CONVERSION OF LIGNOCELLULOSIC BIOMASS INTO ITS MOLECULAR COMPONENTS BY SEQUENTIAL COMBINATION OF ORGANIC ACID AND BASE

A Dissertation in
Energy and Mineral Engineering
by
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Submitted in Partial Fulfillment of the Requirements for the Degree of
Doctor of Philosophy

May 2012
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ABSTRACT

The primary objective of this research is to explore a new concept of converting lignocellulosic biomass into liquid organic products via hydrolysis by sequentially combining acid and base treatments. The concept was examined by studying two-step hydrolytic reactions of biomass (spruce) using oxalic acid (OA) and tetramethylammonium hydroxide (TMAH) at moderate reaction temperatures below 200 °C. Different selectivity of C-O bond cleavage of hemicellulose, cellulose, and lignin between the reactions with OA and TMAH was demonstrated, and the sequential combination of OA and TMAH treatments exhibited an enhancing effect on conversion of biomass, which proves the promise of the proposed concept. A similar enhancing effect of combination was further confirmed in the reactions with mineral acid and base.

Interestingly, characterization of solid residue from reactions of biomass and further investigation of the reactions of commercial cellulose revealed that the A-B sequence (the first reaction with OA and the second with TMAH) enhanced the conversion of cellulose at the second step with TMAH. It was suggested from the NMR and XRD study of solid residues that this enhancement was caused by the reduction of crystallinity of cellulose by the first reaction with OA. This effect was shown to be an interesting feature of A-B treatment sequence for converting lignocellulosic biomass. To improve the yield of monomeric sugars, the effect of adding organic solvents to the system was also studied. No improvement on sugar yield was observed under the explored conditions. However, it was shown that some furans and phenols can be directly formed from the reactions of biomass in the binary solvent system, which may be beneficial for producing more value-added chemicals from biomass.
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ACKNOWLEDGEMENTS

This dissertation is submitted under my name, but the whole process of conducting Ph. D. study including writing manuscripts and this dissertation was never possible without a great amount of support I received from many people. First of all, I gratefully acknowledge Prof. Chunshan Song for having me as one of his graduate students and being a great advisor and mentor throughout these years. I am thankful for his encouragement and patience. He always helped me being motivated for working hard and wisely as well. I deeply thank Prof. Yaw Yeboah for supporting and guiding towards a successful Ph. D. work as a co-advisor. He taught me what is expected for a Ph. D. holder and how to work productively. I also acknowledge Dr. Yongsheng Chen, Dr. Caroline Clifford, and Dr. Robert Rioux for serving as the committee members and giving me meaningful advice from different perspectives. Many fellow students, group members, and technical staffs assisted the actual experimental work. Dr. Ungkana Wonsiriwan was an exchanging student from Thailand in our group, and we worked together closely to start off this biomass research in the group. I am truly thankful for her effort and contribution. As for the analytical work, I would like to thank the following people: Dr. Dania A. Fonseca and Mr. Ron Wasco at the EMS Energy Institute for their kind assistance in instrumental analysis; Dr. Alan Benesi and Dr. Wenbin Luo in the Department of Chemistry for operating NMR; Dr. Maria Klimkiewicz and Dr. Nichole Wonderling at Materials Research Institute for the guidance on SEM and XRD measurements, respectively. I appreciate all the current and former group members of the Clean Fuels and Catalysis Program of the EMS Energy Institute for helpful discussions and support, as well as staffs of Department of Energy and Mineral Engineering and the EMS Energy Institute for their miscellaneous assistance. Finally, I sincerely thank my parents for their love and unconditional support since the day of my birth till today.
Chapter 1

Introduction to Technology and Chemistry of Biomass Conversion

1.1 General Introduction

1.1.1 Biomass as an Alternative Feedstock for Fuels and Chemicals

Today, the world’s primary energy is heavily dependent on fossil fuels such as petroleum, coal and natural gas. The growing demand for these non-renewable fossil fuels has been deepening the concerns on depletion of reserves. Biomass is considered as a renewable alternative energy source as well as a chemical feedstock source. Wise use of biomass can potentially contribute to substantial reduction of greenhouse gas emissions, because it captures CO$_2$ during the process of plant growth, enabling the net-zero CO$_2$ emissions ideally. The Biomass Technical Advisory Committee, a group of experts formed under the guidance of The U.S. Department of Energy, predicted that 20% of transportation fuels and 25% of chemicals could be produced from biomass by 2030.\textsuperscript{1} Total biomass use in this prediction is equivalent to 30% of current petroleum consumption.

Lignocellulosic biomass is considered as the most abundant resource of biomass, and thus processing lignocellulose into useful chemical intermediates such as sugars, furans, and phenols has attracted much attention from researchers worldwide. While ethanol can be produced from corn or sugarcane and biodiesel can be synthesized from feedstock such as soybean oil, their production is not sufficient to achieve the DOE’s goal due to the limited feedstocks.\textsuperscript{2,3}

Therefore, the importance of developing the technology for processing lignocellulosic biomass emerges. Lignocellulose is the main component of the plant cell wall, mostly consisting
of three biopolymers: cellulose, hemicellulose and lignin. Wood, for example, is typically composed of 40-50% cellulose, 20-30% hemicellulose and 25-30% lignin.\(^4\)

![Molecular structure of cellulose](image)

Figure 1-1: Molecular structure of cellulose (Klemm et al.\(^5\)).

Cellulose is a linear homopolysaccharide of D-glucopyranose with \(\beta-(1,4)\)-glycosidic linkages. For wood, a degree of polymerization, the number of this linkages in one linear chain of polymer, reaches to approximately 10,000.\(^5\) It is the main backbone of plant cell wall. Multiple layers of cellulose fibers form a stable bundle as a microfibrils, the exterior of which is associated with hemicellulose. Hemicellulose is the second most abundant polysaccharides in wood after cellulose. Unlike cellulose, hemicellulose is a complex group of amorphous polysaccharides comprised of many types of pentoses and hexoses. Spaces between individual microfibrils are considered to be filled with another biopolymer, lignin. Lignin is a crosslinked and highly-branched biopolymer of phenylpropane units. Lignin has a complex polyaromatic structure, inherently different from polysaccharides, and act as glue strengthening the cell wall structure. Yet, the precise structure and the association with cell wall are not well understood.
1.1.2 Processing Pathways of Lignocellulosic Biomass

Several promising technologies for processing lignocellulose to produce fuels and chemicals have been developed, and they are grouped into two approaches: biological and thermochemical processes. 2, 6 Table 1-1 summarizes the main approaches for lignocellulosic biomass conversion processes. 7
Table 1-1: Biomass Conversion Processes for Fuels and Some of Their Operational Parameters (modified from Behrendt et al.\textsuperscript{7})

<table>
<thead>
<tr>
<th>Processing system</th>
<th>Approach</th>
<th>Products</th>
<th>Solvent</th>
<th>Pressure</th>
<th>Temperature</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biological process</td>
<td>Fermentation</td>
<td>Ethanol</td>
<td>Yes</td>
<td>~ 1 bar</td>
<td>~ 30 °C</td>
<td>Multiple steps are necessary: pretreatment, hydrolysis, fermentation and distillation.</td>
</tr>
<tr>
<td>Gasification</td>
<td>Syn-gas (CO and H\textsubscript{2})</td>
<td>No</td>
<td>&lt; 1-20 bar</td>
<td>700-900 °C</td>
<td>Pure synthetic fuel can be produced by Fischer-Tropsch process.</td>
<td></td>
</tr>
<tr>
<td>Thermo-chemical process</td>
<td>Flash pyrolysis</td>
<td>oxygenated organic liquids (bio-oil)</td>
<td>No</td>
<td>&lt; 1-5 bar</td>
<td>&lt; 500 °C</td>
<td>Upgrading process must be developed due to instability of bio-oil.</td>
</tr>
<tr>
<td>Direct liquefaction</td>
<td>oxygenated organic liquids (bio-oil)</td>
<td>Yes</td>
<td>&lt; 1-240 bar</td>
<td>150-420 °C</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The most popular method among the biological processes is ethanol production using enzymes and microorganisms. Ethanol obtained by this method from lignocellulose is often referred to as cellulosic ethanol (differentiated from ethanol generated from starch). This ethanol production process mainly consists of four unit operations: pretreatment, hydrolysis (enzymatic or non-enzymatic), fermentation (by microorganisms), and distillation (i.e., product separation/purification)\textsuperscript{8,9}. Many biochemists and bioengineers have conducted research on the enzymatic hydrolysis processing of the carbohydrate fraction to monomeric sugars. Enzyme is a bulky molecule and one difficulty of enzymatic hydrolysis is the relatively low degree of enzyme access to the reactive sites\textsuperscript{10}. Therefore, mechanical or chemical pretreatment steps are almost inevitable to enhance the enzymatic hydrolysis. This is achieved by removing the lignin and reducing the cellulose crystallinity. Some of the pretreatment methods include steam explosion, hot water treatment, dilute acid treatment and lime treatment.\textsuperscript{9} Pretreatment is considered as one
of the most expensive processing steps in cellulosic ethanol production as well as enzymatic hydrolysis step.

The thermochemical processes can be categorized as gasification, pyrolysis and direct liquefaction.\(^7\) In gasification, biomass is partially oxidized to generate synthesis gas (syn-gas), which is a mixture of CO and H\(_2\). Fischer-Tropsch process can subsequently convert the syn-gas into pure alkanes with desired carbon chain lengths. The gasification process is presumably the most robust process that can be applied universally to any type of biomass feedstock. However, one disadvantage is the requirement of high operation temperatures (700-900 °C), resulting in relatively low process thermal efficiency (typically 16-50%) of the overall processes (gasification and F-T synthesis) to produce synthetic fuels.\(^11\) The loss of a large amount of energy originally stored in the biomass during the conversion process is inevitable in order to compensate the energy required to heat the system.

Pyrolysis and liquefaction processes are designed to directly produce more liquid products with better efficiency. Pyrolysis is the thermal decomposition of the compounds in the absence of air at typically around 500 °C, while liquefaction is operated at lower temperatures and higher pressures in the presence of solvent.\(^7\) Both processes generate liquid products, and pyrolysis-derived liquid from biomass is often referred as “bio-oil". Bio-oil is a dark brown, complex mixture of different organics, mostly oxygenated compounds.\(^12\) Bio-oil has a high polarity and acidity (pH ~2.5). It is unstable and the composition changes with time mostly due to the presence of oxygenated compounds, thus it cannot be directly used as transportation fuel or source for valuable chemicals. Therefore, feasible upgrading processes for bio-oil must be developed.\(^13, 14\)

Direct liquefaction, on the other hand, uses solvents to liquefy the biomass mostly by delignification and hydrolysis at elevated temperatures.\(^7, 15\) As mentioned previously, bio-oil of similar quality as from pyrolysis is produced. While extensive research has been conducted from
the perspective of paper pulping and wood science, the number of literature studies on direct biomasse liquefaction for fuels and chemicals production is limited. Therefore, the better fundamental understanding of direct liquefaction of biomass using solvents is still needed.

1.1.3 Chemicals from Biomass

While production of cellulosic ethanol is an important part of processing of biomass in the future, a greater variety of chemicals other than ethanol are also expected as valuable fuels/chemicals from lignocellulosic biomass. The high cost of lignocellulose processing also makes it more important to design an integrated process, a so-called biorefinery, to produce various high-value chemicals as well as fuels. Successful production of high-value chemicals from biomass would help the process become more cost competitive. Moreover, some oxygenated chemicals are highly valued in industry, which could even alleviate the requirements of deoxygenation from lignocellulose feedstock compared to the case of fuels production. The U.S. Department of Energy published reports in 2004 and 2008 that identifies the top value added chemicals from biomass feedstocks. All twelve identified building block chemicals from carbohydrates are oxygenated compounds. They include 2,5 furan dicarboxylic acid (C₆H₆O₃), levulinic acid (C₅H₈O₃), 3-hydroxybutyrolactone (C₄H₆O₃) and glycerol (C₃H₈O₃). It should be noted that ethanol was not selected among the top 30 value added chemicals. One reason for this exclusion is the smallness of the molecule (only two carbons) compared to other candidates, which requires additional process of C-C coupling to produce most of fine chemicals. Another reason is a high miscibility with water which make ethanol less attractive as a component of fuel.

Substantial achievements in producing fuels or chemicals from carbohydrates, lignin and model compounds have been made. For example, productions of possible liquid fuels such as C₇-C₁₅ liquid alkanes, dimethylfuran, and ethoxymethylfurcal from carbohydrates have been
reported. Huber et al. reported production of liquid alkanes from carbohydrates by dehydration by acid catalysts and following aldol condensation by solid base catalysts and hydrogenation.\textsuperscript{21} Mascal and co-workers showed that cellulose can be converted into 5-(chloromethyl)furfural, a stable hydrophobic organic liquid, which can be readily transformed into equivalent substance of diesel additives.

On the other hand, lignin, which has the chemically stable polyphenolic structure, can also be processed. Phenols from lignin can be used as building blocks of new synthetic bioplastics or resins.\textsuperscript{24, 25} It is also considered as a source of liquid fuel additives\textsuperscript{26}, while it is not yet commercially realized. An example of explored approaches is hydrotreating of the lignin to obtain phenols and benzenes.\textsuperscript{27, 28}

1.1.4 Need for More Efficient Hydrolysis Approach

Among different potential approaches, hydrolysis has some advantages including the relatively lower temperature requirement and the potential to tailor the product distribution.\textsuperscript{10, 17} Most of current thermochemical approaches such as gasification and pyrolysis intensively involve pyrolytic C-C bond cleavages. Although the processing system can be robust and applicable universally regardless of types of biomass, such decomposition of biomass structure would lower the selectivity of desired reactions. As a result, there are limited opportunities to tailor the products and chemistry. The products would undesirably have a broad distribution as observed in bio-oil.

Thus, it is worth considering whether extensive carbon-carbon bond cleavage is necessary to fractionate the composite of lignocellulosic biomass. The most abundant linkages in lignin structure are $\beta$-O-4 aliphatic aryl ether bonds. Besides, polysaccharides are polymerized
via glycosidic bonds. Accordingly, effective cleavage of carbon-oxygen bonds might be sufficient to liberate useful components out of lignocellulosic biomass.

