# Xylan Removal by Xylanase for the Production of Dissolving Pulp from Bamboo

by

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# Abstract

With α-cellulose content and fiber characteristics similar to those of wood, bamboo is an attractive alternative feedstock for the production of dissolving grade pulp. A high level of hemicellulose in bamboo will lead to substantial complications in downstream processing of dissolving pulps into cellulose derivatives such as viscose, acetates, ethers etc. Xylanase treatment is an environment-friendly method that enables the selective removal of xylan (the major hemicellulose in bamboo) without detrimental effects on cellulose. In this study, we investigated a combination of mechanical refining with xylanase treatment for incorporation into a pre-hydrolysis kraft-based bamboo dissolving pulp production process. Laboratory PFI refining and xylanase treatment were combined to improve the xylan removal efficiency. Refining at 9000 revolutions increased the efficiency of subsequent enzymatic treatment resulting in a 44% removal of beta- plus gamma-cellulose with only 3 h of xylanase treatment. The alpha-cellulose content of bleached pulp prepared following combined refining-xylanase treatments was 93.37% (w/w) while the xylan content was only 2.38%. The properties of refined fibers prior to xylanase treatment, such as freeness, water retention value, fiber size and Scanning Electron Microscopy (SEM) images were investigated to further understand the underlying mechanism of the effect of refining on enzymatic treatment. The brightness, reactivity and viscosity of bleached bamboo dissolving pulp after ECF bleaching (D-EP-D) sequence were also evaluated. These results demonstrated the feasibility of combining refining and xylanase treatment to produce high quality bamboo dissolving pulp.

# Preface

In this work, I was responsible for experimental design, experimental procedures and data analysis. Dr. Martinez and Dr. Beatson supervised the research and reviewed the manuscript. We also include the list of journal and conference contributions.

1. Journal Paper

**Lingfeng Zhao**, Zhaoyang Yuan, Rodger Beatson, Xue Feng Chang, Nuwan S. Kapu, Heather L. Trajano, D. Mark Martinez. Increasing efficiency of enzymatic hemicellulose removal from bamboo for production of high-grade dissolving pulp. Bioresource Technology, 2017, 223, 40-46. This paper presents a version of Chapter 4.

2. Conference Presentation

Nuwan S. Kapu, Zhaoyang Yuan, Xue Feng Chang, **Lingfeng Zhao**, D. Mark Martinez, Rodger Beatson. Developing bamboo as an alternative feedstock for bio-products. Research Day, 2013, Department of Chemical & Biological Engineering, UBC. (Poster)

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# List of Glossary and Nomenclature

| | |
|---|---|
| CCE | Cold caustic extraction. |
| CED | Cuprirethylenediamine. |
| CMC | Carboxymethyl cellulose. |
| CSF | Canadian Standard Freeness, mL. |
| CTH | Controlled temperature and humidity. |
| D | Chlorine dioxide bleaching. |
| DNS | Dinitrosalicylic acid solution. |
| ECF | Elemental chlorine free bleaching. |
| EOP | Extractive oxygen-alkaline peroxide bleaching. |
| EP | Oxidative extraction reinforced with hydrogen peroxide. |
| FQA | Fiber quality analyzer. |
| HPLC | High performance liquid chromatography. |
| ISO | Brightness unit. |
| IUBMB | International Union of Biochemistry and Molecular Biology. |
| K | Kraft cooking. |
| MCC | Microcrystalline cellulose. |
| NCC | Nanocrystalline cellulose. |
| NFC | Nanofibrillated cellulose. |
| NREL | National Renewable Energy Laboratory. |
| O.D. | Oven dried pulp. |
| $O_2D$ | Oxygen-delignification. |

| | |
|---|---|
| P | The primary fibre cell wall layer. |
| PHK | Pre-hydrolysis. |
| $R^2$ | Regression coefficient. |
| S1 | The outer secondary fibre cell wall layer. |
| S2 | The inner secondary fibre cell wall layer. |
| SEM | Scanning electron microscope. |
| TAPPI | Technical association of the pulp and paper industry |
| w/w | Weight by weight. |
| WRV | Water retention value. |
| $X$ | The percentage of alpha-cellulose content in unbleached bamboo oxygen-delignified pulp. |
| $X_\alpha$ | The percentage of alpha-cellulose content in bleached bamboo dissolving pulp. |
| $X_{holo}$ | The percentage of holo-cellulose content in unbleached bamboo oxygen-delignified bamboo pulp. |

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# Chapter 1: Introduction

With the continuing increase in the population and the limited supply of non-renewable fossil-based resources, the proposed biorefinery concept, in which energy and numerous biobased products are produced from lignocellulosic materials, is of great interest. Dissolving pulp, regenerated cellulose and cellulose-based products, made from renewable lignocellulosic biomasses, are at the forefront of this biorefinery concept. Dissolving pulp is the starting material for the manufacture of various cellulose-based products such as viscose rayon, cellulose nitrate, cellulose acetate, methyl cellulose, and carboxymethyl cellulose (CMC) (Christov et al., 2000; Hiett, 1985). Viscose rayon is one of the most important raw materials in the textiles industry. Cellulose nitrate can be used to manufacture explosives while cellulose acetate has been used for the production of cigarette filters, eyeglasses frames and photography films. Methyl cellulose and carboxymethyl cellulose (CMC) are widely utilized in the food, pharmaceuticals, and construction industries (Gübitz et al., 1997; Hiett, 1985; Schild and Sixta, 2011). Moreover, the production of nanofibrillated cellulose (NFC), nanocrystalline cellulose (NCC) and microcrystalline cellulose (MCC) from dissolving pulp has also been extensively studied (Sixta et al., 2013; Wang et al., 2015a).

In general, dissolving pulps are characterized by high alpha-cellulose content and minor amounts of non-cellulosic impurities such as lignin, hemicellulose and ash (Hinck et al., 1985). In addition, uniform molecular weight distribution of alpha-cellulose, high brightness and high reactivity are all important characteristics of dissolving pulp (Hinck et al., 1985, Sixta, 2006). As one of the undesirable components of dissolving pulp,

hemicellulose causes several problems in downstream conversion processes and adversely affects the final quality of cellulose derivatives. For example, during the viscose rayon manufacturing process, when the hemicellulose content in dissolving pulp is higher than 5% (w/w), the xanthation and filtering stages are adversely affected, and the physical properties of viscose are also negatively affected (Christov and Prior, 1993; Rydholm, 1965). In the production of cellulose ethers, hemicellulose dissolved in the steeping lye greatly impairs the conversion process (Gehmayr et al., 2011). In the case of cellulose acetate, hemicellulose levels even as low as 2.8% in the dissolving pulp can severely affect acetate filterability, dispersion, solution color and can cause thermal instability of the product (Funaki et al., 1993; He et al., 2009; Wilson and Tabke, 1974).

For the production of dissolving pulp, cotton linter, softwoods and hardwoods are the primary raw materials. However, due to the limited availability of land for cotton growth and diminishing wood resources in developing countries, non-woody raw materials have attracted increasing attention. Of the non-woods, bamboo, encompassing 1250 species within 75 genera, is a highly abundant lignocellulosic resource covering over 20 million hectares worldwide, of which 18 million hectares of bamboos are grown in Asia (Ding et al., 2008; He et al., 2008). The fast-growing bamboo can be harvested every year for one hundred years after an initial 3-5 years of growth. In addition, the fact that the structure and chemical composition of bamboo is also similar to that of wood, makes bamboo a particularly attractive feedstock for the production of dissolving pulp (Dence, 1992; Okubo et al., 2004; Saka, 2004; Sridhar et al., 2007).

For bamboo pulping, the kraft pulping process is generally preferred because it can

provide an acceptable delignification while maintaining the pulp yield and viscosity (Khristova et al., 2006; Salmela et al., 2008; Yang et al., 2008). To convert the kraft pulp production process into a dissolving pulp production process, a hemicellulose extraction step is generally required. For this purpose, pre-hydrolysis, subjecting lignocellulosic material to hydrothermal (water/steam) or dilute acid treatment prior to kraft pulping has been commercially utilized (Kapu and Trajano, 2014; Liu et al., 2010, Sixta, 2006). Mechanistically, during pre-hydrolysis, hemicelluloses are much more readily degraded than cellulose due to a much lower degree of polymerization (DP), an amorphous structure, and higher hydrophilicity (Sixta, 2006). On the other hand, acetylated polysaccharides in feedstock subjected to hydrothermal treatment release acetic acid, which reduces the pH value of the liquor accelerating the hemicellulose hydrolysis (Kapu and Trajano, 2014). Many studies have suggested adding acid to provide an acidic medium (pH 2-3) could improve hemicelluloses hydrolysis during pre-hydrolysis (Behin et al., 2008; Saeed et al., 2009; Tunc et al., 2008). Moreover, pre-hydrolysis also enhances the reactivity and accessibility of residual solids due to the break of the primary cell wall and the exposure of the secondary wall. The opening up of structure of the feedstock facilitates the delignification in the subsequent alkaline kraft pulping process, and hence significantly reduces the lignin and ash contents of the resulting pulp (Behin and Zeyghami, 2009). In summary, dissolving pulp with satisfactory high cellulose content and low hemicellulose content can be obtained by the introduction of pre-hydrolysis prior to kraft cooking.

Although this pre-hydrolysis step is effective in removing hemicellulose from woody materials, in at least some studies it has been observed to be not as equally effective when treating bamboo (Batalha et al., 2012; Colodette et al., 2011). This lower efficiency of

hemicellulose removal during pre-hydrolysis of bamboo might be due to differences in chemical composition and anatomical structure (Batalha et al., 2012). Furthermore, improvement of hemicellulose removal from bamboo by pre-hydrolysis significantly decreased subsequent kraft pulping yield (Batalha et al., 2012; Colodette et al., 2011; Ma et al., 2011; Santiago and Pascoal Neto, 2007; Vu Mân et al., 2004;). Therefore, to develop bamboo as a raw material for producing dissolving pulp, it is necessary to incorporate a hemicellulose removal step after kraft pulping.

Several methods have been investigated to remove hemicellulose from pulp (Christov et al., 2000; Ibarra et al., 2010; Li et al., 2015). Among the used methods, the environmentally friendly enzyme treatment is considered to be one of the most promising due to its selective reaction with hemicellulose without detrimental effects on cellulose. More than 90% of hemicellulose in bamboo is xylan (Salmela et al., 2008), and previous studies have shown that xylan in hardwood and several non-wood pulps could be removed by applying xylanase (Bajpai and Bajpai, 2001; Christov and Prior 1996; Christov et al., 2000; Gübitz et al., 1997; Ibarra et al., 2010; Köpcke et al., 2010; Roncero et al., 2005; Senior et al., 1988). The objective of this project is to investigate the feasibility of xylanase application for the production of high-grade bamboo dissolving pulp.

# Chapter 2: Literature review

Since Paice and Jurasek (1984) first suggested enzymes can be used to improve pulp manufacturing processes, selective removal of xylan by xylanase for the preparation of dissolving pulp has been expected to have a profound impact on the future technology of the process. In addition, xylanase treatment prior to bleaching has been proven to be a viable application of enzymes in the pulping industry. It is able to boost subsequent bleaching effects, thereby reducing the consumption of bleaching chemicals and costs plus decreasing the formation of toxic materials in effluent (Daneault et al., 1994). As described in previous reviews, biological methods of pulp pre-bleaching using xylanases provide the possibility of selectively removing up to 20% of xylan (Kantelinen et al. 1993), and reducing the pentosan content in wood pulp by as much as 50% (Christov and Prior 1996). However, very limited work on the removal of hemicelluloses from bamboo pulp, derived from pre-hydrolysis kraft pulping process, has been done.

## 2.1 Molecular structure of xylan in bamboo pulp

The contents of the three major polymeric constituents of bamboo, cellulose, hemicellulose and lignin, are similar to wood. As shown in Table 2.1, compared to hardwood and softwood, bamboo contains higher cellulose content, lower hemicellulose and lignin contents (Dence 1992; Saka 2004; Sridhar et al., 2007). The typical cellulose is a homopolysaccharide composed of β-D-glucopyranose units, which are linked together by (1-4)-glycosidic bonds (Borghei-Ghomi, 2001). Hemicellulose is made up of several heteropolysaccharides, such as xylan, xyloglucan (heteropolymer of D-xylose and D-

glucose), glucomannan (heteropolymer of D-glucose and D-mannose), galactoglucomannan (heteropolymer of D-galactose, D-glucose and D-mannose), and arabinogalactan (heteropolymer of L-arabinose and D-galactose) (Shallom and Shoham, 2003). Lignin, the third major component, is a complex polymer built of hydroxyl-phenyl syringyl and guaiacyl units (Gindl, 2002; Wei and Song, 2001; You et al., 2015). During pulping, lignin is the dominant contributor to the dark color of the pulp.

**Table 2.1 Chemical composition of bamboo and different types of woods (Dence, 1992; Okubo et al., 2004; Sridhar et al., 2007).**

| | Cellulose (%) | Hemicellulose (%) | Lignin (%) | Pectin, starch, ash, etc. (%) |
|---|---|---|---|---|
| Bamboo | 57-66 | 20-25 | 11-27 | 1-5.5 |
| Hardwoods | 54-61 | 23-35 | 19-24 | 1-4 |
| Softwoods | 53-62 | 26-31 | 27-33 | 1-3 |

These three polymeric constituents make the plant cell wall. Within the cell wall, cellulose, hemicellulose and lignin are connected compactly together with different bonds such as ester bonds, hydrogen bonds, and covalent bonds (Chen, 2006; Yang et al., 2008). Cellulose and hemicellulose or lignin molecules are mainly coupled by hydrogen bonds. The strong chemical bonds are formed between the polyphenolic units in lignin and carbohydrates of cellulose and hemicellulose. The hemicellulose is found at the interface between lignin and cellulose. They provide fiber cohesion and plant cell wall integrity (Beg et al., 2001).

The pentosan components and structure of hemicellulose vary among different plant

species. In bamboo, more than 90% of hemicelluloses are composed of 4-O-methyl-D-arabinoglucuronoxylan (a β-(1-4)-xylan) (Salmela et al., 2008). Fig. 2.1. shows xylan how the D-xylopyranose, L-arabinofuranose, 4-O-methyl-D-glucopyranosyl uronic acid, and D-glucopyranosyl uronic acid units are linked to form the structure of xylan in bamboo. The arabinoglucuronoxylan consists of a main chain of (1→4)-linked β-D-xylopyranosyl residues to which are directly attached α-L-arabinofuranosyl or α-(2-O-β-D-xylopyranosyl-L-arabinofuranosyl) branches at the O-3 and 4-O-methyl-D-glucuronopyranosyl or D-glucuronopyranosyl branches at the O-2 of the xylosyl residues of the main chain (Yoshida et al., 1998).

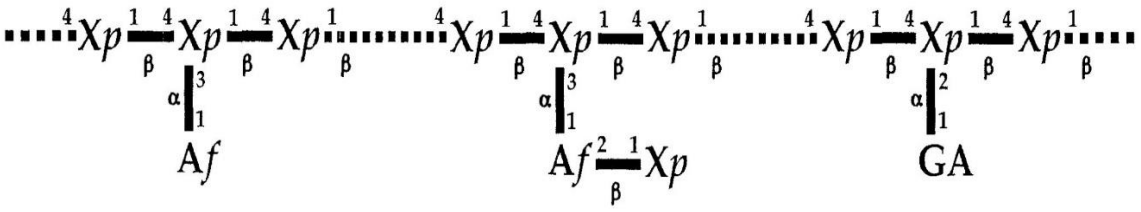



**Fig. 2.1. The structure of bamboo xylan. Af: L-arabinofuranose, GA: 4-O-methyl-D-glucopyranosyluronic acid or D-glucopyranosyluronic acid, Xp: D-xylopyranose. Adapted from Yoshida et al. (1998).**

## 2.2 Molecular structure of xylanase

Microbial xylanases are single subunit proteins that can catalyze the endohydrolysis of 1,4-β-D-xylosidic linkages in xylan. In 1961, the International Union of Biochemistry and Molecular Biology (IUBMB) assigned the enzyme code EC 3.2.1.8 to xylanase (Gupta et al., 2016). Their official name is endo-1,4-β-xylanase, but commonly used synonymous

terms include xylanase, endoxylanase, 1,4-β-D-xylan-xylanohydrolase, endo-1,4-β-D-xylanase, β-1,4-xylanase and β-xylanase. The principal sources of endo-1,4-β-xylanases (EC 3.2.1.8) are bacteria, fungi, algae, protozoa, gastropods and anthropods (Prade, 1995), for example, *Aureobasidium pullulans* (Christove and Prior 1993), *Pyrodictium abyssi* (Andrade et al., 2001), *Saccbaromonospora viridis* (Roberts et al. 1990) and *Thermotoga maritima* (Ihsanawati et al., 2005). Xylanase from different organisms usually has a specific optimum temperature range, which varies between 40 °C and 85 °C. Normally, xylanases are stable over a wide pH range from 3-10, and show an optimum in the pH range of 4 to 7. The composition of xylanases, reported from various sources, indicate that the amino acids consist of predominantly aspartic acid, glutamic acid, glycine, serine, and threonine residues (Kulkarni et al., 1999). Glutamic acid residues and aspartic acid residues are believed to act as catalysts for the hydrolysis of xylan (Coutinho and Henrissat, 1999; Nurizzo et al., 2002).

