Conversion of Lignin to Phenolic Monomers under Alkaline Conditions by using Hydrogen Peroxide with Copper (II) Oxide and Iron (III) Sulfate Catalysts

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การเปลี่ยนลิกนินเป็นสารประกอบฟีนอลิกโมเลกุลเดี่ยวในภาวะด่างโดยใช้ไฮโดรเจนเปอร์ออกไซด์ร่วมกับตัวเร่งปฏิกิริยาคอปเปอร์ (II) ออกไซด์ และไอร์ออน (III) ซัลเฟต

อติวิชญ์ อนันตกิจธำรง

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By Mr.Atiwich Anantkijthamrong
Field of Study Petrochemical Engineering
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Accepted by the Faculty of Engineering, King Mongkut's Institute of Technology Ladkrabang in Partial Fulfillment of the Requirements for the Degree of Bachelor of Engineering (Petrochemical Engineering).

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Abstract

In this study, conversion of lignin to phenolic monomers was performed in a two-stepwise manner. In the first stage, the amorphous components such as hemicelluloses and lignin of extractives-free sugarcane bagasse were hydrothermally liquefied. The effects of sodium hydroxide concentrations in a range of 1.0-4.0%w/v was explored using a 10-ml batch reactor at temperatures 100-200°C for 30 min. In the second stage, the oxidative depolymerization of the first-stage liquefied portion was then performed. Hydrogen peroxide, copper (II) oxide, and iron (III) sulfate were added at this stage, and carried out by varying sodium hydroxide concentration at 150°C for 30 min. The obtained results suggested that some specific values of sodium hydroxide concentration and temperature such as 4.0%w/v at 140°C, 3.0%w/v at 160°C, 2.0%w/v at 180°C, and 1.0%w/v at 200°C could be used as appropriate conditions in the first-stage hydrothermal liquefaction. It was demonstrated that the lower sodium hydroxide concentration was adopted, the higher temperature was required. As treated by those conditions, the removal degrees of hemicelluloses and lignin from sugarcane bagasse were 89.87-92.20wt% and 94.59-98.19wt%, respectively. This finding was confirmed by an increase in the crystallinity indexes of the first-stage residues in comparison with sugarcane bagasse. As for the second-stage oxidation products, three aldehyde phenolic monomers from three monolignol species of lignin were formed, i.e., vanillin, syringaldehyde, and p-hydroxybenzaldehyde. It was revealed that sodium hydroxide concentration played a significant role in lignin depolymerization under alkaline condition in the presence of metal catalysts. A larger amount of monophenolic compounds was produced at 12%w/v sodium hydroxide solution.

Keywords: alkaline pretreatment, delignification, hydrothermal liquefaction, lignocelluloses, oxidative depolymerization, sugarcane bagasse

เรื่อง	การเปลี่ยนลิกนินเป็นสารประกอบฟีนอลิกโมเลกุลเดี่ยวในภาวะด่างโดยใช้ไฮโดรเจนเปอร์ออกไซด์ร่วมกับตัวเร่งปฏิกิริยาคอปเปอร์ (II) ออกไซด์ และเหล็ก (III) ซัลเฟต
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บทคัดย่อ

งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาการผลิตสารประกอบฟีนอลิกโมเลกุลเดี่ยวจากลิกนินในภาวะด่างโดยใช้ไฮโดรเจนเปอร์ออกไซด์และตัวเร่งปฏิกิริยาโลหะ การทดลองใช้ชานอ้อยปราศจากสารสกัดเป็นวัตถุดิบเริ่มต้น โดยแบ่งออกเป็น 2 ขั้นตอน ในขั้นตอนแรก คือ การย่อยสลายองค์ประกอบที่มีโครงสร้างอสัณฐาน ได้แก่ เฮมิเซลลูโลส และลิกนิน เป็นผลิตภัณฑ์ของเหลวด้วยปฏิกิริยาไฮโดรเทอร์มัลโดยใช้สารละลายโซเดียมไฮดรอกไซด์ ปัจจัยที่ศึกษา ได้แก่ ความเข้มข้นของสารละลายโซเดียมไฮดรอกไซด์ในช่วงร้อยละ 1.0-4.0 โดยมวลต่อปริมาตร และ อุณหภูมิตั้งแต่ 100-180 องศาเซลเซียส โดยทำปฏิกิริยาในเครื่องปฏิกรณ์แบบกะขนาด 10 มิลลิลิตร ระยะเวลา 30 นาที ในขั้นตอนที่ 2 เปลี่ยนลิกนินด้วยปฏิกิริยาออกซิเดชันในภาวะด่างเพื่อผลิตสารประกอบฟีนอลิกโมเลกุลเดี่ยว โดยการเติมไฮโดรเจนเปอร์ออกไซด์ คอปเปอร์ (II) ออกไซด์ และเหล็ก (III) ซัลเฟต ลงในผลิตภัณฑ์ของเหลวที่ได้จากขั้นตอนแรก และทำปฏิกิริยาที่อุณหภูมิ 150 องศาเซลเซียส ระยะเวลา 30 นาที จากการทดลองพบว่า ค่าความเข้มข้นของสารละลายโซเดียมไฮดรอกไซด์โดยมวลต่อปริมาตร และอุณหภูมิ ได้แก่ ร้อยละ 4.0 ที่ 140 องศาเซลเซียส ร้อยละ 3.0 ที่ 160 องศาเซลเซียส ร้อยละ 4.0 ที 140 องศาเซลเซียส และร้อยละ 4.0 ที่ 140 องศาเซลเซียส สามารถใช้เป็นภาวะที่เหมาะสมสำหรับปฏิกิริยาไฮโดรเทอร์มัลในขั้นตอนที่ 1 ได้ เมื่อนำกากของแข็งไปวิเคราะห์องค์ประกอบเคมี พบว่าเฮมิเซลลูโลสและลิกนินถูกกำจัดออกไปได้ร้อยละ 89.87-92.20 และ 94.59-98.19 โดยมวล ตามลำดับ นอกจากนี้ ค่าดัชนีความเป็นผลึกของกากของแข็งเพิ่มขึ้นจากร้อยละ 39.46 เป็นร้อยละ 57.40-59.40 ซึ่งบ่งชี้ว่าองค์ประกอบที่มีลักษณะอสัณฐานถูกกำจัดออกไป

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Atiwich Anantkijthamrong

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CHAPTER I

INTRODUCTION

1.1 Background of the research and its significance

Since the global industrialization, extensive amount of energy, mainly from fossil fuels, has been consumed for industrial and transportation usages in the recent decades. Moreover, the global energy demand tends to rapidly increase every year due to the population growth, as well as economic and industrial evolutions. In Thailand, the capability of domestic energy production cannot currently meet the energy demand, similar to many other developing countries. Much amount of energy has been necessarily imported and continued to increase for consecutive years. However, the uncertainty of fossil fuels together with their price fluctuation bring the nation inevitably encounter with the energy problems. Not only the afore mentioned but fossil fuels also cause environmentally effects such as the carbon dioxide emission which leads to the greenhouse effect. In consequence, the other sources of energy have been considered to use as an alternative to fossil fuels. In other words, this current situation brings renewable energy become more important.

Renewable energy is defined as all of the energy forms that obtain from natural resources including hydro energy, wind energy, solar energy, geothermal energy and biomass energy. Using renewable energy is the world promising solution to solve energy crisis and environmental problems. Among renewable energy, biomass is only tangible and versatile substances which can be converted to biofuels and biochemicals, while others are solely used for electricity production. Even though it is normally argued that biomass-to-biofuels conversion process and using itself also cause the carbon dioxide emissions. Nevertheless, biofuels are carbon neutral through carbon fixing process by plant respiration. In comparison with the fossil fuels, using biofuels, thus, does not cause a rapid carbon accumulation in the global atmosphere.

Lignocelluloses are the most abundant biomass resource on earth because they are non-edible, cheap, available in huge amount, and environmentally friendly. Their compositions are made up by cellulose, hemicelluloses, and lignin. Because of the co-existence along with the complicated chemical structure of these three components, the specific pretreatment method is required to fractionate them out for their individual value-added process. In case of cellulose and hemicelluloses, there are various common technologies to convert them to either biofuels or biochemicals, e.g., hydrolysis, fermentation, pyrolysis, etc. However, depolymerization of lignin is rather difficult and still challenging. In fact, during the bioenergy production from cellulose and hemicelluloses, lignin is typically discarded or removed as a residue.

Lignin is a high molecular weight heterogeneous aromatic polymer, which exist in nature. In pulp and paper process, lignin is intentionally eliminated to obtain holocellulose. This is so-called delignification step. The lignin residue obtained from the treatment of lignocellulosic materials is referred as to “technical lignin”. Due to the progress in development of holocellulose utilization, many types of technical lignins are available in industrial scale, e.g., kraft lignin, lignosulfonate lignin, and organosolv lignin. Because of their specific structure, these lignins are possible to be selectively depolymerized to aromatic compounds or used as polymeric materials. Nevertheless, most of the residual lignin obtained has been used to generate energy by combustion, that is, only a few amount is used to produce value-added products. In recent years, a variety of chemical conversion have been developed to liquefy and depolymerize lignin to its derivatives.

To selectively depolymerize lignin, as its unique structure, lignin itself requires a strong oxidant to promote the cleavage of intermolecular in lignin macromolecules. Among various delignification methods, hydrogen peroxide is widely used as an oxidant because it shows high effectiveness to depolymerize lignin even under mild conditions. Moreover, the presence of copper (II) oxide and iron (III) sulfate could significantly enhanced ether bonds cleavage and hydrogen peroxide dissolution, respectively (Xinping et al., 2014). In this study, two-stepwise conversion of lignin to phenolic monomers were investigated. Amorphous portions such as hemicelluloses and lignin in extractives-free sugarcane bagasse were hydrothermally liquefied in the first stage, while oxidative depolymerization of the liquefied portion was conducted in the second stage using copper (II) oxide and iron (III) sulfate catalysts under alkaline conditions. Qualitative and quantitative analyses of the obtained phenolic monomers were performed. This line of study can add more insight into the fractionation techniques of amorphous and crystalline portions in lignocelluloses. Such finding also paves the way for the future biorefinery concepts and highlights their potentials in market share of bio-based aromatics, which are now produced from crude oils merely.

1.2 Objective

To study two-stepwise conversion of lignin to phenolic monomers using hydrogen peroxide with copper (II) oxide and iron (III) sulfate catalysts under alkaline conditions

1.3 Scopes of the Research

1.3.1 To study chemical compositions of extractives-free sugarcane bagasse

1.3.2 To study two-stepwise conversion of lignin to phenolic monomers; first stage: hydrothermal liquefaction of extractives-free sugarcane bagasse under alkaline conditions, second stage: oxidative depolymerization of the obtained liquefied portion from the first stage using copper (II) oxide and iron (III) sulfate catalysts under alkaline conditions

1.3.3 To study the effects of sodium hydroxide concentration and temperature on first-stage hydrothermal liquefaction of lignin in extractives-free sugarcane bagasse

1.3.4 To study effect of sodium hydroxide concentration on the second-stage oxidative depolymerization of the liquefied portion to produce phenolic monomers using hydrogen peroxide as an oxidant with copper (II) oxide and iron (III) sulfate catalysts

1.3.5 To conduct both quantitative and qualitative analyses of phenolic monomers

1.4 Expected Benefits

1.4.1 To acquire the appropriate conditions in first-stage hydrothermal liquefaction in views of degree of the lignin removal and high purity of the residual cellulose fraction

1.4.2 To obtain the appropriate conditions for oxidative depolymerization of lignin to phenolic monomers using hydrogen peroxide with copper (II) oxide and iron (III) sulfate catalysts under alkaline conditions

1.4.3 To highlight a promising method in chemical conversion of lignin to biochemicals and biofuel

CHAPTER II

THEORY AND LITERATURE REVIEWS

In this chapter, chemical compositions of lignocellulosic biomass were briefly described, including their three main components which are cellulose, hemicelluloses, and lignin. In addition, characteristics and structures of technical lignins and thermochemical conversion techniques of lignin to its phenolic monomers were explained. Some relevant literatures related to the delignification methods were accordingly reviewed to pinpoint an appropriate research methodology for this work.

2.1 Biomass

Biomass refers to organic materials derived from living organisms which are considered as the natural energy storage. Biomass can be obtained from three main sources.

1. Plants, e.g., wood, agricultural residues, and wastes from agricultural processing
2. Animals, e.g., livestock, animal's excrement, and fatty oils
3. Human activity, e.g., municipal wastes and wasted paper

Typically, biomass comprises of three main constituents such as

1. Moisture

   Moisture is the water constituents existing in biomass which mainly found in high amount for the biomass from agricultural residues.

2. Combustible substances

   Combustible substances are the hydrocarbon compounds which comprise of carbon, hydrogen and oxygen atoms. Combustible substances can be classified into 2 parts namely volatiles matter and fixed carbon.

3. Non-combustible substance or ash

   Ash is inorganic substances which cannot be burnt to generate energy. Typically, ash content proportion in the biomass is around 1-3wt%.

2.2 Lignocelluloses

Lignocelluloses are the organic matter which exists in plant cell wall, mainly comprised of cellulose, hemicelluloses, and lignin. In general, lignocelluloses can be classified into four major groups based on their sources, which are

1. woody biomass, e.g., softwoods and hardwoods
2. agricultural residues, e.g., sugarcane bagasse, corncob, palm trunk, etc.
3. energy crops, e.g., switchgrass, miscanthus, and short life cycle plants
4. cellulosic waste, e.g., municipal waste and waste from pulping process

Lignocelluloses are different in proportions of their amounts of major constituents. Table 2.1 demonstrates amounts of the main constituents in various types of lignocelluloses. As seen, lignocelluloses mostly contain 40% of cellulose, 30% of hemicelluloses, 20% of lignin, and 10% of others components such as proteins, starch, extractives, and ash.

