Development of Dissolving Pulp from *Phyllostachys pubescens* Stem by Prehydrolysis Soda Cooking with 2-Methylanthraquinone

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CHEM Mouylin

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A Dissertation Submitted to
The Graduate School of Life and Environmental Sciences,
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in Partial Fulfillment of the Requirements
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CHEM Mouylin

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List of Abbreviations

2-MAQ	*2-methylanthraquinone*
AA	*Active alkali*
CCE	*Cold Caustic Extraction*
DP	*Degree of Polymerization*
ECF	*Elemental Chloring-free*
IARC	*International Agency for Research on Cancer*
IC	*Ion Chromatograph*
S_a	*Syringaldehyde*
SAQ	*Soluble Anthraquinone*
V_a	*Vanillin*

Chapter 1. Introduction

1.1 Research background

Bamboo grows mostly in the tropics. It is also found in the subtropical and temperate zones of all continents excluding Europe. Further, it belongs to a group of large woody grasses. There are 1250 species within the 75 genera of bamboo, and most grow rapidly, attaining stand maturity in five years (Scurlock et al., 2000; Sugesty et al., 2015). Bamboo is grown in India, Myanmar, Thailand, Indonesia, China, Japan, the Philippines, Australia, South Africa, South America, and southern North America. Bamboo has many utilities in Asian countries, such as in manufacturing writing instruments, furniture, building materials, weaving materials, household utensils, and agricultural tools. In addition, bamboo is a raw material used in the making of paper, musical instruments, religious articles, medicine, weapons, and other items (Yunnan et al., 1996). *Phyllostachys pubescens* is originally from China, and its plantations cover an area of 20,000 km^2, which accounts for 60% of the total area covered by bamboo in China. It was introduced to Japan around the 1750s. The species has been used as a source of both poles and edible shoots in South-East Asia, and the optimal temperature for its growth is approximately 13–20°C (Qiu et al., 1992).

Bamboo is a non-wood raw material alternative (long fiber) for producing dissolving pulp and is available in large quantities and diverse types (Sugesty et al., 2015). Cellulose, hemicellulose, and lignin are the main components of plant biomass. Cellulose is composed of D-glucose units bond by β-1,4,-glucosidic bonds. Cellulose with a degree of polymerization $\geq 10{,}000$ is arranged in a microfibril structure. The hydrogen bonds in the cellulose structure provide tensile strength, are insoluble in most solvents, and are resistant to microbial degradation (Jørgensen et al., 2007). Hemicelluloses are heterogenous polymers that form different polymers of pentoses, including xylose and arabinose; hexoses, including glucose, mannose, and galactose; and sugar acids. Hemicelluloses can form side chains and exhibit some degree of acetylation when the degree of polymerization is < 200 (Jørgensen et al., 2007; Lau, 2012; Saha, 2003). Lignin is a large molecular structure composed of cross-linked polymers of phenolic monomers, including coniferyl alcohol, coumaryl alcohol, and sinapyl alcohol (Jørgensen et al., 2007). The largest component of the plant cell wall is cellulose, accounting for 35–50 wt% of plant dry mass, with hemicellulose accounting for 20–25 wt% and lignin for 10–25 wt% (Saha, 2003). As **Figure 1-1** shows, all lignocellulosic materials contain three main components: cellulose, hemicellulose, and lignin. However, the processes of extracting the components vary, which is taken into account in the type of cooking reagents adopted.

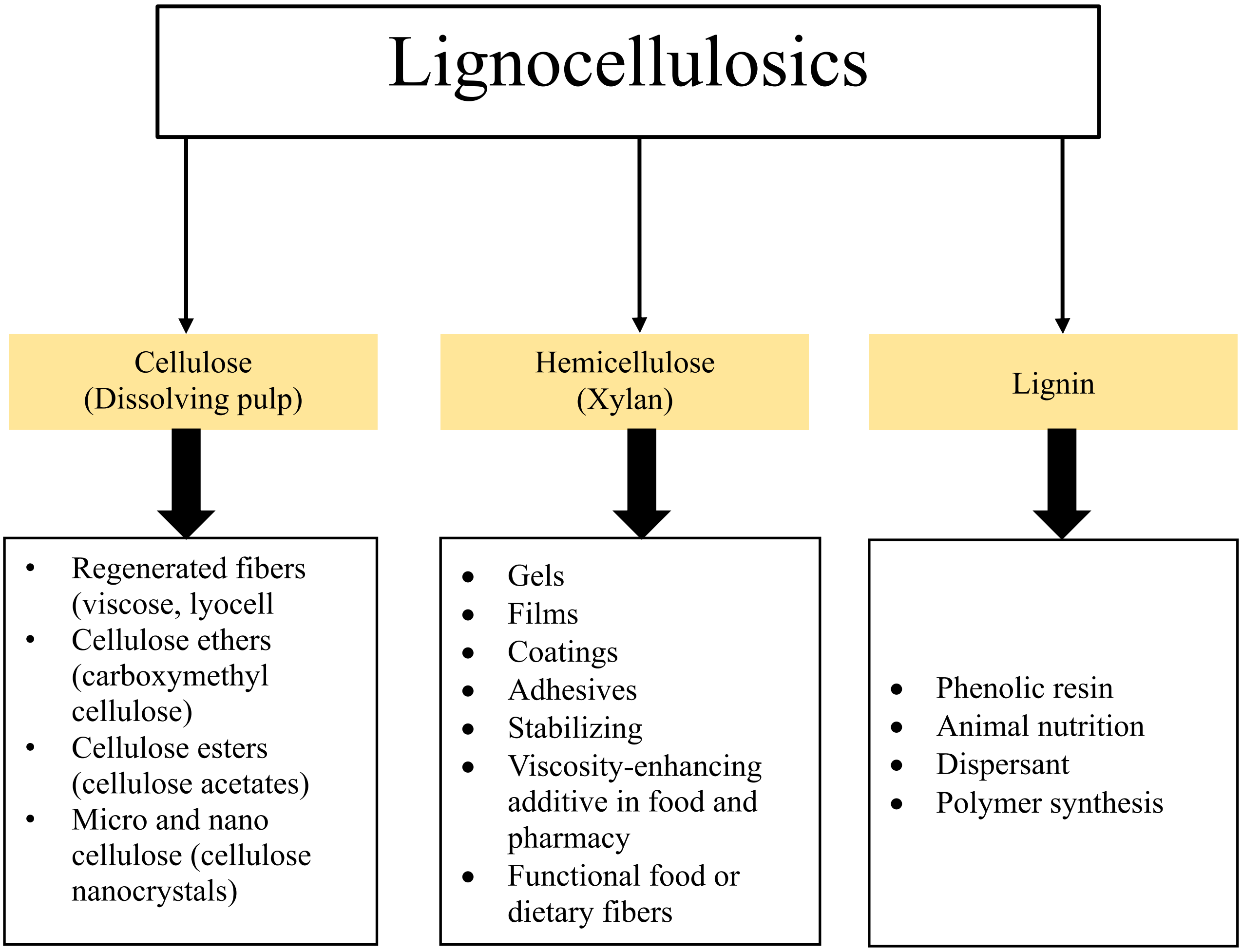


Figure 1-1 Lignocellulosic materials composed of cellulose, hemicelluloses, and lignin (Kumar & Christopher, 2017; Baurhoo et al., 2008; Nadif et al., 2002; Tejado et al., 2007; Bian et al., 2013; Garrote et al., 2008)

In cooking processes of lignocelluloses, cooking can be divided into two main types, as follows: Group I: refers to cooking processes where residue remains in the form of lignin and cellulose (e.g., autohydrolysis, weak acid hydrolysis, and concentrated acid or water-free acid hydrolysis) and Group II: residue remains in the forms of cellulose and cellulose/hemicellulose (e.g., kraft cooking, sulfite cooking, organosolv process, extraction with water, and alkaline cooking) (Kamm et al., 2008).

Dissolving pulp is obtained by chemical processes, and the bleached pulp should be composed of > 90% pure cellulose. Dissolving pulp has many applications, including production of regenerated fibers (viscose, lyocell), cellulose ethers (carboxymethyl cellulose, methyl cellulose, ethyl cellulose, hydroxy ethyl cellulose, and cyanoethyl cellulose), cellulose esters (acetates, propionates, butyrates, and nitrates), micro and nano cellulose (microfibrillated cellulose, microcrystalline cellulose, crystalline nano cellulose, and cellulose nanocrystals), and other materials (cellulose composites, superabsorbent polymers, ion exchange resin, cellulose filters) (Kumar & Christopher, 2017). High-quality dissolving pulp must have high-purity α-cellulose (> 94%), high brightness (> 88%), high viscosity (> 6.2 mPa.s), low ash content (0.15%), low hemicellulose content (2–-4%), and low lignin content (< 0.05%) (Sixta et al., 2006, Sugesty et al., 2015). The raw materials and the cooking process are key factors influencing dissolving pulp quality. Recently, demand for dissolving pulp has increased; similarly, demand for long fiber wood, such as *Pinus merkusii*, and short fiber wood, such as *Eucalyptus* sp., has increased, as they are used for the production of dissolving pulp. However, wood scarcity and environmental issues pose challenges for dissolving pulp production. Bamboo offers good quality fiber for paper making. The heating value of bamboo is lower than the values for many woody species, although it is higher than those of most other agricultural residues, such as grasses and straw (Scurlock et al., 2000).

Autohydrolysis technologies are water-based and steam-based processes. Prehydrolysis has numerous applications, such as cooking process or fractionation (to remove hemicelluloses with limited cellulose degradation and splitting of the α and β aryl ether bonds of lignin), defibration for fiberboard production (using high pressure steam), and the enzymatic hydrolysis of cellulose (Garrote et al., 1999). The basic prehydrolysis steps can be described as follows: (1) heating transfer to the wood tissue; (2) deprotonation of acids or deacetylation and subsequent release of hydrogen ions related to acetic acid, leading to decreased pH; (3) mass transport of hydrogen ions in cells, followed by solubilization of reaction products; (4) mass transport of hydrogen ions and solubilized reaction products out of the cell wall; and (5) further degradation of the dissolved wood constituents in the surrounding liquid (Wojtasz-Mucha et al., 2021). Since prehydrolysis uses only water, the treatment is considered to minimize corrosion, and no sludge is generated. In addition, the cellulose is not easily degraded. Prehydrolysis has several effects on lignocellulosics, such as hemicellulose depolymerization to produce sugar oligomers, hemicellulose sugars, as well as degradation products, and changes in the physicochemical characteristics of cellulose and lignin, which facilitates delignification with alkaline solutions or organo-solvents (Garrote et al., 1999).

In the prehydrolysis process, the depolymerization of hemicellulose occurs, yielding a variety of xylooligomer products. Xylooligomers are a byproduct of prehydrolysis treatment, and they are attractive since their functional ingredients have the potential to promote human health. For example, xylooligomers can stimulate the growth of beneficial gut bacteria and improve the robustness of the immune system (Bian et al., 2013; Garrote et al., 2008). Due to the considerable growth in demand for functional food and feed in markets, the demand of high-quality xylooligomers has increased. (Jain et al., 2015; Lai et al., 2019; Moure et al., 2006). **Figure 1-2** shows the effect of prehydrolysis on the fractionation of xylan-rich by-products, resulting in soluble and degraded xylan-rich fractions, cellulose, and lignin residue. Cellulose is continuously processed for the production of dissolving pulp, whereas lignin can be isolated from black liquor after cooking processes.

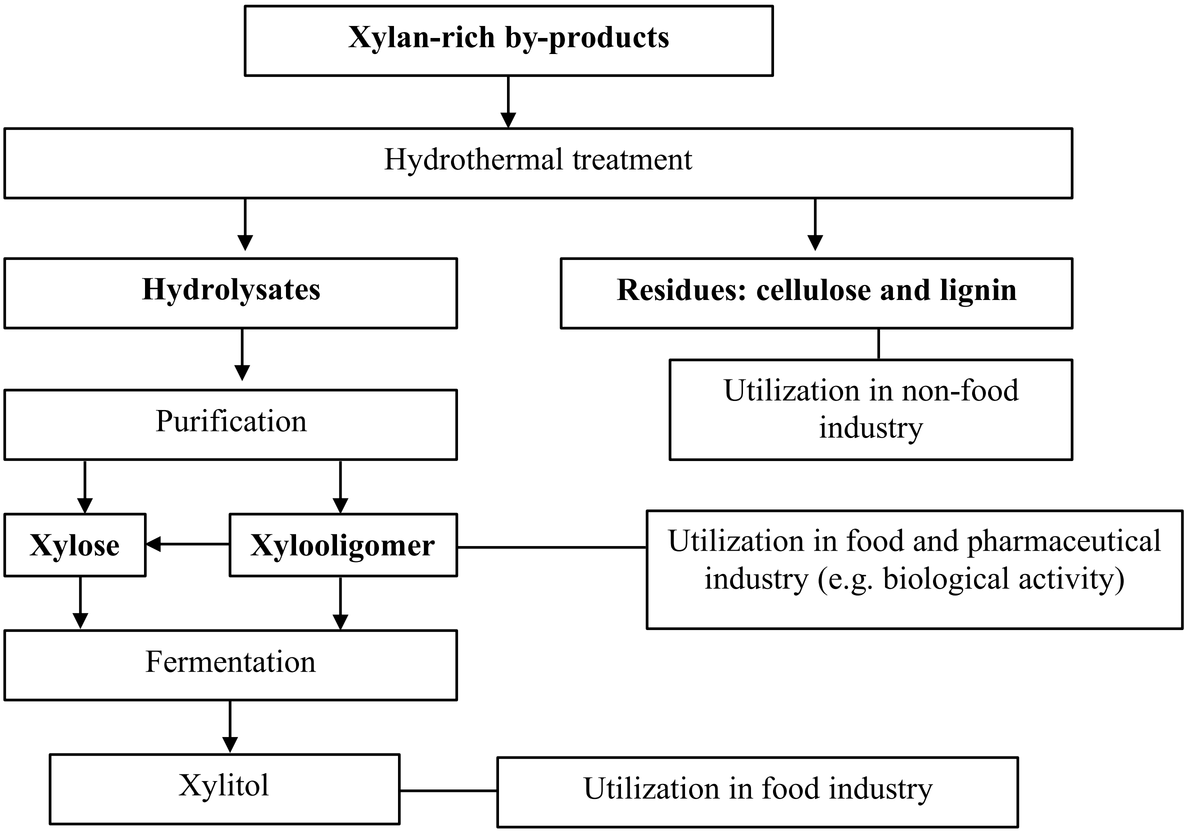


Figure 1-2 Effect of prehydrolysis on the fractionation of xylan-rich by-products resulting in soluble and degraded xylan-rich fraction, cellulose, and lignin residue (Kabel, 2002).

Lignin is the most abundant non-carbohydrate component of natural substances and the largest source of aromatics on earth. During paper production in modern pulp mills, almost all the lignin extracted from lignocellulosic materials is burnt to generate energy and recover chemicals (Sameni et al., 2014). Compared to the use of carbohydrate components, such as cellulose and hemicellulose, lignin exploitation is still challenging because of its natural rigidity, heterogeneity, and complexity (Yoo & Ragauskas, 2021).

