Xylanase production by thermophilic Actinomycete *Thermobifida fusca* PA1-1

Danai Khorprasert, Werasit Sanpamongkolchai, Wirat Vanichsriratana, Mangkorn Rodprapakorn*

Department of Biotechnology, Faculty of Agro-Industry, Kasetsart University, 50 Ngam Wong Wan Rd, Ladyaow, Chatuchak, Bangkok, 10900, Thailand.
**e-mail: mangkorn.r@ku.ac.th*

Abstract

Xylanases have been the focus of much attention due to their extensive applications in the pulp and paper, food industries, animal feed, and bioconversion of agricultural wastes to useful materials. *Thermobifida fusca* PA1-1 is one of actinomycetes that can be produce xylanase. This study focused on optimal condition for production of xylanase using various agricultural wastes as carbon sources instead of xylan in shaking flask. It was found that rice straw was the most potential carbon sources for higher xylanase production compared with other agricultural wastes. The influence of various culture conditions including; fermentation period, initial concentration of carbon sources, initial pH and incubating temperature was studied. The highest xylanase activity (24.87 unit/ml) was observed in 1.5% rice straw for 6 days at 50°C after 6 days with an initial pH of 11.0.

Keywords: actinomycetes, *Thermobifida fusca*, xylanase, rice straw

Introduction

Xylan is the second most abundant biopolymer after cellulose and the major hemicellulosic polysaccharide found in the plant cell wall. (Timell, 1967) It consists of β-1,4-linked xylopyranosyl residues and branches of neutral or uronic monosaccharides and oligosaccharides (Joseleau et al. 1992).

Xylanase (endo-1,4-β-D-xylan xylanohydrolase; EC 3.2.1.8) is an the key enzyme for the break down of xylan. It is an extracellar enzyme, which can degrade xylan to short-chain xylooligosaccharides of varying lengths (Krengel et al. 1996). Xylanases have increased their importance due to their potential application in the pulp and paper, food industries, animal feed, and bioconversion of agricultural wastes (Tucker et al. 1989; Xu et al. 2000; Nunez et al. 2001; Saha, 2003; Moers et al. 2003).

The various biotechnological techniques like submerged and solid state fermentation are employed for xylanase biosynthesis (Cai et al. 1998; Gawande and Kamat 1999; Kansoh and Gammel 2001). The submerged fermentation is most beneficial as compared to other techniques due to more nutrients availability, sufficient oxygen supply and less time required for the fermentation (Hoq et al. 1994; Gomes et al. 1994; Veluz et al. 1999; Gouda 2000). The production of microbial xylanase is preferred over plant and animal sources, because of their availability, structural stability and easy genetic manipulation (Bilgrami and Pandy 1992). Industrial process conditions are generally hostile in terms of extremes of temperature, pH, presence of inhibitors etc., and the enzymes intended to be used for such processes must be withstand such conditions. Majority of the reported xylanases do not meet such criteria (Sharma and Bajaj 2005; Bocchini et al. 2008). Therefore, search for still better enzymes which comply well with the industrial processes is in progress (Bajaj and Abbass 2011; Kumar and Satyanarayana 2011). Another major hurdle for wide range application of enzymes in industries is high cost of their production (Bajaj et al. 2011). Pure substrates being highly expensive cannot be afforded at the industrial-level bulk production of enzymes. Therefore, it is necessary to explore cheap substrates for cost-effective enzyme production. Agricultural residues represent one such cheap raw material for industrial production of

enzymes (Geetha and Gunasekaran 2010; Bajaj and Wani 2011), and are available in abundance in countries with extensive agriculture.
This paper reports the determination of the optimum environmental conditions for the production of xylanase from the thermophilic actinomycete *Thermobifida fusca* PA1-1 which was isolated from palm empty bunch composted in Thailand (Kunpeuk 2011).

Methodology

Microoganism and growth conditions

Thermobifida fusca PA1-1was isolated from palm empty bunch-composted in Thailand (Kunpeuk 2011). It was maintained as a suspension of spores and hyphal fragments in 20% (v/v) glycerol at -20^{o}C and routinely cultures on L-agar plates with subsequent incubation at 50^{o}C for 96 h. The L-agar medium contained 0.5% (w/v) tryptone, 0.5% (w/v) yeast extract, 0.5% (w/v) sodium chloride, 0.1% (w/v) glucose and 1.5% (w/v) agar at pH 8.0. Distilled-water suspensions of sporulating growth were used to inoculate all liquid cultures.

