Optimization of Two-step Lignin Depolymerization from Sugarcane Bagasse to Phenolic Monomer Compounds under Alkaline Conditions

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A Report Submitted in Partial Fulfillment of the Requirements

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Department of Chemical Engineering, School of Engineering,

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การหาภาวะที่เหมาะสมของการย่อยสลายลิกนินจากชานอ้อยเป็นสารประกอบฟีนอลิกมอนอเมอร์แบบ 2 ขั้นตอนภายใต้สภาวะด่าง

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สถาบันเทคโนโลยีพระจอมเกล้าเจ้าคุณทหารลาดกระบัง

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By	Thapakorn Srichan
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Abstract

Optimization of two-step lignin depolymerization from sugarcane bagasse to phenolic monomer compounds under alkaline conditions was investigated. Hydrothermal liquefaction was performed to liquefy lignin from cell wall of sugarcane bagasse in the first stage. The reaction conditions, i.e. NaOH solutions of 0.5-12%w/v and temperatures of 100-210℃, were studied. The liquefied product was subjected to second-stage oxidative depolymerization under alkaline conditions by using hydrogen peroxide coupled with copper (II) oxide and iron (III) sulfate as catalysts in the second stage. As a result, in the first stage, the appropriate conditions to remove lignin from cell wall extractives-free sugarcane bagasse were NaOH concentrations (%w/v) and the corresponding temperatures (℃) of (0.5, 225), (1, 210), (2, 180), (3, 160), (4, 140), and (12, 130). In the second stage, oxidative depolymerization was performed over a temperature range of 130-250℃ whereby NaOH concentration was kept as inherent one in the first stage. hydroxybenzaldehyde, hydroxybenzoic acid, p-coumaric acid, vanillin, vanillic acid, syringaldehyde, and syringic acid were produced. The highest yield phenolic monomers of 11.2 wt% were produced by using liquefied lignin obtained by (4%w/v, 140℃) from the first stage and subsequently treated at 190 ℃ in the second stage. It was revealed that compositions of phenolic monomer compounds varied among first and second stage treatment conditions. At the optimum point, hydroxybenzaldehyde and hydroxybenzoic acid, namely p-type lignin, were found as the major products. This might be derived from hydroxycinnamic acids in lignin-carbohydrate complexes.

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สาขาวิชา วิศวกรรมปิโตรเคมี

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สถาบันเทคโนโลยีพระจอมเกล้าเจ้าคุณทหารลาดกระบัง

บทคัดย่อ

งานวิจัยนี้ศึกษาภาวะที่เหมาะสมของการย่อยสลายลิกนินจากชานอ้อยเป็นสารประกอบฟีนอลิกมอนอเมอร์แบบ 2 ขั้นตอนภายใต้สภาวะด่าง ขั้นตอนที่ 1 การขจัดลิกนินออกจากโครงสร้างผนังเซลล์พืชของชานอ้อยเป็นผลิตภัณฑ์ของเหลวด้วยปฏิกิริยาไฮโดรเทอร์มัล ภาวะที่ศึกษาได้แก่ ความเข้มข้นของสารละลายโซเดียมไฮดรอกไซด์ร้อยละ 0.5-12 โดยมวลต่อปริมาตร ที่อุณหภูมิ 100-210 องศาเซลเซียส และขั้นตอนที่ 2 อุณหภูมิของการเปลี่ยนลิกนินพอลิเมอร์ในผลิตภัณฑ์ของเหลวจากขั้นตอนแรกเป็นสารประกอบฟีนอลิกมอนอเมอร์ด้วยปฏิกิริยาออกซิเดชันภายใต้ภาวะด่างโดยใช้ไฮโดรเจนเปอร์-ออกไซด์เป็นตัวออกซิไดซ์ร่วมกับตัวเร่งปฏิกิริยาคอปเปอร์ (II) ออกไซด์และไอร์ออน (III) ซัลเฟต จากการทดลองพบว่าภาวะที่เหมาะสมในการขจัดลิกนินจากโครงสร้างผนังเซลล์พืชของชานอ้อยที่ปราศจากสารสกัดที่ความเข้มข้นของสารละลายโซเดียมไฮดรอกไซด์และอุณหภูมิดังนี้ ดังนี้ (0.5, 210), (1, 200), (2, 180), (3, 160), (4, 140), (5, 140), (6, 130), (8, 120), (10, 110) และ (12, 100)และเมื่อนำผลิตภัณฑ์ของเหลวที่ได้จากขั้นตอนที่1 ที่ภาวะ (4,140) ทำปฏิกิริยาต่อไปในขั้นตอนที่ 2 ที่อุณหภูมิ 190 องศาเซลเซียสเกิดเป็นสารประกอบฟีนอลิกมอนอเมอร์รวมร้อยละ 11.2 โดยมวล และยังพบว่าไฮดรอกซีเบนซัลดีไฮด์และกรดไฮดรอกซีเบนโซอิกซึ่งเป็นลิกนินประเภทพาราคูมาริลเป็นผลิตภัณฑ์หลักที่เกิดจากการย่อยสลายสารประกอบเชิงซ้อนลิกนิน-คาร์โบไฮเดรต

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Thapakorn Srichan

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CHAPTER I

INTRODUCTION

1.1 Backgrounds of the research and its significance

The unbalance between the higher energy demand and the limited supply of fossil fuels has resulted in the idea of renewable energy. Biorefinery is the concept of using biomass, especially agricultural waste materials, to convert biomass into fuels and chemicals. In general, biorefinery process consists of two steps. First step, biomass is pretreated to make it ready for conversion in the next step. After that, in the second step conversion of biomass to fuel and chemical products are performed.

Biomass is organic, a renewable, sustainable and environmentally friendly source of energy used to create fuels or other forms of power. It means biomass consists of material that produce from living organisms and their waste included agricultural residues (e.g., wheat straw, sugarcane bagasse, corn stove). One of the most abundant a major source of biomass is lignocellulose[1]. Lignocellulosic biomass consists of cellulose, hemicelluloses and lignin, which represent 40, 30 and 30wt%, respectively. The specific pretreatment method is required to fractionate them out for their individual value-added process. In the case of cellulose and hemicelluloses, there are diverse common technologies to convert them into biofuels.

Lignin is an amorphous compound and three-dimensional polymer. Lignin is mainly composed of hydroxyl-phenylpropane units emerged from three monomers *p*-coumaryl, coniferyl and sinapyl alcohol variously distributed depending on vegetal materials. These monomers are linked to each other by ether bonds, which are dominant, and by carbon-carbon bonds. There is various method to delignification, which removed the structural polymer lignin from plant cell wall. For instance, alkaline pretreatment which is performed alkaline solutions such as NaOH, KOH, $Ca(OH)_2$ and $Ba(OH)_2$ can play an important role in the conversion of lignocelluloses. Residual liquid (such as lignin) produced from alkaline pretreatment can even be used to generate a number of other products. For example, distributed to the grid for residential or commercial use as a

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component of phenolic powder resins, polyurethane foams, epoxy resins or as valuable food and industrial products such as vanillin, ferulic acid, or hydroxybenzoic acid[2].

Oxidative degradation of lignin is able to interrupt the network molecular structure of lignin to reduce steric hindrance and expose active groups. To selectively depolymerize lignin, lignin itself requires a strong oxidant to promote the cleavage of intermolecular in lignin macromolecules. Among various oxidation 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 bond cleavage and hydrogen peroxide dissolution, respectively[3].

Two-step conversion of lignin to phenolic monomer compounds was investigated by Techan[4]. In the first-stage, hydrothermal liquefaction was performed liquefied lignin from cell wall of sugarcane bagasse. The appropriate NaOH concentrations (%w/v) and the corresponding temperatures (°C) were (0.5,210), (1,200), (2,180), (3,160), (4,140) and (12,100). In the second stage, the liquefied lignin was then subjected to oxidative depolymerization under alkaline conditions by using hydrogen peroxide coupled with copper (II) oxide and iron (III) sulfate as catalysts at 150 °C. As a result, phenolic monomers were produced from the first stage liquefied lignin. The solid residue from first-stage delignification these methods, composed mainly of cellulose, moreover, did not contaminate with any solid catalyst. However, qualitative and quantitative analyses for phenolic monomer compounds were not performed yet.

Thongue[5] studied the two-step conversion of lignin to phenolic monomer compounds under additional alkaline conditions for the analysis reducing sugars produced by the degradation of cellulose and hemicellulose. Up to and including the effect of temperature of oxidation under alkalinity in second stage was also studied on the yield of phenolic monomer compounds. The experiment was conducted at 130-190°C for 30 minutes. It was found that the delignification in the first stage at the concentration of 4 %w/v sodium hydroxide solution and the temperature of 140°C was followed by oxidative

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lignin decomposition under alkaline conditions in second stage at 190°C produced the highest total yield phenolic monomers of 13.5wt% on lignin basis. Additionally, the analysis of reducing sugars in liquid products, it was found that the effect was less than 1wt% sugarcane bagasse. This indicated that partly hemicellulose and cellulose removed from the plant cell wall structure during two-step conversion of lignin to phenolic monomer compounds occurred in form of complex structure substance and cannot be degraded into reducing sugars.