With advancements in biomass conversion technologies, various types of lignin have been produced, as showed in **Figure 1-3**. Kraft lignin, soda lignin, and lignosulfonate have been produced in the traditional pulp and paper industries. Kraft lignin is type of lignin isolated from black liquor after the cooking process (Froass et al., 1998). Lignosulfonate is a common lignin obtained from pulp and paper manufacturing processes by sulfite cooking with calcium, ammonium, sodium, or magnesium and contains high amounts of sulfur (3.5–8.0%) (Patil et al., 2020). Organosolv lignin (e.g., ethanol organosolv lignin) is isolated from spent liquor by dilution with water containing partially hydrolyzed hemicellulose, furans, and soluble lignin. Afterward, organic solvent is recovered and recycled form the spent liquor (Vaidya et al., 2022). Soda lignin is recovered from sulfur-free cooking processes using sodium hydroxide (Mousavioun & Doherty, 2010). Black liquor can be further fractionated into valuable products. And soda-anthraquinone (SAQ) is the preferable AQ for use in delignification cooking processes, as an additive to decrease carbohydrate degradation as shown in **Figure 1-4** (Takahashi et al., 2011). Further, it is favored due to the absence of sulfur compounds in cooking liquor, which enables the recovery of sulfur-free raw lignin product. The separation of lignin is one alternative process of improve the efficiency of cooking processes and addressing associated environmental challenges (Ghatak, 2008). Soda lignin can be used for the production of phenolic resin, animal nutrition, and dispersants (Baurhoo et al., 2008; Nadif et al., 2002; Tejado et al., 2007). Besides, soda lignin can be used to synthesize polymers and low-molecular weight substances (Wörmeyer et al., 2011). In addition, the soda lignin extraction process is considered sulfur-free, and the obtained chemical components are similar to those of natural lignin, as opposed to those of kraft lignin and lignosulphonate lignin.

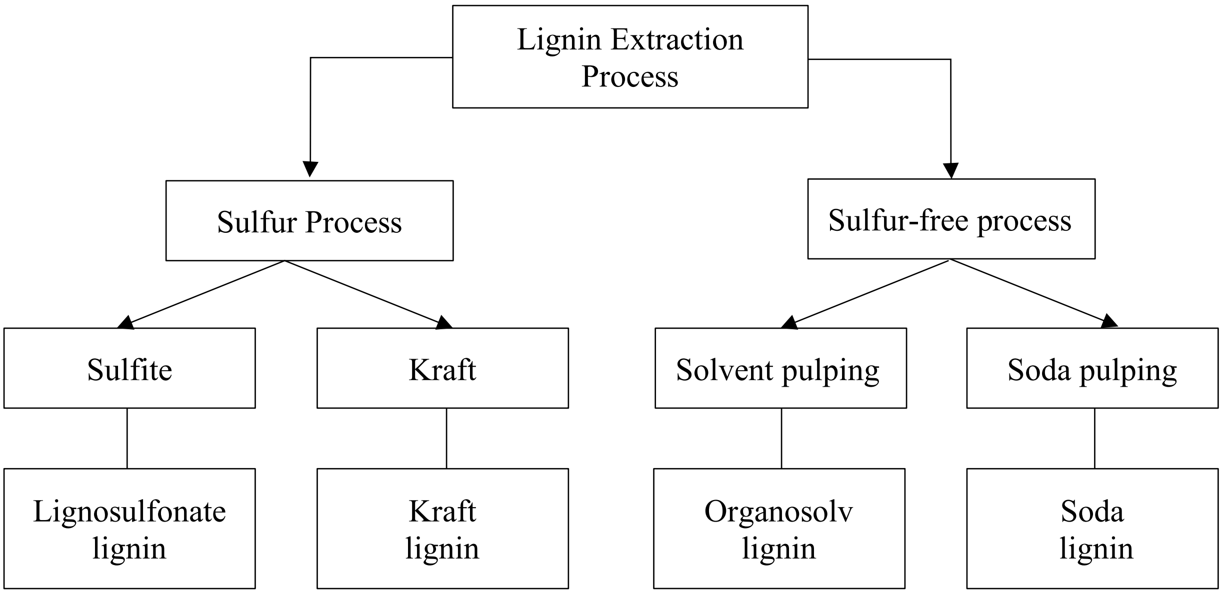


Figure 1-3 Biomass conversion technologies for various types of lignin (Mahmood et al., 2018)

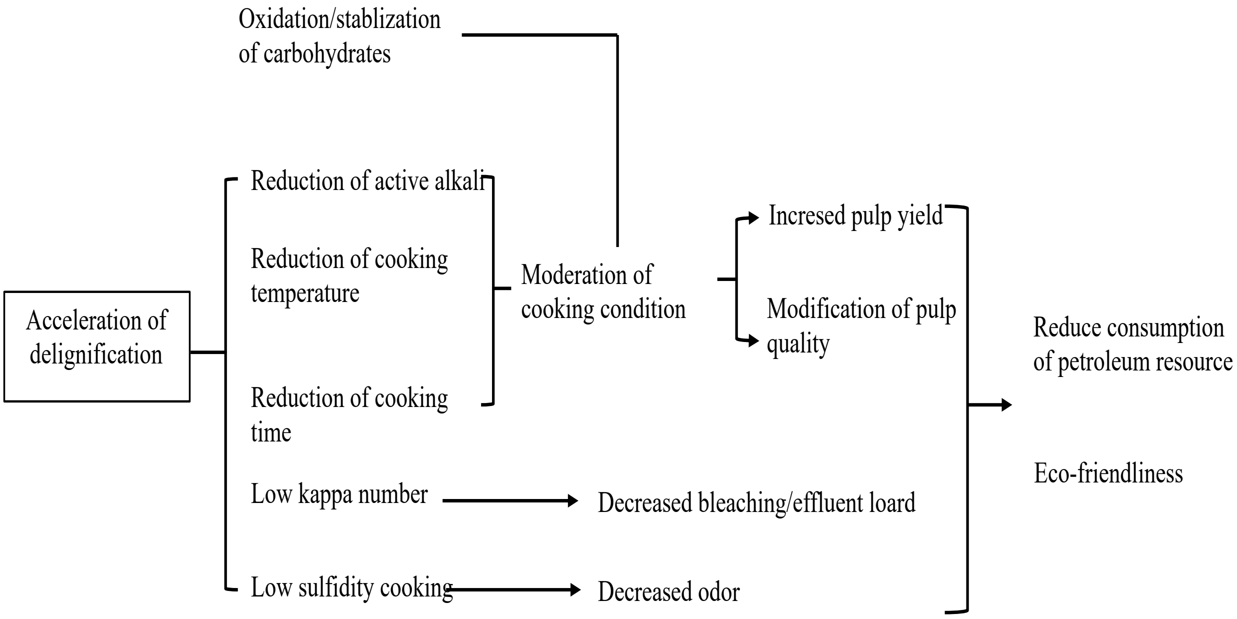


Figure 1-4 Beneficial effects of anthraquinones on cooking (Takahashi et al., 2011)

LignoBoost (Ohman et al., 2016) is a green process that can separate lignin from black liquor. The process begins with obtaining weak black liquor from cooking black liquor, which is then evaporated to a certain concentration. Subsequently, lignin is separated from black liquor using carbon dioxide, which precipitates lignin from alkaline solutions at pH 9–10. Afterward, the solution is filtered, and the resulting product further acidified with sulfuric acid to a final pH value of around 2 (Kouisini & Paleologou, 2015; Ohman et al., 2016). **Figure 1-5** shows a separation method based on the LignooBoost process.

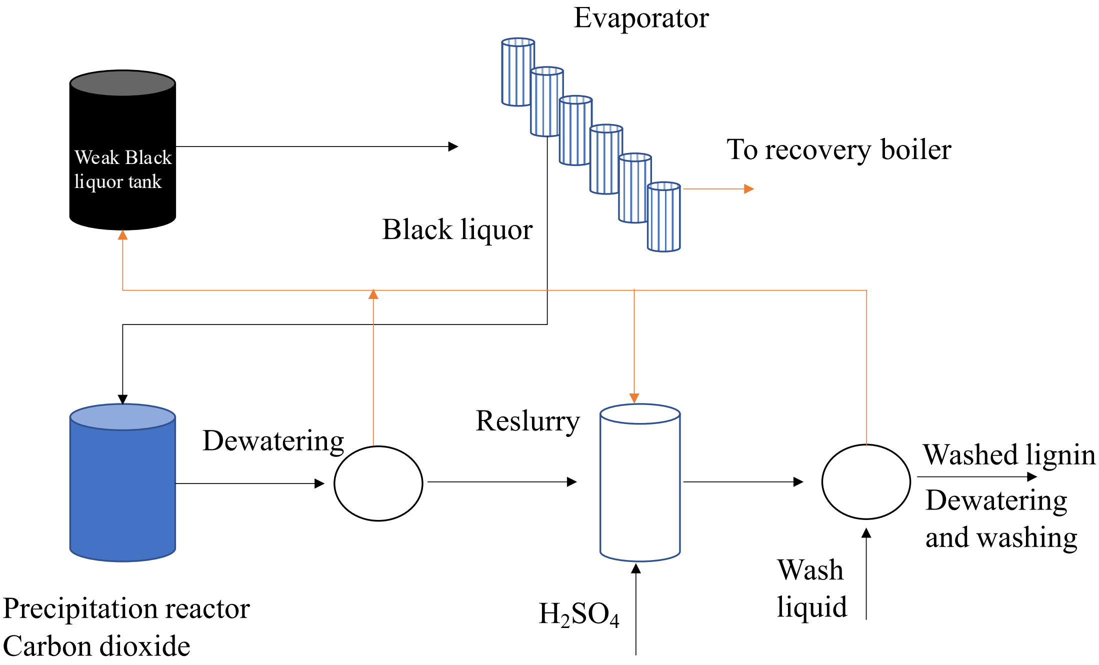


Figure 1-5 Separation method based on LignoBoost process (Ohman et al., 2013).

1.2 Problem statement

Dissolving pulp consists of high-purity cellulose (94%–99%) with low hemicellulose and low lignin contents. Specifically, dissolving pulp has the ability to dissolve in a solvent or to be derived into a homogeneous solution that is a precursor for textile fibers and cellulose derivatives, such as cellulose esters and cellulose ethers (Sixta et al., 2006; Ambjörnsson et al., 2014). A high amount of hemicellulose in dissolving pulp impairs further processing into end products (Behin and Zeyghami, 2009; Ibarra et al., 2010). Therefore, prehydrolysis must be performed prior to cooking to remove hemicellulose, and the xylan residue in prehydrolysate can be an alternative source of xylooligomers. The lignin fraction should be isolated from black liquor based on the biorefinery concept.

1.3 Research objective

The main objective of this study was to produce bamboo dissolving pulp using a combination of prehydrolysis and soda cooking with additives prior to elemental chlorine-free (ECF) bleaching. The characterization of lignin precipitates from the black liquor of prehydrolysis soda cooking was also carried out. The mixture of xylooligomers obtained from bamboo prehydrolysate was also analyzed and discussed.

References

Baurhoo, B., Ruiz-Feria, C. A.,, Zhao, X., 2008. Purified lignin: nutritional and health impacts on farm animals-a review. Anim. Feed Sci Technol. 144, 175–84. https://doi.org/10.1016/j.anifeedsci.2007.10.016.

Bian, J., Peng, F., Peng, X.P., Peng, P., Xu, F., Sun, R.C., 2013. Structural features and antioxidant activity of xylooligosaccharides enzymatically produced from sugarcane bagasse. Bioresour. Technol. 127, 236–241https://doi.org/10.1016/j.biortech.2012.09.112.

Froass, P.M., Ragauskas, A.J., Jiang, J.E., 1998. Nuclear magnetic resonance studies. 4. Analysis of residual lignin after kraft pulping. Ind. Eng. Chem. Res. 37, 3388–3394.. https://doi.org/ 10.1021/ie970812c.

Garrote, G.D.H.P., Dominguez, H., Parajo, J.C., 1999. Hydrothermal processing of lignocellulosic materials.Holz Als Roh - Und Werkstoff 57, 191–202. https://doi.org/10.1007/s001070050039.

Garrote, G., Domínguez, H., Parajó, J.C., 1999. Mild autohydrolysis: an environmentally friendly technology for xylooligosaccharide production from wood. J Chem. Technol. Biotechnol. 74, 1101–9. https://doi.org/10.1002/(SICI)1097-4660(199911)74:11<1101::AID-JCTB146>3.0.CO;2-M.

Garrote, G., Yáñez, R., Alonso, J.L. and Parajó, J.C., 2008. Coproduction of oligosaccharides and glucose from corncobs by hydrothermal processing and enzymatic hydrolysis. Ind. Eng. Chem. Res. 47, 1336–1345. https://doi.org/10.1021/ie071201f.

Ghatak, H. R., 2008. Spectroscopic comparison of lignin separated by electrolysis and acid precipitation of wheat straw soda black liquor. Ind. Crops Prod. 28, 206–212. https://doi.org/10.1016/j.indcrop.2008.02.011.

Jain, I., Kumar, V., Satyanarayana, T., 2015. Xylooligosaccharides: an economical prebiotic from agroresidues and their health benefits. Indian J. Exp. Biol. 53, 131–142.

Jørgensen, H., Kristensen, J.B., Felby, C., 2007. Enzymatic conversion of lignocellulose into fermentable sugars: challenges and opportunities. Biofuels Bioprod. Biorefining. 1, 119–134. https://doi.org/10.1002/bbb.4.

Kabel, M.A., 2002. Characterisation of Complex Xylo-Oligosaccharides from Xylan Rich by-Products. Wageningen University. Ph.D. Thesis.

Kamm, B., Kamm, M., Schmidt, M., Hirth, T., Schulze, M., 2005. Lignocellulose-based chemical products and product family trees, in: Kamm, B., Gruber, P.R., Kamm, M (Eds). Biorefineries-Industrial Processes and Products: Status Quo and Future Directions, WILEY-VCH Verlag GmbH & Co. KGaA, pp. 97–149.

Kouisini, L., Paleologou, M., 2015. Method for separating lignin from black liquor. United States Patent 2(12).

Kumar, H., Christopher, L.P.,. 2017. Recent trends and developments in dissolving pulp production and application. Cellulose. 24, 2347–2365. https://doi.org/10.1007/s10570-017-1285-y.

Lai, C., Jia, Y., Wang, J., Wang, R., Zhang, Q., Chen, L., Shi, H., Huang, C., Li, X., Yong, Q., 2019. Co-production of xylooligosaccharides and fermentable sugars from poplar through acetic acid pretreatment followed by poly (ethylene glycol) ether assisted alkali treatment. Bioresour. Technol. 288. https://doi.org/10.1016/j.biortech.2019.121569.

Lau, C. S., 2012. Characterization and quantification of monomers, oligomers, and by-products from xylan during biomass pretreatment. University of Arkansas, Fayetteville. Ph.D. Thesis.

Mahmood, Z., Yameen, M., Jahangeer, M., Riaz, M., Ghaffar, A., Javid, I., 2018. Lignin as natural antioxidant capacity. Intech Open, 181–205. https://doi.org/10.5772/intechopen.73284

Moure, A., Gullón, P., Domínguez, H., Parajó, J.C., 2006. Advances in the manufacture, purification and applications of xylo-oligosaccharides as food additives and nutraceuticals. Process Biochem. 41, 1913–1923. https://doi.org/10.1016/j.procbio.2006.05.011.

Mousavioun, P., Doherty, W.O.S., 2010. Chemical and thermal properties of fractionated bagasse soda lignin. Ind Crops Prod. 31, 52–58. https://doi.org/10.1016/j.indcrop.2009.09.001.

Nadif, A., Hunkeler, D., Käuper, P., 2002. Sulfur-free lignins from alkaline pulping tested in mortar for use as mortar additives. Bioresour. Technol. 84, 49–55. https://doi.org/10.1016/S0960-8524(02)00020-2.

Ohman, F., Theliander, H., Tomani, P., Axegard, P., 2013. Method for separating lignin from black liquor. United States Patent 2(12).

Ohman, F., Theliander, H., Tomani, P., Axegard, P., 2016. Method for separating lignin from black liquor, a lignin product, and use of a lignin product for the production of fuels or materials. United States Patent 2(12).

Patil, V., Adhikari, S., Cross, P., Jahromi, H., 2020. Progress in the solvent depolymerization of lignin. Renew. Sust. Energ. Rev. 133, 110359. https://doi.org/10.1016/j.rser.2020.110359.

Saha, B.C., 2003. Hemicellulose bioconversion. J. Ind. Microbiol. Biotechnol. 30, 279–291. https://doi.org/10.1007/s10295-003-0049-x.