Table 2.1 Compositions of main components in lignocelluloses (Rabemanolontsa and Saka, 2013)

Lignocellulose	Component (wt%)			
	Cellulose	Hemicelluloses	Lignin	Others*
Cupressaceae (Softwoods)				
- Japanese Cedar	38.67	23.16	33.78	4.39
Fagacceae (Hardwoods)				
- Japanese beech	43.94	28.43	24.02	3.60
Poaceae (Grass)				
- Bamboo	39.80	31.41	20.81	7.98
- Rice Straw	34.53	21.82	20.22	23.42
- Corn cob	34.61	33.10	18.16	14.13
- Sugarcane bagasse	40.92	33.01	22.33	3.74

Remarks: *Others are proteins, starch, extractives, and ash.

2.2.1 Cellulose

Cellulose is a homogeneous biopolymer which is built up with a number of glucose units through β-(1,4) glycosidic bonds, as shown in Figure 2.1. The empirical formula of cellulose is $(C_6H_{10}O_5)_n$ where n is the number of repeating units which can be in a range between 100-10,000. The structure of cellulose is arranged from the intertwining of glucose molecules called “fibril” which mostly of hydroxyl group form many hydrogen bonds. The hydrogen bonds are construct the strength of crystalline structure. As its high crystallinity, hydrolysis of cellulose is difficult to some certain. However, an amorphous structure, so-called para-crystalline cellulose, can be found on the aggregated chains because of its disordered arrangement. These amorphous regions can be depolymerized by either chemical or physical pretreatment easier than crystalline cellulose.

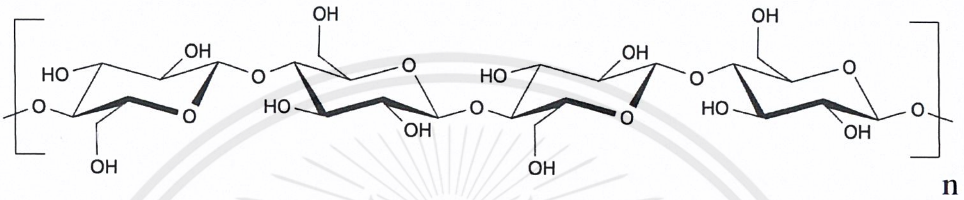


Figure 2.1 Chemical structure of cellulose (Laine, 2005)

2.2.2 Hemicelluloses

Hemicelluloses is a lower molecular weight polymer compared to cellulose. Hemicelluloses is heterogeneous polyoses which contains both of pentose and hexose sugars. Pentose and Hexose are monosaccharides that have 5 and 6 carbon atoms in their molecule, respectively. Figure 2.2 depicts an example of hemicelluloses structure. In plant cell wall, hemicelluloses is build up from xylan and glucomannan which is different in their amount depending on plant species. As for hardwoods and agricultural residue, the major hemicelluloses is xylan main chain which consists of a number of xylose units, and galactose, arabinose and acetic acid in its side chain. This makes the branching structure of hemicelluloses which can be easily decomposed by hydrolysis using either diluted acid or base. Since hemicelluloses is amorphous, its intermolecular bonds are more readily to be cleaved than cellulose. In addition to this, hemicelluloses has strong covalent bonds with lignin, as shown in Figure 2.3. therefore, hydrolysis of hemicellulose generally undergoes together with delignification.

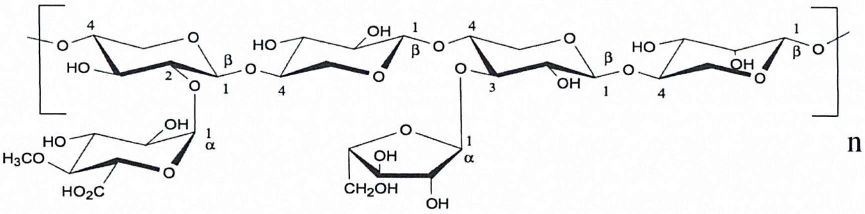


Figure 2.2 Example of chemical structure of hemicelluloses (Laine, 2005)

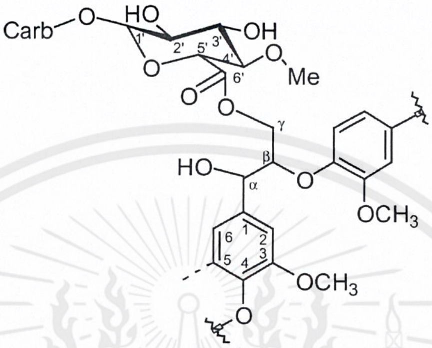


Figure 2.3 Major formation of lignin-carbohydrate bonds (Berlin and Balakshin, 2014)

2.2.3 Lignin

Lignin is a nature-existing aromatic biopolymer. It is mostly found at the edge of plant cell wall. Typically, molecules of lignin randomly bond to form the three- dimensional structure. Figure 2.4 depicts an example of macromolecular structure of lignin. As seen from the figure, a number of in aliphatic and/or aromatic moieties are linked by ether and ester bonds (non-condensed type bond) and carbon-carbon bonds (condensed type bond). The general structure unit of lignin monomers is called "phenyl propane unit", as illustrated in Figure 2.5. It is built up with a phenolic hydroxyl group, methoxy groups, and propyl side chain. Depending on the number of methoxy group (OMe), three different conformations of lignin monomers (monolignols) can be found. Figure 2.6 illustrates their monolignols structure which are *p*-coumaryl alcohol, coniferyl alcohol, and synapyl alcohol. Generally, the proportion of three monolignols is different which depending on plant species. Table 2.2 summarizes three plant families and their monolignols composition in molar ratios.

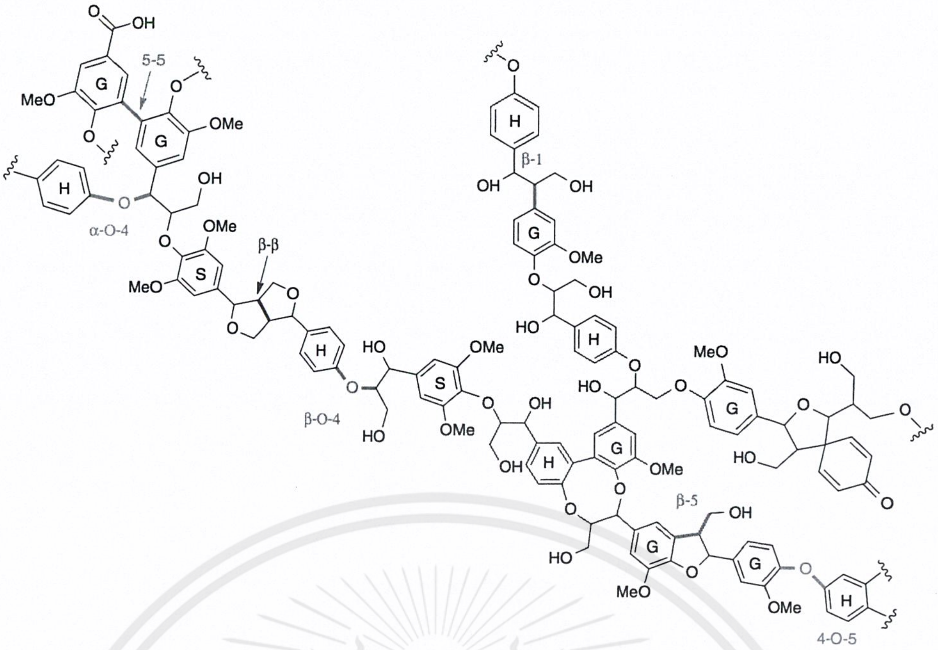


Figure 2.4 Chemical structure of lignin (Joffres et al., 2013)

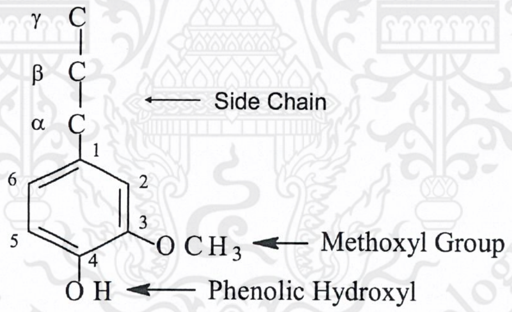
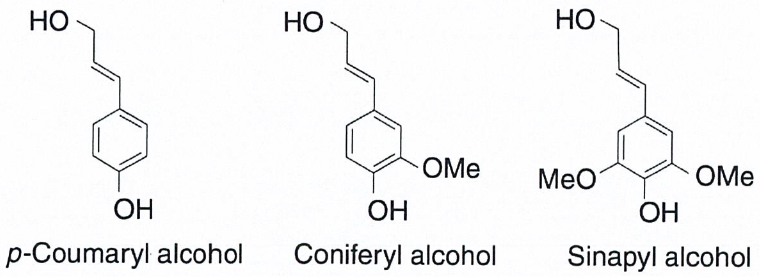


Figure 2.5 Phenyl propane unit nomenclature (Pandey and Kim, 2011)



p-Coumaryl alcohol   Coniferyl alcohol   Sinapyl alcohol

Figure 2.6 Structures of three lignin monomers (Joffres et al., 2013)

Table 2.2 Compositions of phenyl propane monomers in biomass-derived lignin from lignocelluloses (Rabemanolontsa and Saka, 2013)

Lignocellulose	Molar ratio		
	Guaiacyl	Syringyl	*p*-Hydroxyphenyl
Cupressaceae (Softwoods)			
- Japanese Cedar	1.00	0.00	0.05
Fagacceae (Hardwoods)			
- Japanese beech	1.00	2.00	0.00
Poaceae (Grass)			
- Bamboo	1.00	1.28	0.45
- Rice Straw	1.00	0.67	0.52
- Corn cob	1.00	0.64	0.77
- Sugarcane bagasse	1.00	0.80	0.19

2.3 Lignocellulose pretreatments

Lignocelluloses are the warrant carbon feedstocks which can be converted to many useful biochemicals. Pretreatment methods are responsible to fractionate their components and/or make them readily for the subsequent production of value-added compounds. Numerous up-to-date pretreatment technologies aim to delignify and enhance cellulose accessibility, which have a significant profitable advantage on holocelluloses (cellulose and hemicelluloses) usages.

2.3.1 Alkaline pretreatment

Alkaline pretreatment is known as a high-selectivity delignification technique. Sodium hydroxide, potassium hydroxide, ammonium hydroxide and calcium hydroxide are frequently used. However, calcium hydroxide (lime) is reported to be a notable reagent because it is cheap and harmless. Under the alkaline conditions, ester linkages between lignin macromolecules are cleaved which leads to lignin depolymerization. Moreover, hydrolysis of xylan side chain molecules, cleavage of lignin-carbohydrate linkages, swelling of cellulose, and cellulose decrystallization also occur. Mild reaction conditions reveal an outstanding advantage of the alkaline pretreatment although it requires long reaction time. The tedious step of neutralization is a major drawback of alkaline pretreatment process.

2.3.2 Acid pretreatment

Diluted mineral acids such as sulfuric acid and hydrochloric acid are often used in acid pretreatment of lignocelluloses. Organic acids like maleic acid and oxalic acid can also be used. Diluted formic or acetic acid with or without hydrogen peroxide has been reported to account for effective delignification. During acid pretreatment, however, hemicelluloses and cellulose are found to be hydrolyzed through the breaking of chemical bonds between pentose and hexose molecule, but it is ineffective for lignin hydrolysis to obtain phenolic monomers. High concentration of acid, moreover, allows condensation of lignin and deposition om cell wall structure under an elevated temperature. Thus, acid pretreatment is mostly considered as an efficient method for the production of reducing sugars.

2.3.3 Ammonia fiber expansion (AFEX)

Ammonia fiber expansion is the process which lignocelluloses are treated with highly-pressurized liquid anhydrous ammonia at a mild temperature (70-200°C) for a short reaction time. After the rapid pressure releasing, ammonia gas is suddenly expanded and then interrupt carbohydrate interlinkages. The crystalline native cellulose fiber was stated to be torn and transformed to a highly accessible allomorphic cellulose. In addition, anhydrous ammonia is dissociated into water during AFEX process, thus, ammonium and hydroxide ions are formed and acted as an alkaline medium. As a consequence, lignin molecules was substantially degraded, while hemicelluloses was partly hydrolyzed.

2.3.4 Pretreatment by organic solvent

Numerous alcohols/water mixtures, e.g., methanol, ethanol, and glycol are involved in the pretreatment of lignocelluloses with organic solvents. Moreover, minerals acids were sometimes used as a catalyst to reduce the severity of temperature and pressure employed in the reaction, and improve the delignification ability. Hemicelluloses and lignin were degraded and solubilized into organic solvent during such a pretreatment, while cellulose portion retains in solid fraction. Lignin can be subsequently precipitated from organic phase by lowering the pH. From the foregoing, organic pretreatment is a very efficient fractionation method of which yields relatively high purity of the three main fractions of lignocelluloses.

2.4 Industrial delignification processes and their technical lignins

In views of the recent progress in lignocellulose utilization, huge amount of lignin was delignified by pulping process into water-soluble portion. Sources of lignin, delignification method, and recovery process have significantly effected on liquefied-lignin components and their structures. Typically, the chemical structure of native lignin has dramatically degraded and modified during the process.

2.4.1 Kraft pulping process and kraft lignin

Kraft pulping is the delignification process which undergoes under a strong alkaline condition and obtains kraft lignin as a by-product. Generally, kraft process employs sodium hydroxide and sodium sulfide. During the kraft cooking process, most of the existing lignin in wood is degraded into lower molecular weight fragments and dissolved in alkaline aqueous solution. The main reactions of lignin degradation in kraft process are illustrated in Figure 2.7. The β-O-4 bonds are greatly cleaved under quite severe conditions in alkaline medium, however, the phenolic hydroxyl groups are not much increased due to the repolymerization among such phenolic groups. The characteristics of kraft lignin are high contents of condensed-type structure which is recalcitrant to oxidative degradation, and the presence of sulfur impurities in aromatic moieties that may have some consequence on further value-added process.