## 2.3 Mechanism of xylanase action on xylan

Xylanase action on xylan is a kind of catalytic hydrolysis. The catalytic mechanism of xylanase produced from different organisms consists of a double-displacement mechanism and a single-displacement mechanism in all case (Collins et al., 2005; Coutinho and Henrissat, 1999; Rye and Withers, 2000). As shown in Fig. 2.2a, two glutamate residues are involved in this catalytic double-displacement hydrolysis with retention of the configuration at the anomeric carbon (Coutinho and Henrissat, 1999). One type of glutamic acid residue acts as an acid-base catalyst, whereas, the other type is likely to be the nucleophile (Havukainen et al., 1996). While xylan catalysed hydrolysis by xylanase

proceeds in acid-base system, one glutamate residue as the acid-base catalyst residues protonates the oxygen of the substrate, and the second one performs a nucleophilic attack. This leads to the formation of a leaving group and an α-glycosyl xylanase intermediate. In the second step, a proton from a nucleophilic water molecule is abstracted by the first carboxylate group to form general glutamate residue. Meanwhile, -OH ion from water molecule attacks the anomeric carbon forming an oxocarbenium-ion-like transition state which then give rise to product. In addition, a glutamate and an aspartate are believed to be the catalytic residues that participate in the single-displacement reaction (Coutinho and Henrissat, 1999; Nurizzo et al., 2002). As illustrated in Fig. 2.2b, a catalytic acid group provides protonic assistance to the departure of the leaving group, at the same time, the other carboxylate provides a general base catalytic assistance which activates a nucleophilic water molecule to attack the anomeric carbon. Therefore, the glycosidic bond between two xyloses can be cleaved (Collins et al., 2005).

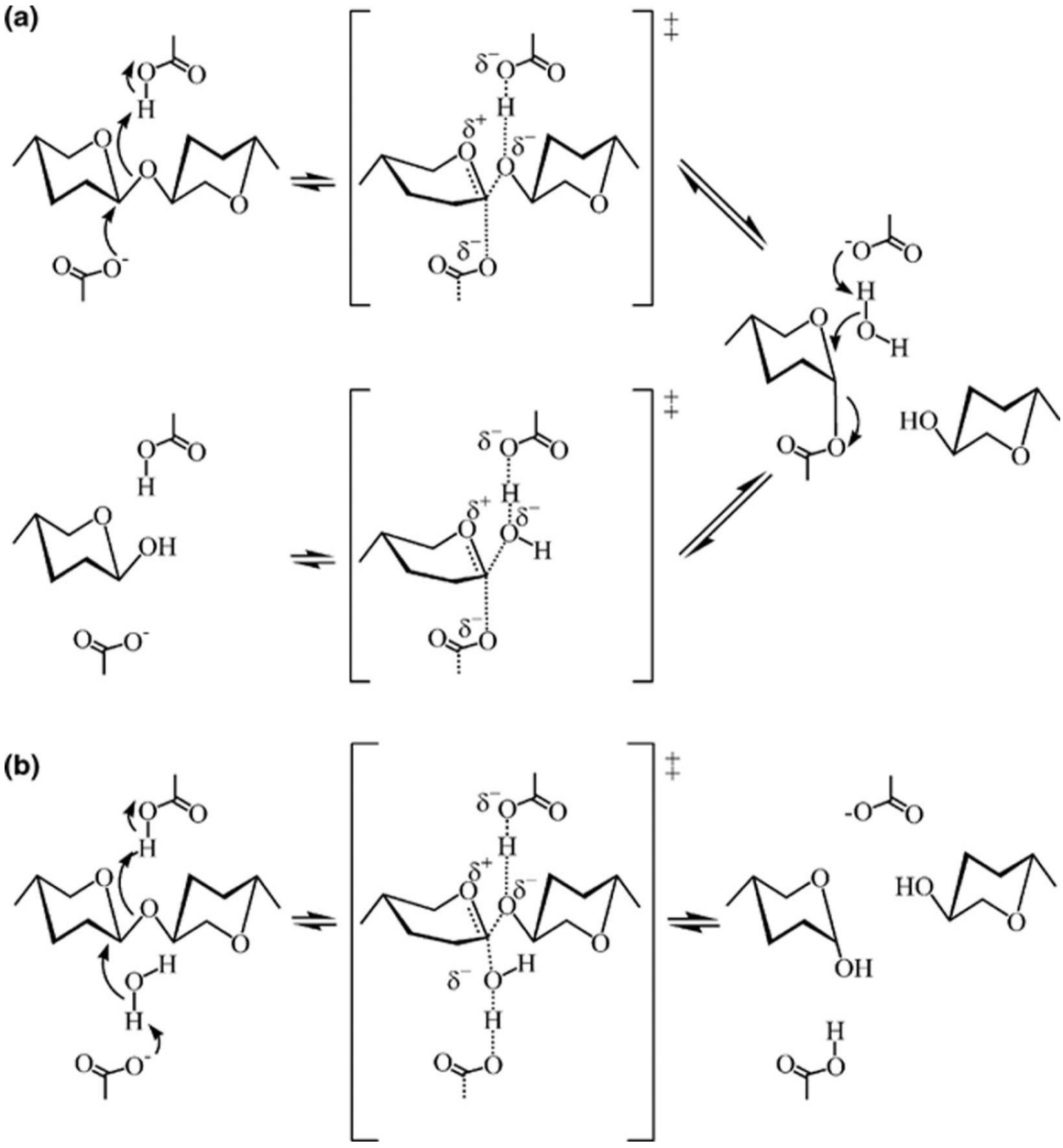



**Fig. 2.2. Reaction mechanism of xylanase with xylan (Collins et al., 2005).**

## 2.4 Mechanism of xylanase action on xylan in bamboo pulp

Xylan is main component of bamboo hemicellulose and the majority of hemicellulose in bamboo pulp after pre-hydrolysis kraft cooking is also xylan. Xylanase, facilitating the cleavage of bonds in xylan molecules with the addition of the elements of water, results in

the formation of xylan oligomers and monomers, which can be easily removed from the pulp. (Campbell and Reece, 2005). Moreover, during xylanase treatment, xylan in lignin-carbohydrate-complexes is also removed, resulting in the improvement of accessibility of lignin to bleaching chemicals (Bhat, 2000; Daneault et al., 1994). Therefore, xylanase treatment of pre-hydrolyzed kraft pulp prior to bleaching could reduce polymeric xylan to oligomeric or monomeric xylan with relatively low molecular weight, and also enhance the permeability of bleaching chemicals to aid removal of lignin during subsequent bleaching operations (Christov and Prior, 1993). Accordingly, high purity cellulosic dissolving pulp may be obtained after the subsequent bleaching sequences (Fig. 2.3).

During xylanase treatment of pulp, temperature, pH, enzyme dosage, reaction time and pulp consistency have been demonstrated to be the main factors that affects the xylan removal efficiency (Daneault et al., 1994). The pulp consistency affects the dispersion of the enzyme that can also be controlled by an appropriate continuous mixing system. The optimal temperature, pH, enzyme dosage and reaction time are mainly dependent on the organism from which the xylanase is obtained. For example, Senior et al., (1988) reported xylanase derived from *Trichoderma harzianum* could remove 54% xylan from hardwood pulp with treatment at temperature 50 °C and pH 5 for 24 hours. Moreover, continuous research is conducted on developing xylanase that has high activity even under hydrothermal and harsh pH conditions, which allows the utilization of xylanase directly after the cooking stage in kraft pulping process. The xylanase generated from *Distyoglomus thermophilum* had highest activity at 85-90 °C and pH 10, moreover, the xylanase derived from mutant of this bacterium can degrade xylan at a temperature as high as 95 °C (Li et al., 2013).

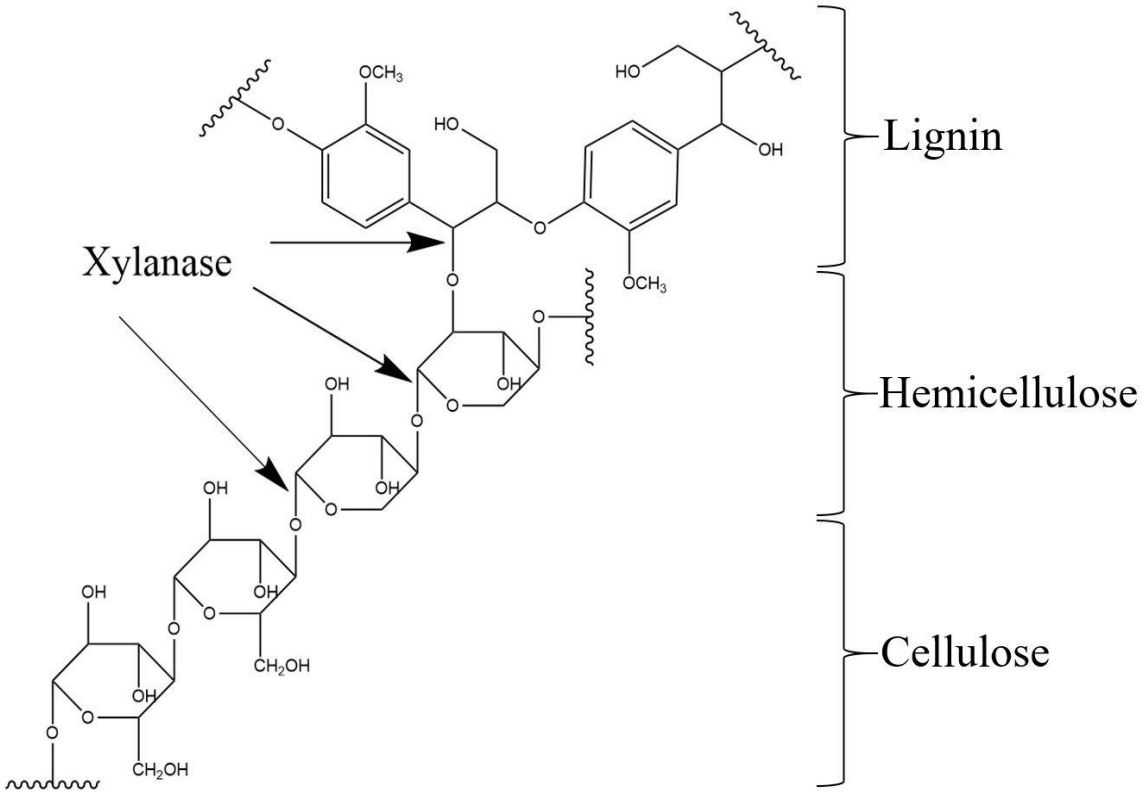



**Fig. 2.3. Possible structure of lignin-carbohydrate complex in unbleached pre-hydrolysis kraft bamboo pulp. Arrows shows possible sites of xylanase hydrolysis (adapted from Daneault et al., 1994).**

## 2.5 Approaches to improve xylanase treatment efficiency

Despite the fast removal of xylan with xylanase, it was found that complete removal of hemicellulose from pulp by enzymatic hydrolysis is difficult to achieve, even using very high loadings of enzymes (Christov et al., 1995; Christov and Prior, 1993, 1996; Jeffries and Lins, 1990; Roberts et al., 1990; Senior et al., 1988). This is probably due to the inaccessible location of the substrate, which hinders the accessibility of xylanase to xylan (Lian et al., 2012a,b; Tian et al., 2014). Thus, a single xylanase treatment step might not

be able to produce dissolving pulp with the very low hemicellulose content (< 2% w/w). To achieve the goal of producing dissolving pulp with very low hemicellulose content, considerable effort has been conducted to further remove hemicellulose by using treatments that combine cold caustic extraction (CCE) with xylanase or combine cellulase with xylanase (Henriksson et al., 2005; Sixta, 2006).

As is well known, the CCE process is an effective way to remove the residual hemicelluloses from pulp fibers. During CCE processing, pulp fibers are swollen by aqueous sodium hydroxide, allowing the interior hemicellulose to be extracted easily through the pores in the fiber wall (Sixta, 2006). Xylan removal by a CCE stage for the production of dissolving pulp from different feedstocks has been investigated; such as softwood sulfite pulps (Gehmayr and Sixta 2012), sugarcane bagasse (Liu et al., 2013) and bamboo (Batalha et al., 2012). Several researches have described the positive effect of combined enzymatic and alkali treatments on extraction of xylan from hardwood kraft pulp and protection of cellulose yield (Gehmayr et al., 2011; Hakala et al., 2013; Jackson et al., 1998). However, there is a challenge in removing residual alkali from the highly swollen pulp after a CCE process, which consequently leads to high capital investment and high chemical consumption (Gehmayr and Sixta, 2012). The utilization of CCE also transfers cellulose I to cellulose II, resulting in lower reactivity of obtained dissolving pulp (Schild and Sixta, 2011; Sixta et al., 2013).

Cellulase, also referred to as glucanase, is an enzyme which randomly hydrolyzes amorphous cellulose located on the fiber surface and in-between the microfibrils, resulting in increase in swelling ability of the fiber, thus increasing the porosity of fiber structures

(Henriksson et al., 2005). The increase in the porosity of fiber structures from the cellulase treatment can lead to the exposure of more accessible xylan toward xylanase treatment. Glucanase performing synergistically with xylanase to increase xylan removal from the dissolving pulp has been investigated by Gübitz (1997), Ibarra (2010), Jackson (1996), Köpcke (2008), etc. Furthermore, several researchers have described the positive effect of a combined glucanase and xylanase treatment on the reactivity of dissolving pulp in the downstream cellulosic conversion process (Engström et al., 2006; Gehmayr et al., 2011; Henriksson et al., 2005; Ibarra et al., 2010; Köpcke et al., 2008).

In general, in either CCE or gluanase combined with xylanase treatments, xylan removal is mainly improved by the opening of the fiber structure making the xylan inside the fiber wall more accessible to xylanase. Based on these findings, it can be concluded that during xylanase treatment, the efficiency of xylan removal is not only depended on treatment conditions, the accessibility of xylanase to xylan on fiber wall is also an important parameter. Mechanical refining is one of the effective methods of modifying fiber morphology. It can lead to fibrillation and could increase the accessibility of xylan, thereby promoting the reaction efficiency of xylanase with the pulp. Within the past several years, development of accessibility of xylan in wood pulp to xylanase by mechanical refining has been reported (Lian et al., 2012a,b; Tian et al., 2014). However, no studies have been published on the application of mechanical refining for improvement of xylanase treatment of bamboo pulp. The present study investigates the proposed concept of combining mechanical refining and enzymatic treatments to remove hemicellulose in order to convert conventional bamboo kraft pulp into high grade dissolving pulp.

## 2.6 Scope, hypothesises and objectives

With regards to an emerging bio-economy, utilization of dissolving pulp for the production of different kinds of biobased products is of great importance. However, as outlined in the foregoing discussion, hemicelluloses in bamboo pose a significant challenge for the production of high quality of dissolving pulp and the preservation of cellulose yield. Xylanase can be used as an additive to remove hemicelluloses efficiently and in an environmental friendly way. Due to the low hemicellulose removal efficiency with xylanase only, the increasing the accessibility of xylanase to xylan in bamboo pulp might be a promising approach to improve the efficiency of xylanase treatment. There is very limited work on xylanase application for the production of high-grade bamboo dissolving pulp, especially, work on treatments that combine mechanical refining with xylanase. Therefore, this thesis is focused on this area.

In order to efficiently remove hemicellulose from pre-hydrolysis kraft bamboo pulp and understand the mechanism, experiments with the commercial bamboo pulp were conducted. The specific objectives of this research are as follows:

1. To assess two commercial xylanases and select the xylanase providing the highest activity for xylan degradation at conditions suitable for treating bamboo pulp (addressed in Chapter 3);
2. To investigate the effect of mechanical refining on xylanase treatment of bamboo unbleached pulp (addressed in Chapter 4).
3. To characterize the change of fibre morphology of bamboo pulp at varied levels of mechanical refining and interpret the relationship between these properties and effect

of xylanase treatment (addressed in Chapter 4).

4. To evaluate the effect of sequential treatment with mechanical refining and xylanase on the quality of final bleached bamboo dissolving pulp (addressed in Chapter 4).

Based on the objectives, the hypotheses of this work are as follows:

1. xylanase can remove hemicellulose from bamboo pulp;
2. refining can improve the xylanase removal efficiency by increasing the accessibility;
3. the combination of refining and xylanase treatment can improve pulp bleaching.

## 2.7 Thesis outline

This thesis is organized into 5 chapters. The motivation and a literature review are given in Chapters 1 and 2, respectively. Chapter 3 is focused on selection of xylanase and finding optimized conditions for the xylanase treatment of bamboo pulp. As to there are many xylanases with different optimum reaction conditions and activity available on the commercial market, a xylanase and its suitable treatment conditions needed to be selected for the subsequent study. The results of enzyme activity of two xylanases are presented. Moreover, that xylanase treatment conditions with bamboo pulp, temperature, time, pH and consistency were investigated. Chapter 4 presents the results of the effect of mechanical refining on xylanase treatment of bamboo pulp. The physical properties of fiber after refining, such as freeness, water retention value, the length of fiber, fines content and scanning electron microscope (SEM) images were investigated to better understand the mechanism of the effect of refining on enzymatic treatment. The xylan content, α-cellulose content, brightness, reactivity and viscosity of bleached bamboo dissolving pulp after D-

EP-D bleaching, in which D is chlorine dioxide and EP is oxidative extraction reinforced with hydrogen peroxide, were also investigated. Chapter 5 presents the contribution to knowledge and recommendations for future research.

# Chapter 3 Selection of xylanase and optimizing the treatment conditions

Xylanases with advantages of being environmentally friendly and effective in reducing chemicals consumption in the bleach plant have been thoroughly investigated as a means to improve product quality and reduce the production cost in the pulping industry. As discussed previously (Chapter 2) in order to use xylanase in the production of bamboo dissolving pulp, a suitable xylanase needs to be identified and the appropriate application conditions need to be determined. Treatment conditions such as temperature, pH, pulp consistency, xylanase dosage and reaction time, are all important factors that could significantly affect the efficiency of xylan removal from bamboo pulp. In this chapter, two commercial xylanases are assessed for use in the production of bamboo dissolving pulp and the suitable treatment conditions are determined.