Sameni, J., Krigstin, S., dos Santos Rosa, D., Leao, A., Sain, M., 2014. Thermal characteristics of lignin residue from industrial processes. BioResources 9, 725–737. https://doi.org/10.15376/biores.9.1.725-737.

Scurlock, J.M., Dayton, D.C., Hames, B., 2000. Bamboo: an overlooked biomass resource?. Biomass and Bioenergy 19, 229–244. https://doi.org/10.1016/S0961-9534(00)00038-6.

Sixta, H., Koch, G., Ressel, J.B., Potthast, A., Krotschek, A.W., 2006. The History of Paper Making. Vol 1. https://doi.org/10.1016/S0268-0890(97)90050-5

Sugesty, S., Kardiansyah, T., Hardiani, H., 2015. Bamboo as raw materials for dissolving pulp with environmental friendly technology for rayon fiber. . Procedia Chem. 17, 194–199. https://doi.org/10.1016/j.proche.2015.12.122

Takahashi, S., Homma, M., Kajiyama, M., Ohi, H., Tanaka, J., 2011. Influence of soluble anthraquinone compound on bleaching load during kraft cooking of various woods. Japan Tappi J. 65, 792–798. https://doi.org/10.2524/jtappij.65.792.

Tejado, A., Peña, C., Labidi, J., Echeverria J.M., Mondragon, I., 2007. Physico-chemical characterization of lignins from different sources for use in phenol–formaldehyde resin synthesis Bioresour. Technol. 98, 1655–1663. https://doi.org/10.1016/j.biortech.2006.05.042.

Vaidya, A.A., Murton, K.D., Smith, D.A., Dedual, G., 2022. A review on organosolv pretreatment of softwood with a focus on enzymatic hydrolysis of cellulose. Biomass Convers. Biorefinery. https://doi.org/10.1007/s13399-022-02373-9.

Wojtasz-Mucha, J., M. Hasani, and H. Theliander. 2021. “Dissolution of Wood Components during Hot Water Extraction of Birch.” *Wood Science and Technology* 55(3):811–35. https://doi.org/10.1007/s00226-021-01283-9.

Wojtasz-Mucha, J., Hasani, M., Theliander, H., 2021. Dissolution of wood components during hot water extraction of birch. Bioresour. Technol. 102, 4157–4164. https://doi.org/10.1016/j.biortech.2010.11.063.

Yoo, C. G., Ragauskas, A.J., 2021. Opportunities and challenges of lignin utilization. ACS Symp. Ser. 1377, 1–12. https://doi.org/10.1021/bk-2021-1377.ch001.

Wang K.L., Xu, J.C., Pei, S.J., Chen, S.Y., 1996. Selected non-timber forest products for natural resources conservation and community development in Southwest Yunnan, China. In *The International Seminar on NTFPs (China Yunnan, Laos, Vietnam)* (pp. 31-38).

Chapter 2. Dissolving Pulp from *Phyllostachys pubescens* Stem by Prehydrolysis Soda Cooking with 2-Methylanthraquinone

2.1 Introduction

Bamboo plays a very important role in both economic and industrial sectors. It is used in agriculture, infrastructure, furniture, food, pulp, and paper (Singha and Hassan, 2017). The main chemical components of moso bamboo (*Phyllostachys pubescens)* stem are cellulose, hemicellulose, and lignin, which account for up to 90% of the total dry mass; the levels of cellulose and hemicellulose are approximately 55%–65% (Scurlock et al., 2000; Do et al., 2020). Moso bamboo can provide pulp with properties similar to pulp from hardwoods by kraft cooking (Runge et al., 2012; Moradbak et al., 2016). Some bamboo producing countries, such as China and India, have produced pulp industrially from bamboo, which has similar or sometimes better quality than pulp made from wood. Another study indicated that prehydrolyzed semantan bamboo (*Gigantochloa scortechinii*) with soda-anthraquinone (AQ) cooking provided high-strength mechanical properties of paper, and soda-AQ cooking was a more environment-friendly cooking process than kraft cooking (Hassan et al., 2013).

Bamboo is a non-wood biorefinery resource that has been targeted for the production of high-purity dissolving pulp in recent studies (Batalha et al., 2012; Ma et al., 2012; Luo et al., 2014; Wu et al., 2014; Sugesty et al., 2015). The biorefinery concept deals with developing a chemical process that isolates biomass components as precisely as possible and processes them further into value-added products (Schild et al., 2010). Prehydrolysis of lignocellulosic materials helps to loosen the pulp matrix and increases the accessibility of lignin to cooking and bleaching chemicals. Moreover, the branched structure and low degree of polymerization of hemicellulose makes it easier to hydrolyze than cellulose (Behin and Zeyghami, 2009). A comparison between steam prehydrolysis and hot water prehydrolysis of bamboo, followed by kraft cooking and bleaching, showed a change in the lignin structure with a significant decrease in the level of methoxy group when hot water prehydrolysis had been performed (Luo et al., 2014). This indicated that hot water prehydrolysis may break down the β-*O*-4 ether bonds of syringyl type lignin. A study on prehydrolysis of corn (*Zea mays*) stalk and Kans grass (*Saccharum spontaneum*) through soda-AQ cooking showed removal of fines, primarily pith, with increased sugars, lignin, and acetic acid in the prehydrolysate, and improved delignification (Jahan and Rahman, 2012). Another study on prehydrolysis has also been conducted to investigate the ability of sulfuric acid addition to extract hemicellulose and concluded that the prehydrolysis temperature and sulfuric acid addition had a significant effect on the properties of dissolving pulp (Jahan, 2009). However, acid prehydrolysis can lead to a loss in brightness which would increase bleaching load (Rudie et al., 2007).

In addition to prior cooking, kraft cooking and soda-AQ cooking also affect the quality of dissolving pulp. Some studies have conducted prehydrolysis prior to kraft cooking and soda-AQ cooking in order to obtain high-quality dissolving pulp (Harsono et al., 2016; Putra et al., 2018; Salaghi et al., 2018). A previous study (Salaghi et al., 2018) compared *Eucalyptus globulus* wood pulp obtained by prehydrolysis soda-AQ cooking with that by prehydrolysis kraft cooking. Soda-AQ cooking prehydrolysis at 150 °C with a low dosage of active alkali (AA) followed by a modified bleaching process was found to result in high removal of hemicellulose and high-quality dissolving pulp (Salaghi et al., 2019). For non-woody materials, soda-AQ cooking, as it is a non-sulfur type of cooking, is preferable to kraft cooking owing to its environment-friendliness. Several studies have been conducted on the production of dissolving pulp from sugarcane (*Saccharum officinarum*) bagasse and oil palm (*Elaeis guineensis*) empty fruit bunch (Andrade and Colodette, 2014; Harsono et al., 2016; Maryana et al., 2017).

Over the past few decades, use of elemental chlorine-free (ECF) bleaching technology has increased. Using mild hot water prehydrolysis improved the bleachability of soda-AQ pulp to a level equal to that of kraft pulp (Francis et al., 2008). Dissolving pulp from bamboo using soda-AQ cooking followed by bleaching yielded a good pulp brightness (Batalha et al., 2012). Harsono et al. (2016) showed that the brightness and α-cellulose content of the empty fruit bunch pulp obtained from ECF bleaching were better than those obtained from totally chlorine-free bleaching, and Yuan et al., (2017) reported that bamboo provided an ECF-bleached dissolving kraft pulp of good quality in the case of a process with xylanase treatment and cold caustic extraction (CCE).

Most studies currently focus on the valorization of cellulose and hemicellulose (FitzPatrick et al., 2010), since lignin has been considered a low-value residual (Cherubini, 2010; Doherty et al., 2011). However, lignin can be converted into valuable products, and the technique to isolate it depends on the used materials, as it is produced during the processes of treating lignocellulosic materials, such as kraft lignin, lignosulphonates, and soda lignin (Gosselink et al., 2004). The process of extraction, which is used to separate lignin from lignocellulosic biomass, considerably influences the lignin structure, properties, and purity. Soda lignin can be applied in the production of phenolic resins, animal nutrition, dispersants, and polymer synthesis, and the weight-average molecular weight, polydispersity, and ash content of soda lignin were 1000–3000 (up to 15000) $g \cdot mol^{-1}$, 2.5–3.5, and 0.7%–3.5%, respectively (Vishtal and Kraslawski, 2011).

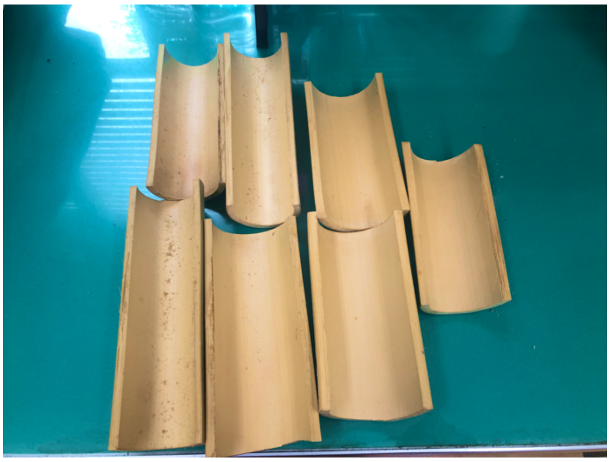
The main objective of this chapter was to produce bamboo dissolving pulp using a combination of prehydrolysis and soda cooking with additive prior to ECF bleaching. The characterization of lignin precipitates from the black liquor of prehydrolysis soda cooking was also studied.

2.2 Materials and Methods

2.2.1 Materials

Two-year-old moso bamboo was harvested in Hitachi-Omiya city, Ibaraki, Japan. The green bamboo was air-dried to a moisture content of 10% with node removal. Afterward, the bamboo stems were cut into chips with the following dimensions: 5 mm thick, 10 mm wide, and 20 mm long. Soluble anthraquinone (SAQ: 1, 4-dihydro-9, 10-dihydroxyanthracene sodium salt) and 2-methylanthraquinone (2-MAQ) as cooking additives were provided by Kawasaki Kasei Chemicals, Kawasaki, Japan and Alfa Aesar, Lancashire, United Kingdom, respectively. For producing peroxymonosulfuric acid (P_{sa}), 98% sulfuric acid (Fujifilm Wako Pure Chemical, Osaka, Japan) was added to a 45% aqueous hydrogen peroxide solution (Mitsubishi Gas Chemical, Tokyo, Japan) at 70 °C, following a previously reported procedure (Rizaluddin et al., 2015; Salaghi et al., 2018).

Bamboo Forest



Dried in direct sunlight to a moisture level of ±10%

Dried bamboo was cut into chips with a size of 0.5 cm thickness *1.0 cm width * 2.0 cm length

Photo 2-1: Bamboo preparation

2.2.2 Prehydrolysis and soda cooking with SAQ and 2-MAQ

Prehydrolysis was conducted on 50 g oven-dried bamboo chips mixed with 245 mL of distilled water (liquid-to-bamboo ratio of 5 mL/g) at a temperature of 150 °C for various durations (3, 5, and 7 h). After the prehydrolysis, 200 mL of prehydrolysate was separated from the mixture. For soda cooking, the active alkali (AA) dosages used were 17%, 20%, 23%, 25%, and 28% with a SAQ dosage of 0.1% or 2-MAQ dosage of 0.06% (based on oven-dried bamboo weight). AA dosage (%) is defined as Na_2O weight (%) based on oven-dried material weight.

As a cooking additive, 2-MAQ was reduced by glucose to be soluble before mixing with soda cooking liquor according to a previously reported procedure (Anita et al., 2021). Cooking temperature and duration were at 160 °C for 3 h, and the liquor-to-bamboo ratio was 5 mL/g.

2.2.3 Oxygen delignification and pulp bleaching

Unbleached pulp was subjected to oxygen delignification (O) with NaOH dosage of 1.0% at oxygen pressure of 0.5 MPa and pulp consistency (PC) of 30%. The temperature was maintained at 120 °C for 60 min. Oxygen-delignified pulp was subjected to ECF bleaching. The sequence and the conditions of each stage for pulp obtained from soda cooking with SAQ (soda-SAQ cooking) were as follows:

1) CCE: PC: 10%, NaOH dosage: 8%, temperature: 45 °C, and time: 30 min.
2) Chlorine dioxide combined with P_{sa} (D_0/P_{sa}): PC: 10%, kappa factor (chlorine dioxide dosage: % as active chlorine ÷ kappa number of pulp): 0.29, P_{sa} dosage: 0.1% (H_2SO_5), pH: 2.8, temperature: 80 °C, and time: 40 min.
3) Alkali extraction with hydrogen peroxide (E_p): PC: 10%, H_2O_2 dosage: 0.42%, NaOH dosage: 0.9%, temperature: 70 °C, and time: 90 min.
4) Final chlorine dioxide (D_1): PC: 10%, ClO_2 dosage: 0.45%, temperature: 70 °C, and time: 100 min.

The sequence for soda-SAQ pulp was O-CCE-D_0/P_{sa}-E_p-D_1. The sequence for pulp obtained from soda cooking with 2-MAQ (soda-2-MAQ cooking) was O-D_0/P_{sa}-E_p-D_1, and the conditions of each stage were the same as those for soda-SAQ pulp.

2.2.4 Chemical analysis of bamboo residue and pulp

The bamboo chips were milled into 40-80 mesh meals, and the meals were extracted with acetone for 8 h to determine the extractives content. The contents of ash and acid-insoluble lignin were measured according to the TAPPI test methods T211 om-93 and T222 om-88, respectively. Acid-soluble lignin was measured by UV-Vis spectrophotometry at 205 nm.

Nitrobenzene oxidation of bamboo meals was conducted to determine syringaldehyde (S_a) and vanillin (V_a) yields according to a previously reported method (Nakagawa-Izumi et al., 2017; Salaghi et al., 2018).

The monosaccharide content of the filtrate obtained from the acid-insoluble lignin measurement of bamboo meals, the residue, and the pulp was determined using a Dionex ICS 3000 Ion Chromatograph (Dionex, Sunnyvale, CA, USA) equipped with a pulsed amperometric detector, single pump model, CarboPac PA1 column (φ 4 mm × 250 mm), CarboPac PA1 guard column (φ 4 mm × 50 mm), and an auto sampler according to a previously reported method (Salaghi et al., 2018). The eluent used in this system was a mixture of 0.15 M NaOH, 0.1 M KOH, 0.125M Na_2CO_3, and filtrated distilled water.

The kappa number and viscosity of the pulp were measured according to the TAPPI test methods T236 cm-85 and T254 om-85, respectively. The pulp brightness was measured using the Tokyo-Denshoku TC-3600 digital color meter (Tokyo Denshoku, Tokyo, Japan).

2.2.5 Chemical analysis of prehydrolysate

The monosaccharide content of the prehydrolysate was determined by the ion chromatography before and after the 72% sulfuric acid hydrolysis, followed by the 4% sulfuric acid hydrolysis for 1 h at 121 °C. The acid-insoluble lignin and acid-soluble lignin were determined according to the procedure described in section **2.2.4**. Oligomers content was estimated by a difference between monosaccharide contents after and before the 72% and 4% sulfuric acid hydrolysis.

2.2.6 Preparation of lignin precipitate from black liquor of soda cooking

The black liquor of soda cooking was freeze-dried. The mixture of organic and inorganic solids was dissolved in distilled water to prepare various solid concentrations in the range of 17%–35%. At 27 °C, 3.0 L of CO_2 (approximately 120 mmol) was charged to 10 g of the solid (29–59 mL of black liquor). The mixture was then washed with 20 mL of carbonated water, centrifuged at 2000 G (3000 rpm, r = 19.9 cm) for 30 min to recover the precipitate, and washed again. The washing procedure was repeated twice, and the lignin precipitate was oven-dried at 45 °C for 18 h.