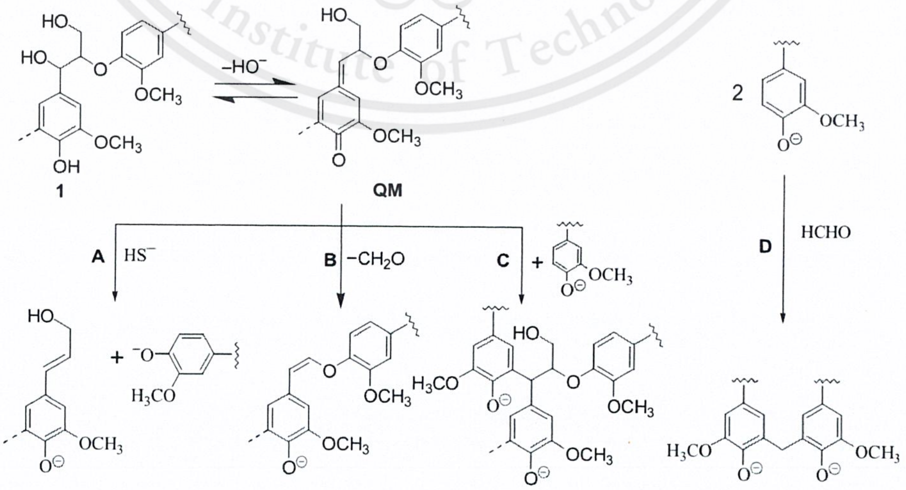


Figure 2.7 Main reactions of lignin degradation in alkaline pulping (Berlin and Balakshin, 2014)

2.4.2 Acidic-sulfite process and lignosulfonate lignin

Lignosulfonate lignin is obtained as a by-product from sulfite pulping process of wood for cellulose production, which is presently supplanted by the kraft process. In sulfite pulping, either sulfites (SO_3^{2-}) or bisulfites (HSO_3^-) is used as a reagent depending on the adopted pH with different ionic cations, e.g., Na^+, Ca^+, and K^+. During the acidic sulfite process, rather than the cleavage of β-O-4 bond, which leads to phenolic hydroxyl group augmentation, the main reaction is the splitting of α-O-4 linkages and inducing of sulfonates on α and β positions of propyl side chain. In the presence of sulfonic groups on lateral positions, as shown in Figure 2.8, hydrophilic property of lignin molecules is increased, which promotes the water solubility and allows them to settle apart from carbohydrate matrixes.

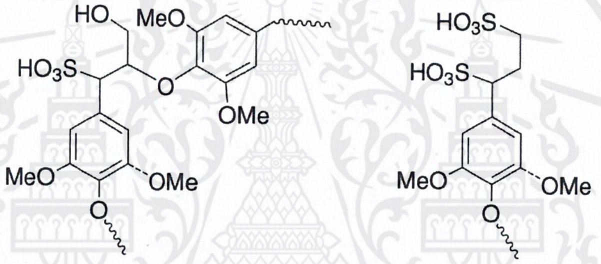


Figure 2.8 Possible phenyl propane structure of lignosulfonate lignin (Rodrigues Pinto et al., 2012)

2.4.3 Organosolv process and organosolv lignin

Organosolv lignin is a by-product of delignification process. In such process, a mixture of organic solvents is combined with acid catalyst as a cooking medium. The reaction is then performed at high temperature and pressure. By those conditions, the α- and β-ester linkages can split off and some chemical bonds between lignin and cell wall components can cleave. The characteristics of organosolv lignin are high in molecular weight and low in content of phenolic hydroxyl groups. Generally, the orgnosolv process leads to less transformation of lignin macromolecule compared to kraft and lignosulfonate lignins. Moreover, the non-existence of organic sulfur in lateral side chain is an outstanding aspect of organosolv lignin. The organic sulfur is incorporated with the aromatic ring instead.

2.4.4 Acid hydrolysis and acid-hydrolyzed lignin

Acid-hydrolyzed lignin is obtained from the delignification process using a diluted acid. Under acidic condition, the cleavage of lignin macromolecules linkages undergoes mainly at β-O-4 ester bond which produces various free phenolic compounds as depicted in Figure 2.9. The products resulted from acidic hydrolysis are referred as “Hibbert Kotones”. Even though the depolymerization of lignin lead to the increasing of monophenolic intermediates, they do not withstand and exist in the liquors because of their low-stability in acidic medium. In addition, during acid-hydrolysis process, the condensation occurs significantly, which consequently ends up with a great amount of condensed-type linkages and high molecular weight lignin.

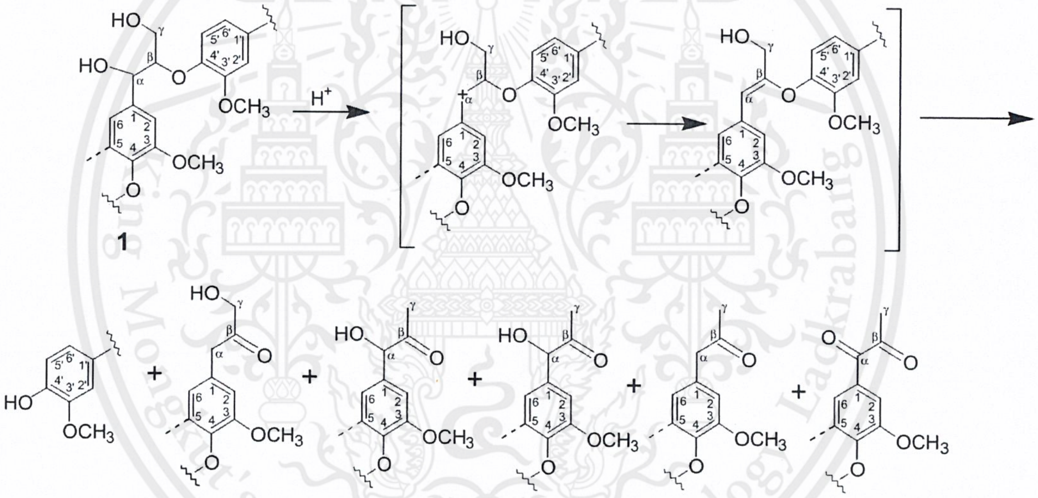


Figure 2.9 Major delignification reactions under acidic condition (Berlin and Balakshin, 2014)

2.5 Thermochemical conversion of lignin

To make use of aromatic products obtained from lignin, a variety of technologies for converting lignin to biofuels and biochemicals have been developed for decades. A well-known chemical conversion technique are thermochemical methods. They include, pyrolysis, gasification, liquefaction, and wet oxidation. In such methods, thermal energy, some appropriate reaction mediums, additives, and catalysts are needed.

2.5.1 Pyrolysis

Pyrolysis is generally a thermal conversion of organic substances by heating them in the absence of oxygen either with or without catalysts. At present, pyrolysis has been widely adopted to produce char, bio-oils, and gases from lignin. The bio-oils (pyrolytic lignin) basically comprise of monolignols, phenolic monomers, and others aromatic oxygenated compounds, where gaseous products are carbon monoxide, carbon dioxide, and others volatiles hydrocarbons. The proportion of product compositions also depends on several parameters, e.g., temperature, pressure, heating rate, and additives. Typically, the more severe conditions are adpoted, the lower molecular weight organic compounds are produced. Lignin pyrolysis has been studied under a wide range of temperatures (400-750°C). The yields of products is around 30-40% in char, 40-60% in pyrolytic liquid, and 8-20% in gaseous. To minimize char formation and increase yields of bio-oils, fast pyrolysis (0.5-10 s reaction time) has been proposed. However, fast pyrolysis necessarily required higher reaction temperature and heating rate (600-1000°C, 10-200°C/s) than regular pyrolysis to proceed the ether bonds cleavage in lignin macromolecules.

2.5.2 Gasification

Gasification of lignin is the endothermic reaction process which converts lignin into gases. Most of the gaseous products are carbon dioxide and portion of fuel gas, e.g., hydrogen, methane, and small amount of ethylene. The gasification process is consecutive and competitive reactions of depolymerization, hydrogenation, methanation, and steam reforming. This process is affected by temperature, pressure, reaction time, and catalyst. Osada et al. (2004) revealed that lignin cannot be completely converted to gaseous products without char formation at a moderate temperature (250-400°C). In this regards, the high temperature around 700°C is required to avoid char formation. The increasing of pressure was, in addition, resulted in higher hydrogen and lower carbon dioxide portions. Moreover, high yield of hydrogen was obtained in the presence of catalysts, e.g., metals. metal salts, and metal oxides.

2.5.3 Liquefaction

Liquefaction of lignin is the production of low oxygen contents bio-oils using water or organic solvents at a moderate temperature and pressure (250-450°C, 20-25 MPa). The liquefied bio-oils are mostly phenolic products such as catechol, cresol, and phenol. The main influences that affects the yields and product types are a temperature and reaction time. The prolonged reaction time leads to a lesser production of high molecular weight molecules, while the low molecular weight products are increased. Besides, a higher temperature shows more alkyl-substituted and less aromatic methoxy products.

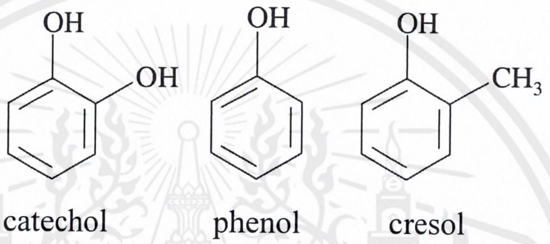


Figure 2.10 Example of phenolic bio-oils from lignin liquefaction (Mukherji et al., 2012)

2.5.4 Wet oxidation

Wet oxidation is a thermochemical conversion process where the lignin macromolecules are oxidized in the presence of aqueous solution, oxidant, and catalyst at a mild temperature and pressure (100-320°C, 0.5-20 MPa). From oxidative degradation of lignin, aromatic aldehydes, e.g., vanillin, syringaldehyde, and *p*-hydroxybenzaldehyde are produced. The presence of catalysts can help increase yields of aldehydes. Moreover, those aldehydes can be further oxidized to phenolic or even organic acids under the conditions of excessive oxidant and longer reaction time. The pH value is also important for lignin degradation and aldehydes production. In alkaline medium, lignin degradation occurs in a high efficiency, while acidic medium causes a reduction in yield of aldehydes.

2.6 Oxidation of lignin

Due to the presence of hydroxyl groups in macromolecules of lignin, it is an oxidative competitor in the degradation reactions that tend to form more phenolic derivatives with additional functionalities (Kim, 2010). The oxidative degradation involves C-C bond cleavage together with ester- and ether- bond cleavages. The prevalent oxidants are nitrobenzene, metal oxides, and hydrogen peroxide. Moreover, various types of catalysts in the presence of oxygen have been widely use in lignin oxidation. The mechanisms involve the oxidation of hydroxyl groups by cation radicals and cleavage of C-C bond by alkyloxy radical to form phenolic aldehydes and other alkyl radicals, as depicted in Figure 2.11. Besides, the lignin molecules could undergo hydrolysis due to the presence of water in aqueous solution. The products from the oxidation reaction of lignin are effected by steric and charge repulsion, which are influence to the product stabilities.

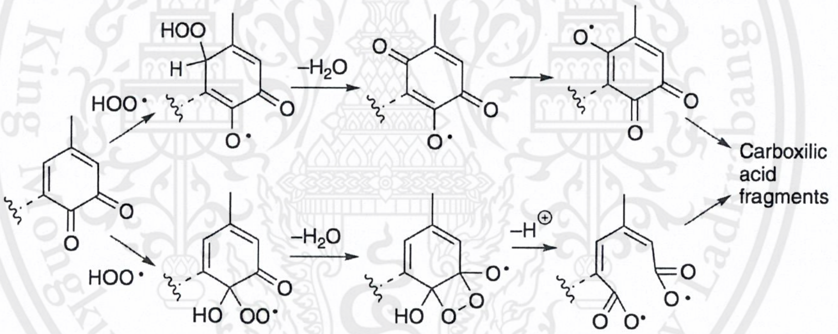


Figure 2.11 Mechanism of oxidation reaction with hydrogen peroxide (Calvo-Flores et al., 2015)

2.7 Utilization of phenolic monomers obtained from lignin

Since lignin is an only existing aromatic biopolymer in nature, it is potentially used as a renewable source for aromatic chemicals, which are presently obtained from petrochemical plants solely. Vanillin (4-hydroxy-3-methoxybenzaldehyde) and syringaldehyde (4-hydroxy-3,5-dimethoxybenzaldehyde) are the examples of phenolic aldehydes which can be produced from the wet oxidation process of lignin. Their molecule are depicted in Figure 2.12. Vanillin and syringaldehyde are used primarily for flavor and fragrance ingredients in foods and beverages. Moreover, they are also used in pharmaceutical, cosmetic, fume, and personal care product manufacturing.

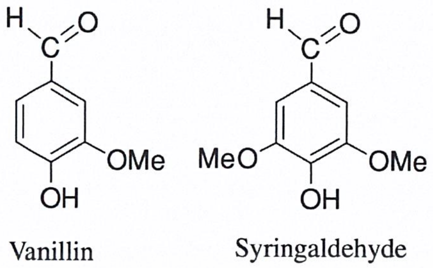


Figure 2.12 Chemical structure of vanillin and syringaldehyde (Rodrigues Pinto et al., 2012)

2.8 Literature Reviews

Over the years, researchers have studied on the lignin conversion in an attempt to produce biofuels and biochemicals. The well-known conversion processes are pyrolysis, gasification, liquefaction, and wet oxidation, which are different in their conditions and products. In this present study, hydrothermal delignification followed by oxidative depolymerization of liquefied lignin at mild temperature and pressure was of interest. The production of phenolic monomers, which are aldehydes and their acids were, in addition, explored. The conditions for delignification and oxidative depolymerization reaction as well as wet oxidation reaction were reviewed.