## 3.1 Materials and methods

### 3.1.1 Raw materials and chemicals

Two commercial xylanases, DP-407 and DP-408, were provided by Iogen Bio-products Corporation. The supplier recommended that the range of both xylanases dosage was 100 to 1500 mL/tonne oven dried (O.D.) pulp, at a pH between 4 and 8. The recommended reaction temperature of DP-407 and DP-408 were 40 to 75 °C and 50 to 80 °C, respectively. Pure birch wood xylan, provided by Sigma-Aldrich, was dissolved to 0.05M solution in sodium citrate buffer at pH levels of 4, 5, 6, 7 and then used for determination of xylanase activity assay.

The wet pre-hydrolysis kraft-based dissolving bamboo pulp, used as the control sample, was provided by Lee & Man Paper Manufacturing Ltd. China. This pulp was prepared by pre-hydrolysis, kraft cooking followed by oxygen-delignification. The obtained pulp was thoroughly washed with distilled water at a consistency of 3.5% with a laboratory mixer to remove impurities and produce a homogeneous stock. The washed pulps were centrifuged to a moisture content of 80% and stored in sealed plastic bags at 4 °C for subsequent treatments and analysis. The control pulp used in this study had a kappa number of approximately 6 and a brightness 43% ISO. It contained 85% (w/w) glucan, 6.6% (w/w) xylan and small amounts of other components such as lignin, other polysaccharides, ash and extractives.

### 3.1.2 Xylanase activity assay

This assay was measured according to a method of determining xylanase activity reported by Bailey et al. (1992). In this method, the xylan substrate in the buffer solutions at pH 4, 5, 6, 7 was incubated with the xylanase for 300 s at 60 °C, 65 °C, 70 °C, 75 °C and 80 °C in a water bath. Dinitrosalicylic acid (DNS) was made up as shown in Table 3.1 and used to determine the sugars released by the action of the xylanase. DNS oxidizes the aldehyde or ketone groups presented in sugar molecules to carboxyl groups, and the 3,5-dinitrosalicylic acid is in its turn reduced to 3-amino and 5-nitrosalicylic acid, which can be detected with a UV-Vis spectrophotometer at 540 nm.

Xylose calibration curve were established using known concentrations of xylose. Four standards of different xylose concentrations, between 0.1 and 1 mg/mL, were prepared with pure xylose and sodium citrate buffer. The absorbance of each sample was measured

according to the DNS assay. Then, the measured absorbance was plotted against the concentration of xylose. The resulting curve was linear, with regression coefficient ($R^2$) around 1, and thus, was used to convert the absorbance to the xylose concentration for this experiment. The standard calibration curve is included in Appendix A.

**Table 3.1. Dinitrosalicylic acid (DNS) recipe.**

| Chemicals | Dosage |
|---|---|
| Distilled water | 1416 mL |
| 3,5-Dinitrosalicylic acid | 10.6 g |
| NaOH | 19.8 g |
| Rochelle salts (Na-K tartarate) | 306 g |
| Phenol (melt at 50 °C) | 7.6 mL |
| Na metabisulfite | 8.3 g |

### 3.1.3 Xylanase treatment of bamboo pulp

The xylanase treatment was conducted in a laboratory water bath. For each sample, 20 g (oven dried weight) of bamboo pulp was treated under various conditions in the polyethylene bag (Table 3.2). Every 30 min, the bags were removed from the water bath and the pulp was kneaded 40 times by hand. After the completion of the xylanase treatment, the samples were filtered, and filtrate from each sample was collected and frozen for subsequent sugar analysis by high performance liquid chromatography (HPLC).

**Table 3.2. Conditions used during xylanase treatment of pre-hydrolysis kraft unbleached bamboo pulp.**

| Temperature (°C) | pH | Pulp consistency (%) | Xylanase dosage (mL/tonne O.D. pulp) | Reaction time (h) |
|---|---|---|---|---|
| 65 | 5 | 10 | 1500 | 3 |
| 70 | 5 | 3 | 1500 | 3 |
| 70 | 5 | 5 | 1500 | 3 |
| 70 | 5 | 10 | 0 | 3 |
| 70 | 5 | 10 | 300 | 3 |
| 70 | 5 | 10 | 600 | 3 |
| 70 | 5 | 10 | 900 | 3 |
| 70 | 5 | 10 | 1500 | 3 |
| 70 | 5 | 10 | 1500 | 6 |
| 70 | 5 | 10 | 1500 | 16 |
| 70 | 5 | 10 | 1500 | 24 |
| 70 | 6 | 10 | 1500 | 3 |
| 70 | 7 | 10 | 1500 | 3 |
| 75 | 5 | 10 | 1500 | 3 |
| 80 | 5 | 10 | 1500 | 3 |

### 3.1.4 Sugar analysis in filtrate

Carbohydrates content of the filtrate was determined using National Renewable Energy Laboratory (NREL) standard protocols (Sluiter et al. 2010). Briefly, filtrate samples were subjected to a two-step sulfuric acid hydrolysis protocol to digest the polysaccharides into monomeric sugars. After hydrolysis, Monomeric sugars were determined using a Dionex ICS 5000+ HPLC system equipped with an AS-AP autosampler and an electrochemical detector (Thermo Fisher Scientific, MA, USA) following the NREL methods (Sluiter et al.

2010). The monomeric sugars were separated on a Dionex Carbopac SA10 analytical column (Thermo Fisher Scientific, MA, USA) at 45 °C using 1 mM NaOH as the mobile phase. Fucose was used as an internal standard.

## 3.2 Results and discussion

### 3.2.1 Effect of temperature and pH on xylanase treatment

According to the xylanase activity experiment with pure birch xylan over a range of temperatures and pH levels (Figs. 3.1 and 3.2), xylanase DP-407 showed higher enzyme activity than DP-408 xylanase. At 70 °C and pH 5, DP-407 had the highest enzyme activity, which was 2050948 nkat/mL, compared to 671234 nkat/mL for DP-408 treatment at 75 °C and pH 5. However, DP-408 xylanase is stable at higher temperatures and can degrade xylan even at temperature above 75 °C. The optimal temperature for DP-408 was between 70 °C to 75 °C, and optimal pH was similar to that of DP-407. In a summary, DP-407 had the highest activity and DP-408 had better stability at high temperature. Both enzymes were selected for the subsequent treatment of bamboo pulp. The effects of temperature and pH on xylanase treatment of bamboo pulp are shown in Figs. 3.3 and 3.4. The optimal temperatures and pH levels are similar to those found in the enzyme activity experiments.

A typical kraft based bamboo dissolving pulp process is conducted with sequential steps of pre-hydrolysis (PHK), kraft cooking (K), oxygen-delignification (O), chlorine dioxide (D) bleaching, oxygen/peroxide reinforced alkaline extraction (EOP), and a final chlorine dioxide (D) bleaching stage. The operating temperature and pH of oxygen-delignification stage are normally around a temperature of 105 °C and pH 11.5-12.5. The oxygen-delignified pulp is washed and then stored for subsequent chlorine dioxide

bleaching, which is generally carried out at 70 °C and pH 3. The pH of washed pulp is close to neutral. According to these operation conditions and the above results for DP-407 and DP-408 xylanase treatment of birch xylan, both xylanases should be suitable for treatment of oxygen-delignified bamboo pulp in its storage tank, in which temperature is 70 °C and pH is 5.

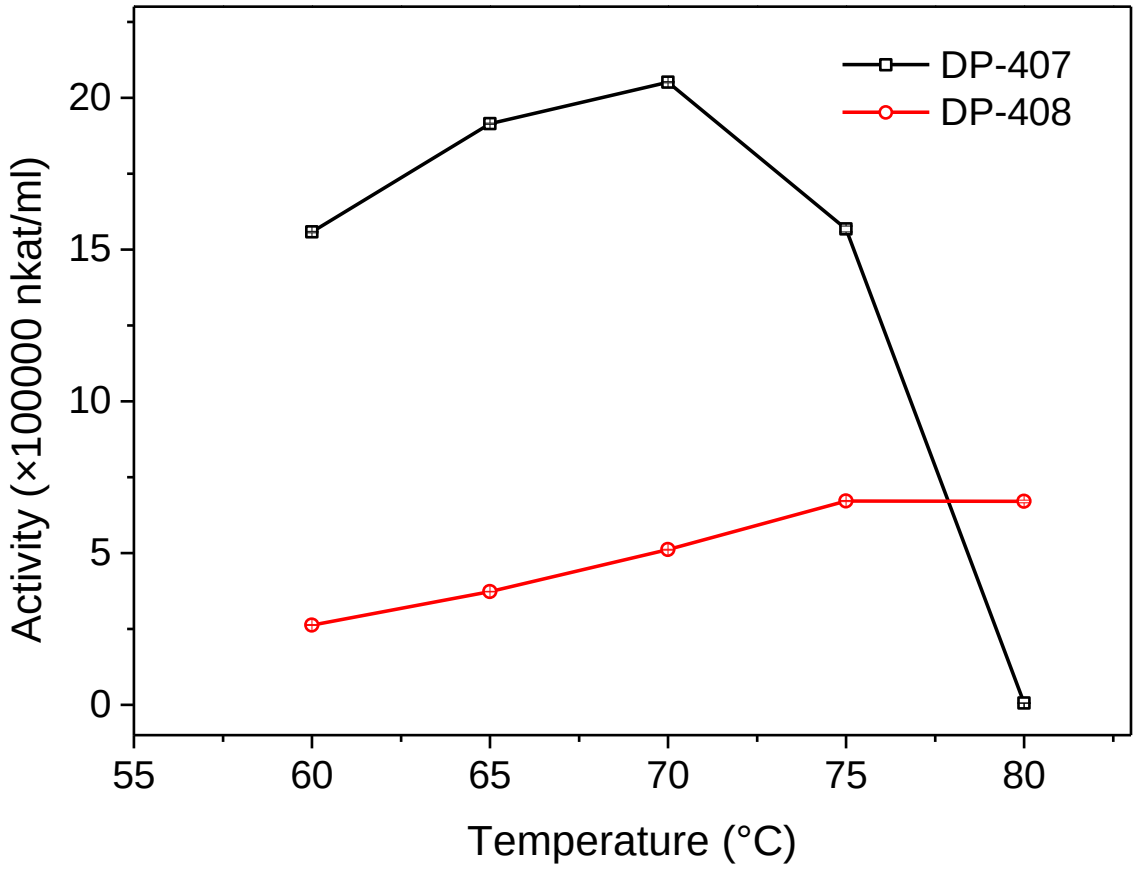



**Fig. 3.1. The effect of temperature on enzyme activity with pure birch xylan at pH 5.**

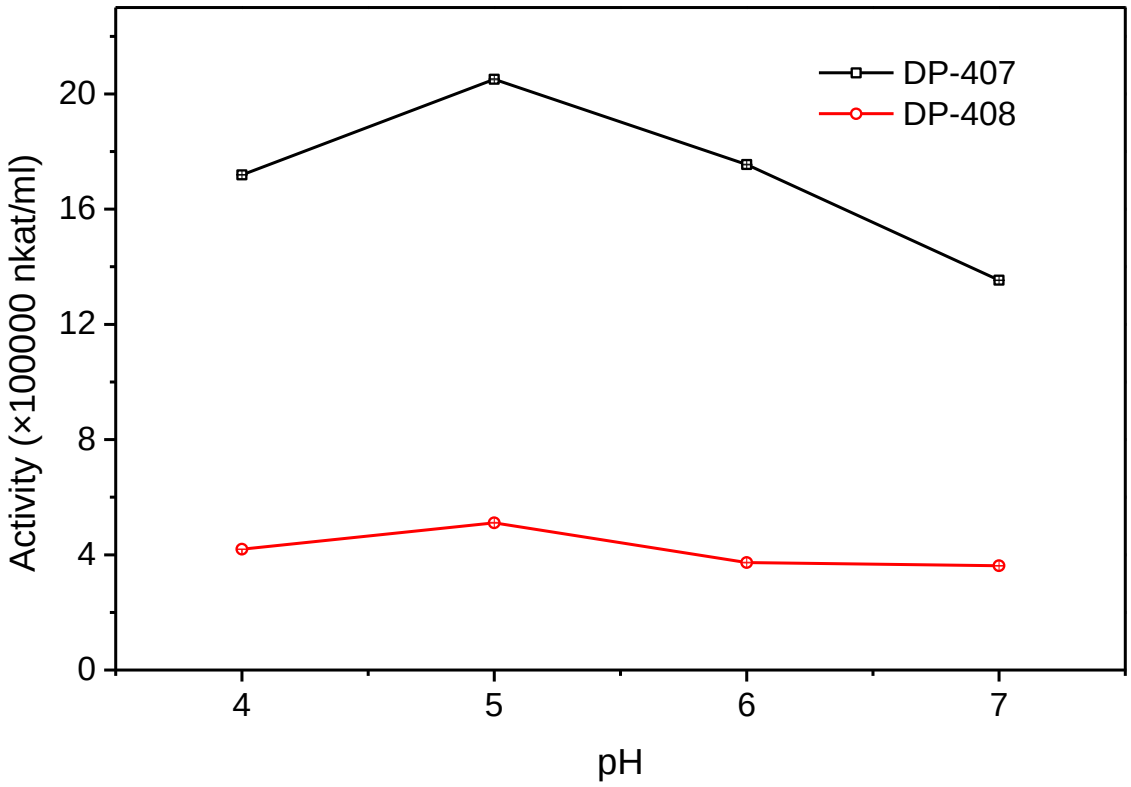



**Fig. 3.2. The effect of pH on enzyme activity with pure birch xylan at 70 °C.**

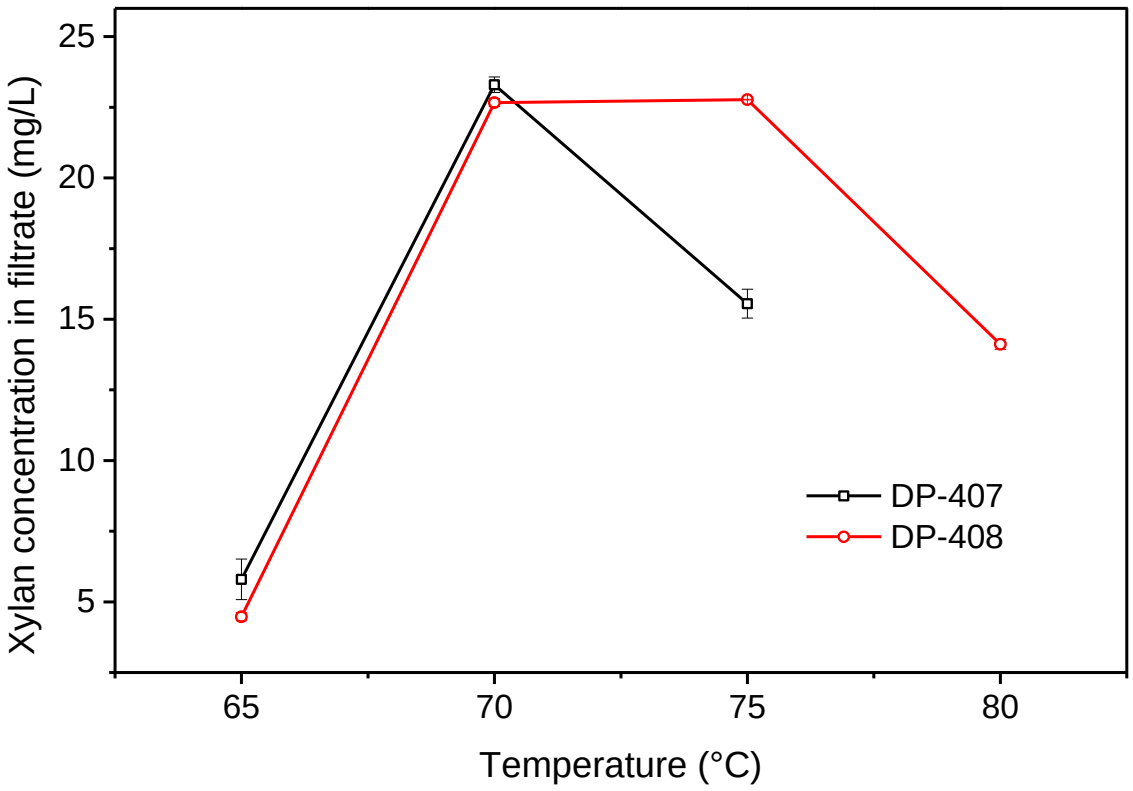



**Fig. 3.3. Effect of xylanase treatment temperature on the reduction of xylan in oxygen-delignified bamboo pre-hydrolysis kraft pulp (pH: 5, pulp consistency: 10%, xylanase dosage: 1500 mL/tonne of O.D. pulp, reaction time: 3 h).**