Subsequently, 7.5 L of CO_2 was charged to 16 g of the solid (45 mL of black liquor) until pH 8.8 was reached. The lignin precipitate was then oven-dried, and referred to as lignin CO_2-precipitate. The lignin CO_2-precipitate was then acidified to approximately pH 2.9 with the addition of 2 mol/L H_2SO_4. The precipitate of the pH 2.9 mixture was recovered by centrifugation and washed with distilled water. The centrifugation and washing procedures were repeated five times. The lignin precipitate was then oven-dried. Hereafter, the precipitate is referred to as lignin CO_2-H_2SO_4-precipitate. The other lignin precipitate was prepared by directly adding 2 mol/L H_2SO_4 into the black liquor until pH 2.9 was reached. The precipitate was then recovered and dried as lignin CO_2-H_2SO_4-precipitate. Hereafter, the precipitate is referred to as lignin H_2SO_4-precipitate.

2.2.7 Molecular weight distribution of soda lignin

The lignin precipitate was dissolved in 0.5 mol/L NaOH and subjected to gel permeation chromatography using a Sephadex G-50 column (φ 18 mm × 500 mm). After separation, each fraction of the sample was detected by UV-Vis spectrophotometry with the absorbance at 280 nm. Polystyrene sulfonic acid, vanillin, and guaiacylglycerol-β-guaiacyl ether were used as the internal standards. Molecular weight (Mw) was obtained by the equation $\text{Log}_{10} M_w = 3.95 - 2.01 \times$ relative retention volume (Ohi et al., 1988; Salaghi et al., 2019).

2.3 Results and Discussion

2.3.1 Chemical composition of moso bamboo material

As shown in **Table 2-1**, the glucan content was 42.2% in 2-year-old moso bamboo stem, which was similar to that of *Acacia crassicarpa* wood and sugarcane bagasse but was lower than that of *E. globulus* wood. However, the xylan content in moso bamboo was 20.2% and higher than those in both hardwoods and sugarcane bagasse.

The lignin content in moso bamboo was 33.1%, and the nitrobenzene oxidation indicated that the combination of S_a and V_a yield and S_a/V_a ratio for moso bamboo stem were 1.37 mmol/g-lignin and 1.22, respectively, which were lower than those for *E. globulus* (3.04 mmol/g-lignin and 5.81) but were higher than those for *A. crassicarpa* and sugarcane bagasse.

The high S_a/V_a ratio of lignocellulosic material results in good alkaline delignification, and the high combination yield of S_a and V_a indicates a high content of non-condensed lignin (Akiyama et al., 2005; Jin et al., 2012). Therefore, moso bamboo may lead to better delignification than *A. crassicarpa* and sugarcane bagasse.

Table 2-1 Comparison of chemical composition between moso bamboo (*Phyllostachys pubescens*) stem, plantation hardwoods, and sugarcane (*Saccharum officinarum*) bagasse

	Moso bamboo	*Acacia crassicarpa*[c]	*Eucalyptus globulus*[d]	Sugarcane bagasse[e]
Glucan (%)	42.2 ± 2.42	41.1	49.4	39.3
Xylan (%)	20.2 ± 1.24	10.9	15.3	18.0
Acid-insoluble lignin (%)	30.4 ± 0.07	29.6	22.2	23.7
Acid-soluble lignin (%)	2.71 ± 0.26	1.89	5.4	2.3
Ash (%)	1.23 ± 0.25	0.22	0.2	1.8
Acetone extractives (%)	2.59 ± 0.01	2.08	0.6	2.0
Unknown (%)	0.9[a]	14.2	7.1	12.9
S_a/V_a[b] molar ratio	1.22 ± 0.07	0.74	5.81	0.84
S_a and V_a yields (mmol/g-lignin)	1.37 ± 0.09	1.24	3.04	1.30

Note: [a]: Arabinan, mannan, and galactan

[b]: S_a/V_a: Syringaldehyde/vanillin obtained by nitrobenzene oxidation.

[c]: (Utami et al., 2021)

[d]: (Salaghi et al., 2018, 2019)

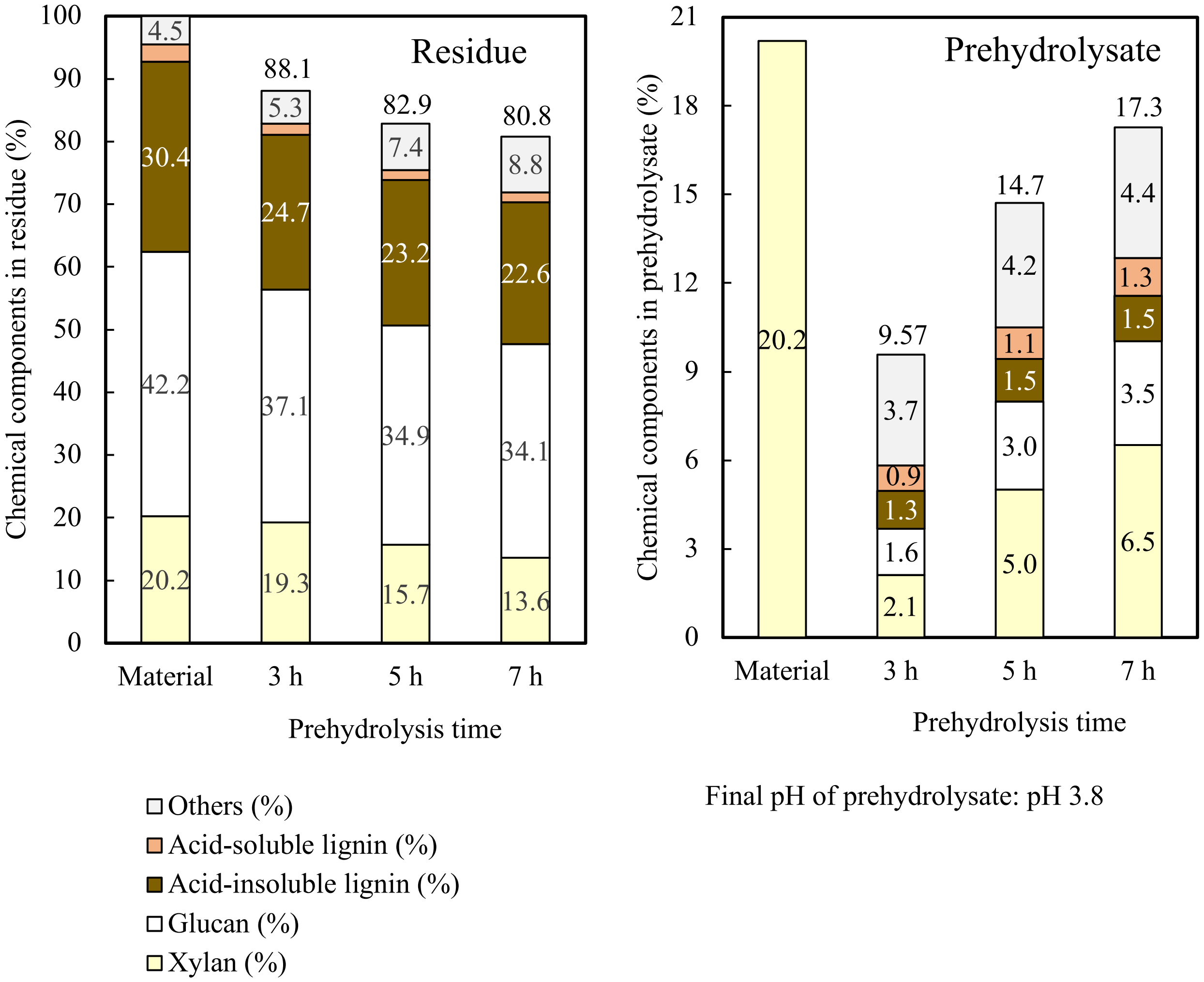
[e]: (Maryana et al., 2017)

2.3.2 Characterization of residue and prehydrolysate during prehydrolysis

Prehydrolysis has a low environmental impact, and the degradation of cellulose can be controlled to preserve the degree of polymerization of cellulose (Garrote et al., 2001; Wan Rosli et al., 2003). Water hydrolysis can degrade some of the components of lignocellulosic material and form acetic acid and the necessary acidity to catalyze the hydrolysis, which results in the dissolution of hemicellulose.

Figure 2-1 shows the chemical composition of residue and prehydrolysate after prehydrolysis of moso bamboo at 150 °C for 3, 5, and 7 h, and the contents of xylan as a main hemicellulose decreased to 19.3%, 15.7%, and 13.6% (based on untreated weight), which corresponded to decrements of approximately 4%, 22%, and 33% of the xylan material that dissolved into the prehydrolysate, respectively. Moreover, the content of xylan that depolymerized into the prehydrolysate increased from 2.1%, 5.0%, and 6.5% (based on untreated weight) after the prehydrolysis for 3, 5, and 7 h, respectively. In addition, the data revealed that glucan decreased to 37.1%, 34.9%, and 34.1%, which accounted for approximately 12%, 17%, and 19% of the glucan in the wood chip. The results are consistent with the findings of previous reports, in which xylan structures and glucan in wood consists of two parts, including a fraction solubilized easily and a fraction difficult to extract (Borrega et al., 2011; Gil Garrote et al., 1999). In addition, the glucan was observed to be more stable than xylan when the wood chips were subjected to prehydrolysis. According to the analysis results presented in **Figure 2-1**, both xylan and glucan contents in the prehydrolysate were significantly lower than those in solid residue. The findings are consistent with the findings of several studies (Leschinsky et al., 2009; Shi et al., 2019; Tunc & Van Heiningen, 2008), which can be explained by further conversion of soluble sugars to other products, for example furans. In addition, the physical size of the wood chip could have a diffusion effect, which are the products of depolymerization of carbohydrates that remain in the wood chips.

As shown in **Figure 2-2**, xylose did not increase significantly with the extension of the prehydrolysis time, whereas xylooligomer content increased from 1.51% to 5.68%. Approximately 28% of the bamboo xylan was dissolved into the prehydrolysate as xylooligomers at the final pH 3.8. This result showed that the prehydrolysate obtained from 7 h prehydrolysis was an alternative source of the xylooligomer products.



Final pH of prehydrolysate: pH 3.8

Figure 2-1 Separation of xylan from moso bamboo during prehydrolysis (150 °C, liquor-to-bamboo ratio: 5 mL/g)

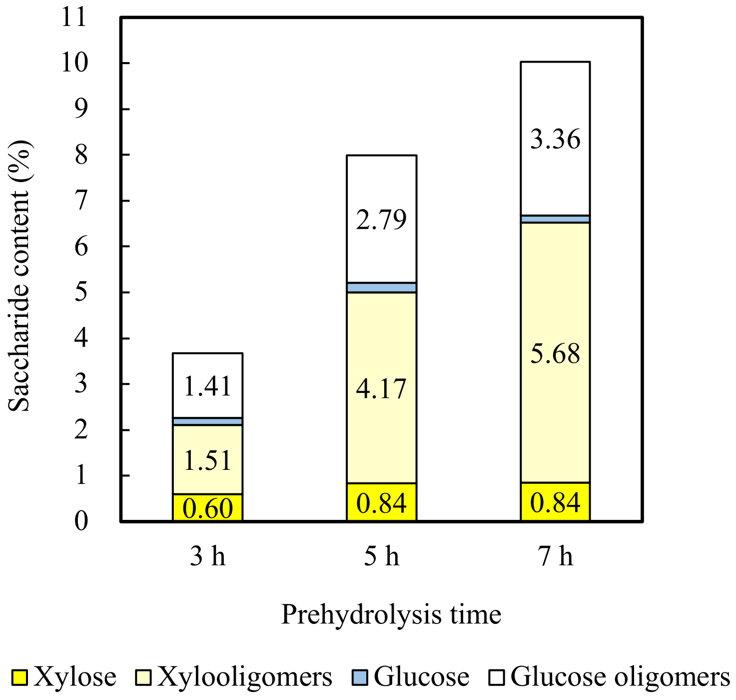


Figure 2-2 Contents of mono- and oligosaccharides in prehydrolysate (150 °C, liquor-to-bamboo ratio: 5 mL/g)

Prehydrolysis has a low environmental impact, and the degradation of cellulose can be controlled to preserve the degree of polymerization of cellulose (Garrote et al., 2001; Wan Rosli et al., 2003). Compared to untreated bamboo, prehydrolysis may change the lignin structure rather than affect its distribution (Ma et al., 2011, 2012). Prehydrolysis helps the penetration of chemicals in the subsequent alkali cooking by creating pores in the chips for further reactions (Jahan et al., 2015; Martino et al., 2015), which improves the delignification process. Furthermore, prehydrolysis can break down the α-benzyl ether bond between lignin and carbohydrate (Garrote et al., 2001; Wan Rosli et a*l*., 2003). In addition to the hydrolysis of xylan to xylose or xylooligomers, the prehydrolysis process depolymerized a part of lignin possibly through homolytic cleavage of the β-*O*-4 ether bond at high temperatures, which resulted in an increase in lignin dissolution during the subsequent cooking and provided the pulp with a lower kappa number (Leh et al., 2008). In this study, approximately 33% xylan was extracted from moso bamboo chips. This suggests that a low chemical dosage is required during the soda cooking process compared to non-prehydrolysis (Chang and Holtzapple, 2000).

Prehydrolysis for 7 h enabled the removal of approximately 33% xylan based on xylan material, and most of xylan was recovered in the prehydrolysate. Therefore, for the development of bamboo dissolving pulp with high-quality and products from xylan, such as furfural, xylose, and xylooligomers, prehydrolysis at 150 °C for 7 h was considered for further treatment in this study.

2.3.3 Effect of soda-AQ on prehydrolyzed bamboo

It is difficult for liquor to penetrate bamboo during cooking. The structure, thick cell wall, high hybrid cell content, and wax are the main factors affecting the difficulty of liquor penetration (Ma et al., 2012). Therefore, varied AA dosages (17%, 20%, and 23%) were applied to soda cooking.

Table 2-2 indicates that increasing the AA dosage decreased the pulp yield and the kappa number. Overall, the pulp yields from different prehydrolysis times and AA dosages ranged between 30.6% and 34.0%, whereas the kappa number ranged between 10.9 and 22.9. The brightness increased with increasing AA dosage and the removal of lignin. Pulp with a kappa number of 10.9, pulp yield of 30.6%, and brightness of 45.0% was obtained after 7 h of prehydrolysis soda-SAQ cooking with an AA dosage of 25%.

Under the same conditions (prehydrolysis: 3 h, AA: 20%), kappa number of pulps for moso bamboo, *A. crassicarpa*, and sugarcane bagasse were 17.5, 13.2 (Utami et al., 2021), and 10.1 (Maryana et al., 2017), whereas pulp yields were 33.0%, 42.6%, and 37.6%, respectively. Contrary to our expectation in section **2.3.1**, moso bamboo did not show better delignification than *A. crassicarpa* and sugarcane bagasse. There may be some amount of ferulic acid in bamboos (Syamsu et al., 2019), which consumes sodium hydroxide (active alkali). Another explanation could be due to the higher lignin content in bamboo (33.1%) than *A. crassicarpa* and sugarcane bagasse, as shown in **Table 2-1**. Meanwhile, Syamsu et al. (2019) conducted cooking comparison on non-wood material such as bamboo, sago pith waste, and water hyacinth, and showed that bamboo consumed higher dosage of sodium hydroxide and needed longer cooking time, since the bamboo contained highest lignin. The higher lignin content in the material required more chemical substances and longer cooking time (Sharma et al., 2018).

Table 2-2 Yield and kappa number of moso bamboo pulp obtained by prehydrolysis soda-soluble anthraquinone (SAQ) cooking (Prehydrolysis: 150 °C, cooking: 160 °C for 3 h, SAQ dosage: 0.1%, liquor-to-bamboo ratio: 5 mL/g)

Prehydrolysis time (h)	Active alkali dosage (%)	Kappa number	Pulp yield (%)	Brightness (ISO %)
3	17	22.9	34.0	34.5
3	20	17.5	33.0	37.3
3	23	12.8	32.6	41.8
3	25	11.8	31.4	42.8
5	20	12.8	32.1	41.8
5	23	12.4	31.6	42.6
5	25	11.6	31.2	43.1
7	20	12.7	31.0	42.5
7	23	11.0	30.8	43.8
7	25	10.9	30.6	45.0

Figure 2-3 demonstrates that xylan, acid-insoluble lignin, and acid-soluble lignin decreased with increasing AA dosage and prolonged prehydrolysis time. Prehydrolysis for 7 h with an AA dosage of 25% provided pulp with a xylan content of 5.65%, which would be the most suitable for the production of dissolving pulp.