For the hydrothermal delignification step, Wang et al. (2010) reported the alkaline pretreatment of coastal Bermuda grass using 0.5-3.0%w/v sodium hydroxide concentrations at 121°C using an autoclave. The results revealed that 85.41wt% of lignin and 60.52wt% of xylan were removed using 3.0%w/v of sodium hydroxide for 90 min. The effect of reaction time on lignin removal was, however, found to be insignificant. There was not much different in percentage removal of lignin for 60 and 30 min reaction time, which were 85.81wt% and 82.41wt%, respectively.

Xue-Fei Zhou (2014) explored the hydrothermal degradation of kraft lignin to value-added products. The reaction was performed in 250-ml batch reactor, which was filled with 5.0 g of kraft lignin and 30 ml of deionized water, and then treated at 130, 180, and 230°C. Each experiment was proceeded for 15 and 60 min. The results revealed that an increase in temperature from 130°C to 180°C for 15 min reaction time leaded to an increase in degradation degree from 13.5 to 17.6wt%. A further increase in temperature to 230°C resulted in the pronounced degradation, which has been raised up to 23.7wt%. However, the yields of liquid products tended to decrease with the increasing temperature.

Xinping et al. (2014) studied the proper conditions of lignin oxidative degradation for producing monophenolic compounds with hydrogen peroxide, copper(II) oxide, and iron(III) sulfate under microwave irradiation. The results indicated that the degradation of alkaline lignin and yield of monophenolic compounds were relatively low when using only copper(II) oxide as an oxidant. Nevertheless, using hydrogen peroxide as a single oxidant resulted in high degree of degradation (93.17wt%) but still ended up with low yield of monophenolic compounds (4.01wt%). The optimal conditions, where the highest degradation degree (94.47wt%) and maximum yield of monophenolic compounds (6.97wt%), were found when using hydrogen peroxide as the oxidant with copper(II) oxide and iron(III) sulfate catalysts at 180°C for 90 min reaction time.

Moreover, Xinping et al. (2016) have recently explored the hydrothermal oxidation of lignin using hydrogen peroxide as an oxidant in the presence of copper(II) oxide and iron(III) sulfate in 100-ml stainless steel autoclave. Various types of solvents were used, namely, deionized water, methanol, and tetrahydrofuran. Effects from the differences in methanol to water ratio and mixtures between organic substances and water on lignin degradation and phenolic monomers production were demonstrated. A high range of phenolic monomers yields (15-18wt%) and intense degree of polymerization were successfully found.

From the oxidation mechanism of lignin, organic acids could be produced during the intermolecular bond cleavage of lignin. Acetic acid and formic acid were produced by the oxidation through aliphatic side chains, while succinic acid was formed by aromatic ring rupturing at quite severe conditions. Hasegawa et al. (2011) studied the organic acid production by lignin wet oxidation of lignin using 0.1wt% hydrogen peroxide as an oxidant. It was revealed that when hydrogen peroxide started to decompose (~100°C), yields of acetic acid and formic acid were increased significantly. Besides, succinic acid was readily to be produced at 150°C.

In this study, at first, the delignification of extractives-free sugarcane bagasse and organosolv lignin was explored using 1.0-4.0%w/v of sodium hydroxide solution at 120-200°C for 30 min. The amorphous portion, e.g., hemicelluloses and lignin, were expected to be liquefied at this stage. The obtained residue would then contained high content of cellulose. The liquefied portion as received from the first-stage appropriate conditions was further subjected to lignin oxidative depolymerization in the second step. At this stage, the experiment was performed in the presence of hydrogen peroxide with copper(II) oxide and iron(III) sulfate catalysts under alkaline conditions to convert lignin in the liquefied portion to phenolic monomers. This work would help and add more insight into the fractionation of amorphous portions of plant cell wall, particularly lignin, and demonstrate the conversion behavior of lignin to phenolic monomers in views of the effects of treatment conditions.

CHAPTER III

RESEARCH METHODOLOGY

This two-stepwise lignin conversion study attempted to selectively fractionate the amorphous cell wall component, which are hemicelluloses and lignin, so-called delignification in the first step, and convert the liquefied lignins to phenolic monomers in the second stage. Extractives-free sugarcane bagasse and organosolv lignin were used as the samples. Preparation methods of extractives-free sugarcane bagasse, first-stage delignification, and second-stage oxidative depolymerization experimental procedures, as well as analytical procedures were described in these following sections.

3.1 Preparation of extractives-free sugarcane bagasse

3.1.1 Size exclusion

Sugarcane bagasse (Singapore species) were washed with deionized water and dried at room temperature. The bagasse was oven-dried at 60°C for 12 hours. Then dried bagasse was cut into small pieces (around 1 cm) and grinded with a food blender to reduce more its size. Afterwards, the bagasse was size-screened by mesh sieving. The bagasse sample of which size is in the range of 425-850 μm was collected and used for further preparation steps.

3.1.2 Acetone extraction

To remove all non-polar extractives, e.g., fat and oils, the size-screened bagasse sample in 3.1.1.1 was subjected to acetone extraction by using Soxhlet apparatus for 8 hours. The extracted bagasse was left in fume hood for 12 hours to allow an excessive amount of acetone to volatilize, and dried in the oven at 60°C for 12 hours.

3.1.3 Hot water extraction

The acetone-extracted sugarcane bagasse was further extracted with hot water to eliminate the polar extractives, e.g., remaining free sugars, ions, and starch. The bagasse was immersed in a beaker of deionized water. A plate heater equipped with magnetic stirrer was employed. The temperature was kept constant at 60°C for 1 hour with vigorous mixing. The mixture was then cooled down to room temperature, and subsequently filtered with vacuum filtration apparatus. The obtained solid fraction was oven-dried at 105°C for 24 hours. This extractives-free sugarcane bagasse was kept dried in a zip-lock bag and used in this study. Prior to experiments or analysis, the extractives-free sample was dried again at 105°C for 2 hours, cooled down in a desiccator, and recorded its weight. All calculations of percentage conversion or yields through this study were reported on this oven-dried extractives-free sugarcane bagasse basis, unless stated otherwise.

3.2 Chemical composition analysis by Klason lignin determination method

Chemical composition analysis of extractives-free sugarcane bagasse as well as the solid residue as treated by appropriate condition from the first-stage were performed to quantify the amount of their major components, namely, cellulose, hemicelluloses, and lignin, by using Klason lignin determination method.

3.2.1 Materials and chemicals

1. Extractives-free sugarcane bagasse or the residues as treated by appropriate conditions from first-stage hydrothermal liquefaction
2. 72wt% of sulfuric acid solution
3. Deionized water

3.2.2 Equipment

1. 100-ml amber glass bottle
2. 50-ml beaker
3. 10-ml glass pipette
4. Autoclave
5. Desiccator
6. Vacuum filtration apparatus (vacuum pump, ceramic Buchner funnel, rubber ring and filter flask)
7. Filter paper (Whatman no.540)

3.2.3 Experimental procedures

1. Dry extractives-free sugarcane bagasse and the residues obtained from the appropriate conditions in the oven at 105°C for 4 hours. After that, cool down to room temperature in the desiccator
2. Weight 0.2 g of the oven-dried sample and put into 50-ml beaker
3. Add 2 ml of 72wt% sulfuric acid solution by using a glass pipette into beaker, stir by glass rod periodically for a well mixing, and keep the reaction at room temperature for 2 hours
4. Measure 75 ml of deionized water and put in a separated beaker. After 2 hours, add a certain amount of water into the acid solution beaker in step 3. to terminate the reaction
5. Pour the mixture into a 100-ml amber glass bottle. Rinse out the beaker with the remaining portion of the measured deionized water in step 4 for many times and transfer all the solid and the washing liquid into the glass bottle
6. Tighten the bottle cap well and wrap the whole glass bottle with aluminum foil
7. Put it in an autoclave, heat up to 121°C, and keep at this temperature for 60-90 min
8. Turn off the heater and release pressure inside the autoclave. Allow the autoclave temperature to decrease for a few minutes. Then bring the glass bottle out, and cool down to room temperature
9. Fitter the mixture by vacuum filtration to separate hydrolysates from solid residue
10. Analyze the amounts of glucose and xylose in hydrolysates. The obtained glucose and xylose yields (wt%) for the amount cellulose and hemicelluloses, respectively
11. Dry the solid residue at 105°C for 24 hours, cool down to room temperature, and define its weight as the amount of lignin

3.3 One-step conversion of lignin to phenolic monomers

In this experiment, extractives-free sugarcane bagasse was subjected to conversion of lignin in 12%w/v sodium hydroxide solution using hydrogen peroxide as an oxidant with copper (II) oxide and iron (III) sulfate catalysts. Organosolv lignin was additionally experimented for comparison. The reaction conditions used in this step were adapted from Xinping et al. (2016), which reported as the optimum conditions for the production of monophenolic compounds. The one-step conversion was done for using as the comparison with two-step conversion established in this study.

3.3.1 Materials and Chemicals

1. Oven-dried extractives-free sugarcane bagasse or organosolv lignin
2. 12%w/v of Sodium hydroxide solution
3. 0.75 M of hydrochloric acid
4. 30wt% of hydrogen peroxide
5. Copper (II) oxide
6. Iron (III) sulfate
7. Ethyl acetate

3.3.2 Equipment

1. 10-ml batch reactor equipped with thermocouple
2. Heater (furnace) and controller experimental set-up
3. Vacuum filtration apparatus
4. Filter paper (Whatman grade 540)
5. Iced-water bath

3.3.3 Experimental procedure

1. Load 0.05 g of a lignin sample, 0.008 g of copper (II) oxide, and 0.001 g of iron (III) sulfate into the reactor
2. Add 4 ml of sodium hydroxide and 0.4 ml of hydrogen peroxide into batch reactor. Tighten the reactor cap and check leakage by pressurizing with nitrogen gas (2.5 MPa) at room temperature
3. Install the batch reactor to heater (furnace) and controller board, set the desired temperature, and switch on the system
4. Let the reaction undergo for 60 min, then cool the reactor temperature down by quenching in iced-water bath for 10 min
5. Open a reactor and filter the mixture by vacuum filtration.
6. Neutralize the filtrate with 3wt% of hydrochloric acid

7. Extract the obtained filtrate with ethyl acetate, then take the ethyl acetate layer for phenolic monomer analysis
8. Analyze types and amounts of phenolic monomers as well as other by-products by gas chromatography–mass spectrometry (GC/MS) technique

3.4 First-stage hydrothermal liquefaction of extractives-free sugarcane bagasse

Extractives-free sugarcane bagasse was hydrothermally liquefied using 1.0-4.0%w/v of sodium hydroxide solution at 100-200°C for 30 min.

3.4.1 Materials and Chemicals

1. Oven-dried extractives-free sugarcane bagasse
2. 1.0-4.0%w/v of sodium hydroxide solution

3.4.2 Equipment

1. 10-mL batch reactor equipped with thermocouple
2. Heater (furnace) and controller experimental set-up
3. Vacuum filtration assembly
4. Filter paper (PTFE membrane, 0.4 μm)
5. Iced-water bath

3.4.3 Experimental procedure

1. Load 0.1 g of extractives-free sugarcane bagasse into the reactor.
2. Add 6 ml of sodium hydroxide solution into batch reactor. Tighten the reactor cap and check leakage by pressurizing with nitrogen gas (2.5 MPa) at room temperature
3. Install the batch reactor to heater (furnace) and controller board, set the desired temperature, and switch on the system
4. Let the reaction undergo for 30 min, then cool the reactor temperature down by quenching in iced-water bath for 10 min
5. Release pressurized nitrogen in the reactor by gently open the reactor valve
6. Open a reactor and filter the mixture by vacuum filtration
7. Collect the filtrates at 4°C for the second-stage experiment and analysis

8. Wash the reactor with deionized water many times to recover all of the solid residue deposited in the reactor
9. Oven-dry the solid residue at 105°C for 4 hours and then store in dessicator for further chemical composition analysis

3.5 Second-stage oxidative depolymerization of liquefied lignin to phenolic monomers

3.5.1 Materials and Chemicals

1. Liquefied portion (filtrate) from the first-stage hydrothermal liquefaction (section 3.3)
2. 30wt% of hydrogen peroxide
3. Copper (II) oxide
4. Iron (III) sulfate
5. 0.05 mg/ml of 1,3-diphenoxybenzene in ethyl acetate
6. 0.75 M hydrochloric acid solution

3.5.2 Equipment

1. 10-ml batch reactor equipped with thermocouple
2. Heater (furnace) and controller experimental set-up
3. Vacuum filtration apparatus
4. Filter paper (PTFE membrane, 0.4 μm)
5. Iced-water bath

3.5.3 Experimental procedure

1. Load 4 ml of liquefied portion from the first-stage, 0.02 g of copper (II) oxide, and 0.002 g of iron (III) sulfate into the reactor
2. Add 0.4 ml of hydrogen peroxide into batch reactor. Tighten the reactor cap and check leakage by pressurizing with nitrogen gas (2.5 MPa)
3. Install the batch reactor to the shaker equipped with a furnace, set the desired temperature, and switch on the system
4. Let the reaction undergoes for 60 minutes, then cool the reactor temperature down by quenching in in iced-water bath for 20 minutes
5. Release pressurized nitrogen in the reactor by gently open the reactor valve

6. Open a reactor and filter the mixture by using vacuum filtration
7. Neutralize the filtrate with 0.75 M hydrochloric acid
8. Extract the obtained filtrate with ethyl acetate, then take the ethyl acetate layer for phenolic monomer analysis
9. Analyze types and amounts of phenolic monomers by gas chromatography–mass spectrometry (GC-MS) technique

3.6 Analytical procedures

3.6.1 Quantification of reducing sugars in hydrolysates after Klason lignin determination method

Reducing sugars in hydrolysates from Klason lignin determination method comprise of monosaccharides from cellulose and hemicelluloses, which are glucose and xylose, respectively. The amounts of such monosaccharides were analyzed by using high performance liquid chromatography (HPLC) under the following conditions:

HPLC column:	Bio-Rad, Aminex HPX-87H
Detector:	Refractive index (RI)
Eluent:	0.005 M of sulfuric acid
Eluent flow rate:	0.60 ml/min
Column Temperature:	65°C

Each reducing sugar standard solutions, which are glucose, xylose, and arabinose, were firstly analyzed by HPLC for individual identifications based on their retention times. Calibration curves of the standard solutions were constructed by plotting between the concentrations and peak areas obtained from the HPLC chromatograms. At least two known concentrations of the reducing sugar standard solutions were incorporated. The curves were used for quantification of the reducing sugars in hydrolysates from their peak areas in chromatograms measured by HPLC. Sugar yields were reported in percentage on the oven-dried extractives-free sugarcane bagasse basis.

