.

Eriksson, K. E. L. 1990. Biotechnology in the pulp and paper industry. *Wood science technology,* **24**: 79-101.

Fadel, M. and Fouda, M. S. 1993. Physiological studies on xylanase production by *Penicillium fumiculosum* on some agricultural wastes. *Microbiol*ogy, **148**: 304-312.

Fang, H. Y., Changa, S. M., Hsieh, M. C. and Fang, T. J. 2007. Production, optimization growth conditions and properties of the xylanase from *Aspergillus carneus* M34. *Journal of Molecular Catalysis B: Enzymatic,* **49**: 36-42.

Fattah A. F. A., Doaa, A. R. Mahmoud, S. M. Shash, F. M. P. and Sherien, M. M. A. 2011. Production of xylanase by free and immobilized cells of *Cladosporium macrocarpum* NRC 15. *World Applied Sciences Journal,* **15**(10): 1376-1381.

Fawole, O. B. and Odunfa, S. A. 2003. Some factors affecting production of pectin enzymes by *Aspergillus niger*. International *Biodeterioration*, **51**: 223-227.

Goyal, M., Kalra, K. L., Sareen, V. K. and Soni, G. 2008. Xylanase production with xylan rich lignocellulosic wastes by a local soil isolate of *Trichoderma viride*. *Brazilian Journal of Microbiology*, **39**: 535-541.

Gupta, U. and Kar, R. 2009. Xylanase production by a thermo-tolerant *Bacillus* species under solid-state and submerged fermentation. *Brazilian Archieves of Biology and Technology*, **52**(6): 1363-1371.

Gupta, V. K., Gaur, R., Yadava, S. K. and Darmwal. N. S. 2009. Optimization of xylanase production from free and immobilized cells of *Fusarium solani* F7. *BioResourses,* **4**(3): 932-945.

Haltrich, D., Nidetzky, B., Kulke, K.D., Steiner, W. and Zupanie, S. 1997. Production of fungal xylanases. *Bioresource Technology*, **58**: 137 -161.

Hakim, A. R. 2006. Study on the effect of substrates concentration in the production of xylanase from *Aspergillus niger* using palm kernel cake. Thesis: University College of Engineering and Technology, Malaysia.

Haq, I., Khan, A., Butt, W.A., Ali, S. and Qadeer, M.A. 2002. Effect of carbon and nitrogen sources on xylanase production by mutant strain of *Aspergillus niger* GCBMX-45. *Journal of Biological Science*, **2**: 143-144.

Haq, I., Tasneem, M. , Raana, K., Khan, A., Mukhtar, H. and Javed, M. 2004. Optimization of cultural conditions for the production of xylanase by chemically mutated strain of *A. niger* GCBCX-20. *International Journal of Agriculture and Biotechnology,* **6**: 1115-1118.

Hoq, M., Hempel, C., and Deckwer, W. D. 1994. Cellulase-free xylanase by *Thermomyces lanuginosus* RT9: effect of agitation, aeration, and medium components on production. *Journal of Biotechnology*, **37**: 49-58.

Horikoshi, K. 1996. Alkaliphiles: from an industrial point of view. FEMS *Microbiology Reviews*, **18**: 259-270.

Ivanova, V., Yankov, D., Kabaivanova, L. and Pashkkoulov, D. 2001. Simultaneous biosynthesis and purification of two extra cellular *Bacillus* hydrolases in aqueous two alpha amylase. *Journal of Biochemical Engineering*, **8**: 61-81.

Kansoh, A. L. and Gammel, A. 2001. Xylanolytic activities of *Streptomyces sp.1*, taxonomy production, partial purification and utilization of agricultural wastes. *Acta Microbiologica et Immunologica Hungarica*, **48**: 39-52.

Kaur, H., Dutt, D. and Tyagi, C. H. 2011. Production of novel alkali-thermo-tolerant cellulose-poor xylanases from *Coprinopsis cinerea* HK-1 NFCCI-2032. *Bioresources*, **6**(2): 1376-1391.

Kavya, V. and Padmavathi, T. 2009. Optimization of growth conditions for xylanase production by *Aspergillus niger* in solid state fermentation. *Polish Journal of Microbiology*, **58** (2): 125-130.

