Effect of acidity on hydrolysis and depolymerization of lignocellulose in one-pot lignin-first biorefinery

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Engineering in Chemical Engineering
Department of Chemical Engineering
FACULTY OF ENGINEERING
Chulalongkorn University
Academic Year 2020

ผลของความเป็นกรดต่อการเกิดปฏิกิริยาไฮโดรไลซิสและการดีพอลีเมอไรเซชันของลิกโนเซลลูโลสในกระบวนการลิกนินเฟิร์สไบโอรีไฟเนอรีในขั้นตอนเดียว

น.ส.รัตนาภรณ์ แก้วกล่ำ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิศวกรรมศาสตรมหาบัณฑิต
สาขาวิชาวิศวกรรมเคมี ภาควิชาวิศวกรรมเคมี
คณะวิศวกรรมศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2563

Thesis Title	Effect of acidity on hydrolysis and depolymerization of lignocellulose in one-pot lignin-first biorefinery
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Field of Study	Chemical Engineering
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Accepted by the FACULTY OF ENGINEERING, Chulalongkorn University in Partial Fulfillment of the Requirement for the Master of Engineering

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รัตนาภรณ์ แก้วกล่ำ : ผลของความเป็นกรดต่อการเกิดปฏิกิริยาไฮโดรไลซิสและการดีพอลีเมอไรเซชันของลิกโนเซลลูโลสในกระบวนการลิกนินเฟิร์สไบโอรีไฟเนอรีในขั้นตอนเดียว. (Effect of acidity on hydrolysis and depolymerization of lignocellulose in one-pot lignin-first biorefinery) อ.ที่ปรึกษาหลัก : รศ. ดร. วรงค์ ปวราจารย์

ในการทำลิกนินเฟิร์สไบโอรีไฟเนอรี เป็นวิธีการสำหรับการดึงและดีพอลีเมอไรเซชันลิกนิกในลิกโนเซลลูโลสให้เกิดเป็นผลิตภัณฑ์ที่มีมูลค่า งานวิจัยนี้ได้มีการใช้ตัวทำละลายผสมระหว่างน้ำที่มีกรดและเอทีลีนไกลคอลในการทำลิกนินเฟิร์สไบโอรีไฟเนอรีเพื่อพัฒนาความสามารถในการทำลายพันธะของลิกนินควบคู่กับการดีพอโลเมอไรเซชันเซลลูโลสและเฮมิเซลลูโลสในขั้นตอนเดียว โดยงานวิจัยนี้ใช้ทะลายปาล์มเป็นชีวมวลตั้งต้นและทำการทดลองที่ 180-250°C ภายใต้ความดัน 30 บาร์ของแก๊สไฮโดรเจนเป็นเวลา 1-6 ชั่วโมง การทดลองนี้แสดงให้เห็นว่าการมีกรดในระบบตัวทำละลายผสมช่วยทำให้ผลได้ของน้ำมันลิกนินสูงถึง 60% โดยน้ำหนัก โดยที่ในน้ำมันลิกนิกประกอบไปด้วยโมโนเมอร์สำคัญ เช่น ฟีนอล ไกวอะซิล และ ไซรินกิล เป็นต้น ยิ่งไปกว่านั้นในการทดลองที่พีเอช 4 ยังทำให้ผลได้ของโมโนเมอร์สูงถึง 4.5% เมื่อทำการวิเคราะห์ผลิตภัณฑ์ที่อยู่ในน้ำ พบว่ามี กลูโคส ไซโลส และ อะราบิโนส ซึ่งเป็นผลิตภัณฑ์น้ำตาลที่ได้จากการไฮโดรไลซิสเซลลูโลสและเฮมิเซลลูโลส แสดงให้เห็นว่า กระบวนการลิกนินเฟิร์สไบโอรีไฟเนอรีขั้นตอนเดียว สามารถแยกทั้งลิกนิก เซลลูโลส และเฮมิเซลลูโลส ออกจากชีวมวลตั้งต้น และดีพอลีเมอไรเซชันกลายเป็นมอนอเมอร์ที่มีมูลค่าสูงขึ้นได้ นอกจากนี้ยังมีการศึกษาผลของอุณหภูมิและเวลาในการทำปฏิกิริยาซึ่งพบว่าในสภาวะที่รุนแรงจะส่งผลให้ได้ผลได้ของลิกนินสูง แต่ทำให้ผลได้ของน้ำตาลลดลง งานวิจัยได้ศึกษาบทบาทของตัวทำละลายผสมในการทำลิกนินเฟิร์สไบโอรีไพเนอรีขั้นตอนเดียวพบว่าน้ำที่มีกรดไม่เพียงแต่ดีพอลีเมอไรซ์คาร์โบไฮเดรต แต่ยังมีส่วนในการดีพอลิเมอไรซ์ลิกนินอีกด้วย เอทิลีนไกลคอลช่วยในการละลายลิกนินออกจากชีวมวล และตัวเร่งที่เป็นของแข็งช่วยทำให้ลิกนิกที่ว่องไวมีความเสถียรมากขึ้น

สาขาวิชา วิศวกรรมเคมี ลายมือชื่อนิสิต .....

ปีการศึกษา 2563 ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

6170254321 : MAJOR CHEMICAL ENGINEERING

KEYWORD: Lignin-first biorefinery; Lignin depolymerization; Hydrolysis of carbohydrate; lignocellulose

Rattanaporn Kaewklam : Effect of acidity on hydrolysis and depolymerization of lignocellulose in one-pot lignin-first biorefinery . Advisor: Assoc. Prof. VARONG PAVARAJARN, Ph.D.

In lignin-first biorefinery, extraction and depolymerization of lignin from lignocellulosic materials provide value-added lignin monomers. In this study, a mixture of acid-containing water and ethylene glycol was introduced to the lignin-fist biorefinery for simultaneously improving lignin cleavage, together with the depolymerization of cellulose and hemicellulose within one-pot. The experiments were conducted at 180-250°C under the hydrogen pressure of 30 bar for 1-6 h using oil palm empty fruit bunch (OPEFB) as a biomass source. The presence of acid and mixed solvent in the reaction provided the lignin oil yield up to 60 wt% comprising of the main monomers such as phenol, guaiacyl, and syringyl. Moreover, the acidity at pH 4 leads to the lignin monomer yield of 4.5 wt%. Glucose, xylose, and arabinose from hydrolysis of cellulose and hemicellulose in water phase were identified to clarify that the one-pot lignin-first biorefinery simultaneously depolymerize lignin and hydrolyze carbohydrate. The effects of temperature and reaction time were investigated. The results revealed that the harsh conditions result in high potential of lignin depolymerization but diminish the sugar units of carbohydrate. This study demonstrate that ethylene glycol participates in the extraction of lignin from OPEFB, while acid-containing water participates not only in depolymerization of carbohydrate but also in depolymerization of lignin. On the other hand, acid-catalyst stabilizes the reactive lignin.

Field of Study:	Chemical Engineering	Student's Signature .....
Academic Year:	2020	Advisor's Signature .....

ACKNOWLEDGEMENTS

I would like to express my very great appreciation to Associate Professor Dr. Varong Pavarajarn, my research advisor, for his patient guidance, honest kindness, enthusiastic encouragement, and constructive recommendation throughout my master's degree. Moreover, I would also like to thank Dr. Chalida Klaysom, Dr. Rungthiwa Methaapanon, and Dr. Nawin Viriya-empikul for their comments and supervision as the thesis examination committee.

I would like to extend my thanks to all members of the Center of Excellence in Particle and Material Processing Technology (CEPT), Department of Chemical Engineering, Chulalongkorn University. Further thanks to all staff in the Department of Chemical Engineering, Chulalongkorn University.

Furthermore, I would like to acknowledge Green technology research public company limited for the instrumental analysis support and Prof. Dr. Navadol Laosiripojana and all members of the Joint Graduate School of Energy and Environment (JGSEE), who have shared their precious time throughout the entire graduate degree for the valuable laboratory techniques.

Eventually, I wish to thank my family for their endless amounts of love, important moral support and always being there for me throughout my life.

Rattanaporn Kaewklam

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CHAPTER I

INTRODUCTION

1.1 Introduction

Biorefinery, the process converting biomass to valuable chemicals, fuel, and power, get more attention. Many researchers improved and studied biorefinery technology to reduce residues from agriculture, plants, industries, and animals [1]. Lignocellulose, one of the feedstocks in the biorefinery process, is well-known because it is available. Lignocellulose consists of cellulose, hemicellulose, and lignin. Cellulose is polysaccharides, which comprise only glucose units but hemicellulose comprises many types of sugar unit such as xylose arabinose and xylose. Lignin is a natural polymer used for the main source of aromatic.

Delignification is the process that removes lignin from lignocellulose to receive cellulose and hemicellulose used in bioethanol, pulp, and fiber additives production, etc. [2]. The amounts of recovery lignin decreased because they are destroyed during the removing process. Besides, the recovery lignin has a high molecular weight (M_w) so difficult to use in the industry [3]. Thus, the new process was created to reduce the loss of lignin quantity and obtain low molecular weight lignin.

Recently, lignin-first biorefinery is the technology focusing on lignin. The aim of the lignin-first biorefinery is lignin separation from lignocellulose in the form of lignin monomers such as syringol, guaiacol, and phenol via reductive catalytic fractionation (RCF). RCF is the approach that combines lignocellulose fractionation with lignin depolymerization-stabilization operating under H_2 pressure. Moreover, the solid residues obtained from the RCF process, cellulose and hemicellulose, are retained [4]. In the RCF, the solvents affect lignin solubility and hydrogen solubility or efficiency of hydrogen donating if the solvent was used as a hydrogen source. The solvent acts as a nucleophile that cleaves β-O-4 bond linkage in the lignin, therefore,

an increasing in their polarities increases the separation efficiency of lignocellulose. For this reason, the good solvents for RCF must be alcohols such as methanol, ethanol, and ethylene glycol [5]. Apart from the solvent, the reductive catalyst also presents the main role in the RCF process. The reductive catalyst stabilizes the lignin monomers, which were broken by the solvent. The popular reductive catalyst is a nickel catalyst because it is available and a good stabilizer for active lignin monomer [6]. The carbohydrate pulp derived after the RCF is determined the amount of sugar recovery using acid hydrolysis to convert cellulose and hemicellulose to sugar units. Sulfuric acid is a strong acid widely used in the hydrolysis step because the precipitation of lignin occurs at low pH [7].

According to the lignin-first biorefinery, the method to determine the lignin monomers and sugar unit quantities contain many steps complicated both lignin extraction and hydrolysis of carbohydrate after the RCF process. Therefore, this work studies the combination of the RCF process with acid hydrolysis in one-pot lignin first biorefinery in the batch reactor for obtaining lignin monomers and sugar units in one step using weak acid instead of the strong acid in the hydrolysis to prevent the lignin precipitation. Mixture solvent was used in the reaction, acid-contained water was used for hydrolysis of cellulose and hemicellulose while ethylene glycol was used for fractionation of lignin. Moreover, the biomass used in this study is available in Thailand such as oil palm empty fruit bunches.

1.2 Objective

To study the effect of acidity on hydrolysis and depolymerization of lignocellulose in one-pot lignin-first biorefinery.

1.3 Scope of work

1.3.1 Study in batch reactor using oil palm empty fruit bunch (OPEFB).

1.3.2 Study the effect of acid by varying the types of acid such as sulfuric acid and acetic acid

1.3.3 Study the effect of pH in the pH range 3-5

1.3.4 Study the effect of water (acid): ethyleneglycol volumetric ratio (1:1, 1:2, and 1:3)

1.3.5 Study the effect of temperature at 180, 200, 220, and 250°C

1.3.6 Study the effect of reaction time in the time range 1, 2, 3, and 6 hrs.

CHAPTER II

FUNDAMENTAL THEORY AND LITERATURE REVIEWS

This chapter represents the basic knowledge and reviews the research which relate to lignin-first biorefinery including lignin applications, extraction of lignin and reductive catalytic fractionation

2.1 Fundamentals

2.1.1 Biomass

Biomass is one of renewable energy sources. Biomass is used as feedstock of the industry. Moreover, biomass is used to replace the fossil fuel, which release CO_2. There are many sources of biomass such as forestry crops and residues, agricultural crops and residues, sewage, municipal solid waste, animal residues, and industrial residues, etc. The most popular biomass is lignocellulose which refers to plant biomass [8].

2.1.2 Lignocellulose

Lignocellulose, the complex matrix in plants, consists of 40-50% cellulose, 25-30% hemicellulose, and 15-25% lignin [8]. Lignocellulose is known as waste biomass in many industries. Recently, lignocellulose is used as the source of energy such as biofuels, bioethanol, fine chemicals, etc. The amount of each component depends on plant type and the part of the plants.

Table 1Chemical composition of selected lignocellulosic biomass (dry weight % basis) [9]

Biomass	Cellulose, %	Hemicellulose, %	Lignin, %
White cotton	94-96	1-2	<1
Brown cotton	85-88	2-3	5-7
Flax	85-88	5-6	3-5
Softwood	36-38	20-23	27-28
Hardwood	44-46	25-27	22-25
Bagasse	37-39	23-25	19-21
Corn stalks	35-37	28-30	18-20
Corn cobs	34-36	36-38	9-11
Corn stover	35-37	28-30	18-20
Wheat straw	34-36	28-30	15-17
Rice straw	36-38	25-27	7-9
Switchgrass	36-38	26-28	17-19

Cellulose, the most abundant biopolymer found in plant cell walls, comprises 3000 or more glucose units linked by beta-1,4 glycosidic bonds (Figure 1). Cellulose is long straight, unbranched chains forming H-bonds with the adjacent chains. Cellulose is insoluble cause it is a crystalline. In the industry, cellulose obtained from wood pulp is used as a source of fiber in paper production, stabilizer in the food additive industry and feedstock for the production of ethanol [10].

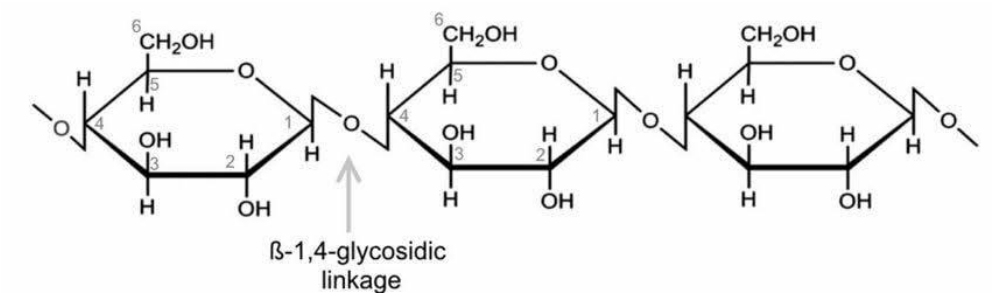


Figure 1 β-1,4 glycosidic bond of a cellulose unit [11].

Hemicellulose is polysaccharides, which is composed of different sugar units like xylose, arabinose, glucose and mannose in different proportions and positions. Unlike cellulose, hemicellulose is an amorphous structure (Figure 2) easily dissolved in water. Both cellulose and hemicellulose are hydrolyzed to obtain sugar, which is the feedstock of bioethanol production [12].

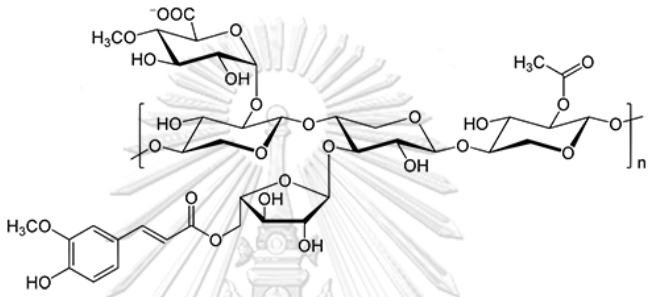


Figure 2 Structure of hemicellulose [12].

Lignin, one of the components of lignocellulose, is the polymer contained phenolic monomers such as p-coumaryl alcohol, coniferyl alcohol and, sinapyl alcohol (Figure 3). For this reason, lignin is the major source of aromatic compounds in nature. Lignin is usually fractionated from the pulp in order to remove the lignin from cellulose.

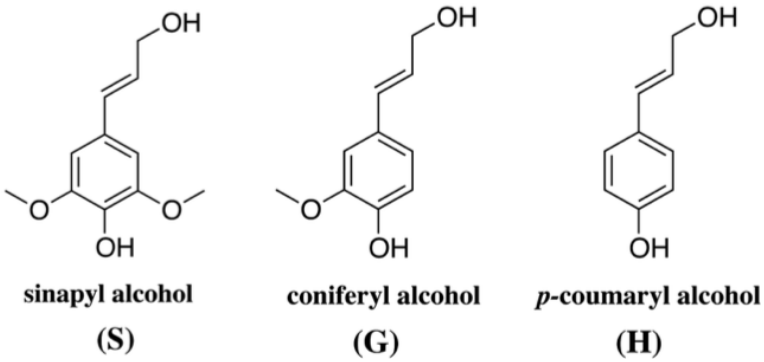


Figure 3 Representative structures of lignin monomers [13].

Hence, lignin is waste from many industries. Currently, the well-known application of lignin is vanillin industrial. Moreover, lignin is used to produce biofuel, phenolic compound, carbon fiber, activated carbon, dispersant, etc. [13]. Before using the lignin in the industry, lignin is broken the beta-O-4 linkage (Figure 4), which held the lignin monomer stick together to obtain the valuable phenolic monomers.

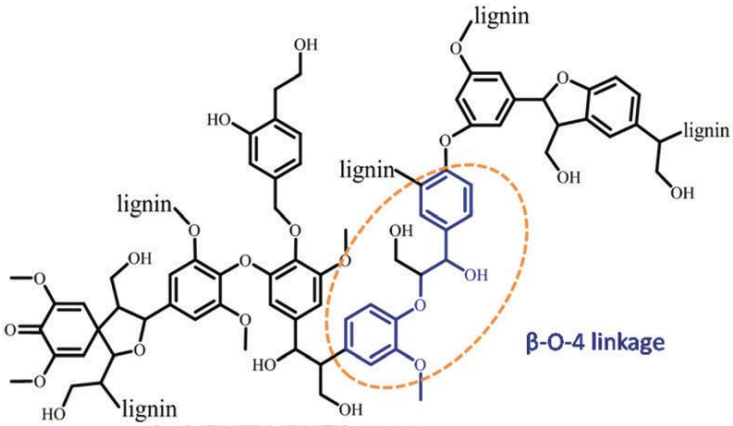


Figure 4 The chemical structure of a lignin fragment with the β-O-4 linkage [14].

2.1.3 Biorefinery

A biorefinery is a facility that merging biomass conversion processes and technology to produce fuels, power, and chemicals. The biorefinery was developed for reduced the exhaustible natural resource using biomass as an alternative resource. Since the biomass can be converted to valuable products and high quality, it is a good alternative resource [1].

2.1.4 Lignin-first biorefinery

Lignin-first biorefinery is the recently approach concerning on yield and quality of lignin obtained from the fractionation process of lignocellulose. Lignin-first biorefinery was developed process for the selectivity, quality, and yield of both lignin, cellulose, and hemicellulose for replacing the standard pretreatment in biorefinery technology [15]. Besides, the concept of lignin-first biorefinery is fractionate lignin and polysaccharides (cellulose and hemicellulose) via Reductive Catalytic Fractionation (RCF). Lignocellulose pretreatment almost uses for deconstruct cell wall into its compositions but pretreatment method. Reductive catalytic fractionation or RCF of lignocellulose integrates fractionation and depolymerization of lignin in one step in the reactor to reduce the operating time. Moreover, one step RCF prevents repolymerization of small molecule lignin cause immediately depolymerize after fractionating. There are 3 elementary steps in RCF of lignocellulose for easy to understanding, which comprise 1) lignin extraction, 2) solvolytic and catalytic depolymerization, and 3) stabilization. The main compositions of RCF are biomass, alcohol solvents, and heterogeneous reductive catalyst. The general conditions are high temperature range of 180-250°C. and high pressure of hydrogen gas for 2-6 hours. Lignin extraction from lignocellulose was induced by heating. The ether bonds in the lignin are cleaved to small molecule lignin in this step but these small molecules of lignin occurred are active. Then, the reductive catalyst (e.g. Ni/Al_2O_3, Pd/C, and Ru/C) stabilizes these active molecules [6].

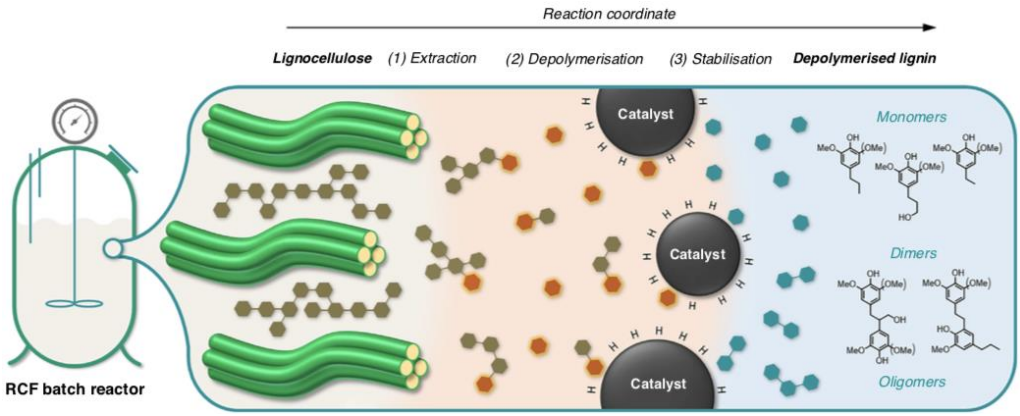


Figure 5 Schematic representation of RCF in batch mode displaying the three elementary steps: lignin extraction, depolymerization and stabilization [6].

The advantages of RCF are increasing the total monomer yield, easy extraction of the lignin monomers, reducing time, steps and cost, and prevention of repolymerization of lignin monomer. After RCF, solid residues are separated and the solvent is evaporated to obtain the lignin monomer in the dark brown oil form. The solid residues or carbohydrate pulp are hydrolysis to investigate the amount of cellulose and hemicellulose.