Xylanase production

Distilled-water suspensions of sporulating growth were used to inoculate into 250 ml shake flasks containing 50 ml of minimal salts-yeast extract nutrient medium (MSYE), pH 8.0 as described previously (Ramachandra et al. 1988), supplemented with 0.5% (w/v) xylan. The liquid medium contained: 0.6% (w/v) yeast extract, 0.01% (w/v) ammonium sulphate, 0.03% (w/v) sodium chloride, 0.01% (w/v) magnesium sulphate, 0.02% (w/v) calcium carbonate and 1 ml of trace-elements solution, pH 8.0. The trace-elements solution contained 0.1% (w/v) ferrous sulphate, 0.09% (w/v) zinc sulphate and 0.02% (w/v) manganese sulphate, pH 8.0. The culture was incubated in the rotary shaking incubator at 250 rpm and 50˚C. The samples were taken at every 24 h for 7 days, all sample were centrifuged at 4^{o}C and the supernatant was assayed as crude enzyme for xylanase activity.

Effect of carbon source and concentration on xylanase production

To determine the effect of various carbon sources on xylanase production, 0.5% (w/v) xylan was replaced by 1.0% (w/v) agricultural wastes, such as corn husk, corncob, rice straw, rice husk and sawdust. The carbon source, which showed the highest xylanase activity was selected and its concentration was varied at 0.5, 1.0, 1.5 and 2.0% (w/v)

Optimization of initial pH and cultivation temperature

Xylanase production was studied by varying the initial pH of fermentation media ranging from 5.0 to 14.0 using above mentioned carbon sources. For optimization of cultivation temperature, the xylanase production was performed at various temperatures (40, 45, 50 and 55˚C).

Xylanase assay

The xylanase activity was measured by determining the amount of reducing sugars from soluble xylan using 3,5-dinitro-salicylic acid (DNS) method (Miller, 1959). The reaction mixture consisted of 0.1 ml of xylan solution (1% Beechwood xylan as substrate) in 50 mM phosphate buffer (pH 7.0) and 0.1 ml of suitably diluted enzyme solution. The reaction was run at 50˚C for 30 min and stopped by adding DNS reagent. Then the treated samples were

boiled in boiling water for 5 min, cooled on ice for 5 min and spectrophotometrically measured at 540 nm. One unit of xylanase activity is defined as an amount of enzyme releasing 1 µmol of reducing sugar using xylose as standard under the assayed condition.

Results and Discussion

Effect of carbon source on xylanase production

The time course of the xylanase production was studied by using various carbon sources, such as 0.5% (w/v) xylan and 1.0% (w/v) of corn husk, corncob, rice straw, rice husk and sawdust as shown in Fig. 1.
The maximum of xylanase production of *T. fusca* PA1-1 was observed after 6 days at 16.74 unit/ml when rice straw was used as a carbon source. Further incubation after this did not show any increment in the level of enzyme activity.