Khan, A. W., Tremblay, D. and LeDuy, A., 1986. Assay of xylanase and xylosidase activities in bacterial and fungal cultures. *Enzyme and microbial technology*, **8**: 373-377.

Khandeparkar, R. and Bhosle, N. B. 2007. Application of thermoalkalophilic xylanase from *Arthrobacter* sp. MTCC 5214 in biobleaching of kraft pulp. *Bioresources Technology*, **98**: 897-903.

Li, K., Azadi, P., Collins, R., Tolan, J., Kim, J.S. and Eriksson, K. E. L. 2000. Relationships between activities of xylanases and xylan structures. *Enzyme microbial technology,* **27**: 89-94.

Li, X., Sun, B. Zhao, J., Lv, Y., Li, H. S. E. and Zhu, Y. 2011. Production and improved bleaching abilities of a thermostable xylanase from a newly isolated *Streptomyces chartreusis* strain. *African journal of Biotechnology,* **10**(64): 14132-14142.

Li, X. T., Jiang, Z. Q., Li, L.T., Yang, S. Q., Feng, W. Y., Fan, J. Y. and Kusakabe, I. 2005. Characterization of a cellulase-free, neutral xylanase from *Thermomyces lanuginosus* CBS 288.54 and its biobleaching effect on wheat straw pulp. *Bioresource Technology,* **96**: 1370-1379.

Loera, O. and Cordova, J. 2003. Improvement of xylanase production by a parasexual cross between *Aspergillus Niger* strains. *Brazilian Archives of Biology and Technology,* **46**(2): 177-181.

Miller, G., 1959. Use of dinitrosalicyclic acid reagent for determination of reducing sugar. *Analytical Chemistry*, **31**: 3.

Mimura, H. and Shinichi, N. 1999. Physiological Characteristics of *Bacillus* sp. *Biocontrol Science and Technology,* **4**: 105-8.

Murugan, S., Arnold, D., Pongiya, U. D. and Narayanan, P. M. 2011. Production of Xylanase from *Arthrobacter sp.* MTCC 6915 Using Saw Dust As Substrate under Solid State Fermentation. *Enzyme Research,* 1-7.

Muthezhilan, R., Ashok, R. and Jayalakshmi, S. 2007. Production and optimization of thermostable alkaline xylanase by *Penicillium oxalicum* in solid state fermentation. *African Journal Microbiology,* **1**(2): 20-28.

Nair, S. G., Sindhu, R. and Shashidhar, S. 2008. Fungal xylanase production under solid state and submerged fermentation conditions. *African Journal of Microbiology,* **2**: 082-086.

Nascimento, R. P., Coelho, R. R. R., Marques, S., Alves, L., Girio, F. M., Bon, E. P. S. and Amaral-Collaco, M. T. 2002. Production and partial characterization of xylanase from *Streptomyces* sp. strain AMT-3 isolated from Brazilian cerrado soil. *Enzyme and Microbial Technology*, **31**(4): 549-555.

Nelson, N. A. 1944. Photometric adaptation of somogyi method for the determination of glucose. *Journal of Biological Chemistry*, **153**: 375-380.

Oshoma, C. E., Imarhiagbe, E. E., Ikenebomeh, M. J. and Eigbaredon, H. E. 2010. Nitrogen supplements effect on amylase production by *Aspergillus niger* using cassava whey medium. *African Journal of Biotechnology*, **9**(5): 682-686.

Pal, P. and Kaushik, P. 2012. Effect of carbon and nitrogen sources on the growth of *Rhizoctonia solani* isolated from *Rhynchostylis retusa* blume. *International Journal of Life Sciences,* **1**(2): 16-18.

Park, Y. S., Kang, S. W., Lee, J. S., Hong, S. I. and Kim, S. W. 2002. Xylanase production in solid state fermentation by *Aspergillus niger* mutant using statistical experimental designs. *Applied Microbiology and Biotechnology*, **58**: 761-766.

Prasertsan, P. Kittikul, A. H., Kunghae, A., Maneesri, J. and Oi, S. 1997. Optimization for xylanase and cellulose production from *Aspergillus niger* ATTC 6275 in palm oil mill wastes and its application. *World Journal of Microbiology and Biotechnology*, **13**: 555-559.