2.1.5 Beta-O-4 bond cleavage

β-O-4 bond cleavage mechanism for lignin is the important step of RCF breaking the C-O bonds (ether bonds) of lignin to the lignin monomers. The main substrate is solvent, which should selective with ether bond in order to obtain the uniform products from the cleavage step. The solvent widely-used in the extraction step in the RCF is alcohols such as methanol, ethanol, ethylene glycol, and isopropanol. The alcohol solvent acts as a source of hydrogen donors (Figure 6). At high temperature, the alcohol solvent dehydrogenated. Then, the hydrogen from the dehydrogenation of alcohol solvent is the reagent in the β-O-4 bond cleavage of lignin [16]. This reaction is called catalytic transfer hydrogenolysis.

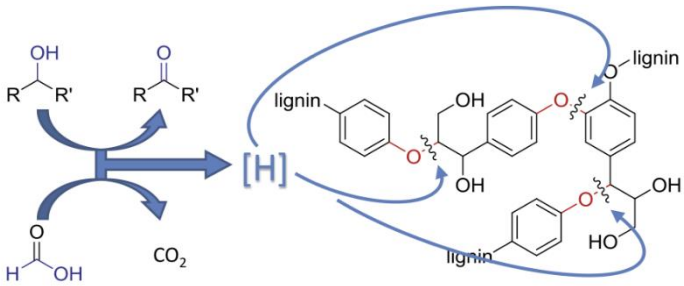


Figure 6 Transfer hydrogenolysis of ligninether bond with alcohol as hydrogen donor [16].

2.1.6 Hydrolysis of carbohydrate

Hydrolysis reaction is a reaction using water breaks down the bonds of the substrate. In general, there are 3 types of hydrolysis reaction including salts, acid, and base as shown in Figure 7.

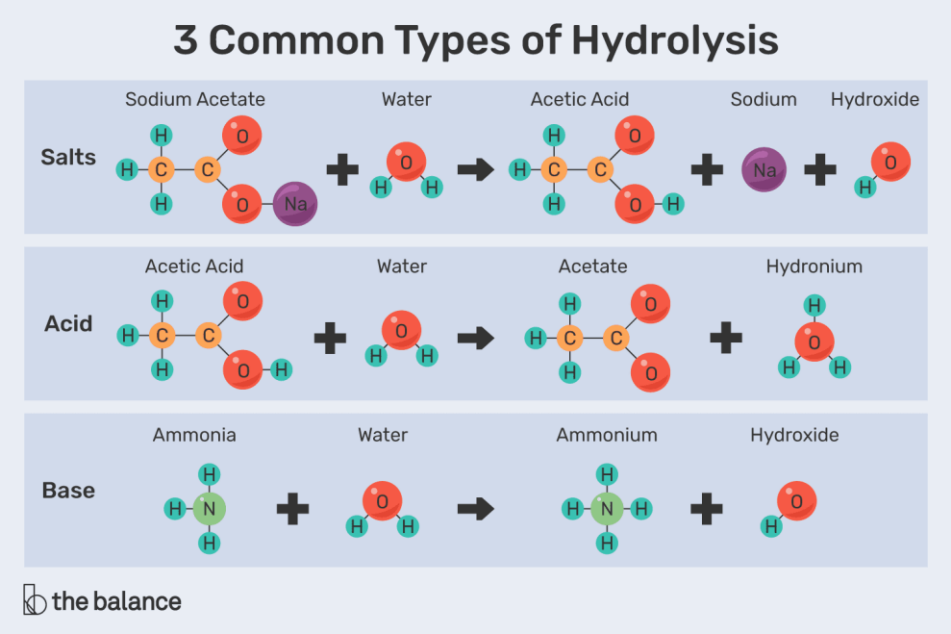


Figure 7 Types of hydrolysis reaction [17]

For carbohydrates, polysaccharides are broken down by water via heating with strong acids. Acid hydrolysis is widely used to convert the polysaccharides to monosaccharides by breaking the glycosidic bonds (ether bond) which link the monomer unit stick together [18] as shown in Figure 8. Strong acid such as HCl and H_2SO_4 is used as a catalyst. After the hydrolysis reaction, the monosaccharides are obtained then types and amount of sugars in the carbohydrate are investigated.

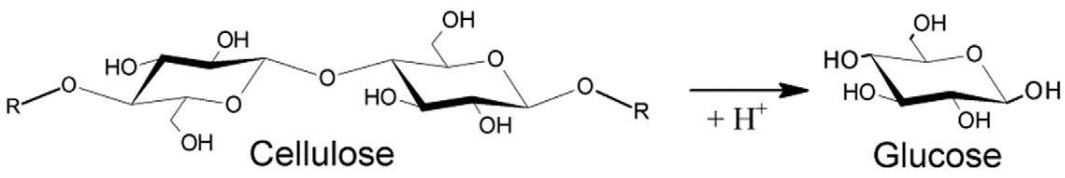


Figure 8 Mechanism of acid-catalyzed cellulose hydrolysis to glucose [18]

2.2 Literature reviews

2.2.1 Delignification

In 2013, Patricia S. B. and co-workers have characterized the lignin extracted from Kraft black liquor which collected from a pulp industry located in Southern Brazil [19]. The lignin was precipitated using sulfuric acid (98%w/w) and hydrochloric acid (37%w/w). The pH of the solution was adjusted from pH 6 to 2. The results indicated that the percentage of precipitated lignin increased with a decrease of pH. Lignin performed the highest phenol content (39.2%) when precipitated with hydrochloric at pH 6. Unlike sulfuric acid, the phenol content was the lowest at the same pH. This study demonstrated that the types of acid and pH which were used are the significant factors for the precipitation of lignin.

2.2.2 Selective hydrogenation catalyst

In 2008, Kou et al. have reported that the hydrogenation of lignin using a noble metal catalyst such as Pt, Ru, Pd, and Rh supported on activated carbon under 4 MPa H_2 provides 46 wt% monomeric phenols [20]. In 2015, Klein et al. have studied the efficient of heterogeneous Ni/C catalyst in lignin depolymerization for converting lignin from different wood to monomer products such as 4-(3-hydroxypropyl)-2-methoxyphenol (DHE-OH), 2,6-dimethoxy-4- propyl-phenol (DMPP), propenyl derivatives isoeugenol (i-EuOH), and methoxyisoeugenol (Mi- EuOH). From this study, it demonstrated that Ni/C was an excellent catalyst for one-pot providing high yield lignin monomer, highly active and selective in lignin conversion to monomeric phenols [21]. Moreover, the selectivity and lignin oil yield are depended on the types of native lignin, conditions, and solvent used. Table 2 showed that the different types of biomass consist of different monomer units and bond.

Table 2 Depolymerization results of birch, poplar and eucalyptus wood with 5 and 10 wt% Ni/C catalysts in methanol [21].

Entry	Substrate, Ni/C catalyst loading	DHE[a]	i-EuOH[a]	DMPP[a]	Mi-EuOH[a]	DHE-OH[a,b]	Total % yield[c]	Final pressure (bar)[d]
1	Birch, 5 wt%	—	8	—	12	—	20	3.5
2	Poplar, 5 wt%	—	2	—	2	2	6	2.4
3	Eucalyptus, 5 wt%	—	6	—	8	2	16	3.6
4	Birch, 10 wt%	10	1	18	3	—	32	9.4
5	Poplar, 10 wt%	1	8	2	15	—	26	5.5
6	Eucalyptus, 10 wt%	6	3	8	11	—	28	8.5

Reaction conditions: wood biomass = 1.0 g, catalyst = 0.05 g (5 wt%) or 0.1 g (10 wt%), T = 200 °C, 2 bar N_2 and 6 h reaction time.[a] Yields (%) are calculated from the theoretical lignin content in wood, and the mass of the products was quantified by GC-FID (ESI†). Lignin analysis data of birch, eucalyptus and poplar are shown in Table S1.[†] [b] DHE-OH = 4-(3-hydroxypropyl)-2-methoxyphenol. [c] Total yields (%) represent the sum of all lignin-derived products. [d] Pressure inside the reactor after cooling down the reactor to ambient temperature after 6 h reaction time.

2.2.3 Lignin-first biorefinery (Reductive catalytic fractionation)

2.2.3.1 Effect of solvent

In 2015, Schutyser et. al. have studied the effect of bio-based solvents on reductive catalytic fractionation of birch wood using Pd/C as a catalyst. The various solvents used in reductive delignification of lignocellulose were water, dioxane, methanol, ethylene glycol, ethanol, 2-propanol, 1-butanol, tetrahydrofuran (THF), as well as hexane. The birch sawdust, Pd/C catalyst, and solvent were placed in a stainless-steel batch reactor operating at 200°C with 30 bar H_2 pressure. This experiment showed that the increasing of polarity increases the degree of delignification [5] as shown in Figure 9 because the polar solvent exhibit the ability to donate hydrogen bond then they could easier penetrate the lignocellulose matrix. However, increasing of polarity of solvent induces decreasing of carbohydrate pulp due to the solubility.

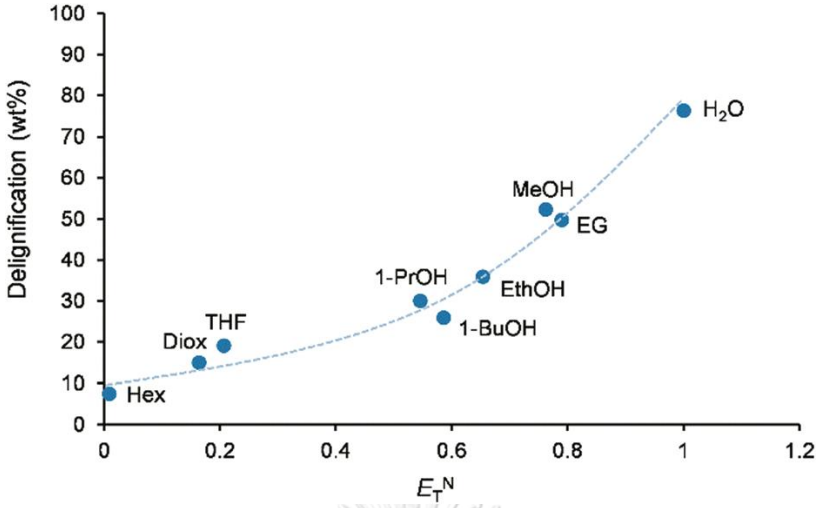


Figure 9 Birch delignification versus solvent polarity as described by the Reichardt parameter (E_T^N) [5].

Although dioxane is a good solvent for lignin, this experiment indicated methanol, ethylene glycol, and water provide a higher degree of delignification it means the solubility of lignin does not affect the degree of delignification in the RCF process. Moreover, Schutyser et. al. have measured the amount of lignin product and carbohydrate retention (C5 and C6 sugar) to investigate the good solvent, which provided high yield both lignin and sugar.

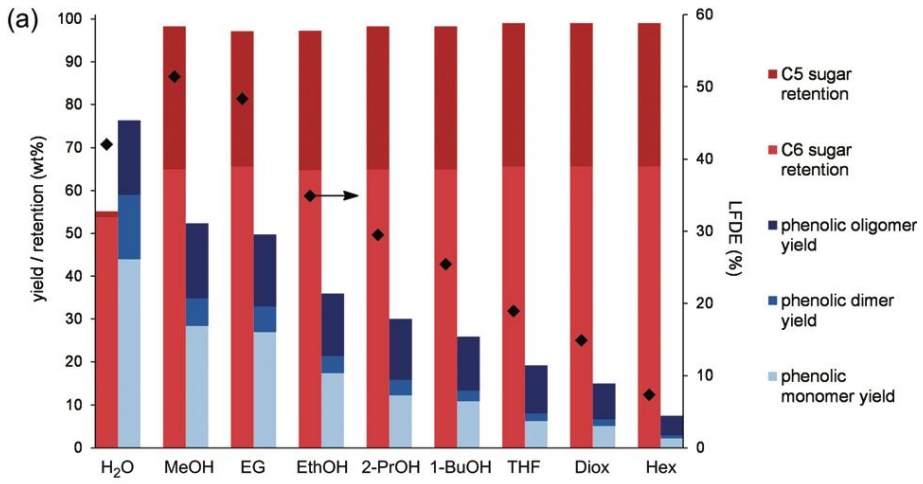


Figure 10 Lignin product yield (phenolic monomers-, dimers- and oligomers), carbohydrate retention (C5 and C6 sugars), 'lignin-first delignification efficiency (LFDE) [5]

Figure 10, the appropriate solvents were methanol and ethylene glycol because both solvents retained high yield of lignin monomer and retention of sugar. The operating pressure of methanol was higher than ethylene glycol. Furthermore, methanol was easier to remove from lignin by evaporation than ethylene glycol. However, the ethylene glycol pulp comprised absolutely separated fiber cells while the methanol pulp constituted the particles of aggregated fiber cells due to high surface accessibility of the ethylene glycol, which was expected to enhance their chemical reactivity [5].

2.2.3.2 Role of the catalyst

In 2017, Van der Bosch and coworkers have studied the roles of Ni-Al_2O_3 pellets in the basket during lignin-first fractionation using methanol as a solvent and birch wood as a lignocellulose source [22]. The roles of Ni-Al_2O_3 was described in 3 steps including solubilization of lignin, depolymerization of lignin, and stabilization of reactive lignin.

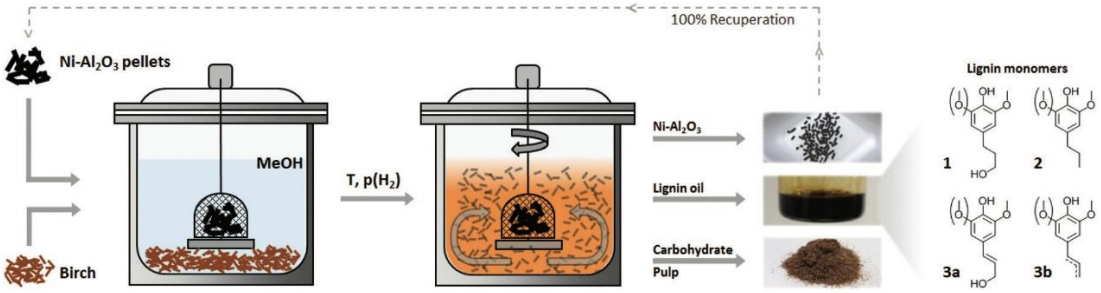


Figure 11 Reductive catalytic fractionation (RCF) of lignocellulose with commercial Ni-Al_2O_3 catalyst pellets (1.2 × 3 mm trilobe) in a catalyst basket (Ø = 0.4 mm), resulting in a monomer-enriched lignin oil, a catalyst-free carbohydrate pulp and a quantitative recuperation of the catalyst. Main monomeric products in the lignin oil: (1) 4-n-propanol-G/S, (2) 4-n-propyl-G/S, (3a) coniferyl/sinapyl alcohol and (3b) 4-n-propenyl-G/S [22].

For the disentanglement and solubilization of lignin step, the lignin oil yield obtained from RCF between the presence and absence of catalyst were no significant difference in the range of 0-6 h. It shown that the catalyst does not participated in this step

In the depolymerization of lignin step, 2D NMR spectra of lignin product and intermediate at 0, 0.5, and 3 hours were recorded both in the presence and absence of a catalyst (Ni-Al_2O_3). The NMR spectra of both demonstrate the β-O-4 bond cleavage but the NMR spectra of the presence the catalyst was faster decreasing of the β-O-4 bond. This situation indicated that the presence of catalyst gently enhances the rate of β-O-4 bond cleavage. The products from β-O-4 bond cleavage as coniferyl/sinapyl alcohol units were formed

For the last step, stabilization of reactive lignin or intermediate, the gel permeation chromatography was used to investigate the molecular weight of the lignin oil during the RCF process at 0, 0.5, 3, and 6 h both the presence as well as absence the catalyst. Gel permeation chromatograms of the lignin in the catalyst-free batch, the monomers and dimers were formed in the 0.5 h but at 6 h the monomers which were formed decrease. On the other hand, the gel permeation chromatogram

of the lignin in the catalyst batch, the signal of monomers and dimers occurred in the first 0.5 h and remained at 6 h. This evidence shown that the catalyst is important in the stabilization step. This study investigated that the catalyst serves a stabilizing role through hydrogenation of the unsaturated solubilized lignin intermediates and prevents the repolymerization of the reactive lignin.

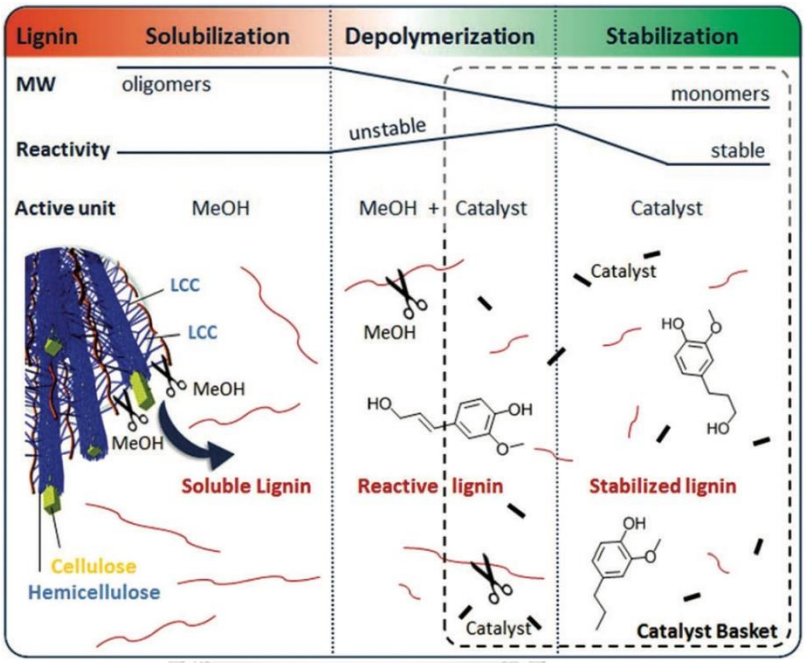


Figure 12 An overview of the catalyst function with respect to lignin solubilization, depolymerization and stabilization during reductive cata- lytic fractionation (RCF) of lignocellulosic biomass. LCC is the abbreviation for lignin carbohydrate complexes. The schematic lignocellulose representation was reproduced with permission [22].

After the role of a catalyst was clarified, the carbohydrate pulp obtained from the catalyst-free conditions was hydrolyzed to convert the carbohydrate to sugar units (e.g. glucose, xylose, and arabinose). Then, the amount of sugars were analyzed by HPLC to assure that the carbohydrate from lignin-first biorefinery are usable.

In 2018, Yong Huang and co-workers have reported the lignin monomers obtained from lignin-first biorefinery of apple wood using Ru/SiC as a catalyst was used for the feedstock of jet fuel aromatic [23]. The degree of delignification of apple wood over

Ru/SiC as a catalyst equals to 72.9 wt%. The resulting lignin from lignin-first biorefinery was extracted using hexane and about 50 wt% of the hexane extracted was obtained. After that, the lignin in hexane extracted was converted to aromatic hydrocarbons via hydrodeoxygenation using MoO_3 as a catalyst under ambient pressure operating. Then, the resulting aromatic hydrocarbons were used as a blend for jet biofuels. Moreover, the Figure 13 showed that lignin could be applied in the polymer industry.

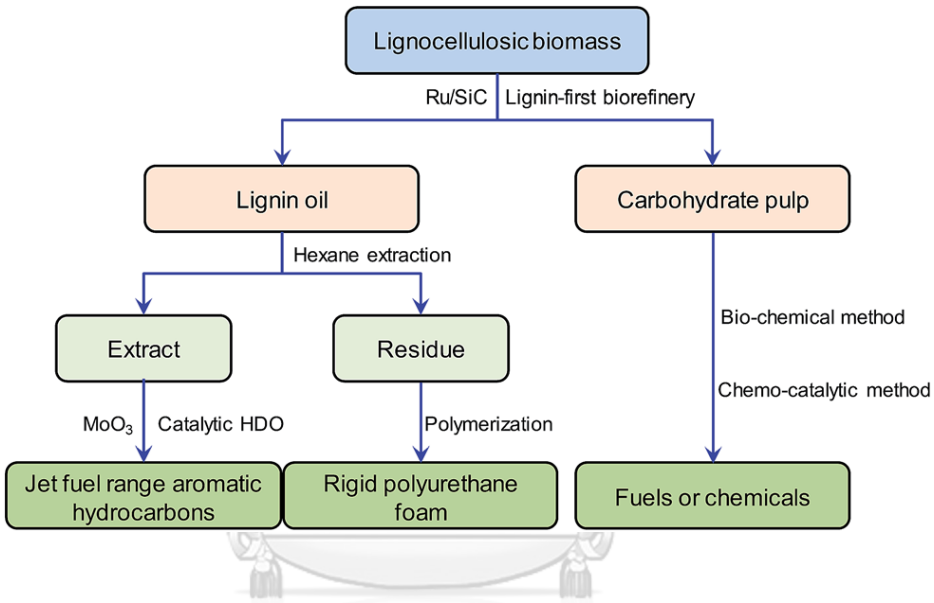


Figure 13 Conceptual design of the lignin-first biorefinery studied in this paper [23].

In addition, the residues from hexane extraction were dimers and oligomers containing β-5, β- β, and α-O-4 bonds. Furthermore, this residue was filled with -OH indicated that is high potential residue in replacement of polyols. Then, the residues were used for preparation of rigid polyurethane foam (RPF).

Moreover, the carbohydrate pulp from the filtration of product after lignin-first biorefinery was hydrolyzed to obtain sugars, which were used in fuel and chemical industries.

In 2019, Xue Liu and colleagues have studied the lignin-first biorefinery of eucalyptus using Ni@ZIF-8 as a chemodivergent catalyst for improving the selectivity of phenolic compounds [24]. Metal-organic frameworks (MOFs) promised functional

catalyst support. The major properties of MOFs: high porosity and large surface area made the MOFs become good support. Besides, the ZIF-8 framework represented the large surface area and high resistance of chemical and thermal so this study used nickel immobilized on ZIF-8 as a catalyst which was good catalytic for hydrogenation in the RCF process to convert lignin into the phenolic compounds. For RCF of eucalyptus sawdust, methanol was used as a solvent, and Ni@ZIF-8 was used as a catalyst at 220°C under 3 MPa H_2 for 4 hours. The lignin oil weight referred to the degree of delignification of eucalyptus. The degree of delignification was 66% based on Klason lignin weight that almost equaled the theoretical maximum. The chromatogram from GC indicated that the syringly/guaiacyl ratio was 2.2:1 and the monomer yield was 32 wt%.