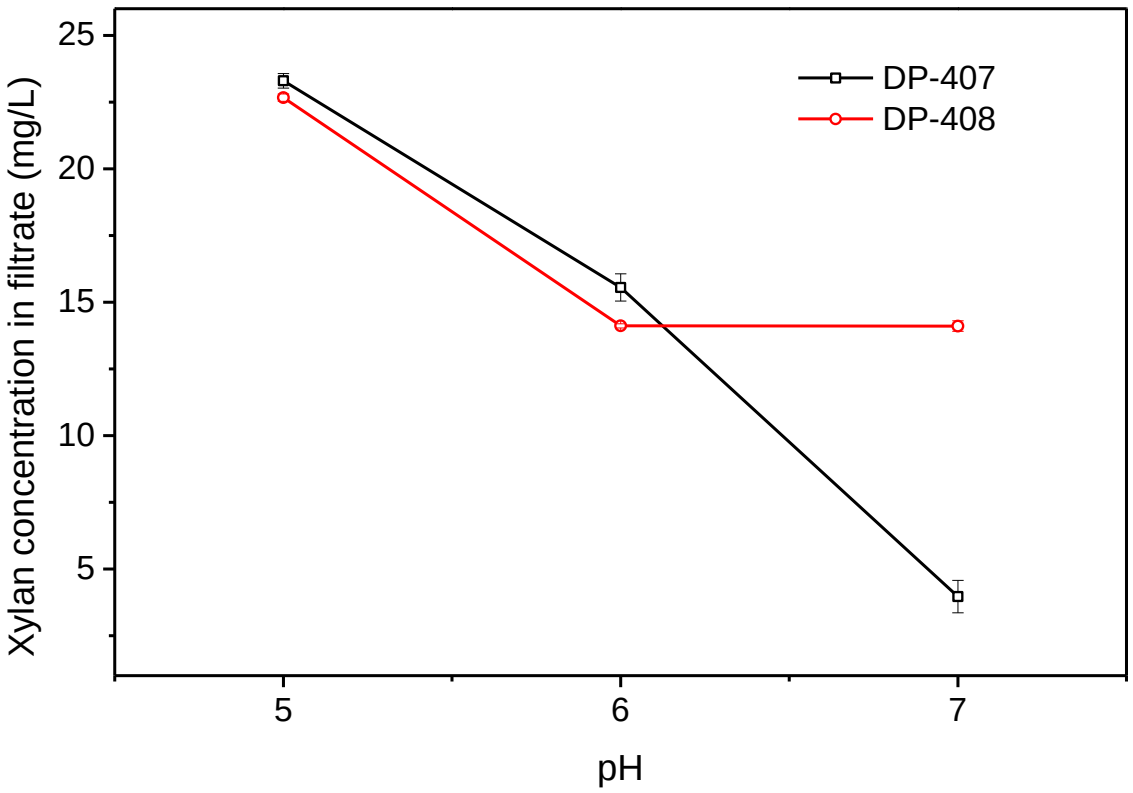



**Fig. 3.4. Effect of xylanase treatment pH on the reduction of xylan in oxygen-delignified bamboo pre-hydrolysis kraft pulp (temperature: 70 °C, pulp consistency: 10%, xylanase dosage: 1500 mL/tonne of O.D. pulp, reaction time: 3 h).**

### 3.2.2 Effect of pulp consistency

Treatments were conducted at different pulp consistencies to assess the impact of changing consistency on xylanase treatment of bamboo pulp. All experiments were carried out under the same conditions and the kneading method. Results showed that pulp consistency at 10% gave better xylan removal than 3% or 5% pulp consistency (Table 3.3.). This might be because at higher consistency the rate of hydrolysis is increased by an increase in xylanase concentration and perhaps an increase in “xylan” concentration

although the latter is in solid form. (Le Costaouec et al., 2013; Liu et al., 2011; Várnai et al., 2013; Wang et al., 2015b). In a modern pulp and paper mill, the medium technology (a pulp consistency of 8-14%), or even high consistency technology (20-35% pulp consistency) are often practiced, because of reduced water use and decreased operating cost (Zhang et al., 2009). Thus, xylanase treatment a consistency of 8-10% is appropriate pulp for pulp mill applications.

**Table 3.3. Effect of consistency on xylan removal from oxygen-delignified bamboo pre-hydrolysis kraft pulp (temperature: 70 °C, pH: 5, xylanase dosage: 1500 mL/tonne of O.D. pulp, reaction time: 3 h).**

| Pulp consistency (%) | Xylan removal (%) | |
|---|---|---|
| | DP-407 | DP-408 |
| 3 | 1.07 | 0.56 |
| 5 | 0.26 | 0.15 |
| 10 | 2.06 | 2.02 |

### 3.2.3 Effect of enzyme dosage and reaction time on xylanase treatment of bamboo pulp

Based on above results, xylanase treatment of the pulp was carried out at the optimized conditions for temperature, pH and pulp consistency. Fig. 3.5 shows the concentration of released xylan in the filtrate generated by treating the pulp with different xylanase dosages for 3 h at pH 5, a temperature of 70 °*C* and a consistency of 10%. As shown in Fig. 3.5, the content of released xylan increased with increase of the xylanase dosage for both xylanases, DP-407 and DP-408. Moreover, the DP-407 xylanase showed slightly higher xylan degradation efficiency than DP-408 (Fig. 3.5). For example, the concentration of released

xylan in filtrate by 1500 mL/tonne of O.D. pulp DP-407 and DP-408 xylanase treatment were 23.30 mg/L and 22.67 mg/L, respectively.

Fig. 3.6 shows the effect of reaction time on the xylan concentration in filtrate. It illustrates that the xylan concentration in the filtrate increase rapidly during the first 3 hours on treating the pulp with either the DP-407 or the DP-408 xylanase. After 3 h of treatment, the xylan degradation rate decreased with a plateau in xylan concentration being reached around 6h (Fig. 3.6). One likely reason might be that the xylanases lost enzyme activity continuously during the reaction with substrate (Borghei-Ghomi, 2001). However, considering that the DP-407 xylanase had a much higher enzyme activity than DP-408 (Fig. 3.1 and Fig. 3.2), it is interesting to observe that of the course of treatment both xylanases removed similar amounts of xylan from the pulp. This might be attributed to the removal of other components that accelerates the removal of xylan with DP-408, the one with lower activity.

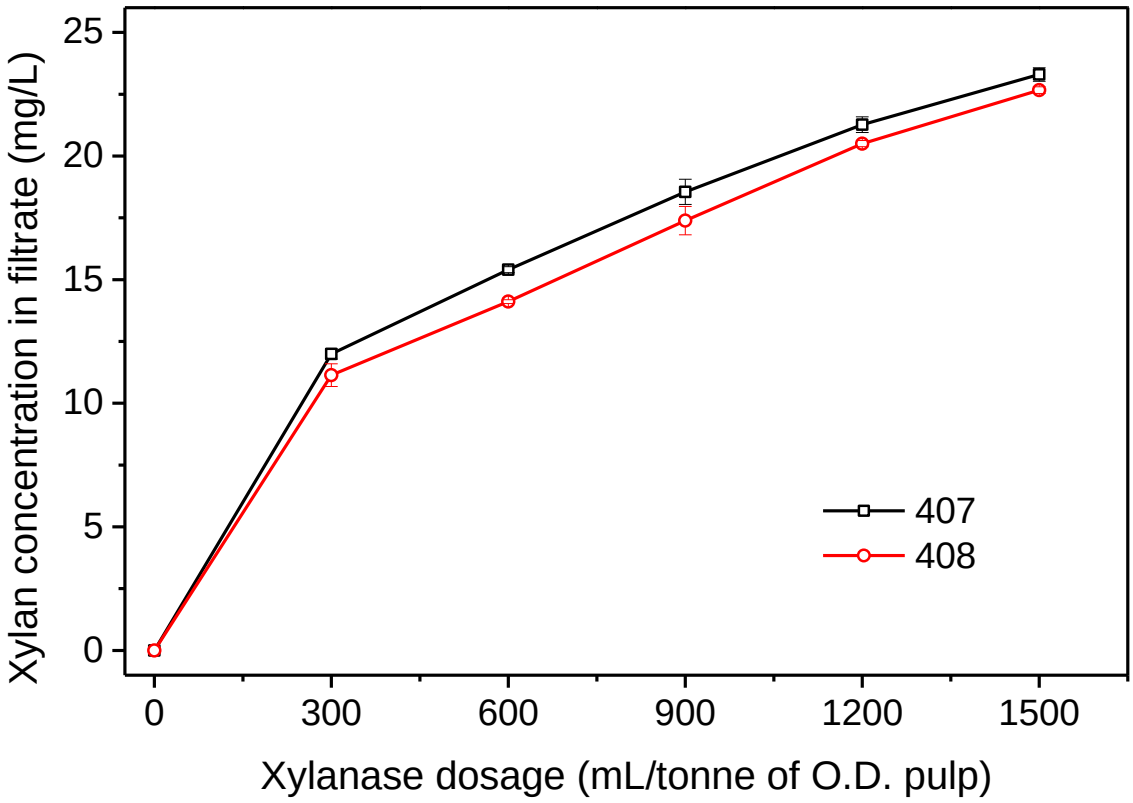



**Fig. 3.5. Effect of xylanase dosage on removal of xylan from oxygen-delignified bamboo pre-hydrolysis kraft pulp (temperature: 70 °C, pH: 5, pulp consistency: 10%, reaction time: 3 h).**

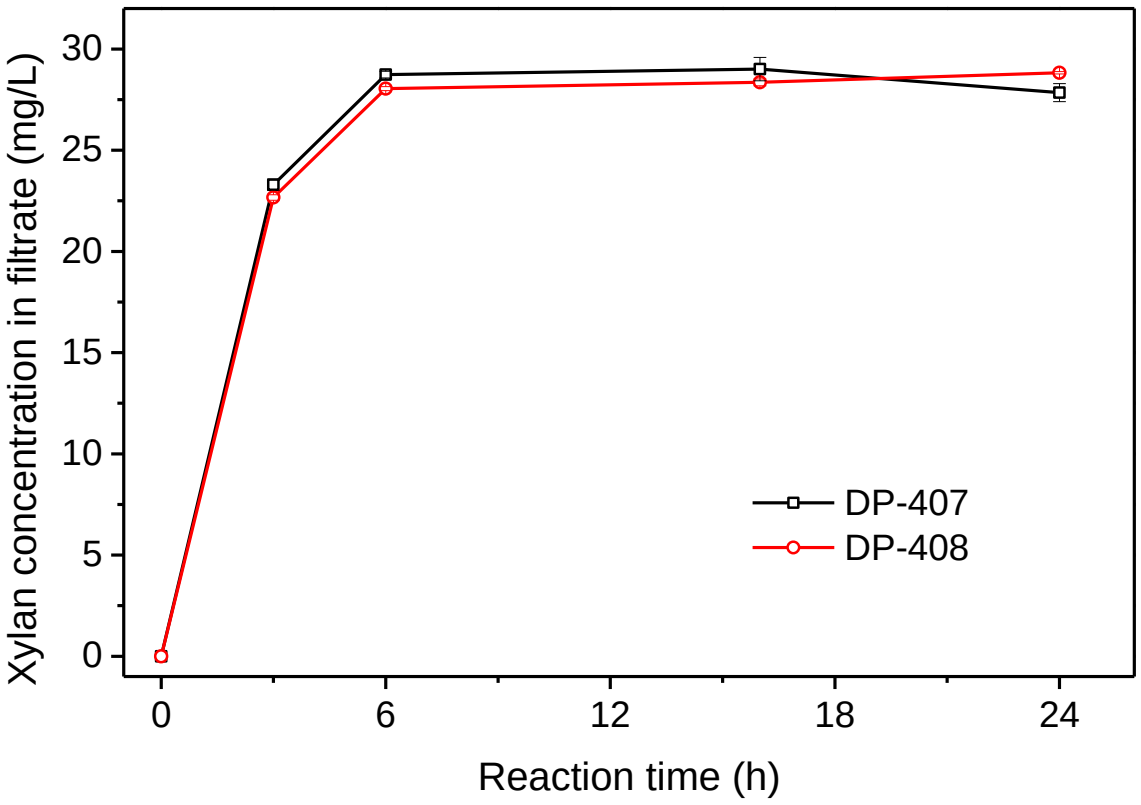



**Fig. 3.6. Effect of reaction time on removal of xylan from oxygen-delignified bamboo pre-hydrolysis kraft pulp (temperature: 70 °C, pH: 5, pulp consistency: 10%, xylanase dosage: 1500 mL/tonne of O.D. pulp).**

After xylanase treatment, no other pentosan than xylose was detected by HPLC in the filtrate, but some glucan was released during xylanase treatment. As Fig. 3.7. and Fig. 3.8. show, the concentration of released glucan in filtrate was increased with increase in xylanase dosage and reaction time. An accepted reason is that some lignin-carbohydrate complexes containing cellulose fractions are released during xylanase treatment (Du et al., 2014). However, the DP-408 xylanase released more glucan than DP-407. It could be that these two commercial xylanases, DP-407 and DP-408, are not pure endo-1,4-β-xylanase. An organism does not produce only one enzyme from its body, and commercial xylanase

products go through separation, concentration and purification from the raw extract (Yasinok et al., 2010). It may also be the case that DP-407 and DP-408 xylanase might contain some other enzymes synergistically act on bamboo fiber cell wall to help remove xylan.

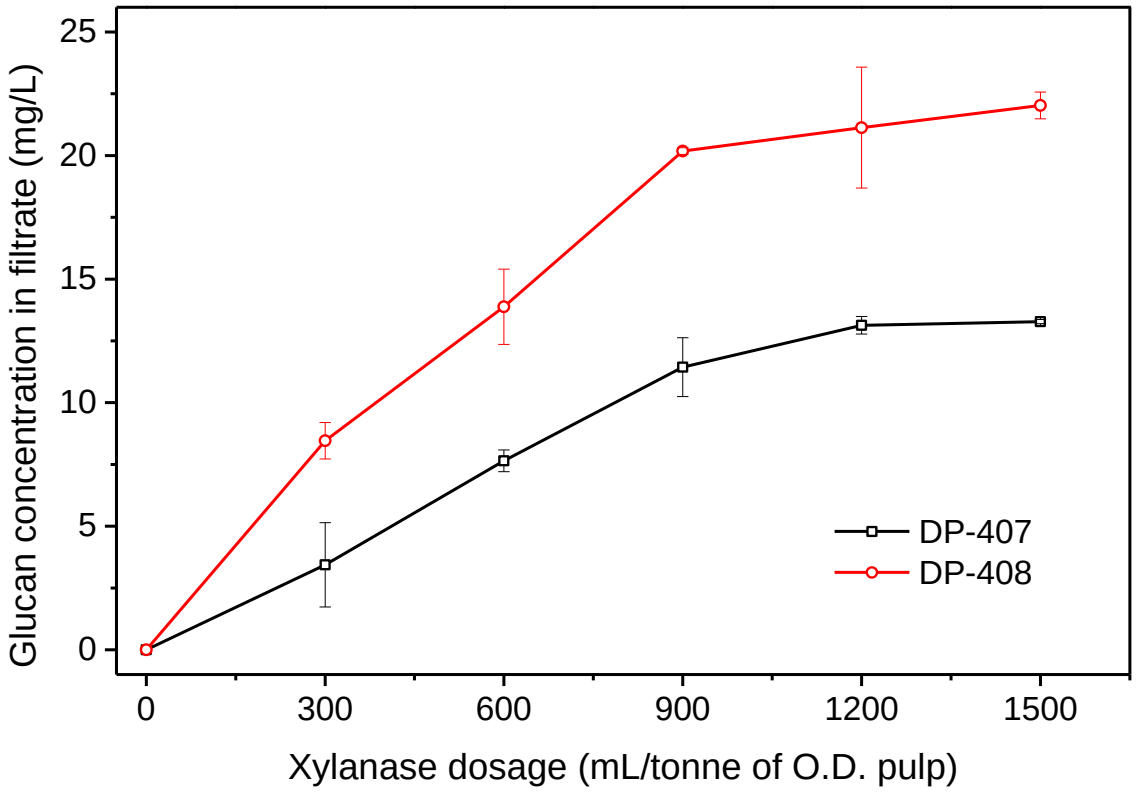



**Fig. 3.7. Effect of xylanase dosage on removal of glucan from oxygen-delignified bamboo pre-hydrolysis kraft pulp (temperature: 70 °C, pH: 5, pulp consistency: 10%, reaction time: 3 h).**

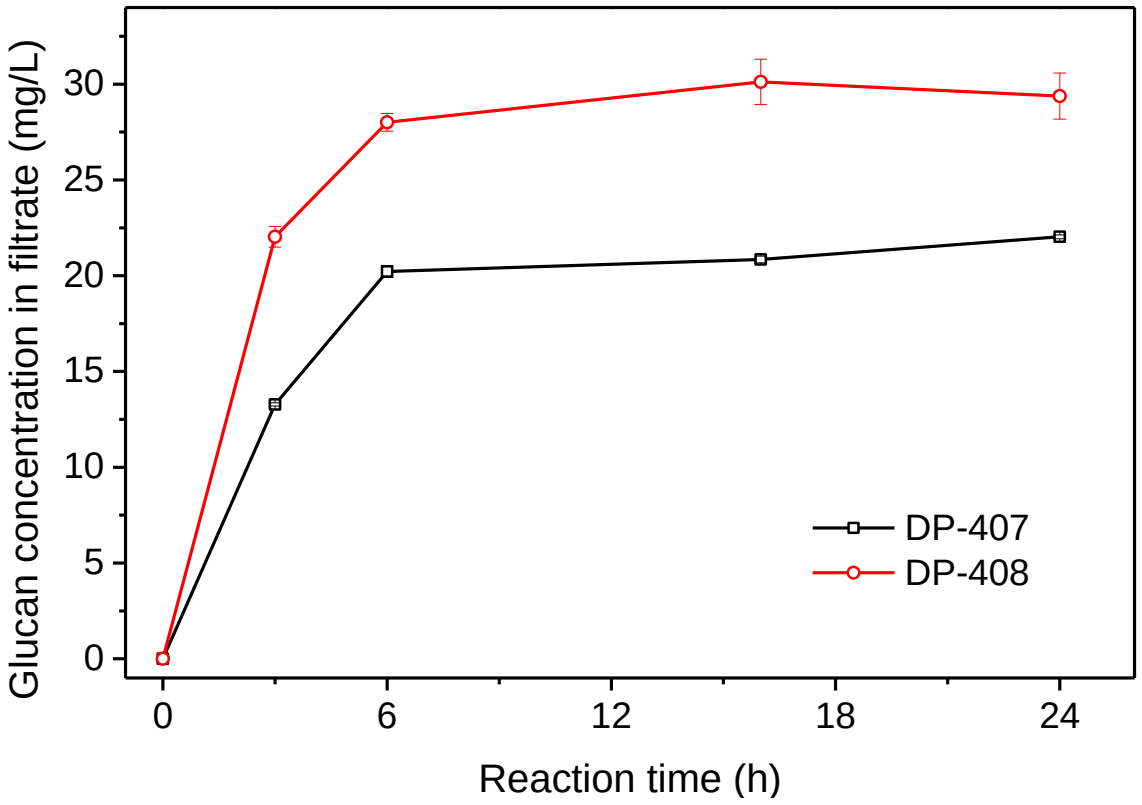



**Fig. 3.8. Effect of reaction time on removal of glucan from oxygen-delignified bamboo pre-hydrolysis kraft pulp (temperature: 70 °C, pH: 5, pulp consistency: 10%, xylanase dosage: 1500 mL/tonne of O.D. pulp).**

## 3.3 Conclusion

Two Iogen xylanases, DP-407 and DP-408, showed great potential to effectively remove xylan from bamboo pulp. Both enzymes could achieve good xylan removal within 3 h during treatment at 70 °C and pH 5 with a high xylanase dosage (1500 mL/tonne of O.D. pulp). The higher pulp consistency (10% versus 3 and 5%) provides higher xylan removal perhaps related to the increase in xylanase concentration and the increase in "xylan" concentration although the latter is in solid form when using higher pulp consistency. The

differences between DP-407 and DP-408 included enzyme activity, working temperature and pH range. DP-407 showed higher enzyme activity than DP-408, but DP-408 gave similar extents of xylan removal from bamboo pulp even at temperatures higher than 70 °C. That could be due to fact that DP-408 removed more glucan than DP-407, resulting in increased accessibility of the xylan for the xylanase. Since the preservation of cellulose yield is one important during the production of bamboo dissolving pulp and DP-407 exhibited an acceptable xylan removal, while maintaining cellulose yield, DP-407 selected for the subsequent refining experiments described in Chapter 4.