In this regard, hydrolysis approaches are seemingly more flexible and favorable in the context of biorefinery and cost competitiveness, especially as the initial step for lignocellulose processing. As already mentioned above, many efforts on hydrolysis of lignocellulosic biomass have been directed toward ethanol production by pretreatment, hydrolysis of cellulose, and glucose fermentation. Nevertheless, ethanol is presumably not the most attractive target chemical from biomass (further discussed in Section 2.1) In regard to biomass refinery, only recently researchers seek other promising candidates such as 5-hydroxymethylfurfural that can be produced from carbohydrates.22

Despite these successful research activities on processing each component of lignocellulose (i.e., carbohydrates or lignin), there remains the most challenging issue to be solved: deconstructing the resistant lignocellulose structure, rather than single constituent, and effectively producing stable compounds from lignocellulose. Conversion of real biomass is more difficult compared to that of any single constituent. Reaction pathways and products may greatly vary due to the complexity of the macromolecular structure. Some salts present in biomass may also affect the reactions. Thus, the study of single constituent or model compounds alone does not necessarily explain the chemistry behind converting real biomass. This gap is the challenge that more research activities must address to, and the understanding of lignocellulose processing needs to be advanced. This research is designed to address this principal key issue.

1.1.5 Hydrolysis by Base

The most established process to hydrolyze lignocellulosic biomass can be found in the paper industry: the Kraft pulping process.29 The Kraft process selectively hydrolyzes the lignin
and hemicellulose constituents using a basic solution, leaving cellulosic fibers for paper. In this process, an aqueous solution of sodium hydroxide and sodium sulfide, known as white liquor, is reacted with wood chips at about 170 °C for several hours. Delignification is the major part of this process. The lignin macromolecule is fragmented into smaller water/alkali-soluble molecules during the process, primarily through the cleavage of α-aryl ether bonds in phenolic aryl-propane units. In kraft pulping, β-aryl ether bonds can also be effectively cleaved with the aid of sulfur species in while liquor. The mechanism proposed for the cleavage of β-aryl ether bonds in this case is illustrated in Figure 1-3. Most of the carbon-carbon linkages survive during the pulping process. In a conventional softwood kraft pulping, 95-96 % (by weight) of lignin is removed. A variety of organic solvents have also been studied for pulping applications. Similarly to kraft pulping, the cleavage of the α-aryl ether bonds primarily occurs in organosolvents pulping. Pulping with organic acids such as formic acid and acetic acid are also investigated. In acidic systems, β-aryl ether bonds are also subject to cleavage.

![Figure 1-3: Proposed mechanism of sulfidolytic cleavage of β-aryl ether bonds in phenolic arylpropane units in kraft pulping.](image)

Figure 1-3: Proposed mechanism of sulfidolytic cleavage of β-aryl ether bonds in phenolic arylpropane units in kraft pulping.
In this study, tetramethylammonium hydroxide (TMAH), is initially examined. TMAH, with the formula of (CH₃)₄NOH, is a strong basic organic solvent primarily applied in the photolithography process.³² It is also used as an additive in the study of thermochemolysis of lignin during pyrolysis GC analysis.³³, ³⁴ Clifford et al.³³ reported in their study of lignin characterization that TMAH catalyzes hydrolysis and also cause subsequent methylation of the acidic hydroxyl groups in the fragmented lignin at elevated temperatures above 300 °C. If such reactive hydroxyl groups are immediately capped with methyl groups through this mechanism, controlling the subsequent reactions after hydrolysis might be possible. This should help the detailed investigation of the base hydrolysis and the subsequent reaction paths. TMAH is highly toxic and requires precautions to handle. One way to treat the TMAH is decomposition by oxidation process.³²

### 1.1.6 Hydrolysis by Acid

Acid hydrolysis is more practiced and studied for hydrolysis of polysaccharides to liberate monomeric sugars.³ Hydrolysis of cellulose is often the focus of research among the processing for depolymerization of lignocellulose, and it is commonly known to proceed as follows.³⁵, ³⁶ Dissociated H⁺ ion attacks the β-1,4-glycosidic bonds which leads to the cleavage of the cellulose chain. The carbonium ion is formed after this cleavage, and this reacts with a water molecule to liberate H⁺. Thus, mechanistically acid catalyzes the hydrolysis and is not consumed. Figure 1-4 illustrates this proposed mechanism.
The cleavage of the β-glycosidic linkage in the cellulose structure is a critical challenge due to its high crystallinity. Because of this physical structure of cellulose, the accessibility of glycosidic bonds for water molecules and H\(^+\) ions to react with is often hindered.

On the other hand, further degradation of liberated sugars under acidic conditions also occurs to generate products such as furfural and hydroxymethylfurfural. This is not desirable for cellulosic ethanol production, which requires sufficiently isolated glucose for following fermentation step. Nevertheless, furfural is commercially manufactured by using dilute sulfuric acid.\(^9\) Hemicellulose in biomass is readily hydrolyzed to xylose and further broken down to form furfural. This presents a good example of value-added chemicals that can be produced through a well-tailored hydrolysis approach.

For cellulose depolymerization via hydrolysis, treatment with inorganic acid such as H\(_2\)SO\(_4\), HCl, or H\(_3\)PO\(_4\) with a variety of concentrations has been extensively investigated.\(^{37-42}\) Compared to enzymatic hydrolysis of cellulose, acid hydrolysis has lower theoretical glucose yields and requires higher temperature. In fact, acid hydrolysis reportedly showed lower glucose yields, mainly due to the decomposition of glucose, formation of oligomer intermediates, and so
forth. In most cases using batch reactors with mineral acids, 55-60% glucose yield have been achieved.\textsuperscript{37, 38, 41} When a bed-shrinking flow-through reactor that can compress the biomass bed as the reaction proceeds, the glucose yield as high as 90% has been claimed using extremely low sulfuric acid (0.07 wt%) at \textgreater 200 °C.\textsuperscript{39} Yet, there are critical drawbacks of these approaches such as acid recovery, corrosion, and separation.

In general, stronger acid (low pKa) can depolymerize cellulose more effectively, but more readily leads to further degradation of liberated sugars. Other than mineral acids, some organic acids are also able to hydrolyze cellulose. Especially, dicarboxylic acids are of interest because it is suggested that they might mimic the mechanism of biocatalysts such as glycosidase.\textsuperscript{33, 44} For example, Mosier et al showed that maleic acid can hydrolyze cellulose as much as dilute sulfuric acid with minimal glucose degradation under conditions of 30 min at 175 °C.\textsuperscript{45} In this study dicarboxylic acid is used and compared with mineral acid to examine if there exists any advantages of organic acid.

Oxalic acid, a simple dicarboxylic acid with the formula of C\textsubscript{2}H\textsubscript{2}O\textsubscript{4}, was chosen as an acid catalyst. This is because it is known as the strongest among organic acids. Also, it generates no inorganic salts and thus it is less likely to degrade the quality of the organic products, even if the catalyst (i.e., oxalic acid) is not completely recovered. Study of oxalic acid can be found in literature,\textsuperscript{46-48} but it is only investigated as a pretreatment of biomass for subsequent enzymatic hydrolysis. Only recently, vom Stein et al. applied oxalic acid to hydrolysis of cellulose directly, focusing on the effect of addition of salt.\textsuperscript{44} Use of oxalic acid as an acid catalyst to depolymerize polysaccharides, therefore, should be an interesting approach.

Moreover, the solubility of oxalic acid dihydrate is well dependent on temperatures (e.g., solubility values S: \textasciitilde 4 g (COOH)\textsubscript{2}/100 g water at 5 °C, \textasciitilde 31 g/100g water at 60 °C).\textsuperscript{49} Since oxalic acid dihydrate is a white powder solid at an ambient temperature, it might be worth designing a
catalyst recovery process based upon heat exchange. Finally, oxalic acid is a naturally occurring substance and known to aid in lignin and cellulose biodegradation by wood-rotting fungi.46

1.2 Objective of This Research

The principal objectives of this research are:

- To understand the effect of acidic and basic conditions on the characteristics of hydrolysis of lignocellulosic biomass
- To explore the concept of a novel method of converting lignocellulosic biomass into liquid organic products via hydrolysis by sequentially combining acid and base conditions.

Hypotheses behind this work are as follows:

1. In hydrolytic reactions of lignocellulosic biomass, pH conditions (in acidic or basic) affect the selectivity of C-O bond cleavage of hemicellulose, cellulose, and lignin. Specifically, the cleavage of β-(1,4)-glycosidic bonds in polysaccharides is preferred under acidic conditions over that of bonds in lignin, while the cleavage β-O-4 aliphatic aryl ether bonds in lignin is preferred under basic conditions.

2. The combination of acidic and basic conditions can achieve higher conversion of biomass than the case with acid or base alone.

3. Addition of organic solvents to the system helps the transfer of hydrophobic products to organic phase and thus inhibit subsequent reaction of them.
1.3 References


Chapter 2

Conversion of Spruce by Sequential Combination of Acid and Base into Its Molecular Components

2.1 Introduction

Developing effective pathways for processing lignocellulosic biomass to produce chemicals and fuels has become of great interest as a means of replacing nonrenewable feedstocks.\textsuperscript{1-5} The critical challenge in processing lignocellulosic biomass arises from two chemical and physical characteristics: the complexity and the recalcitrance of the structure comprised of cellulose, hemicellulose, and lignin. This nature of lignocellulosic biomass, consequently, requires harsh reaction conditions for processing, which often leads to the trade-off between a high conversion and good selectivity to desired products.\textsuperscript{6} On the other hand, the variety of constituents, polysaccharides and lignin, is a potential benefit since it could generate a wide range of chemical products, which makes the concept of biorefinery attractive.\textsuperscript{3}

Converting lignocellulose into its molecular components such as monomeric sugars and phenolics is important to maximize its versatility as a feedstock. While there are various approaches for converting lignocellulosic biomass as summarized in Chapter 1, hydrolysis approach is considered to have some advantages: for example, the relatively lower temperature requirement and the potential to tailor the product distribution.\textsuperscript{7-9} In this regard, hydrolysis of biomass has long been investigated. Such approaches include dilute or concentrated acid hydrolysis,\textsuperscript{10-12} alkaline hydrolysis,\textsuperscript{13} hydrolysis in hot-compressed water,\textsuperscript{14,15} and enzymatic hydrolysis.\textsuperscript{16,17} In general, hydrolysis reaction of biomass can be promoted either in acidic or basic conditions, although the use of acid has gained more attention.
No study, however, was reported regarding combining the use of acid and base in hydrolysis of biomass. Thus, this present work explored the potential of sequential combination of organic acid and base treatments at modest temperatures below 200 °C for converting spruce, a lignocellulosic biomass substrate, using two aqueous solutions: oxalic acid (OA) solution and tetramethylammonium hydroxide (TMAH) solution.

2.2 Experimental

2.2.1 Materials

White spruce, a species of lignocellulosic biomass, is chosen as a representative of softwood material. Spruce samples were provided by Mr. Lee Stover and Dr. Nicole Brown at School of Forest Resources at Pennsylvania State University. Wood chips are milled by a Wiley mill and sieved using a 40 × 20 mesh sieve. The size of the resulting wood particles is between 420-841 µm. Before reaction, samples are dried at 80 °C in a vacuum oven overnight (more than 9 hours) and stored in a desiccator. The chemicals used in the study as catalysts are oxalic acid dihydrate (99+%, Sigma-Aldrich), tetramethylammonium hydroxide solution (TMAH; 10 wt % in H₂O, Sigma-Aldrich). Hydroxylamine hydrochloride (99%, Alfa Aesar), aniline (99+%, Alfa Aesar), N,O-bis(trimethylsilyl)trifluoroacetamide with trimethylchlorosilane (99% BSTFA with 1% TMCS, Sigma-Aldrich), hexane (98.5%+, Alfa Aesar), and 2-(hydroxymethyl)phenyl-β-d-glucopyranoside (salicin; 99+%, Sigma-Aldrich) are used for derivatization of sugars in analytical procedures. Sugar standards used for identification are d(+)-xylose (>99%, Fluka), l(+)-arabinose (98%, EMD Biosciences), d(+)-glucose (99%, Alfa Aesar), d(+)-mannose (99%, Alfa Aesar), d(+)-galactose (98%, Alfa Aesar), and d(+)-cellobiose (98%, EMD Biosciences). All chemicals were used as-received without further purification.
2.2.2 Reactions

Solid sample (spruce or cellulose, 0.5 g) and 10 wt% oxalic aqueous solution or 10 wt% TMAH aqueous solution (10 g) were added into a batch micro-reactor (25 mL). The reactor was purged with nitrogen gas. The initial pressure in the reactor was atmospheric pressure at room temperature. Then the reactor was placed in a sand bath (Techne, SBL-2D) preheated at a desired temperature, and vertically shaken at a rate of 250 cycles/min. As for comparison of reactor systems, some experiments were also carried out in a glass tube batch reactors. Figure 2-1 shows these two batch reactor systems.

After the reaction, the liquid and solid products/residues were separated by filtration. The weight of solid was measured after drying in a vacuum oven at 80 °C for more than 12 h. The conversion of biomass was calculated as loss of solid sample from the initial sample weight (0.5 g). In typical experiments of two-step reactions, the second step was applied to the solid residue from the first step reaction. For the sake of consistency of the conditions, the residues were collected from several runs of reactions and accumulated to allow the second step to use the same amount of substrate (e.g., 0.5 g). The recovered solid residue was stored in a desiccator after drying. This scheme of two-step reactions is illustrated in Figure 2-2. The overall conversion by a two-step reaction experiment \( X_{\text{overall}} \) was calculated from conversions at the first and second steps, denoted as \( X_1 \) and \( X_2 \), respectively, as follows:

\[
X_{\text{overall}} = 1 - (1 - X_1)X_2
\]

(e.q.2-1)
Figure 2-1: Two types of batch-reactor systems: (a) stainless-batch reactor and (b) glass-tube reactor system.

Figure 2-2: General scheme of two-step reactions.

Typical conditions: 150 °C, 1 atm initial N₂, 2 h for each step

A: 10% Oxalic acid
B: 10% TMAH (Tetramethylammonium Hydroxide)
W: DI water

Two-step reaction: A-A, A-B, B-A, B-B

Liquid analysis
- GC-FID of oxime-TMS derivatized polysaccharide monomers

Solid analysis
- Py-GC-MS
- ¹³C-NMR
2.2.3 Analysis of Liquid Products: Gas Chromatography (GC) with Flame Ionization Detector (FID) of Derivatized samples.