In addition, Sinsatitporn[6] studied more first-stage conditions by using NaOH concentrations in the rage of 5-10%w/v. It was found that the appropriate conditions NaOH concentrations and the corresponding temperatures (°C) were (5,140), (6,130), (8,120), (10,110) and. In the second stage, the liquefied lignin was then subjected to oxidative depolymerization under alkaline conditions by using hydrogen peroxide coupled with copper (II) oxide and iron (III) sulfate as catalysts of 110-190°C for 30 minutes. The highest total yield of phenolic monomers at 11.2wt% of lignin were obtained when using NaOH concentration of 4%w/v and temperature of 140°C in the first stage followed by using temperature of 190°C in the second stage.

However, the tendency of the highest total phenolic monomers yield remained increase as the temperature was built up in second stage. This research investigates the optimum conditions by extending the temperature range in the second stage whereby NaOH concentration was kept as inherent one in the first stage.

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1.2 Objectives

1.2.1 To study the effect of second-stage temperature to phenolic monomers yield.

1.2.1 To identify the optimum conditions of two-step lignin depolymerization from sugarcane bagasse to phenolic monomer compounds.

1.3 Scopes of Work

1.3.1 To study 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 portion from the first stage using copper (II) oxide and iron (III) sulfate catalysts under alkaline conditions.

1.3.2 To study effect of NaOH concentration in 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.

1.3.3 To study effect of temperature from 210°C to 250°C in the second stage on phenolic monomers yield.

1.3.4 To conduct both quantitative and qualitative analyses of phenolic monomer compounds

1.4 Expected Outputs

1.4.1 To explain effect sodium hydroxide (NaOH) concentration from liquefied lignin and temperature for oxidative decomposition lignin on second stage to produce phenolic monomer compounds.

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1.4.2 To produce phenolic monomer compounds from lignin and conduct quantity of the phenolic monomer compounds is used as a guide to utilize as a fuel or other chemicals that have added value.

1.4.3 To obtain the appropriate conditions for delignification and oxidation of lignin to the yield of phenolic monomer compounds using hydrogen peroxide with copper (II) oxide and iron (III) sulfate catalysts under alkaline conditions.

1.4.4 To separate solid residue from first stage, composed mainly of cellulose, without contaminate any solid catalyst.

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CHAPTER II

THEORY AND LITERATURE REVIEWS

In this chapter, compositions and components of the lignocellulose were described, including the delignification from structural of lignocellulose. In addition, thermochemical conversion techniques of lignin to phenolic monomer components, condensation of lignin and literature reviews that related to this work were mentioned as following.

2.1 Biomass

Biomass refers to organic materials that come from living things which are used to generate energy. Biomass can be obtained from three main sources as plants, animals, and human activity, respectively.

2.2 Lignocelluloses

Lignocelluloses are the organic compound which are mainly consists of cellulose, hemicellulose and lignin of cell wall. Typically, lignocelluloses are classified as woody biomass, agricultural residues, energy crops and cellulose waste. Lignocelluloses are mainly composed of proportional to cellulose, hemicellulose, and lignin as 40:30:30, respectively.

2.2.1. Cellulose

Cellulose is a biopolymer that linkage between the glucose molecules by β-(1,4) glycosidic bonds. The chemical 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 as shown in Figure 2.1. The molecules are totally arranged in long straight chains and have a strong tendency to form intra- and intermolecular hydrogen bonds. Bundling of cellulose leads to formation of microfibrils which in turn to form fibers.

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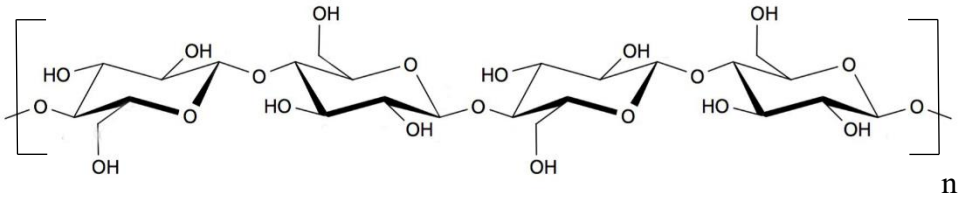

Figure 2.1 Chemical structure of cellulose[7]

2.2.2 Hemicelluloses

Hemicelluloses are a branched polyoses. The structural of hemicelluloses is amorphous as shown in Figure 2.2. Mostly, linkage between pentose sugars which are xylose and arabinose and hexose sugars which are glucose, galactose, and mannose by β-(1,4) glycosidic bonds.

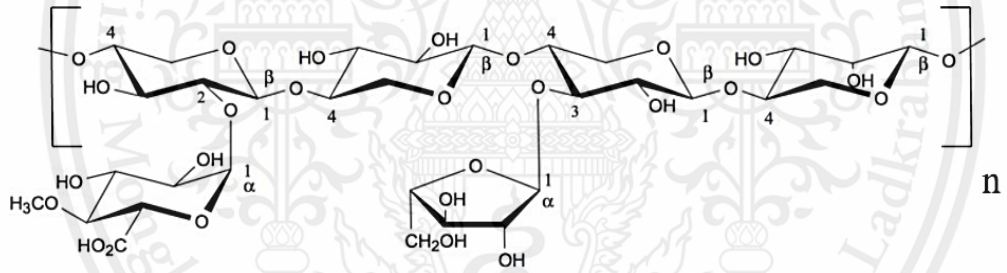

Figure 2.2 Example of chemical structure of hemicelluloses[7]

2.2.3 Lignin

Lignin is an aromatic biopolymer. Normally, linking between cellulose and hemicellulose, and contributes to increased strength of wood tissue. The general structure unit of lignin monomers is called phenyl propane unit. It is composed of phenolic hydroxyl group, methoxy groups, and propyl side chain. The differential conformations of lignin monomers (monolignols) depended on number of methoxy group (OMe). Theirs are *p*-coumaryl alcohol (P-type), coniferyl alcohol (G-type), and synapyl alcohol (S-type) as

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demonstrated in Figure 2.3. Typically, molecules of lignin randomly bond to form the three-dimension structure. An example of macromolecular structure of lignin as shown in Figure 2.4.

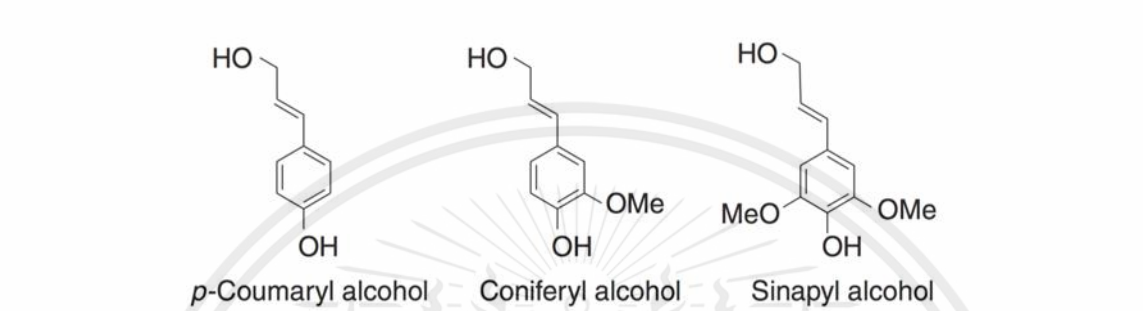

Figure 2.3 Structures of three lignin monomers[8]

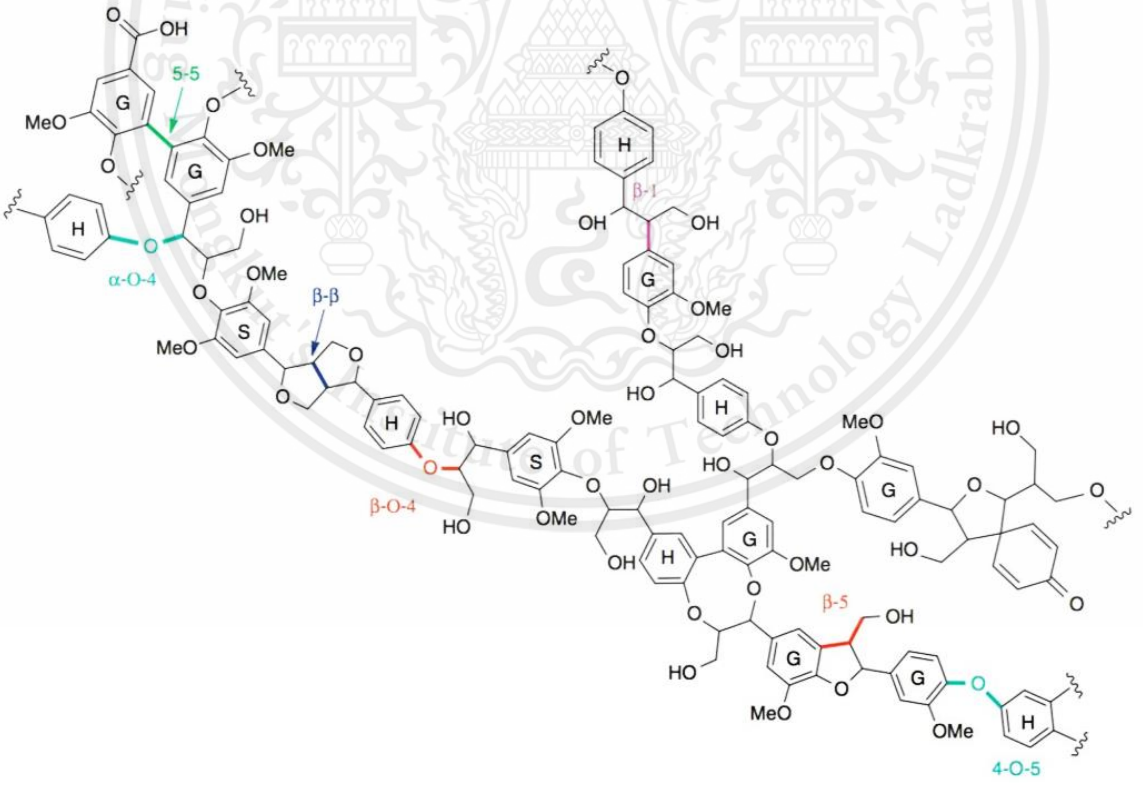

Figure 2.4 Chemical structure of lignin[9]

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2.2.4. Lignin-carbohydrate complexes

Lignin-carbohydrate complexes are linkage between lignin and carbohydrates which are hemicelluloses by hydroxycinnamic acids that consist of ferulic acid that linkage lignin with ether bond and p-coumaric acids that linkage lignin with ester bond via ester and ether bonds as bridges as shown in Figure 2.5. Theoretically, ester bonds are prone to cleavage by alkaline delignification. However, ether bonds are easily cleaved by acid hydrolysis.