# Chapter 4 Increasing efficiency of enzymatic hemicellulose removal from bamboo by mechanical refining for the production of high-grade dissolving pulp

According to foregoing results and discussion, xylanases have shown to be an effective way to remove xylan from bamboo pulp. However, during xylanase treatment of bamboo pulp, similar to many studies with other lignocellulosic feedstocks, the efficiency of xylanase treatment is not as high as when treating isolated birch xylan (Lian et al., 2012a,b; You et al., 2009a,b). This is probably due to the low accessibility of xylan inside the fibre wall to xylanase. Based on past studies (Lian et al., 2012a,b; Tian et al., 2014), we hypothesized that mechanical refining can lead to fibrillation and increase the accessibility of xylan, thereby promoting the reaction efficiency of xylanase with pulp. The present study used the proposed concept of combining mechanical refining and enzymatic treatments to remove hemicellulose to convert conventional bamboo kraft pulp into high grade dissolving pulp.

## 4.1. Material and methods

### 4.1.1. Raw materials and chemicals

The wet pre-hydrolysis kraft-based dissolving bamboo pulp, used as the control sample, was provided by Lee & Man Paper Manufacturing Ltd. China. This pulp was prepared by pre-hydrolysis, kraft cooking and oxygen-delignification. The obtained pulps were thoroughly washed with distilled water at a consistency of 3.5% with a laboratory mixer to

remove impurities and produce a homogeneous stock. The washed pulps were centrifuged to a moisture content of 80% and stored in sealed plastic bags at 4 °C for subsequent treatments and analysis. The control pulp used in this study had a kappa number of approximately 6 and a brightness 43% ISO. It contained 87% (w/w) glucan, 3.5% (w/w) xylan and small amounts of other components such as lignin, other polysaccharides, ash and extractives. A commercial xylanase was provided by Iogen Bio-products Corporation. The supplier recommended a range of xylanase dosage (100-1500 mL/ tonne O.D. pulp), reaction temperature (40-75 °C) and pH value (5-8). Its highest activity (2050928 nkat/mL) occurred at 70 °C and pH 5.

### 4.1.2. Mechanical refining and grinding

The mechanical treatment was performed using a PFI refiner according to TAPPI standard T248 sp-00. A 24 g (equivalent to oven dried) pulp sample was refined at a 10% pulp consistency for 0, 3000, 6000, 7000, 9000, 12000, and 15000 revolutions.

Pulp powder was prepared by grinding bamboo pulp dried for two days in the controlled temperature and humidity (CTH) room in a Wiley mill. The pulp powder which passed through a 20 mesh screen was collected for subsequent xylanase treatment and analysis.

### 4.1.3. Xylanase treatment

The xylanase treatment was conducted in a water bath. For each sample, 20 g (oven dried weight) of bamboo pulp or pulp powder was treated with 1500 mL xylanase per tonne oven dried (O.D.) pulp at 10% pulp consistency for 1-8 h at 70 °C in citrate buffer (pH 5) in a polyethylene bag. Every 30 min, the samples were removed from the water bath and

kneaded 40 times by hand. After the completion of the xylanase treatment, the samples were placed in hot water to boil for 15 min to denature the xylanase, and subsequently filtered and washed with 1 liter deionized water three times. The xylanase treated pulp and powder samples were stored at 4 °C for subsequent analyses.

### 4.1.4. Pulp bleaching

The dissolving pulp was bleached to full brightness with a D-EP-D sequence, in which D is chlorine dioxide and EP is oxidative extraction reinforced with hydrogen peroxide. Table 4.1 shows the conditions used for each bleaching stage.

**Table 4.1. The D-EP-D bleaching conditions.**

| Condition | D0 | EP | D1 |
|---|---|---|---|
| Consistency (%) | 10 | 10 | 10 |
| Temperature (°C) | 70 | 80 | 80 |
| Time (min) | 90 | 60 | 180 |
| ClO2 as Cl2 (% of O.D. pulp) | 1.3 | - | 0.5 |
| NaOH (% of O.D. pulp) | - | 0.6 | - |
| H2O2 (% of O.D. pulp) | - | 0.3 | - |
| Final pH | 2.4 | 10.8 | 4.4 |

### 4.1.5. Compositional analysis

The moisture content of solid samples was measured by drying at 105 ± 2 °C to constant weight. The extractives content of bamboo culm samples and bamboo chips was determined using a Soxhlet extractor according to TAPPI T 204 cm-97.

Carbohydrates and lignin content of the solids was determined after air drying. Lignin

content was calculated as the sum of Klason lignin and acid soluble lignin. Klason lignin content of the pulp was determined using National Renewable Energy Laboratory (NREL) standard protocols (Sluiter et al. 2010). Briefly, 40-60 mesh samples were subjected to a two-step sulfuric acid hydrolysis protocol to digest the polysaccharides into monomeric sugars. After hydrolysis, Klason lignin was separated through filtration and weighed after drying at 105 ± 2 °C. Acid soluble lignin in the hydrolysate (after removing Klason lignin) was measured at wavelength 205 nm using a UV-Vis. spectrophotometer (Dence, 1992). Monomeric sugars were determined using a Dionex ICS 5000+ HPLC (high performance liquid chromatography) system equipped with an AS-AP autosampler and an electrochemical detector (Thermo Fisher Scientific, MA, USA) following the NREL methods (Sluiter et al. 2010). The monomeric sugars were separated on a Dionex Carbopac SA10 analytical column (Thermo Fisher Scientific, MA, USA) at 45 °C using 1 mM NaOH as the mobile phase. Fucose was used as an internal standard.

### 4.1.6. Measurement of alpha-cellulose content

For the measurement of alpha-cellulose content, lignin must be removed from the pulp sample prior to the analysis. A 2 g sample of unbleached oven-dried bamboo pulp was mixed with 65 mL deionized water, 0.5 mL acetic acid and 0.6 g sodium chlorite in a conical flask. The covered flask was placed in a 75 °C water bath and shaken at 150 rpm for 5 h. Every hour, 0.5 mL acetic acid and 0.6 g sodium chlorite were added until the sample became white. The sample was filtered and washed with a large amount of water in a 30 mL medium-porosity fritted glass crucible to neutralize acid, then the sample was washed by acetone before placing the sample into the oven to dry prior to determination of

the holo-cellulose content ($X_{holo}$).

Subsequently, the bleached bamboo pulp sample was subjected to 17.5% sodium hydroxide in 20 °C water bath for 45 min. The sample was filtered in a 30 mL medium-porosity fritted glass crucible, and was then washed sequentially with 9.5% sodium hydroxide (NaOH), large amount of deionized water, and 2 mol/L acetic acid. The oven dried sample was weighed to determine percentage of alpha-cellulose content in bleached bamboo dissolving pulp ($X_{\alpha}$).

The percentage of alpha-cellulose content in unbleached bamboo pulp samples (X) was calculated according to:

$$X = X_{holo} \times X_{\alpha}$$

### 4.1.7. Pulp properties determination

The freeness and water retention value (WRV) were measured according to the TAPPI T227 om-04 and TAPPI UM256 um-99, respectively. The mean fiber length and percentage of fines content in bamboo dissolving pulp sample were measured by fiber quality analyzer (FQA) and all data is reported as the length weighted fiber length (TAPPI method T233 cm-00 and T261 cm-00). The brightness was measured according to the TAPPI T452 om-08. The viscosity of all samples was measured according to TAPPI T230 om-04 using cuprirethylenediamine (CED) solution as solvent. All measurements were carried out in duplicate. The reactivity of samples was determined based on a method reported by Östberg et al., (2012). For the SEM observation of pulp samples, the dissolving pulp samples were freeze-dried and coated with gold film in order to observe the surface

morphology with a HITACHI S-3000N scanning electron microscope (SEM).

## 4.2. Results and discussion

### 4.2.1. Impact of PFI refining on xylanase treatment

Fig. 4.1 shows the effect of refining on xylan removal by xylanase treatment for oxygen-delignified bamboo pre-hydrolysis-kraft pulp. As shown in Fig. 4.1, mechanical refining affected xylan content slightly, which is in agreement with previous studies using hardwood pulp (Miao et al., 2014). When mechanical refining preceded xylanase treatment, xylan removal from pulp was increased from negligible for non-refined pulp to 17.4% after refining for 7000 revolutions. Refining beyond 7000 revolutions did not lead to a further substantial decrease in xylan content. With PFI refining to 9000 revolutions, the xylan content in the bamboo pulp decreased from 3.5% to 2.73% (w/w) by 3 h xylanase treatment, i.e. a 22% decrease. Refining to 15000 revolutions led to about 25% xylan removal with a final xylan content of 2.6%. This could be attributed to the fact that mechanical refining increased the surface area and pore volume of fibre, thereby increasing the accessibility of xylan to enzymes during the subsequent enzymatic treatment.

The alpha-cellulose content is another important parameter of dissolving pulp. As illustrated in Table 4.2, the alpha-cellulose content increased with increasing refining revolutions while beta- plus gamma-cellulose content in pulp decreased. This is because beta- plus gamma-cellulose can be classified as total hemicelluloses, and would include the xylan and any other hemicelluloses that might be removed along with xylan during xylanase treatment. The mass fraction of alpha-cellulose, and beta- plus gamma-cellulose

in pulp after 9000 PFI refining revolutions and xylanase treatment were 90.54% and 4.89%, respectively. The remaining 4.57% of treated bamboo pulp is likely comprised of lignin, ash, extractives and residual hemicelluloses. Thus, refining can improve xylan removal thereby increasing alpha-cellulose content of treated pulp. Taken together the results shown in Fig. 4.1 and Table 4.2 indicate that, under the xylanase treatment conditions used, the number of useful PFI refining revolution for enhanced xylan removal is in the 7000-9000 range.

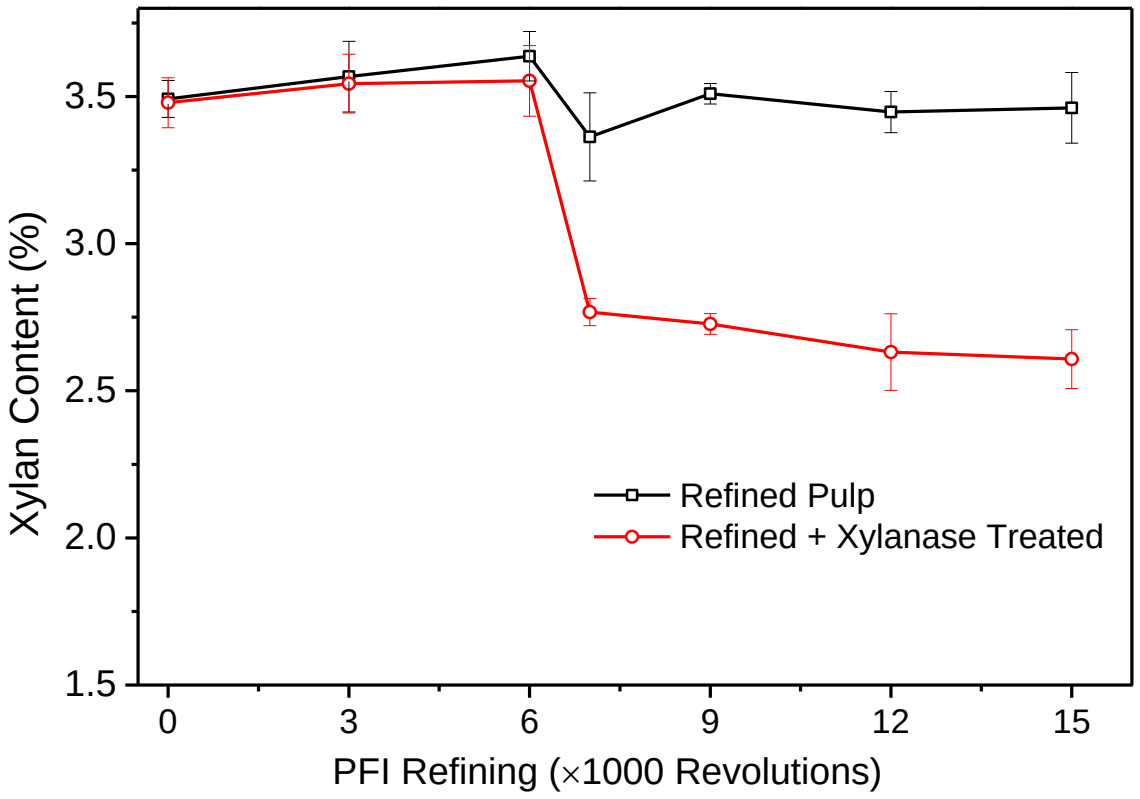



**Fig. 4.1. Effect of refining of oxygen-delignified bamboo pre-hydrolysis kraft pulp on xylan removal during treatment with xylanase (dosage of xylanase: 1500 mL/tonne O.D. pulp; temperature: 70 °C; pH: 5; reaction time: 3 h).**

**Table 4.2. Cellulose content of oxygen-delignified bamboo pre-hydrolysis kraft pulp after sequential refining and xylanase treatments (dosage of xylanase: 1500 mL/tonne O.D. pulp; temperature: 70 °C; pH: 5; reaction time: 3 h).**

| Refining revolutions | alpha-cellulose (%) | beta- plus gamma-cellulose (%) |
|---|---|---|
| 0 | 87.00 | 8.67 |
| 3000 | 87.02 | 8.65 |
| 6000 | 88.42 | 8.24 |
| 7000 | 89.91 | 5.73 |
| 9000 | 90.54 | 4.89 |
| 12000 | 91.77 | 3.91 |
| 15000 | 91.92 | 3.82 |

### 4.2.2. Effect of the duration of xylanase treatment on xylan removal from bamboo

For industrial pulp production, treatment time is a critical parameter for increasing production efficiency and reducing operational costs. In this series of experiments, original pulp, pulp after 9000 refining revolutions and pulp powder (< 20 mesh) were used as substrates for xylanase treatment (Fig. 4.2). Both grinding (< 20 mesh) and refining improved xylan removal during enzyme treatment. For example, after 3 h of treatment, the xylan content of raw pulp, pulp refined to 9000 revolutions and pulp powder were 3.51%, 2.73% and 2.55% (w/w), respectively. With increasing enzymatic treatment time, the xylan content of all three substrates decreased. However, in the case of 9000 revolutions pulp, the extension of xylanase treatment to 8 h only resulted in pulp with 2.44% xylan, representing only 0.29% additional xylan removal beyond the 3 h treatment. Neither the increase of enzyme treatment time to 8 h nor grinding to pass 20-mesh resulted in complete

xylan removal. Though grinding and 8 h enzyme treatment produced the best results in terms of xylan removal, these conditions are too energy intensive for industrial application. While a technoeconomic evaluation is beyond the scope of this work, a 3 h xylanase treatment preceded by refining to 7000-9000 revolutions appears to be suitable for further testing for industrial application. Mechanical refining can lead to fibrillation and disruption of fibers, thereby increasing the specific surface area, the fibre pore size and the fines content (Grönqvist et al., 2014; Tian et al., 2014). These changes can improve the enzyme-substrate contact and penetration of xylanase through the fibre wall, thus enhancing the hemicellulose removal efficiency during xylanase treatment of bamboo pulp. In addition, mass transfer of xylan hydrolysis products is likely improved by the structural changes caused by refining. Leveling off of the xylan removal beyond 7000 revolutions can be attributed to a saturation of the increase in fibre surface area and pores available for contact with xylanase. Grinding causes cutting and breakage of fibre, in turn increasing the number of exposed fibre ends and pores for xylanase to contact xylan (Mou et al., 2013; Tian et al., 2014). Results in Fig. 4.2 demonstrated that even with bamboo powder, not all xylan could be removed with xylanase treatment for up to 8 h. This could be due to molecular interactions between xylan and the lignocellulose matrix which cannot be hydrolyzed by the xylanase enzyme preparation used. Moreover, it has been suggested that the xylan structure has some crystallinity, which limits its accessibility to xylanase (Qing and Wyman, 2011).