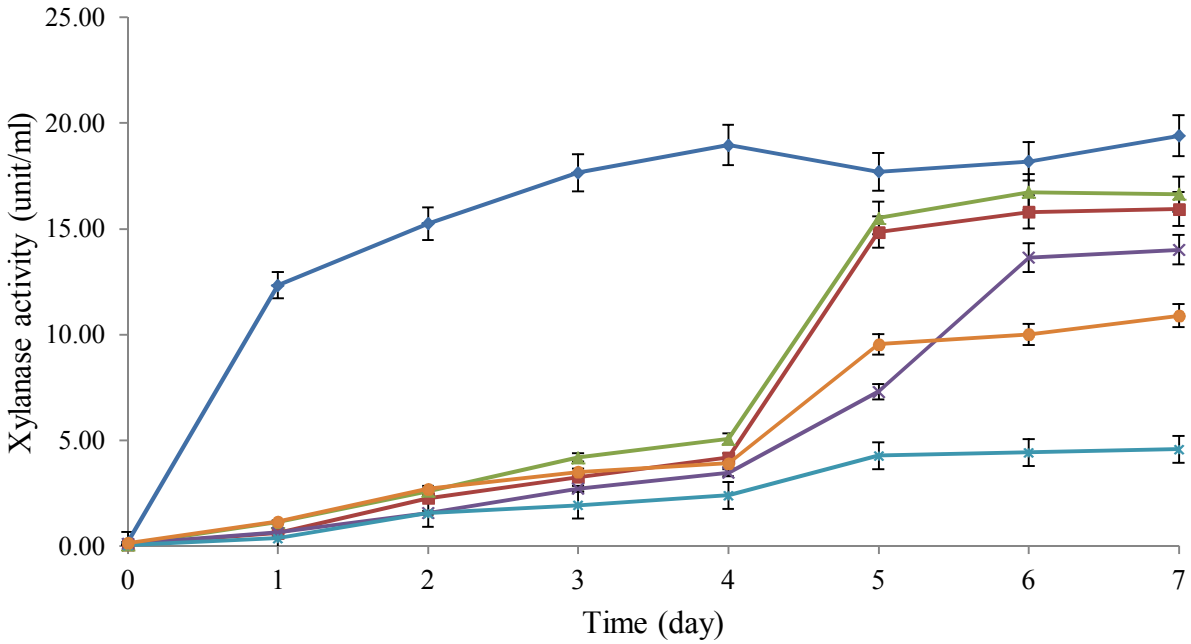

Figure 1 Time course of xylanase production of *Thermobifida fusca* PA1-1 from various carbon sources at 50˚C and 250 rpm for 7 days.
(◆ xylan, ▲ rice straw, ■ corn husk, × corncob, ● rice husk, ✳ sawdust)

Rice straw was found to be the best carbon source when compared with other tested agricultural wastes. It may be due to the hemicellulose (xylan) content and structure of xylan in rice straw which easy to be digested by xylanase from *T. fusca* PA1-1 (Sanghi et al. 2009; Yang et al. 1995; Sa´ Pereria et al. 2002). On the other hand, other carbon sources such as corn husk, corncob, rice husk and sawdust contain more lignin than rice straw (Kuhad 1993; Howard et al. 2003; Kuhad et al. 2011). And lignin protects the xylan from the attack by hydrolytic enzymes (Goyal et al. 2008).

Effect of carbon source concentrations on xylanase activity

Effect of carbon source concentrations for xylanase production by *T. fusca* PA1-1 was investigated (Fig. 2). The concentration of rice straw at 1.5% (w/v) gave the maximum

activity of xylanase (16.53 unit/ml). However, when the concentration of rice straw was increased to 2.0% (w/v), the xylanase activity was decreased. It is possibly due to larger amount of carbon sources reduce dissolved oxygen in the culture medium, which in turn affect the growth of microorganism (Leartslarus et al. 2002).

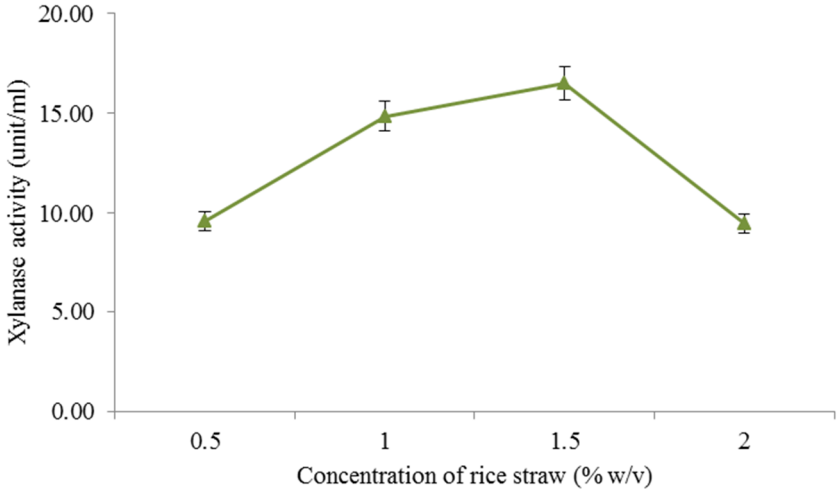

Figure 2 Production of xylanase by *Thermobifida fusca* PA1-1 using rice straw as a carbon sources at various concentrations.