For analysis of sugars by gas chromatography, liquid products were derivatized to oxime-trimethylsilyl (oxime-TMS) forms prior to the injection. The oxime-TMS derivatization technique has been recently studied and shown to be effective for analysis by gas chromatography.\textsuperscript{18-21} The derivatization method was adapted from the literature by Rojas-Escudero et al.\textsuperscript{22} A 200 µL aliquot of the aqueous solution was taken in a vial and 50 µL of internal standard solution (400 µg/mL salicin in water) was added. The solution was then dried under nitrogen flow at room temperature. For oxime formation, 50 mg of hydroxylamine hydrochloride and 1 mL of aniline were added to the vial, and it was placed in a water bath at 60 °C for 10 min. Next, 250 µL of BSTFA (N,O-bis(trimethylsilyl) trifluoroacetamide) containing 1% TMCS (trimethylchlorosilane) was added and placed under sonication at room temperature for 10 min as the silylation step. The final silylated products were extracted with 1 mL of hexane before the injection into gas chromatography. Figure 2-3 presents the transformation of glucose molecule as an example to oxime-TMS form by this derivatization method. Further details are described in Appendix B.

![Figure 2-3: Derivatization of glucose molecule into oxime-TMS form.](attachment:image)

The applied method focuses on analysis of monosaccharides, and cellobiose disaccharide can also be identified. Other dimers, trimers, and oligomers may exist in liquid products, but are not detectable by the current GC method. After derivatization, a total of 1 ml of hexane-extracted
sample was injected into GC-FID (Varian CP-3800 with VF-5ms column: 30 m × 0.25 mm capillary column, 0.25 mm thickness). The column oven temperature was set as follows: initial temperature 160 °C, held for 1 min, and increased to 172 °C at a rate of 2 °C/min, to 210 °C at a rate of 10 °C/min, to 320 °C at a rate of 30 °C, and then held for 2 min. Temperatures of injector and detector were 250 and 320 °C, respectively. The flow rate of carrier gas was set at 2.7 mL/min. These profile was based upon the literature and prior work with the instrument.

2.2.4 Characterization of Solid Products

Solid-State $^{13}$C Cross-Polarization Magic Angle Spinning Nuclear Magnetic Resonance (CPMAS NMR). The $^{13}$C cross polarization magic angle spinning (CPMAS) spectra with total suppression of spinning sidebands (TOSS) were obtained at room temperature on a Bruker AV-300 solid state NMR spectrometer operating at 75.55 MHz. A total of 2048 scans were accumulated. A rectangular contact was used with a contact time of 3 msec, and the spinning rate was 5000 Hz. The relaxation delay was 5 sec. Spinal 128 $^1$H decoupling was used during acquisition. The spectra were referenced indirectly to the aromatic $^{13}$C shift of hexamethylbenzene ($d = 132.2$ ppm).

X-ray diffraction (XRD). Powder X-ray diffraction (XRD) measurements were carried out at room temperature using an automated Scintag Pad V powder diffractometer (Cu Kα radiation: $\lambda = 1.54059$ Å) over the scanning range of $5^\circ < 2\theta < 50^\circ$ with a rate of $2.5^\circ \text{ min}^{-1}$ at 30 mA and 35 kV.

Pyrolysis-Gas Chromatography-Mass Spectrometry (Py-GC-MS). Py-GC-MS is capable of analyzing solid organic substances by thermally decomposing the sample under inert gas environment (pyrolysis) with a high heating rate (typically within a few seconds of total operation time) into small gaseous fragments, which can be introduced to GC-MS equipment. Py-GC-MS was used to characterize the chemical composition of residual solids after reactions. A small amount of sample (less than 100 µg) was placed into a quartz sample tube. The tube was then inserted to the
coil of the filament rod, where the pyrolysis was conducted. The pyrolysis unit is Pyroprobe 1000, and the GC-MS instrument specifications are HP 5890 Series II equipped with HP 5971A mass selective detector and Rxi-5 ms column (30 m × 0.25 mm, 0.25 mm thickness). Pyrolysis profile was heating at a rate of 5 °C/ms to 610 °C and holding at the final temperature for 10 s. The temperature of GC oven was programmed to rise from 35 °C to 300 °C at a rate of 4 °C/min, and held for 15 min. The temperature of injection and interface was 280 °C. Mass detection was performed at the m/z range of 40-550. Peak identification was made based on Wiley standard library as well as information from literature. Scanning Electron Microscopy.

Scanning electron microscopic images were taken using a Hitachi S-3500N instrument. Samples were covered with a thin layer of gold using a vacuum sputter-golder for better conductivity of the samples.

2.3 Results and Discussion

2.3.1 Enhancement of Conversion by Combination

Table 2-1 and Figure 2-4 summarize the conversions of spruce samples by single- and two-step reactions at 150 °C. The general trend of the conversion for the single-step reactions (water < Acid < Base) observed in this study is consistent with those in the literature. For the two-step hydrolysis reactions, it was interestingly found that the sequence of two reactions with acid and base achieved comparable or greater conversion of the biomass sample, compared with prolonged reactions with acid or base alone. Such type of study on two-step hydrolysis with acid and base treatments was not reported so far in literature.

As previously described in Section 2.2.1, the overall biomass conversion by two-steps reaction experiments is calculated as $X_{overall} = 1 - (1 - X_1)X_2$, where each $X$ represents conversion at each step. For example, if the first step and second step have both 50% of conversion, then the
overall conversion would be 75%, considering the fact that the second step was only applied for the solid residue from the first step. The overall conversion increased in the order of A-A < B-A ≈ B-B < A-B. Although B-B (run 3, 60.5%) showed slightly higher conversion than B-A (run 4, 59.6%), the difference between the two was not significant with a typical standard error range of 0.2-3.0%. These results indicate some portion of biomass can be hydrolyzed either in acidic or basic conditions to a similar degree. B-A, B-B, and A-B sequences achieved higher than 50% overall conversion. In these cases, it is clear that the treatment converted more than one of three major components of biomass (cellulose, hemicellulose, and lignin), because any of them accounts for 20-40% of the biomass composition and does not exceed 50%. However, although it is well known that cellulose is usually the most resistant to hydrolysis treatment, it is still not clear how much of each of the three major components was converted by the reactions.

Overall, A-B sequence achieved the highest conversion of biomass (run 2, 67.1%). Clearly seen from Table 2-1, the positive effect of the first treatment by acid on the next step by base is evidenced in conversions at the second step. Conversion by base after acid, A-B (run 2, 51.1%) was greater than that of the first reaction by base (run 3 or 4, 46.2%). On the other hand, conversion by acid after base, B-A (run 4, 25.0%), was lower than that of the first reaction by acid (run 1 or 2, 32.7%), resulting in a similar overall conversion (run 4, 59.6%) with that of B-B (run 3, 60.5%). This enhancement by combination in the case of A-B treatment sequence suggests the different characteristics between acid and base treatments. In order to understand how this enhancement is caused, analysis of liquid and solid products were performed and discussed in the following sections.
Table 2-1: Conversions of Biomass (Spruce) by Single- and Two-Step Reactions at 150 °C for 2 h

<table>
<thead>
<tr>
<th>Run</th>
<th>Sequence</th>
<th>Conversion (%)</th>
<th>First-step</th>
<th>Second-step</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A-A</td>
<td>32.7</td>
<td>9.9</td>
<td>39.3</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>A-B</td>
<td>32.7</td>
<td>51.1</td>
<td>67.1</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>B-B</td>
<td>46.2</td>
<td>26.5</td>
<td>60.5</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>B-A</td>
<td>46.2</td>
<td>25.0</td>
<td>59.6</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Water</td>
<td>18.5</td>
<td>-</td>
<td>18.5</td>
<td></td>
</tr>
</tbody>
</table>

Figure 2-4: Conversions of spruce by single and two-step reactions at 150 °C for 2 h.
In order to study the effect of reactor system, the same set of two-step experiments were performed in a glass-tube reactor system at the same condition of 150 °C for 2 h. Table 2-2 and Figure 2-5 present the conversion results. As a general trend, conversions were lower than the cases in a stainless reactor. This decrease was most prominent in the case of single step by TMAH, where conversion was almost half (46.2% versus 24.9%). This may be attributed to the difference of reactor design. As shown in Figure 2-1, a glass tube reactor has a long tube shape but only about an inch at the bottom of the reactor is actually in direct contact with the heater. This should be worse heat transfer to the reactants compared to the stainless-reactor case where the whole reactor is immersed in the sand bath. Despite these lower conversions, a similar enhancing effect of sequential combination was demonstrated with A-B sequence achieving the highest conversion of 50.3%. Overall conversions increase in the order of A-A < B-B < B-A < A-B.

Table 2-2: Conversions of Biomass (Spruce) by Single- and Two-Step Reactions at 150 °C for 2 h in a Glass-Tube Batch Reactor

<table>
<thead>
<tr>
<th>Run</th>
<th>Sequence</th>
<th>First-step</th>
<th>Second-step</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A-A</td>
<td>28.0</td>
<td>8.0</td>
<td>33.8</td>
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<tr>
<td>2</td>
<td>A-B</td>
<td>28.0</td>
<td>31.0</td>
<td>50.3</td>
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<tr>
<td>3</td>
<td>B-B</td>
<td>24.9</td>
<td>16.0</td>
<td>36.9</td>
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<tr>
<td>4</td>
<td>B-A</td>
<td>24.9</td>
<td>32.0</td>
<td>48.9</td>
</tr>
</tbody>
</table>
2.3.2 Analysis of Liquid Products

GC-FID analysis of derivatized liquid products identified different products from single-step reactions with acid and base as shown in Figure 2-6. Observed compounds are summarized in Table 2-3 with their retention time. From a control experiment of reaction of biomass with water, only xylose and arabinose were confirmed at retention time of 6.6 and 6.8 min, respectively. Both of these sugars are pentoses, which can only be produced from hemicellulose fraction of polysaccharides.

Figure 2-5: Conversions of spruce by single and two-step reactions at 150 °C for 2 h in a glass-tube reactor.
Figure 2-6: GC-FID chromatograms of liquid products from the single-step reactions of biomass at 150 °C for 2 h.
From the reaction with acid at 150 °C, the main products are some hexoses (galactose, mannose, glucose), hexose derivatives, and cellobiose, as well as pentoses (xylose and arabionose). While glucose is the monomeric unit of cellulose, it is also one of the monosaccharides in hemicellulose. Therefore, glucose may be liberated from either of hemicellulose or cellulose, or both. In general, however, hemicellulose is more hydrolyzable than

<table>
<thead>
<tr>
<th>Compound</th>
<th>Retention Time (min)</th>
<th>Observed in reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xylose</td>
<td>6.6</td>
<td>W, A</td>
</tr>
<tr>
<td>Arabinose</td>
<td>6.8</td>
<td>W, A</td>
</tr>
<tr>
<td>2-keto-D-gluconic acid</td>
<td>8.6</td>
<td>B</td>
</tr>
<tr>
<td>2-keto-D-gluconic acid</td>
<td>8.8</td>
<td>B</td>
</tr>
<tr>
<td>Galactose</td>
<td>10.4</td>
<td>A</td>
</tr>
<tr>
<td>Manose</td>
<td>10.5</td>
<td>A</td>
</tr>
<tr>
<td>Glucose</td>
<td>10.6</td>
<td>A</td>
</tr>
<tr>
<td>Hexose derivatives</td>
<td>13</td>
<td>A</td>
</tr>
<tr>
<td>cellobiose</td>
<td>14.2</td>
<td>A</td>
</tr>
</tbody>
</table>

Figure 2-7: Keto-gluconic acid: identified molecule in reaction of biomass with TMAH.

From the reaction with acid at 150 °C, the main products are some hexoses (galactose, mannose, glucose), hexose derivatives, and cellobiose, as well as pentoses (xylose and arabionose). While glucose is the monomeric unit of cellulose, it is also one of the monosaccharides in hemicellulose. Therefore, glucose may be liberated from either of hemicellulose or cellulose, or both. In general, however, hemicellulose is more hydrolyzable than
cellulose due to its non-crystalline nature, as confirmed in the control experiment above. A semi-quantitative analysis of glucose liberated from hemicellulose was attempted. In hemicellulose of white spruce, galactoglucomannnan is the major constituent, consisting of 17-18% of biomass. Galactoglucomannnan is a polymer consisting of galactose, mannose, and glucose. The ratio β-D-glucopyranose to β-D-mannopyranose (glucose to mannose) can be considered to be constant at 1:3. According to this information, the amount of glucose units liberated from hemicellulose is estimated as equal to 1/3 of mannose observed. After subtracting the allocation of glucose from hemicellulose, the excess glucose was assumed to be from cellulose portion. The result of this estimation was summarized in Table 2-4. Note that sugars other than glucose were all considered to be from hemicellulose, and summed up to give the total amount of sugars produced. The quantity of sugars was presented as the weight percentages on the basis of the total initial mass of biomass. In all cases tested except A-A sequence, glucose derived from hemicellulose was always greater than that from cellulose, which is reasonable since hemicellulose is known as readily hydrolysable while cellulose contains a high-crystalline structure and much more resistant to depolymerization. In clear contrast, A-A sequence (Run 3) yielded less sugars from hemicellulose (2.9%), while more from cellulose (5.6%) than the case of A. Hemicellulose appears to be depolymerized readily in the reaction with OA in a single step at 150 °C, and an additional reaction step was able to attack more cellulose fractions. Meanwhile, a slight decrease of sugars from hemicellulose when the reaction with OA at 150 °C prolonged from 1 to 2 hrs (Run 1 and 2) suggests the subsequent reaction of sugars. Increasing reaction temperature to 180 °C (Run 5) also resulted in decrease of sugars from hemicellulose, but slight increase of that from cellulose. This suggest the degradation of sugars at higher temperature, but increasing temperature also has a positive effect for relaxing the highly crystalline cellulose structure.
By reaction with TMAH, on the contrary, two peaks were observed at retention time of 8.6 and 8.8 min. According to identification by mass spectroscopy, 2-keto-D-gluconic acid (KGA) is suggested (the same identification was given to two peaks), the structure of which is shown in Figure 2-7. This compound is presumably produced from degradation of liberated sugars. To confirm the origin of KGA, the reactions of commercial standard sugars with TMAH at the same conditions were carried out. Glucose, galactose, mannose, arabinose, and xylose were examined. KGA was produced from all three tested hexoses (glucose, galactose, and mannose), but not from pentoses (arabinose and xylose). Figure 2-8 illustrates GC-FID chromatograms of the products from some of these model reactions. This clearly demonstrates that KGA is formed from hexoses liberated from biomass in the reaction with TMAH. The presence of KGA from the reaction of biomass evidences the hydrolysis of polysaccharides under basic conditions. This is also supported by another set of experiments using commercial cellulose (discussed in Chapter 3) and TMAH, where the formation of KGA was confirmed. Yet, it is not clear at this stage whether monomeric sugars are first produced and subsequently degraded, or the product was directly formed when the unit is released from the chains of hemicellulose and cellulose. While reaction involved with base (TMAH) exhibited high conversions, monomeric sugars such as glucose were not observed in final liquid products. There is no literature reporting the mechanism of

<table>
<thead>
<tr>
<th>Run</th>
<th>Experiments</th>
<th>From Hemicellulose Weight %</th>
<th>From Cellulose Weight %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A, 150 °C, 1 h</td>
<td>15.4</td>
<td>1.9</td>
</tr>
<tr>
<td>2</td>
<td>A, 150 °C, 2 h</td>
<td>13.1</td>
<td>3.4</td>
</tr>
<tr>
<td>3</td>
<td>A-A, 150 °C, 2 h</td>
<td>2.9</td>
<td>5.6</td>
</tr>
<tr>
<td>4</td>
<td>B-A, 150 °C, 2 h</td>
<td>10.0</td>
<td>3.3</td>
</tr>
<tr>
<td>5</td>
<td>A, 180 °C, 2 h</td>
<td>3.3</td>
<td>2.5</td>
</tr>
</tbody>
</table>
depolymerization of polysaccharides by TMAH. The study of TMAH on converting polysaccharides, especially cellulose, is further conducted in Chapter 3.