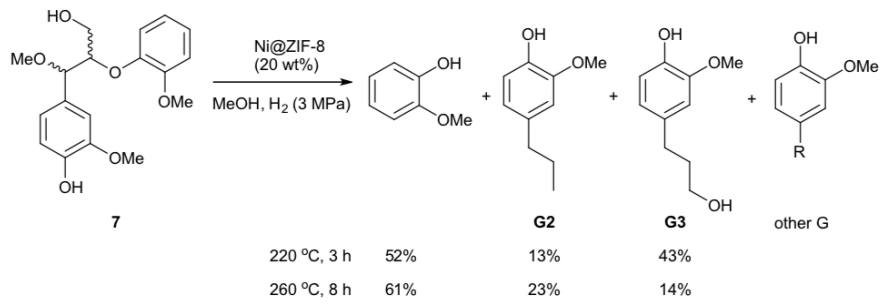


Figure 14 Represent the reductive catalytic fractionation of eucalyptus sawdust with Ni@ZIF-8 under 3 MPa H_2 pressure [24].

In addition, the conditions of RCF varied investigated that decreasing of temperature significant decreased both the degree of delignification and monomer yield. Moreover, at 220°C under 1 atm N_2 pressure was observed the decreasing of lignin monomers as well as the degree of delignification. This result indicated that H_2 was the main role in the RCF process using Ni@ZIF-8 as a catalyst.

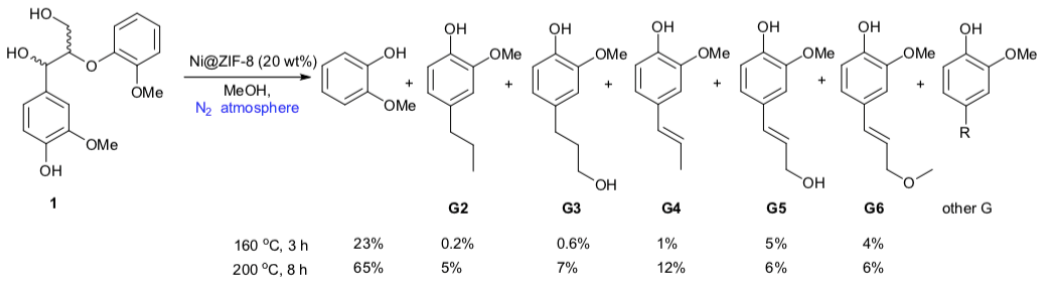


Figure 15 Represent the reductive catalytic fractionation of eucalyptus sawdust with Ni@ZIF-8 under 1 atm N_2 pressure [24].

CHAPTER III

EXPERIMENTAL

3.1 General procedures

3.1.1 Analytical instruments

Two dimensions Heteronuclear Single Quantum Coherence Nuclear magnetic resonance (2D HSQC NMR) spectra were performed with a Varian Mercury Plus 400 spectrometer and Bruker Advance 400 MHz spectrometer using DMSO as a solvent. UV-Vis absorption spectra in the range of 200-600 nm was measured by UV-Vis spectrophotometer. Fourier Transform Infrared Spectrophotometer (FT-IR) was used to measured infrared spectrum of transmittance. Analysis of molecular weight of lignin was recorded by Gel Permeation Chromatography (GPC) using PLgel 10 μm mixed B 2 column (Mw resolving range= 500-10,000,000). High Performance Liquid Chromatography (HPLC) was performed with an HPX-87X column and a refractive index detector. Gas Chromatography (GC) was composed of HP5-column (Agilent 6890 series) and Flame ionization detector (GC-FID). GC was performed an Agilent 6890 series HP1-MS capillary column and an Agilent 5973 series Mass Spectroscopy detector.

3.1.2 Materials

Oil palm empty fruit bunch were obtained from Energy Absolute Public Company Limited. All reagents were AR grade purchased from Quality Reagent Chemical and there are not purification processes before they were used. Nylon 66 membrane (pore size 0.45 μm) in Gel permeation chromatography measurement was

purchased from Sigma Aldrich. Filter paper (pore size 13 μm) was purchased from filtraTech. Stainless-steel batch reactor was performed a controller (Figure 16).

Figure 16 Stainless-steel batch reactor (50mL, Parr instrument) with controller.

3.2 One-pot lignin first biorefinery of OPEFB

Added 2 g of biomass, which were dried to drain water and organic compound out at 100 °C for 24 h, 40 mL mixture of acid-contained water/ethylene glycol and 0.2 g of 16% Ni/Al_2O_3 (surface area 259.47 m^2/g, pore volume 0.52 cm^3/g, and pore size 8.06 nm) into the stainless-steel batch reactor (50mL, Parr instrument). The solution was measured the pH by pH meter. Then N_2 gas was purged to remove air and the reactor was filled with 3MPa H_2 gas. The reactor was heated to 250°C for 3h. After that, cooled the temperature down and removed H_2 gas out. After the reaction, the solid residues were separated from the product mixture using vacuum filtration. Next, lignin oil was separated from mixture solvent by using liquid-liquid extraction. Then 150 mL deionized water was added to the product mixture to form a homogeneous mixture and added dichloromethane (DCM) to extract the lignin oil. The DCM-extracted phase was evaporated to obtain lignin oil and its weight determined the degree of delignification (based on Klason lignin). The solid phase

was characterized by TGA, and SEM. Additionally, water phase was identified the sugar unit by HPLC equipped with AMINEX column, RI detector, and PDA detector.

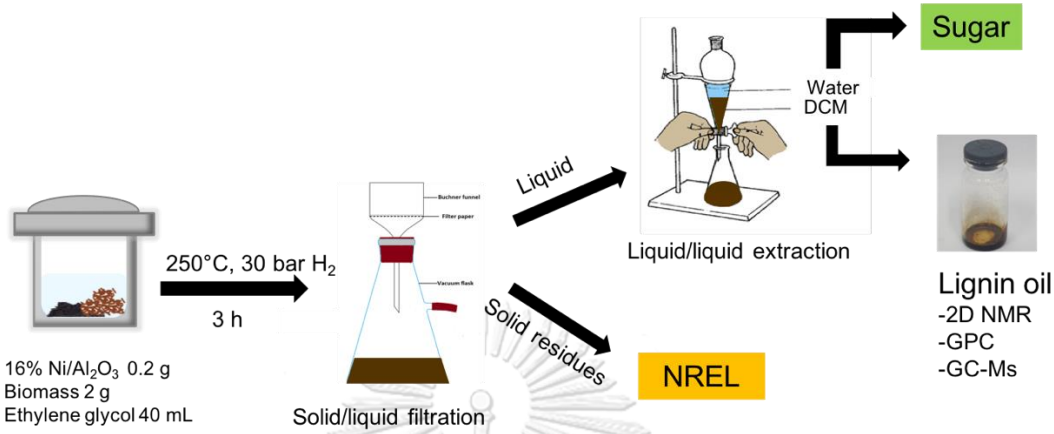


Figure 17 Schematic of the method for lignin-first biorefinery.

3.3 Characterization of lignin

To measure the molecular weight distribution of lignin oil, A sample of lignin oil was dissolved in tetrahydrofuran (THF) (2 mg mL^{-1}) and then filtered using nylon 66 membrane (pore size 0.45 μm) to remove any particulate matter before injected into GPC.

Qualitative analysis of lignin oil using 2D HSQC NMR to identify beta-O-4 bond and lignin monomer types. A sample of lignin oil was solubilized in dimethyl sulfoxide (DMSO)

To determine the functional groups of lignin oil, lignin oil mixed with potassium bromine (KBr) was measured FT-IR spectra as transmittance versus wavenumber range of 500-4500 cm^{-1}

Gas chromatography was used to analyze the lignin oil using HP5-column and flame ionization detector (FID). The operating conditions were used: injection temperature of 573 K, column temperature program: 50°C (2 min), 15 °C.min^{-1} to 150°C , 10 °C.min^{-1} to 220°C and 20 °C.min^{-1} to 290°C (12 min), detection

temperature of 300°C. Sample preparation, 0.2 g of lignin oil was added internal standard 2-isopropylphenol to increase their volatile. After that, dried with N_2 flow and added 0.5 mL of pyridine and 0.5 mL of N-methyl-N-(trimethylsilyl) trifluoroacetamide. The adjusted sample was placed in an oven at 353 K for 30min.

GC-MS is used to investigate the lignin monomer. GC was performed an Agilent 6890 series HP1-MS capillary column and an Agilent 5973 series Mass Spectroscopy detector. The operating conditions were used: injection temperature of 523 K, column temperature program: 60 °C (2 min), 10 °C.min^{-1} to 280 °C (13 min), detection temperature of 290 °C.

3.4 Composition analysis of water phase

The water phase which was separated from the DCM phase was adjusted pH in the range of 6-7 using calcium carbonate ($CaCO_3$). Then, filtered the small particle by syringe filters (pore size 0.2 μm) and injected into the HPLC with an autosampler, 0.5 mM H_2SO_4 was used as a mobile phase, flow rate 0.6 ml/min. The separation was executed at 65°C using a Refractive index detector (RI detector) and a Photodiode array detector (PDA detector).

3.5 Determine the composition in OPEFB

The amount of sugar and lignin in OPEFB was investigated by NREL method. First, put 3 g of solid residues, which were dried at 105°C for 3 days into a pressure tube and gradually dropped 3 mL 72% sulfuric acid (H_2SO_4) onto the sample. After that, mixed the sample by vortexer every 15 minutes for 1 h. The mixture was diluted to 4% H_2SO_4 in 84 mL deionized water for small molecules of sugar digesting. Next, the pressure tube was heated in an autoclave at 121°C for 1h. After that, cooled the pressure tube to room temperature using water. The amount of acid-soluble lignin (ASL) was measured by UV-Vis spectroscopy (absorption wavelength at 240 nm). Before HPLC analysis, neutralized the solution with $CaCO_3$ and filtered solid

matter using syringe filters (pore size 0.2 μm). Put acid-insoluble residues that were filtered from the solution into a crucible and dried overnight at 105℃ to find lignin and ash content. Then the crucible was placed in the furnace to remove lignin

.

Furnace Temperature Ramp Program:

ramp from room temperature to 105 ℃ and hold at 105 ℃ for 12 minutes

ramp to 250 ℃ at 10 ℃ / minute and hold at 250 ℃ for 30 minutes.

ramp to 575 ℃ at 20 ℃ / minute and hold at 575 ℃ for 180 minutes

cooled the temperature. Next, removed the crucibles from the furnace. After that, the weight of crucible and ash content was measured to calculate the acid-insoluble lignin content of biomass [25].

Figure 18 Autoclave and sterilizer [25].

CHAPTER IV

RESULTS AND DISCUSSION

4.1 One-pot lignin-first biorefinery and hydrolysis of carbohydrate

The one-pot lignin-first biorefinery process using oil palm empty fruit bunches (OPEFB) as a biomass source, 16% Ni/Al_2O_3 as a catalyst, and a mixture of acid-contained water and ethylene glycol as solvent at 250 ℃, 30 bar H_2 (the final pressure was 70 bar) converted the lignin into lignin monomers and carbohydrate into the sugar units. The product obtained from the reaction was filtrated to divide the liquid phase from the solid residues. Then, the liquid phase was extracted with deionized water and dichloromethane to separate the lignin oil from sugar-contained mixture solvents. In the organic phase, after evaporating dichloromethane, the lignin oil obtained was dark brown and viscous as shown in Figure 19a. Another phase, the water phase was yellow aqueous comprising the sugar units. The solid phase which was separated from the liquid phase was the biomass residues and 16% Ni/Al_2O_3 (Figure 19b).

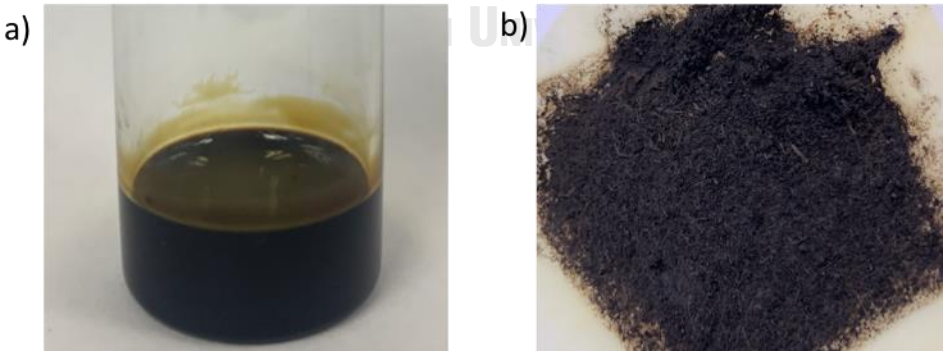


Figure 19 Product obtained from the reaction (a) lignin oil, and (b) solid residues.

4.2 Determination of Oil Palm Empty Fruit Bunches (OPEFB)

The composition of Oil palm empty fruit bunches was analyzed by the National Renewable Energy Laboratory (NREL) standard biomass analytical procedure. Table 3 showed the cellulose, hemicellulose, and lignin content of OPEFB which was used in this work compare to the previous research [26]. The amount of each composition was slightly different from the previous research probably as a result of growing in different places. Lignin in our OPEFB that was composed of lignin-soluble acid (2.5 wt%) and lignin-insoluble acid (36.34%) was the main component.

Table 3 Table Composition of oil palm empty fruit bunches (OPEFB)

Composition	content (wt%)	
	This work	Saifuddin *et al*., (2013)
Cellulose	20.6	22.5
Hemicellulose	9.8	24.5
Lignin	38.8	24.0
Ash	2.7	0.4
Moisture and other components	20.0	22.0

4.3 Characterization

4.3.1 Characterization of lignin

4.3.1.1 Two-dimensional nuclear magnetic resonance (2D-NMR) analysis

The lignin oil derived from OPEFB via one-pot lignin first biorefinery was characterized by ^{13}C NMR, ^{1}H NMR, and 2D HSQC NMR analysis to identify the lignin inter-linkages and the chemical structures of lignin which were contained mixtures of monomers, dimers, and oligomers. The ^{1}H NMR spectra in Figure 20a shows a strong signal at 3.4 ppm assigned to the proton attached to the hydroxy group. The

methoxyphenol was observed at 3.4, 3.7, 6.7, and 7.2 ppm which are the signals of proton in the methanol group, the methoxy group, the proton of aromatic ring neighboring methoxy group side chain, and aromatic proton, respectively. Moreover, the ^{13}C NMR spectra in Figure 20b. demonstrated the signals of the methoxy group in the range of 55-70 ppm and the signals of the aromatic rings at 115, 120, and 130 ppm. Both ^{1}H NMR and ^{13}C NMR spectra indicated that the main components of lignin oil are the aromatic compounds which have methoxy group as side chains. Additionally, the correlation signals of lignin oil were investigated base on the reported literature [27] to identify the possible structure of the mixture components in the lignin oil. Figure 21a showed the cross-signals (HSQC) corresponding to the inter-linkage of lignin at δ_C/δ_H 55-70/3.2-4.2. Methoxyl and β-O-4 aryl ether linkage were observed at δ_C/δ_H 55.6/3.73. The C_γ-H_γ in structure A were discovered at δ_C/δ_H 59.5/3.5. The signal at δ_C/δ_H 65.1/4.1 represented C_γ-H_γ in the side chain of S/G units as a structure B. Moreover, Figure 21b represented the cross-signals in the regions of δ_C/δ_H 110-135/6.3-7.2. The S and G units of lignin were investigated at δ_C/δ_H 106/6.6 and 112/6.4 respectively. Besides, $C_{2,6}$,$H_{2,6}$ of H units was observed at δ_C/δ_H 128/7.1. According to NMR analysis, both 1D-NMR and 2D-NMR confirmed that the lignin-first biorefinery work for extracting and depolymerizing lignin. The signal of the β-O-4 linkage which was the ether bond between lignin monomers confirmed that this work could extract lignin from OPEFB into the lignin oil and the signals of S, G, and H units also confirmed that the lignin was depolymerized into the monolignols.

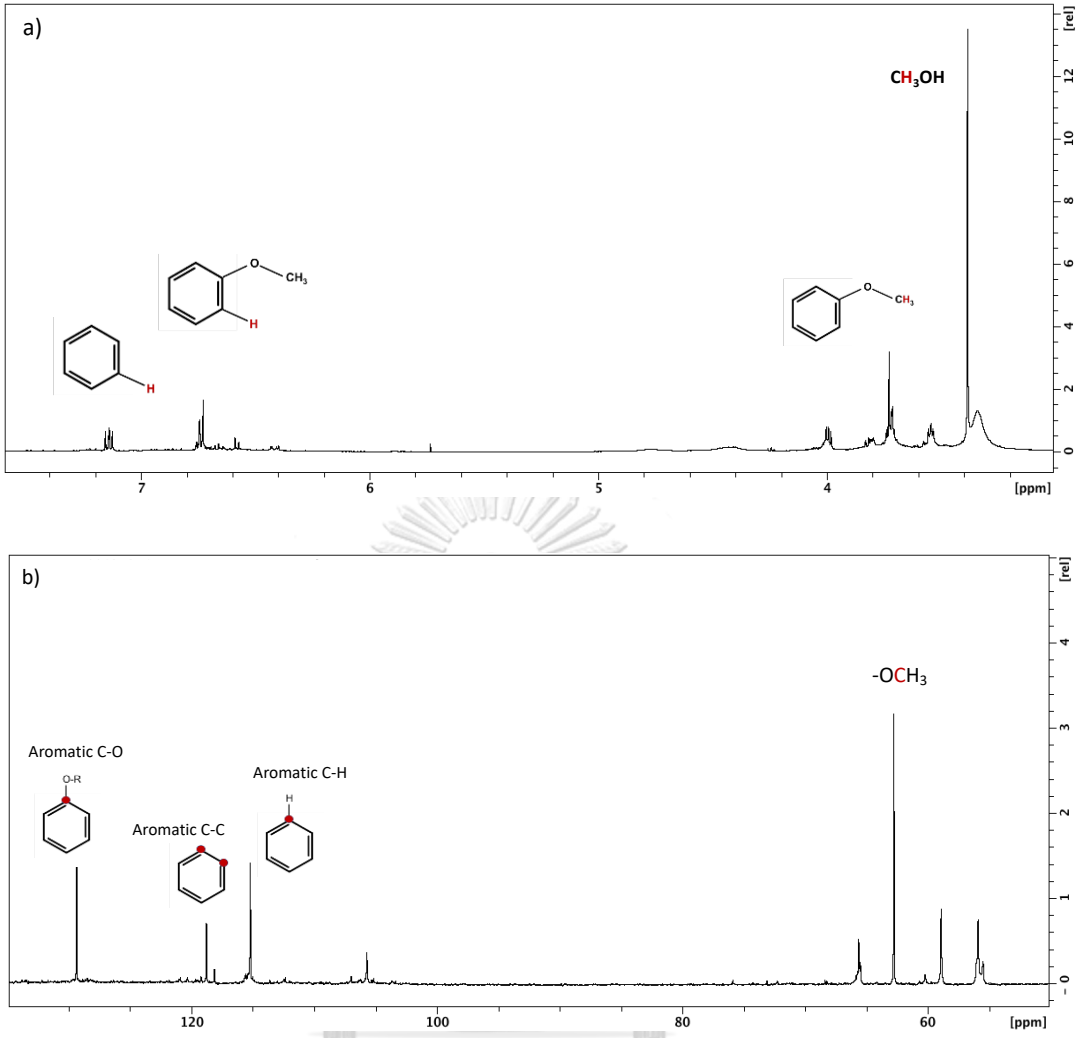


Figure 20 NMR analysis of the lignin oil (500 MHz, d_6-DMSO) a) ^{1}H-NMR and b) ^{13}C-NMR.

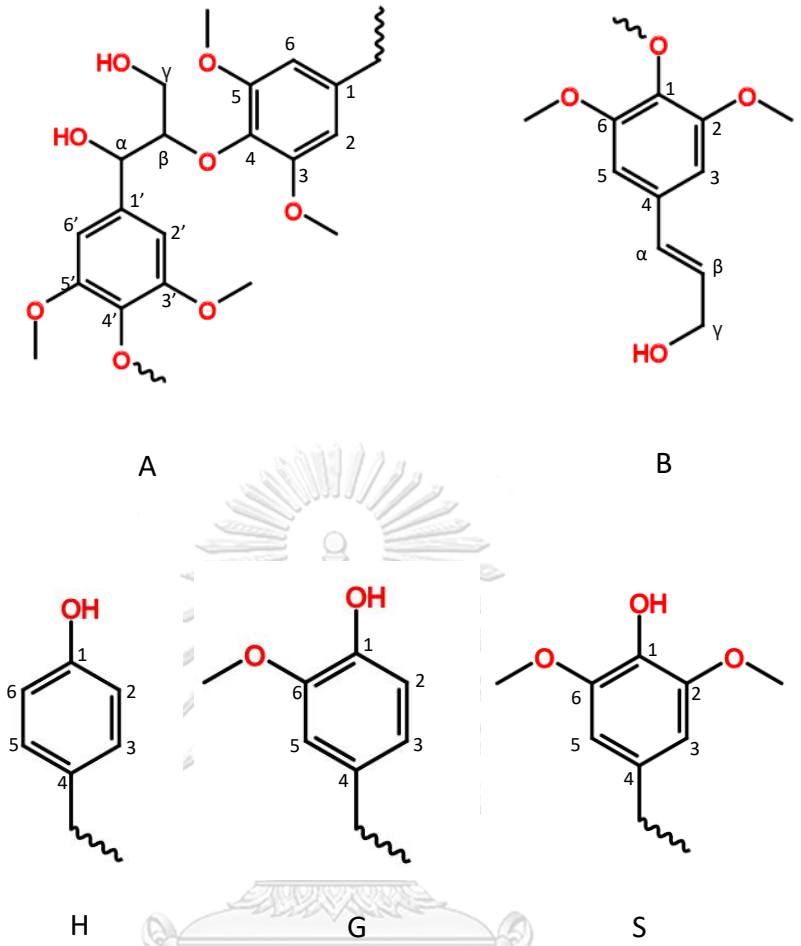


Figure 21 The interunit linkages and main monomers of lignin, involving different side-chain linkages, identified by 2D NMR of lignin oil: (A) β-O-4 aryl ether linkages; (B) p-hydroxycinnamyl alcohol; (H) p-hydroxyphenyl units (G) guaiacyl units; (S) syringyl units.