Pucci, L. E. A., Rodrigues, P. B., Borges, P. and Freitas, R. T. F. 2003. Oil levels and enzymatic complex addition in broiler diets. *Zootechnology,* **32**: 909-917.

Rashid, A. N. 1999. Characterization of xylanase produced by *Bacillus pumilus* strain PJ19. *Journal of Microbial Biotechnology*, **9**(2): 157-162.

Reid, I. D. 1989. Solid-state fermentation for biological delignification. *Enzyme and microbial technology,* **11**: 786-803.

Rifaat, H. M., Nagieb, Z. A. and Ahmed, Y.M. 2005. Production of xylanase by *Streptomyces* species and the bleaching effect on rice straw pulp. *Applied Ecology and Environmental Research,* **4**(1): 151-160.

Sati, S. C. and Bisht, S. 2006. Utilization of various carbon sources for the growth of waterborne conidial fungi. *Mycologia,* **98**(5): 678-681.

Seyis, I. and Aksoz, N. 2005. Effect of carbon and nitrogen sources on xylanase production by *Trichoderma harzianum* 1073 D3. *International Biodeterioration & Biodegradation,* **55**: 115-119

Shahriarinour, M., Wahab, M. N. A., Mohamad, R., Mustafa, S. and Ariff, A. B. 2005. Effect of medium composition and cultural condition on cellulase production by *Aspergillus terreus. African Journal of Biotechnology*, **10**(38): 7459-7467.

Simoes, M. L. G., Tauk-Tornisielo, S. M. and Tapia, D. M. P. 2009. Screening of culture condition for xylanase production by filamentous fungi. *African Journal of Biotechnology*, **8**(22): 6317-6326.

Singh, S., Tyagi, C. H., Datt, D. and Upadhyaya, J. S. 2009. Production of high level of cellulose-poor xylanases by wild strains of white-rot fungus *Coprinellus disseminatus* in solid-state fermentation. *New Biotechnology*, **26**: (3/4).

Somogyi, M. 1952. Notes on sugar determination. *Journal of Biological Chemistry*, **195**: 19-23.

Subramaniyan, S. and Prema, P. 2002. Biotechnology of microbial xylanases: Enzymology, molecular biology and application. *Critical Reviews in Biotechnology,* **22**: 33-64.

Suresh, P. V. and Chandrasekaran, M. 1999. Impact of process parameters on chitinase production by an alkalophilic marine *Beauveria bassiana* in solid state fermentation. *Process Biochemistry*, **34**: 257-267.

Takahashi, K., Inoue, H., Sakai, K., Kohama, T., Kitahara, S., Takishima, K., Tanji, M., Athauda, S., Takahashi, T. and Akanuma, H. 1991. The primary structure of *Aspergillus niger* acid proteinase A. *The Journal of Biological Chemistry*, **266**: 19480-19483.

Tallapragada, P. and Venkatesh, K. 2011. Isolation, identification and optimization of xylanase enzyme produced by *Aspergillus niger* under submerged fermentation. *Journal of Microbiology and Biotechnology Research,* **1**(4): 137-147.

Techapun, C., Poosaran, N., Watanabe, M. and Sasaki, K. 2003. Thermostable and alkaline tolerant microbial cellulase-free xylanases produced from agricultural wastes and the properties required for use in pulp bleaching bioprocesses: A review. *Process Biochem,* **38**: 1327-1340.

Thakur, V. V., Jain, R. K. and Mathur, R. M. 2012. Atudies on xylanase and laccase enzymatic prebleaching to reduce chlorine-based chemicals during CEH and ECF bleaching. *Bioresources,* **7**(2): 2220-2235.

Wang, S. L., Yen, Y. H., Shih, I. L., Chang, A. C., Chang, W. T., Wu, W. C. and Chai, Y. D. 2003. Production of xylanases from rice bran by *Streptomyces actuosus* A-151. *Enzyme and Microbial Technology,* **33**: 917-925.

Wong, K. K. Y., Tan, L. U. L. and Saddler, J. N. 1988. Multiplicity of β-1,4 xylanase in microorganisms: functions and applications. *Microbiology Reviews*, **52**: 305-317.

Yang, R., Xu, S., Wang, Z. and Yang, W. 2005. Aqueous extraction of corncob xylan and production of xylooligosacchrids. **38**: 677-682.