2.3.3 Effect of Reaction Parameters

First, the effect of pH conditions on the reaction of biomass was studied using different concentrations of OA and TMAH. The conversion and yield of the products are shown in Figure 2-8. Initial and final pH (after the reaction) for each condition are summarized in Table 2-5. As expected, higher conversion was observed at higher and lower initial pH, reflecting the hydrolysis reaction is accelerated under either acidic or basic condition. Correspondingly, yield of hexose and KGA are higher with higher concentration of OA and TMAH, respectively.

Figure 2-8: GC-FID chromatograms of derivatized product of (a) glucose standard solution and liquid products from reaction of (b) glucose solution, (c) cellulose, and (d) biomass with 10% TMAH at 150 °C for 2 h.
Concentration higher than 10% is not yet examined, and this remains as a recommended future work.

Figure 2-9: Effect of concentration of OA and TMAH on conversion of biomass and yields of the products at 150 °C for 2 h.
Figures 2-10 and 2-11 represent the effect of temperature and reaction time of reaction with OA on conversion and yield of total sugars. It is demonstrated that although the higher temperature substantially improves the conversion, it was detrimental to sugar recovery. Among the examined conditions, 150 °C achieved the highest total sugar yield, suggesting a severe degradation under acidic condition at above 180 °C. Prolonging the reaction time also showed a similar result. With extending the reaction to 8 hrs, the conversion was slightly improved, but the effect was not as high as changing the temperature. On the other hand, the sugar yield sharply dropped when the time is extended from 4 to 8 hrs. Considering the difference of sugar yield between 1-4 hrs reaction time is not significant, shorter reaction time should be further explored in order to achieve a better sugar recovery suppressing the subsequent degradation.

Table 2-5: Conversions of Biomass and pH Changes before and after the Reaction with Varying Concentration of OA and TMAH at 150 °C for 2 h

<table>
<thead>
<tr>
<th>Condition</th>
<th>Conversion (%)</th>
<th>Initial pH</th>
<th>Final pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>10% OA</td>
<td>32.7</td>
<td>1.1</td>
<td>1.3</td>
</tr>
<tr>
<td>0.1% OA</td>
<td>27.8</td>
<td>3.0</td>
<td>2.7</td>
</tr>
<tr>
<td>0.001% OA</td>
<td>18.8</td>
<td>4.5</td>
<td>4.2</td>
</tr>
<tr>
<td>DI (water)</td>
<td>18.5</td>
<td>6.8</td>
<td>4.5</td>
</tr>
<tr>
<td>0.001% TMAH</td>
<td>11.3</td>
<td>9.0</td>
<td>4.5</td>
</tr>
<tr>
<td>0.1% TMAH</td>
<td>14.9</td>
<td>12.4</td>
<td>5.2</td>
</tr>
<tr>
<td>5% TMAH</td>
<td>37.7</td>
<td>14.2</td>
<td>13.5</td>
</tr>
<tr>
<td>10% TMAH</td>
<td>46.2</td>
<td>15.0</td>
<td>14.6</td>
</tr>
</tbody>
</table>
Figure 2-10: Effect of temperature of reaction with OA on conversion of biomass and yield of total sugars at 150 °C for 2 h.
As a comparison with mineral acid and base, 2% sulfuric acid and 10% NaOH were used to conduct the same set of experiments. Table 2-6 and Figure 2-12 show the conversion of biomass with these mineral acid and base treatment. The overall conversion increases in the order of A-A < B-B ~ B-A < A-B. The same enhancement of conversion in the A-B sequence indicates the effect of sequential combination on conversion of biomass is not unique to the use of organic acid and base. Figure 2-13 illustrates GC-FID chromatograms of liquid products from biomass by a single-step with 2% sulfuric acid (SA) and 10% NaOH together with those of OA and TMAH cases. It clearly demonstrates the indifference between the organic and mineral acid and base used in the study on the characteristics of the products.
Table 2-6: Conversions of Biomass (Spruce) by Two-Step Reaction with 2% Sulfuric acid and 10% NaOH at 150 °C for 2 h

<table>
<thead>
<tr>
<th>run</th>
<th>Sequence</th>
<th>First-step</th>
<th>Second-step</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A-A</td>
<td>29.3</td>
<td>15.0</td>
<td>39.9</td>
</tr>
<tr>
<td>2</td>
<td>A-B</td>
<td>29.3</td>
<td>49.8</td>
<td>64.5</td>
</tr>
<tr>
<td>3</td>
<td>B-B</td>
<td>40.3</td>
<td>23.3</td>
<td>54.2</td>
</tr>
<tr>
<td>4</td>
<td>B-A</td>
<td>40.3</td>
<td>25.1</td>
<td>55.3</td>
</tr>
</tbody>
</table>

Figure 2-12: Conversions of spruce by single and two-step reactions with 2% sulfuric acid and 10% NaOH at 150 °C for 2 h.
Figure 2-13: GC-FID chromatograms of derivatized liquid products from biomass by single-step reactions with (A) 10% OA and 2% sulfuric acid and (B) 10% TMAH and 10% NaOH at 150 °C for 2 h.
2.3.4 Analysis of Solid Residues

In order to further understand different features of treatments, physical and chemical information of biomass solid residue were obtained. First, scanning electron microscopy was used to characterize the physical morphology of the samples. SEM images of raw spruce, spruce after acid treatment (A-A), and that after base treatment (B-B) are shown in Figure 2-14. There were no major differences in the particle size of biomass. Presumably the bulk structure of original biomass sample was preserved after these treatments. Nevertheless, the more fibrous structure was found in the sample after B-B treatment (Figure 2-14 (c)), compared to the original biomass and that after A-A treatment. Considering the fact that cellulose is the main backbone of the fiber of biomass, with surrounding hemicellulose and lignin that further cements the structure, B-B treatments appears to be able to fractionate hemicellulose and lignin from cellulose fractions. To prove this speculation, further characterization of solid residues were carried out.
Figure 2-14: Scanning Electron Microscopic images of selected biomass samples: (a) spruce, (b) after A-A treatment, and (c) after B-B treatment.
Solid residues were also analyzed by Pyrolysis (Py)-GC-MS and $^{13}$C NMR for chemical information. Interestingly, it showed significant contrasts among single- and two-step reactions that could explain the positive effect of sequential combination of acid and base. Figure 2-15 illustrates an example of Pyrolysis-GC-MS chromatogram from the single-step reaction (OA, 150 °C for 2 h). Together with the total ion chromatogram at the top, some selected ion chromatograms (GC chromatogram with a specified m/z value from Mass spectrometer) were extracted and presented in the figure. In all samples analyzed, a broad peak appeared at around the retention time of 28-31 min, 1,6-anhydro-β-D-glucopyranose, is dominant in peak area of polysaccharides, whereas various peaks of phenolic compounds from lignin with similar intensities were observed. To make a comparison between samples, peaks in the Py-GC-MS chromatogram were grouped into two categories: carbohydrates (polysaccharides) and lignin-derived phenolic compounds.

Assuming the response factors are equal among different compounds, the ratio of peak area of these two groups (denoted as P/L ratio) was calculated as semi-quantitative representative of solid residue composition. The resulting P/L ratios from these estimations are shown in Table 2-7. The P/L ratio of the original spruce biomass sample is 1.6 (run 1). This is 20-50% lower than the expected value of 2-3.2, which was derived using the information of typical biomass (40-50% cellulose, 20-30% hemicellulose and 25-30% lignin). Thus, in this analysis only the trend of P/L ratios is discussed and the absolute value was assumed not scientifically significant. In single-step reactions, P/L ratio after acid and base reaction at 150 °C for 2 h was 3.2 (run 3) and 9.9 (run 4), respectively. This evidences that base reaction effectively depolymerizes the lignin structure greater than acid, resulting in the higher P/L ratio. The same trend was observed in much more contrast for A-A (run 5, 2.9) and B-B (run 8, 42.0). Because removal of lignin in the first step can increase the exposure of crystalline cellulose fiber to the second reagent, this is presumably one factor to aid the second reaction, particularly for B-A sequence.
Figure 2-15: Pyrolysis-GC-MS chromatogram of biomass sample after the single-step acid reaction at 150 °C for 2 h, with some selected ion chromatograms.
Figure 2-16 presents $^{13}$C CPMAS NMR spectra of solid residues after two-step reactions of biomass at 150 °C for 2 h compared with the original spruce. The NMR signal assignments to wood constituents were made according to the literature. The most noticeable feature in Figure 2-16 is in A-B residue, where the lignin signals at 56, 147, and 137 ppm become much more intense, and surprisingly, the cellulose signals at 63, 74, 89, and 105 ppm almost disappear. The P/L ratio of A-B correspondingly decreased to 1.3 (run 6 in Table 2-3). This result suggests the effect of acid and base combination may arise not only from lignin removal but also physical/chemical change in cellulose structure.

Table 2-7: Polysaccharide/Lignin (P/L) Ratio of Solid Residues from Reactions of Biomass Based on the Peak Area by Pyrolysis-GC-MS Analysis

<table>
<thead>
<tr>
<th>Run</th>
<th>sample, conditions of treatment</th>
<th>P/L ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>spruce</td>
<td>1.6</td>
</tr>
<tr>
<td>2</td>
<td>W, 150 °C, 2 h</td>
<td>2.9</td>
</tr>
<tr>
<td>3</td>
<td>A, 150 °C, 2 h</td>
<td>3.2</td>
</tr>
<tr>
<td>4</td>
<td>B, 150 °C, 2 h</td>
<td>9.9</td>
</tr>
<tr>
<td>5</td>
<td>A-A, 150 °C, 2 h</td>
<td>2.9</td>
</tr>
<tr>
<td>6</td>
<td>A-B, 150 °C, 2 h</td>
<td>1.3</td>
</tr>
<tr>
<td>7</td>
<td>B-A, 150 °C, 2 h</td>
<td>30.9</td>
</tr>
<tr>
<td>8</td>
<td>B-B, 150 °C, 2 h</td>
<td>42.0</td>
</tr>
<tr>
<td>9</td>
<td>W, 215 °C, 1 h</td>
<td>3.8</td>
</tr>
<tr>
<td>10</td>
<td>A, 215 °C, 1 h</td>
<td>0.9</td>
</tr>
<tr>
<td>11</td>
<td>B, 215 °C, 1 h</td>
<td>24.2</td>
</tr>
</tbody>
</table>
2.3.5 Analysis of Gaseous Products from the Reaction with OA

The gas evolution was only observed in the reaction with OA. After the reaction, the pressure was around 200 psi at room temperature. By measuring the weight of the reactor before and after the gas release, the gas product is estimated at around 0.2-0.3 g. The control reaction of OA solution at the same condition also generated a comparable amount of gaseous products, which is indicative of the decomposition of oxalic acid at this temperature. Table 2-8 shows the composition of the gaseous products from the reactions with and without biomass. It was confirmed that CO and CO₂ were produced by the decomposition of OA, regardless of the presence of biomass in the reaction system. Additional gas evolution from the biomass is
indicated by the higher concentration of CO and CO$_2$ as well as lower concentration of N$_2$ compared to the control case. As for decomposition of OA, Kakumoto et al. reported that two main decomposition pathways of OA were CO$_2$ + HCOOH and CO$_2$ + CO + H$_2$O. Interestingly, however, hydrogen was confirmed in the present work, which might be another product from OA. If produced, hydrogen might also be involved in the reaction of biomass under the present hydrolytic conditions, which is interesting to be explored. Lower temperature and shorter reaction time can be suggested for an effort to minimize such undesired decomposition of OA.

Table 2-8: Composition of Gaseous Products from the Reaction of Oxalic Acid Solution at 150 °C for 2 h

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Conc. (mole %)</th>
<th>Without biomass</th>
<th>With biomass</th>
</tr>
</thead>
<tbody>
<tr>
<td>H$_2$</td>
<td>8.7</td>
<td>6.0</td>
<td></td>
</tr>
<tr>
<td>O$_2$</td>
<td>2.9</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>N$_2$</td>
<td>26.5</td>
<td>13.0</td>
<td></td>
</tr>
<tr>
<td>CO</td>
<td>28.0</td>
<td>35.0</td>
<td></td>
</tr>
<tr>
<td>CO$_2$</td>
<td>33.5</td>
<td>44.8</td>
<td></td>
</tr>
</tbody>
</table>

2.4 Conclusions

In this chapter, the enhancing effect of sequential combination of organic acid (oxalic acid, OA) and base (TMAH) was studied. First, by analyzing the liquid products of single-step reactions with acid and base, it was shown that reactions with OA and TMAH can both promote depolymerization of polysaccharides, but generating two different products, glucose from that with OA and keto-gluconic acid from that with TMAH. From two-step reaction experiments,
sequence of A-B, the reaction with acid followed by that with base, exhibited the highest conversion (67.1%) and the positive effect was clearly confirmed from the enhancement of the conversion in the second step. This verifies the hypothesis 2. Pyrolysis-GC-MS and $^{13}$C CPMAS NMR of solid residues clearly demonstrated that reaction with TMAH more effectively removed lignin fraction compared to that with OA, which proved the hypothesis 1. Surprisingly, however, these analyses also revealed that the sample after A-B sequence contains much less cellulose. Removal of lignin by base treatment did not fully explain the mechanism of positive effect by the sequential combination, which is beyond the initial hypotheses. This unexpected implication leads us to further continue the work on reactions of cellulose.