Effect of initial pH on xylanase production

The production of xylanase by *T. fusca* PA1-1 was studied by varying initial pH of the fermentation media ranging from 5.0 to 14.0 (Fig. 3). Maximum production of xylanase (25.88 Unit/ml) was achieved when the initial pH of MSYE nutrient medium was 11.0. When the pH was increased or decreased from the optimum value, the production of xylanase was greatly decreased due to cultivation of the microorganism at an unfavorable pH may limit the growth and xylanase production, and subsequently reduce substrate accessibility (Bajpai 1997).

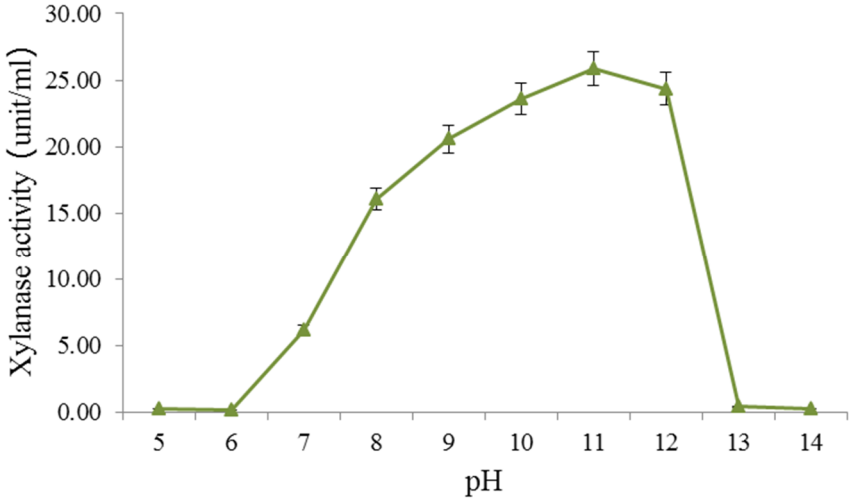

Figure 3 Effect of initial pH on xylanase production by *Thermobifida fusca* PA1-1.

Effect of temperature on xylanase production

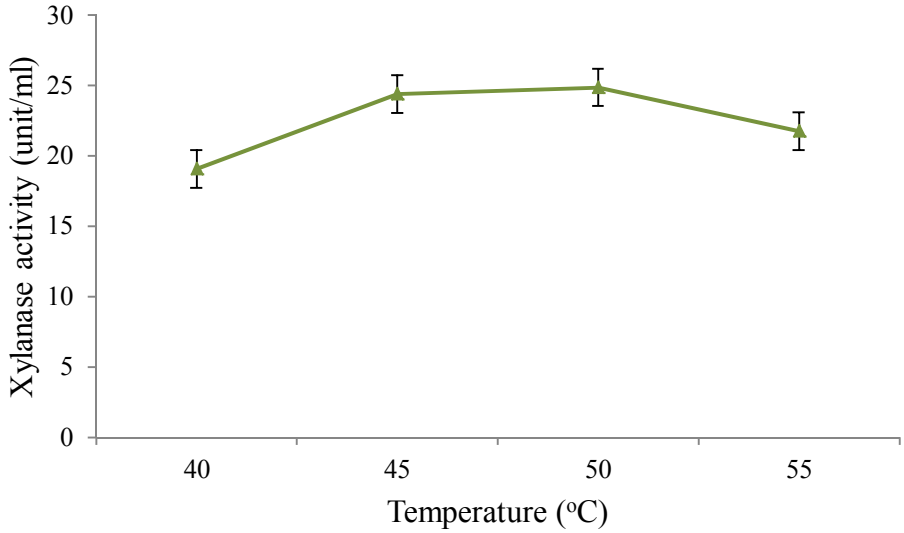

Figure 4 Effect of cultivation temperature on xylanase production by *Thermobifida fusca* PA1-1.

The effect of temperature on the production of xylanase by *T. fusca* PA1-1 was investigated by growing the organism in the temperature range of 40°C to 55°C. The results revealed that the optimum temperature for xylanase production (24.87 unit/ml) was between 45-50°C which was similar to xylanase activity from *Thermobifida fusca* (Yang et al. 2007) and *Streptomyces* sp. Ab106 (Techapun et al. 2002). The optimum temperature range of xylanase produced by thermophilic microorganism are generally thermostable and also stable in presence of denaturing agents and organic solvents (Zakariya 2008) which differ from xylanase produced by mesophilic microbes.