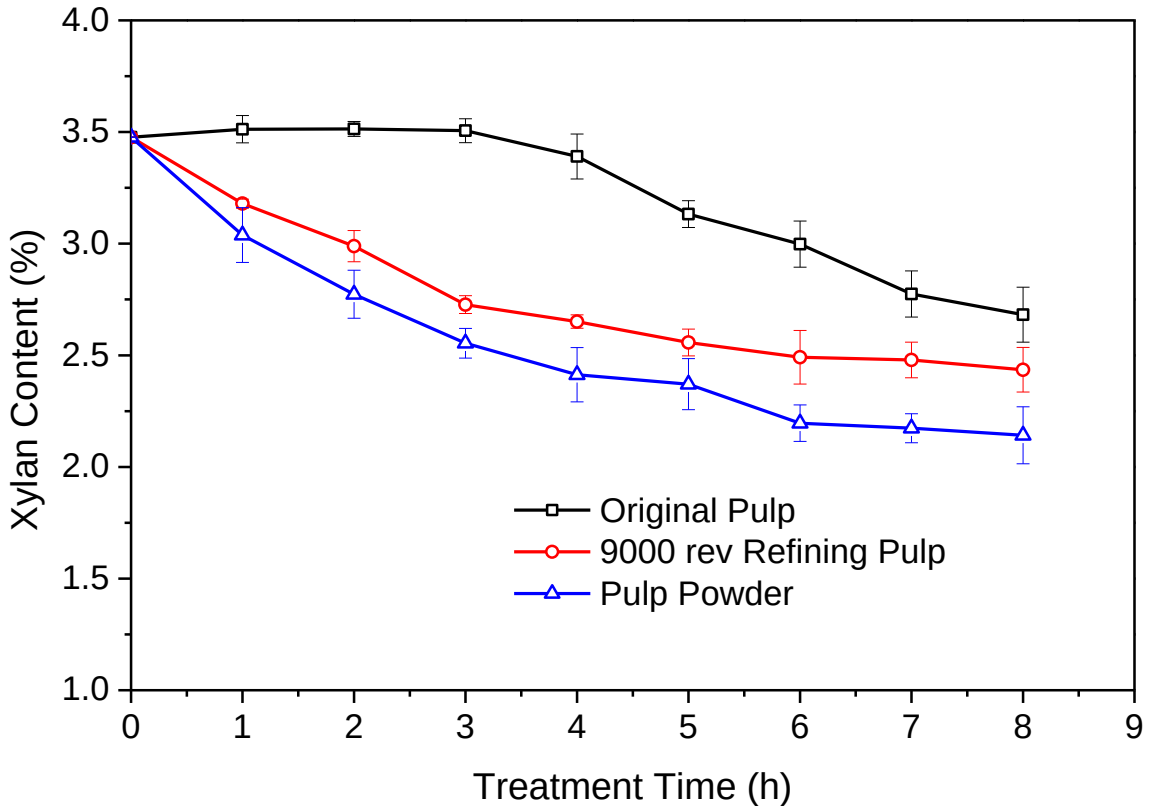



**Fig. 4.2. Effect of refining and xylanase treatment on the xylan content of oxygen-delignified bamboo pre-hydrolysis kraft pulp (refining: 9000 revolutions, pulp powder: < 20 mesh; enzyme treatment: xylanase charge of 1500 mL/tonne O.D. pulp, pH 5, temperature 70 °C).**

### 4.2.3. Effect of PFI refining on fibre physical properties

To gain further insight into the underlying mechanism of the effect of mechanical treatment, the physical properties of pulp samples were analyzed. The fibre lengths and fines contents of original pulp, pulp refined to 9000 and 15,000 revolutions are compared to those of pulp powder in Table 4.3. Pulp powder had the lowest average fibre length and the highest fines content, likely due to extensive fibre cutting during grinding.

Freeness, fibre length, fines content and water retention value (WRV) are parameters often used to describe pulp properties, and Fig. 4.3 shows these properties of oxygen-delignified bamboo pre-hydrolysis kraft pulp after different levels of PFI refining. As shown in Fig. 4.3a, pulp freeness (CSF) decreased with increasing refining (beating energy). Fibre length of pulp significantly decreased while the fines content greatly increased at higher refining revolutions (Figs. 4.3b and c). WRV, representing fibre flexibility and internal fibrillation of the pulp, increased with increased refining. Moreover, all the physical properties shown in Fig. 4.3 underwent rapid changes up to 6000-7000 revolutions, and thereafter changed at a slower rate with increasing refining. This paralleled the leveling off of xylan content after xylanase treatment depicted in Fig. 4.1. In agreement with literature, these results collectively support the idea that fibre specific surface area, short-fibre fraction and the degree of micro-pore formation increased with increasing refining energy (Miao et al., 2014). Shorter fibres pose an interesting case: even though the short-fibre fraction has a larger specific surface area than the long fibres, they have slightly smaller pores (Bäckström et al., 2008; Li et al., 2015). The small-size pores hinder the removal of xylan due to the lower penetration rate of chemicals (including biomolecules such as enzymes) (Li et al., 2015) as well as the poor diffusion of xylan hydrolysis products out of the fibre. Thus, improved enzymatic hydrolysis of xylan would depend on optimal refining conditions that result in a balance between fibre specific surface area and pore size.

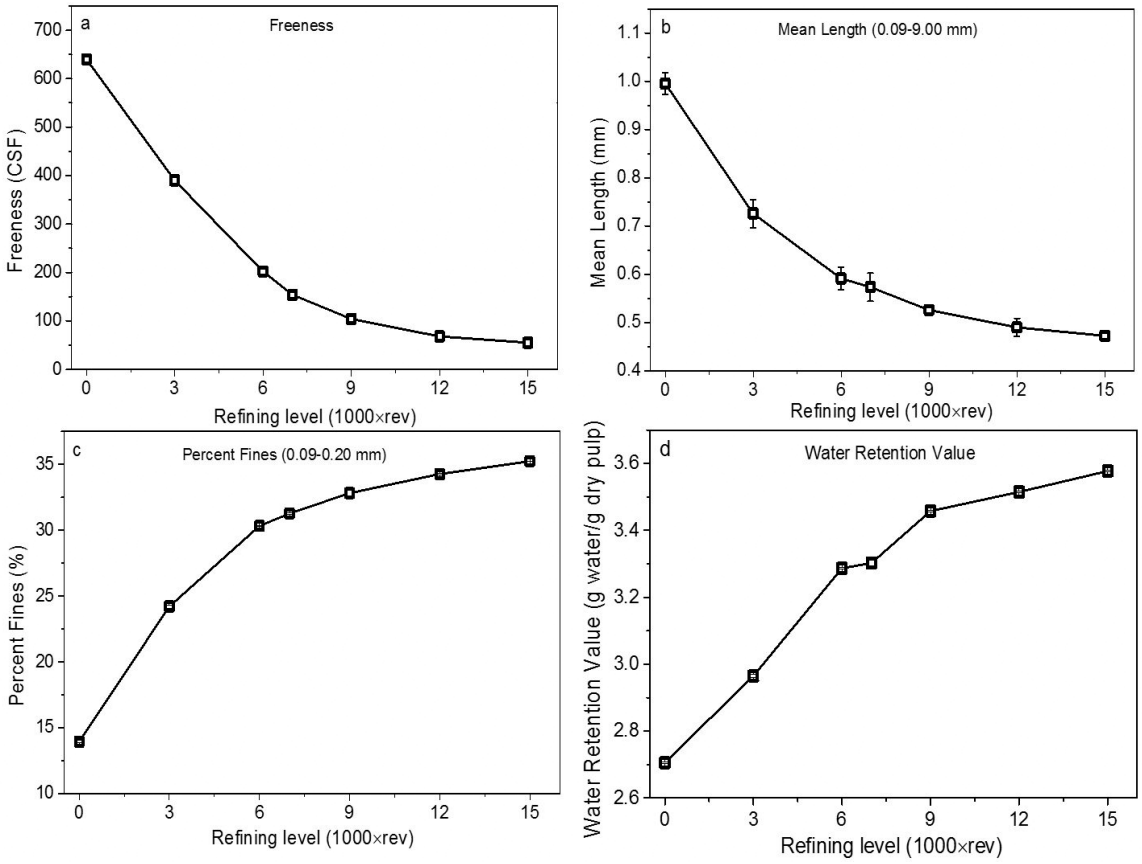



**Fig. 4.3. Properties of oxygen-delignified bamboo pre-hydrolysis kraft pulp at different refining revolutions. (a: freeness, b: fiber length, c: percentage of fines, d: water retention value (WRV)).**

**Table 4.3. Fibre length and fines content of original pulp, refined pulp and pulp powder (< 20 mesh).**

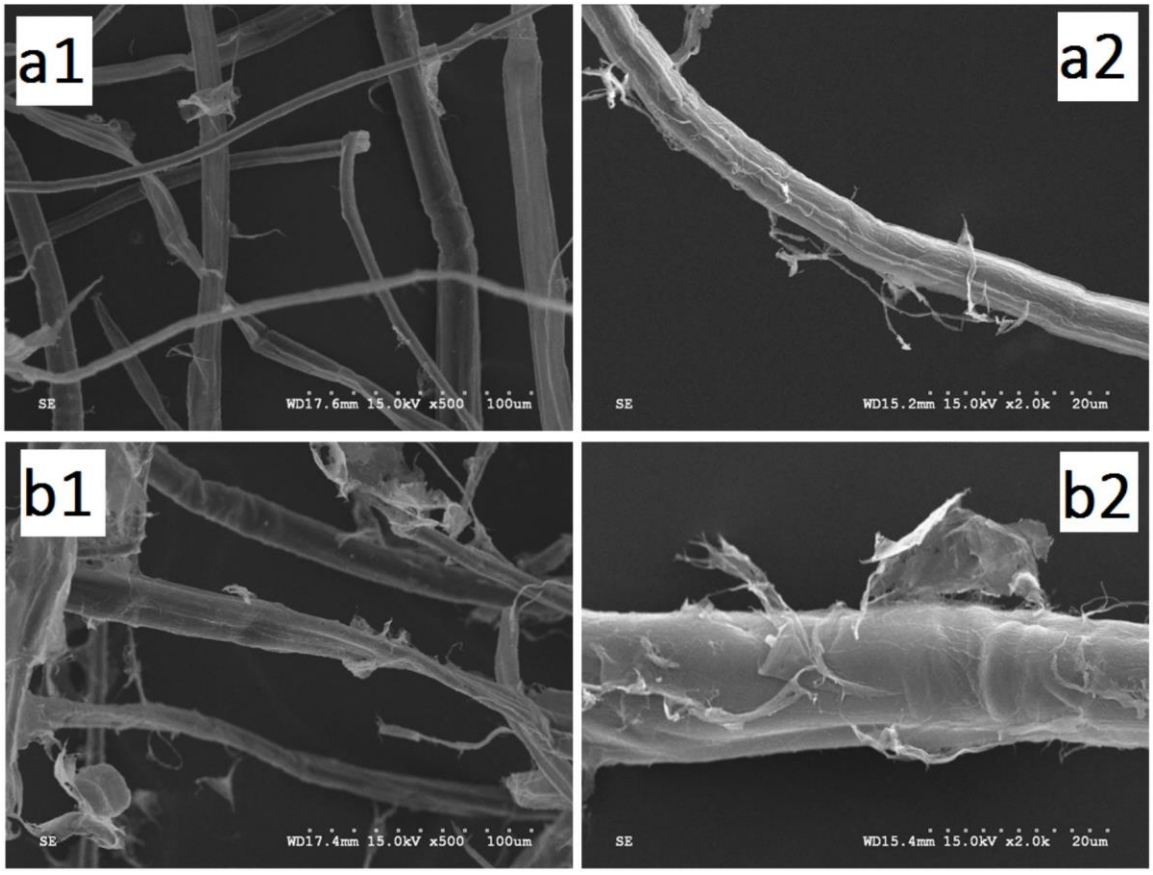
| Sample | Fibre mean length (mm) | Fines content (%) |
|---|---|---|
| Original pulp | 1.00 | 13.95 |
| 9000 refining pulp | 0.53 | 32.81 |
| 15000 refining pulp | 0.47 | 35.24 |
| Pulp powder | 0.26 | 55.10 |

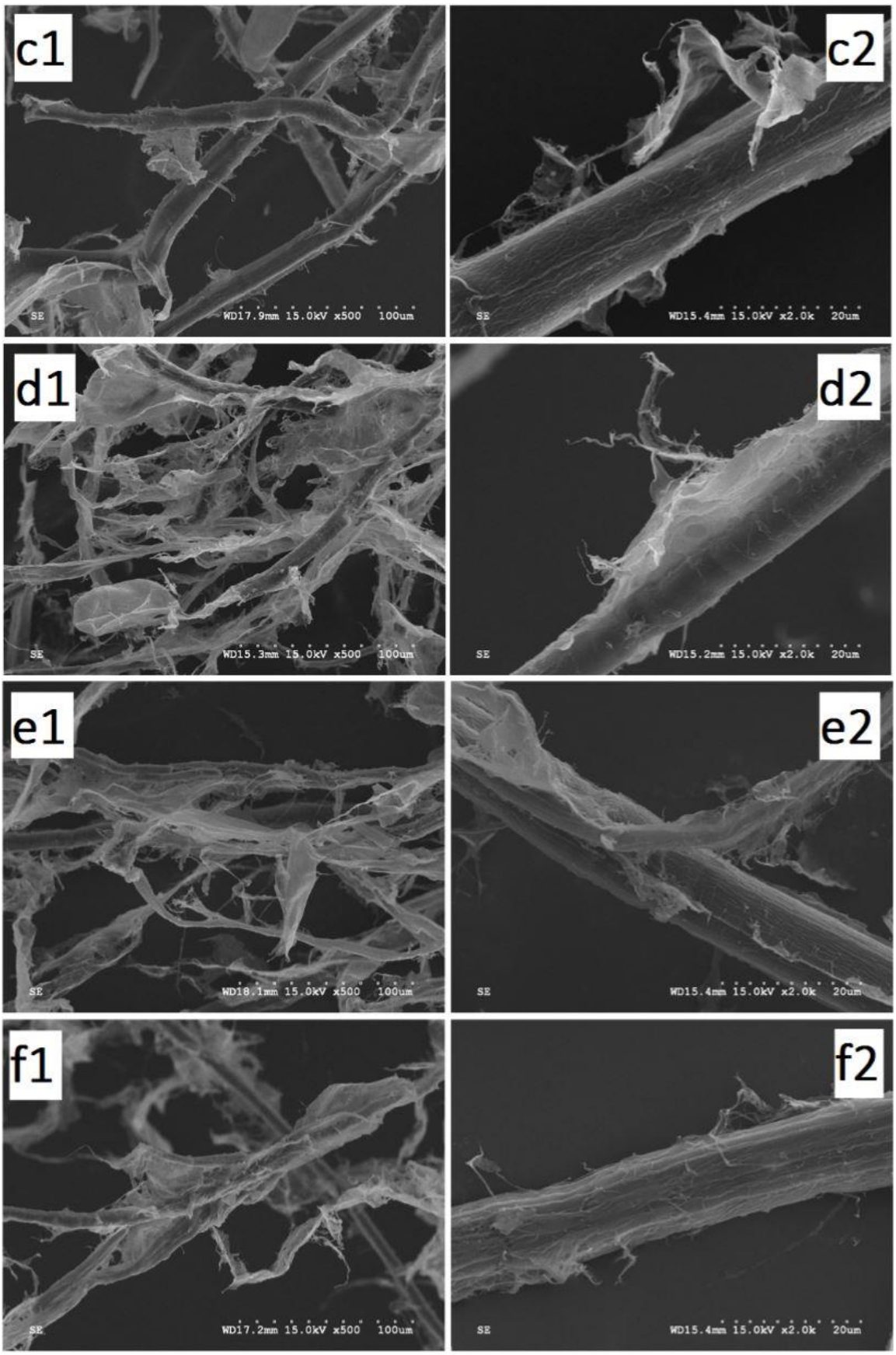
### 4.2.4. Effect of PFI refining on fibre morphology: scanning electron microscopy (SEM)

Results to this stage provided evidence that refining-induced physical changes in fibres caused substantial improvements in the enzymatic removal of xylan from bamboo pulp. We performed SEM imaging of fibres to determine if this was indeed the case. As shown in Fig. 4.4, significant morphological changes occurred. The unrefined pulp exhibited smooth and rigid fibre surfaces (Figs. 4.4a1-2). The fibres of pulp refined to 3000 to 15000 revolutions appeared to become substantially rougher on the surface (Figs. 4.4b-g). In a PFI mill, pulp fibres are exposed to repeated compression and shear forces. In the fibre cell wall, the primary (P) and outer secondary (S1) wall layers are gradually delaminated and peeled off, leaving the inner secondary wall (S2) (Torres et al., 2012; Zhang et al., 2013). Our observations with fibres refined to 3000-6000 revolutions showed similar changes to fibre morphology (Fig. 4.4b-c). At 7000 revolutions, fibrillation appears to include to the inner secondary cell wall (S2 layer), and more fibrils, fibre swelling and delamination were observed (Figs. 4.4d1-2). After refining to 9000 revolutions (Figs. 4.4e1-2), fibrillation seemed to decelerate, as evidenced by somewhat smoother fibre surfaces (Zhang et al.,

2013). Refining to as high as 15000 revolutions did not result in additional changes in the fibre surface beyond those observed with refining to 9000. This indicated that the secondary wall (S2) fibre wall is much harder to disrupt by PFI refining (Torres et al., 2012).