2.5 References


Chapter 3

Conversion of Cellulose by Sequential Combination of Acid and Base

3.1 Introduction

Depolymerization of cellulose is a critical step of converting lignocellulose. Hydrolysis of cellulose to produce glucose has been extensively studied using enzymes,\textsuperscript{1,2} supercritical water,\textsuperscript{3,4} and dilute acids.\textsuperscript{1,5} Nevertheless, report on combination of acid and base conditions for hydrolysis of cellulose could not be found with a extensive literature survey. The objective of this present work is to examine the benefit of combination for converting cellulose, and also clarify the mechanism of the positive effect by the combination observed in reactions of biomass discussed in Chapter 2.

For hydrolysis of biomass, while inorganic acids (e.g., H\textsubscript{2}SO\textsubscript{4}, HCl, H\textsubscript{3}PO\textsubscript{4}) and enzymes have been extensively explored, organic dicarboxylic acids arise as an interesting candidate. In fact, oxalic,\textsuperscript{6-8} maleic,\textsuperscript{9-11} and fumaric acids\textsuperscript{9,10} have been recently recognized as effective in pretreatment for fermentation of lignocellulose, primarily by removing hemicellulose. OA, among the strongest organic acids, is thought to play some role in biological wood decays by serving as a proton donor.\textsuperscript{12} This supports the choice of OA in this study. Use of base is also expected to affect the hydrolysis of cellulose, since basic conditions is applied for delignification in Kraft pulping process of paper industry and known to attack polysaccharides as well.\textsuperscript{13}

With two-step experiments of biomass as shown in Chapter 2, it was interestingly revealed that sequential combination of OA and TMAH effectively converts the biomass sample greater than the use of acid or base alone. Main products were monomeric sugars and their derivatives. It was revealed that OA provides proton to hydrolyze mainly polysaccharides (hemicellulose and cellulose) but less portion of lignin, while TMAH can effectively promote
cleavage of β-O-4 aliphatic aryl ether bonds in lignin as well as polysaccharides. Among different combinations, A-B sequence achieved the highest conversion. Analysis of solid residues suggested, in the case of A-B, the first step with OA enhanced the conversion of cellulose fractions in the biomass at the second step with TMAH. Although this effect may be one of the reasons for the positive effect of the sequential combination, the mechanism behind this effect was not fully understood. Hence this present study attempts to elucidate the cause of this positive effect particularly in A-B sequence on cellulose.

### 3.2 Experimental

#### 3.2.1 Materials

Microcrystalline cellulose was purchased from Alfa Aesar. The chemicals used in this experiment are oxalic acid dehydrate (99+%, Sigma-Aldrich), tetramethylammonium hydroxide solution (TMAH; 10 wt. % in H₂O, Sigma-Aldrich), hydroxylamine hydrochloride (99%, Alfa Aesar), phenylbenzene (aniline; 99+%, Alfa Aesar), N,O-Bis(trimethylsilyl)trifluoroacetamide with trimethylchlorosilane (99% BSTFA with 1% TMCS, Sigma-Aldrich), hexane (98.5%+, Alfa Aesar), 2-(Hydroxymethyl)phenyl-β-D-glucopyranoside (salicin; 99+%, Sigma-Aldrich), D(+)-glucose(99%, Alfa Aesar), and D(+)–xylose (>99%, Fluka). All the chemicals were used without further purification.

#### 3.2.2 Reactions

A stainless-steel 25 ml horizontal batch micro-reactor was used to carry out the reactions. 0.5 g of cellulose and 10 wt% OA aqueous solution or 10 wt% TMAH aqueous solution (10 g) were added into a reactor, and the reactor was purged with nitrogen gas. The initial pressure in
the reactor was atmospheric pressure at room temperature. Then the reactor was placed in a preheated fluidized bed sand bath (Techne, SBL-2D), and vertically agitated at a rate of 250 cycles/minute during the reaction.

After the reaction, the liquid and solid products/residues were separated by filtration. The weight of solid was measured after drying in a vacuum oven at 80 °C for more than 12 h. Conversion of substrate was calculated as loss of solid from the initial weight.

Sequential combination of acid and base was studied by two-step reactions. Solid residues from several runs of the first step were accumulated. Then the second step reaction was performed using 0.5 g of these solids and 10 g of a fresh reagent of acid or base, which makes the concentration of substrate and reagent consistent over the two steps. In this study, a reaction with water, acid, or base are denoted as W, A, or B, respectively. Also, a combination of them is used to represent the second step of two-step reactions; for instance, A-B represents the base reaction in the second step that is carried out using the residue from the acid reaction in the first step.

3.2.3 Analysis of Liquid Products: Gas Chromatography (GC) with Flame Ionization Detector (FID) of Derivatized samples.

Analysis of liquid products was performed using GC-FID (Varian CP-3800 with VF-5ms column: 30 m × 0.25 mm capillary column, 0.25 mm thickness) after derivatization. The derivatization method was adapted from literature, and the exact procedures as well as detailed GC conditions are described in Chapter 2.

3.2.4 Characterization of Solid Products

The techniques and conditions which were the same as those in the study of biomass in Chapter 2 are only briefly described below. Solid-State $^{13}$C Cross-Polarization
Magic Angle Spinning Nuclear Magnetic Resonance (CPMAS NMR). The $^{13}$C cross polarization magic angle spinning (CPMAS) spectra with total suppression of spinning sidebands (TOSS) were obtained at room temperature on a Bruker AV-300 solid state NMR spectrometer operating at 75.55 MHz. 256-8224 scans were accumulated depending upon the samples.

X-ray diffraction (XRD). Powder X-ray diffraction (XRD) measurements were carried out at room temperature using an automated Scintag Pad V powder diffractometer (Cu Kα radiation: $\lambda = 1.54059$ Å) over the scanning range of $5^\circ < 2\theta < 50^\circ$ with a rate of $2.5^\circ \text{min}^{-1}$ at 30 mA and 35 kV.

Pyrolysis-Gas Chromatography-Mass Spectrometry (Py- GC-MS). Py-GC-MS was also used to characterize the chemical composition of residual solids after reactions. The pyrolysis unit is Pyroprobe 1000, and the GC-MS instrument specifications are HP 5890 Series II equipped with HP 5971A mass selective detector and Rxi-5 ms column (30 m $\times$ 0.25 mm, 0.25 mm thickness). Pyrolysis profile was the same as that in Chapter 2.

Scanning Electron Microscopy. Scanning electron microscopic images were taken using a Hitachi S-3500N instrument. Samples were covered with a thin layer of gold using a vacuum sputter-golder for better conductivity of the samples.

Elemental Analysis. Elemental analysis (C, H, and N) was performed by LECO Tru Spec CHN analyzer.

3.3 Results and Discussion

3.3.1 Conversion of Cellulose

Single- and two-step reactions of cellulose were carried out first to verify the positive effect of combination on conversions. Table 3-1 and Figure 3-1 summarize conversions of two-step reactions; a reaction condition for each step was at 150 °C for 2 h. In the first-step reactions, the conversions increased in the order of water (3.8%) < Acid (29.1%) < Base (58.1%). Both 10% OA and 10% TMAH were shown to promote depolymerization of cellulose under our hydrolysis
conditions. TMAH was more effective than OA in converting cellulose. Further, overall conversions after two-step reactions converted cellulose in the order of A-A (36.5%) < B-B (61.9%) < B-A (68.3%) < A-B (73.0%). Interestingly, the greater conversions were achieved by combining acid and base (Sequences B-A and A-B) than the case of prolonged reaction with acid or base alone. Control reactions without acid or base (denoted as W) were conducted as the first step (Run 5-7 in Table 1). None of these exhibited a conversion as high as B-A or A-B. Another control reaction was examined in a single step reaction with a 1:1 weight-ratio mixture of 10% OA and 10% TMAH (pH 3~4), which resulted in a low conversion (7.1%; Run 8 in Table 1). These results confirm that the enhancing effect can be only achieved when acid and base is sequentially combined, not by combination with water or a mere combination of acid and base in a single step.

We also carried out the two-step reactions using mineral acid and base for comparison. 2% sulfuric acid and 10% sodium hydroxide were used to make the same initial pH conditions (pH~0.7 and ~14, respectively). Table 3-2 presents the conversion of cellulose. Whereas the overall conversions were higher by 4-10%, the same trend was observed: A-A (41.4%) << B-B (71.8%) < B-A (76.0%) ~ A-B (77.2%). Similar to the case with OA and TMAH, sequential combination achieved higher conversion than the reaction with acid or base alone, indicating the enhancing effect is not unique to the use of organic acid and base. However, the margin between B-A and A-B is even smaller and not very significant in this case compared to that with OA and TMAH.

This general trend of conversions by two-step reactions with OA and TMAH is similar to the results for biomass (spruce) in Chapter 2. In hydrolysis of lignocellulose by the sequential combination, the enhancing effect was due to several factors at the first step, including the removal of hemicellulose by the reaction with either OA or TMAH and the removal of lignin by that with TMAH. Consequently, the chemical and physical structure of the residue after the first step became more susceptible to the following treatments. On the other hand, in hydrolysis of pure
cellulose, such factors are no longer relevant and there should be a different mechanism that makes the combination effective. Such an effect on the structure of cellulose might also be responsible for the enhancing effect by the sequential combination of OA and TMAH on biomass. Further clarification of the mechanism of such effect is attempted by the following analyses of liquid products and solid residues.

Table 3-1: Conversions of Cellulose by Two-Step Reaction with 10% Oxalic Acid and 10% TMAH at 150 °C for 2 h

<table>
<thead>
<tr>
<th>run</th>
<th>Sequence</th>
<th>First-step</th>
<th>Second-step</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A-A</td>
<td>29.1</td>
<td>10.4</td>
<td>36.5</td>
</tr>
<tr>
<td>2</td>
<td>A-B</td>
<td>29.1</td>
<td>61.9</td>
<td>73.0</td>
</tr>
<tr>
<td>3</td>
<td>B-B</td>
<td>58.1</td>
<td>9.2</td>
<td>61.9</td>
</tr>
<tr>
<td>4</td>
<td>B-A</td>
<td>58.1</td>
<td>24.4</td>
<td>68.3</td>
</tr>
<tr>
<td>5</td>
<td>W-W</td>
<td>3.8</td>
<td>6.1</td>
<td>9.7</td>
</tr>
<tr>
<td>6</td>
<td>W-A</td>
<td>3.8</td>
<td>23.2</td>
<td>26.1</td>
</tr>
<tr>
<td>7</td>
<td>W-B</td>
<td>3.8</td>
<td>55.7</td>
<td>57.4</td>
</tr>
<tr>
<td>8</td>
<td>(A+B)*</td>
<td>7.1</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*1:1 mixture of OA and TMAH
Figure 3-1: Conversions of cellulose by two-step reactions with 10% OA (A) and 10% TMAH (B) at 150 °C for 2 h.

Table 3-2: Conversions of Cellulose by Two-Step Reaction with 2% Sulfuric acid (A) and 10% NaOH (B) at 150 °C for 2 h

<table>
<thead>
<tr>
<th>run</th>
<th>Sequence</th>
<th>First-step</th>
<th>Second-step</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A-A</td>
<td>22.1</td>
<td>24.7</td>
<td>41.4</td>
</tr>
<tr>
<td>2</td>
<td>A-B</td>
<td>22.1</td>
<td>70.8</td>
<td>77.2</td>
</tr>
<tr>
<td>3</td>
<td>B-B</td>
<td>61.9</td>
<td>25.9</td>
<td>71.8</td>
</tr>
<tr>
<td>4</td>
<td>B-A</td>
<td>61.9</td>
<td>36.8</td>
<td>76.0</td>
</tr>
</tbody>
</table>
3.3.2 Glucose and Its Derivatives in Liquid Products

The liquid product was analyzed by GC-FID after derivatization. As mentioned earlier, the derivatization method in this study was optimized for identification of monosaccharides. Most of oligosaccharides are not detected by the current method. Figures 3-2 and 3-3 illustrate GC-FID chromatograms of liquid products from cellulose by a single-step and two-step reactions, respectively.

Reaction with water without OA or TMAH was performed as a benchmark experiment, where only the peak of the internal standard was observed (RT: 13.5-13.6 min). Interestingly, as shown in Figure 3-2, distinct differences in products from acid and base reactions were found. Glucose (RT: 10.6 and 10.8 min) was identified in the products from acid reaction. Glucose gives two peaks of α, β-anomers with this derivatization method. Base reaction did not generate glucose, but 2-keto-D-gluconic acid (KGA, RT: 8.7 and 8.8 min), which was identified using GC-MS. Both glucose and 2-keto-D-gluconic acid were identified as their oxime-trimethylsilyl forms due to analytical derivatization. Several peaks in the chromatogram of acid reaction (RT: 12.9-13.1 min) are considered to be the hexose-derived compounds formed during the analytical derivatization procedure, not during the hydrolysis reaction itself, since these peaks were also confirmed from the analysis of standard monomeric sugar solutions.
As described in Chapter 2, in acid reaction of biomass (spruce) using OA, several pentoses, hexoses, and cellobiose were generated. From semi-quantitative analysis of ratio of pentose and hexose in the product, it was concluded that, for carbohydrates, OA mainly depolymerizes hemicellulose, but some hydrolysis of cellulose fraction also occurs. Reaction of cellulose in this study further demonstrated that OA depolymerizes cellulose at 150 °C, even though a low conversion (29.1%) indicates that a complete hydrolysis is difficult due to high crystallinity. This result reflects the fact that OA is weaker than typical mineral acids, which may be a challenge for efficient hydrolysis with organic acid. On the other hand, vom Stein and co-workers reported the addition of salt (NaCl) enhanced hydrolysis of cellulose with dicarboxylic acids.\textsuperscript{15} In addition, partial decomposition of OA at the current condition was confirmed from the generation of some gaseous products, mostly CO and CO\textsubscript{2}. This was observed in the previous work of biomass as
shown in Chapter 2. The blank test of OA solution alone at 150 °C produced the gas in the same order of magnitude as those from the reactions of biomass or cellulose. For better use of OA and possible recovery, lowering temperature below 150 °C should be investigated in our future study.