Conclusions

The production of xylanase by *T. fusca* PA1-1 was conducted by using agricultural wastes as carbon source in shaking flask. The agricultural wastes such as corn husk, corncob, rice straw, rice husk and sawdust were used for the xylanase production. The results showed that 1.5% (w/v) rice straw was the best carbon source. The optimization for highest xylanase production was observed at incubation temperature of 50˚C for 6 days in minimal medium (pH 11.0). Further experiments on effect of nitrogen sources for xylanase production and characterization of xylanase must be carried out to claim its potential for industrial processes.

References

Bajaj, B.K., Abbass, M. (2011) Studies on analkali-thermostable xylanase from *Aspergillus fumigatus* MA28. 3Biotech. 1:161–171.

Bajaj, B.K., Sharma, M., Sharma, S. (2011) Alkalistableendo-b-1,4-xylanase production from a newly isolated alkalitolerant *Penicillium* sp. SS1 using agro-residues. 3Biotech. 1:83–90.

Bajaj, B.K., Wani, M.A. (2011) Enhanced production of a phytase with potentially suitable attributes for animal feed application from *Nocardia* sp. MB36 by employing agro-residues as substrates. Eng.LifeSci.11:620–628.

Bajpai P. (1997) Microbial xylanolytic enzyme system: properties and applications. Adv. Appl. Microbiol. 43:141-194.

Bilgrami, K.S., Pandy A.K. (1992) In: Jain, E.S.K. (ed.), Industry and Fermentation in Introduction to Biotechnology. 149–165.

Bocchini, D.A., Gomes, E., DaSilva, R. (2008) Xylanase production using *Bacillus circulans* D1 using maltose as carbon source. Appl.Biochem.Biotechnol. 146:29–37.

Cai, J.M., Ke W., Jie Z., Ruipen R. (1998) Production, properties and application of xylanase from *Aspergillus niger* A3. Annl. N.Y. Acad.Sal. 864:214–218.

Gawande P.V., Kamat M.Y. (1999) Production of *Aspergillus* xylanase by lignocellulosic waste fermentaion and its application. J. Appl.Microbiol. 87:511–519.

Geetha, K., Gunasekaran, P. (2010) Optimisation of nutrient medium containing agricultural wastes for xylanase production by *Bacillus pumilus* B20. Biotechnol. BioprocessEng. 15:882–889.

Gomes D.J., Gomes J., Stiener W. (1994) Production of highly thermostable xylanase by a wild strain of thermophilic fungus *Thermoascus aurantiacus* and partial characterization of the enzyme. J. Biotech., 37:11–22.

Gouda, M.K. (2000) Purification and partial characterization of cellulose free xylanase produced in solid state and submerged fermentation by *Aspergillus tamarii*. Adv. Food Sci. 22:31–37.

Goyal M., Kalra K.L., Sareen V.K., Sonic G. (2008) Xylanase production with xylan rich lignocellulosic wastes by a local soil isolate of *Trichoderma viride.* Braz J Microbiol. 39(3):535-541

Hoq M.M, Hempel C., Deckwer W.D. (1994) Cellulase free xylanase by *Thermomyces lanuginosus* RT9; Effects of aeration, agitation and medium components on production. J. Biotechnol. 37:49–58.

Howard, R.L., Abotsi E., Jansen van Rensburg E.L., Howard S. (2003) Lignocellulose biotechnology: issues of bioconversion and enzyme production. Afr. J. Biotechnol. 2:602-619.

Joseleau J.P., Comtat J., Ruel K. (1992) Chemical structure of xylans and their interactions in the plant cell walls. In: Visser J, Beldman G, vanSomeren M.A.K., Voragen A.G.J. (eds) Xylans and xylanases. Elsevier Amsterdam 1–15.

Kansoh, A.L., Gammel A. (2001) Xylanolytic activities of *Streptomyces* sp. 1, taxonomy production, partial purification and utilization of agricultural wastes. Acta Microbiol. Immunol. Hungarica. 48:39–52.

Krengel, U., Dijkstra B.W., Bioson A.F. (1996) Three–dimensional structure of endo–1, 4– beta –xylanase I from *Aspergillus niger* :Molecular basis for its low pH optimum. J. Mol. Biol. 263:70–8.

Kuhad, R.C. (1993) Lignocellulose Biotechnology: current and future prospects. Crit. Rev. Biotechnol. 13 (2):151-172.