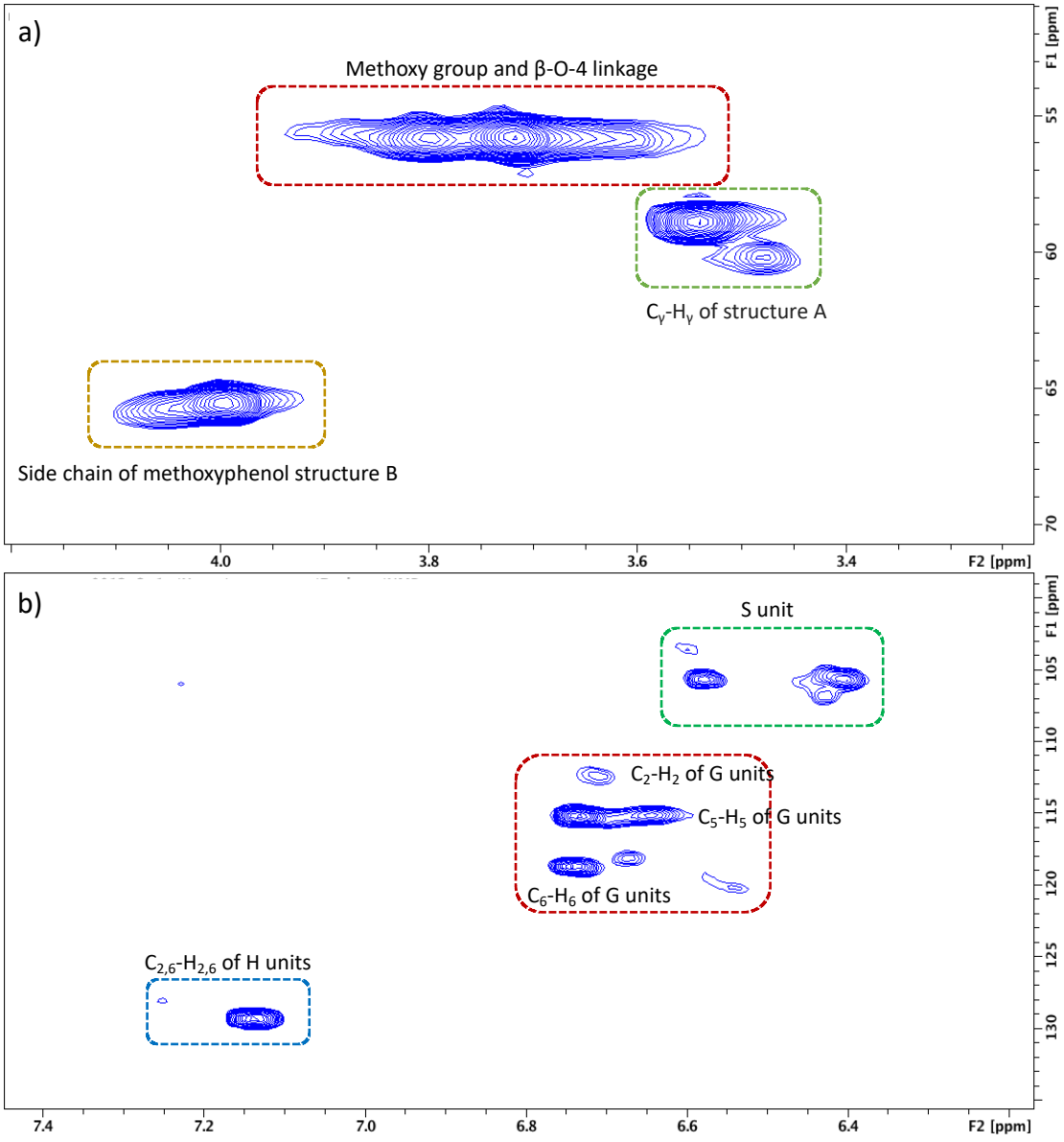


Figure 22 2D-NMR analysis of the lignin oil product (500 MHz, d_6-DMSO) a) ^{1}H NMR and b) ^{13}C NMR.

4.3.1.2 Gel permeation chromatography (GPC) analysis

The molecular weight distribution and the average molecular weight of lignin oil were investigated by gel permeation chromatography (GPC) performed with PLgel 10 μm mixed B2 columns (M_w resolving range = 500-10,000,000) using

tetrahydrofuran as a solvent. The average molecular weight of lignin oil derived from OPEFB was out of the standard calibration curve rang (500-10,000,000 Da). Therefore, it was calculated from extrapolation of calibration curve. Table 3 shows that the weight-average molecular weight of lignin oil was 500 Daltons and the number-average molecular weight of lignin oil was 410 Daltons. The molecular weight of lignin oil was low indicated that the main component in lignin oil was small molecules. Since the lignin was solved from biomass and depolymerized into small molecules such as monomers, dimers, and oligomers. Moreover, there was a shoulder peak at 250 Daltons as shown in the chromatogram (Figure 23) denoted that there are many molecules that have a molecular weight of about 250 Daltons. According to the dimer molecule of lignin also has a molecular weight of around 250 Daltons, the lignin product may have the dimer molecules including in lignin oil. Additionally, the polydispersity index (PDI) of lignin oil was 1.2 shows that the distribution of molecular weight was slightly broad. Even though the distribution of molecular weight was not the monodispersity, the molecular weight of lignin oil and the distribution of the molecule was smaller than the previous work which used a similar process [22].

Table 4 Molecular weight of lignin oil product.

M_n	M_w	Polydispersity
410	500	1.2

*M_n is the number-average molecular weight and M_w is weight-average molecular weight

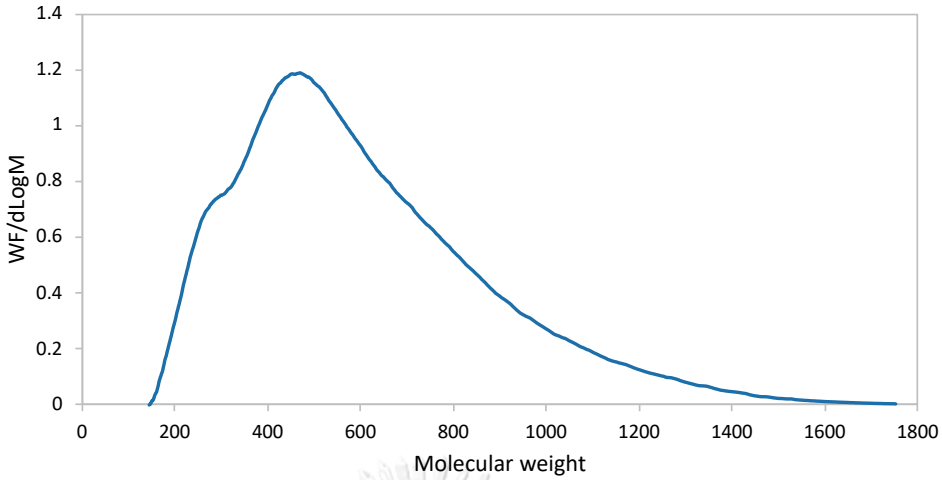


Figure 23 Gel permeation chromatograms (GPC) of the lignin oil.

Lignin oil product was characterized by GC-MS that was performed in DB-1 column. The lignin oil was resolubilized with dichloromethane before injecting into GC-MS. According to the GC-MS chromatograms (Figure 23), there were 4 aromatic compounds at retention time 4.260, 5.850, 8.869, and 9.726 that represents phenol, 2-methoxyphenol (guaiacol), 4-ethylphenol, and 2,6-dimethoxyphenol (syringol) respectively. The monomers investigated were the important lignin monomers. In addition, the amounts of dimer-, and oligomer of lignin which were detected at the retention time more than 15 min were low.

4.3.1.3 Gas chromatography-mass spectrometry (GC-MS) analysis

The characterization of lignin oil with NMR, GPC, and GC-MS clarified that the one-pot lignin first biorefinery of OPEFB using 16%Ni/Al_2O_3 as a catalyst and a mixture of acid-contained water and ethylene glycol as a solvent works for extracting lignin from the biomass and depolymerizing the large molecule of lignin into small molecules such as monomers- and dimers.

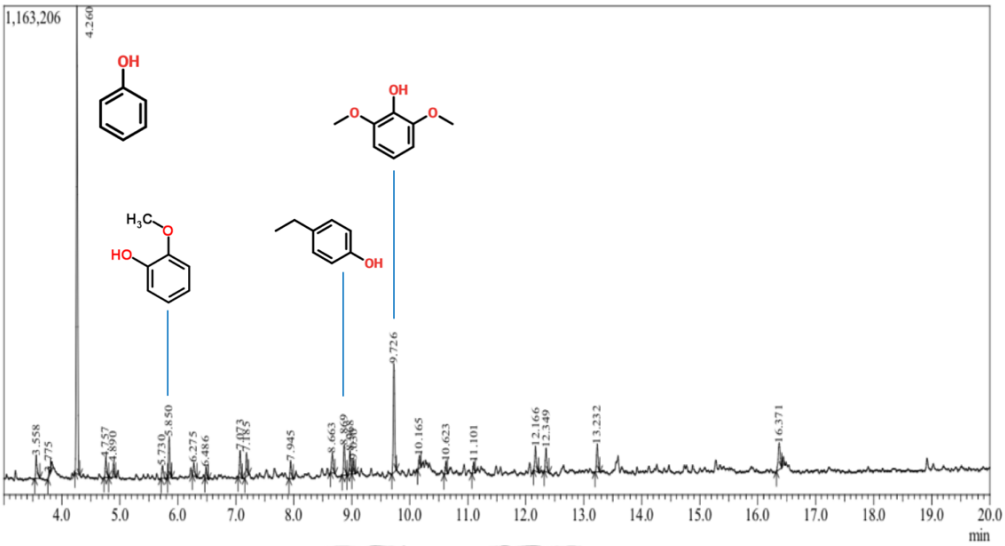


Figure 24 GC-MS chromatogram of lignin oil.

4.3.2 Characterization of sugar in water phase

Sugar units and by-products in the water phase from one-pot lignin first biorefinery of OPEFB were identified by HPLC that performed with Aminex column and RI detection. HPLC chromatogram comprised the peaks of sugar units such as glucose, xylose, and arabinose. Besides, by-products which were lactic acid, acetic acid, formic acid, and hydroxymethylfurfural (HMF) also were detected by HPLC. This result confirmed that our process was work to extract and depolymerize the cellulose and hemicellulose from lignocellulose into the sugar units via hydrolysis reaction.

4.3.3 Characterization of solid residues

After the reaction, the product was divided into 2 phases which were liquid and solid. The solid residues were characterized by SEM-EDS technique and TGA/DTG analysis.

4.3.3.1 Scanning Electron Microscopy- Energy Dispersive X-ray spectroscopy (SEM-EDS)

The morphology of OPEFB and solid residues were investigated by SEM technique. As shown in Figure 25a,b the morphology of OPEFB was covered with the chemicals component such as wax, oil, lignin, and hemicellulose which prevent the fiber from enzymatic hydrolysis [28, 29]. The orientation of OPEFB was slightly crystalline. In contrast, the surface morphology of the solid residue (Figure 25c,d) was rough and amorphous. Moreover, the substances which covered its surface was removed. These results demonstrated that the one-pot lignin first biorefinery converts the structure of OPEFB.

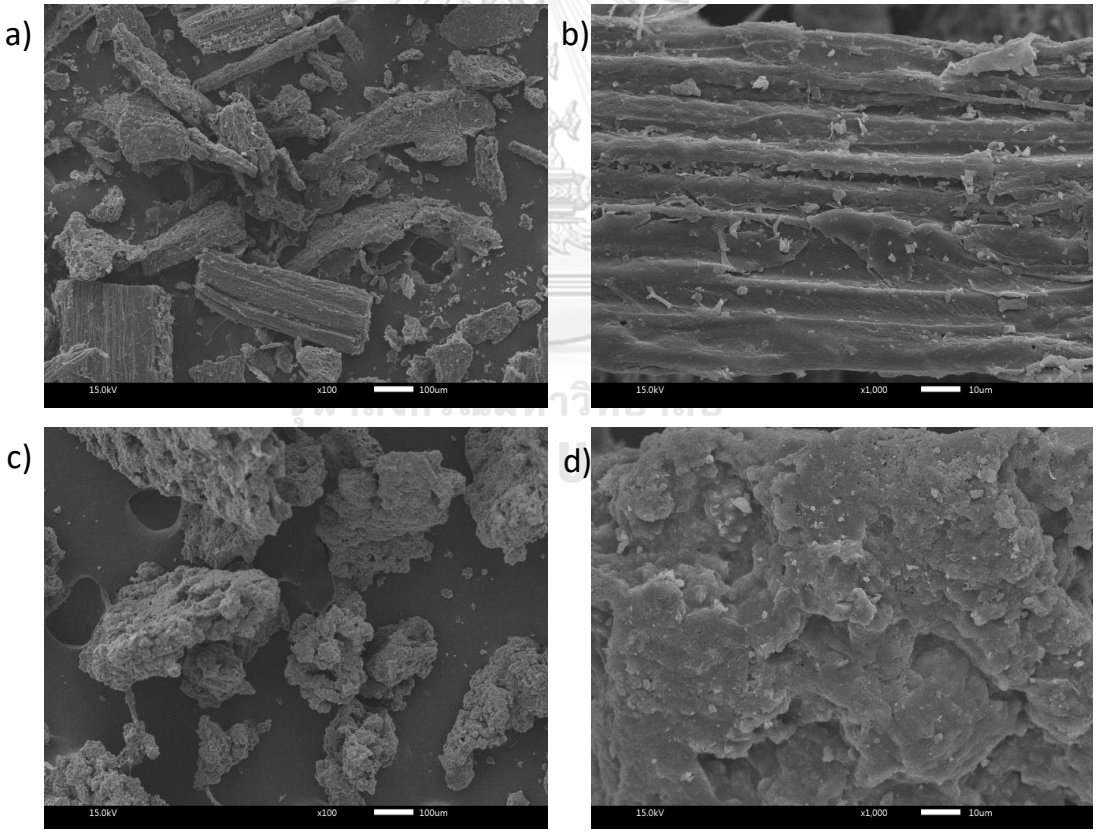


Figure 25 SEM images of a) raw OPEFB 100x, b) raw OPEFB 1000x, c) solid residues 100x, and d) solid residues 1000x.

The element composition was analyzed by EDS as shown in Table 5. Comparing the element composition in the solid residue to OPEFB, the amount of carbon of solid residue increased. Even though the solid residue included the catalyst which was composed of oxygen, the amount of oxygen decreased.

Table 5 EDS analysis of OPEFB and solid residue

Element	The element composition (wt%)	
	Raw OPEFB	Solid residue
C	46.43	63.56
O	48.82	33.77

4.3.3.2 Thermogravimetry analysis and Derivative thermogram analysis (TGA/DTG)

In this study, TGA and DTG of raw OPEFB and solid residues obtained from the reaction were used to analyze the thermal stability between 20-900℃ as shown in Figure 26. Considering the TGA curve of raw OPEFB, there was weight loss in the range of 60-100℃ corresponding to the evaporation and dehydration of moisture and light chemical compounds. Next, the decomposition of cellulose and hemicellulose occurred in the weight loss stage between 230-380℃ [30, 31]. In this stage, the weight loss rapidly decreased with increasing temperature indicated that there was a high content of cellulose and hemicellulose. In the temperature range 380 -600℃ as the third stage, this stage was the lignin degradation. Since lignin was composed of the complex network and cross-linked aromatic, the weight loss of this stage would gradually decrease with temperature increase. After the third stage, the solid remaining 20% was char content that was not decomposed. On the other hand, in the TGA curve of solid residue after the reaction, the curve of the solid residue was gradually decreased with increasing temperature. Besides, there was low moisture content in the first stage (60-100℃). In the second stage, the rate of weight loss was

slow, and volatile mass loss was low compare to raw OPEFB. Moreover, the solid residue remained 50% of char content. According to the DTG curve (Figure 26b), there were 2 peaks. The first peak at 60°C coincided with the weight loss of moisture stage in the TGA curve. Next, a sharp peak at 320°C with a slight left shoulder at 290°C was detected which was a peak of the cellulose and hemicellulose decomposition, respectively [32]. For DTG of solid residue, moisture decomposition peak at 60°C disappeared and the peak of cellulose and hemicellulose decomposition shifted to higher temperature (from 320°C to 360°C). Besides, the peak at 290°C of solid residue was broader than OPEFB in the same position. In addition, there was a peak at 420°C that was the peak of the decomposition of lignin and complex component. Changes in the TGA curve and DTG peaks such as loss of moisture decomposition peak, the transition of peak position to the higher temperature, and the broadening of the peak, also indicated that the OPEFB was lost the cellulose and hemicellulose or probably the stable compounds were formed. Additionally, the peaks of solid residue in the DTG curve were in agreement with the peak of hydrochar which was the carbonaceous material formed by the thermal decomposition of biomass [33].

According to the results of element and thermogravimetry analysis, the carbon content and complex compound increased while the volatile compounds decreased which indicated that the one-pot lignin first biorefinery resulting in the change of both morphology and thermal stability of OPEFB. Otherwise, the OPEFB probably was changed into the hydrochar.

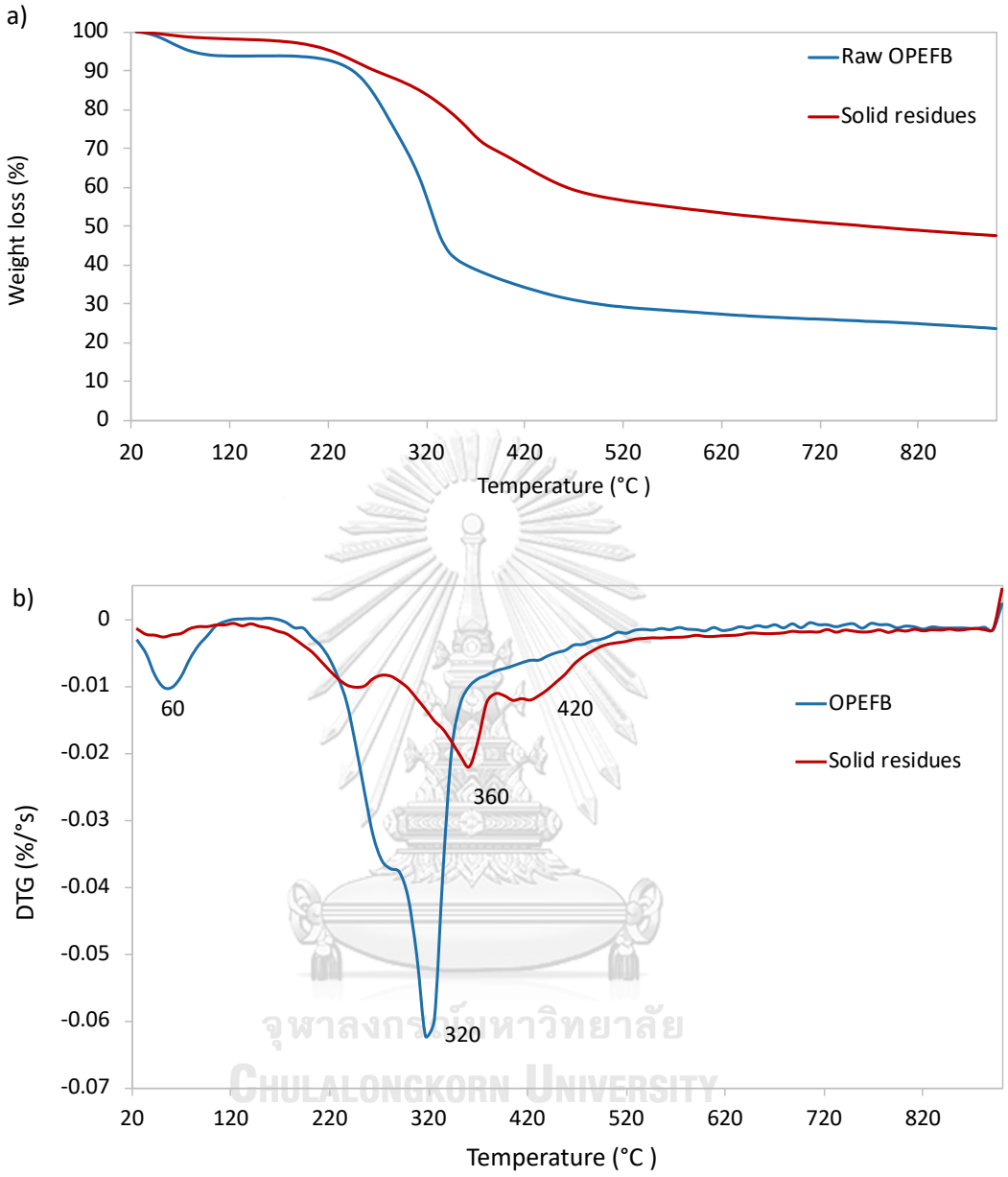
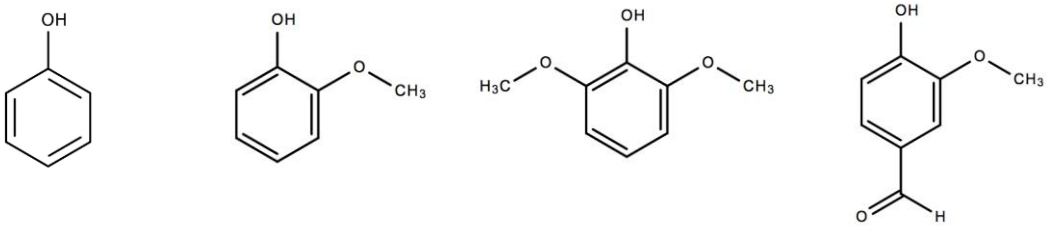


Figure 26 TGA and DTG analysis of (a) raw OPEFB and (b) solid residues.