Figure 3-3: GC-FID chromatograms of derivatized liquid products from microcrystalline cellulose by single-step and two-step reactions with (A) 10% OA and (B) 10% TMAH at 150 °C for 2 h.
In the reaction with TMAH, 2-keto-D-gluconic acid (KGA) (RT: 8.7 and 8.8 min) was identified, as the same product from biomass reaction. From an additional experiment of glucose standard reaction with TMAH (0.5 g of sugar and 10 g of 10% TMAH aqueous solution at 150 °C for 2 hrs; the chromatogram not shown), it was confirmed that KGA is formed from glucose, which suggests a subsequent reaction of glucose in basic condition. Hydrolysis in alkaline solution is generally known to exhibit a high reaction rate, but monomeric sugar is severely attacked by alkalis even at temperatures below 100 °C (e.g., with 18% NaOH).\textsuperscript{16} Similar degradation process presumably occurs in the reaction with TMAH. Although KGA is considered to be the product of glucose degradation, a potential value may be generated, since gluconic acid can be produced by hydrogenation. It is worth noting that gluconic acid is one of the top 30 value-added chemicals from sugars.\textsuperscript{17}

Yield and selectivity of glucose for different sequences are summarized in Table 3-3. The yields at the first and second step are calculated based upon the initial amount of the reactant at each step. Overall yields are defined as follows:

$$Y_{overall} = Y_1 + Y_2(1 - X_1)$$

In the equation, X and Y represent conversion and yield, respectively. This overall yield is a hypothetical value in a case such that the second step was carried out using the exact amount of the solid residue from one run of experiment of the first step (e.g., 1-X1). As described earlier, the actual experiments at the second step was conducted using the solid samples (0.5 g) accumulated from several runs of the first step reactions in order to minimize the experimental errors.
Table 3-3: Glucose Yield and KGA Yield from Cellulose by Two-Step Reaction with 10% OA and 10% TMAH at 150 °C for 2 h

<table>
<thead>
<tr>
<th>run</th>
<th>Sequence</th>
<th>Glucose yield (%)</th>
<th></th>
<th>Selectivity (%)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>First-step</td>
<td>Second-step</td>
<td>Overall</td>
<td>First-step</td>
</tr>
<tr>
<td>1</td>
<td>A-A</td>
<td>6.2</td>
<td>8.0</td>
<td>11.9</td>
<td>21.4</td>
</tr>
<tr>
<td>2</td>
<td>A-B</td>
<td>6.2</td>
<td>-</td>
<td>6.2</td>
<td>21.4</td>
</tr>
<tr>
<td>3</td>
<td>B-B</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>B-A</td>
<td>-</td>
<td>17.4</td>
<td>7.3</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>run</th>
<th>Sequence</th>
<th>KGA yield (%)</th>
<th></th>
<th>Selectivity (%)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>First-step</td>
<td>Second-step</td>
<td>Overall</td>
<td>First-step</td>
</tr>
<tr>
<td>1</td>
<td>A-A</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>A-B</td>
<td>-</td>
<td>13.7</td>
<td>9.7</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>B-B</td>
<td>11.8</td>
<td>0.4</td>
<td>12.0</td>
<td>20.3</td>
</tr>
<tr>
<td>4</td>
<td>B-A</td>
<td>11.8</td>
<td>-</td>
<td>11.8</td>
<td>20.3</td>
</tr>
</tbody>
</table>

Glucose yields for A, A-A, and B-A reactions were determined as 6.2%, 8.0%, and 17.4%, respectively. GC-FID chromatograms for these three are presented in Figure 3-3-A. Yields at the second step (both A-A and B-A) are higher than the first step with OA. These increased yields at the second steps were interestingly in contrast to their lower conversions: 10.4% (A-A) and 24.4% (B-A) against 29.1% (A) as shown in Table 3-1. Higher selectivity also reflects this contrast. This might be explained by the varied degree of crystallinity or polymerization of microcrystalline cellulose sample. Relatively unstable amorphous region should be readily depolymerized during the first step compared to more rigid high-crystalline region. At the same time, the first treatment either by OA or TMAH presumably partially loosens the structure of remaining solid to help the second reaction. These could have negative and positive effect on conversion at the second step, respectively. The enhanced yields by A-A and B-A suggest the change in the structure of solid
sample by the first step with OA and TMAH, which will be further discussed in the later section of solid analysis. The sequence A-A exhibited the highest overall glucose yield (11.9%), obviously due to the fact that any base-involved sequence generated KGA instead of glucose. Low selectivity of glucose at the first step (21.4%) strongly suggests the presence of oligomers and sugar derivatives in the liquid products. Oligomers present in solution can be further hydrolyzed in acid to glucose, but the subsequent degradation of monomeric sugar also occurs. Experimental parameters and reaction medium system must be further explored to maximize the glucose yield in a single-step. In contrast, however, the selectivity significantly improved to >70% at the second step in both A-A and B-A sequences. The reason behind this improvement is currently under investigation and not clear yet, but could possibly be attributed to the faster rate of glucose production at the second step than that at the first step.

Yield and selectivity of KGA for B, B-B, and A-B were obtained in the same way as glucose (Table 3-3). Figure 3-3-B shows GC-FID chromatograms. KGA from B-B sequence was below 5% of that from the first step with B, which is reasonably consistent to the low conversion of 9.2% (run 3 in Table 3-1). In the A-B sequence, the production of KGA at the second step was about 15% greater than that from the single reaction with TMAH. This improvement is similar in trend to the glucose yield from A and B-A, also supporting the effect of sequential combination. Low selectivity of KGA at the first step (20.3%), which is comparable with the selectivity of glucose in the case with OA, indicates that there are also oligomers and other types of sugar derivatives in the liquid products after reactions with TMAH.
Figure 3-4: GC-FID chromatograms of derivatized liquid products from microcrystalline cellulose by single-step and two-step reactions with (A) 10% OA and 2% sulfuric acid and (B) 10% TMAH and 10% NaOH at 150 °C for 2 h.
Table 3-4: Glucose Yield and KGA Yield from Cellulose by Two-Step Reaction with 2% Sulfuric Acid and 10% NaOH at 150 °C for 2 h

<table>
<thead>
<tr>
<th>run</th>
<th>Sequence</th>
<th>Glucose yield (%)</th>
<th>KGA yield (%)</th>
<th>Selectivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>First-step</td>
<td>Second-step</td>
<td>Overall</td>
</tr>
<tr>
<td>1</td>
<td>A-A</td>
<td>4.1</td>
<td>2.5</td>
<td>6.0</td>
</tr>
<tr>
<td>2</td>
<td>A-B</td>
<td>4.1</td>
<td>-</td>
<td>4.1</td>
</tr>
<tr>
<td>3</td>
<td>B-B</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>B-A</td>
<td>-</td>
<td>5.2</td>
<td>2.0</td>
</tr>
</tbody>
</table>

As a comparison with mineral acid and base, GC-FID chromatograms of liquid products from cellulose by a single-step with sulfuric acid (SA) and NaOH were illustrated in Figure 3-4, together with those of OA and TMAH cases. First, the similar nature of hydrolysis was indicated by the result that glucose and KGA were the identified products from reactions with SA and NaOH, respectively. Table 3-4 summarizes yield and selectivity of glucose and KGA in two-step reactions. In reactions with acid, glucose yield was always higher for those with OA by 2-12%. This trend is consistent with the reported result by Lee and Jeffries\textsuperscript{18}, where the reaction of corncob with dicarboxylic acid (maleic and oxalic acid) at 170 °C for 18 min produced greater amount of xylose and glucose than that with sulfuric acid at the same initial pH value. In addition, the selectivity did not improve at the second step unlike the OA case. These differences might imply the potential benefit of use of organic acid. Nevertheless, glucose yields by either OA or SA at the single step reaction are less than 10%. In literature, as mentioned in Chapter 1, 55-60% glucose
yield is reported in case of batch reactors. Since studying the effect of combination is the focus of the work, optimization of the reaction conditions for glucose yield still remains to be achieved. Although in reactions of biomass, 1 h and 2 h did not show much significant difference as shown in Figure 2-11. Shorter reaction times less than 60 min should be considered for future work of both biomass and cellulose.

In reactions with base, at the first step, yield of KGA by the reaction with NaOH was comparable with the TMAH case. At the second step, only 0.9% of yield was obtained in A-B sequence, which is much lower than the TMAH case (13.7% in A-B). Considering the fact of comparable KGA yields at the first step, 11.8% with TMAH and 11.4% with NaOH, the cause of this result might be related to unknown different feature of OA and SA treatment at the first step.

Through the product analysis above, it was shown that both OA and TMAH promote cleavage of β-(1,4)-glycosidic linkages in cellulose to liberate monomeric sugar unit, but to a different degree, producing different compounds. The subsequent study was directed to elucidate what makes the sequential combination of acid and base effective in conversion of cellulose and biomass.

### 3.3.3 Effect of the Treatment with Water, Acid, or Base on the Structure of Cellulose

In order to clarify the cause of the enhancing effect in sequential combination, structures of cellulose after treatments with OA and TMAH were studied. In this section, simple denotation of A and B are used to describe the treatment with 10% OA and 10% TMAH at 150 °C for 2 h, respectively. First, scanning electron microscopy was used to characterize the morphology of the samples after treatments. Figure 3-5 shows SEM images of raw cellulose, cellulose after acid treatment (A-A), and that after base treatment (B-B). No significant change in the particle size or major features of the particles were seen, indicating the A or B treatment depolymerize and
solubilize the cellulose from the exposed surfaces and did not completely destroy the structure in the bulk. Yet, it is worth noting that the sample after B-B treatment (Figure 3-5 (c)) appears to have relatively more fibrous structure. This might be interpreted, together with the result of higher conversion by the treatment with TMAH than that with OA, that this treatment converted most of the less-ordered amorphous regions and thus the remaining residue consists of more highly-ordered region. This hypothesis will be further addressed in the discussion with the results of NMR and XRD study. In addition, Energy dispersive X-ray spectroscopy was conducted for some selected samples to obtain elemental information of the surface, which revealed an issue of corrosion of the stainless reactor in case of the treatment with OA. This issue was addressed in Appendix C.
Figure 3-6 shows $^{13}$C CPMAS NMR spectra of cellulose samples after treatments.

Assignments of peaks in $^{13}$C CPMAS NMR spectra are made according to the literature.$^{19-22}$

Carbon positions in the unit structure of cellulose are shown in Figure 3-7. In the NMR spectra of
cellulose, the first region of resonances around 60-70 ppm is contribution from C6 of the primary alcohol group. The base resonances between 70 and 81 ppm can be attributed to C2, C3, and C5 ring carbons that do not anchor the glycosidic linkages. That of 80-93 ppm originates from C4, and 102-108 ppm from C1, the anomeric carbon.
Figure 3-6: $^{13}$C CPMAS NMR spectra of raw and treated celluloses. A: (a) raw cellulose and the samples after (b) water, (c) acid, and (d) base treatment at 150 °C for 2 h. B: (a) the raw cellulose and the samples after (c) acid, (e) A-A, and (f) A-B treatments. C: (a) the raw cellulose and the samples after (d) base, (g) B-B, and (h) B-A treatments.
NMR can distinguish chemically equivalent carbons if they are magnetically not equivalent. In case of cellulose, different degree of packing of cellulose chains or distinct physical conformation can lead to such situations. Usually for NMR study of cellulose, C4 signals are used to distinguish crystalline and amorphous regions. Among the region of 80-93 ppm for C4 carbon, the assignment of signals around at 87-93 ppm to the crystalline structure is generally accepted, and region of 80-87 ppm can be attributed to the amorphous domains. In Figure 3-6-A, it is shown that the crystalline structure of cellulose is not significantly altered after a single treatment with water or base. Only the acid-treated sample, on the contrary, exhibited broadening of all peaks characteristic to cellulose, indicating increase of disorder in cellulose structure. Furthermore, with broadening of C4 peak (88.5 ppm), resonances at this region shifted to lower ppm (80-87 ppm) and the shoulder of the peak became greater, which must be the contribution from the increase of amorphous regions. Crystallinity index is often used to discuss the degree of crystallinity quantitatively. One method to obtain crystallinity index (CrI) from NMR spectra is calculating the ratio of the peak area of crystalline region in C4 to the total peak area of C4. Based upon this method, CrIs were calculated using the equation below for four samples: raw, water-treated (W), OA-treated (A), and TMAH-treated (B) cellulose.

\[
\text{CrI} = \frac{\text{peak area of crystalline region in C4 (87-93 ppm)}}{\text{peak area of C4 (80-93 ppm)}}
\]
They are represented in Table 3-5 together with conversions by the corresponding treatment. In the single step reactions, only the treatment with acid caused a reduction in CrI from 0.61 to 0.40, while those with water and base slightly increased CrI from that of original raw cellulose. After the second steps, CrI of three samples decreased except that of A-B that was not determined due to very low intensity of the peak. Decrease of CrI was more significant for samples after A-A and B-A than B-B, in agreement with the result of the single step.

Table 3-5: Crystallinity and Conversion of Cellulose after Reactions with 10% OA and 10% TMAH at 150 °C for 2 h

<table>
<thead>
<tr>
<th>Sample</th>
<th>Conversion (%)</th>
<th>Crystallinity Index&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Mean size of crystalline domain (nm)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw</td>
<td>-</td>
<td>0.61</td>
<td>4.8</td>
</tr>
<tr>
<td>Water-treated (W)</td>
<td>3.8</td>
<td>0.73</td>
<td>5.3</td>
</tr>
<tr>
<td>Acid-treated (OA)</td>
<td>29.1</td>
<td>0.40</td>
<td>4.6</td>
</tr>
<tr>
<td>Base-treated (TMAH)</td>
<td>58.1</td>
<td>0.68</td>
<td>5.2</td>
</tr>
<tr>
<td>A-A</td>
<td>36.5</td>
<td>0.10</td>
<td>4.3</td>
</tr>
<tr>
<td>A-B</td>
<td>73.0</td>
<td>n.d.**</td>
<td>4.5</td>
</tr>
<tr>
<td>B-B</td>
<td>61.9</td>
<td>0.60</td>
<td>5.1</td>
</tr>
<tr>
<td>B-A</td>
<td>68.3</td>
<td>0.31</td>
<td>4.6</td>
</tr>
</tbody>
</table>

<sup>a</sup>Estimated from (a) NMR spectra and (b) XRD patterns.