Kuhad, R.C., Gupta R., Khasa Y.P. (2011) Bioethanol production from lignocellulose: an overview, pp. 53-106. *In* B. Lal and P.M. Sarma, ed. Wealth from waste: Trends and technologies. TERI Press, India.

Kumar, V., Satyanarayana, T. (2011) Applicability of thermo-alkali-stable and cellulase-free xylanase from a novel thermo-halo-alkaliphilic *Bacillus halodurans* TSEV1 in producing xylooligosaccharides. Biotechnol. Lett. 33: 2279–2285.

Kunpeuk, W. (2011) Screening, Characterization and Optimization for Carboxymethylcellulase Production by Thermophilic Bacteria from Soil. M.S. Thesis, Kasetsart University.

Leartslarus C., Ratanakhanokchai K., Kyu L.K. (2002) Optimization of extracellular cellulose-free xylanase production by alkaliphilic *Bacillus firmus* K-1. KMUTT Research & Development Journal. 27: 3-17.

Miller G.M. (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem 31: 426-428.

Moers K., Courtin C.M., Brijs K., Delcour J.A. (2003) A screening method for endo–beta–1,4–xylanase substrate selectivity. Anal. Biochem. 319: 73–7.

Nunez L.Z., Rodriguez R., Baez M.A. (2001) Gas retention mechanism in wheat bread doughs. The role of xylanase and arabinoxylan fractions. J. Biochem. 56: 31–3.

Ramachandra M., Craeford D.L., Hertel G. (1988) Characterization of an extracellular lignin peroxidase of the lignocellulolytic actinomycete *Streptomyces viridosporus*. Applied and Environmental Microbiology 54: 3057–3063.

Sa´ Pereria P.,Mesquita A., Duarte J.C., Barros M.R.A., Costa-Ferreira M. (2002) Rapid production of thermostable cellulase-free xylanase by a strain of *Bacillus subtilis* and its properties. EnzymeMicrob.Technol. 30:924–933.

Saha B.C. (2003) Hemicellulose bioconversion. J. Indian Microbiol.Biotechnol. 30:279–91.

Sanghi A., Garg N., Kuhar K., Kuhad R.C., Gupta V.K. (2009) Enhanced production of cellulase-free xylanase by alkalophilic *Bacillus subtilis* ASH and its application in biobleaching of kraft pulp. BioResource4 3:1109–1129.

Sharma P., Bajaj B.K. (2005) Production and partial characterization of alkali-tolerant xylanase from analkaliphilic *Streptomyces* sp.CD3. J.Sci.Ind.Res. 64:688–698.

Techapun, C., Charoenrat T., Watanabe M., Sasaki K., Poosaran N. (2002) Optimization of thermostable and alkaline-tolerant cellulase-free xylanase production from agricultural waste by thermotolerant *Streptomyces* sp. Ab106, using the central composite experimental design. Biochem. Eng. J. 12:99-105.

Timell T.E. (1967) Recent progress in the chemistry of wood hemicelluloses. Wood Sci Technol. 1:45-70.

Tucker M.P., Mohagheghi A., Grohmann K., Himmel M.E. (1989) Ultra–thermostable cellulases from *Acidothermus cellulolyticus*: Comparison of temperature optima with previously reported cellulases. Biotechnology 7: 817–20.

Veluz G., Taksuo K., Hiroshi M., Yusaku F. (1999) Screening *Rhizopus* sp. J. Fac. Agric. 43:419–423.

Xu Z., Bai Y., Sun W., Tao W. (2000) Screening and fermentation conditions of a bacterial strain over–producing xylanase. Wei Sheng Wu Xue Bao. 40:440–3.

Yang, C.H., Yang S.F., Liu W.H. (2007) Production of xylooligosaccharides from xylans by extracellular xylanases from *Thermobifida fusca*. J. Agric. Food Chem**.** 55: 3955-3959.

Yang V.W., Zhuang Z., Elegir G., Jeffries T.W. (1995) Alkaline active xylanase produced by an alkalophilic *Bacillus* sp. Isolated from kraft pulp. J.Ind.Microbiol. 15:434–441.

Zakariya M.N.B. (2008) Isolation of xylan degrading microorganism from local hot spring. Bachelor Thesis, University Malaysia Pahang