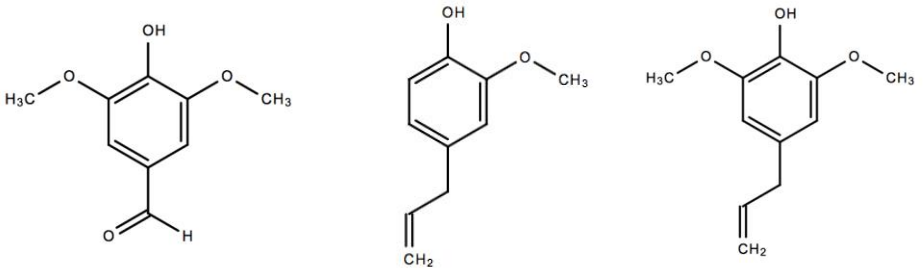
The characterization of the product including lignin oil, water phase, and solid residue from one-pot lignin first biorefinery of OPEFB confirmed that the reaction could extract and depolymerize lignin, cellulose, and hemicellulose into the monomers. Unfortunately, the partial of OPEFB probably was changed into the hydrochar.

4.4 The effect of catalyst and hydrogen gas on lignin extraction

The absence and the presence of 0.2 g 16% Ni/Al_2O_3 in the one-pot lignin first biorefinery at 250°C, 3 h, and 30 bar H_2 were studied to investigate the effect of catalyst. As shown in figure 27, the lignin oil yield in the reaction with and without catalyst was 34.80%, and 24.70%, respectively. Demonstrated that the presence of catalyst resulting in the higher lignin oil yield than the absence. Furthermore, the reaction with catalyst provided a monomer yield of 4.41% while the monomer yield of the reaction without catalyst was 0.67%. Besides, the lignin oil from the reaction with catalyst was composed of 7 main monomer types such as phenol, guaiacol (G), syringol (S), vanillin (ald-G), syringaldehyde (ald-S), 2-methoxy-4-(1-propenyl)-phenol (isoeugenol; propenyl-G), and 2,6-dimethoxy-4-(2-propenyl)-phenol (methoxyeugenol; propenyl-S) as shown structure below. Meanwhile, the lignin oil from the reaction without catalyst lacked ald-S and propenyl-G. These results revealed that the free catalyst reaction could also extract the lignin from OPEFB but low efficiency of lignin cleavages. The presence of catalyst increased the efficiency of lignin cleavage and solubility of lignin. Probably that catalyst participated in hydrogenolysis reaction and improved the lignin linkage cleavage then provided more soluble-lignin fragment [22]. Otherwise, the catalyst participated in the hydrogenation of lignin fragments to prevent the repolymerization of reactive molecules [34].



Phenol G S Ald-G



Ald-S Propenyl-G Propenyl-S

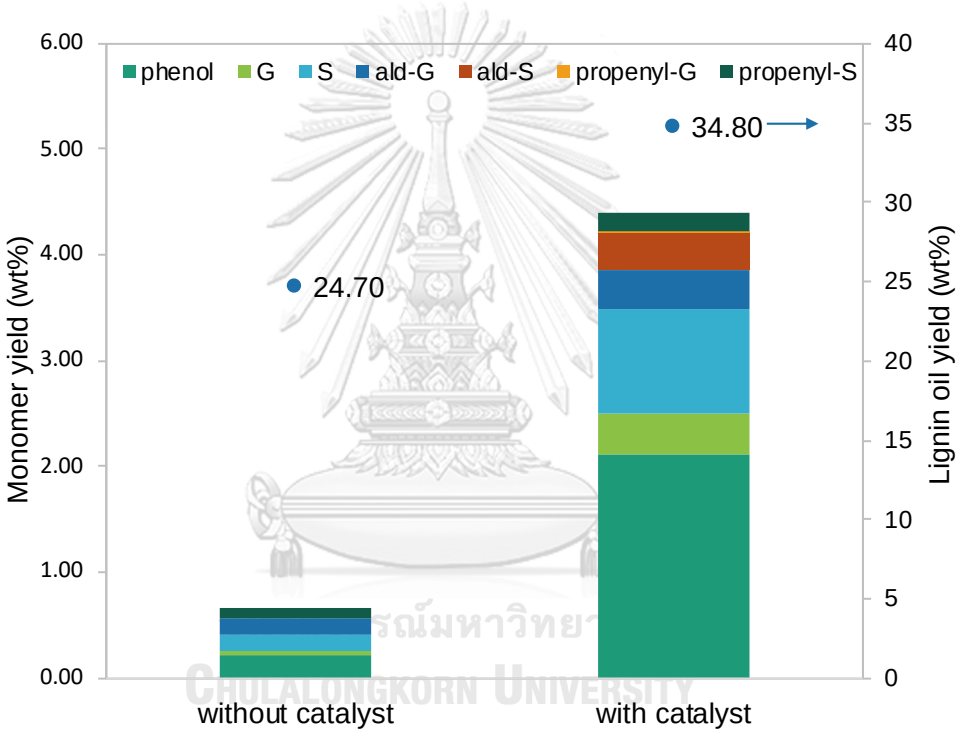


Figure 27 Representation of 7 main monomers (phenol, G, S, ald-G, ald-S, propenyl-G, and propenyl-S) and plot of Monomer yield and lignin oil yield obtained from lignin-first biorefinery in the absence and the presence of catalyst.

One-pot lignin-first biorefinery is composed of 3 parts which were extraction of lignin from biomass, depolymerization of lignin into the lignin monomers, and stabilization of the reactive lignin fragments to the stable fragments. The depolymerization part was studied to identify the cleavage mechanism. Although the actual mechanism had not been clarified, the popular proposed mechanism was the

Catalytic Transfer Hydrogenolysis (CTH) [16, 22, 23]. CTH is the reaction using an alcohol solvent as a hydrogen source that provides hydrogen for breaking the ether bond between lignin monomers. The alcohol solvent attached to the surface of the catalyst then it was dehydrogenated to produce the hydrogen. The hydrogen obtained has participated in the depolymerization of lignin via hydrogenolysis reaction. Then, the absence of a catalyst experiment should significantly diminish the lignin cleavage while the absence of external hydrogen source or hydrogen gas should not affect the lignin cleavage. This work has studied the effect of catalyst and hydrogen gas on lignin depolymerization to prove the proposed cleavage mechanism of lignin in the lignin-first biorefinery. Ethylene glycol and water in the ratio of 1:1 was used as a co-solvent for the reaction. The lignin oil yield of the reaction without catalyst was 24.7% while the lignin oil yield of the reaction without hydrogen gas was 35.6%. The lignin oil which was composed of lignin monomers was identified by GC-MS. Table 4 shows that the composition of lignin oil obtained from the reaction without 16% Ni/Al_2O_3 as a catalyst contains more kind of the lignin monomers than the lignin oil obtained from the reaction without hydrogen gas. Moreover, the amount of each lignin monomers in lignin oil obtained from the reaction without catalyst was higher than the reaction without hydrogen (Figure 28). Then, the reaction without catalyst could break the lignin linkage more than the reaction without hydrogen gas. Although the catalyst for producing hydrogen via dehydrogenation of alcohol solvent has not participated in the reaction, lignin was broken into the lignin monomer. While, the hydrogen gas free reaction but still remain an alcohol solvent or ethylene glycol and catalyst which can produced the hydrogen for breaking lignin bond, the lignin was hardly broken into the monomers. These results show that the lignin linkage was not broken by the hydrogen produced from the dehydrogenation of alcohol solvent. Then, the Catalytic Transfer Hydrogenolysis was not the main route to cleavage the lignin into the monomer for this work.

Table 6 The composition of lignin oil in different conditions.

Chemicals	Without H_2	Without Catalyst
Phenol	+	+
Guaiacol	-	+
Syringol	+	+
Vanillin	-	+
Syringaldehyde	-	-

*using a mixture of deionized water and ethylene glycol (1 : 1, volume ratio) as a solvent, 250 ℃, 3 h.

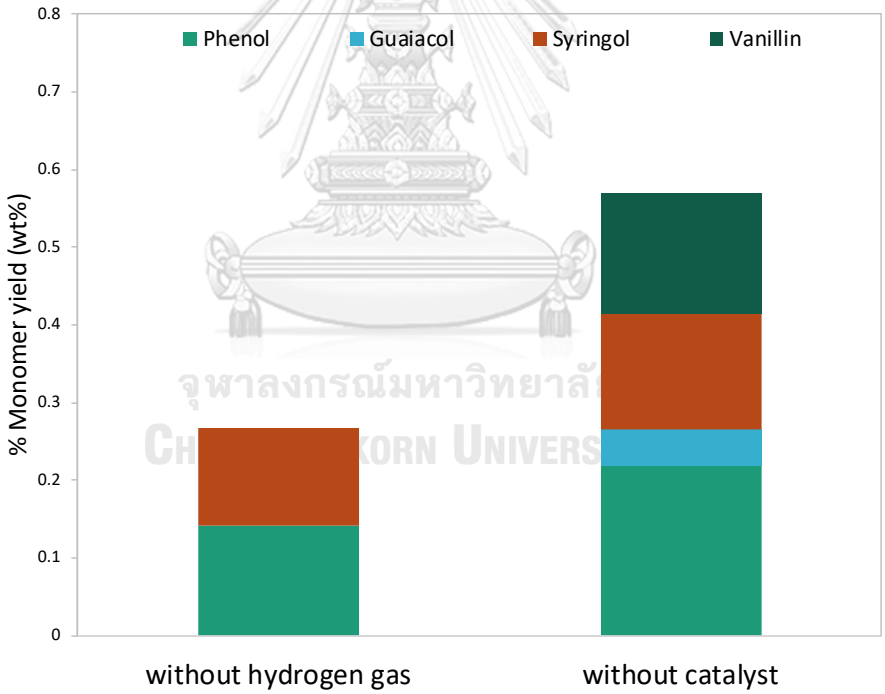


Figure 28 Monomer yield of lignin oil obtained from lignin-first biorefinery in the absence of catalyst compare to in the absence of hydrogen gas.

4.5 The ratio effect of solvent mixtures

In general, lignin-first biorefinery focus on the lignin extraction and depolymerization into the monolignol. The pure alcohol solvent was used for extracting lignin from biomass and depolymerizing lignin into small molecules. While cellulose and hemicellulose insoluble in alcohol solvent resulting in both cellulose and hemicellulose remained in the solid residues. This work used solvent mixtures in the one-pot lignin first biorefinery. Not only lignin was extracted and depolymerized into the monomers but also cellulose and hemicellulose. Water-ethylene glycol mixture in the volume ratio of 1:1, 1:2, and 1:3 was used to study the ratio effect of solvent mixture on lignin oil yield. Since the ethylene glycol (EG) has the ability for dissolving lignin fragments[35]. Also, water could break the inter-linkage in cellulose and hemicellulose to obtained the sugar units via hydrolysis reaction[36]. As the results in Figure 29, the lignin oil yield of 1:1, 1:2, and 1:3 volume ratio of water/EG conditions were 34.80%, 39.41%, and 60.46%, respectively. The results show that the increase in water proportion induced the yield of lignin oil decreased due to the ability of EG for dissolving lignin. Consider the solubility of lignin in water compares to EG, lignin fragments insoluble in water but soluble in EG. Corresponding to the previous research has found that the methoxy groups of lignin interrupt water from diffusing into the hydroxy group where water bind with lignin via hydrogen bond. Besides, the methoxy group of lignin that was more hydrophobic than the hydroxyl group resulting in lignin molecule was not surrounded by water, therefore lignin was not soluble in water [37]. Moreover, the study of the interaction simulation between lignin fragments and EG in the presence of water in solution investigated that EG could bind with lignin via hydrogen bonding. The presence of water in mixtures provided the bond length of the hydrogen bond between lignin and EG longer than in the absence of water so the presence of water resulting in the hydrogen bond of EG and lignin was weak [35].

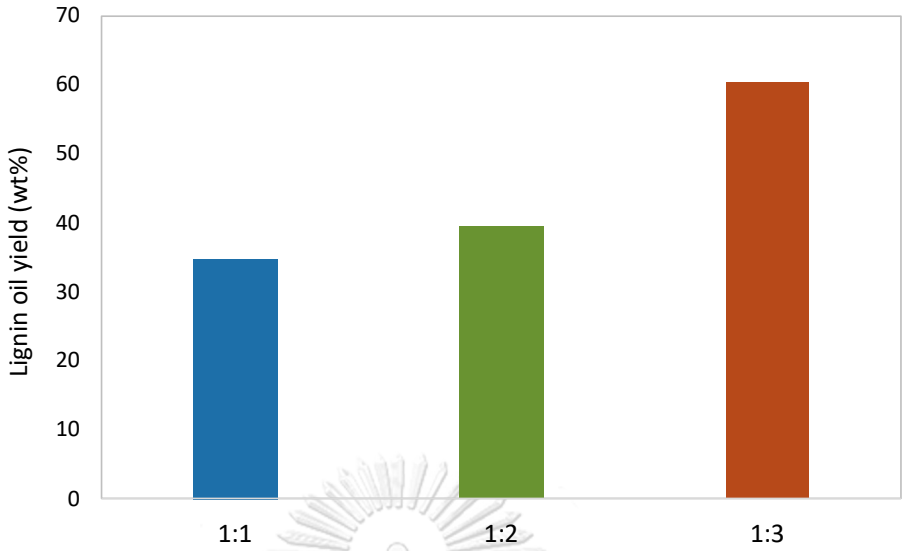


Figure 29 Lignin monomer yield from one-pot lignin first biorefinery using water-EG mixture as a solvent (250°C, 3h, and 30 bar H_2)

Apart from lignin oil yield, the monomer yield of lignin was also analyzed to study the ratio effect of the solvent mixture. The Figure 30 represented that the total monomer yield of 1:1, 1:2, and 1:3 volume ratio conditions were 4.41%, 3.82%, and 3.78%, respectively. These results implied that increasing the water proportion increases the monomer yield of lignin. Furthermore, the volume ratio of 1:1 condition provided the main monomers such as phenol, G, S, ald-G, ald-S, propenyl-G, and propenyl-S while the volume ratio of 1:2 and 1:3 provided 6 main monomers without propenyl-G. The changing of water proportion affected the lignin monomer yield denoted that water contributed to the depolymerization of lignin. These results also supported that the lignin probably was depolymerized by solvent through the solvolysis reaction, not CTH reaction because the changing of solvent ratio affects the lignin cleavage. As EG contained hydrogen sources more than water, the high proportion of EG should provide high efficiency for breaking lignin into monomers. In contrast, our results showed that the high proportion of EG rendered the low lignin monomer. In conclusion, one-pot lignin first biorefinery using water-EG mixture as a solvent work for extracting and depolymerizing lignin. Water participated in the lignin bond cleavage, while EG participated in the dissolution of lignin fragments therefore

a high proportion of water provided high monomer yield but low lignin oil yield. According to the results, we proposed the mechanism that lignin was cleaved into the small molecules via acid-catalyzed hydrolysis then, they were dissolved into EG and hydrogenated by the catalyst and hydrogen gas to stabilize the active lignin fragment.

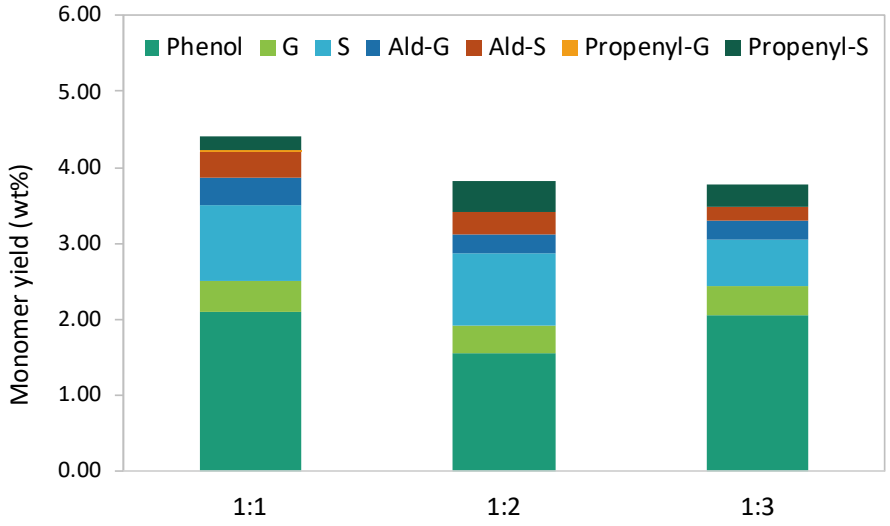


Figure 30 Lignin monomer yield from one-pot lignin first biorefinery using water-EG mixture as a solvent (250°C, 3h, and 30 bar H_2).

4.6 The study of effect of acidity

Since the water was used to depolymerize the cellulose and hemicellulose into sugar units via hydrolysis, acid was necessarily added into the solvent to catalyze the hydrolysis reaction. To study the effect of acidity on the lignin part, we have varied the acid types and pH range to understand the role of acid in the lignin extraction and depolymerization step. As the lignin precipitated at the extreme acid condition [43], we used the pH range of 3-5 that was not too acidic and still hydrolyzed the cellulose and hemicellulose.

4.6.1 Effect of acid types on lignin oil and monomer yield

Sulfuric acid and acetic acid were used as a homogeneous catalyst to evaluate the effect of acid types on lignin. This experiment used the water/EG volume ratio of 1:1 at 250 °C for 3 hours with 30 bar H_2 because this condition contained equal water and EG that easy to observe the behavior of both phases. In addition, the pH was adjusted by the proton concentration calculation based on the equilibrium constant of the dissociation reaction of an acid (Ka). The lignin oil yield in the absence of acid was 34.80% while the lignin oil yield in the presence of acetic acid at pH of 3,4, and 5 were 35.24%, 35.64%, 38.94%, respectively. Also, the lignin oil yield of sulfuric acid at pH of 3, 4, and 5 conditions were 42.23%, 46.26%, 45.62%, respectively (Figure 31). The presence of acids increased the lignin oil yield. Lignin-first biorefinery reaction with sulfuric acid showed a significantly higher lignin oil yield than the reaction with acetic acid. Moreover, the reaction with acetic acid showed that an increase of acidity (pH decrease) decreased the lignin oil yield.

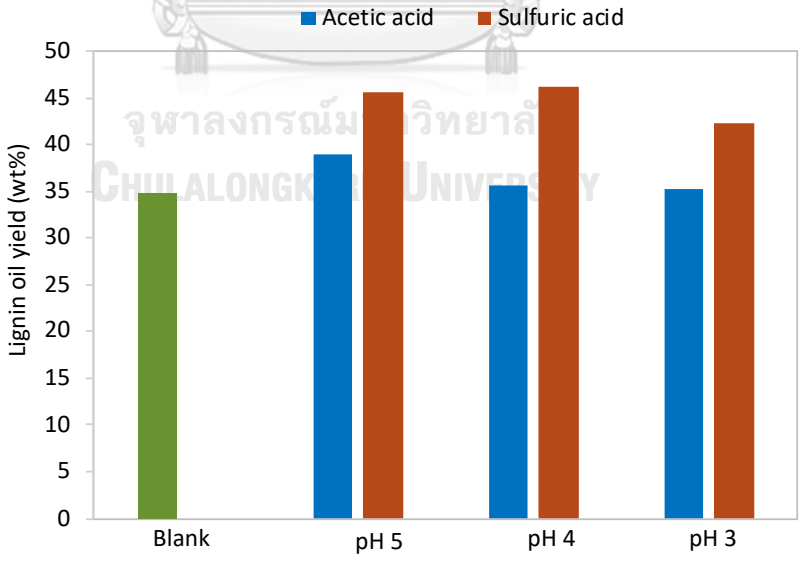


Figure 31 Lignin oil yield from one-pot lignin first biorefinery using water-EG mixture (1:1) as a solvent at 250℃, 3h, and 30 bar H_2.

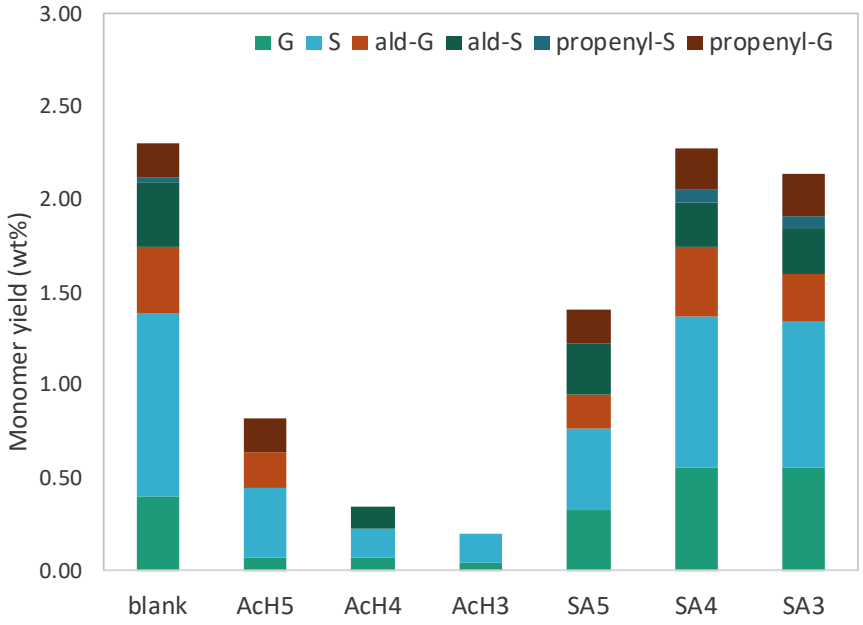


Figure 32 Lignin monomer yield from one-pot lignin first biorefinery using water-EG mixture (1:1) as a solvent at 250°C, 3h, and 30 bar H_2.