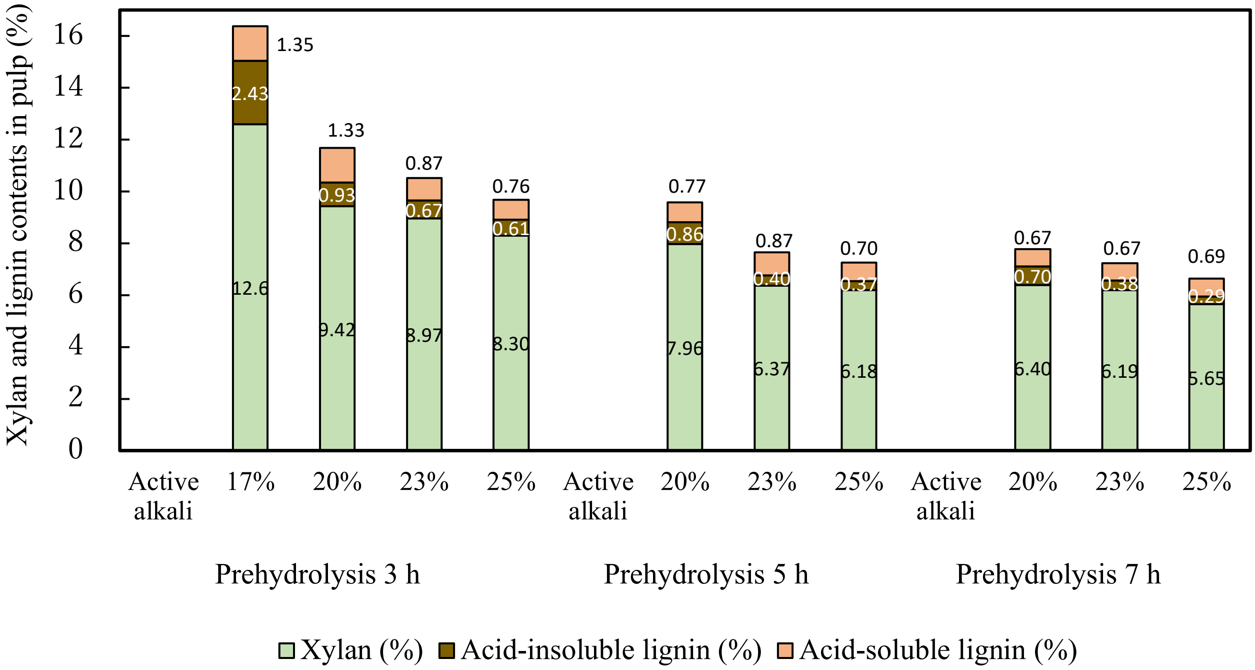


Figure 2-3 Xylan, acid-insoluble lignin, and acid-soluble lignin of moso bamboo pulp obtained by prehydrolysis soda-soluble anthraquinone (SAQ) cooking (Prehydrolysis: 150 °C, cooking: 160 °C for 3 h, SAQ dosage: 0.1%, liquor-to-bamboo ratio: 5 mL/g)

The most suitable pulp with a xylan content of 5.65% was selected for the bleaching process. However, the xylan content in the unbleached pulp may be still a little high compared to dissolving pulp quality (α-cellulose content: >94%). In dissolving pulp production, the cellulose should contain minimal residual hemicellulose and lignin to minimize cellulose degradation. To remove hemicellulose further, the additional treatment of CCE was employed with 8% NaOH aqueous solution, and then the xylan residue in the pulp decreased from 5.65% to 5.19%.

Therefore, an increase in the AA dosage to 28% with 2-MAQ 0.06% was applied to produce prehydrolysis soda pulp for high-grade dissolving pulp. **Table 2-3** shows the data from soda cooking with SAQ and 2-MAQ at dosages of 0.1% and 0.06%, respectively. It was inferred that soda pulp with AA dosage of 28% might provide a lower pulp yield, kappa number, and xylan content than with AA dosage of 25%. However, the pulp yield obtained by soda cooking with 2-MAQ did not decrease significantly. With 2-MAQ of 0.06%, the xylan content reduced to 4.00%, which did not require further treatment of CCE to remove xylan from the pulp.

To catalyze soda cooking of loblolly pine (*Pinus taeda*), 2-MAQ enabled a lower kappa number and higher pulp yield than AQ when used with 0.1% (Leyva et al., 1998). In another study, Francis et al. (2006) compared soda-AQ cooking with soda-2-MAQ cooking for sugar maple (*Acer Saccharum*) and found that soda-AQ pulp was of low quality, whereas soda-2-MAQ pulp was equivalent to kraft pulp. A report by the International Agency for Research on Cancer (IARC) studied whether the potentially mutagenic or carcinogenic properties of AQ, which may impact human health, were caused by the impurities of AQ (IARC- International Agency for Research on Cancer, 2012). Recently, Anita et al. (2021) reported that the residual amounts of 2-MAQ in eucalyptus pulp obtained from kraft cooking with 2-MAQ were trace and that Ames tests indicated that 2-MAQ was not mutagenic. Therefore, our study selected 2-

MAQ of 0.06% to replace AQ, and 2-MAQ was shown to be a more effective catalyst than AQ for prehydrolysis soda cooking of moso bamboo.

Table 2-3 Xylan content of pulp obtained from soda-soluble anthraquinone (SAQ) cooking and soda-2-methylanthraquinone (2-MAQ) cooking (Prehydrolysis: 150 °C for 7 h, cooking: 160 °C for 3 h, liquor-to-bamboo ratio: 5 mL/g)

Cooking condition	Pulp yield (%)	Kappa number	Xylan (%)	Brightness (% ISO)
Soda-SAQ 0.1%, 25% active alkali	30.6	10.9	5.65	45.0
Soda-2-MAQ 0.06%, 28% active alkali	29.5	9.1	4.00	47.4

2.3.4 Bleaching of pulp obtained from prehydrolysis soda cooking with SAQ and 2-MAQ

As shown in **Table 2-4**, bleached pulp from prehydrolysis soda-2-MAQ 0.06% with AA dosage 28% (O-D_0/P_{sa}-E_p-D_1) showed similar yield and α-cellulose content (25.8% and 95.0%, respectively) compared to that obtained from prehydrolysis soda-SAQ 0.1% with AA dosage 25% (O-CCE-D_0/P_{sa}-E_p-D_1). The α-cellulose content of moso bamboo pulp was higher than those of *A. crassicarpa* pulp (Utami et al., 2021) and sugarcane bagasse (Maryana, et al., 2017). According to Yuan et al., 2017, the bamboo dissolving pulp which was produced by treating with 12% NaOH at 95 °C for 150 min followed by kraft cooking with effective alkali 22%, sulfidity 25% at 170°C for 80 min, then employed with xylanase treatment (8 U/g) and cold caustic extraction (at 6% NaOH) before ECF bleaching sequence provided dissolving pulp with α-cellulose content 97%, hemicellulose 1.36 %, viscosity 6.25 mPa.s, and brightness 87.8 % ISO. Although our study showed a marginally lower α-cellulose and higher hemicellulose, the viscosity and the brightness were higher by only water prehydrolysis soda-2-MAQ 0.06% with AA dosage 28% without xylanase treating and cold caustic extraction before ECF bleaching (O-D_0/P_{sa}-E_p-D_1).

These results demonstrate that increasing the AA dosage to 28% in soda-2-MAQ 0.06% cooking of the prehydrolysis moso bamboo chips can produce good quality dissolving pulp without CCE. The brightness, α-cellulose content, viscosity, and ash content of the dissolving pulp were 89.9% ISO, 95.0%, 8.8 mPa·s, and 0.0%, respectively.

Table 2-4 Bleaching ability of prehydrolysis soda-soluble anthraquinone (SAQ) and soda-2-methylanthraquinone (2-MAQ)

	Soda-SAQ 0.1%, active alkali 25%				Soda-2-MAQ 0.06%, active alkali 28%			
	Brightness (% ISO)	Viscosity (mPa·s)	Yield (%)	α-Cellulose content (%)	Brightness (% ISO)	Viscosity (mPa·s)	Yield (%)	α-Cellulose content (%)
O	62.4	18.2	28.3	-	64.6	15.7	27.7	-
CCE	64.1	17.7	27.5	-	-	-	-	-
D_0/P_{sa}	72.6	12.2	27.0	-	71.9	11.1	26.9	-
E_p	85.0	10.4	26.2	-	84.5	8.9	26.3	-
D_1	89.7	10.2	25.9	95.3	89.9	8.8	25.8	95.0

Note: [a]Moso bamboo pulp (Prehydrolysis: 150 °C for 7 h, cooking: 160 °C for 3 h, liquor-to-bamboo ratio: 5 mL/g)

[b]Ash content of final bleached pulps: 0.00%

Some bamboo species have high ash contents, which have adverse effects on processing machinery. Therefore, mineral removal before cooking and bleaching processes are critical for dissolving pulp production processes. The mineral contents of bamboo mostly consist of inorganic mineral, such as silica, calcium, and potassium, whereas manganese and magnesium are the two common minerals (Li, 2004). Due to the links of metal cations to carboxyl groups of wood components, and lowered prehydrolysis pH, most of the groups become protonated, which causes the release of metal ions (Lindgren et al. 2002). Another factor that leads to the removal of minerals at acidic pH is metal salt dissolution. As in **Table 2-5**, prehydrolysis also had a significant effect on ash content from the material; approximately 60% of ash content was removed during prehydrolysis.

Table 2-5 The removal of ash during pre-hydrolysis, cooking and bleaching

Sample	Ash content (%)
3 h Prehydrolysis wood residue	0.53
5 h Prehydrolysis wood residue	0.48
7 h Prehydrolysis wood residue	0.48
Unbleached pulp[a]	0.05
Bleached pulp[a,b]	0.00

Note: [a] Moso bamboo pulp (Prehydrolysis: 150 °C for 7 h, Soda-2-MAQ cooking: 160 °C for 3 h, liquor-to-bamboo ratio: 5 mL/g)

[b] O-D_0/P_{sa}-E_p-D_1

2.3.5 Characterization of lignin precipitate obtained by prehydrolysis soda cooking

According to the concept of biorefinery, a part of the dissolved products after the soda cooking should be recovered for conversion into green chemicals and fuel. The solid content of organic and inorganic compounds providing a high yield of lignin precipitate was in the range of 27%–30% for softwood black liquor, whereas that for hardwood black liquor was 30%–35% (Tomani, 2010).

The results in **Table 2-6** show the yield of the lignin precipitate from the bamboo black liquor after CO_2 charging. The ash content of the freeze-dried solid was 52.6%, and the solid contents of organic and inorganic compounds in the prepared black liquor were 17%, 31%, and 35%, with precipitate yields of 20%, 38%, and 42%, respectively. The yield of lignin precipitate was observed to be strongly influenced by the total solid content in the black liquor.

Uloth (1989) compared three procedures of lignin isolation (acid precipitation using sulfuric acid, carbon dioxide precipitation, and ultrafiltration), and reported that acid precipitation resulted in high lignin amounts. The three procedures were used to separate lignin with low ash content; the first was precipitated at pH 8.8 by CO_2 charging (lignin CO_2-precipitate). After washing with water, the pH of the mixture increased slightly to 9.9. The second was precipitated by CO_2 followed by H_2SO_4 washing (lignin CO_2-H_2SO_4-precipitate), and the third was precipitated at pH 2.9 by H_2SO_4 (lignin H_2SO_4-precipitate). As shown in **Table 2-6**, lignin CO_2-precipitate had higher ash content than lignin CO_2-H_2SO_4-precipitate and lignin H_2SO_4-precipitate. Lignin CO_2-H_2SO_4-precipitate showed good results, with low xylan and ash levels.

Table 2-6 Lignin precipitate yield and chemical composition of lignin precipitate obtained from black liquor of prehydrolysis soda-2-methylanthraquinon (2-MAQ) cooking

Precipitation	Solid concentration (%)	Yield (%)	Ash (%)	Acid-insoluble lignin (%)	Acid-soluble lignin (%)	Xylan (%)
CO_2 (pH 8.8–9.9)	17	20	35.7	-	-	-
	31	38	37.1	-	-	-
	35	42	38.3	-	-	-
CO_2 (pH 8.8) and H_2SO_4 (pH 2.9)	35	-	0.6	87.6	7.7	2.7
H_2SO_4 (pH 2.9)	35	-	0.9	86.7	9.9	3.0

Molecular weight distribution is an important property of polymers. The molecular weight distribution of lignin CO_2-H_2SO_4 precipitate is shown in **Figure 2-4**. Lignin precipitated at pH 8.8 by CO_2 showed a marginally higher weight-average molecular weight (M_w) and number-average molecular weight (M_n) than lignin CO_2-H_2SO_4-precipitate and H_2SO_4-precipitate (**Table 2-7**). This result is in agreement with those of previous studies (Pakkanen and Alén, 2012; Kumar et al., 2016) on hardwood lignin from soda-AQ black liquor. A part of lignin precipitated by CO_2 charging contains ionically bound-sodium in comparison with the acidified lignin fraction because the pK_a values of phenolic hydroxyl groups are 9–11 and those of carboxylic acid groups are 3–5. Generally, at higher pH, the lignin precipitate fraction had a higher molecular weight, whereas at lower pH, lignin precipitates had a lower molecular weight (García et al., 2009; Santos et al., 2014; Kumar et al., 2016; Zhu et al., 2015).

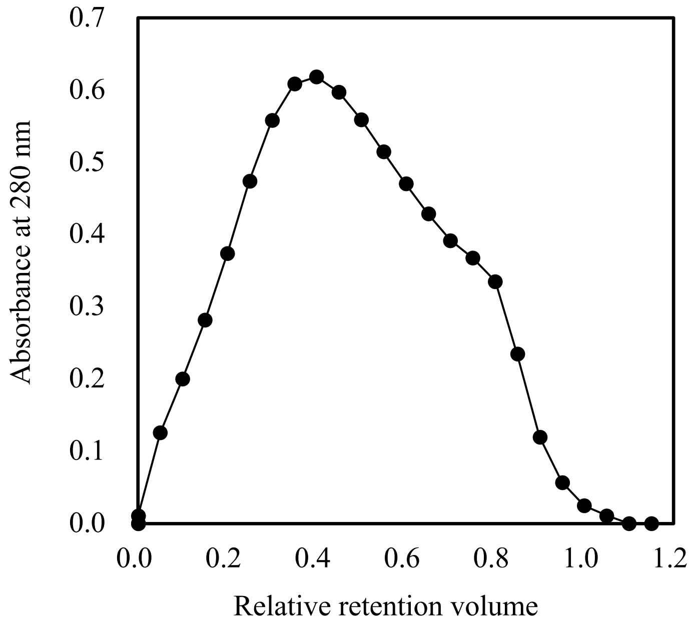


Figure 2-4 Molecule-weight distribution of lignin precipitate (by CO_2-H_2SO_4) obtained from prehydrolysis soda-2-methylanthraquinone cooking of moso bamboo

(Sephadex G-50 column: φ 18 mm × 500 mm, Effluent: 0.5 mol/L NaOH)

Table 2-7 Weight-average molecular weight (M_w), number-average molecular weight (M_n), and polydispersity (M_w/M_n) of lignin precipitate obtained from black liquor of prehydrolysis soda-2-methylanthraquinone cooking of moso bamboo

Precipitation	M_w	M_n	M_w/M_n
CO_2 (pH 8.8)	1752	640	2.74
CO_2 (pH 8.8) and H_2SO_4 (pH 2.9)	1566	598	2.62
H_2SO_4 (pH 2.9)	1505	605	2.49

According to **Figure 2-5**, the contents of glucan, xylan, and lignin in 100 g moso bamboo material, which consisted of prehydrolyzed residue (81 g) and hydrolysate (17 g), were 42 g, 20 g, and 30 g, respectively. The hydrolysate contains xylan fragment, which can be separated into each xylooligomer (6 g) and used for generating furfural. The black liquor from soda-2-MAQ cooking and oxygen-delignification contains approximately 53 g of bamboo components, including dissolved lignin (28 g), other organic compounds (25 g), and inorganic compounds (58 g as ash content), and provides 46 g of precipitates (containing 18 g ash) by carbon dioxide treatment. Approximately 25 g of lignin can be isolated by carbon dioxide and sulfuric treatments. Furthermore, the soda lignin can be obtained without emission of any sulfur compounds. Dissolving pulp with good qualities is obtained with a yield of 26 g, whereas organic components of 40 g in the hydrolysate and black liquor can be used as bioenergy in a recovery boiler. The bleaching effluent contains a small amount (2 g) of organic compounds.