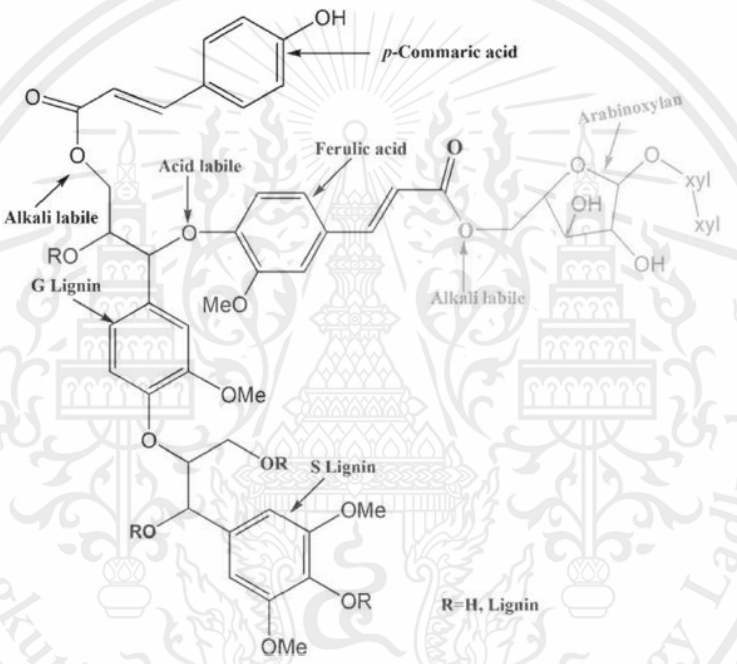

Figure 2.5 Structure of lignin-carbohydrate complexes[2]

2.3 Lignocellulose pretreatments

Chemical pretreatment of lignocellulose is purposed to improve the structure of lignocellulose and separate the components of cellulose, hemicellulose and lignin. Normally, chemical pretreatment methods of lignocellulose including acid pretreatment, alkaline pretreatment, steam explosion pretreatment and organic solvent pretreatment.

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2.3.1 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 of cell wall structure under an elevated temperature. Thus, acid pretreatment is mostly considered as an efficient method for the production of reducing sugars.

2.3.2 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.

2.3.3 Steam explosion pretreatment

Steam explosion pretreatment is a method to improve the structure and increase the crystalline surface area of lignocellulose. Typically, this method is treated with hot steam (160-260°C) under pressure (0.69-4.83 MPa) followed by an explosive decompression of the biomass that results in a break the biomass fibers rigid structure. The immediate pressure released, resulting in the water changing from liquid phase to vapor phase and

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expanding rapidly. These methods resulted the destruction of the lignocellulose structure and the degradation of cellulose, hemicelluloses, and lignin. The products from this method can be further hydrolyzed to produce reducing sugars. However, the steam explosion may cause inhibitors of enzymes such as furfural, for example, if the reducing sugar obtained with the enzyme to produce ethanol, the resulting furfural will inhibit its activity.

2.3.4 Organic solvent pretreatment

Numerous alcohols/water mixtures 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 solvent pretreatment is a very efficient fractionation method of which yields relatively high purity of the three main fractions of lignocelluloses.

2.4 Delignification

2.4.1 Kraft pulping process

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. Structure of Kraft lignin as shown in Figure 2.6.

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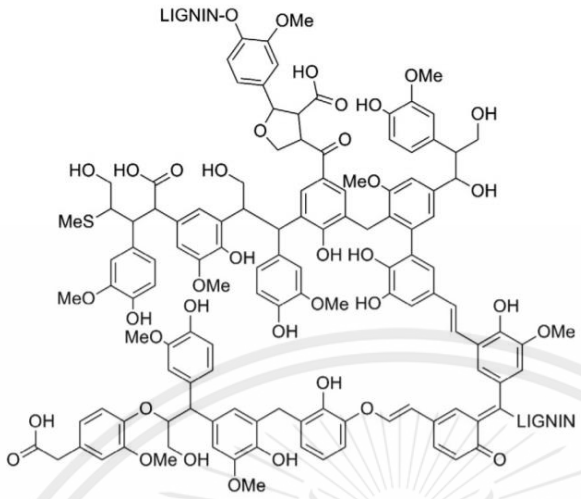

Figure 2.6 Structure of Kraft lignin[10]

2.4.2 Sulfite pulping process

Sulfite pulping process produced lignosulfonate lignin as a by-product which is presently supplanted by the kraft process. In sulfite pulping, either sulfites (SO_3^{2-}) or bisulfites (HSO_3^-) is used as are agent 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 2.7, hydrophilic property of lignin molecules is increased, which promotes the water solubility and allows them to settle apart from carbohydrate matrixes.

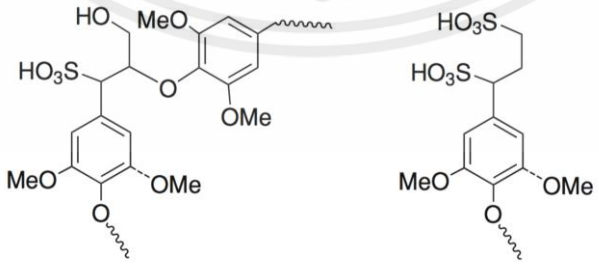

Figure 2.7 Possible phenylpropane structure of lignosulfonate lignin[10]

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2.4.3 Organosolv process

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 lignin. 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.

2.5 Oxidation of lignin

Oxidative degradation of lignin is used in the production of lignin monomers. Oxidizing agents such as hydrogen peroxide, metal oxides, nitrobenzene, etc. Degradation of lignin involves the breaking of the bonds between two carbon atoms (C-C linkage) and the carbon-oxygen bond (C-O linkage), which is the bonding between the monomers lignin and obtained the monomers as shown in Figure 2.8.

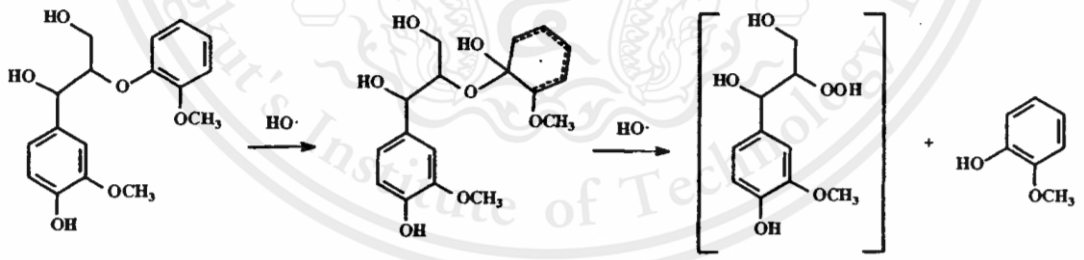

Figure 2.8 Mechanism of oxidation reaction[11]

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2.6 Literature reviews

Wang et al[12]. 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 for90 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.

Zhou[13] studied 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.

Xin-ping et al.[3] studied the proper conditions of lignin oxidative degradation for producing mono phenolic compounds with hydrogen peroxide, copper (II) oxide, and iron (III) sulfate under microwave irradiation. The results indicated that the degradation of wheat straw alkaline lignin and yield of mono phenolic 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 mono phenolic compounds (4.01wt%). The optimal conditions, where the highest degradation degree (94.47wt%) and maximum yield of mono phenolic 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.

Anantkijthamrong[14] studied conversion of lignin to the phenolic monomers by two-step. In the first stage, extractives-free sugarcane bagasse was hydrothermally liquified. The effect sodium hydroxide concentrations in a range of 1-4%w/v were explored using a

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10-ml batch reactor at temperatures 100-200°C for 30 min. As results, the removal degrees of hemicelluloses and lignin from sugarcane bagasse were 89.87-92.20wt% and 94.59-98.19wt%, respectively. As the second stage oxidation products, phenolic monomers were produced.

Techan[4] studied the conversion of lignin to a two-step phenolic monomer under the same alkaline conditions as Anantkijthamrong[14] but increased the value of the experimental conditions including concentration range 0.5-12%w/v and temperature 80-240°C. In first stage, the removal degrees of lignin from extractive-free sugarcane bagasse were 94.6-98.2wt%. In second stage, the highest total yield of phenolic monomers was found at 3.09wt% by using liquefied lignin from the first stage as treated by NaOH 3%w/v at 160°C.