Subsequently, the monomer yield of lignin oil (wt%) in each condition was shown in Figure 32. AcH and SA in the Figure 32 referred to acetic acid and sulfuric acid, respectively, and the number after AcH and SA referred to pH value. The lignin monomer yield of the reaction with acetic acid was lower than the lignin monomer yield of the reaction with sulfuric acid. Besides, monomer yield decreased by the amount of acetic acid increased. Additionally, the reaction with sulfuric acid provided various monomer types more than the reaction with acetic acid. These results represented that the increase of acetic acid not only diminish lignin oil yield but also diminish monomer yield. These results were consistent with previous research by Xuebing Zhao e.al, (2013) [38] who studied the simulation of acid-catalyzed delignification of lignin by acetic acid. They found that the lignin could bind with acetic acid to form the larger molecule via condensation reaction as shown in Figure 33. Therefore, the aggregation of lignin and acetic acid resulting in the low lignin oil yield and monomer yield. Considering the catalyze ability of acid in the hydrolysis reaction, moreover, as the ether bond of lignin was cleaved via acid hydrolysis [39] as shown in Figure 34, weak acid was not widely used because the bond between H-

X of weak acid was stronger than strong acid. Besides, the leaving group of acetic acid or acetate was the poor leaving group while the leaving group of sulfuric or HSO_4^- was the excellent leaving group[40]. This evidence clarified that acetic acid was not suitable for breaking the ether bond of lignin.

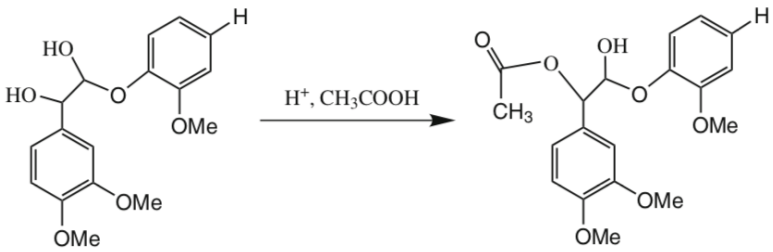


Figure 33 Possible condensation reactions of lignin molecule and AcH [38].

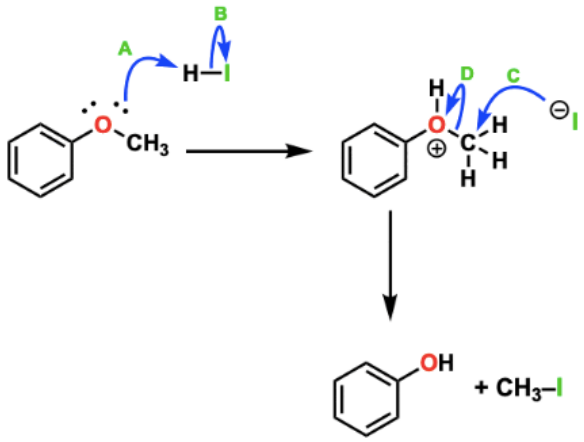


Figure 34 An acidic cleavage of ethers (S_N2) mechanism [39].

4.6.2 Effect of acidity on lignin oil yield

Since the sulfuric acid improved the efficiency of lignin dissolve and cleavage, it was used to study the effect of acidity on lignin oil yield and monomer yield using the water/EG volume ratio of 1:1, 1:2, and 1:3 at 250°C, 3h, and 30 bar H_2. The effect of acidity was studied in the pH range of 3-5 and pH of blank condition was around 6. As shown in Figure 35, the lignin oil yield in the water/EG ratio of 1:3 was higher

than the lignin oil yield in the water/EG ratio of 1:2 and 1:1, respectively because EG provided high lignin solubility as mentioned at the topic of ratio effect of mixture solvent. Then the lignin oil yield increased with the proportion of EG increased. In addition, Figure 35 represented the presence of sulfuric acid leads to the change of lignin oil yield. For the lignin oil yield in the water/EG ratio of 1:2, the presence of acid increased the lignin oil yield. Although the lignin oil yield at pH 4 decreased. Unlike, the water/EG ratio of 1:3 conditions showed the presence of acid did not increase the lignin oil yield. At pH 3 and pH 5, the lignin oil yield had a similar value to the blank condition due to it might be the highest lignin content which can be dissolving in the EG. Unfortunately, the lignin oil yield at pH 4 in every mixture ratio remained constant. It might be the acidity at pH 4 did not suitable for dissolving lignin fragment into the ethylene glycol. For the water/EG ratio of 1:1, the lignin oil yield slightly increased with an increase in acidity. Since the presence of acid participated in the lignin bond cleavage via acid-catalyzed hydrolysis then the lignin fragments will be less polar and become dissolved in the ethylene glycol. Moreover, at pH 5 of each water/EG ratio provided high lignin oil yield due to the less acidity decreased the protonation of lignin fragment then it becomes more hydrophilic [41] (but still less than water) leads to high efficiency soluble into the EG.

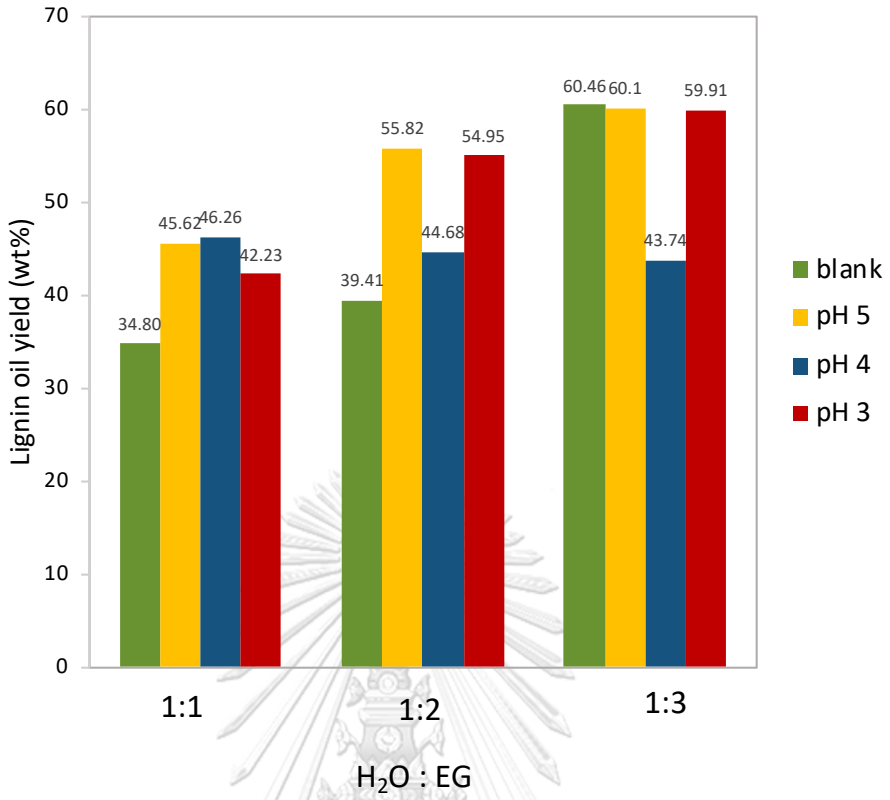


Figure 35 Lignin oil yield from one-pot lignin first biorefinery with sulfuric acid at 250°C, 3h, and 30 bar H_2.

Apart from lignin oil yield, the lignin monomer yield was also investigated to study the effect of acidity on the capability of lignin cleavage. Figure 36a showed the phenol was the main monomer obtained from every condition. Considering the change in water/EG ratio of each pH did not affect the lignin monomer yield. There was propanyl-G only occurred in the water/EG ratio of 1:1. Moreover, the lignin monomer yield obtained from the reaction with the water/EG ratio of 1:1 was higher than the reaction with the water/EG ratio of 1:2 and 1:3 in every pH condition because the high proportion in water resulting in high efficiency of the lignin cleavage then the monomer obtained increased. These results were similar to the ratio effect of the water/EG mixture which was explained above in 4.5. In the present, there are studies which reported that acid improved the lignin bond cleavage of both β-O-4 and α-O-4 bond via acid-catalyzed hydrolysis or acidolysis reactions [42, 43]. In

contrast, some studies reported that concentrated acid induced the repolymerization and participation of lignin fragments [44, 45]. According to the results, as shown in Figure 36, lignin monomer yield at pH 3 was inferior to pH 4. Although the acidity participated in the breaking of lignin linkage, an increase of acidity also induced the precipitation of lignin. When the pH decreased, high molecular weight lignin was precipitated [43, 46]. Also, the lignin fragment such as phenolic compound was protonated then the electrostatic repulsive force between lignin fragment reduced and become more hydrophobic resulting in the precipitation [41]. Besides, the GPC chromatogram (Figure 36b) was in agreement with the high acidity induced the lignin repolymerization. Due to the GPC of lignin in the presence of acid provided a long tail peak that represents the high amount of large molecule. Fortunately, the presence of acid also provided a high lignin monomer yield similar to the lignin monomer yield in the absence of an acid condition. For each water/EG ratio, the lignin monomer yield at pH 5 was lowest compare to others. Though the lignin oil yield at pH 5 was the highest, the lignin monomer yield was not. It probably resulting of the presence of less acidity improved the lignin solubility then, the lignin fragments which may be the dimer- were dissolved by EG before it was cleaved into the monomer. For pH 4, even the lignin oil yield at pH 4 was lowest, the monomer yield was highest. This pH was probably not suitable for dissolving lignin but it was selective for breaking the lignin bond.

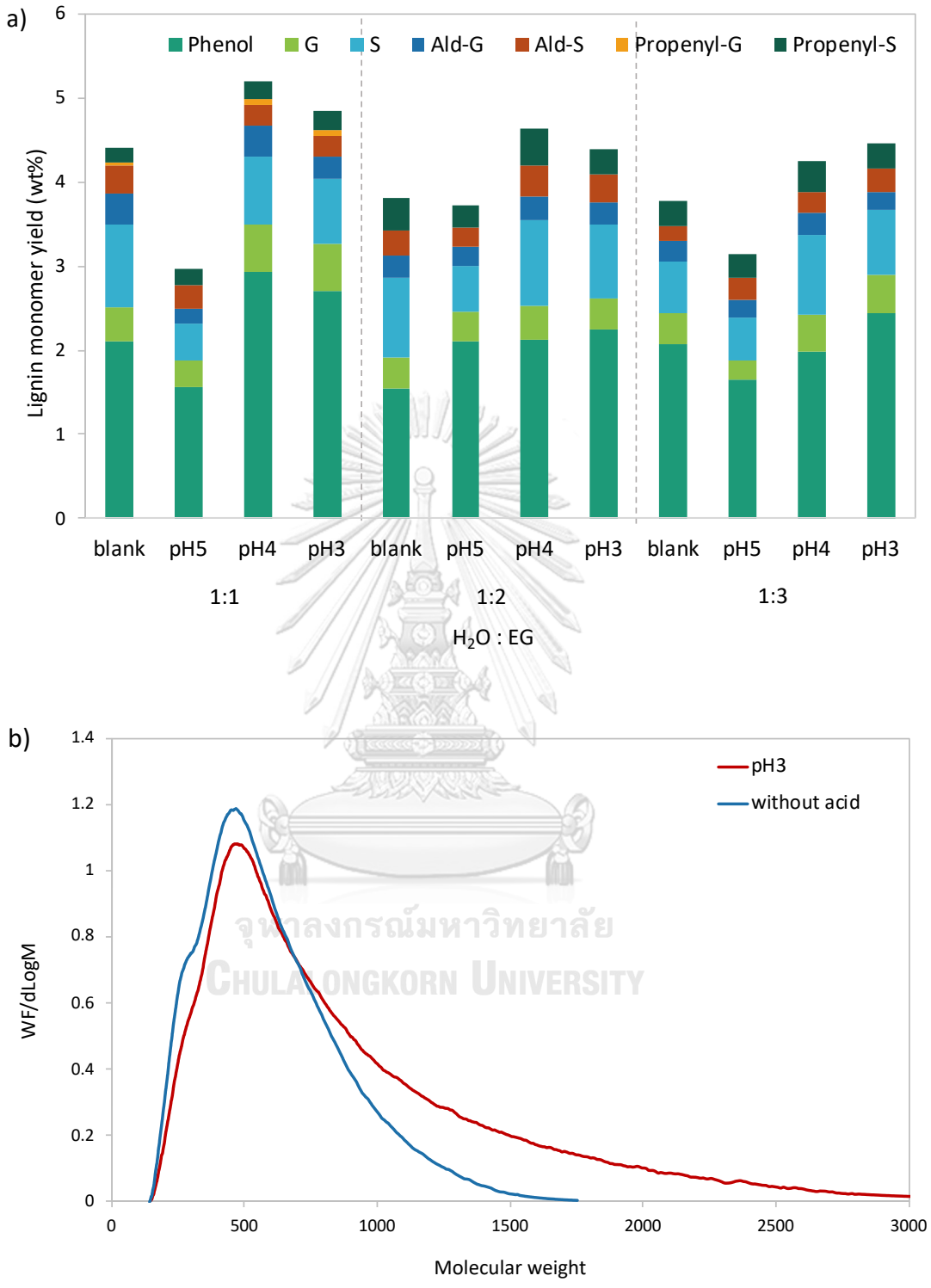


Figure 36 a) Lignin monomer yield from one-pot lignin first biorefinery with sulfuric acid at 250°C, 3h, and 30 bar H_2. And b) GPC chromatogram of lignin oil with and without the sulfuric acid participated in the reaction.

According to the effect of acidity on lignin oil yield and monomer yield, it can be concluded that the reaction with pH 5 condition provided high efficiency of lignin solubility while the reaction with pH 4 provided high efficiency of lignin depolymerization. Besides, the reaction with pH 3 provided high efficiency both lignin solubility and lignin depolymerization.

4.6 Effect of reaction temperature

4.6.1 Effect of reaction temperature on lignin

As the reaction at pH 3 with a water/EG ratio of 1:1 was successful for acid-catalyzed hydrolysis lignin and provided high monomer yield, this condition was used to study the effect of temperature. The lignin oil yield at 180, 200, 220, and 250°C were 12.97%, 25.84%, 37.82%, and 42.23%, respectively. The trend was observed in Figure 37 that the lignin oil yield increased when the reaction temperature increased from 180 to 250°C. As expected, in the one-pot lignin first biorefinery performed at 180-250°C, the lignin depolymerization increased. Increasing temperature not only weaken the linkage between lignin and others component in biomass but also weaken the bond between lignin molecules [35]. Then, the lignin linkage was easy to break into the lignin fragments which small molecules, then the fragments were dissolved by EG.

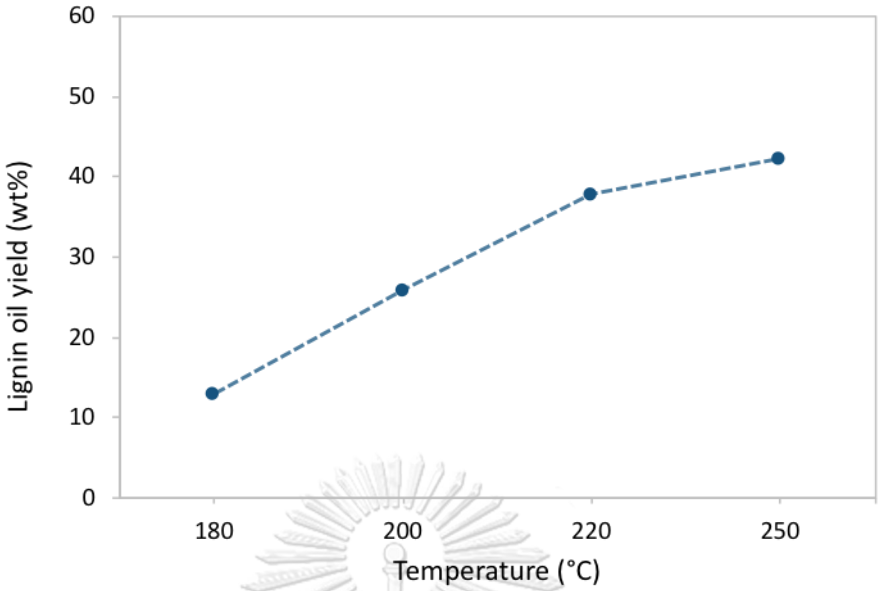


Figure 37 Lignin oil yield from one-pot lignin first biorefinery with the sulfuric acid-contained water/EG ratio of 1:1 (pH3), 3h, and 30 bar H_2 at different temperature.

In the part of the effect of temperature on lignin monomer yield, Figure 38 showed that S/G monomer increased with temperature, ald-S/G, and propenyl decreased with temperature while phenol increased in the temperature range of 180-220°C and decreased at 250°C. At high temperature, the substituted phenolic compounds such as alkyl-side chain substituted as propenyl-S/G and aldehyde-side chain were eliminated via the hydrogenation followed dealkylation reaction into the S/G [47]. Owing to the bond dissociation energy of the side chain of the phenolic compound was high then a high temperature was required [48]. Moreover, this result was in agreement with the previous study which reported that the unsubstituted phenolic compound was observed at high temperature while the substituted phenolic compound was observed at low temperature (<180°C) [3]. For the phenol part, yield increased in the temperature range of 180-220°C then slightly decreased after 220°C. Generally, lignin linkage including C-C bond such as **β**-**β**, **β**-1, **β**-5 and 5-5, and C-O bonds such as **β**-O-4, **α**-O-4, and 4-O-5. The most abundant linkage was **β**-O-4 follow by the **α**-O-4 bond [42]. The C-O bond was easy to break and provided a high-value phenolic compound, there were several studies than the C-C bond. The

main product of α-O-4 aryl-ether bond was phenol while the main product of β-O-4 was H, G, and S units. Considering the bond dissociation energy (BDE) of each linkage obtained from the computation, the BDE of β-O-4 (<80 kcal/mol) was higher than α-O-4 (<60 kcal/mol) resulting in α-O-4 was easier to break than β-O-4 [49]. The previous results corresponding to our study that S and G units were observed at high temperatures while phenol was detected at a lower temperature (<220°C).

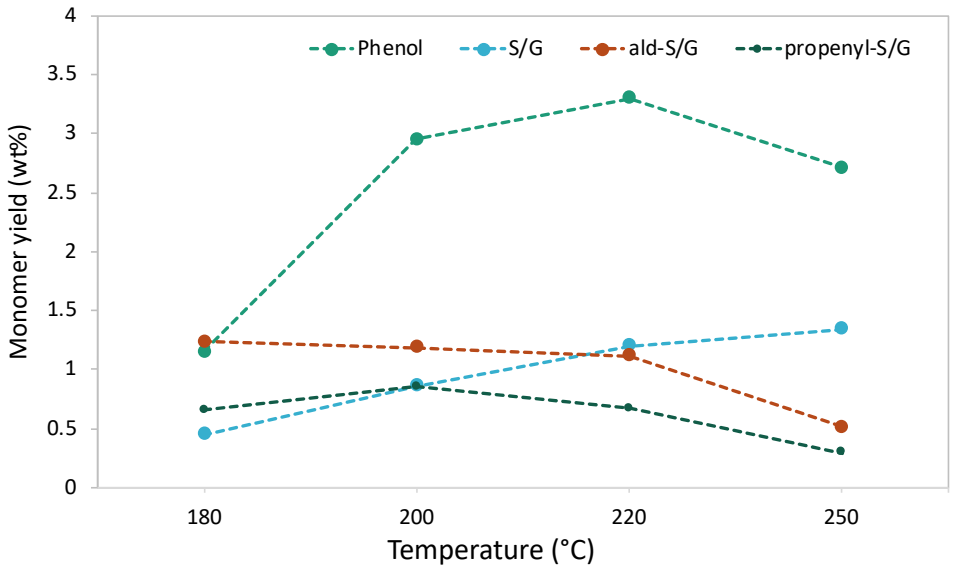
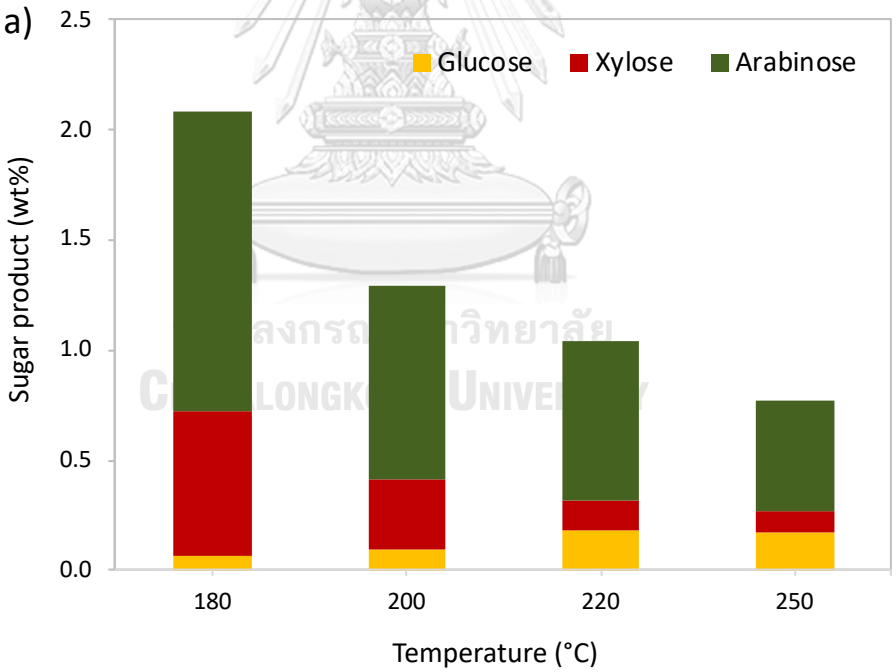


Figure 38 Lignin monomer yield from one-pot lignin first biorefinery with the sulfuric acid-contained water/EG ratio of 1:1 (pH3), 3h, and 30 bar H_2 at different temperature.

4.6.1 Effect of reaction temperature on cellulose and hemicellulose hydrolysis

One-pot lignin first biorefinery using sulfuric acid-contained water/EG ratio of 1:1 at pH 3, 3h, and 30 bar H_2 was used to study the effect of temperature on cellulose and hemicellulose hydrolysis reaction. The sugar product yield was calculated based on starting OPEFB. After the reaction, the liquid phase was divided into. 2 phases which were the DCM phase containing lignin fragment and the water

phase containing sugar fragments. The composition in the water phase was identified by HPLC analysis performed with the AMINEX column. There were 3 mains sugar units including glucose, xylose, and arabinose were observed. Corresponding to the product obtained from the hydrolyzed of cellulose and hemicellulose. Cellulose was cleaved β-1,4-glycosidic bond into glucose while hemicellulose was cleaved both β-1,4-glycosidic bond and β-1,3-glycosidic bond into xylose and arabinose. Figure 39a showed the quantity of each component at different temperatures. The trend was observed that the all of sugar product yield decreased with the temperature increased. Besides, the amounts of sugar units found were low. In contrast, by-products also were detected in high content which were lactic acid, acetic acid, formic acid (FA), and 5-hydroxymethylfurfural (HMF) as shown in Figure 39b. The byproduct yield increased with the temperature increased.