The results of the present study reveal that prehydrolysis soda-2-MAQ cooking has an advantage over the kraft process as a bamboo biorefinery process.

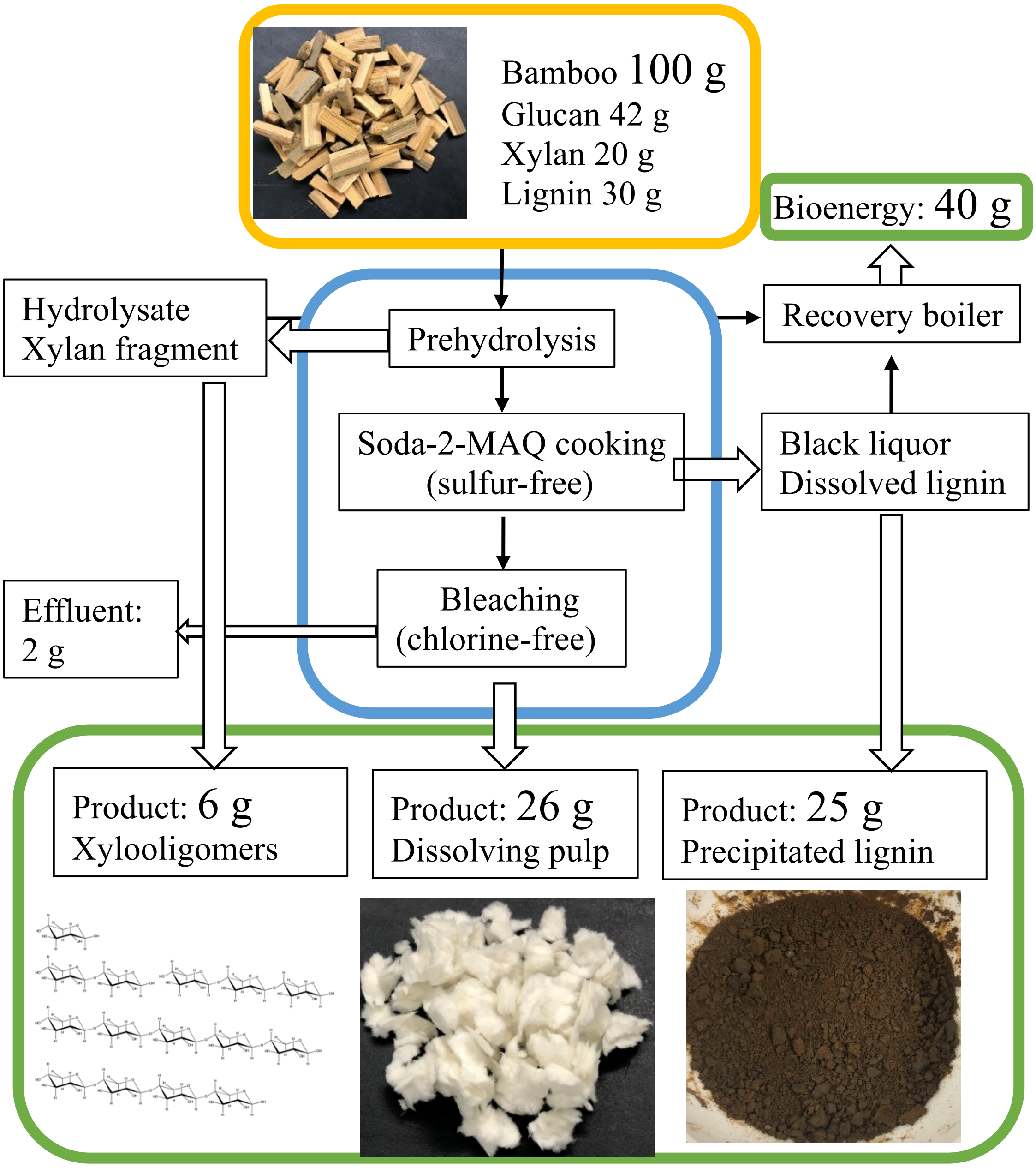


Figure 2-5 Bamboo biorefinery by prehydrolysis soda-2-methylanthraquinone (MAQ) cooking

2.4 Conclusion

In this study, we attempted to simplify the process of cooking especially with respect to using bamboo as the source material instead of wood. Prehydrolysis for 7 h efficiently dissolved hemicellulose from bamboo with little degradation of cellulose. The pulp obtained by the combination of 7 h prehydrolysis with soda-2-methylanthraquinone (2-MAQ) cooking with active alkali dosage of 28% and 0.06% 2-MAQ for 3 h at 160 °C provided a final α-cellulose content of 95.0%, brightness of 89.9%, and viscosity of 8.8 mPa·s for dissolving pulp. In addition, the lignin precipitate by CO_2 charging and H_2SO_4 washing showed low ash and xylan contents compared with that by H_2SO_4 washing alone. The weight-average molecular weight (M_w) and number-average molecular weight (M_n) were 1566 and 598, respectively.

References

Akiyama, T., Goto, H., Nawawi, D.S., Syafii, W., Matsumoto, Y., Meshitsuka, G., 2005. Erythro/threo ratio of β-*O*-4-structures as an important structural characteristic of lignin. Part 4: Variation in the erythro/threo ratio in softwood and hardwood lignins and its relation to syringyl/guaiacyl ratio. Holzforschung. 59(3), 276–281. doi:10.1515/HF.2005.045

Ambjörnsson, H.A., Östberg, L., Schenzel, K., Larsson, P.T., Germgard, U., 2014. Enzyme pretreatment of dissolving pulp as a way to improve the following dissolution in NaOH/ZnO. Holzforschung. 68(4), 385–391. doi:10.1515/hf-2013-0070

Andrade, M.F., Colodette, J.L., 2014. Dissolving pulp production from sugar cane bagasse. Ind Crops Prod. 52, 58–64. doi:10.1016/j.indcrop.2013.09.041

Anita, Y., Putra, A. S., Tanifuji, K., Nakagawa-izumi, A., Ohi, H., Evelyn, E., 2021. Kraft cooking with teak wood extract and determining residual 2-methylanthraquinone in eucalyptus pulp. Japan Tappi J. 75(2), 153–163. doi: 10.2524/jtappij.75.153

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. BioResources. 7(1), 640–651. doi:10.15376/biores.7.1.0640-0651

Behin, J., Zeyghami, M., 2009. Dissolving pulp from corn stalk residue and waste water of Merox unit. Chem Eng J. 152(1), 26–35. doi:10.1016/j.cej.2009.03.024

Borrega, M., Nieminen, K., Sixta H., 2011. Degradation kinetics of the main carbohydrates in birch wood during hot water extraction in a batch reactor at elevated temperatures. Bioresour Technol. 102(22), 10724–10732. doi:10.1016/j.biortech.2011.09.027

Chang, V.S., Holtzapple, M.T., 2000. Fundamental factors affecting biomass enzymatic reactivity. Appl Biochem Biotechnol - *Part A Enzym Eng Biotechnol*. 84–86, 5–37.

doi:10.1385/abab:84-86:1-9:5

Cherubini, F., 2010. The biorefinery concept: Using biomass instead of oil for producing energy and chemicals. Energy Conversion and Management. 51(7), 1412–1421. doi: 10.1016/j.enconman.2010.01.015.

Do, V.T., Tanifuji, K., Jin, G., Ohi, H., 2020. Neutral sulfite semi–chemical pulping of moso–bamboo. Japan Tappi J. 74(4), 379–387. doi:10.2524/jtappij.74.379

Doherty, W.O.S., Mousavioun, P. and Fellows C. M., 2011. Value-adding to cellulosic ethanol: Lignin polymers. Industrial Crops and Products. 33(2), 259–276.

FitzPatrick, M., Champagne, P., Cunningham, M.F. and Whitney, R.A., 2010. A biorefinery processing perspective: treatment of lignocellulosic materials for the production of value-added products. Bioresource technology. 101(23), 8915–8922.

Francis, R.C., Shin, S.J., Omori, S., Amidon, T.E., Blain, T.J., 2006. Soda pulping of hardwoods catalyzed by anthraquinone and methyl substituted anthraquinones. J Wood Chem Technol. 26(2), 141–152. doi:10.1080/02773810600701737

Francis, R.C., Bolton, T.S., Abdoulmoumine, N., Lavrykova, N., Bose, S.K., 2008. Positive and negative aspects of soda/anthraquinone pulping of hardwoods. Bioresour Technol. 99(17), 8453–8457. doi:10.1016/j.biortech.2008.02.055

García, A., Toledano, A., Serrano, L., Egüés, I., González, M., Marín, F. and Labidi, J., 2009. Characterization of lignins obtained by selective precipitation. Separation and Purification Technology. 68(2), 193–198.

Garrote, G., Domínguez, H., Parajó, J.C., 2001. Generation of xylose solutions from *Eucalyptus globulus* wood by autohydrolysis-posthydrolysis processes: Posthydrolysis kinetics. Bioresour Technol. 79(2), 155–164. doi:10.1016/S0960-8524(01)00044-X

Garrote, G., Domínguez, H., Parajó, J.C., 1999. Mild autohydrolysis: an environmentally friendly technology for xylooligosaccharide production from wood. J Chem Technol Biotechnol. 74, 1101–1109. doi:10.1002/(SICI)1097-4660(199911)74:11<1101::AID-JCTB146>3.0.CO;2-M

Gosselink, R.J.A., Abächerli, A., Semke, H., Malherbe, R., Käuper, P., Nadif, A. and Van Dam, J.E.G., 2004. Analytical protocols for characterisation of sulphur-free lignin. Industrial Crops and Products. 19(3), 271–281.

Harsono, H., Putra, A.S., Maryana, R., Rizaluddin, A. T., H'ng, Y. Y., Nakagawa-izumi, A., Ohi, H., 2016. Preparation of dissolving pulp from oil palm empty fruit bunch by prehydrolysis soda-anthraquinone cooking method. J Wood Sci. 62(1), 65–73. doi:10.1007/s10086-015-1526-3

Hassan, N.H.M., Muhammed, S., Ibrahim, R., 2013. Effect of soda-anthraquinone pulping conditions and beating revolution on the mechanical properties of paper made from *Gigantochloa scortechinii* (Semantan bamboo). Malaysian J Anal Sci. 17(1), 75–84.

IARC- International Agency for Research on Cancer. 2012. Some chemicals present in industrial and consumer products, food and drinking-water. IARC Monogr Eval Carcinog Risks Hum. 101, 9–549.

Ibarra, D., Köpcke, V., Larsson, P.T., Jääskeläinen, 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(19), 7416–7423. doi:10.1016/j.biortech.2010.04.050

Jahan, M.S., 2009. Studies on the effect of prehydrolysis and amine in cooking liquor on producing dissolving pulp from jute (Corchorus capsularis). Wood Sci Technol. 43(3–4), 213–224. doi:10.1007/s00226-008-0213-6

Jahan, M.S., Rahman, M.M., 2012. Effect of pre-hydrolysis on the soda-anthraquinone pulping of corn stalks and Saccharum spontaneum (kash). Carbohydr Polym. 88(2), 583–588. doi:10.1016/j.carbpol.2012.01.005

Jahan, M.S., Islam, M.A., Rahman, M.M., Nayeem, J., Ahmed, S., Quaiyyum, M.A., 2015. Steam and hot water prehydrolysis of bamboo and its effect on residual lignin structureand pulping. Cellul Chem Technol. 51(5–6), 455–463.

Jin, Z., Jin, G., Shao, S., Katsumata, K.S., 2012. Lignin characteristics of bast fiber and core in kenaf, bark and wood of paper mulberry and mulberry. J Wood Sci. 58(2), 144–152. doi:10.1007/s10086-011-1228-4

Kumar, H., Alén, R., Sahoo, G., 2016. Characterization of hardwood soda-AQ lignins precipitated from black liquor through selective acidification. BioResources. 11(4), 9869–9879. doi:10.15376/biores.11.4.9869-9879

Leh, C.P., Rosli, W.D.W., Zainuddin, Z., Tanaka, R., 2008. Optimisation of oxygen delignification in production of totally chlorine-free cellulose pulps from oil palm empty fruit bunch fibre. Ind Crops Prod. 28(3), 260–267. doi:10.1016/j.indcrop.2008.02.016

Leschinsky, M., Sixta, H., Patt, R., 20009. Detailed mass balances of the autohydrolysis of eucalyptus globules at 170ºC . BioResources. 4(2), 687–703.

Leyva, A., Dimmel, D. R., Pullman, G. S., 1998. Teak extract as a catalyst for the pulping of loblolly pine. TAPPI J. 81(5), 237–240.

Li, X.B., 2004. Physical, chemical, and mechanical properties of bamboo and its utilization potential for fiberboard manufacturing. LSU Master's Theses. 866

Lindgren, K., Berglin, N., Brelid, H., 2002. Effects of the use of an NPE chip Kidney in a closed bleach plant. TAPPI International Pulp Bleaching Conference, 2002.

Luo, X., Liu, J., Wang, H., Huang, L., Chen, L., 2014. Comparison of hot-water extraction and steam treatment for production of high purity-grade dissolving pulp from green bamboo. Cellulose. 21(3), 1445–1457. doi:10.1007/s10570-014-0234-2

Ma, X., Huang, L., Chen, Y., Cao, S., Chen, L., 2011. Preparation of bamboo dissolving pulp for textile production. Part 1. Study on prehydrolysis of green bamboo for producing dissolving pulp. BioResources. 6(2), 1428–1439. doi:10.15376/biores.6.2.1428-1439

Ma, X., Huang, L., Cao, S., Chen, Y., Luo, X., Chen, L., 2012. Preparation of dissolving pulp from bamboo for textile applications. Part 2. Optimization of pulping conditions of hydrolyzed bamboo and its kinetics. BioResources. 7(2), 1866–1875. doi:10.15376/biores.7.2.1866-1875

Martino, D.C., Colodette, J.L., Jardim, J.M., Chandra, R.P., Saddler, J.N. Hot water pretreatment to enhance the production of a eucalypt dissolving pulp. Published online 2015.