Thongue[5] studied the two-step conversion of lignin to phenolic monomer compounds under additional alkaline conditions for the analysis reducing sugars produced by the degradation of cellulose and hemicellulose. Up to and including the effect of temperature of oxidation under alkalinity in second stage was also studied on the yield of phenolic monomer compounds. The experiment was conducted at 130-190°C for 30 minutes. It was found that the delignification in the first stage at the concentration of 4 %w/v sodium hydroxide solution and the temperature of 140°C was followed by oxidative lignin decomposition under alkaline conditions in second stage at 190°C produced the highest total yield phenolic monomers of 13.5wt% on lignin basis. Additionally, the analysis of reducing sugars in liquid products, it was found that the effect was less than 1wt% sugarcane bagasse. This indicated that partly hemicellulose and cellulose removed from the plant cell wall structure during two-step conversion of lignin to phenolic monomer compounds occurred in form of complex structure substance and cannot be degraded into reducing sugars.

In addition, Sinsatitporn[6] studied more first-stage conditions by using NaOH concentrations in the rage of 5-10%w/v. It was found that the appropriate conditions NaOH concentrations and the corresponding temperatures (°C) were (5,140), (6,130), (8,120), (10,110) and. In the second stage, the liquefied lignin was then subjected to oxidative

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depolymerization under alkaline conditions by using hydrogen peroxide coupled with copper (II) oxide and iron (III) sulfate as catalysts of 110-190°C for 30 minutes. The highest total yield of phenolic monomers at 11.2wt% of lignin were obtained when using NaOH concentration of 4%w/v and temperature of 140°C in the first stage followed by using temperature of 190°C in the second stage.

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CHAPTER III

RESEARCH METHODOLOGY

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^{o}C for 24 hours then 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 425-850μm was collected.

3.1.2 Acetone extraction

The sample bagasse from size exclusion was subjected to acetone extraction by using Soxhlet for 8 hours. After that the extracted bagasse was left in fume hood for 24 hours to allow an excessive amount of acetone to volatilize and dried in the oven at 60^{o}C for 24 hours.

3.1.3 Hot water extraction

The sample bagasse from acetone extraction was immersed in a beaker of deionized water. A plate heater equipped with magnetic stirrer was employed. The temperature was kept constant at 60^{o}C for 1 hour with vigorous mixing. The mixture was then cooled down to room temperature, and consequently filtered with vacuum filtration. The obtained solid fraction was oven-dried at 105^{o}C for 24 hours. This extractives-free sugarcane bagasse was kept dried in a zip-lock bag and used in this study. However, previously experiments, the extractives-free sugarcane bagasse was dried again at 60^{o}C for 24 hours and immediately recorded its weight. All calculations of percentage conversion or yields through this study were reported on this oven-dried extractives-free sugarcane bagasse basis.

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3.2 Chemical composition analysis by Klason lignin determination method

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

3.2.1 Materials and chemicals

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

3.2.2 Equipment and apparatus

1. 100-ml amber glass bottle
2. 50-ml beaker
3. 1-ml glass pipette
4. Vacuum filter
5. Autoclave
6. Desiccator
7. Filter paper (Whatman no.540)
8. Glass rod
9. Glass funnel

3.2.3 Experimental procedures

1. Dry extractives-free sugarcane bagasse and the residues obtained from first stage in the oven at 60^{o}C for 24 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

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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 put it in an autoclave, heat up to 121^{o}C, and keep at this temperature for 1 hour then bring the glass bottle out and cool down to room temperature
7. Filtrate the mixture by vacuum filter to separate hydrolysates from solid residue
8. Analyze the amounts of glucose and xylose in hydrolysates. The obtained glucose and xylose yields(wt%) for the amount cellulose and hemicelluloses, respectively
9. Dry the solid residue at 60^{o}C for 24 hours, cool down to room temperature and calculate its weight as the amount of lignin from sample

3.3 Two-step conversion of lignin to phenolic monomers

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

Oven-dried extractives-free sugarcane bagasse used to liquefaction for delignification from lignocellulose under alkaline condition. The appropriate conditions from Techan[4] and Sinsatitporn[6] are used which are 0.5-12%w/v sodium hydroxide solution and temperature among range 80-220^{o}C.

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3.3.1.1 Materials and Chemicals

1. Oven-dried extractives-free sugarcane bagasse
2. 0.5-12.0%w/v of sodium hydroxide solution
3. 99.99%w nitrogen gas

3.3.1.2 Equipment and apparatus

1. 10-mL batch reactor equipped with thermocouple
2. Heater (furnace) and controller experimental set-up
3. Vacuum filter
4. Filter paper (PTFE membrane, 0.45 μm)
5. 1-ml glass pipette
6. Iced-water bath

3.3.1.3 Experimental procedure

1. Load 0.1 g of extractives-free sugarcane bagasse into the batch reactor.
2. Add 6 ml of sodium hydroxide solution into batch reactor. Tighten the reactor cap then pressurizing with nitrogen gas at 2.5 MPa and check leakage
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 30 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^{o}C for the second-stage experiment and analysis
8. Oven-dry the solid residue at 60^{o}C for 24 hours and then store in desiccator for further chemical composition analysis

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

3.3.2.1 Materials and Chemicals

1. Liquefied portion from the first-stage hydrothermal liquefaction (section 3.3.1)
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. 72wt% of sulfuric acid
7. 99.99%w nitrogen gas

3.3.2.2 Equipment and apparatus

1. 10-ml batch reactor equipped with thermocouple
2. Heater (furnace) and controller experimental set-up
3. Vacuum filter
4. Filter paper (PTFE membrane, 0.45 μm)
5. 1-ml glass pipette
6. Iced-water bath

3.3.2.3 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

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4. Let the reaction undergoes for 30 minutes, then cool the reactor temperature down by quenching in in iced-water bath for 30 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 filter
7. Neutralize the filtrate with 72wt% of sulfuric 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

3.4 Extraction of phenolic monomer compounds with ethyl acetate

3.4.1 Materials and Chemicals

1. Neutralize liquefied portion from two-step conversion of lignin to phenolic monomers
2. 0.05 mg/ml of 1,3-diphenoxybenzene in ethyl acetate (internal standard solution)

3.4.2 Equipment and apparatus

1. 1-ml glass pipette
2. Pasteur pipette
3. 5 ml test tube

3.4.3 Experimental procedures

1. Load 1.5 ml of neutralize liquefied portion from two-step conversion of lignin to phenolic monomers into test tube
2. Add 0.3 ml of 0.05 mg/ml of 1,3-diphenoxybenzene in ethyl acetate (internal standard solution) and add enough sodium sulfate into test tube
3. Tighten the test tube and shake test tube 100 times by hand then left to separate the solution and acetate layers

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4. Using a pasteur pipette, suction the ethyl acetate layer and add it to the sampling via for analysis by gas chromatography–mass spectrometry (GC-MS) technique or it was used to conduct a sillylate derivative reaction before the gas chromatography technique was analyzed

3.5 Derivatization of phenolic monomer compounds prior to GC-MS analysis

Schummer et al[16]reported methods for analyzing the type and quantity of phenolic monomer compounds. The silylate derivatives were treated with N,O-bis(trimethylsilyl) trifluoroacetamide, BSTFA before being analyzed by gas chromatography. A phenolic monomer compound with higher vaporization and thermal stability is obtained. As a result, the detection ability and the chromatograms of the substance can be better separated.

3.5.1 Materials and Chemicals

1. The liquefied product from two-step depolymerization in ethyl acetate layer
2. N,O-bis(trimethylsilyl) trifluoroacetamide
3. Pyridine
4. Sodium sulfate

3.5.2 Equipment and apparatus

1. Micro syringe 20, 50 and 100 µl
2. 5 ml test tube
3. Test tube for GC-MS

3.5.3 Procedures

1. Add 20 µl of N,O-bis(trimethylsilyl) trifluoroacetamide and 5 µl of pyridine into test tube for GC-MS
2. Add 40 µl liquefied production ethyl acetate layer from two-step depolymerization into test tube for GC-MS then waiting for 2 hours

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3. 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:	Shodex KS-801
Detector:	Refractive index Detector(RI)
Eluent:	deionized water
Eluent flow rate:	1 ml/min
Column Temperature:	80^{o}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

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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 Quantification of phenolic monomers in liquefied

Phenolic monomer compounds formed by the conversion of lignin to phenolic monomer compounds in two stages and through the extraction with ethyl acetate can be analyzed by using Gas Chromatography (GC) technique to identify the type and quantity of Phenolic monomer compounds formed By using the condition of analysis tools as follows

GC column:	HP5-MS
Carrier gas:	Helium gas
Injection temperature:	200^{o}C
Column Temperature:	40^{o}C for 5 minutes then increasing temperature 5^{o}C every 1 minutes until to 300^{o}C and maintain the temperature at 300^{o}C for 5 minutes

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Specify the type of phenolic monomer compound using the standard graph of phenolic monomer compounds were standardized and quantified by the ratio between the area under the chromatogram of the phenolic monomer compound to the area under the chromatogram of internal standard compound (1,3-diphenoxibenzene) as equation 3.4 when using the ratio of the area under the curve to the standard curve, the amount of phenolic monomer compounds will be known.

$$\text{Ratio of the area under the graph} = \frac{\text{Peak area of phenolic monomer}}{\text{Peak area of internal standard compound}} \qquad 3\text{-}4$$

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CHAPTER IV

RESULTS AND DISCUSSION

In this chapter, the compositions of cellulose, hemicelluloses and lignin in extractives free-sugarcane bagasse were described, including, the results of two-step lignin depolymerization. In addition, the amounts of phenolic monomers yield from the second-stage oxidative depolymerization were mentioned as following.