In a specific case of chemical composition analysis in residues by Klason lignin determination method, the weight percentage of cellulose retention, as well as the weight percentage of hemicelluloses and lignin removals, were calculated and reported on the oven-dried extractives-free sugarcane bagasse basis by using the following formula:

Cellulose retention (wt%)

$$= \frac{\text{Amount of cellulose in solid residue (g)}}{\text{Amount of cellulose in extractives-free sugarcane bagasse (g)}} \times 100 \qquad (3\text{-}1)$$

Hemicelluloses removal (wt%)

$$= \frac{\text{Amount of hemicelluloses in solid residue (g)}}{\text{Amount of hemicelluloses in extractives-free sugarcane bagasse (g)}} \times 100 \qquad (3\text{-}2)$$

Lignin removal (wt%)

$$= \frac{\text{Amount of lignin in solid residue (g)}}{\text{Amount of lignin in extractives-free sugarcane bagasse (g)}} \times 100 \qquad (3\text{-}3)$$

3.6.2 Calculation of cellulose crystallinity index in residues

The crystalline structure of cellulose was analyzed using X-ray diffractometer (XRD). The samples used in the XRD analysis were oven-dried at 105°C for 2 hours. Each of samples weight was 0.02 g. Samples were scanned from $2\theta = 5^{\circ}$ to $2\theta = 40^{\circ}$. Crystallinity index (CrI) was determined by using equation 3-4. (Segal et al., 1959)

$$\text{Crystallinity index (CrI)} = \frac{(I_{002} - I_{am})}{I_{002}} \qquad (3\text{-}4)$$

where I_{002} is the intensity at $2\theta = 22^{\circ}$

and I_{am} is the intensity at $2\theta = 18^{\circ}$

3.6.3 Identification of phenolic monomers in liquefied products

The ethyl acetate-soluble portions of both one-step and two-step lignin conversion were analyzed by using gas chromatograph-mass spectrometer (GC/MS) to identify possible phenolic monomer products by following condition.

Capillary column:	CP-Sil DB-5, 30 m × 0.25 mm × 0.25 μm
Carrier gas:	Helium
Injector temperature:	250°C
Temperature profile:	40°C for 5 min then 10°C/min to 250°C and hold at 250°C for 5 min

The results were compared with the mass fragmentation data library for their identifications.

3.6.4 Estimation of phenolic monomer amounts in liquefied products

The amount of phenolic monomers in ethyl acetate-soluble portion were estimated by comparing their peak areas with an internal standard (1,3-diphonoxybenene) obtained from GC/MS chromatograms. The comparative peak ratios of phenolic monomers were reported. The calculation followed equation 3-5.

$$\text{Comparative peak ratio} = \frac{\text{Peak area of phenolic monomer}}{\text{Peak area of 1,3-diphenoxybenzene}} \qquad (3\text{-}5)$$

CHAPTER IV

RESULTS AND DISCUSSION

In this chapter, the obtained results were discussed including (4.1) chemical composition analysis of extractives-free sugarcane bagasse, (4.2) one-step lignin oxidative degradation of sugarcane bagasse under alkaline conditions and (4.3) two-step conversion of lignin to phenolic monomers; first stage: hydrothermal liquefaction of extractives-free sugarcane bagasse under alkaline conditions, second stage: oxidative depolymerization of the obtained liquefied portions from the first stage using copper (II) oxide and iron (III) sulfate catalysts under alkaline conditions.

4.1 Chemical compositions of extractives-free sugarcane bagasse

Chemical compositions of extractives-free sugarcane bagasse were analyzed using Klason lignin determination method. The obtained fractions were hydrolysates and solid residue. The oven-dried solid residue was referred to amount of lignin, while the filtrates were further subjected to the quantification of reducing sugars. As a result, the solid residue of 21.36wt% was found, which could be represented to the total amount of lignin in extractives-free sugarcane bagasse. Amounts of monosaccharides in the filtrate were shown in Table 4.1. Their yields were 47.28, 29.98, and 2.22wt%, which were glucose, xylose, and arabinose, respectively.

Table 4.2 illustrates the cell wall compositions of sugarcane bagasse. Amount of cellulose was calculated by multiplying glucose yield with 0.9 dehydrated factor, while the amount of hemicelluloses was approximated from multiplying xylose and arabinose yields with 0.88 dehydrated factor. It was elucidated that the major cell wall components of extractives-free sugarcane bagasse used in this study were 42.55wt% of cellulose, 28.34wt% of hemicelluloses, and 21.36wt% of lignin. In comparison with the values reported by Rabemanolontsa and Saka (2013), the obtained cell wall components could be confirmed and used as a calculation basis in further steps. Ash and others components, however, were not analyzed. They were presented in trace amount, and neglected in this study.

Table 4.1 Amounts of cell wall constituent monosaccharides in extractives-free sugarcane bagasse

Sample	Monosaccharides yield (wt%)				
	Glucose	Xylose	Arabinose	Mannose	Galactose
Rabemanolontsa and Saka (2013)	45.47	27.8	1.6	0.0	0.0
Present work	47.28	29.98	2.22	-	-

Table 4.2 Cell wall compositions of extractives-free sugarcane bagasse

Sample	Cell wall component (wt%)			
	Cellulose	Hemicelluloses	Klason lignin	Others
Rabemanolontsa and Saka (2013)	40.92	33.01	22.33	1.61
Present work	42.55	28.34	21.36	-

4.2 One-step lignin oxidative depolymerization under alkaline conditions

The oxidative depolymerization of extractives-free sugarcane bagasse and organosolv lignin was performed under alkaline conditions in the presence of copper (II) oxide and iron (III) sulfate catalysts in one-step manner. Reaction conditions used in this step experiment were 150°C for 60 min, which were reported to be optimum by Xinping wt. al (2016). GC-MS chromatograms of oxidation products from extractives-free sugarcane bagasse and organosolv lignin were illustrated in Figures 4.1 and 4.2, respectively. The labeled numbers in the figures situate the products peaks. Their details, structures, and retention times were described in Table 4.3. The major products were found to be phenolic aldehydes and phenolic acids from the three monolignol species of lignin, e.g., coniferyl alcohol, synapyl alcohol, and *p*-coumaryl alcohol. Phenolic aldehydes, which are vanillin, syringaldehyde, and *p*-hydroxybenzaldehyde, were recovered as the main products in oxidative depolymerization of both extractives-free sugarcane bagasse and organosolv lignin. In case of organosolv lignin, a higher amount of synapyl alcohol than coniferyl alcohol was observed. This suggested that the organosolv lignin sample used in this study was probably isolated from hardwoods.

However, the presence of *p*-coumaryl alcohol derivatives such as *p*-hydroxyacetophenone, *p*-hydroxybenzoic acid, and *p*-hydroxycinnamic acid might be due to demethylation reaction of coniferyl and synapyl alcohols.

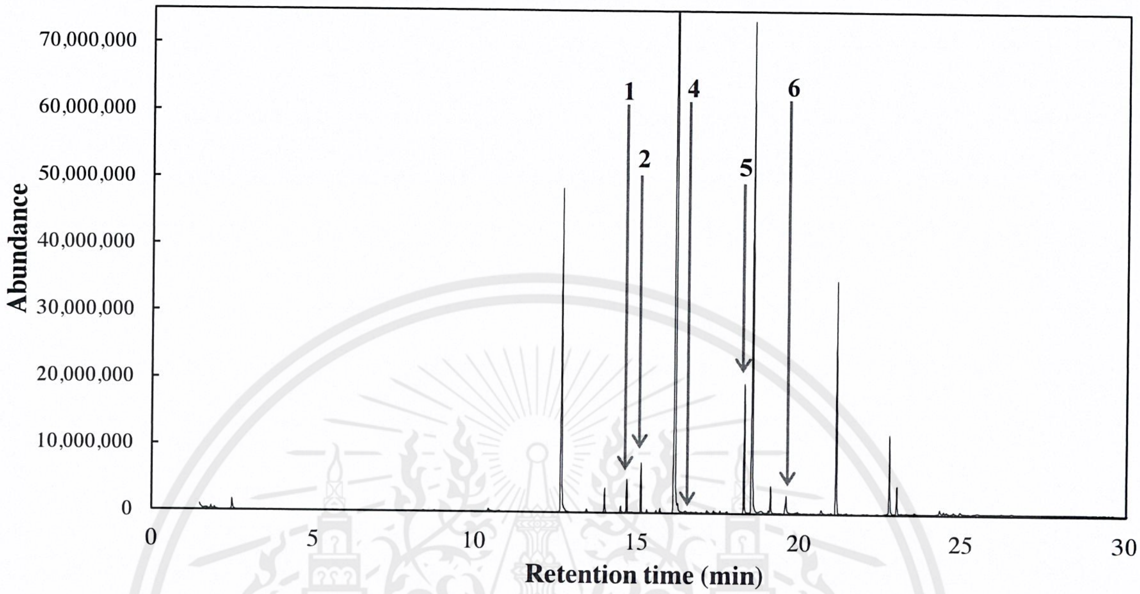


Figure 4.1 GC-MS chromatogram of oxidation products in ethyl acetate-soluble portion from one-step oxidative depolymerization of extractives-free sugarcane bagasse. The labeled numbers refer to the compounds in Table 4.3.

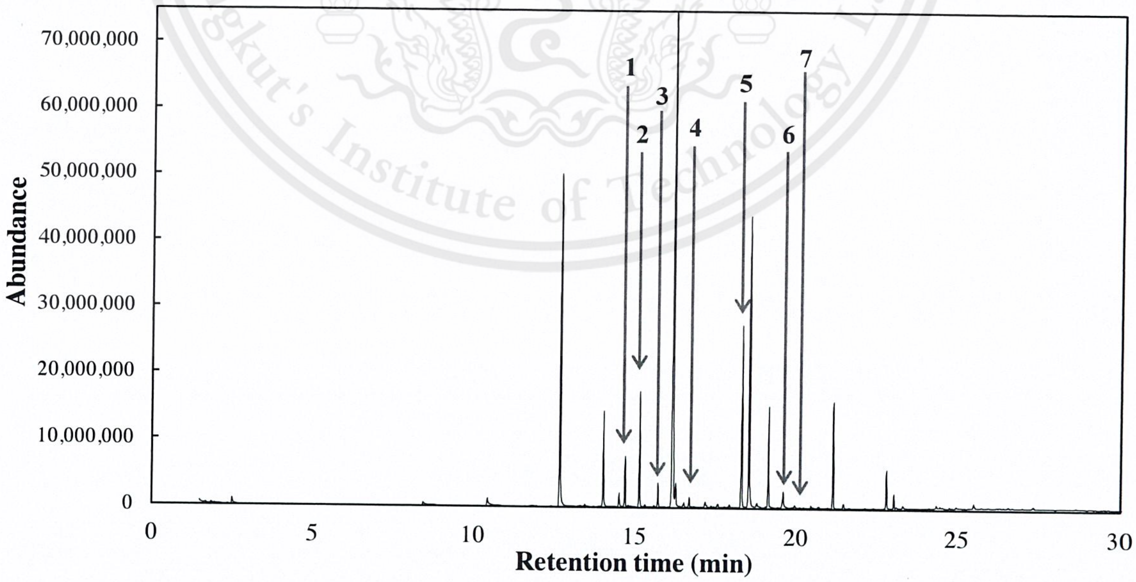


Figure 4.2 GC-MS chromatogram of oxidation products in ethyl acetate-soluble portion from one-step oxidative depolymerization of organosolv lignin. The labeled numbers refer to the compounds in Table 4.3.

Table 4.3 Monophenolic compounds in ethyl acetate-soluble portion obtained from one-step oxidative depolymerization, and their structures and retention times

No.	Monophenolic compound	Structure*	Retention time
1	*p*-Hydroxybenzaldehyde	H-CHO	14.69
2	Vanillin	G-CHO	15.13
3	*p*-Hydroxyacetophenone	H-OCH_3	15.71
4	*p*-Hydroxybenzoic acid	H-COOH	16.52
5	Syringaldehyde	S-CHO	18.32
6	*p*-hydroxycinnamic acid	H-CH_2CH_2COOH	19.31
7	Syringic acid	S-COOH	19.94

Remarks: *G refers to guaiacyl (2-methoxyphenol), S refers to syringol (2,6-dimethoxyphenol) and H refers to *p*-hydroxyl phenol.

The one-step oxidative depolymerization of lignin to phenolic monomers underwent two major mechanisms; (1) hydrothermal liquefaction and (2) lignin oxidative depolymerization. As treated by one-step method, some of amorphous components such as hemicelluloses and lignin were separated into the liquid portion. It was found that the solid residue was 56.92wt%. Based on cellulose content in extractives-free sugarcane bagasse (42.55wt%), it might be anticipated that this residue was mainly composed of cellulose, and some remaining hemicelluloses and lignin. The undissolved metal catalysts were, in addition, found to contaminate in the cellulose-rich fraction, which caused serious drawbacks in further utilization of the residual cellulose. In this regard, two-stepwise conversion of lignin to phenolic monomers was hence proposed. In the first stage, hemicelluloses and lignin in plant cell wall were hydrothermally liquefied under the alkaline conditions without additions of metal catalysts and hydrogen peroxide. In the second stage, liquefied portions obtained from the first-stage delignification were subjected to oxidative depolymerization by using hydrogen peroxide as an oxidant and copper (II) oxide and iron (III) sulfate catalysts. The similar treatment conditions as one-step conversion or milder were applied. By this two-stepwise method, the liquefied lignin could be separated from the solid residue, which was expected to contain cellulose with high purity, in the course of first-stage hydrothermal liquefaction without using metal catalysts. Furthermore, the homogeneous mixing of the liquefied lignin substrate and alkaline medium in the second-stage oxidative depolymerization could enhance the chemical activity. Thus, higher yields of phenolic monomers could be possibly obtained compared to the one-step method.