<sup>b</sup>Not determined due to very low intensity of the peaks for estimation at the noise level.

To further support this trend in crystallinity, XRD measurements were also carried out and the obtained patterns are shown in Figure 3-8. Two main peaks at $2\theta = 20.0^\circ$ and $22.3^\circ$ in raw cellulose are attributed to the crystalline plane of cellulose, and the strongest peak at $2\theta = 22.3^\circ$ corresponds to the crystalline place (002).<sup>25, 27, 28</sup> The intensities of these two main peaks significantly decreased only after acid treatment. This decrease in intensity is indicative of the
reduction of crystallinity, which is also confirmed by the mean size of crystalline domain as shown in Table 3-5. The size slightly decreased from 4.8 nm in the acid-treated sample (4.6 nm), in contrast with the water- and base-treated samples in which the size slightly increased. This trend is consistent with the change in crystallinity index obtained from NMR spectra. In XRD patterns of the sample after acid treatment, some new peaks were found at 20 = 17.5°, 17.9°, 28.7°, and 33.5°. These were identified as iron oxalate Fe(C2O4) or its dihydrate. It is very likely that iron originates from the materials of stainless steel reactor used in the present study. In general, use of homogeneous acid catalyst often encounters the issue of corrosions under certain conditions, and it certainly is a critical challenge for hydrolytic reaction of biomass, too. This issue was further discussed in Appendix B.

Both XRD and NMR measurements revealed the first treatment with acid can reduce the crystallinity of cellulose. It is noteworthy that the conversion by the reaction with OA is almost half of that with TMAH, but more effective in respect to reduction of crystallinity. It is very likely that acid treatment caused rearrangement of the intra- and inter-chain hydrogen bonds in the crystalline cellulose, creating more disorder of the physical structure in the residue. Although cleavage of readily accessible glycosidic bonds in the reaction with OA is evidenced by formation of glucose, low conversion and yield of glucose indicates that the reaction with OA under our condition does not completely convert the majority of crystalline cellulose into soluble mono-/oligo-saccharides, but it rather generates the more disordered amorphous-like structure in the insoluble products.
In contrast, treatment with base did not cause significant change in conformations of cellulose despite its greater conversion. This suggests that, in the reaction with TMAH, greater amount of glycosidic bonds are efficiently cleaved and produces soluble mono-/oligo-saccharides and their derivatives. Nevertheless, it is also indicated that hydrolysis primarily occurs in the amorphous region of cellulose sample, leaving more of the crystalline cellulose in the insoluble residue. This can cause the increase of CrI and the size of crystalline domain observed by NMR and XRD analysis, which is consistent with the alkaline-treated cellulose fiber. Alkaline treatment is also known to cause structural change in crystalline forms from metastable cellulose I to stable cellulose II. Although our XRD study did not confirm such change in crystalline phase due to the broadness of the main peak, such transition might be responsible for making the residue after B treatment more resistant compared to that after A treatment.

Figure 3-8: XRD patterns of (a) the raw cellulose and the samples after (b) water, (c) acid, and (d) base treatment at 150 °C for 2 h.
Figures 3-6-B and 3-6-C show $^{13}$C CPMAS NMR spectra of samples after the second-step treatments compared with the raw cellulose and those after the first step. Again, broadening of peaks was only observed after acid treatment (A-A and B-A sequences), while prolonged base treatment did not cause further change in the structure (B-B sequence). An exception, however, is the sample after A-B sequence, (Figure 3-6-B). Despite the increased number of scans, no characteristic feature of cellulose was obtained in the spectrum. For this sequence, a solid sample was prepared again and NMR measurement was repeated, which resulted in the same feature of spectrum. Several possibilities to explain this result include (1) complete loss of organic substance and (2) presence of magnetic impurities that interferes NMR signal. It is not yet clear what caused significant decrease of carbon signal to almost negligible level, the complete loss of the original feature must be relevant to the greatest conversion in this sequence of A-B.

Together with the distinct results of solids after the first step with acid and base, it is suggested that in our approach of sequential combination, the rearrangement of hydrogen bonds by the first step with OA increases the disordered structure in the remaining solid with reducing the crystallinity, and thus generating more reactive cellulose for the second step with TMAH. TMAH treatment was shown to be more destructive in our conditions for converting cellulose, but it did not cause as much disorder in the remaining solid as OA treatment. This explains the highest conversion by A-B sequence in biomass and cellulose reactions. TMAH treatment might cause the structural change in cellulose and make the residue more stable, which might be relevant to the difference in conversions of A-B and B-A. It still remains to be answered whether additional reaction with OA can further convert the more resistant residue after A-B treatment. If it does, sequences of more than just two steps appears to be more promising. Moreover, while the change of physical structure such as reduction of crystallinity is shown to be a cause of the enhanced
conversion, the change of chemical nature of the cellulose structure might also be responsible for the enhancement, which is an important aspect to be addressed in a future study.

For the study beyond the structural information of cellulose, Py-GC-MS measurement was performed to characterize the chemical composition of residual solids after reactions. Figures 3-9-A and 3-9-B present total ion chromatograms of solids after single-step and second-step reactions, respectively. In the chromatogram of raw cellulose, a broad peak at 27-30 min was observed, which is identified as levoglucosan (LGA; 1,6-anhydro-β-D-glucopyranose), an anhydro form of glucose unit. LGA is known as a major product from pyrolysis of carbohydrates.\textsuperscript{30,31} A peak at 17.3 min is identified as heptanal, but other small peaks were not identified with sufficient match in the library. They are mostly suggested as alcohols, aldehydes, and ketones in the range of C2 to C7. Also, at the omitted range of retention time earlier than 5 min, smaller compounds such as CO\textsubscript{2} gave a great number of small peaks. Similarly to the raw cellulose, LGA is a major product in all samples after single-step and second-step reactions.

As a quantitative analysis, four categories are defined and their compositions based on the total peak area are represented in Figure 3-10. They are heptanal, LGA, other C6 species, and other compounds including CO\textsubscript{2}. Any of the first treatment with water, acid, or base at 150 °C increased the yield of LGA from 48.9\% to 72.2\%, 72.4\%, and 55.5\%, respectively. A similar trend was reported in case of the cellulose treated with a dilute H\textsubscript{2}SO\textsubscript{4}.\textsuperscript{31} Nonetheless, whether such increase was due to removal of inorganic impurities, catalytic effect by acid or base during pyrolysis, or structural change of cellulose by treatment was not clarified. In our experiments, the yields of LGA from the samples after water and acid treatments were in a comparable extent. Therefore it is fair to rule out the possibility of catalytic effect by residual acid in the solids during pyrolysis.
Figure 3-9: Py-GC-MS total ion chromatograms of the raw and treated cellulosics. A: (a) the raw cellulose and the samples after (b) water, (c) acid, and (d) base treatment at 150 °C for 2 h. B: the samples after (e) A-A, (f) A-B, (g) B-B, and (h) B-A treatments.
Less extent of increase in the case with TMAH than that with water and OA might be attributed to the higher content of more resistant crystalline structure, since positive effect on LGA yield by treatments could be offset once the treatment already converted significant amount of solid. The decreasing trend in LGA yield after the two-step reactions might also reflect this phenomenon. LGA yield decreased in order of A-A (61.8%) > B-B (46.9%) > B-A (33.5%) > A-B (30.5%), which is the same order of the decreasing amount of cellulose residue after the two-step reactions as previously shown in Table 3-1. Presumably, the higher the conversion by the two-step reactions, the more resistant the residual solid becomes and the less LGA could be produced from pyrolysis of this residue. This match of the trend demonstrates that A-B sequence is unique in the sense of compositions of residual solids. Lastly, the presence of the organic compounds, including Figure 3-10: Compositional changes in Py-GC-MS analyses of cellulose after different treatment.
the monosaccharide, in the solid after A-B sequence was confirmed as same as other samples. Thus the absence of significant signal of carbon in $^{13}$C CPMAS NMR does not indicate the absence of organic substances. This peculiar feature after A-B sequence will require further investigation including the possible effect of impurities in cellulose on NMR spectrum.

Solid analyses support the effectiveness of sequential combination of acid and base for converting biomass or cellulose, but it is necessary to study more cycles of sequence. In addition, it is also important to develop effective ways for recovering the catalysts to make the process attractive. Recovering oxalic acid from solution by crystallization is an interesting approach, which should be addressed in a future study. To maximize the value of this process and optimize the product yield and selectivity, kinetic aspect of hydrolysis with OA and TMAH will be suggested for future work.

3.4 Conclusion

The concept of sequential combination of organic acid and base was applied to hydrolysis of cellulose as a new approach for efficient depolymerization of cellulose. Hydrolysis of cellulose was performed at moderate reaction temperature of 150 °C using aqueous solutions of oxalic acid (OA), tetramethylammonium hydroxide (TMAH), or both. Two-step experiments revealed the enhancing effect by the sequential combination on conversion (A-B, 73.0% > B-A, 68.3% > B-B, 61.9% > A-A, 36.5%). While the reaction with OA produced glucose, that with TMAH produced 2-keto-D-gluconic acid, which was likely to be formed from a subsequent reaction of glucose. These results clearly demonstrated that OA and TMAH facilitate the cleavage of the glycosidic bonds in cellulose. NMR and XRD study of solid residues revealed the reduction of crystallinity after the reaction with OA, resulting in the more disordered amorphous-like structure in the solid compared to raw cellulose and the sample after the reaction with TMAH. Meanwhile, TMAH
treatment did not significantly alter the crystallinity of residual cellulose, despite the high conversion in the single-step. This different characteristic of hydrolysis with OA can be the main cause of high conversion of cellulose/biomass in A-B treatment sequence. Compositional changes in solid residue after reactions were investigated further by Py-GC-MS, and it showed that levoglucosan (LGA; 1,6-anhydro-b-D-glucopyranose) was a major product from the pyrolysis of cellulose samples at 610 °C. For the samples from the two-step reactions, levoglucosan yield decreased in the order of A-A (61.8%) > B-B (46.9%) > B-A (33.5%) > A-B (30.5%), which is also the order of the decreasing amount of cellulose residue after two-step reactions. This result also indicates the uniqueness of A-B treatment sequence. This present work demonstrates that sequential combination of organic acid and base is effective for hydrolysis of cellulose, and it can be extended to conversion of lignocellulosic biomass.

3.5 References


Chapter 4

Effect of Organic Solvent on Conversion of Spruce with Acid and Base

4.1 Introduction

Hydrolytic conversion of lignocellulose generates a variety of products including monomeric- and oligomeric sugars, sugar derivatives such as organic acids, and aromatics from lignin. In the previous studies presented in Chapters 2 and 3, the reactions of biomass and cellulose in the aqueous phase with acid, base, and their combination were examined, where only monomeric sugars and some sugar derivatives were successfully analyzed. Further analysis of the products is helpful to obtain better understanding of the complexity of the present reactions. In this regard, the study of the reactions of biomass in binary solvent systems is of great interest. In addition, subsequent reaction of sugars into sugar derivatives might be affected by the presence of organic solvent.

Reactions of biomass and model compounds for biomass in binary systems have been extensively studied. For example, Chheda and co-workers studied dehydration of mono- and poly-saccharides in biphasic system consisting of an aqueous phase and an organic extracting phase of 7:3 (w/w) methylisobutyl ketone (MIBK)-2-butanol mixture. Crossley et al. examined hydrogenation and condensation reactions of some biomass-derived model compounds in the emulsion of water and decalin over carbon-nanotube-inorganic hybrid catalyst. In the reactions of biomass, the main purpose of binary solvent systems is to separate the desirable organic products from the aqueous phase that is the main reaction phase. The present study explored the experiments of reaction in binary solvent systems in order to study whether the addition of organic solvents to the system helps the transfer hydrophobic compounds to the organic phase and inhibits the subsequent reaction, which addresses hypothesis 3.
4.2 Experimental

4.2.1 Materials

To be consistent with the study in single-phase reaction experiments, the same biomass sample of white spruce was chosen for the present study. Detailed information of the sample and the procedures of the pretreatment are described previously in Section 2.2.1. The chemicals used in the study as catalysts are oxalic acid dihydrate (99+%, Sigma-Aldrich), tetramethylammonium hydroxide solution (TMAH; 10 wt% in H$_2$O, Sigma-Aldrich). Hydroxylamine hydrochloride (99%, Alfa Aesar), aniline (99+%, Alfa Aesar), N,O-bis(trimethylsilyl)trifluoroacetamide with trimethylchlorosilane (99% BSTFA with 1% TMCS, Sigma-Aldrich), hexane (98.5+%, Alfa Aesar), and 2-(hydroxymethyl)phenyl-β-d-glucopyranoside (salicin; 99+%, Sigma-Aldrich) are used for derivatization of sugars in analytical procedures. Toluene (BDH, 99.5%), 1-butanol (99.4+%, Sigma-Aldrich), and methylisobutyl ketone (MIBK, 4-methyl-2-pentanone, 99.5+%, Sigma-Aldrich) were used as organic solvents. All chemicals were used as-received without further purification.

4.2.2 Reactions

Solid sample (spruce or cellulose, 0.5 g), 10 wt% oxalic aqueous solution or 10 wt% TMAH aqueous solution (10 g), and organic solvent (toluene, butanol, or MIBK; 2.5 g) were added into a batch micro-reactor (25 mL). The reactor was purged with nitrogen gas. The initial pressure in the reactor was atmospheric pressure at room temperature. Then the reactor was placed in a sand bath (Techne, SBL-2D) preheated at a desired temperature, and vertically shaken at a rate of 250 cycles/min. After the reaction, the liquid and solid products/residues were separated by filtration. The weight of solid was measured after drying in a vacuum oven at 80 °C.
for more than 12 h. In the same way as that of Chapter 1, the conversion of biomass was calculated as the weight loss of the solid sample from the initial sample weight (0.5 g).