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 referred to values reported by Sinsatitporn[6]. 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 20.4wt% 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 44.0, 34.7, and wt%, 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 amounts 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 39.6wt% of cellulose, 30.5wt% of hemicelluloses, and 20.4wt% of lignin which correspond to Rabemanolontsa and Saka[15].

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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[15]	45.5	27.8	1.6	0.0	0.0
Sinsatitporn[6]	44.0	34.7	0.0	0.0	0.0

Table 4.2 Cell wall chemical compositions of extractives-free sugarcane bagasse

Sample	Cell wall chemical compositions (%wt)		
	Cellulose	Hemicelluloses	Lignin
Rabemanolontsa and Saka[15]	40.9	33.0	22.3
Techan[4]	38.4	31.4	20.9
Sinsatitporn[6]	39.6	30.5	20.4

4.2 Two-step lignin oxidative depolymerization under alkaline conditions

4.2.1 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 0.5-12.0%w/v and temperatures of 80-210^{o}C for 30 min. From the experiment, the graph of the relationship between the concentration of the sodium

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hydroxide solution and other values which were 0.5-10wt/v%, volume, temperature and weight of solid residue was obtained.

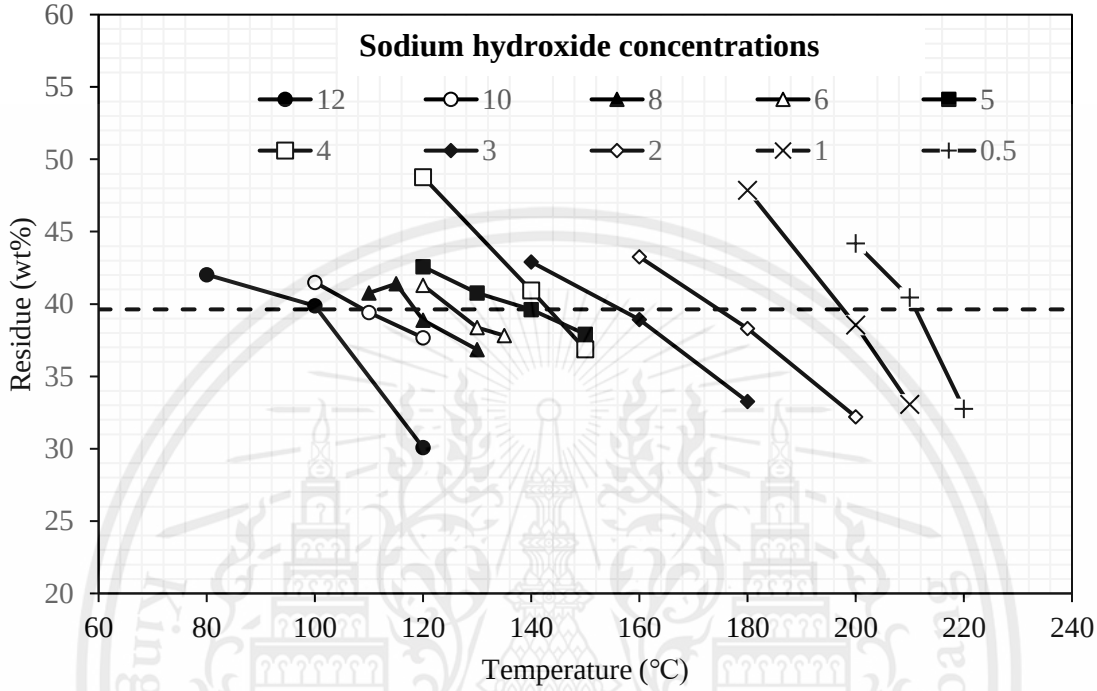

Figure 4.1 Changes in solid residue weights as treated by first-stage hydrothermal liquefaction using 0.5-12%w/v of sodium hydroxide solutions at 80-220°C for 30 min[6]

However, this study was considered optimal conditions from value that close to the cellulose content in the extractives-free sugarcane bagasse at 39.6 %wt. It was found that the concentration of sodium hydroxide solution in units by w/v% and temperature in units by °C for the removal of lignin from the cell wall structure at the first-stage delignification were (0.5, 210), (1, 200), (2, 180), (3, 160), (4, 140), (5, 140), (6, 130), (8, 120), (10, 110) and (12, 100). Moreover, at these reaction conditions, it was found that the %wt of solid residues left from the reaction were 40.5, 38.6 38.3 38.9 40.9 39.6 38.4 38.9 39.4 and 39.9 %wt, respectively. In the order between the concentration of sodium hydroxide solution and temperature, it was found that the concentration of the solution was inversely proportional to the temperature.

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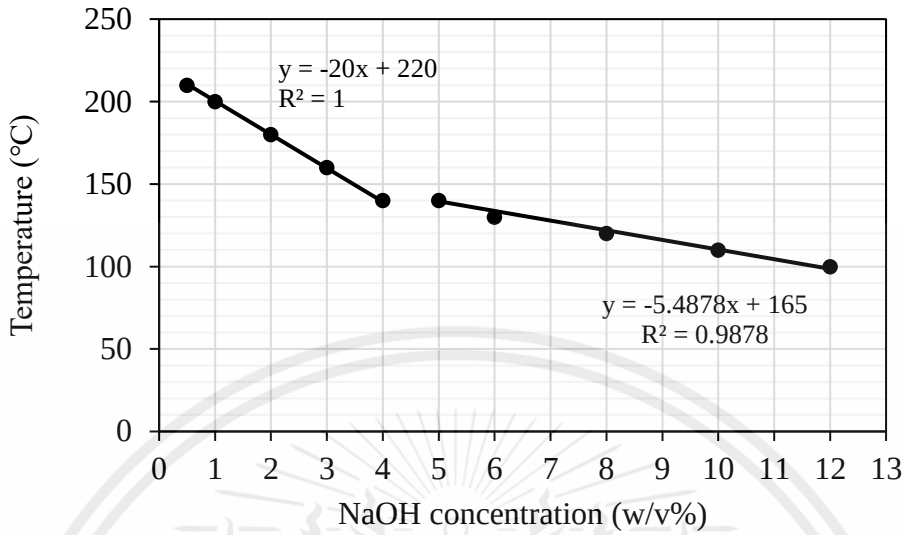

Figure 4.2 Co-linearity plot of two independent variables, namely concentration and temperature, in a the first-stage hydrothermal delignification[6]

Figure 4.2 shows the co-linearity plot of two independent variables, namely concentration and temperature, in a the first-stage hydrothermal delignification. Each data point in this graph represents the coordinate of NaOH concentration and corresponding temperature whereby the delignification is satisfactorily done with cellulose-rich residue remained. As seen, the NaOH concentration and temperature are inversely proportional to each other. This graph can be divided into 2 parts, the slope of the first part, sodium hydroxide concentration 0.5-4 %w/v, was greater than that the second part, sodium hydroxide concentration 5-12 %w/v. It is apparently seen that increasing NaOH concentration in the first part has more effective result in reduction of delignification temperature than that in the second part.

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4.2.2 Second-stage oxidative depolymerization of the obtained liquefied portions

The objective of second-stage oxidative depolymerization was converted the lignin polymer in the liquefied portions from first-stage delignification to phenolic monomer compounds by oxidation under alkaline conditions. Hydrogen peroxide as an oxidizer was used with copper (II) oxide and iron (III) sulfate as a catalyst, reacting at 210-250 °C for 30 min.