Maryana, R., Nakagawa-izumi, A., Kajiyama, M., Ohi, H., 2017. Environment-Friendly Non-Sulfur Cooking and Totally ChlorineFree Bleaching for Preparation of Sugarcane Bagasse Cellulose. J Fiber Sci Technol. 73(8), 182–191. doi:10.2115/fiberst.2017-0025

Moradbak, A., Tahir, P.M., Mohamed, A.Z., Halis, R., 2016. Alkaline sulfite anthraquinone and methanol pulping of bamboo (*Gigantochloa scortechinii*). BioResources. 11(1), 235–248. doi:10.15376/biores.11.1.235-248

Nakagawa-Izumi, A., H’ng, Y.Y., Mulyantara, L.T., Maryana, R., Do, V.T., Ohi, H., 2017. Characterization of syringyl and guaiacyl lignins in thermomechanical pulp from oil palm empty fruit bunch by pyrolysis-gas chromatography-mass spectrometry using ion intensity calibration. Ind Crops Prod. 95, 615–620. doi:10.1016/j.indcrop.2016.11.030

Ohi, H., Ishizu, A., Nakano, J., 1988. Behavior of lignin during alkaline sulfite-quinone cooking. Japan Tappi J. 42(9), 861–870. doi:10.2524/jtappij.42.861

Pakkanen, H., Alén, R., 2012. Molecular mass distribution of lignin from the alkaline pulping of hardwood, softwood, and wheat straw. J Wood Chem Technol. 32(4):279–293. doi:10.1080/02773813.2012.659321

Putra, A.S., Nakagawa-izumi, A., Kajiyama, M., Ohi, H., 2018. Peer Reviewed Biorefinery of Oil Palm Empty Fruit Bunch by Nitric Acid Prehydrolysis Soda Cooking. Production of Furfural and Dissolving Pulp. Japan Tappi J. 72(6), 641–649. doi:10.2524/jtappij.1701

Rizaluddin, A.T., Liu, Q., Panggabean, P.R., Ohi, H., Nakamata, K., 2015. Application of peroxymonosulfuric acid as a modification of the totally chlorine-free bleaching of acacia wood prehydrolysis-kraft pulp. J Wood Sci. 61(3), 292–298. doi:10.1007/s10086-015-1465-z

Rudie, A., Reiner, R., Ross-Sutherland, N., Kenealy, W. Acid Prehydrolysis of Wood. Published online 2007.

Runge, T., Houtman, C., Heinricher, J., 2012. Timber bamboo pulp. *2012 TAPPI PEERS Conf Build a Sustain Futur*. 12(2), 315–322.

Salaghi, A., Putra, A.S., Maryana, R., Kajiyama, M., Ohi, H., 2018. Preparation of dissolving pulp by totally chlorine-free bleaching: Roles of hardwood syringyl and guaiacyl lignins. Japan Tappi J. 72(10), 1167–1175.

Salaghi, A., Putra, A.S., Rizaluddin, A.T., Kajiyama, M., Ohi, H., 2019. Totally chlorine-free bleaching of prehydrolysis soda pulp from plantation hardwoods consisting of various lignin structures. J Wood Sci. 65(1). doi:10.1186/s10086-019-1785-5

Santos, P.S.B. do., Erdocia, X., Gatto, D.A., Labidi, J., 2014. Characterisation of Kraft lignin separated by gradient acid precipitation. Ind Crops Prod. 55, 149–154. doi:10.1016/j.indcrop.2014.01.023

Schild, G., Sixta, H., Testova, L., 2010. Multifunctional alkaline pulping, delignification and hemicellulose extraction. Cellul Chem Technol. 44(1–3), 35–45.

Scurlock, J.M.O., Dayton, D.C., Hames, B., 2000. Bamboo: An overlooked biomass resource? Biomass and Bioenergy. 19(4), 229–244. doi:10.1016/S0961-9534(00)00038-6

Sharma, N., Godiyal, R.D., Thapliyal, B.P. and Anupam, K., 2018. Pulping and bleaching of hydro distillation waste of citronella grass (Cymbopogon winterianus Jowitt) for papermaking. Waste and biomass valorization. 9(3), 409–419.

Shi, H., Zhou, M., Jia, W., Li, N., Niu, M., 2019. Balancing the effect of pretreatment severity on hemicellulose extraction and pulping performance during auto-hydrolysis prior to kraft pulping of acacia wood. Biotechnol Prog. 35, e2784. doi:10.1002/btpr.2784

Singha, B.L., Hassan, Y., 2017. Analysis of carbohydrate, moisture content and specific gravity of *Bambusa tulda* with special reference to its harvesting season. Int J Sci Res. 6(1), 279–283. doi:10.21275/art20163989

Sixta, H., Koch, G., Ressel, J.B., Potthast, A., Krotschek, A.W., 2006. The History of Paper Making. Vol 1. doi:10.1016/S0268-0890(97)90050-5

Sugesty, S., Kardiansyah, T., Hardiani, H., 2015. Bamboo as raw materials for dissolving pulp with environmental friendly technology for rayon fiber. Procedia Chem. 17(December):194–199. doi:10.1016/j.proche.2015.12.122

Syamsu, K., Fahma, F. and Pari, G., 2019. Structure analysis of three non-wood materials for liner paper. Nordic Pulp & Paper Research Journal. 34(4), 453–466.

Tomani, P., 2010. The lignoboost process. Cellul Chem Technol. 44(1-3), 53–58.

Tunc, M.S., Heiningen, A.V., 2008. Hydrothermal dissolution of mixed southern hardwoods. Holzforschung. 62, 539–545. doi:10.1515/HF.2008.100

Uloth, V., 1989. “Kraft Lignin Recovery: Acid Precipitation Versus Ultrafiltration, Part I: Laboratory Test Results”, by V.C. Uloth and J.T. Wearing, Pulp and Paper Canada, 90 (9): T310–T314.

Utami, S.P., Tanifuji, K., Putra, A.S., Nakagawa-Izumi, A., Ohi, H., Evelyn, E., 2021. Effects of soluble anthraquinone application on prehydrolysis soda cooking of *Acacia crassicarpa* wood. Japan Tappi J. 75(4), 373–379. doi:10.2524/jtappij.75.373

Vishtal, A. and Kraslawski, A., 2011. Challenges in industrial applications of technical lignins. BioResources, 6(3), 3547–3568. doi: 10.15376/biores.6.3.vishtal.

Wan Rosli, W.D., Leh, C.P., Zainuddin, Z., Tanaka, R., 2003. Optimisation of soda pulping variables for preparation of dissolving pulps from oil palm fibre. Holzforschung. 57(1), 106–113. doi:10.1515/HF.2003.017

Wu, C., Zhou, S., Zhao, C., Wang, D., 2014. Improved reactivity of bamboo dissolving pulp for the viscose process: Post-treatment with beating. BioResources. 9(2), 3449–3455. doi:10.15376/biores.9.2.3449-3455

Yuan, Z., Wen, Y., Kapu, N.S., Beatson, R., Mark Martinez, D., 2017. A biorefinery scheme to fractionate bamboo into high-grade dissolving pulp and ethanol. Biotechnol Biofuels. 10(1), 1–16. doi:10.1186/s13068-017-0723-2

Zhu, W., Westman, G., Theliander, H., 2015. The molecular properties and carbohydrate content of lignins precipitated from black liquor. Holzforschung. 69(2), 143–152. doi:10.1515/hf-2014-006

Chapter 3. Behaviors of xylan during bamboo biorefinery

3.1 Introduction

Generally, hemicellulose in hardwood is xylan, whereas it is glucomannan in softwood. Many plant xylan materials are heteropolysaccharides with homopolymeric backbone chains of 1,4-linked β-D-xylopyranose units (Saha, 2003). During hemicellulose isolation from lignocellulosic material, it can be transformed into many forms through partial hydrolysis. In hydrolysis of hemicelluloses, other than the expected products, some products might also be detected. Xylooligomer (degree of polymerization from 2-7) products after hydrolysis of hemicelluloses are considered the important products for functional food or dietary fibers and are used as food supplements for health benefits (Bhaumik & Dhepe, 2016).

Xylooligomers are used as food ingredients and are classified as nutraceuticals, which are mainly bioactive compounds and are considered non-digestible oligosaccharides, non-carcinogenic in humans, and have important biological properties. Xylooligomers are also used as dietary sweeteners in diet food and by diabetic individuals (Choque Delgado et al., 2011; Nabarlatz et al., 2007). In recent years, there has been increasing interest in novel prebiotics such as xylooligomers, which are produced by hydrolyzing xylan. Xylooligomers are oligomers containing 2–10 xylose molecules linked by β-1-4 bonds (Mäkeläinen et al., 2010).

Extraction of hemicellulose by hydrothermal processing (prehydrolysis) is an alternative method for the valorization of bamboo under the biorefinery approach. Prehydrolysis is an effective pretreatment approach that enables the recovery of soluble saccharides in oligomeric form. In prehydrolysis, only water is added without any chemicals, which confers an important benefit for the recovery process (Chem et al., 2022).

As in **Figure 2-2**, **Chapter 2** presents the saccharide content (%) data in prehydrolysate after 3, 5, and 7 h of prehydrolysis. Extension of the prehydrolysis time did not significantly increase xylose, whereas xylooligomer content increased from 1.51% to 5.68%. This indicates that approximately 28% of the bamboo xylan was dissolved into the prehydrolysate as xylooligomers. According to the results, the prehydrolysate obtained from 7 h prehydrolysis was an alternative source of the xylooligomer products. To determine the difference in the behavior of xylooligomers under different prehydrolysis times, the xylooligomers of the prehydrolysates were analyzed using ion chromatography.

This chapter mainly describes the analysis of the xylooligomers of bamboo prehydrolysate.

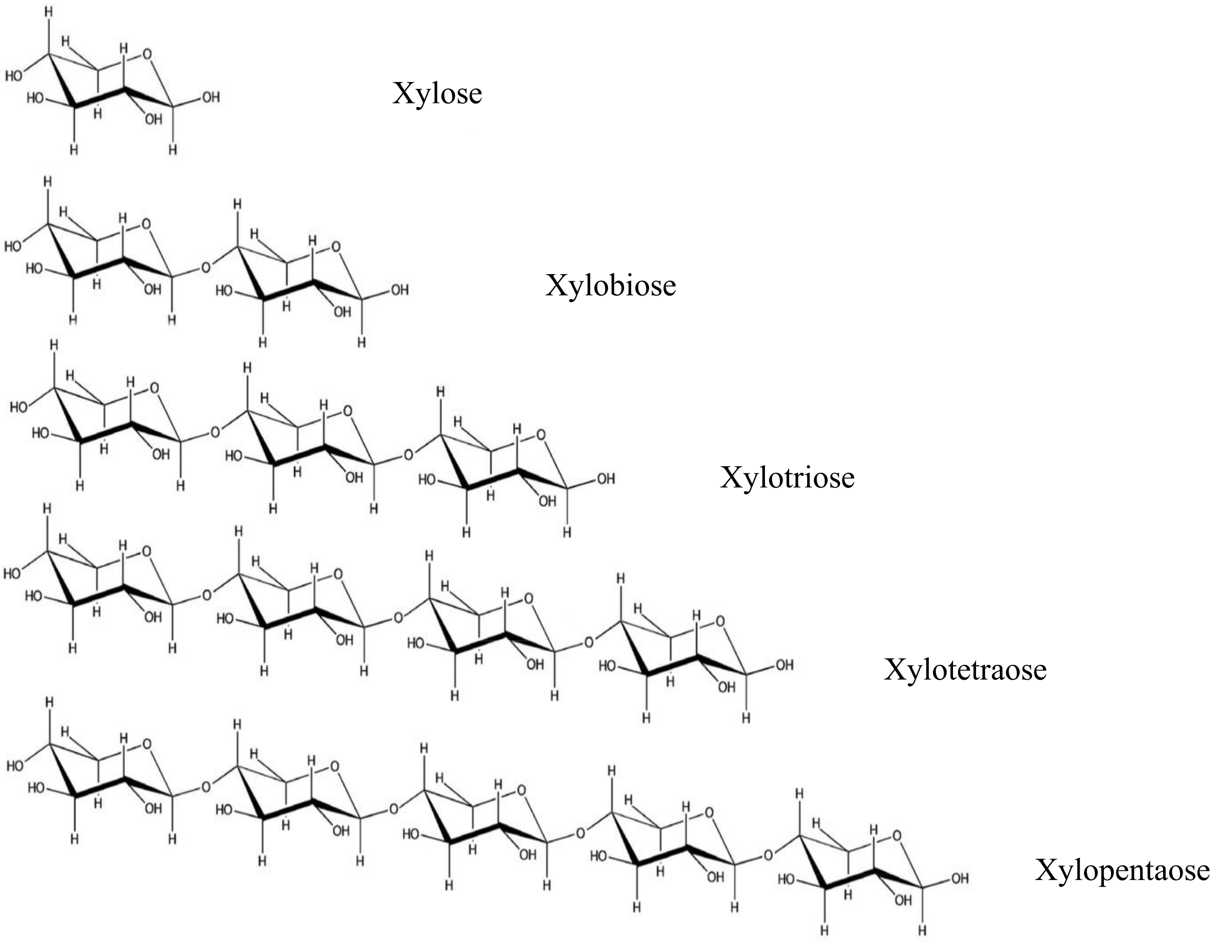


Figure 3-1 Chemical schematic structure of xylooligomer

3.2 Materials and Method

3.2.1 Materials

Prehydrolysis was conducted on 50 g oven-dried bamboo chips mixed with 245 mL of distilled water (liquid-to-bamboo ratio of 5 mL/g) at a temperature of 150 °C for various durations (3, 5, and 7 h). After the prehydrolysis, 200 mL of prehydrolysate was separated from the mixture. Subsequently, the prehydrolysate was kept at 4°C until further experiments.

3.2.2 Degree of polymerization analysis of prehydrolysate xylooligomers

The prehydrolysate liquid was passed through a 0.2 μM syringe filter and then analyzed using an ion chromatograph (IC) system to determine the xylooligomer chains. Specifically, the xylooligomer mixture was investigated using a Dionex ICS 3000 ion chromatography system (Dionex). The Dionex ICS 3000 Ion system was equipped with a pulsed amperometric detector, a single pump model, and a CarboPac PA1 column (φ 4 mm × 250 mm), CarboPac PA1 guard column (φ 4 mm × 50 mm). The column was equilibrated with 100 mM NaOH at 35 ℃ at a flow rate of 1 mL/min. Gradient elution was performed using 100 mM NaOH and 100 mM NaOH/1M CH_3COONa.

3.3 Results and Discussion

The standards of fucose and xylose were prepared for ion chromatography analysis to obtain the retention time. As shown in **Figure 3-2 (A),** the peak of fucose appeared at a retention time of 3.2 min, while that of the xylose standard appeared at a retention time of 5.4 min. The xylooligomer from prehydrolysate was obtained following 3 h of prehydrolysis. At a retention time of 5.4 min, xylose appeared, followed by other xylooligomers. Recently, Dionex corporation has been used to analyze oligosaccharides profiles. A study by Yang and Wyman on the separation of xylooligomer from corn stover by hydrothermal pretreatment showed that using a Dionex with a Carbopac PA100 column yielded well-separated peaks. In the study, xylose was detected, followed by other xylooligomers (xylobiose, xylotriose, xylotetraose, etc.) (Yang & Wyman, 2008). **Figure 3-2 (C)** shows the xylooligomers for prehydrolysate after 5 h of prehydrolysis. The peak corresponding to the xylooligomers increased compared to that observed after prehydrolysis for 3 h, and several peaks of xylooligomers appeared. **Figure 3-2 (D)** shows the xylooligomers observed for the prehydrolysate after 7 h of prehydrolysis. The extension of the time of prehydrolysis to 7 h yielded high peak values for xylooligomers. The results are consistent with the data illustrated in **Figure 2-2**. As discussed in **Chapter 2**, prehydrolysis time had a significant effect on xylooligomers.