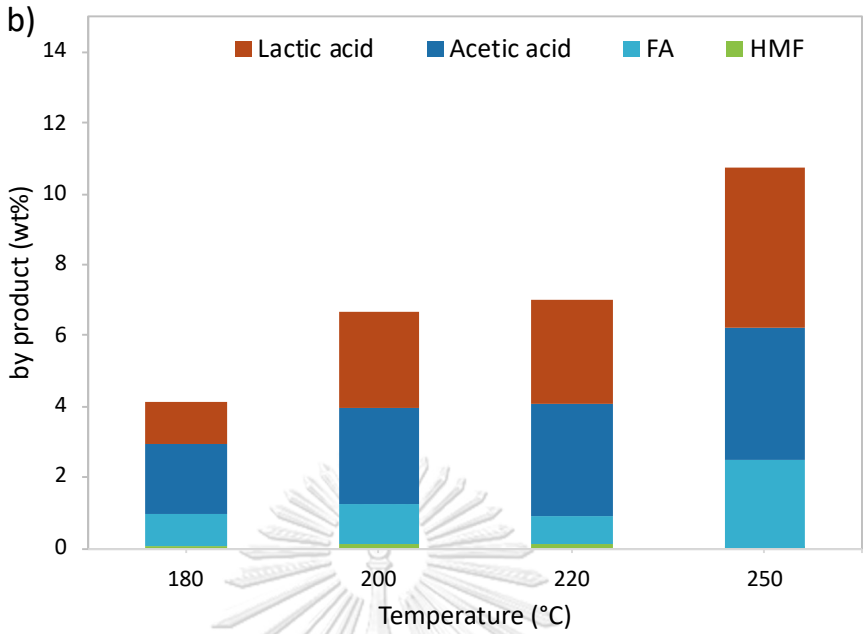


Figure 39 Product yield a) main product and b) byproduct in the water phase from one-pot lignin first biorefinery with the sulfuric acid-contained water/EG ratio of 1:1 (pH3), 3h, and 30 bar H_2 at different temperature.

Considering the conversion pathway of main product to by-product. For cellulose, it was cleaved the β-1,4-glycosidic bond into the water-soluble fragments or glucose units via acid-catalyzed hydrolysis. Then glucose units could be converted to HMF with 2 routes, the first is direct dehydration of glucose to produce HMF and another is the isomerization of glucose into fructose before dehydrated into HMF. Besides, HMF also could be converted to formic acid and levulinic acid though rehydration reaction [50]. The possible reaction pathway of cellulose conversion was shown in Figure 39.

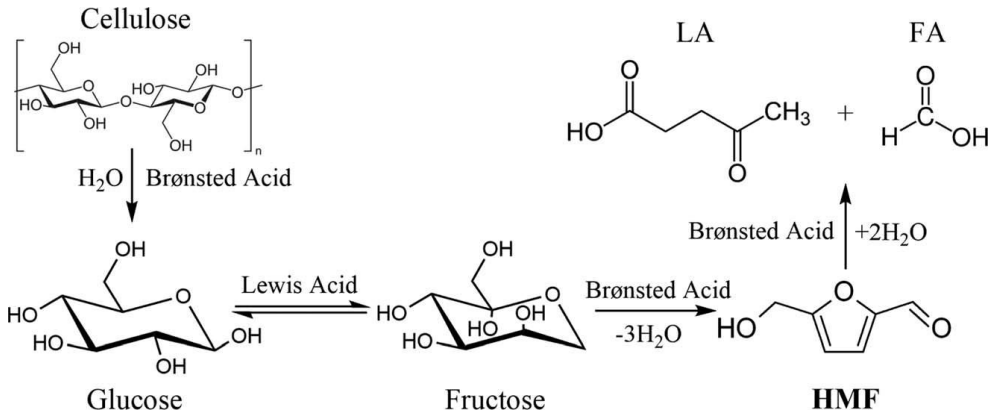


Figure 40 Reaction pathways of glucose to HMF [50].

Like cellulose, hemicellulose was also cleaved β-1,4 and β-1,3-glycosidic bond become the soluble fragments including acetic acid, xylose, and arabinose. Consequently, the pentose was converted to furfural via acid-catalyzed dehydration. Moreover, furfural could be decomposed into formic acid [51] as shown in Figure 41.

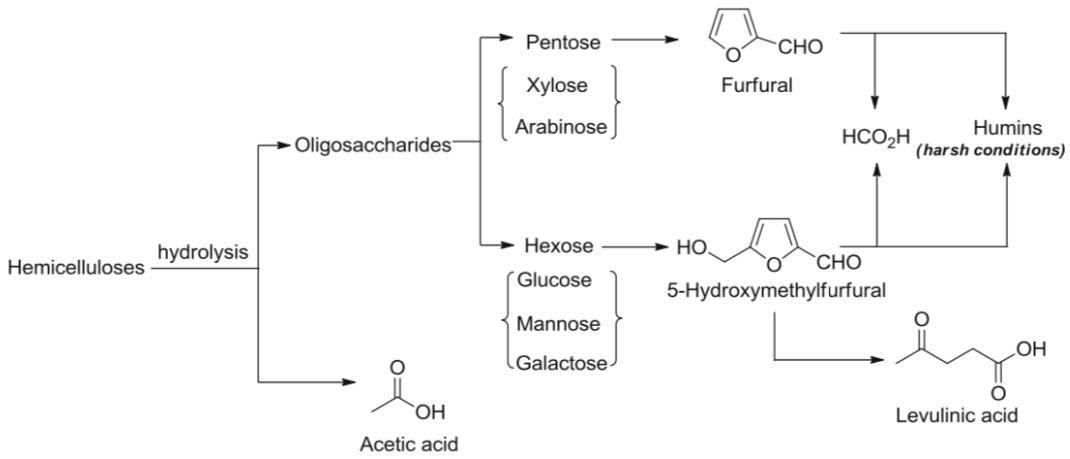


Figure 41 Reaction pathways of hemicellulose [51].

According to the experimental results, temperature significantly affected the hydrolysis of cellulose and hemicellulose. The major products including glucose, xylose, and arabinose preferred low temperature while by-products including HMF, lactic acid, acetic acid, and FA preferred high temperature. These results

demonstrated that the reaction at low temperature, the hydrolysis of cellulose and hemicellulose were predominated. Meanwhile, at high temperatures, dehydration, rehydration, and degradation of the major products were predominant. In agreement with the previous study which reported that an increase in temperature resulting in the forward reaction to occur [51-53].

Depolymerization of lignin and hydrolysis of cellulose and hemicellulose provided the highest yield in a different condition. Lignin depolymerization preferred a high temperature for breaking bonds into the monomers. In contrast, elevated temperature also decomposition the sugar units into the by-product. Besides, a low temperature suitable for hydrolysis of cellulose and hemicellulose into sugar units could not break the lignin linkage into the lignin monomers.

4.7 Effect of reaction time

4.7.1 Effect of reaction time on lignin oil yield

One-pot lignin first biorefinery processing under the sulfuric-contained water/EG ration of 1:1 at pH4, 250°C, and 30 bar H_2 was used to study the effect of time. The lignin oil yield was investigated as shown in Figure 42a, the reaction at 1, 2, 3, and 6 h were 40.96%, 44.52%, 46.26%, and 43.89%, respectively. The lignin oil yield gradually increased for the first 2 h and reached the maximum at 3 h then, slightly decreased at 6 h. At shorter reaction times, lignin fragments were more solubilized by EG. Owing to, at reaction time 1h and 2h, lignin was fast cleaved and solubilized resulting in a high lignin oil yield. However, some lignin fragments were not completely stabilized then they could repolymerized into the large molecule lignin. As shown in Figure 42b, during the liquid-liquid extraction after solid-liquid filtration at reaction time 1h and 2 h, there was the precipitate occurred. Meanwhile, at 3 h and 6 h, there were not precipitation during the liquid-liquid extraction (Figure 42c). In agreement with the lignin fragments which active could repolymerized at the

short reaction time and extended time led to the stabilization of active lignin fragment.

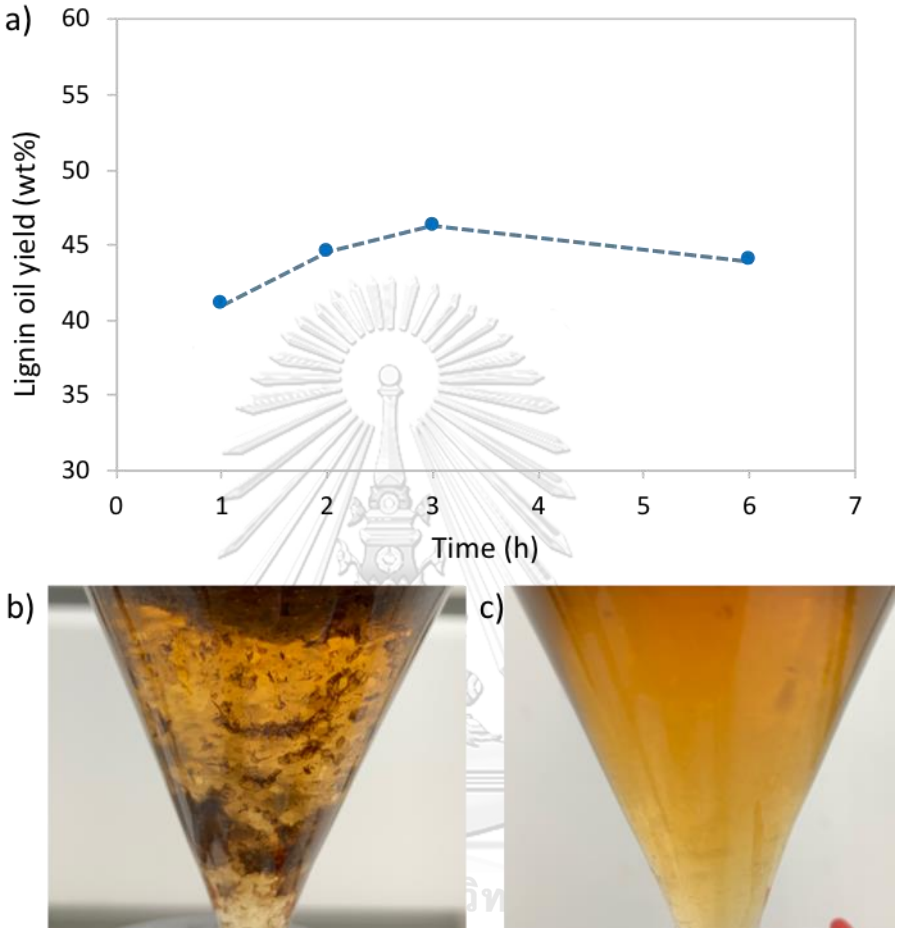


Figure 42 a) Lignin oil yield at different reaction time, b) Lignin precipitate during the liquid-liquid extraction of lignin product obtained from the experiment with reaction time 1h, and c) 3 and 6 h, processing via one-pot lignin first biorefinery with the sulfuric acid-contained water/EG ratio of 1:1 (pH4), 250°C, and 30 bar H_2.

The lignin monomers at different reaction time were investigated as shown in Figure 43. Although extended time increased the lignin oil yield, extended time did not increase all of the lignin monomers. Considering at a shorter time (1-2 h), the substituted phenolic compound (ald-S/G and propenyl-S/G) decreased while unsubstituted phenolic compounds such as phenol and S/G units increased. Starting,

the lignin polymer was cleaved into the side chain phenolic compounds subsequently, the side chains on phenolic compounds were removed. These results indicated the fast hydrogenation, decarbonylation, and dealkylation of the substituted phenolic monomer occurred by H_2 and the catalyst. Corresponding to the previous report that the Ni catalyst provided high-efficiency hydrogenation of unsaturated side chain of phenolic compound to the saturated side chain phenolic compound [54] and the side chain phenolic compound was removed after the lignin was cleaved [55]. Next, at the 3 h, extended reaction time led to a decrease in lignin monomers due to the lignin monomer were stabilized and some active fragments were repolymerized into large molecule. At 6 h, S/G monomers dramatically increased while substituted phenolic compound decreased, and phenol unchanged. An increase in reaction time led to the breaking of lignin oligomers, converting of unsaturated lignin to saturated and stabilizing the active lignin fragment to the S/G units [56]. Even though the S and G units could be converted into phenol by H_2 and catalyst, this transformation required high temperature (<300°C) and long reaction time due to a high bond energy between phenol and substituted methoxy group [57].

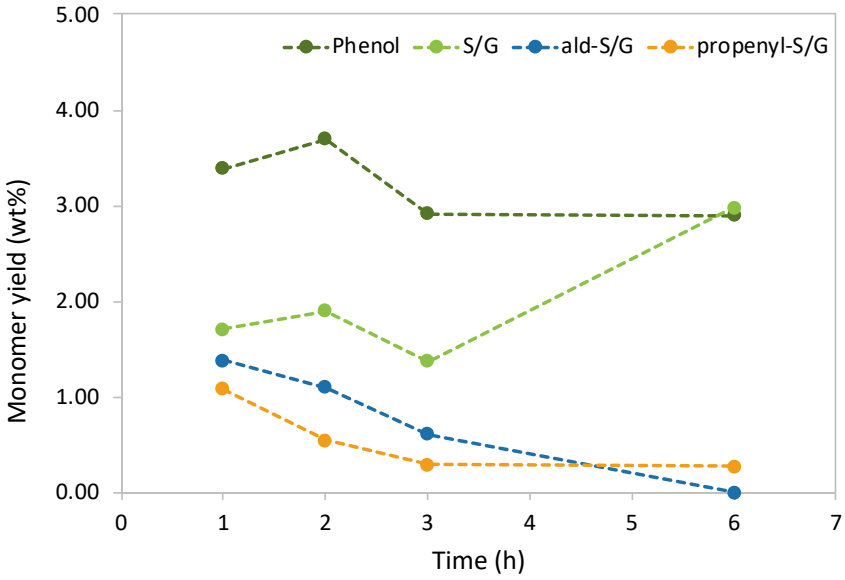


Figure 43 Lignin monomer yield from one-pot lignin first biorefinery with the sulfuric acid-contained water/EG ratio of 1:1 (pH4), 250°C, and 30 bar H_2 at different reaction time.

According to the results, at shorter reaction time, the dissolved lignin was high monomer content but low stability. Meanwhile, at longer reaction time, the dissolved lignin was high yield and high stability. Besides, the lignin obtained at longer time was selective for S and G units.

CHAPTER V

CONCLUSION

5.1 Conclusion

In this study, one-pot lignin first biorefinery which combined the depolymerization of lignin with the hydrolysis of cellulose and hemicellulose in one step was successful for extracting and breaking the lignocellulose into the valuable product such as guaiacol, syringol, vanillin, glucose, xylose, and arabinose. This processing is performed in a mixture of sulfuric cid-contained water and ethylene glycol at 250°C, 3 h, and 30 bar H_2 using OPEFB as a biomass source and 16% Ni/Al_2O_3 as a reductive catalyst. The presence of the catalyst and hydrogen gas clarified that the lignin linkage was cleaved by the solvent via acid-catalyzed hydrolysis.

The ratio of mixture solvent affects the lignin cleavage and solubility, water participated in the lignin cleavage while ethylene glycol participated in the lignin solubility. The suitable acid was sulfuric acid at pH 3, which provided high lignin oil yield and remain the capability in lignin depolymerization. Moreover, temperature and reaction time were significant factors for depolymerization of both lignin, cellulose, and hemicellulose. Harsh conditions, high temperatures with long reaction times improve the lignin depolymerization, solubility, and stabilization. In contrast, the harsh condition reduced the sugar units in the water phase and led to the transformation of sugar units into by-products. In addition, the characterization of solid residue revealed that the solid obtained from the reaction become the hydrochar.

5.2 Recommendation

5.2.1 Processing under mild condition to improve the hydrolysis of cellulose and hemicellulose.

5.2.3 Reduce the pressure of the reaction for applying to the industry because this mixture solvent probably did not require the high.

5.2.4 Put the solid catalyst into the basket for easy separation from the solid residue.

5.2.5 Varying the solid catalyst to improve the selectivity of lignin cleavage.

5.2.6 Use the lignin model compound for studying the deep mechanism in this reaction condition.

APPENDIX

Equation

$$\text{Lignin oil yield (wt\%)} = \frac{\text{weight of oil (g)}}{\text{Klason lignin content} \times \text{starting OPEFB (g)}} \times 100$$

$$\text{Lignin monomer yield (wt\%)} = \frac{\text{weight of monomer (g)}}{\text{weight of oil (g)}} \times 100$$

$$\text{Sugar product (wt\%)} = \frac{\text{weight of eachsugar product (g)}}{\text{weight of starting OPEFB (g)}} \times 100$$

Lignin monomer properties

Table 7 Properties of lignin monomers

Chemicals	Boiling point (°C)	Melting point (°C)	Solubility in water
Phenol	182	40-42	Soluble (8.3 g/100 ml at 20°C)
Syringol	261	50-56	Slightly soluble
Guaiacol	204-206	27-29	Slightly soluble
Vanillin	170	81-84	Soluble (1 g/100 ml at 25°C)
Syringaldehyde	192	113	Insoluble

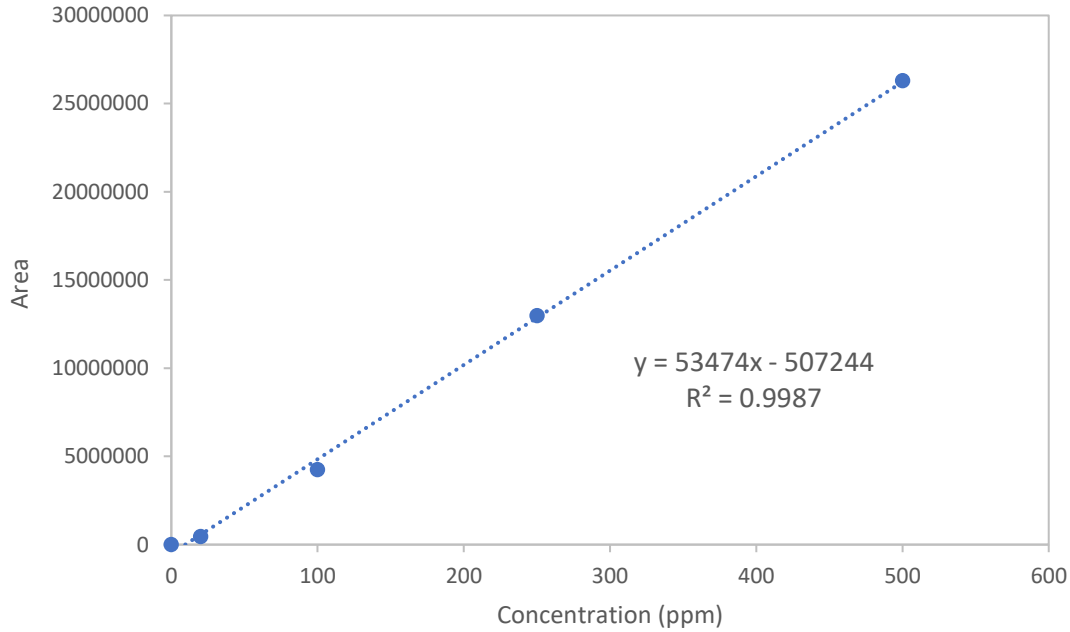
Catalyst characterization

Table 8 Result of BET characterization of 16% Ni/Al_2O_3

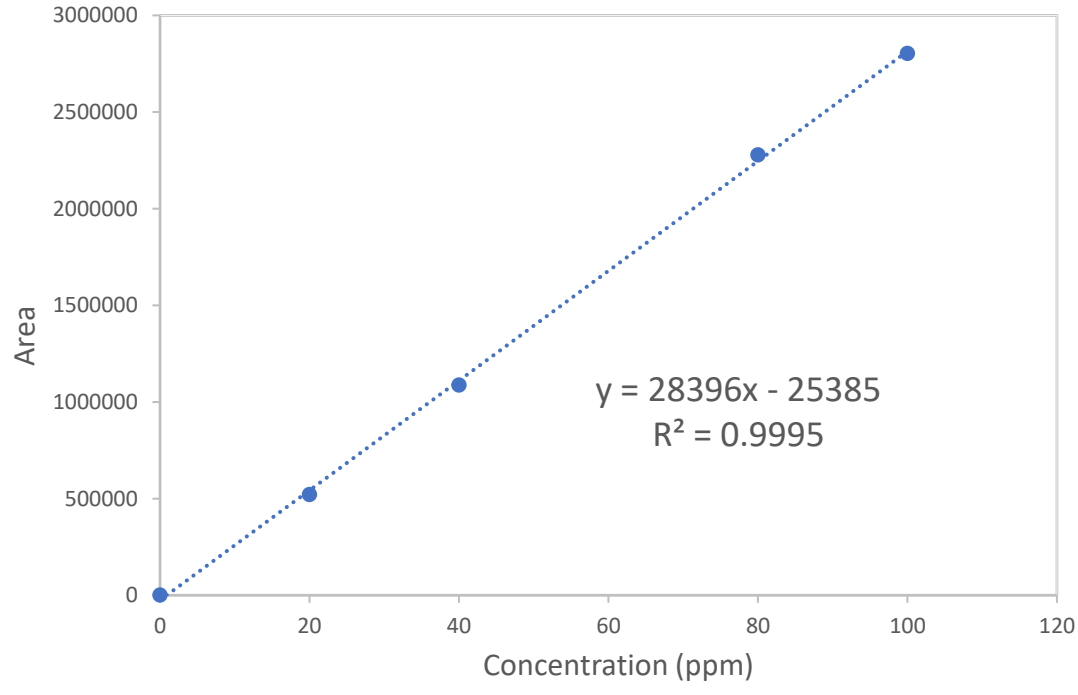
Catalyst	Surface area (m^2/g)	Pore size (nm)	Pore volume (cm^3/g)
16% Ni/Al_2O_3	259.47	8.06	0.52

Lignin monomer calibration curve (GC-MS)