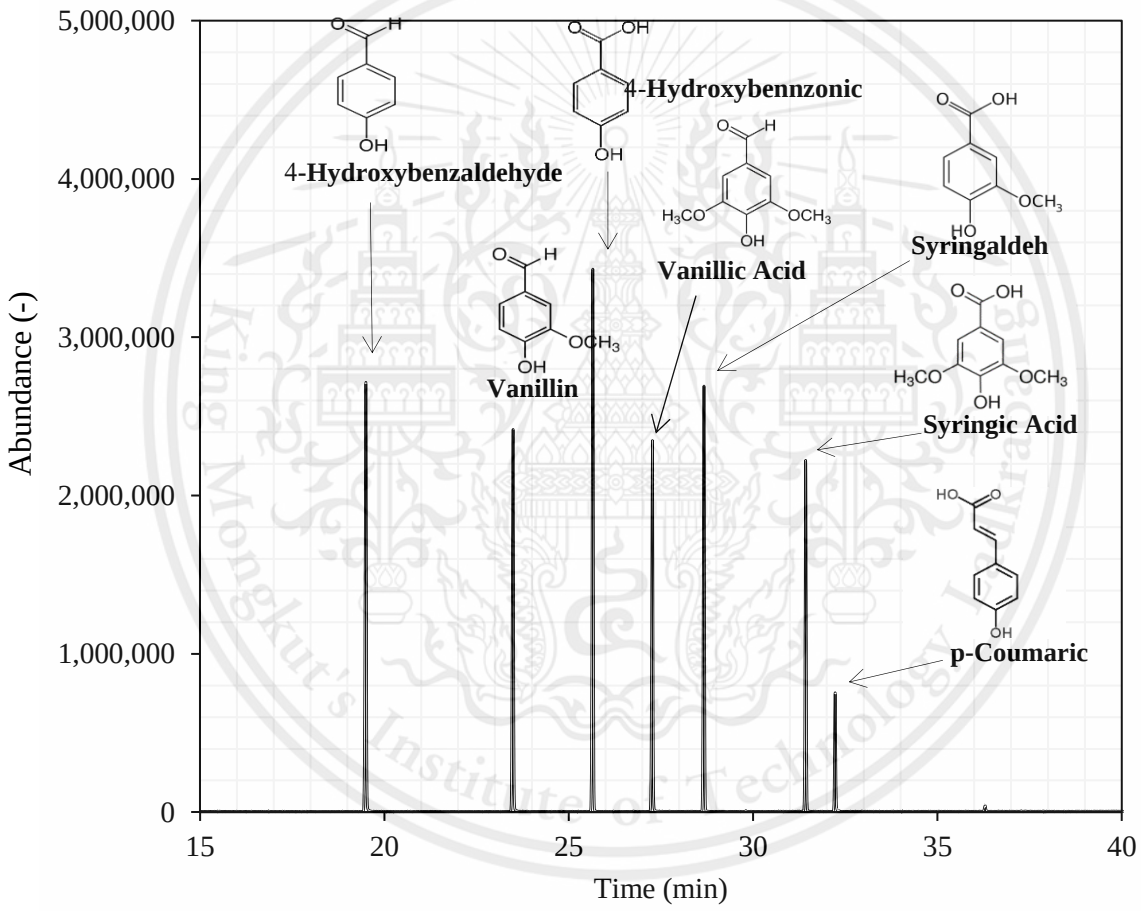

Figure 4.3 Example of GC-MS chromatogram indicating phenolic monomer compounds

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The extractives-free sugarcane bagasse was reacted with hydrothermal liquefaction in first-stage delignification using the optimal conditions for removing lignin from the cell wall structure and the liquid product from the first step is used to further reacted in the second stage. The liquefied product from this stage is extracted with ethyl acetate and reacted with silylate derivatization with N,O-bis(trimethylsilyl) trifluoroacetamide and pyridine before being analyzed by GC-MS to specify the type and quantity of phenolic monomer compounds that formed. Figure 4.3 shown a chromatogram of a standard solution containing phenolic monomer compounds found in the liquefied product of the oxidation reaction in second-stage oxidative depolymerization.

Comparison of lignin removal from cell wall structure in first-stage delignification with the percentage yield of phenolic monomer compounds formed in second-stage oxidative depolymerization was shown in Table 4.3, 4.4, 4.5 and 4.6. It was found that the higher the temperature in the second-stage oxidative depolymerization, the less phenolic compounds are obtained.

Table 4.3 Phenolic monomers yield from 2nd-stage oxidative depolymerization at 210°C for 30 min

Appropriate conditions for removing lignin in 1st-stage		Phenolic monomers yield in 2nd-stage (%wt on lignin basis)							
NaOH concentrations (%w/v)	Temperature (°C)	H	HA	p-CA	V	VA	S	SA	Total
3	160	3.8	-	-	0.2	-	-	-	4.0
4	140	2.2	-	-	-	-	-	-	2.2

Remarks: H refers to 4-hydroxybenzaldehyde, HA refers to 4-hydroxybennzonic acid
p-CA refers to p-coumaric acid , V refers to vanillin
VA refers to vanillic acid , S refers to syringaldehyde
SA refers to syringic acid

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Table 4.4 Phenolic monomers yield from 2nd-stage oxidative depolymerization at 230°C for 30 min

Appropriate conditions for removing lignin in 1st-stage		Phenolic monomers yield in 2nd-stage ((%wt on lignin basis)							
NaOH concentrations (%w/v)	Temperature (°C)	H	HA	p-CA	V	VA	S	SA	Total
3	160	1.5	-	-	-	-	-	-	1.5
4	140	0.7	-	-	-	-	-	-	0.7

Remarks: H refers to 4-hydroxybenzaldehyde, HA refers to 4-hydroxybennzonic acid
p-CA refers to p-coumaric acid , V refers to vanillin
VA refers to vanillic acid , S refers to syringaldehyde
SA refers to syringic acid

Table 4.5 Phenolic monomers yield from 2nd-stage oxidative depolymerization at 250°C for 30 min

Appropriate conditions for removing lignin in 1st-stage		Phenolic monomers yield in 2nd-stage ((%wt on lignin basis)							
NaOH concentrations (%w/v)	Temperature (°C)	H	HA	p-CA	V	VA	S	SA	Total
3	160	-	-	-	-	-	-	-	0.0
4	140	0.1	-	-	-	-	-	-	0.1

Remarks: H refers to 4-hydroxybenzaldehyde, HA refers to 4-hydroxybennzonic acid
p-CA refers to p-coumaric acid , V refers to vanillin
VA refers to vanillic acid , S refers to syringaldehyde
SA refers to syringic acid

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Table 4.6 Summary of phenolic monomer yield obtained from the two-step oxidative depolymerization using various conditions (combined with the data from Sinsathaporn[6])

Appropriate conditions for removing lignin in 1st-stage		Phenolic monomers yield in 2nd-stage at various temperature (%wt on lignin basis)							
		Temperature (°C)							
NaOH concentration (%w/v)	Temperature (°C)	110	130	150	170	190	210*	230*	250*
0.5	210	0.0	0.0	0.0	0.2	0.0	-	-	-
1	200	0.0	0.8	0.8	1.3	0.0	-	-	-
2	180	0.0	2.0	1.7	3.1	2.0	-	-	-
3	160	0.0	2.7	2.0	3.3	0.0	4.0	1.5	0.0
4	140	0.7	4.4	4.2	6.5	11.2	2.2	0.7	0.1
5	140	0.7	0.3	4.1	6.4	9.9	-	-	-
6	130	0.0	0.1	0.3	4.6	0.3	-	-	-
8	120	0.0	0.3	0.0	0.0	0.1	-	-	-
10	110	0.0	0.3	0.0	0.0	0.1	-	-	-
12	100	0.0	1.1	0.0	0.0	5.5	-	-	-

Remarks * Extended experimental conditions performed in this present study

Moreover, when combining the data from Sinsatitporn[6], it was found that the phenolic monomers yield decreased when the temperature above 210°C as shown Table 4.6. Therefore, it was concluded that in optimization of two-step lignin depolymerization from sugarcane bagasse to phenolic monomer compounds under alkaline conditions were 4 w/v% of sodium hydroxide solution at 140 °C in the first-stage delignification and at 210 °C in the second-stage oxidative depolymerization. In addition, Figure 4.4 illustrate to determine the optimum point of two-step oxidative depolymerization, the concentration

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and temperature were also inversely proportional to the same as in the first-stage and the optimum point where the highest phenolic monomers yield were obtained when first-stage NaOH concentrations (%w/v) and the corresponding the second-stage temperatures (℃) of (3,210), (4,190) and (6,130) were used.

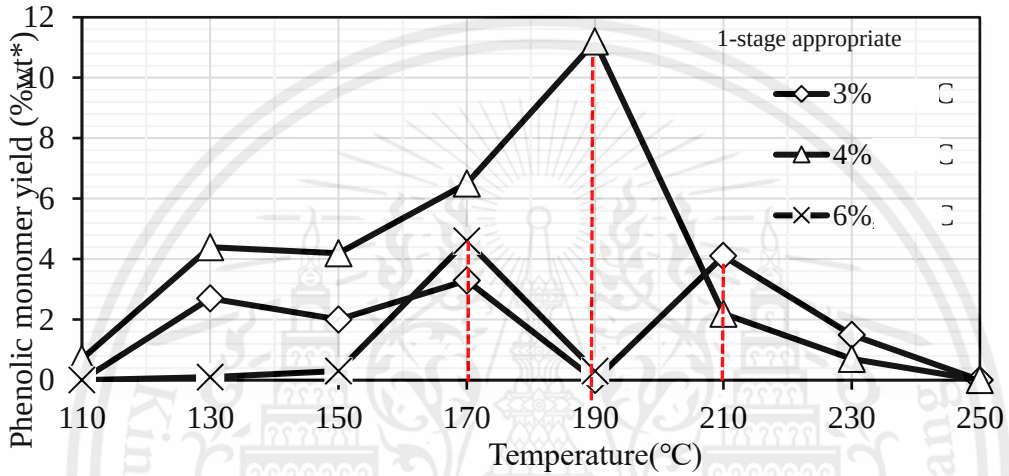

Figure 4.4 Comparative phenolic monomers yield obtained from the 2-stage oxidative depolymerization using various conditions

From the experiment, the chemical compositions can be classified as type in Table 4.7. The highest yield of p-type phenolic monomers such as hydroxybenzaldehyde, hydroxybenzoic acid was obtained at 6.9wt% on lignin basis, which agrees with total p-type of 7.2wt% on lignin basis from Rabemanolonsta and Saka[15]. In addition, comparing these results with one-step[4] depolymerization, the phenolic monomers yield of 9.2wt% lignin was lower than two-step depolymerization of 11.2wt% in this study.

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Table 4.7 Comparison between sugarcane bagasse lignin compositions by types[15] and phenolic monomer products by types from two-step oxidative depolymerization of lignin at optimum conditions (1^{st} -stage: 4%w/v NaOH, 140 C; 2^{nd}-stage: 190 C)

Data source	Lignin composition by types (%wt on lignin basis)		
	P-type	G-type	S-type
Rabemanolonsta and Saka[15]	7.2	47.4	45.4
Present study	6.9	3.5	1.3

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CHAPTER V

CONCLUSION

5.1 Conclusion

In this study, optimization of two-step lignin depolymerization from sugarcane bagasse to phenolic monomer compounds under alkaline conditions using hydrogen peroxide with copper (II) oxide and iron (III) sulfate catalysts. This study was conducted to confirm and added conditions to the experiment reported by Sinsatitporn[6]. The concentration of sodium hydroxide solution 0.5-12 %w/v and the temperature in first-stage delignification from 110-210°C and the temperature in second-stage oxidative depolymerization were studied from 210-250°C considering the appropriate solid residue recovery conditions of cellulose.