The results shown in Figs. 4.3-4.4 and Tables 4.2-4.3 depict a consistent picture: refining leads to fibrillation and delamination of the fibre wall as well as a reduction in fibre length. Moreover, these changes in the fibre initially increased rapidly with increasing refining and then leveled off at 6000-7000 revolutions. Increase in the efficacy of the subsequent xylanase treatment also saturated at 6000-7000 revolutions.




c1
SE WD17.9mm 15.0kV x500 100um
c2
SE WD15.4mm 15.0kV x2.0k 20um
d1
SE WD15.3mm 15.0kV x500 100um
d2
SE WD15.2mm 15.0kV x2.0k 20um
e1
SE WD18.1mm 15.0kV x500 100um
e2
SE WD15.4mm 15.0kV x2.0k 20um
f1
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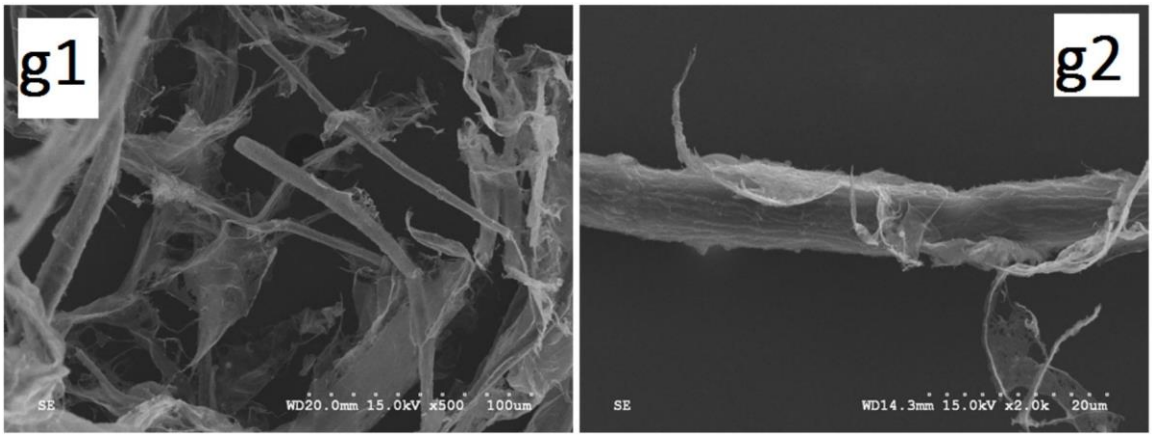


**Fig. 4.4. SEM images of oxygen-delignified bamboo pre-hydrolysis kraft pulp after several levels of PFI refining. a1-2: 0 revolutions, b1-2: 3000 revolutions, c1-2: 6000 revolutions, d1-2: 7000 revolutions, e1-2: 9000 revolutions, f1-2: 12000 revolutions, g1-2: 15000 revolutions.**

### 4.2.5. Bleaching of refining-xylanase treated pulp

To investigate whether the combination of mechanical refining and xylanase treatment can improve pulp bleaching and produce high quality dissolving pulp, the pulp samples obtained by sequential refining (9000 revolutions)-xylanase treatment were subjected to the elemental chlorine free (ECF) bleaching sequence, D-EP-D. As shown in Table 4.4, the treatment combination significantly improved the quality of dissolving pulp, resulting in alpha-cellulose and xylan contents of 93.4% and 2.38%, respectively. Small improvements were also observed with xylanase treatment alone. Brightness of pulp was also increased (Table 4.4). Addition of a xylanase treatment step improved the bamboo dissolving pulp Fock's reactivity, a measure of the reactivity of pulp with xanthation chemicals used in the viscose process, from 21.6 to 31.77%. This value could be increased to 32.42% by the sequential refining-xylanase treatment. Viscosity of dissolving pulp represents the degree

of polymerization of cellulose. Xylanase treatment and refining-xylanase treatment both led to a decrease in the viscosity with the lowest at recorded at 6.1 mPa·s (Table 4.4).

Previous studies reported that xylanase treatment enhanced bleaching effects (Christov and Prior, 1996). Bhat (2000) discussed improved accessibility of lignin to bleaching chemicals following xylanase treatment. We argue that the opening-up of the fibre and the lignocellulose matrix by refining-xylanase treatments reduces mass transfer limitations and enhances the permeability of bleaching chemicals into and dissolved lignin out of bamboo fibres, leading to improved brightness. Our observation of the increase in Fock's reactivity by xylanase (Gehmayr et al., 2011; Köpcke et al., 2008; Rahkamo et al., 1998) and refining (Tian et al., 2014) are in good agreement with the available literature. It has been suggested that xylanase treatment removes xylan deposited on the surface of the cellulose fibrils thereby improving the accessibility of cellulose to xanthation chemicals (Krässig, 1984). Moreover, mechanical refining prior to xylanase treatment provides additional accessible cellulose due to newly exposed fiber ends, pores and fiber surface area (Tian et al., 2014). The decrease in viscosity with xylanase treatment alone is rather surprising. Whether the removal of xylan from the fibres resulted in increased cellulose degradation during the subsequent bleaching stages remains to be tested. Further drops in viscosity when pulp subjected to both refining and xylanase treatment was bleached could be due to mechanical damage to fibre/cellulose that occurred during refining.

Without any attempt at optimizing the bleaching conditions we have used a sequential refining-xylanase treatment combination to produce dissolving pulp of improved quality: 93.4% α-cellulose and 2.38% xylan. While the quality standards vary depending on end-

use, bamboo pulp with comparable composition has been previously used to produce cellulose acetate (He et al., 2008, 2009). However, the 32.42% Fock's reactivity value does not meet the quality standards of high-grade dissolving pulp (Sixta, 2006; Sixta et al., 2013). Therefore, other approaches such as glucanase application could be tested to improve the reactivity in future work (Gehmayr et al., 2011; Krässig, 1993).

**Table 4.4. Xylan content, alpha-cellulose content, brightness, reactivity and viscosity of with/without PFI refining in bleached (D-EP-D) bamboo dissolving pulp.**

| Sample | Xylan Content (%) | Alpha-cellulose content (%) | Brightness (ISO) | Viscosity (mPa·s) | Fock reactivity (%) |
|---|---|---|---|---|---|
| Control | 3.25±0.06 | 92.31±0.03 | 86.2±0.1 | 6.83±0.01 | 21.60±0.90 |
| Xylanase Only | 2.92±0.08 | 92.54±0.04 | 86.7±0.1 | 6.18±0.02 | 31.77±0.24 |
| Refining & Xylanase | 2.38±0.03 | 93.40±0.01 | 87.2±0.1 | 6.10±0.03 | 32.42±0.45 |

Note: PFI refining of 9000 revolutions.

### 4.2.6. Proposed process for upgrading dissolving pulp from bamboo

Based on our findings, we propose a process for the production of high grade dissolving pulp resulted from oxygen-delignified bamboo pre-hydrolysis kraft pulp (Fig. 4.5). In the proposed process, the oxygen-delignified pulp is subjected to a sequential treatment process of mechanical refining (9000 PFI revolutions) and xylanase treatment (1500 mL/tonne of O.D. pulp) to open up the fibre structure and remove xylan. The treated pulp is bleached with an ECF bleaching sequence (D-EP-D) to manufacture final dissolving

pulp product. According to the proposed process flow, no additional chemicals except for xylanase are required, so the integration of these mechanical refining and xylanase treatment stages could be achieved without major capital investment.

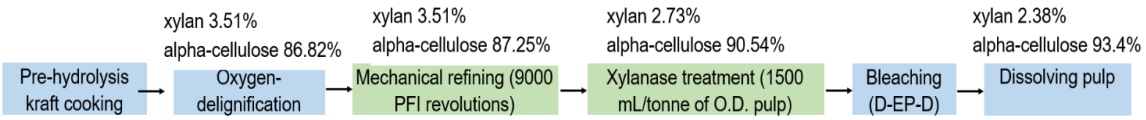


**Fig. 4.5. Proposed process for upgrading bamboo dissolving pulp by mechanical refining and xylanase treatment.**

## 4.3. Conclusions

The feasibility of upgrading oxygen-delignified bamboo kraft pulp by PFI refining and xylanase treatment was investigated. Refining improved enzymatic xylan removal with a concomitant increase in the alpha-cellulose content. Brightness and Fock's reactivity of bamboo dissolving pulp were also increased after combined mechanical refining and xylanase treatment. Experiments conducted to investigate the underlying mechanism of the impact of refining provided evidence that fibrillation and disruption of fiber by mechanical force give xylanase improved access to xylan in bamboo pulp. Furthermore, the changes brought about by refining-xylanase treatment increase the accessibility of residual lignin to bleaching chemicals and facilitate the diffusion of reaction products out of the fibres. Collectively our results indicate that refining combined with xylanase treatment is a promising approach for the production of high-grade bamboo dissolving pulp.

# Chapter 5: Conclusions and recommendations for future work

## 5.1 Conclusions

This study set out to utilize xylanase for hemicellulose removal from bamboo pulp and to improve efficiency of the xylanase treatments for application in the production of bamboo dissolving pulp. Efforts have been made to find the answers to a number of key questions including 1) Which commercial xylanase is most suitable for removing hemicellulose for the production of bamboo dissolving pulp and what are the appropriate treatment conditions? 2) What are effects of mechanical refining on xylanase treatment of bamboo pulp? 3) How does mechanical refining improve xylanase treatment? 4) What are the effects of combined mechanical refining/xylanase treatment on the final quality of bamboo dissolving pulp?

In an evaluation of two commercially available xylanase products Iogen DP-407 xylanase was found to be most effective to removing xylan from bamboo pulp. It should be utilized at its Appropriate conditions for the xylanase treatment for the production of bamboo dissolving pulp were determined to be, a temperature of 70 °C, pH 5, xylanase dosage of 1500 mL/tonne of O.D. pulp, pulp consistency of10% and a reaction time of 3 hours. The effect of combining PFI refining with DP-407 xylanase treatment on xylan removal from oxygen-delignified bamboo kraft pulp was investigated. Refining improved enzymatic xylan removal by providing mechanical forces that fibrillated and delaminated the fiber, thereby, improving access of xylanase to the xylan in the bamboo pulp. The alpha-

cellulose content, brightness and Fock's reactivity of bamboo dissolving pulp were also increased after combined mechanical refining and xylanase treatment. Consequently, refining combined with xylanase treatment is a promising approach for the production of high-grade bamboo dissolving pulp.

## 5.2 Recommendations for future work

The study was conducted using polyethylene bag in water bath as reactor. The refined pulp and xylanase were mixed by hand kneading for consistent time periods. It could be more appropriate to use a reactor where the xylanase could be added during pulp refining, and then the reaction continued in the same vessel with stirring and temperature control. After a series of experiments in this reactor, a kinetic model to simulate xylan removal by xylanase treatment with mechanical refining from bamboo pulp could be built. As a consequence of using this methodology, the laboratory results would be more relevant to a practical industrial process.

A means of increasing in reactivity of bamboo in the dissolving pulp process would also be worthwhile exploring. Perhaps an endoglucanase post-treatment would aid in achieving this goal.

# References


Andrade, C.M., Aguiar, W.B., Antranikian, G. 2001. Physiological aspects involved in production of xylanolytic enzymes by deepsea hyperthermophilic archaeon Pyrodictium abyssi. Appl. Biochem. Biotechnol. 91-93, 655-669.

Bäckström, M., Kolar, M.C., Htun, M. 2008. Characterisation of fines from unbleached kraft pulps and their impact on sheet properties. Holzforschung. 62, 546–552.

Bailey, M.J., Biely, P., Poutanen, K. 1992. Interlaboratory testing of methods for assay of xylanase activity. J. Biotechnol. 23, 257-270.

Bajpai, P., Bajpai, P.K. 2001. Development of a process of the production of dissolving kraft pulp using xylanase enzyme. Appita J. 54, 381–384.

Batalha, L.A.R., Colodette, J.L., Gomide, J.L., Barbosa, L.C.A., Maltha, C.R.A., Gomes, F.J.B. 2012. Dissolving pulp production from Bamboo. Bioresour. 7, 640–651.

Beg, Q.K., Kapoor, M., Mahajan, L., Hoondal, G.S. 2001. Microbial xylanases and their industrial applications: a review. Appl. Microbiol. Biotechnol. 56, 326-338.

Behin, J., Mikaniki, M., Fadaei, Z. 2008. Dissolving pulp (alpha-cellulose) from corn stalk by kraft process. Iranian J. Chem. Eng. 5, 14-28.

Behin, J., Zeyghami, M. 2009. Dissolving pulp from corn stalk residue and waste water of Merox unit. Chem. Eng. J. 152, 26-35.

Bhat, M.K. 2000. Celluloses and related enzymes in biotechnology. Biotechnol. Adv. 18, 355-383.

Borghei-Ghomi, M. 2001. Application of immobilized xylanase in pulp biobleaching. Thesis for degree of master of applied science. Department of chemical engineering and applied chemistry in University of Toronto.

Campbell, N.A., Reece, J.B. 2005. Biology. 7th ed. San Francisco.

Chen, H. 2006. Ecological high value-added theory and application of crop straws. Chemical Industry Press, Beijing, China.

Christove, L.P., Prior, B.A. 1993. Xylan removal from dissolving pulp using enzymes of Aureobasidium Pullulas. Biotechnol. Lett. 15, 1269–1274.

Christove, L.P., Akhtar, M., Prior, B.A. 1995. Proceedings of the 6th International Conference on Biotechnology in the Pulp and Paper Industry: Recent advances in applied and fundamental research, Vienna, Austria, June 11-15. pp. 625-628.

Christove, L.P., Prior, B.A. 1996. Repeated treatments with Aureobasidium pullulans hemicellulases and alkali enhance biobleaching of sulphite pulps. Enzyme. Microb. Technol. 18, 244–250.

Christov, L.P., Biely, P., Kalogeris, E., Christakopoulos, P., Prior, B.A., Bhat, M.K. 2000. Effects of purified endo-β-1,4-xylanase of family 10 and 11 and acetyl xylan esterases on eucalypt sulfite dissolving pulp. J. Biotechnol. 83, 231–244.

Collins, T., Gerday, C., Feller, G. 2005. Xylanases, xylanase families and extremophilic xylanases. FEMS Microb. Rev. 29, 3-23.

Colodette, J.L., Loungue, Jr., D., Pedrazzi, C., Oliveira, R.C., Gomide, J.L., Gomes, F.J.B. 2011. Pulpability and bleachability of xylan-depleted eucalyptus wood chips. Ind. Eng. Chem. Res. 50, 1847–1852.

Coutinho, P.M., Henrissat, B. 1999. Carbohydrate-active enzyme server (CAZY) at URL: http://afmb.cnrs-mrs.fr/~cazy/CAZY.

Daneault, C., Leduc, C., Valade, J.L. 1994. The use of xylanase in kraft pulp bleaching: a review. Tappi J. 77, 6.

Dence, C.W. 1992. The determination of lignin. In: Dence, C.W. (Eds), Methods in Lignin Chemistry. Springer-Verlag, Berlin. pp. 33–61.

Ding, T., Zhou, J., Wan, D., Chen, Z., Wang, C., Zhang, F. 2008. Silicon isotope fractionation in bamboo and its significance to the biogeochemical cycle of silicon. Geochimica et Cosmochimica Acta. 72, 1381–1395.

Du, X., Pérez-Boada, M., Fernández, C., Rencoret, J., Río, J.C., Jiménez-Barbero, J., Li, J., Gutiérrez, A., Martínez, A.T. 2014. Analysis of lignin–carbohydrate and lignin–lignin linkages after hydrolase treatment of xylan–lignin, glucomannan–lignin and glucan–lignin complexes from spruce wood. Planta. 239, 1079-1090.

Engström, A.C., Ek, M., Henriksson, G. 2006. Improved accessibility and reactivity of dissolving pulp for the viscose process: pretreatment with monocomponent endoglucanase. Biomacromolecules 7, 2027-2031.

Funaki, Y., Ueda, K., Saka, S., Soejima, S. 1993. Characterization of cellulose-acetate in acetone solution-studies on prehump-II in GPC pattern. J. Appl. Polym. Sci. 48, 419-424.

Gehmayr, V., Schild, G., Sixta, H. 2011. A precise study on the feasibility of enzyme treatments of a kraft pulp for viscose application. Cellulose 18, 479–491.

Gehmayr, V., Sixta, H. 2012. Pulp properties and their influence on enzymatic degradability. Biomacromolecules 13, 645-651.

Gindl, W. 2002. Comparing mechanical properties of normal and compression wood in Norway spruce: The role of lignin in compression parallel to the grain. Holzforschung 56, 395-401.

Grönqvist, S., Hakala, T.K., Kamppuri, T., Vehviläinen, M., Hänninen, T., Liitiä, T., Suurnäkki, A. 2014. Fibre porosity development of dissolving pulp during mechnanical and enzymatic processing. Cellulose 21, 3667–3676.

Gübitz, G.M., Lischnig, T., Stebbing, D., Saddler, J.N. 1997. Enzymatic removal of hemicellulose from dissolving pulps. Biotechnol. Lett. 19, 491–495.

Gupta, V.K., Sharma, G.D., Tuohy, M.G., Gaur, R. 2016. The handbook of microbial bioresourses. CABI, Wallingford, England.

Hakala, T.K., Liitiä, T., Suurnäkki, A. 2013. Enzyme-aided alkaline extraction of oligosaccharides and polymeric xylan from hardwood kraft pulp. Carbohydr. Polym. 93, 102-108.