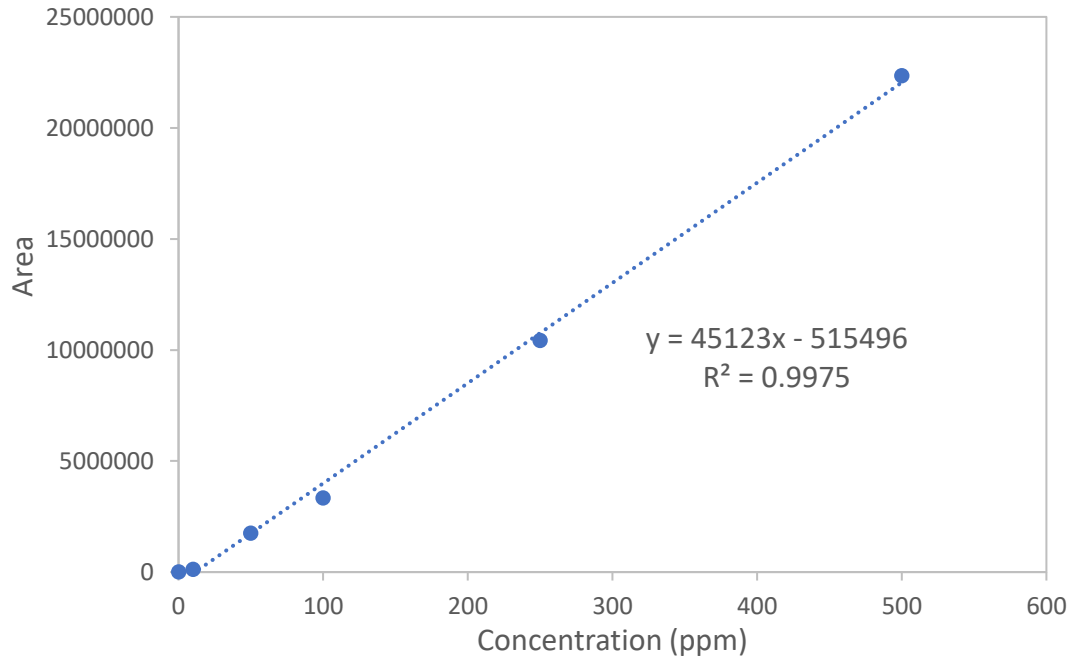
Phenol



Guaiacol



Syringol



Vanillin

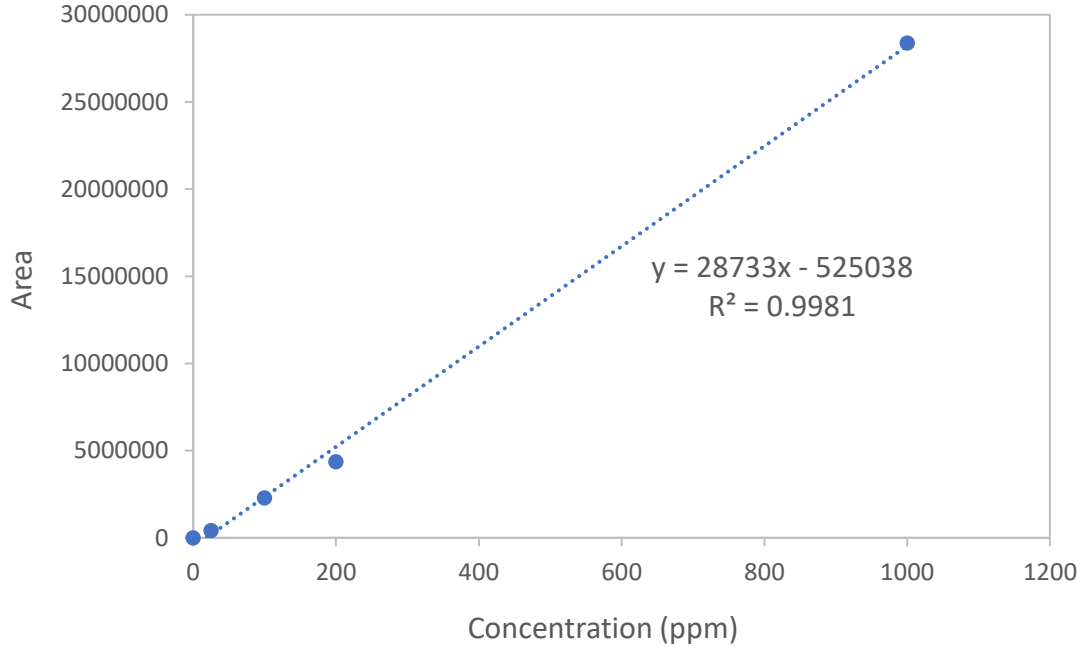


Figure A1 GC-MS calibration curve of main aromatic compound

Table A 1 Detailed phenolic monomer yields of all reaction condition

Conditions		Monomer yield (wt%)						
		Phenol	G	S	Ald-G	Ald-S	Propenyl-G	Propenyl-S
1:1	Blank	2.11	0.4	0.99	0.36	0.34	0.04	0.18
	pH3	2.71	0.55	0.78	0.26	0.24	0.06	0.23
	pH4	2.92	0.56	0.81	0.37	0.24	0.07	0.22
	pH5	1.55	0.33	0.44	0.18	0.28	ND	0.18
1:2	Blank	1.54	0.36	0.95	0.26	0.30	ND	0.40
	pH3	2.24	0.37	0.88	0.26	0.34	ND	0.31
	pH4	2.13	0.40	1.02	0.27	0.37	ND	0.43
	pH5	2.10	0.35	0.55	0.22	0.23	ND	0.27
1:3	Blank	2.06	0.38	0.61	0.25	0.18	ND	0.30
	pH3	2.43	0.46	0.78	0.21	0.28	ND	0.29
	pH4	1.98	0.44	0.96	0.26	0.24	ND	0.38
	pH5	1.64	0.24	0.51	0.20	0.27	ND	0.27

REFERENCES

1. Fernando, S., Adhikari, S., Chandrapal, C., & Murali, N. (2006). Biorefineries: Current Status, Challenges, and Future Direction. *Energy & Fuels, 20*(4), 1727-1737. doi:10.1021/ef060097w
2. Putro, J. N., Soetaredjo, F. E., Lin, S., Ju, Y., & Ismadji, S. (2016). Pretreatment and conversion of lignocellulose biomass into valuable chemicals. *RSC Advances, 6*(52), 46834-46852. doi:10.1039/c6ra09851g
3. Rinaldi, R., Woodward, R., Ferrini, P., & Rivera, H. (2018). Lignin-First Biorefining of Lignocellulose: The Impact of Process Severity on the Uniformity of Lignin Oil Composition. *Journal of the Brazilian Chemical Society*. doi:10.21577/0103-5053.20180231
4. Beckham, G. T. (2018). *Lignin valorization: Emerging approaches*. London, UK: Royal Society of Chemistry.
5. Schutyser, W., Bosch, S. V., Renders, T., Boe, T. D., Koelewijn, S., Dewaele, A., . . . Sels, B. F. (2015). Influence of bio-based solvents on the catalytic reductive fractionation of birch wood. *Green Chemistry, 17*(11), 5035-5045. doi:10.1039/c5gc01442e
6. Renders, T., Bossche, G. V. D., Vangeel, T., Aelst, K. V., & Sels, B. (2019). Reductive catalytic fractionation: state of the art of the lignin-first biorefinery. *Curr Opin Biotechnol*, *56*, 193-201. doi: 10.1016/j.copbio.2018.12.005
7. García, A., Toledano, A., Serrano, L., Egüés, I., González, M., Marín, F., & Labidi, J. (2009). Characterization of lignins obtained by selective precipitation. *Separation and Purification Technology, 68*(2), 193-198. doi:10.1016/j.seppur.2009.05.001
8. Zafar, S. (2020, January 26). *Biofuels from Lignocellulosic Biomass*. BioEnergy Consult. https://www.bioenergyconsult.com/what-is-lignocellulosic-biomass/
9. Michael, I. (2013). *Plant Biomass as a Renewable Source of Biofuels and Biochemicals* (Illustrated ed.). LAP Lambert Academic Publishing.
10. Denault, J. (2015, November 23). *Cellulosic ethanol | biofuel*. Encyclopedia Britannica. https://www.britannica.com/technology/cellulosic-ethanol

11. BD Editors. (2019, October 5). *Polysaccharide*. Biology Dictionary. https://biologydictionary.net/polysaccharide/.
12. The Editors of Encyclopaedia Britannica. (2010, September 11). *Hemicellulose | chemical compound*. Encyclopedia Britannica. https://www.britannica.com/science/hemicellulose.
13. Irmer, J. (2017, March 20). *Lignin – a natural resource with huge potential*. Bioeconomy. https://www.biooekonomie-bw.de/en/articles/dossiers/lignin-a-natural-resource-with-huge-potential
14. Liu, S., Bai, L., Muyden, A. P., Huang, Z., Cui, X., Fei, Z., & Dyson, P. J. (2019). Oxidative cleavage of β-O-4 bonds in lignin model compounds with a single-atom Co catalyst. *Green Chemistry, 21*(8), 1974-1981. doi:10.1039/c9gc00293f
15. Cao, Z., Dierks, M., Clough, M. T., Castro, I. B., & Rinaldi, R. (2018). A Convergent Approach for a Deep Converting Lignin-First Biorefinery Rendering High-Energy-Density Drop-in Fuels. *Joule, 2*(6), 1118-1133. doi:10.1016/j.joule.2018.03.012
16. Zhang, J. (2018). Catalytic transfer hydrogenolysis as an efficient route in cleavage of lignin and model compounds. *Green Energy & Environment, 3*(4), 328-334. doi:10.1016/j.gee.2018.08.001
17. *What You Need to Know About Hydrolysis*. (2019, December 2). ThoughtCo. https://www.thoughtco.com/what-is-hydrolysis-375589
18. Achinas, S., & Euverink, G. J. (2016). Consolidated briefing of biochemical ethanol production from lignocellulosic biomass. *Electronic Journal of Biotechnology, 23*, 44-53. doi:10.1016/j.ejbt.2016.07.006
19. Risanto, L., Hermiati, E., & Sudiyani, Y. (2014). Properties of Lignin from Oil Palm Empty Fruit Bunch and Its Application for Plywood Adhesive. *Makara Journal Of Technology, 18*(2), 67-75. doi:10.7454/mst.v18i2.2944
20. Yan, N., Zhao, C., Dyson, P. J., Wang, C., Liu, L. T., & Kou, Y. (2008). Selective degradation of wood lignin over noble-metal catalysts in a two-step process. *ChemSusChem*, *1*(7), 626–629. doi:10.1002/cssc.200800080

21. Klein, I., Saha, B., Abu-Omar, & M. M. (2015). Lignin depolymerization over Ni/C catalyst in methanol, a continuation: effect of substrate and catalyst loading. *Catalysis Science & Technology*, *5*(6), 3242-3245. doi: 10.1039/c5cy00490j
22. Van den Bosch, S., Renders, T., Kennis, S., Koelewijn, S. F., Van den Bossche, G., Vangeel, T., Sels, B. F. (2017). Integrating lignin valorization and bio-ethanol production: on the role of Ni-Al2O3catalyst pellets during lignin-first fractionation. *Green Chemistry, 19*(14), 3313-3326. doi:10.1039/c7gc01324h
23. Huang, Y., Duan, Y., Qiu, S., Wang, M., Ju, C., Cao, H., & Tan, T. (2018). Lignin-first biorefinery: a reusable catalyst for lignin depolymerization and application of lignin oil to jet fuel aromatics and polyurethane feedstock. *Sustainable Energy & Fuels, 2*(3), 637-647. doi:10.1039/c7se00535k
24. Liu, X., Li, H., Xiao, L.-P., Sun, R.-C., & Song, G. (2019). Chemodivergent hydrogenolysis of eucalyptus lignin with Ni@ZIF-8 catalyst. *Green Chemistry, 21*(6), 1498-1504. doi:10.1039/c8gc03511c
25. Determination of Structural Carbohydrates and Lignin in ... (n.d.). Retrieved December 9, 2020, from https://www.nrel.gov/docs/gen/fy13/42618.pdf
26. Nomanbhay, S. M., Hussain, R., & Palanisamy, K. (2013). Microwave-Assisted Alkaline Pretreatment and Microwave Assisted Enzymatic Saccharification of Oil Palm Empty Fruit Bunch Fiber for Enhanced Fermentable Sugar Yield. *Journal of Sustainable Bioenergy Systems, 03*(01), 7-17. doi:10.4236/jsbs.2013.31002
27. Yuan, T. Q., Sun, S. N., Xu, F., & Sun, R. C. (2011). Characterization of lignin structures and lignin-carbohydrate complex (LCC) linkages by quantitative 13C and 2D HSQC NMR spectroscopy. *J Agric Food Chem, 59*(19), 10604-10614. doi:10.1021/jf2031549
28. Kabir, M. M., Wang, H., Lau, K. T., & Cardona, F. (2012). Chemical treatments on plant-based natural fibre reinforced polymer composites: An overview. *Composites Part B: Engineering, 43*(7), 2883-2892. doi:10.1016/j.compositesb.2012.04.053
29. Latip, N. A., Sofian, A. H., Ali, M. F., Ismail, S. N., & Idris, D. M. N. D. (2019). Structural and morphological studies on alkaline pre-treatment of oil palm

empty fruit bunch (OPEFB) fiber for composite production. *Materials Today: Proceedings, 17*, 1105-1111. doi:10.1016/j.matpr.2019.06.529

30. Ramli, R., Yunus, R. M., Beg, M. D. H., & Prasad, D. M. R. (2011). Oil palm fiber reinforced polypropylene composites: effects of fiber loading and coupling agents on mechanical, thermal, and interfacial properties. *Journal of Composite Materials, 46*(11), 1275-1284. doi:10.1177/0021998311417647
31. Yiin, C. L., Yusup, S., Quitain, A. T., Uemura, Y., Sasaki, M., & Kida, T. (2018). Thermogravimetric analysis and kinetic modeling of low-transition-temperature mixtures pretreated oil palm empty fruit bunch for possible maximum yield of pyrolysis oil. Bioresour Technol, 255, 189-197. doi:10.1016/j.biortech.2018.01.132
32. Gao, P., Zhou, Y., Meng, F., Zhang, Y., Liu, Z., Zhang, W., & Xue, G. (2016). Preparation and characterization of hydrochar from waste eucalyptus bark by hydrothermal carbonization. *Energy,* 97, 238-245. doi:10.1016/j.energy.2015.12.123
33. Nizamuddin, S., Qureshi, S., Baloch, H., Siddiqui, M., Takkalkar, P., Mubarak, N., Dumbre, D., Griffin, G., Madapusi, S., & Tanksale, A. (2019). Microwave Hydrothermal Carbonization of Rice Straw: Optimization of Process Parameters and Upgrading of Chemical, Fuel, Structural and Thermal Properties. *Materials*, *12*(3), 403. doi.org/10.3390/ma12030403
34. Anderson, E. M., Katahira, R., Reed, M., Resch, M. G., Karp, E. M., Beckham, G. T., & Román-Leshkov, Y. (2016). Reductive Catalytic Fractionation of Corn Stover Lignin. *ACS Sustainable Chemistry & Engineering*, *4*(12), 6940–6950. doi.org/10.1021/acssuschemeng.6b01858
35. Yu, O., Yoo, C. G., Kim, C. S., & Kim, K. H. (2019). Understanding the Effects of Ethylene Glycol-Assisted Biomass Fractionation Parameters on Lignin Characteristics Using a Full Factorial Design and Computational Modeling. *ACS Omega*, *4*(14), 16103–16110. doi.org/10.1021/acsomega.9b02298

36. Laca, A., Laca, A., & Díaz, M. (2019). Hydrolysis: From cellulose and hemicellulose to simple sugars. *Second and Third Generation of Feedstocks*, 213–240. https://doi.org/10.1016/b978-0-12-815162-4.00008-

37. Vu, T., Chaffee, A., & Yarovsky, I. (2002). Investigation of Lignin-water interactions by molecular simulation. *Molecular Simulation*, *28*(10–11), 981–991. doi.org/10.1080/089270204000002610

38. Zhao, X., & Liu, D. (2012). Kinetic Modeling and Mechanisms of Acid-Catalyzed Delignification of Sugarcane Bagasse by Aqueous Acetic Acid. *BioEnergy Research*, *6*(2), 436–447. doi.org/10.1007/s12155-012-9265-4

39. Master Organic Chemistry. (2020, September 21). *Acidic cleavage of ethers (SN2)* –. https://www.masterorganicchemistry.com/reaction-guide/acidic-cleavage-of-ethers-sn2-reaction/.

40. Ashenhurst, J. (2019, December 20). *What makes a good leaving group?* Master Organic Chemistry. https://www.masterorganicchemistry.com/2011/04/12/what-makes-a-good-leaving-group/

41. Ukiwe, L. N., Nwoko, C. I., & Akalezi, C. O. The Role of pH and Acids in the Extraction and Precipitation of Lignin in Cashew (Anacardium occidentale). *African Journal of Plant Science and Biotechnology, 3*(1), 86-88.

42. Jasiukaityte-Grojzdek, E., Hus, M., Grilc, M., & Likozar, B. (2020). Acid-catalysed alpha-O-4 aryl-ether bond cleavage in methanol/(aqueous) ethanol: understanding depolymerisation of a lignin model compound during organosolv pretreatment. *Sci Rep, 10*(1), 11037. doi:10.1038/s41598-020-67787-9

43. Zhu, W., & Theliander, H. (2015). Precipitation of Lignin from Softwood Black Liquor: An Investigation of the Equilibrium and Molecular Properties of Lignin. *BioResources*, *10*(1), 1696–1714. doi.org/10.15376/biores.10.1.1696-1715

44. Klein, S. E., Rumpf, J., Kusch, P., Albach, R., Rehahn, M., Witzleben, S., & Schulze, M. (2018). Unmodified kraft lignin isolated at room temperature from aqueous solution for preparation of highly flexible transparent polyurethane coatings. *RSC Advances*, *8*(71), 40765–40777. doi.org/10.1039/c8ra08579j

45. Van den Bosch, S., Koelewijn, S.-F., Renders, T., Van den Bossche, G., Vangeel, T., Schutyser, W., & Sels, B. F. (2018). Catalytic Strategies Towards Lignin-Derived Chemicals. *Topics in Current Chemistry*, *376*(5), 36. doi.org/10.1007/s41061-018-0214-3

46. Oriez, V., Peydecastaing, J., & Pontalier, P.-Y. (2020). Lignocellulosic Biomass Mild Alkaline Fractionation and Resulting Extract Purification Processes: Conditions, Yields, and Purities. *Clean Technologies*, *2*(1), 91–115. doi.org/10.3390/cleantechnol2010007

47. Hu, J., Shen, D., Wu, S., Zhang, H., & Xiao, R. (2014). Effect of temperature on structure evolution in char from hydrothermal degradation of lignin. *Journal of Analytical and Applied Pyrolysis*, *106*, 118–124. doi.org/10.1016/j.jaap.2014.01.008

48. Shaw, A., & Zhang, X. (2019). Density functional study on the thermal stabilities of phenolic bio-oil compounds. *Fuel*, *255*, 115732. doi.org/10.1016/j.fuel.2019.115732

49. Kim, S., Chmely, S. C., Nimlos, M. R., Bomble, Y. J., Foust, T. D., Paton, R. S., & Beckham, G. T. (2011). Computational Study of Bond Dissociation Enthalpies for a Large Range of Native and Modified Lignins. *The Journal of Physical Chemistry Letters*, *2*(22), 2846–2852. doi.org/10.1021/jz201182w

50. Zhao, Y., Wang, S., Lin, H., Chen, J., & Xu, H. (2018). Influence of a Lewis acid and a Brønsted acid on the conversion of microcrystalline cellulose into 5-hydroxymethylfurfural in a single-phase reaction system of water and 1,2-

dimethoxyethane. *RSC Advances*, *8*(13), 7235–7242. doi.org/10.1039/c7ra13387a

51. Shi, Z., Jin, L., Zhou, Y., Li, Y., & Hu, H. (2017). Effect of hydrothermal treatment on structure and liquefaction behavior of Baiyinhua coal. *Fuel Processing Technology*, *167*, 648–654. doi.org/10.1016/j.fuproc.2017.08.015
52. Marianou, A. A., Michailof, C. C., Ipsakis, D., Triantafyllidis, K., & Lappas, A. A. (2019). Cellulose conversion into lactic acid over supported HPA catalysts. *Green Chemistry*, *21*(22), 6161–6178. doi.org/10.1039/c9gc02622c
53. Cui, J., Tan, J., Deng, T., Cui, X., Zhu, Y., & Li, Y. (2016). Conversion of carbohydrates to furfural via selective cleavage of the carbon–carbon bond: the cooperative effects of zeolite and solvent. *Green Chemistry*, *18*(6), 1619–1624. doi.org/10.1039/c5gc01948f
54. Bjelić, A., Grilc, M., Huš, M., & Likozar, B. (2019). Hydrogenation and hydrodeoxygenation of aromatic lignin monomers over Cu/C, Ni/C, Pd/C, Pt/C, Rh/C and Ru/C catalysts: Mechanisms, reaction micro-kinetic modelling and quantitative structure-activity relationships. *Chemical Engineering Journal*, *359*, 305–320. doi.org/10.1016/j.cej.2018.11.107
55. Raikwar, D., Majumdar, S., & Shee, D. (2019). Thermocatalytic depolymerization of kraft lignin to guaiacols using HZSM-5 in alkaline water–THF co-solvent: a realistic approach. *Green Chemistry*, *21*(14), 3864–3881. doi.org/10.1039/c9gc00593e
56. Shen, X., Meng, Q., Mei, Q., Liu, H., Yan, J., Song, J., Tan, D., Chen, B., Zhang, Z., Yang, G., & Han, B. (2020). Selective catalytic transformation of lignin with guaiacol as the only liquid product. *Chemical Science*, *11*(5), 1347–1352. doi.org/10.1039/c9sc05892c

57. Lan, X., Hensen, E. J. M., & Weber, T. (2018). Hydrodeoxygenation of guaiacol over Ni2P/SiO2–reaction mechanism and catalyst deactivation. *Applied Catalysis A: General*, *550*, 57–66. doi.org/10.1016/j.apcata.2017.10.018

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