In the first-stage delignification, it was found the appropriate conditions that removing lignin from cell wall structure at concentrations of sodium hydroxide solutions by units w/v% and the temperature by units °C were (0.5, 210), (1, 200), (2, 180), (3, 160), (4, 140), (5, 140), (6, 130), (8, 120), (10, 110) and (12, 100). In the second stage, oxidative depolymerization was performed over a temperature range of 130-250°C whereby NaOH concentration was kept as inherent one in the first stage. hydroxybenzaldehyde, hydroxybenzoic acid, p-coumaric acid, vanillin, vanillic acid, syringaldehyde, and syringic acid were produced. The highest yield phenolic monomers of 11.2 wt% were produced by using liquefied lignin obtained by (4%w/v, 140°C) from the first stage and subsequently treated at 190 °C in the second stage. It was revealed that compositions of phenolic monomer compounds varied among first and second stage treatment conditions. At the optimum point, hydroxybenzaldehyde and hydroxybenzoic, namely p-type lignin, were found as the major products. This might be derived from hydroxycinnamic acids in lignin-carbohydrate complexes

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5.2 Recommendations

5.2.1 Due to a lack of time, it is recommended to performed additional experiments by studying on NaOH concentrations at 0.5-2 %w/v and 5-12 %w/v with the second-stage temperature at 210-250°C to complete the whole set of experimental conditions.

5.2.2 The liquefied products from the first-stage delignification were not clearly understood their chemical structure yet. Then to clarify this point, characterizations of lignin structure such as molecular weight distributions and elemental analysis (C-H ratio) are recommended.

5.2.3 In addition, the difference of the first-stage liquefied lignin structure can be clarified by adjusting concentration of all liquefied product from first-stage delignification to 4 %w/v and treating with 190 ℃. If similar phenolic monomer yield is obtained, this might be inferred that the first-stage liquefied lignin structure is alike.

5.2.4 It is recommended to study the other alkaline solutions such as KOH, $Ca(OH)_2$ and $Ba(OH)_2$ in two-step lignin depolymerization to phenolic monomer compounds under alkaline conditions to explore the differences in the first-stage delignification and production of phenolic monomers in the second-stage oxidative depolymerization.

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APPENDICES

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APPENDIX A
PREPARATIONS OF CHEMICALS

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

NaOH concentration (%w/v)	Weight of pellets NaOH (g)
0.125	0.5
0.250	1
0.500	2
0.750	3
1.000	4
1.250	5
1.500	6
2.000	8
2.500	10
3.000	12

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3. Preparation of phenolic monomers standard solution

The phenolic monomer compound used as a standard solution for gas chromatographic analysis is 4-hydroxybenzaldehyde, 4-hydroxybennzonic acid, vanillin, vanillic acid, syringaldehyde. and syringic acid. First, preparing a standard solution by weighing approximately 0.01 g of phenolic monomer compound. Note the exact weight and dissolve 1,3-diphenoxybenzene in ethyl acetate at a concentration of 0.05 mg/mL. A volume of 5 ml yields a solution of phenolic monomer with a concentration of 2 mg/ml. to create a standard graph.

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APPENDIX B

RAW DATA

1. Temperature profile

A two-step lignin depolymerization from sugarcane bagasse to phenolic monomer compounds under alkaline conditions in this study was conducted using a batch reactor, during which the first 30 min was the time to raise the temperature to desired value in reaction. After that, the reaction time was counted. An example of the reaction temperature pattern was shown in Figure B.1.

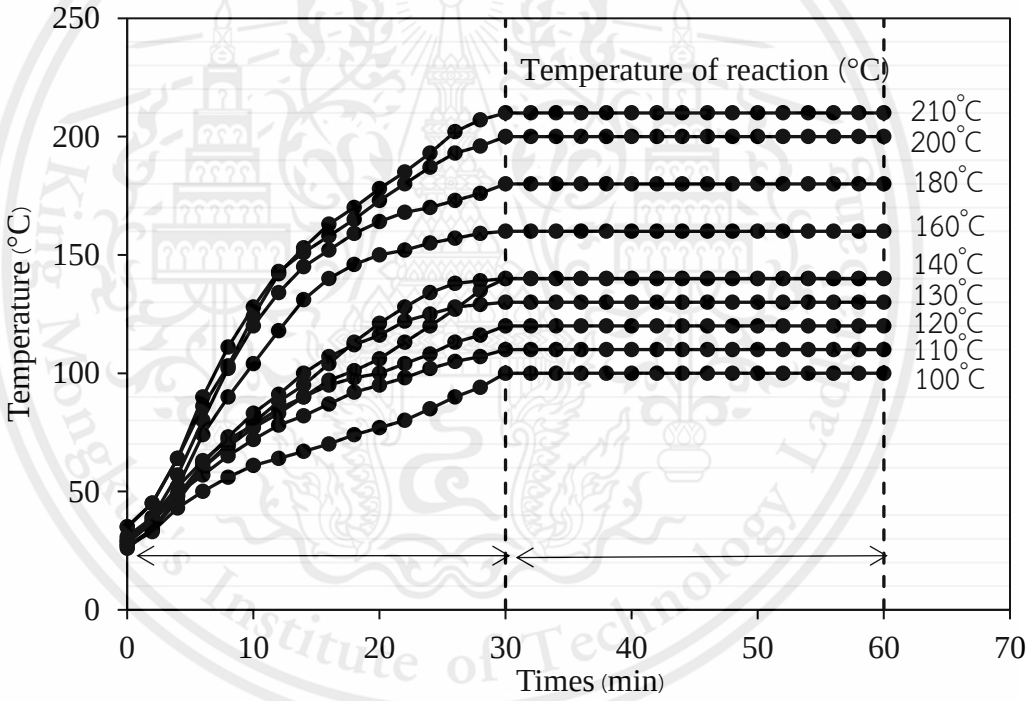

Figure B.1 Temperature profile in first-stage hydrothermal reaction

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2. Calibration curves for calculations of the phenolic monomers concentration analyzed by GC-MS

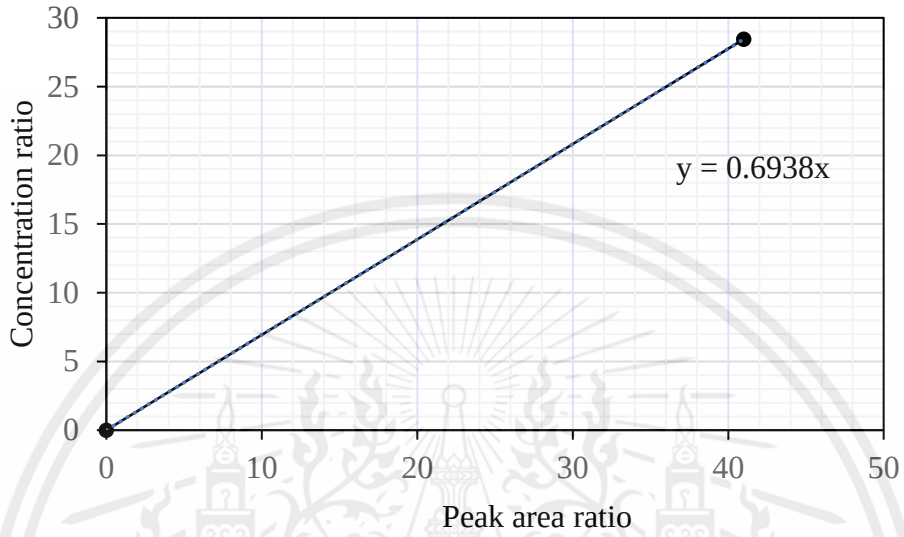

Figure B.2 Calibration curve of peak area from GC-MS chromatogram and concentration of 4-hydroxybenzaldehyde standard solution

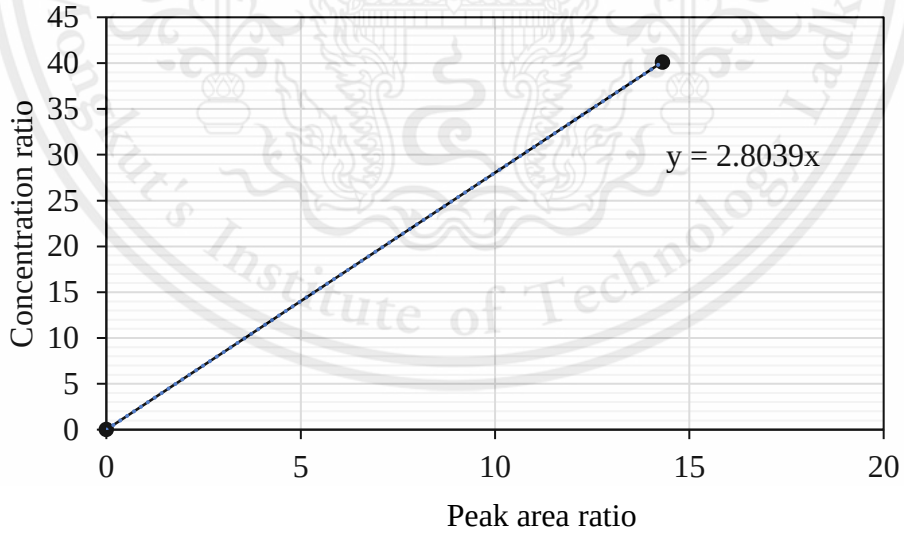

Figure B.3 Calibration curve of peak area from GC-MS chromatogram and concentration of 4-hydroxybennzonic acid standard solution