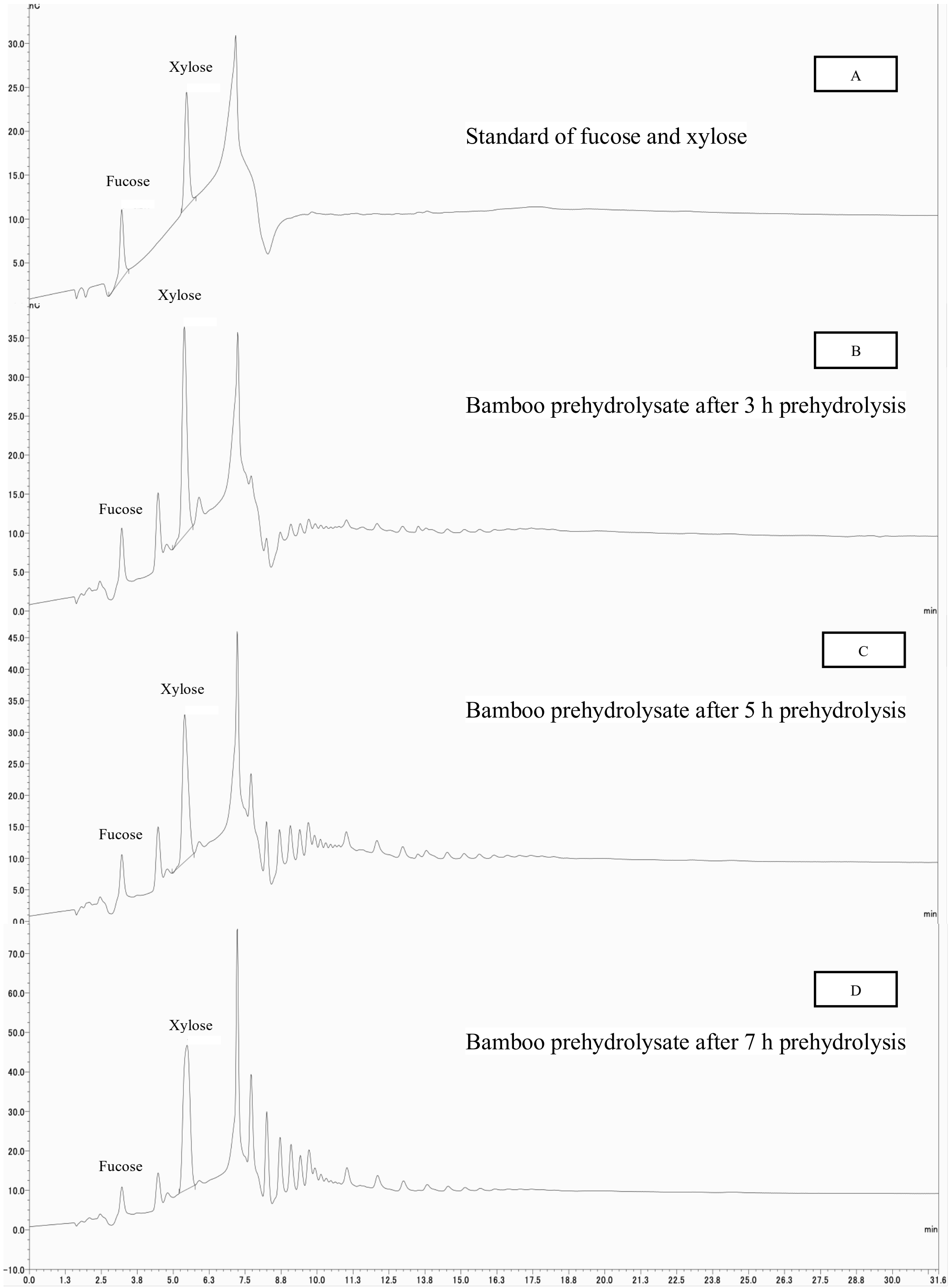


Figure 3-2 Peak area of ion chromatograph of xylooligomer.

Xu et al., (2013) conducted a study on xylooligomers using high-performance anion-exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD). The (HPAEC-PAD) analysis was performed using a Dionex ICS-3000 system (Dionex) equipped with an anion-exchange column, CarboPac PA200 (3 mm × 250 mm), in combination with a guard column, CarboPac PA200 (3 mm × 50 mm), at 30 °C. Two elutions were employed using 0.1M NaOH and 0.5 M CH_3COONa containing 0.1 M NaOH. The results showed that within 30 min, the standard peaks of xylose (X1), xylobiose (X2), xylotriose (X3), xylotetraose (X4), xylopentaose (X5), and xylohexaose (X6) were identified (Xu et al., 2013). Another study identified xylooligomers using a HPAEC-PAD system, using the Dionex ICS-5000 (Dionex) equipped with a CarboPac PA200 guard column and a CarboPac PA200 analytical column at 35 °C. The eluents were 100 mM sodium hydroxide and 100 mM/320 mM NaOH/CH_3COONa (Lau, 2012). In a study by Septiningrum et al., (2015), HPAEC-PAD was applied with a CarboPac PA-100 column (250 mm × 4 mm) and a PA-100 guard column (25 mm × 3 mm) at 30 °C. Gradient elution was performed with 100 mM NaOH and 100 mM NaOH/1 M CH_3COONa at a flow rate of 1 mL/min. The different retention times of xylooligomers in each study are showed in **Table 3-1**.

The Dionex ICS 3000 (Dionex) was also used in the present study to identify xylooligomers from bamboo prehydrolysate. However, the retention times of xylooligomers X2–X6 from our study were quite different from those in other studies (Lau et al., 2012; Xu et al., 2013; Septiningrum et al., 2015). The result might be explained based on the varying concentrations of eluents or types of CarboPac PA used for detection. According to Xu et al., (2013), xylose and xylobiose peaks appeared at retention times of 4.25 min and 5.68 min, respectively, as showed in **Table 3.1**. The delta retention time (Δt) in Xu et al., (2013) and the result of the present study showed similar trends, with xylobiose peaks appearing in at a retention time of 7.72 min, as showed in **Figure 3-3** and **Table 3-2**.

Table 3-1 Retention times (min) of xylooligomers products from literature

Product	Retention time (min) (Xu et al., 2013)	Retention time (min) (Lau, 2012)	Retention time (min) (Septiningrum et al., 2015)
Xylose (X1)	4.25	2.40	2.80
Xylobiose (X2)	5.68	2.70	
Xylotriose (X3)	8.08	3.30	4.90
Xylotetraose (X4)	10.82	4.30	
Xylopentaose (X5)	13.08	6.00	
Xylohexaose (X6)	15.10	9.00	

Several peak areas were identified in the present study. Although each peak area cannot clarify the type of each xylooligomer, in the present study, fucose and xylose standards were used to obtain the peak areas in **Fig 3-2 (A)**. **Table 3-2** presents the peak area ratios of oligomers with fucose obtained by 3, 5, and 7 h prehydrolysis. It clearly shows the peak area ratio of each peak, which increases with the prolongation of the time of prehydrolysis to 7 h. Total peak area ratios of xylooligomers were 0.732, 2.520, and 6.419 at 3, 5, and 7 h prehydrolysis. We consider most of the peak areas obtained in the present study as xylooligomer products since the results in **Chapter 2 (Figure 2-2)** demonstrate that xylooligomer products were obtained following 7 h of prehydrolysis. Besides the xylooligomer products, we may obtain peaks of other products, such as hexenuronosyl xylotriose (ΔX3) (Septiningrum et al., 2015).

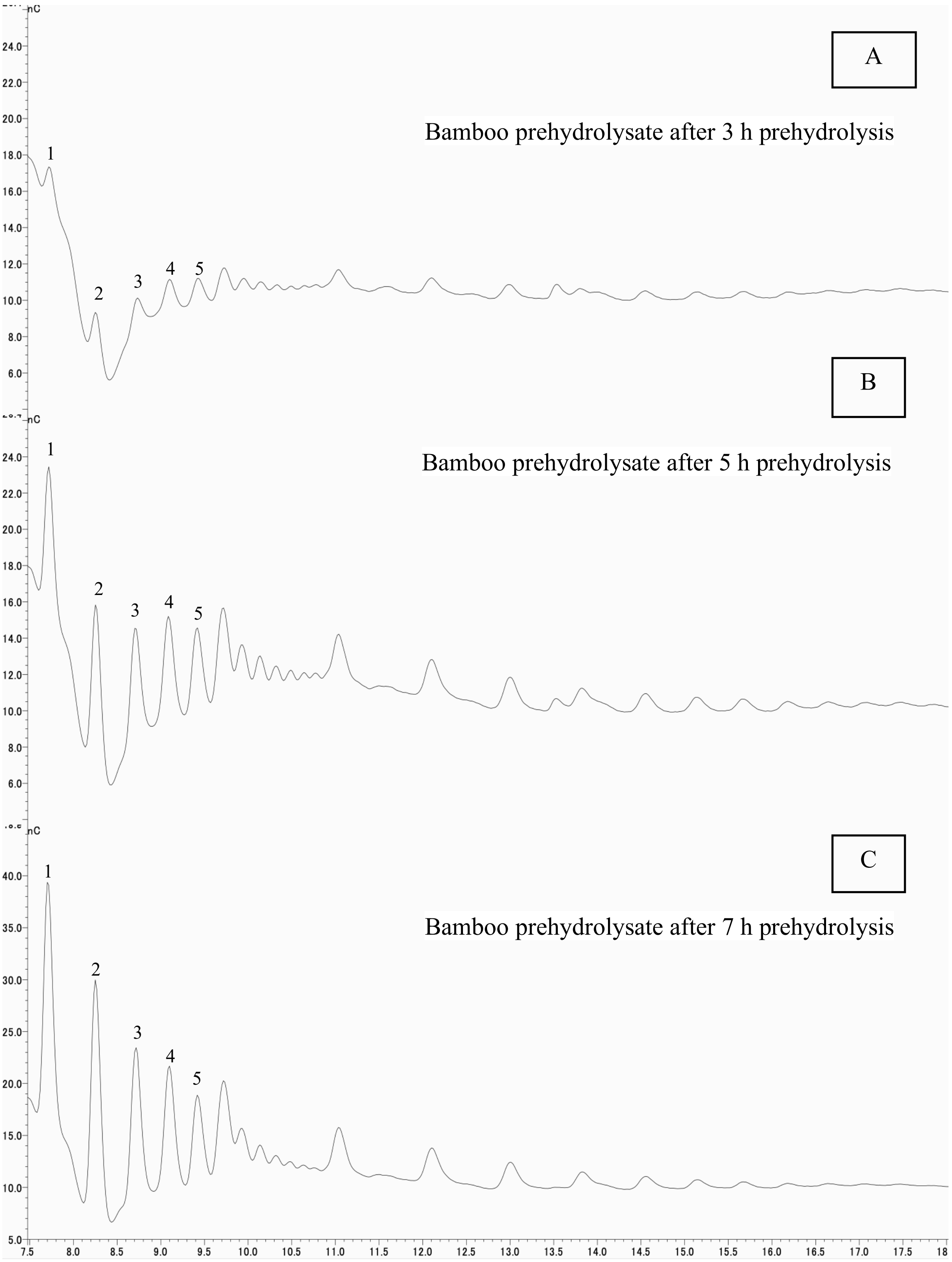


Figure 3-3 Peak areas of ion chromatograph of xylooligomers of bamboo prehydrolysate

Table 3-2 Peak area ratios of bamboo prehydrolysate with fucose standard.

Number of peaks	Retention time (min)	3h	5h	7h
Fucose	3.22	1.000	1.000	1.000
Xylose	5.40	3.415	3.181	5.830
1 (X2)	7.72	0.133	0.555	2.050
2 (X3)	8.25	0.119	0.616	1.619
3 (X4)	8.73	0.195	0.534	1.182
4 (X5)	9.10	0.163	0.467	0.950
5 (X6)	9.43	0.122	0.348	0.618
Total		0.732	2.520	6.419

3.4 Conclusion

Approximately 28% of the bamboo xylan was dissolved into the prehydrolysate as a mixture of xylooligomers at the final pH of 3.8. In addition, IC system analysis of the xylooligomers in bamboo prehydrolysate showed that 7 h of prehydrolysis achieved higher peaks. The results showed that the prehydrolysate obtained from 7 h of prehydrolysis was an alternative source of the xylooligomer products.

References

Bhaumik, P., Dhepe, P.L., 2016. Conversion of biomass into sugars. in: Biomass Sugars for Non-Fuel Applications. Green Chem. 44.

Chem, M., Tanifuji, K., Utami, S.P., Putra, A.S., Ohi, H., Nakagawa-Izumi, A., 2022. Development of dissolving pulp from *Phyllostachys pubescens* stem by prehydrolysis soda cooking with 2-methylanthraquinone. . Ind. Crops Prod. 178, 114570. https://doi.org/10.1016/j.indcrop.2022.114570.

Delgado, G.T.C., Tamashiro, W.M.D.S.C., Junior, M.R.M., Moreno, Y.M.F., Pastore, G.M., 2011. The putative effects of prebiotics as immunomodulatory agents. Food Res. Int. 44, 3167–73. https://doi.org/10.1016/j.foodres.2011.07.032.

Lau, C. S., 2012. Characterization and Quantification of Monomers, Oligomers, and by-Products from Xylan during Biomass Pretreatment. University of Arkansas, Fayetteville. Ph.D. Thesis.

Mäkeläinen, H., Forssten, S., Saarinen, M., Stowell, J., Rautonen, N., Ouwehand, A., 2010. Xylo-oligosaccharides enhance the growth of bifidobacteria and *Bifidobacterium lactis* in a simulated colon model. . https://doi.org/10.3920/BM2009.0025.

Nabarlatz, D., Ebringerová, A., Montané, D., 2007. Autohydrolysis of agricultural by-products for the production of xylo-oligosaccharides.Carbohydr. Polym. 69, 20–28. https://doi.org/10.1016/j.carbpol.2006.08.020.

Saha, B.C., 2003. Hemicellulose bioconversion. J Ind Microbiol Biotechnol 30(5):279–91. https://doi.org/10.1007/s10295-003-0049-x.

Septiningrum, K., Ohi, H., Waeonukul, R., Pason, P., Tachaapaikoon, C., Ratanakhanokchai, K., Sermsathanaswadi, J., Deng, L., Prawitwong, P., Kosugi, A., 2015. The GH67 α-glucuronidase of Paenibacillus curdlanolyticus B-6 removes hexenuronic acid groups and facilitates biodegradation of the model xylooligosaccharide hexenuronosyl xylotriose. Enzyme Microb. Technol. 71, 28–35. . https://doi.org/10.1016/j.enzmictec.2015.01.006.

Xu, Y., Fan, L., Wang, X., Yong, Q., Yu, S.Y., 2013. Simultaneous separation and quantification of linear xylo-and cello-oligosaccharides mixtures in lignocellulosics processing products on high-performance anion-exchange chromatography coupled with pulsed amperometric detection. BioResources 8, 3247–3259. https://doi.org/10.15376/biores.8.3.3247-3259.

Yang, B., Wyman, C.E., 2008. Characterization of the degree of polymerization of xylooligomers produced by flowthrough hydrolysis of pure xylan and corn stover with water. Bioresour. Technol. 99, 5756–5762. https://doi.org/10.1016/j.biortech.2007.10.054.

Chapter 4. Conclusive Remarks

Moso bamboo (*Phyllostachys pubescens)* is a non-woody and non-edible biorefinery resource and it is extremely important for the development of dissolving pulp as a material of regenerated cellulose fiber. However, the bamboo contains high xylan amounts when compared to hardwoods. The aim of the present study was to determine optimal conditions for the production of good quality dissolving pulp from the moso bamboo stem using a combination of prehydrolysis non-sulfur alkali cooking and elemental chlorine-free bleaching, and separation of lignin products from the black liquor in the cooking process. Prehydrolysis at 150 °C for 7 h using soda cooking at 160 °C for 3 h with 28% active alkali and 0.06% 2-methylanthraquinone had a favorable outcome. The xylan contents of the pulp were reduced to 4.0%, and further treatment with cold caustic extraction to remove xylan was not required for the production of dissolving pulp of good quality, although the pulp yield was approximately 30%. The pulp was then bleached to obtain a dissolving pulp with α-cellulose content of 95.0%, brightness of 89.9%, and viscosity of 8.8 mPa·s. Lignin dissolved in the soda cooking black liquor was easily precipitated by CO_2 when at a 35% solid concentration and had a yield of 42%. The precipitation by CO_2 followed by purification at pH 2.9 provided a lignin product with weight-average molecular weight, polydispersity, and ash content of 1566, 2.62, and 0.6%, respectively. Future studies may focus on xylooligomers obtained from prehydrolysate, as they are potentially valuable products and potential biorefinery sources.

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