4.3 Two-stepwise conversion of lignin to phenolic monomers

4.3.1 First-stage hydrothermal liquefaction of extractives-free sugarcane bagasse

First-stage alkaline hydrothermal liquefaction aimed to hydrothermally liquefy the amorphous components, which are hemicelluloses and lignin, in extractives-free sugarcane bagasse. At this stage, the experiments were explored over the ranges of sodium hydroxide concentrations of 1.0-4.0%w/v and temperatures of 100-200°C for 30 min. As seen from the temperature profiles in Figure 4.3, the reaction time was defined after 30 min of heating up.

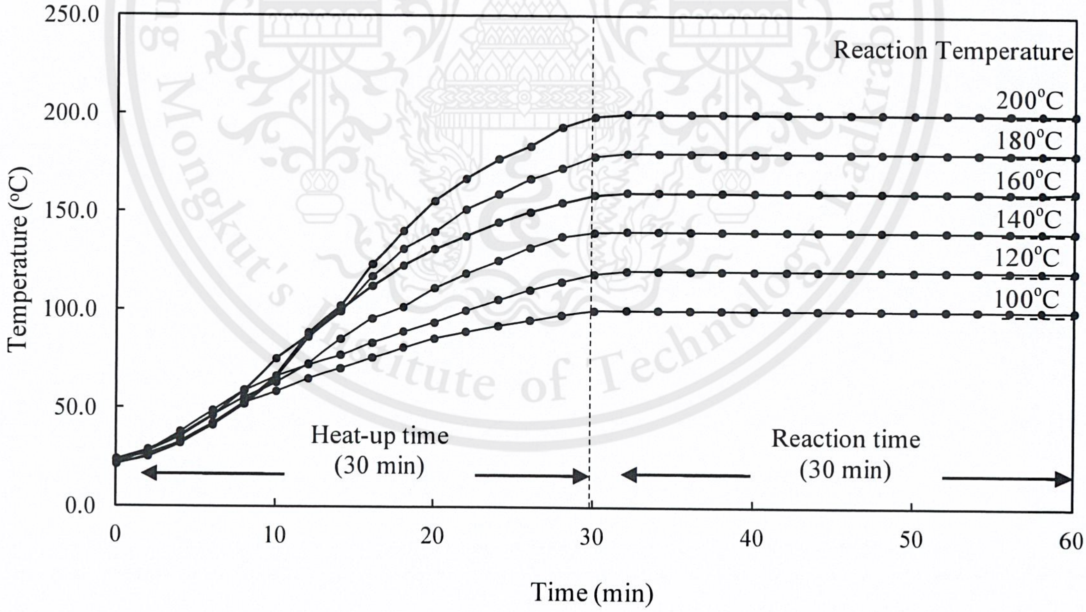


Figure 4.3 Temperature profiles of the batch-type reactions in this study

4.3.1.1 Effects of sodium hydroxide concentrations and temperatures on hydrothermal liquefaction

It was demonstrated that, residue weights decreased when either reaction temperature or sodium hydroxide concentration were increased, as depicted in Figure 4.4

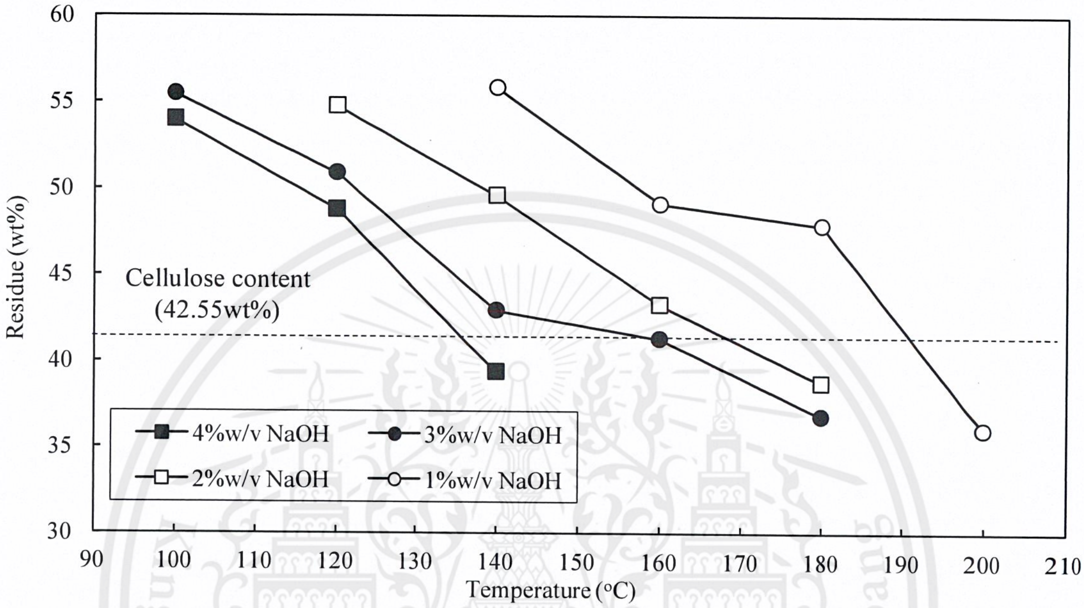


Figure 4.4 Changes in solid residue weights as treated by first-stage hydrothermal liquefaction using 1.0-4.0%w/v of sodium hydroxide solutions at various temperatures for 30 min

The residue weights fell nearly or even lower than 42.55wt% of cellulose content in extractives-free sugarcane bagasse when treating under some conditions, as seen from those points which are near or lower to the dashed line in the figure. The remaining residue weights of 39.36, 41.28, 38.73, and 36.78wt% were found at some specific values of sodium hydroxide concentration and temperature such as 4.0%w/v at 140°C, 3.0%w/v at 160°C, 2.0%w/v at 180°C, and 1.0%w/v at 200°C, respectively. These 4 points of solid residues might contain high purity of cellulose as the approximately 60wt%, which account for the amorphous portion in plant cell wall, were liquefied. Based on the cellulose content in sugarcane bagasse, those pinpointed alkaline concentrations and temperatures during the liquefaction step could probably be considered as the appropriate conditions.

4.3.1.2 Chemical compositions of the solid residues obtained from the appropriate conditions

Chemical compositions of the solid residues obtained from the appropriate conditions in the first-stage hydrothermal liquefaction were analyzed by Klason lignin determination method. The remaining cell wall components were found. Their compositions were reported on the oven-dried extractives-free sugarcane bagasse basis, as summarized in Table 4.4.

Table 4.4 Chemical compositions of residues as treated by 1.0-4.0%w/v sodium hydroxide solutions at 140-200°C for 30 min

First-stage condition	Residue (wt%)	Cell wall component of residue (wt%)		
		Cellulose	Hemicelluloses	Klason lignin
1%w/v NaOH at 200°C	36.78	31.17	4.58	1.03
2%w/v NaOH at 180°C	38.73	31.38	5.29	2.06
3%w/v NaOH at 160°C	41.28	33.41	5.43	2.44
4%w/v NaOH at 140°C	39.36	31.95	4.47	2.94

It was demonstrated that the solid residues were mainly composed of cellulose. The results suggested that under such employed alkaline conditions, lignin macromolecules were depolymerized through ester and ether bond cleavages. Hemicelluloses were, in addition, could be hydrolyzed while cellulose portion was partially preserved due to its crystalline structure. Apart from that, the depolymerization reactions involved with the lignin-carbohydrate bond fragmentation, leading to hemicelluloses and lignin removal. The removed portions from cell wall structure were, as a consequence, recovered in liquid phase, while the remains resided as a solid. Table 4.5 elucidated the percentages of each cell wall component removal.

Table 4.5 Cell wall component removals during the first-stage hydrothermal liquefaction using 1.0-4.0%w/v sodium hydroxide solutions at 140-200°C for 30 min

First-stage condition	Removal (wt%)		
	Cellulose	Hemicelluloses	lignin
1%w/v NaOH at 200°C	26.74	92.20	98.19
2%w/v NaOH at 180°C	26.25	90.74	96.27
3%w/v NaOH at 160°C	21.48	89.87	95.28
4%w/v NaOH at 140°C	24.91	92.03	94.59

The studied first-stage hydrothermal liquefaction showed high efficiency in both high hemicelluloses and lignin removals. Approximately 89.87-92.20wt% and 94.59-98.19wt% of hemicelluloses and lignin were liquefied, respectively. Hemicelluloses and lignin depolymerization played a major role in total solid loss during the studied first-stage hydrothermal liquefaction. The more severe conditions, in terms of either temperature or sodium hydroxide concentration, however, did not significantly result in higher removal of hemicelluloses. Nevertheless, an increase in temperature from 140°C to 200°C could enhance the degree of lignin removal from 94.59wt% to 98.19wt%, even though the sodium hydroxide concentration were lower, namely, 1%w/v. Besides, the cellulose removal was of 21.48-26.74wt%. It is an evidence that cellulose was partially hydrolyzed during first-stage hydrothermal liquefaction because cellulose itself also comprise of the amorphous portions, so-called para-crystalline cellulose. As a consequence, around 75wt% of cellulose was preserved in solid residues.

4.3.1.3 Change in crystallographic property of sugarcane bagasse during hydrothermal liquefaction

Crystallographic property of cellulose-rich residual fractions was explored by using X-ray diffractometer. Figure 4.5 illustrates the crystallinity indexes of residues as treated by 3%w/v sodium hydroxide solution at various temperatures. The indexes increased from 47.30-61.17% over the range of temperatures from 100-180°C. The results revealed that an increase in temperature could enhance the amorphous portion removal, which could be evident by the elevation of the crystallinity indexes.

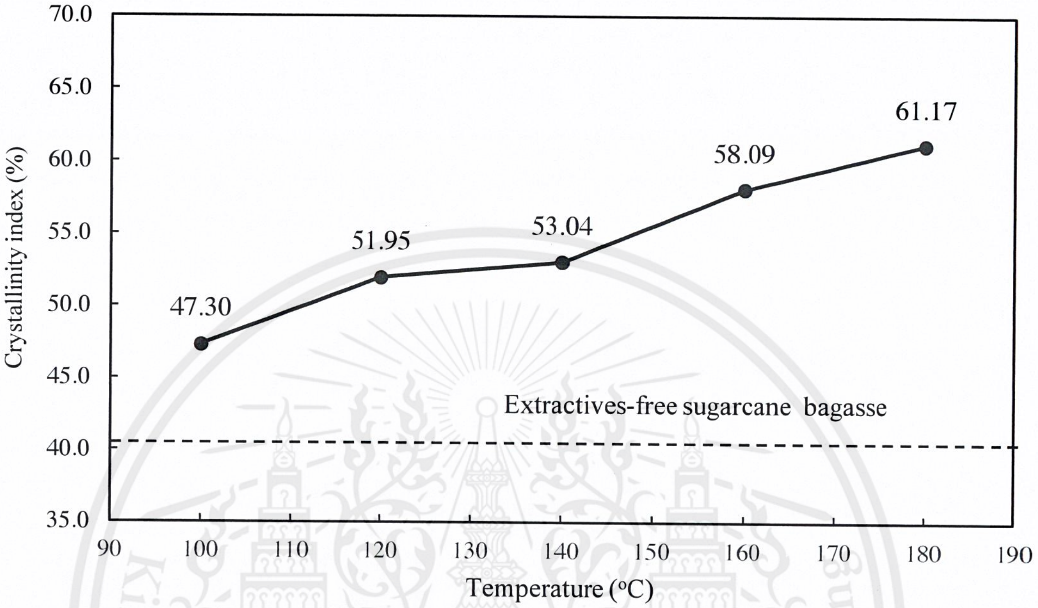


Figure 4.5 Change in crystallinity indexes of residues as treated with 3.0%w/v of sodium hydroxide solution at various temperatures for 30 min

As seen from the figure, the crystallinity index of the residue at 180°C was higher than that of 160°C, which was considered as the appropriate temperature for the first-stage hydrothermal liquefaction at 3%w/v NaOH. It was revealed that additional amount of the amorphous portions could be slightly eliminated when increasing temperature. In addition to that, some amount of cellulose was, however, hydrolyzed in the course of further treatment under higher temperature, resulting in the reduction of residue weight. Therefore, the results of cell wall component analysis in residue by Klason lignin determination method ensured that the pinpointed appropriate conditions could be used for the first-stage hydrothermal liquefaction in views of both high degrees of hemicelluloses and lignin removal, and high recovery of cellulose.

4.3.1.4 X-ray diffractograms and crystallinity indexes of the residues obtained from the appropriate conditions

The crystallinity indexes of extractives-free sugarcane bagasse residues as treated by appropriate conditions were increased compared to the untreated one. Figure 4.6 shows the X-ray diffractogram of solid residues as treated by the appropriate sodium hydroxide concentrations and temperatures in the first-stage hydrothermal liquefaction.