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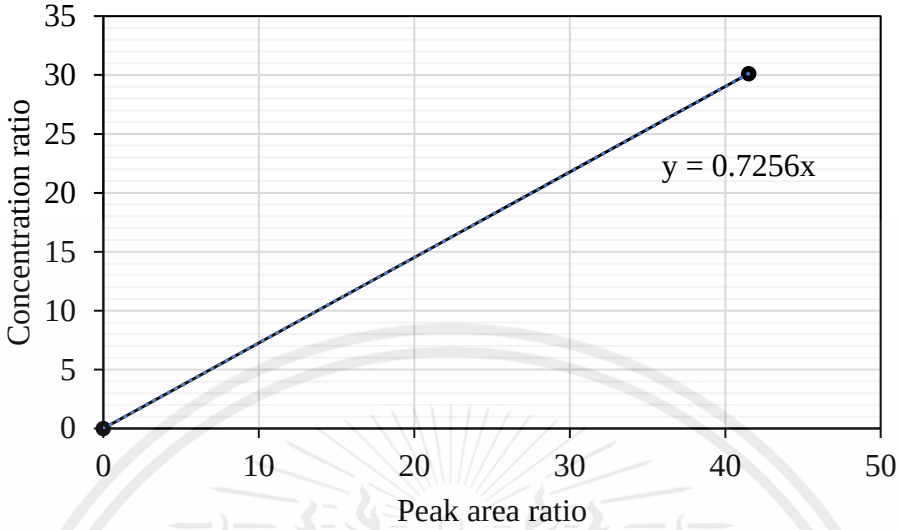

Figure B.4 Calibration curve of peak area from GC-MS chromatogram and concentration of vanillin standard solution

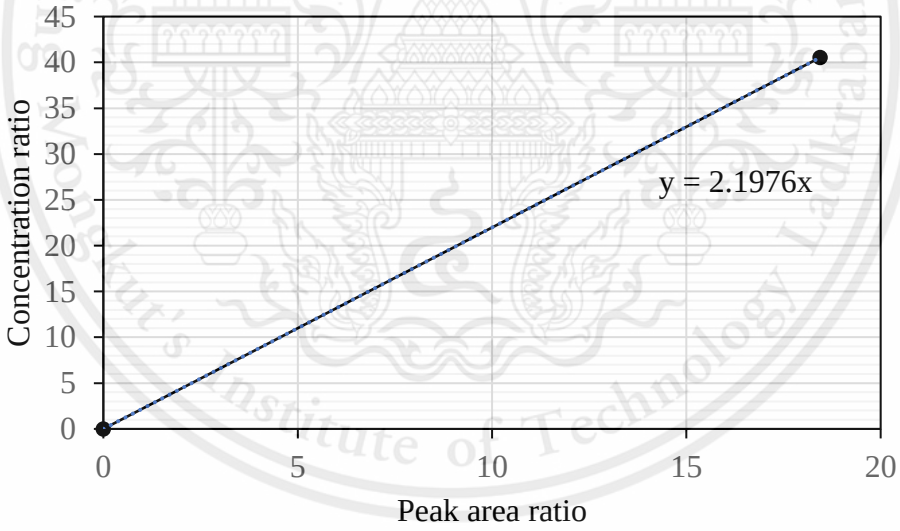

Figure B.5 Calibration curve of peak area from GC-MS chromatogram and concentration of vanillic acid standard solution

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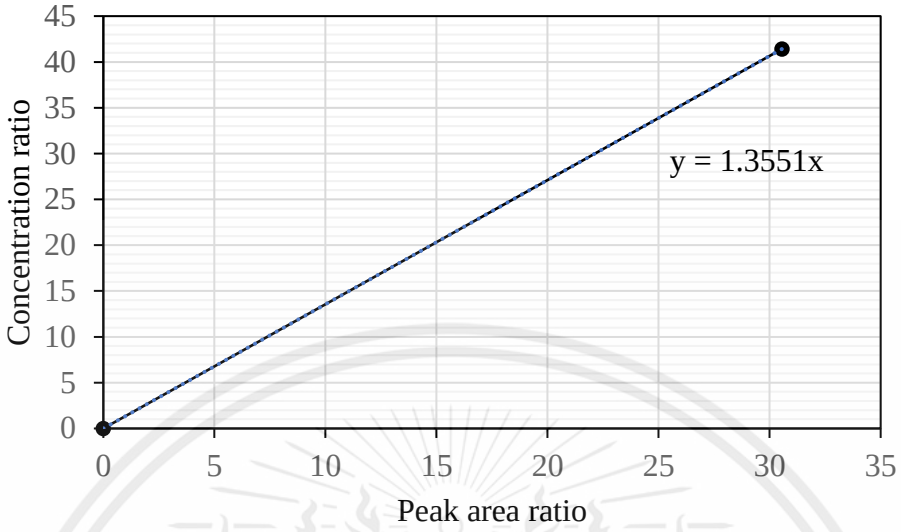

Figure B.6 Calibration curve of peak area from GC-MS chromatogram and concentration of syringaldehyde standard solution

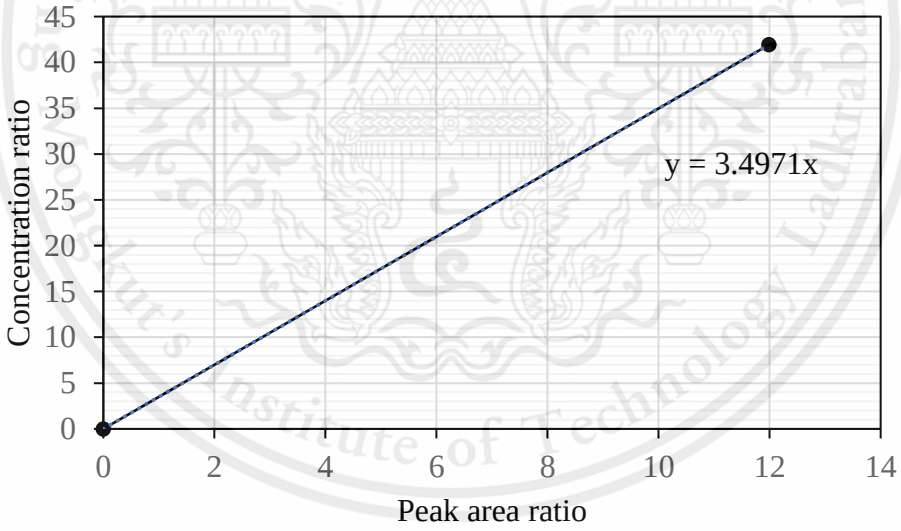

Figure B.7 Calibration curve of peak area from GC-MS chromatogram and concentration of syringic acid standard solution

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APPENDIX C
CALCULATION

1. Table C 1 Raw data from GC-MS chromatogram

Sample		Peak area							
NaOH concen-tration	Tempe -rature	H	HA	p-CA	V	VA	S	SA	Internal standard
3%w/v	210	$2.2x10^5$	$3.8x10^4$	-	$4.0x10^4$	-	-	-	$1.2x10^5$
	230	$9.3x10^5$	-	-	-	-	-	-	$1.3x10^5$
	250	-	-	-	-	-	-	-	$1.2x10^5$
4 %w/v	210	$1.2x10^6$	-	-	-	-	-	-	$1.1x10^5$
	230	$4.0x10^5$	-	-	-	-	-	-	$1.3x10^5$
	250	$2.5x10^5$	-	-	-	-	-	-	$1.4x10^5$

2. Calculation of chemical compositions phenolic monomers from GC-MS

Example Calculation of the amount of phenolic monomers in liquefied product obtained from delignification as treated by 4%w/v at 140℃ for 30 min and the 0xidative depolymerization temperature at 210℃.

Peak area of 4-hydroxybenzaldehyde standard solution obtained from GC-MS chromatogram was 112,111and peak area of 4-hydroxybenzaldehyde 1,191,742.

Peak area ratio = 1,191,742/112,111

= 10.6

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From the standard curve between the area ratio under the curve (x) and the concentration ratio of the 4-hydroxybenzaldehyde standard solution to the standard substance (y).

$$y = 0.6938x$$

$$y = 0.6938(10.6)$$

$$= 7.35428$$

Due to gas chromatography analysis, the standard concentration of 1,3-diphenoxybenzene was 0.05 mg/mL, volume 0.3 ml, and the reaction in second-stage, the silylate derivative reacted to dilute the solutions 4 times.

Therefore, the amount of 4-hydroxybenzaldehyde was produced by the second-stage oxidative depolymerization.

$$= 7.35428 \times 0.05 \times 0.3 \times 4$$

$$= 0.44 \text{ mg}$$

In addition, the %wt of 4-hydroxybenzaldehyde was calculated as compared to the weight of lignin in the extractives-free sugarcane bagasse of 0.02035 g

$$\%\text{wt of 4-hydroxybenzaldehyde} = \frac{0.44}{0.02035 \times 1000} \times 100 = 2.16$$

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