Havukainen, R., Torronen, A., Laitinen, T., Rouvinen, J. 1996. Covalent binding of three expoxyalkyl xylosides to the active site of endo-1,4-xylanase II from Trichoderma reesei. Biochem. 35, 9617-9624.

He, J., Cui, S., Wang, S. 2008. Preparation and crystalline analysis of high-grade bamboo dissolving pulp for cellulose acetate. J. Appl. Polym. Sci. 107, 1029-1038

He, J., Zhang, M., Cui, S., Wang, Y. 2009. High-quality cellulose triacetate prepared from bamboo dissolving pulp. J. Appl. Polym. Sci. 113, 456–465.

Henriksson, G., Christiernin, M., Agnemo, R. 2005. Monocomponent endoglucananse treatment increases the reactivity of softwood sulphite dissolving pulp. J. Ind. Microb. Biotechnol. 32, 211-214.

Hiett, L.A. 1985. Dissolving cellulose: its present position and prospects for future development. Tappi J. 68, 42–48.

Hinck, J.F., Casebier, R.L., Hamilton, J.K. 1985. Dissolving Pulp manufacture. In: Kocurek, M.J., Ingruber, O.V., Al-Wong, P.E. (Eds.), Sulfite science & technology, 3rd ed. TAPPI, CPPA, Atlanta. pp. 213-243.

Ibarra, D., Köpcke, V., Larsson, P.T., Jaaskelainen, A-S, Ek, M. 2010. Combination of alkaline and enzymatic treatments as a process for upgrading sisal paper-grade pulp to dissolving-grade pulp. Bioresour. Technol. 101, 7416-7423.

Ihsanawati, K.T., Kaneko, T., Morokuma, C., Yatsunami, R., Sato, T., Nakamura, S., Tanaka, N. 2005. Structural basis of the substrate subsite and the highly thermal stability of xylanase 10B from Thermotoga maritima MSB8. Proteins. 61, 999-1009.

Jackson, L.S., Heitmann Jr., J.A., Joyce, T.W. 1998. Production of dissolving pulp from recovered paper using enzymes. Tappi J. 81, 171-178.

Jeffries, T.W., Lins, C.W. 1990. In Biotechnology in Pulp and Paper Manufacture, Kirk, T.K., Chang, H.M., (Eds). Butterworth-Heinemann, Boston, MA. pp. 191-202.

Kantelinen, A., Hortling, B., Sundquist, J., Linko, M., Viikari, L. 1993. Proposed mechanism of the enzymatic bleaching of kraft pulp with xylanase. Holzforschung 47, 318-324.

Kapu, N.S., Trajano, H.L. 2014. Review of hemicellulose hydrolysis in softwoods and bamboo. Biofuels. Bioprod. Bioref. 8, 857–870.

Khristova, P., Kordsachia, O., Patt, R., Karar, I., Khider, T. 2006. Environmentally friendly pulping and bleaching of bagasse. Ind. Crops Prod. 23, 131–139.

Köpcke, V., Ibarra, D., Ek, M. 2008. Increasing accessibility and reactivity of paper grade pulp by enzymatic treatment for use as dissolving pulp. Nordic Pulp Pap. Res. J. 23, 363–368.

Köpcke, V., Ibarra, D., Larsson, PT., Ek, M. 2010. Optimization of treatment sequences for the production of dissolving pulp from birch kraft pulp. Nordic. Pulp. Pap. Res. J. 25, 31–38.

Krässig, H.A. 1984. Struktur und Reaktivität von Cellulosefasern. Das. Papier 38, 571-582.

Krässig, H.A. 1993. Cellulose: structure, accessibility, and reactivity. Polymer Monographs, Vol 11. Gordon and Breach Science, Yverdon, Switzerland, Philadelphia, pp. 47–95.

Kulkarni, N., Shendye, A., Rao, M. 1999. Molecular and biotechnological aspects of xylanases. FEMS Microbiol. Rev. 23, 411-456.

Le Costaouec, T., Pakarinen, A., Varnai, A., Puranen, T., Viikari, L. 2013. The role of carbohydrate binding module (CBM) at high substrate consistency: comparison of Trichoderma reesei and Thermoascus aurantiacus Cel7A (CBHI) and Cel5A (EGII). Bioresour. Technol. 143, 196-203.

Li, H., Kankaanpää, A., Xiong, H., Hummel, M., Sixta, H., Ojamo, H., Turunen, O. 2013. Thermostabilization of extremophilic Dictyoglomus thermophilum GH11 xylanase by an N-terminal disulfide bridge and the effect of ionic liquid [EMIM]OAc on the enzymatic performance. Enzyme Microb. Technol. 53, 414-419.

Li, J., Zhang, H., Duan, C., Liu, Y. Ni, Y. 2015. Enhancing hemicelluloses removal from a softwood sulfite pulp. Bioresour. Technol. 192, 11–16.

Lian, H., You, J., Lian, Z. 2012a. Effect of prior mechanical refining on biobleaching of wheat straw pulp with laccase/xylanase treatment. Bioresour. 7, 3113–3124.

Lian, H., You, J., Huang, Y., Li, Z. 2012b. Effect of refining on delignification with laccase/xylanase treatment. Bioresour. 7, 5268–5278.

Liu, H., Zhu, J., Chai, X. 2011. In situ, rapid, and temporally resolved measurements of cellulase adsorption onto lignocellulosic substrates by UV-vis spectrophotometry. Langmuir 27, 272-278.

Liu, Y., Liu, Y., Wang, Z., Peng, J. 2013. Alkaline hydrolysis kinetics modeling of bagasse pentosane dissolution. Bioresour. 9, 445-454.

Liu, Z., Fatehi, P., Jahan, M.S., Ni, Y. 2010. Separation of lignocellulosic materials by combined processes of pre-hydrolysis and ethanol extraction. Bioresour. Technol. 102, 1264-1269.

Ma, X., Huang, L., Chen, Y., Chen, L. 2011. Preparation of bamboo dissolving pulp for textile production: Part 1. Study on pre-hydrolysis of green bamboo for producing dissolving pulp. Bioresour. 6, 1428-1439.

Miao, Q., Chen, L., Huang, L., Tian, C., Zheng, L., Ni, Y. 2014. A process for enhancing the accessibility and reactivity of hardwood kraft-based dissolving pulp for viscose rayon production by cellulose treatment. Bioresour. Technol. 154, 109-113.

Mou, H., Li B., Heikkila, E., Iamazaki, E., Zhan, H., Fardim, P. 2013. Low consistency refining of eucalyptus pulp: effects on surface chemistry and interaction with FWAs. Bioresour. 8, 5995-6013.

Nurizzo, D., Turkenburg, J.P., Charnock, S.J., Roberts, S.M., Dodson, E.J., McKie, V.A., Taylor, E.J., Gilbert, H.J., Davies, G.J. 2002. Cellvibrio japonicas alpha-L-arabinanase 43A has a novel five-blade beta-propeller fold. Nat. Struct. Biol. 9, 665-668.

Okubo, K., Fujii, T., Yamamoto, Y. 2004. Development of bamboo-based polymer composites and their mechanical properties. Composites: Part A. 35, 377-383.

Östberg, L., Hakansson, H., Germgard, U. 2012. Some aspects of the reactivity of pulp intended for high-viscosity viscose. Bioresour. 7, 743-755.

Paice, M.G., Jurasek, L. 1984. J. Wood Chem. Technol. 4, 187-198.

Prade, R.A. 1995. Xylanases: from biology to biotechnology. Biotech. Genet. Eng. Rev. 13, 100-131.

Qing, Q., Wyman, C.E. 2011. Supplementation with xylanase and β-xylosidase to reduce xylo-oligomer and xylan inhibition of enzymatic hydrolysis of cellulose and pretreated corn stover. Biotechnol. Biofuels. 4, 18-29.

Rahkamo, L., Viikari, L, Buchert, J., Paakkari, T., Suortti, T. 1998. Enzymatic and alkaline treatments of hardwood dissolving pulp. Cellulose 5, 79-88.

Roberts, J.C., McCarthy, A.J., Flynn, N.J., Broda, P. 1990. Enzyme Micob. Technol. 12, 210-213.

Roncero, M.B., Torres, A.L., Colom, J.F., Vidal, T. 2005. The effect of xylanase on lignocellulosic components during the bleaching of wood pulps. Bioresour. Technol. 96, 21-30.

Rydholm, S.A. 1965. Pulping Processes. Interscience Publishers, John Wiley & Sons, New York, pp. 3-90.

Rye, C.S., Withers, S.G. 2000. Glycosidase mechanisms. Curr. Opin. Chem. Biol. 4, 573-580.

Saeed, A., Jahan, M.S., Li, H., Liu, Z., Ni, Y., Van Heiningen, A.P.R. 2009. International Biorefinery Conference: Mass balance of hemicelluloses and other components in the prehydrolysis kraft-based dissolving pulp production process. Syracuse, New York.

Saka, S. 2004. Wood as natural raw materials for cellulose acetate production. Macromol. Symp. 208, 7-28.

Salmela, M., Alén, R., Vu, M.T.H. 2008. Description of Kraft cooking and oxygen–alkali delignification of bamboo by pulp and dissolving material analysis. Ind. Crops Prod. 28, 47-55.

Santiago, A.S., Pascoal Neto, C. 2007. Review. Assessment of potential approaches to improve Eucalyptus globulus kraft pulping yield. J. Chem. Technol. Biotechnol. 82, 424-430

Schild, G., Sixta, H. 2011. Sulfur-free dissolving pulps and their application for viscose and lyocell. Cellulose. 18, 1113-1128.

Senior, D.J., Mayers, P.R., Miller, D., Sutcliffe, R., Tan, L., Saddler, J.N. 1988. Biotechnol. Lett. 10, 907-912.

Shallom, D., Shoham, Y. 2003. Microbial hemicellulases. Curr. Opin. Microbiol. 6, 219-228.

Sixta, H. 2006. Handbook of pulp. Wiley-VCH, Weinheim, pp. 325-365 and 1009-1067.

Sixta, H., Lakovlev, M., Testova, L., Roselli, A., Hummel, M., Borrega, M., Schottenberger, H. 2013. Novel concepts of dissolving pulp production. Cellulose 20, 1547-1561.

Sluiter, J.B., Ruiz, R.O., Scarlata, C.J., Sluiter, A.D., Templeton, D.W. 2010. Compositional anaylsis of lignocellulosic feedstocks. 1. Review and description of methods. 58, 9043-9053.

Sridhar, G., Subbukrishna, D.N., Sridhar, H.V., Dasappa, S., Paul, P.J., Mukunda, H.S. 2007. 15th European Biomass Conference & Exhibition: Torrefaction of bamboo. Berlin, Germany, May. pp. 7-11.

Tian, C., Zheng, L., Miao, Q., Cao, C., Ni, Y. 2014. Improving the reactivity of kraft-based dissolving pulp for viscose rayon production by mechanical treatments. Cellulose 21, 3647-3654.

Torres, C.E., Negro, C., Fuente, E., Blanco, A. 2012. Enzymatic approaches in paper industry for pulp refining and biofilm control. Appl. Microbiol. Biotechnol. 96, 327-344.

Tunc, M.S., Van Heiningen, A.R.P. 2008. Hemicellulose extraction of mixed southern hardwood with water at 150 °C: effect of time. Ind. Eng. Chem. Res. 47, 7031-7037.

Várnai, A., Siika-aho, M., Viikari, L. 2013. Carbohydrate-binding modules (CBMs) revisited: reduced amount of water counterbalances the need for CBMs. Biotechnol. Biofuels 6, 1-12.

Vu Mân, T.H., Pakkanen, H., Alén, R. 2004. Delignification of bamboo (Bambusa procera acher). Ind. Crops Prod. 19, 49-57.

Wang, Q., Liu, S., Yang, G., Chen, J., Ni, Y. 2015a. Cationic polyacrylamide enhancing cellulose treatment efficiency of hardwood kraft-based dissolving pulp. Bioresour. Technol. 183, 42-46.

Wang, Q., Liu, S., Yang, G., Chen, J., Ni, Y. 2015b. High consistency cellulose treatment of hardwood prehydrolysis kraft based dissolving pulp. Bioresour. Technol. 189, 413-416.

Wilson, J.D., Tabke, R.S. 1974. Influences of hemicelluloses on acetate processing in high catalyst systems. Tappi J. 57, 77-80.

Yang, Z., Xu, S., Ma, X., Wang, S. 2008. Characterization and acetylation behavior of bamboo pulp. Wood Sci. Technol. 42, 621-632.

Yasinok, A.E., Biran, S., Kocabas, A., Bakir, U. 2010. Xylanase from a soil isolate, Bacillus pumilus: gene isolation, enzyme production, purification, characterization and one-step separation by aqueous-two-phase system. World J. Microb. Biotechnol. 26, 1641-1652.

Yoshida, S., Kuno, A., Saito, N., Aoyama, M., and Kusakabe, I. 1998. Structure of xylan from culms of bamboo grass (Sasa senanensis Rehd.). J. Wood Sci. 44, 457-462.

You, J., Wang, Y. 2009a. Study on surface characteristics of the Masson pine pulp enzymelyzed with three laccase systems. Trans. China Pulp Pap. 24, 27-41.

You, J., Wang, Y., Tong, G., and Ou, Y. 2009b. Comparison between laccase/xylanase system from white rot fungus and composite laccase/xylanase in delignification ability. China Pulp Pap. 28, 1-5.

You, T., Zhang, L., Zhou, S., Xu, F. 2015. Structural elucidation of lignin-carbohydrate complex (LCC) preparations and lignin from Arundo donax Linn. Ind. Crops Prod. 71, 64-74.

Zhang, N., Zhang, M., Xia, X. 2013. A morphology study on the behavior of bamboo pulp fibers in the PFI refining process. Paper Sci. Technol. 32, 4-7.

Zhang, X., Qin, W., Paice, M.G., Saddler, J.N. 2009. High consistency enzymatic hydrolysis of hardwood substrates. Bioresour. Technol. 100, 5890-5897.

# Appendix A. Xylose standard calibration curve

For conversion of xylose concentration from the measured absorbance values, a standard calibration curve was plotted using results from the xylanase activity assay described in section 3.1.2. Fig. A.1. show that the calibration rate is linear (Y=0.0327X-0.0139), with a regression coefficient ($R^2$) of 0.99 for the average results of two replicated experiments.

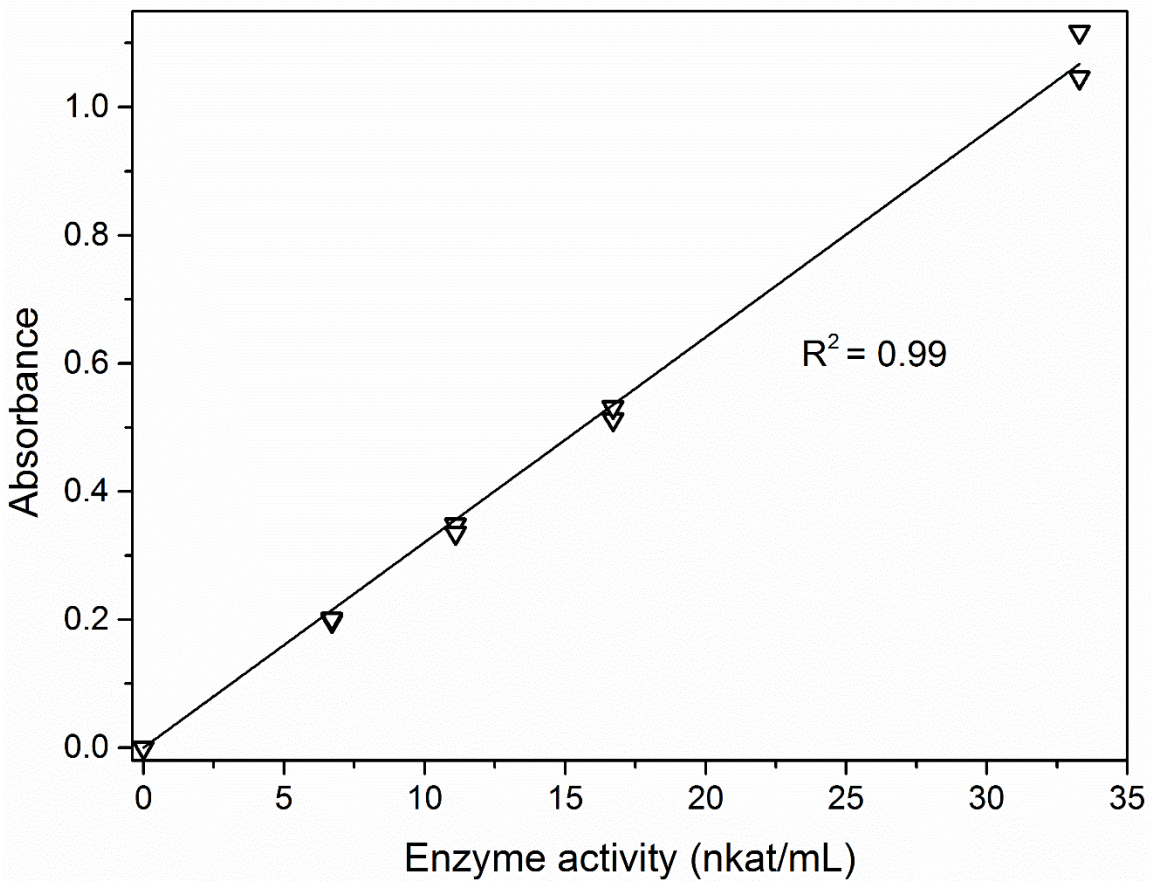


Fig. A.1. Xylose standard calibration curve.