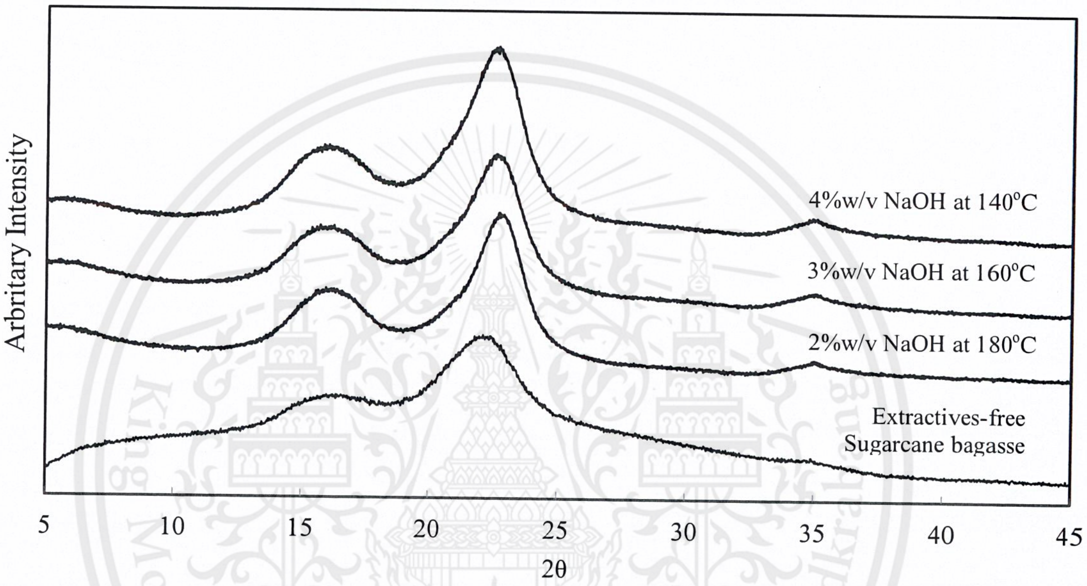


Figure 4.6 X-ray diffractograms of residues as treated by the first-stage appropriate conditions

Compared to the extractives-free sugarcane bagasse, the crystalline structure of cellulose became more prominent as a result of amorphous portion elimination. The crystallinity indexes of those residues, as demonstrated in Table 4.6, increased from 39.46% to 57.40-59.40%. However, crystallinity indexes of solid residues were still lower than the index of microcrystalline cellulose, which were isolated from cotton linter (77.93%). This might be due to the transformation of crystalline structure of native cellulose in extractives-free sugarcane as treated by the first-stage hydrothermal liquefaction. Otherwise, the microcrystalline cellulose might be prepared from different method so that its crystalline structure is greatly different from the one of extractives-free sugarcane bagasse residues.

Table 4.6 Crystallinity indexes of residues as treated by first-stage hydrothermal liquefaction under appropriate conditions

Sample	Crystallinity index (%)
Extractives-free sugarcane bagasse	39.46
Residue as treated by 4%w/v NaOH at 140°C	59.40
Residue as treated by 3%w/v NaOH at 160°C	57.40
Residue as treated by 2%w/v NaOH at 180°C	58.09
Residue as treated by 1%w/v NaOH at 200°C	59.02
Microcrystalline cellulose (cotton linter)	77.93

4.3.2 Second-stage oxidative depolymerization of the obtained liquefied portions

The obtained liquefied portion as treated by 1%w/v and 4%w/v of sodium hydroxide solution in the first stage were subsequently used for oxidative depolymerization in this step. The liquefied portion as treated by 4%w/v of sodium hydroxide was, in addition, adjusted its concentration to 12%w/v and then subjected to the second-stage treatment to compare the resulting monophenolic products to that from the one-step oxidative depolymerization. Figures 4.7, 4.8 and 4.9 depict GC/MS chromatograms of the oxidative products in ethyl acetate-soluble portions using 1%w/v, 4%w/v, and 12%w/v of sodium hydroxide solutions, respectively. The labeled numbers in the figures situate the products peaks. Their details, structures, and retention times were described in Table 4.7. Phenolic aldehydes, e.g., vanillin, syringaldehyde, and *p*-hydroxybenzaldehyde, were the major products from the second-stage depolymerization.

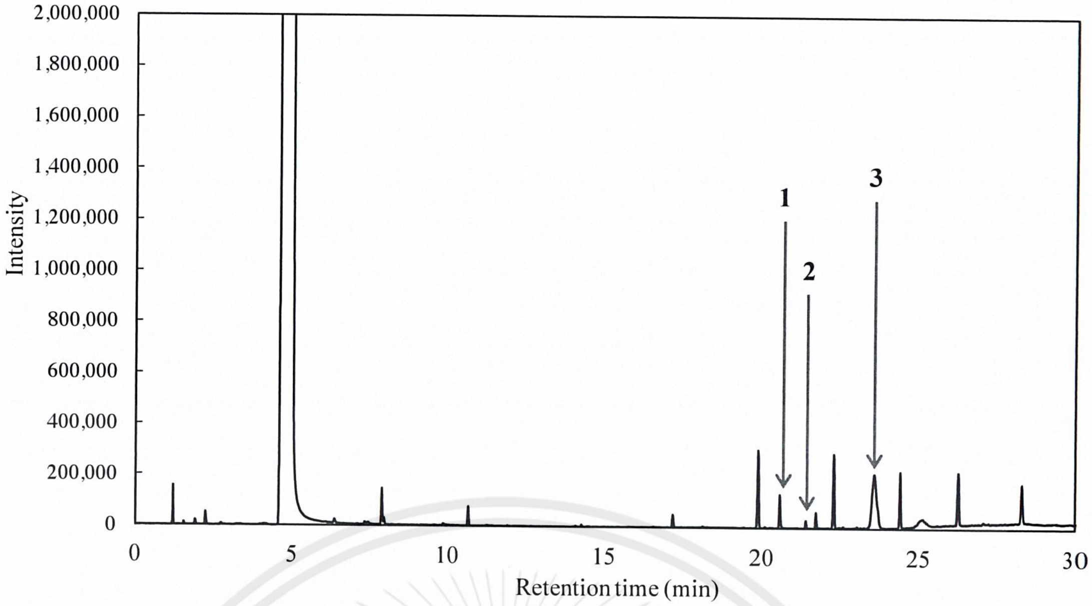


Figure 4.7 GC-MS chromatogram of oxidation products in ethyl acetate-soluble portion obtained from the second-stage depolymerization using 1%w/v sodium hydroxide solution. The labeled numbers refer to the compounds in Table 4.7.

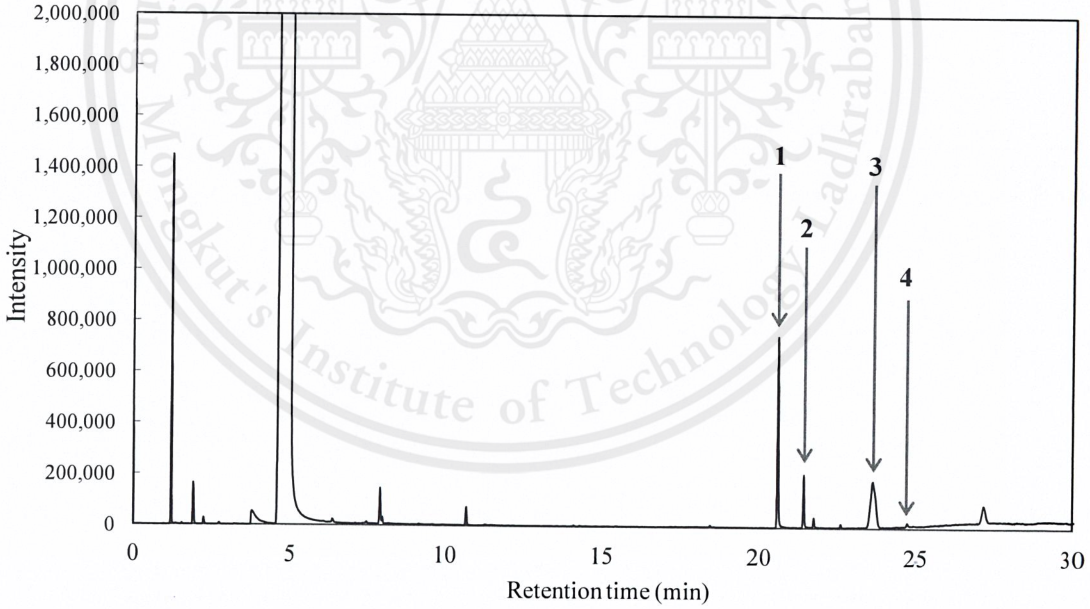


Figure 4.8 GC-MS chromatogram of oxidation products in ethyl acetate-soluble portion obtained from the second-stage depolymerization using 4%w/v sodium hydroxide solution. The labeled numbers refer to the compounds in Table 4.7.

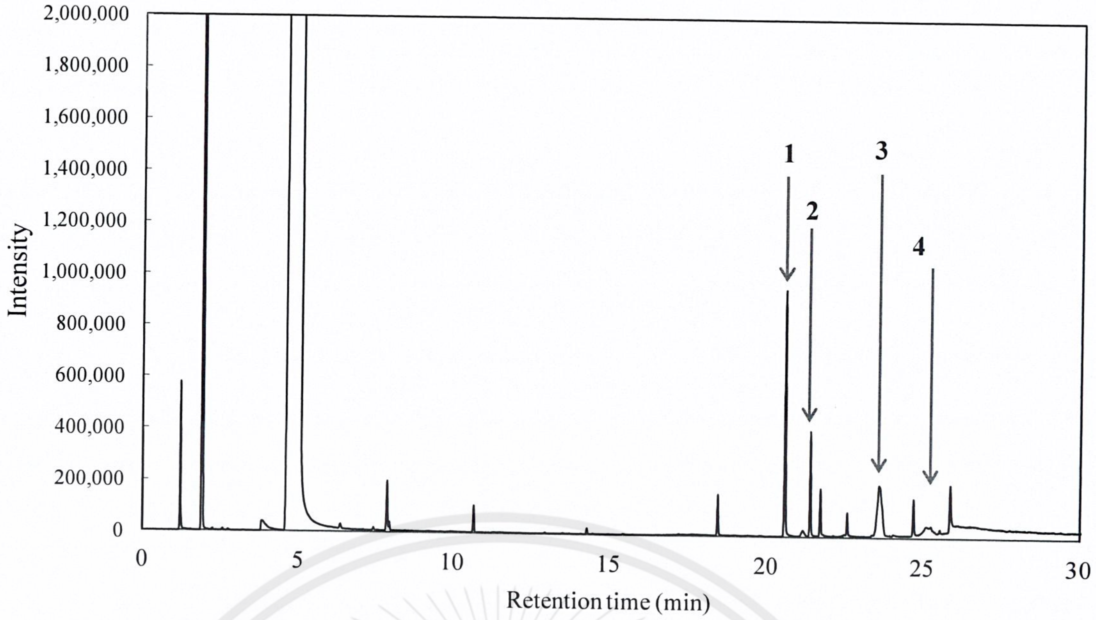


Figure 4.9 GC-MS chromatogram of oxidation products in ethyl acetate-soluble portion obtained from the second-stage depolymerization using 12%w/v sodium hydroxide solution. The labeled numbers refer to the compounds in Table 4.7.

Table 4.7 Monophenolic compounds in ethyl acetate-soluble portion obtained from the second-stage oxidative depolymerization and structures and retention times

No.	compound	Structure*	Retention time
1	*p*-Hydroxybenzaldehyde	H-CHO	20.58
2	Vanillin	G-CHO	21.39
3	1,3-Diphenoxybenzene**	-	23.60
4	Syringaldehyde	S-CHO	24.71

Remarks: * G refers to guaiacyl (2-methoxyphenol), S refers to syringol (2,6-dimethoxyphenol) and H refers to *p*-hydroxyl phenol.

** 1,3-Diphenoxybenzene is used as an internal standard.

The results revealed that sodium hydroxide concentration played a significant role in lignin oxidative depolymerization under alkaline condition in the presence of copper (II) oxide and iron (III) catalysts. A larger amount of monophenolic compounds could be found at 12%w/v sodium hydroxide solution, as seen in Figure 4.10.

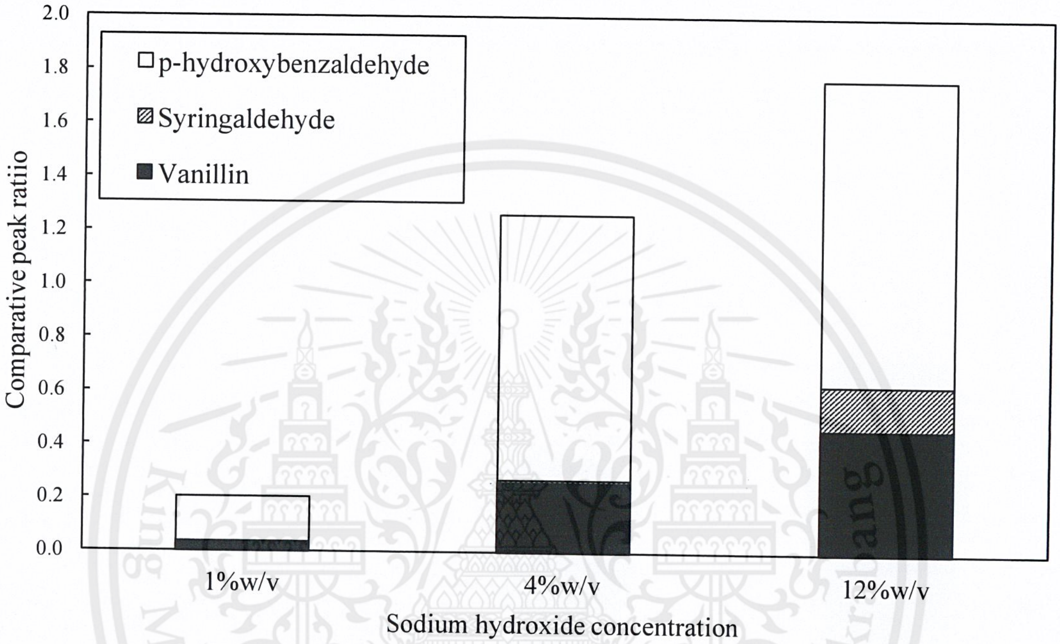


Figure 4.10 Comparative peak ratios of monophenolic compounds obtained from the second-stage oxidative depolymerization using 1%w/v, 4%w/v, and 12%w/v of sodium hydroxide solutions

CHAPTER VI

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In this study, the conversion of lignin to phenolic monomers under alkaline conditions using hydrogen peroxide with copper (II) oxide and iron (III) sulfate catalysts were explored in a two-stepwise manner. As a preliminary study for making a comparison, one-step conversion method was conducted in accordance with the appropriate conditions reported by Xinping et. al (2016). Extractives-free sugarcane bagasse and organosolv lignin were used as samples. As a result, the obtained products were phenolic aldehydes from three monolignols species of lignin, i.e., coniferyl alcohol, synapyl alcohol, and *p*-coumaryl alcohol. The undissolved metal catalysts were, in addition, found to contaminate in the cellulose-rich residue, which caused serious drawbacks in further utilization of the residual cellulose. In this regard, two-stepwise manner for the conversion of lignin to phenolic monomers was hence proposed. In the first stage, hemicelluloses and lignin in plant cell wall were hydrothermally liquefied under the alkaline condition without additions of metal catalysts and hydrogen peroxide using 1.0-4.0%w/v sodium hydroxide solutions at 100-200°C for 30 min. In the second stage, liquefied portions obtained from the first-stage delignification were subjected to oxidative depolymerization by using hydrogen peroxide as an oxidant and copper (II) oxide and iron (III) sulfate catalysts. The similar treatment conditions as one-step conversion or milder were applied. The obtained results suggested that some specific values of sodium hydroxide concentrations and temperatures such as 4.0%w/v at 140°C, 3.0%w/v at 160°C, 2.0%w/v at 180°C, and 1.0%w/v at 200°C which obtain 39.36, 41.28, 38.76, and 36.78wt% of residue, respectively, could be used as appropriate conditions in the first-stage hydrothermal liquefaction. It was revealed that the lower sodium hydroxide concentration was adopted, the higher temperature was required. As treated by those conditions, the removal degrees of hemicelluloses and lignin from sugarcane bagasse were 89.87-92.20wt% and 94.59-98.19wt%, respectively. As a consequence, around 75wt% of cellulose was preserved in solid residues. The crystallinity indexes of extractives-free sugarcane bagasse residues as treated by those appropriate conditions in the first-stage were increased from 39.46% to 57.40-59.40%, which confirmed the elimination of amorphous portion in plant cell wall. However, crystallinity indexes of solid residues were still lower than the index of microcrystalline cellulose, which are isolated from cotton linter (77.93%). This might be due to the transformation of crystalline structure of native cellulose in extractives-free sugarcane during the first-stage hydrothermal liquefaction under the studied alkaline conditions. As for the second stage, the major oxidation products were phenolic aldehydes, e.g., vanillin, syringaldehyde, and *p*-hydroxybenzaldehyde. The results revealed that sodium hydroxide concentration played a significant role in lignin oxidative depolymerization under alkaline conditions in the presence of copper (II) oxide and iron (III) catalysts. A larger amount of monophenolic compounds could be found at 12%w/v sodium hydroxide solution.

In conclusion, the proposed two-step lignin conversion method could successfully fractionate amorphous and cellulosic portions. A relatively pure cellulose content in residue of extractives-free sugarcane bagasse was obtained in the first stage hydrothermal liquefaction, while the liquefied portion could end up with monophenolic aldehydes in the second-stage oxidative depolymerization in the presence of metal catalysts. Those fractionated portions and products were readily to be converted to other value-added biochemical and biofuels. This finding paves the way for the utilization of lignocelluloses and highlights their potentials in market share of bio-based aromatics which are now mostly produced from the crude oils.

5.1 Recommendation

Quantitative analysis of phenolic monomers should be done by using gas chromatography. This method is more precise than comparative calculation of peak ratios with internal standard obtained from GC/MS analysis. Monophenolic compounds and their weights can be reported. More insightful discussion on product yields, lignin structure of the samples, and chemical conversion behavior would be possible.

In addition, derivertization of the monophenolic compounds in ethyl acetate-soluble portion with silylation agent such as N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) should be performed prior to the qualitative and quantitative analyses. Schemmer et al. (2009) reported that the derivertized products were increased in both volatility and thermal stability. The derivertized products would, moreover, reduce the interactions between compounds and GC capillary column, resulting in higher detectability and more accurate than the non-derivertization method.

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APPENDIX

APPENDIX A

PREPARATIONS OF CHEMICALS AND EXAMPLES OF CALCULATIONS

1. Preparation of 72wt% sulfuric acid solution

Measure 66 ml of 97wt% sulfuric acid solution. Slightly pour it into 100-ml volumetric flask filled with a few of deionized water. Keep its temperature low while mixing by immersing the flask into an iced-water bath. Finally, add deionized water to bring the final volume to 100 ml.

2. Preparation of sodium hydroxide solution

Weight some amount of sodium hydroxide anhydrous pellets, as stated in Table A.1 and put into 25-ml volumetric flask. Keep its temperature low while mixing in iced-water bath. Then, add a few amounts of deionized water and swirl the flask gently. Once all the sodium hydroxide pellets are completely dissolved, add deionized water to bring the final volume to 25 ml.

Table A.1 Weights of pellet sodium hydroxide used for the preparation of sodium hydroxide solutions

Sodium hydroxide concentration (%w/v)	Weight of pellets sodium hydroxide (g)
1	0.25
2	0.50
3	0.75
4	1.00

3. Preparation of glucose standard solution

3.1 10 mg/ml glucose standard solution

Weight 0.01 g of glucose and dissolve in 1 ml of deionized water.

3.2 5 mg/ml glucose standard solution

Measure 0.5 ml of glucose standard solution obtained in 3.1. Dilute its concentration to 5 mg/ml by adding 0.5 ml of deionized water.

4. Preparation of xylose standard solution

The preparation method is as described in 3.

5. Calculation of chemical compositions in hydrolysates from Klason lignin determination method

Example Calculation of the amount of xylose in hydrolysates obtained from Klason lignin determination of the residue as treated by 4%w/v at 140°C for 30 min

Peak area obtained from HPLC chromatogram was 1,859. From the calibration curve between peak area and concentration of xylose standard solution, as depicted in figure B.2, the relation was $Y = (2.97872\times10^{-6})\, X$

where Y = concentration of xylose standard solution (mg/ml)

X = peak area obtained HPLC chromatogram

Thus, $Y = (2.97872\times10^{-6}) \times 1{,}859$

$Y = 0.0055$ mg/ml

According to klason lignin procedure, the total liquid volume was brought up to 77 ml and diluted for 10 times for HPLC analysis

Thus, Amount of xylose $= 0.0055 \times 77 \times 10$

$= 4.235$ mg

$= 0.0042$ g

6. Calculation of phenolic monomers peak ratios

Example Calculation of comparative peak ratio of vanillin obtained from the second-stage oxidative depolymerization using 12%w/v sodium hydroxide solution

Peak area of vanillin and 1,3-dephenoxybenzene (internal standard) obtained from GC/MS chromatogram was 919,932 and 1,983,451, respectively. Concentration of 1,3-dephenoxybenzene in ethyl acetate was 0.05 mg/ml.

Thus, comparative peak ratio of vanillin $= \dfrac{919{,}932}{1{,}983{,}451}$

$= 0.46$

APPENDIX B

RAW DATA

1. Calibration curves for calculations of the reducing sugar concentration analyzed by HPLC

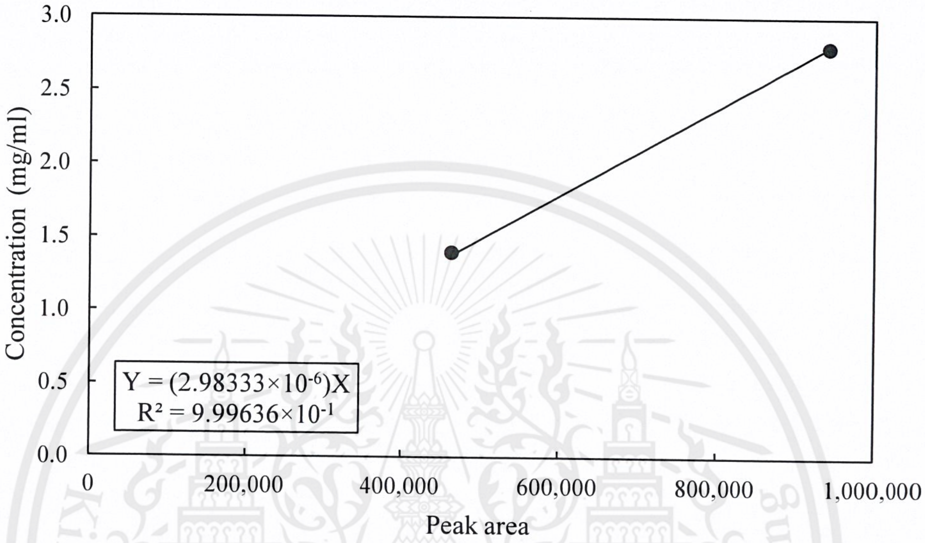

Figure B.1 Calibration curve of peak area from HPLC chromatogram and concentration of glucose standard solution

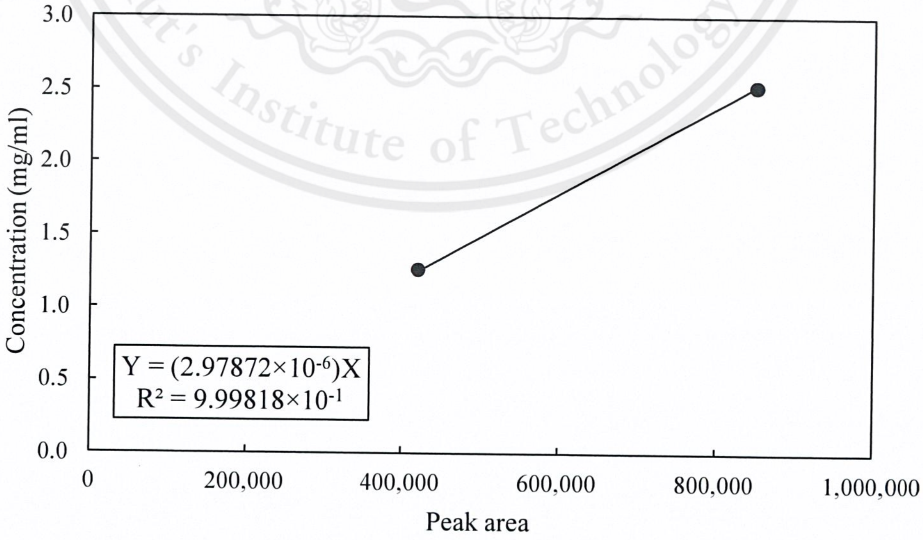

Figure B.2 Calibration curve of peak area from HPLC chromatogram and concentration of xylose standard solution

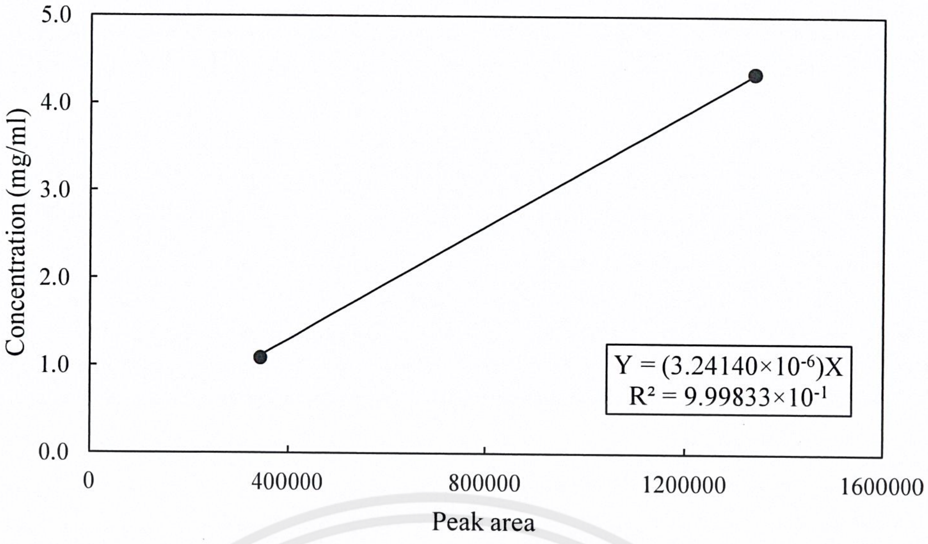

Figure B.3 Calibration curve of peak area from HPLC chromatogram and concentration of arabinose standard solution

2. Raw date of the solid residue of extractives-free sugarcane bagasse after the first-stage hydrothermal liquefaction

Table B.1 Weight percentage of solid residues as treated by 1.0-4.0%w/v at 100-200°C for 30 min at first-stage hydrothermal liquefaction reported on extractives-free sugarcane bagasse basis

NaOH concentration	Temperature (°C)					
	100	120	140	160	180	200
4%w/v	53.97	48.78	39.36	-	-	-
3%w/v	55.45	50.88	42.91	41.28	36.78	-
2%w/v	-	54.74	49.57	43.26	38.73	-
1%w/v	-	-	55.83	49.11	47.87	36.43

3. Peak areas of phenolic monomers and internal standard obtained from GC/MS chromatograms

Table B.2 Peak areas of vanillin, syringaldehyde, p-hydroxybenzaldehyde, and 1,3-diphenoxybenzene (Internal standard) obtained from GC/MS chromatograms

NaOH concentration	Peak area		
	1%w/v	4%w/v	12%w/v
Vanillin	71974	-	334247
Syringaldehyde	480730	21503	1842084
p-hydroxybenzaldehyde	919932	326134	2261654
1,3-diphenoxybenzene	2,012,890	1,859,125	1,982,451

4. Comparative peak ratios of phenolic monomers

Table B.3 Comparative peak ratios of vanillin, syringaldehyde, and *p*-hydroxybenzaldehyde obtained from second-stage oxidative depolymerization using 1%w/v, 4%w/v, and 12%w/v

NaOH concentration	Comparative peak ratio		
	Vanillin	Syringaldehyde	*p*-hydroxybenzaldehyde
1%w/v	0.04	0.00	0.17
4%w/v	0.26	0.01	0.99
12%w/v	0.46	0.16	1.14

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