### 4.2.3 Analysis of Liquid Products: Gas Chromatography (GC) with Flame Ionization Detector (FID) of Derivatized samples and Gas Chromatography (GC) with Mass Spectrometer (MS).

GC-FID (Varian CP-3800 with VF-5ms column: 30 m × 0.25 mm capillary column, 0.25 mm thickness) was used for the analysis of sugars in the liquid products. Monomeric sugars were derivatized to oxime-trimethylsilyl (oxime-TMS) forms by the same analytical derivatization method used in the study in Chapters 2 and 3, based on the method reported by Rojas-Escudero. The column oven temperature was set as follows: initial temperature 160 °C, held for 1 min, and increased to 172 °C at a rate of 2 °C/min, to 210 °C at a rate of 10 °C/min, to 320 °C at a rate of 30 °C, and then held for 2 min. Temperatures of injector and detector were 250 and 320 °C, respectively. The flow rate of carrier gas was set at 2.7 mL/min.

GC-MS analysis of the organic solvent fraction of the liquid product was carried out using a Shimadzu GC-17A gas chromatograph (Column: Rxi-5 ms, 30 m × 0.25 mm i.d. × 0.25 μm film thickness) coupled with a Shimadzu QP-5000 mass spectrometer. Identification was conducted according to the installed matching software according to the National Institute of Standards and Technology (NIST) standard library.
4.3 Results and Discussion

4.3.1 Conversions

The effect of adding toluene, methylisobutyl ketone (MIBK), and butanol to the reaction system on the conversions of biomass were first studied. Properties of these three solvents are summarized in Table 4-1. The polarity increases in the order of toluene < butanol < MIBK.

Table 4-1: Properties of Toluene, Butanol, and Methylisobutyl Ketone (MIBK)

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Formula</th>
<th>Boiling point [°C]</th>
<th>Dielectric constant*</th>
<th>Dipole moment [D]*</th>
<th>Polarity index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toluene</td>
<td>C₆H₅CH₃</td>
<td>111</td>
<td>2.38</td>
<td>0.36</td>
<td>2.4</td>
</tr>
<tr>
<td>Butanol</td>
<td>C₄H₹OH</td>
<td>118</td>
<td>18</td>
<td>1.63</td>
<td>3.9</td>
</tr>
<tr>
<td>MIBK</td>
<td>(CH₃)₂CHCH₂C(O)CH₃</td>
<td>117</td>
<td>13.11</td>
<td>4.2</td>
<td>4.2</td>
</tr>
</tbody>
</table>

* at 25 °C

Conversions of biomass by single-step reactions in binary systems at 150 °C for 2 h are shown in Table 4-2. As a general trend, conversions increased slightly in every binary system both with acid and base, except the case of MIBK with base. However, considering that the observed experimental error ranges between 1-2%, the slight differences in case of TMAH is not significant. Among reactions with acid, the increase in conversions was significant for the reaction with MIBK and that with butanol. This might be attributed to the effect of solvolysis by the added solvent, where the solvent molecule acts as a nucleophile to cleave the chemical bonds. Organosolv process, a proposed technique as an alternative to the Kraft pulping process, has been extensively investigated, and it is well known that the processing biomass with a mixture of water and organic solvent at 140-220 °C can effectively remove lignin and hemicellulose. In the present study, the concentration of the organic solvent was kept constant at 20%, which was lower than that of the typical conditions of organosolve process (40-80%). Reactions with higher concentration of organic solvent are expected to achieve the higher conversions, yet such
investigation is not included, since the present study attempts to focus on the products by hydrolysis in the aqueous phase.

Table 4-2: Conversions of Biomass by Single-Step Reactions in Binary Systems at 150 °C for 2 h

<table>
<thead>
<tr>
<th></th>
<th>Water</th>
<th>Acid</th>
<th>Base</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single phase</td>
<td>18.5</td>
<td>32.7</td>
<td>46.2</td>
</tr>
<tr>
<td>Binary (Toluene)</td>
<td>24.4</td>
<td>33.7</td>
<td>46.2</td>
</tr>
<tr>
<td>Binary (MIBK)</td>
<td>No data</td>
<td>40.0</td>
<td>49.6</td>
</tr>
<tr>
<td>Binary (Butanol)</td>
<td>No data</td>
<td>40.2</td>
<td>45.2</td>
</tr>
</tbody>
</table>

**4.3.2 Analysis of Liquid Products by GC-MS: Case of Toluene**

Compounds in the organic solvent were analyzed by GC-MS. In the case of the addition of toluene, the reaction with OA at 150 °C for 2 h produced furfural, 5-methylfurfural, and two phenolic compounds, 2-methoxy-4-propyl-phenol and 1-(4-hydroxy-3-methoxyphenyl)-2-propanone. On the other hand, no compound was confirmed by GC-MS in the product from the reaction with TMAH. GC-MS chromatograms of the toluene fraction after the single-step reactions with OA and TMAH are shown in Figure 4-1. The compounds found in the product of the reaction with OA were also summarized in Table 4-3 with their retention times and their possible origins, and Figure 4-2 shows their structures. Furfural and 5-methylfurfural can be derived from pentose and hexose, respectively, via subsequent reaction of monomeric sugars such as dehydration. Two phenolics should be formed from lignin, which consists of polyaromatic structure. The presence of phenolics indicates that reaction with OA can also cleave the aryl ether bonds in lignin.
Figure 4-1: GC-MS chromatograms of toluene fraction after the single-step reactions at 150 °C for 2 h with OA and TMAH.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Retention time [min]</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Furfural</td>
<td>5.1</td>
<td>Pentose</td>
</tr>
<tr>
<td>Ethylbenzene</td>
<td>5.7</td>
<td>Impurity of toluene</td>
</tr>
<tr>
<td>1,2-dimethylbenzene</td>
<td>5.9</td>
<td>Impurity of toluene</td>
</tr>
<tr>
<td>Methylfurfural</td>
<td>8.1</td>
<td>Hexose</td>
</tr>
<tr>
<td>2-methoxy-4-propyl-phenol</td>
<td>19.8</td>
<td>Lignin</td>
</tr>
<tr>
<td>1-(4-hydroxy-3-methoxyphenyl)-2-propanone</td>
<td>21.3</td>
<td>Lignin</td>
</tr>
</tbody>
</table>
To examine the addition of sorbent to the reaction system helps the transfer of the hydrophobic compounds, stated in hypothesis 3, the concentration of the product from the reaction with OA in binary system (aqueous and toluene) was compared with the case of the post-extraction, where 2.5 g of toluene was added to the aqueous solution after the single-phase reaction with OA at $150^\circ$C for 2 h. Figure 4-3 illustrates the results, clearly indicating that more than twice amount of furfural was recovered in the case of binary system. This proves the hypothesis 3. On the other hand, the sugar in the aqueous portion was greater in the post-extraction case, as summarized in Table 4-4. While the addition of toluene to the reaction system helps the transfer of some hydrophobic molecule such as furfural, it did not improve the overall efficiency of depolymerization of biomass in terms of sugar yield. This requires further investigation to clarify whether there is a potential benefit of reactions in binary systems. In the following section, plausible mechanisms for the formation of furfural, methylfurfural and the phenolics are discussed.

![Identified compounds in the toluene fraction after the single-step reactions at 150 °C for 2 h with OA (impurities of toluene excluded).](image)

Figure 4-2: Identified compounds in the toluene fraction after the single-step reactions at 150 °C for 2 h with OA (impurities of toluene excluded).

(1) Furfural  (2) 5-methylfurfural  (3) 2-methoxy-4-propyl-phenol  (4) 1-(4-hydroxy-3-methoxyphenyl)-2-propanone
Figure 4-3: Concentrations of furfural and methylfurfural in toluene: (a) Extract with toluene from the aqueous solution after the single-phase reaction with OA and (b) toluene phase from the reaction with OA in binary system at 150 °C for 2 h.

Table 4-4: Furans and Sugars Concentration in (a) Extract with Toluene from the Aqueous Solution after the Single-Phase Reaction with OA and (b) Toluene Phase from the Reaction with OA in Binary System at 150 °C for 2 h

<table>
<thead>
<tr>
<th></th>
<th>Total furans</th>
<th>Sugars in aqueous solution</th>
<th>Furans and sugars</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wt% of biomass</td>
<td>Wt% of biomass</td>
<td>Wt% of biomass</td>
</tr>
<tr>
<td>Post-extract</td>
<td>0.8</td>
<td>16.3</td>
<td>17.3</td>
</tr>
<tr>
<td>Binary system</td>
<td>2.6</td>
<td>13.0</td>
<td>16.1</td>
</tr>
</tbody>
</table>
4.3.3 Subsequent Reactions of Sugars to Furfural and Methylfurfural under Acidic Condition

Furfural is an important value-added chemical obtainable from pentose in hemicellulose. Demand of furfural as a key intermediate is increasing in various fields including plastics, pharmaceuticals, and agrochemicals. Furfural can be produced by acid-catalyzed dehydration of pentose. A simplified scheme of this reaction is illustrated in Figure 4-4. The first step, the hydrolysis of pentosan in hemicellulose to pentose, is much faster than the second step, the dehydration of pentose to furfural. It is clearly demonstrated that this relatively slow step of dehydration also occurred under the present condition of 150 °C for 2 h with 10% OA solution. Several mechanisms were proposed for the formation of furfural from a representative pentose, xylose. In general, the reaction proceeds as follows: (1) the proton attacks oxygen atom of a hydroxyl group to form H₂O⁺ group, (2) one water molecule is liberated and the positive charge remains at the neighboring carbon atom, (3) formation of double bonds or rearrangement of the ring to form another H₂O⁺ group, and (4) repeat this cycle for liberation of two more water molecule. In stoichiometry, it is described as C₅H₁₀O₅ → C₅H₄O₂ + 3H₂O.

![Figure 4-4: Simplified scheme of acid-catalyzed reactions of pentosan to furfural. (Reproduced from the reference)](image)

Methylfurfural (MF), on the other hand, has 6 carbons, different from 5 of furfural. It is reported that MF can be produced from a 6-deoxy sugar or hexose. In the field of food chemistry, it is well known that furfural and MF are among the major products from the thermal treatment of mixture of sugars, called non-enzymatic browning reaction. Hurd et al. were...
the first to report the formation of MF from the reaction of rhamnose with 1.3-5.2 N HCl (4.7-
18.8 wt%) or 3.8-12.1 N H₂SO₄ (16-52 wt%) at 100-130 °C. Rhamnose is a 6-deoxy sugar and
can also be classified as methyl-pentose. A deoxy sugar is a sugar in which one hydroxyl group
was replaced by a hydrogen atom. No detailed mechanism of dehydration of rhamnose to MF was
found after a thorough literature survey, but it is reasonable to assume the reaction proceeds in the
similar pathway as that for furfural from pentose. A general scheme of this dehydration of
rhamnose is shown in Figure 4-5-a. Rhamnose is present in hemicellulose typically as much as
~5% of xylose.¹⁹

Another possible source of MF is hexose. Chidambaram and Bell studied dehydration of
glucose into hydroxymethylfurfural (HMF) and further conversion of HMF into 2,5-dimethylfuran
(DMF) in ionic liquids.³ They reported that MF was the major product of hydrogenation of HMF
over carbon-supported metal catalyst with the selectivity of 51-83%. One oxygen atom is
removed from HMF to form MF, thus this is hydrodeoxygenation process. This pathway of
formation of MF is illustrated in Figure 4-5-b. Chidambaram also confirmed MF can be further
hydrogenated to DMF, which is a high-energy content product and expected as a promising
component of fuel or fuel additives. MF is therefore an interesting intermediate for fuel
applications.

Comparing two possible origins of MF, it is likely that MF observed in the present study
was primarily generated from rhamnose, since the latter pathway requires the hydrogenation step
and an active hydrogenation catalyst. However, this possibility cannot be ruled out because the
presence of hydrogen in the gas products after the reaction biomass with OA was confirmed as
discussed in Chapter 2. It requires further work to provide direct evidence to determine the exact
pathways of the formation of MF.
4.3.4 Conversion of Lignin Fraction

In Chapter 1, the NMR and Pyrolysis GC-MS analysis of solid residues of biomass demonstrated that the significant reduction of lignin content after the reaction with TMAH, greater than the case with OA. Therefore, it was expected to observe phenolic compounds from lignin from the reaction with TMAH. Nevertheless, as shown above in Figure 4-1, phenolic compounds were only observed from the reaction with OA, and not from that with TMAH at 150 °C. This counter-intuitive result implies that, the reaction with TMAH converted major part of lignin only into oligomeric compounds, which still are large molecules with high polarity and prefer to exist in aqueous phase, rather than in organic phase.

On the other hand, 2-methoxy-4-propyl-phenol and 1-(4-hydroxy-3-methoxyphenyl)-2-propanone were observed from the reaction with OA. Both of these have the structure of guaiacyl
propane carbon skeleton. The structure indicates these phenols originate from coniferyl alcohol, which is one of three main monomers of lignin. The formation of low-molecular-weight phenols is reported from the study of reflux of biomass with 0.2 M HCl in 9:1 dioxane-water mixture. Degradation of lignin under acidic conditions is known to occur cleaving α- and β-aryl ether bonds, but the competing condensation reaction of lignin fragments is difficult to avoid. In the case of OA treatment, the presence of monophenol structure in the liquid products and the relatively lower reduction in lignin content in the residual solid might be relevant to this possible condensation reactions. Further study of conversion of lignin was attempted through the experiments using the isolated lignin (organosolv lignin) and summarized in Appendix A.

4.3.5 Effect of Types of Solvent

Addition of butanol (BuOH) and MIBK as well as toluene were also examined as binary solvent system for the reaction of biomass with OA or TMAH at 150 °C for 2 h. Identified compounds are summarized in Table 4-5. In the case of BuOH addition to the reaction with OA, some ethers and esters as well as furfural were produced. It was found that all of these ethers or esters have butyl group, indicating that the solvent BuOH itself partially reacted under the present conditions possibly via partial dehydration. Among these products, dibutyl ether and dibutyl ester ethanedioic acid were also observed from the black test reaction without biomass (BuOH/water mixture with OA) at the same condition. 1,2-di-2-furyl-1,2-ethanediol has a dimer structure of furfural, and is considered to be formed from the dimerization of furfural. From the reaction with TMAH and BuOH, no compounds were observed, the same result as that with toluene.

When MIBK was added as the organic solvent, such reaction of the solvent itself occurred in basic condition, but not in acidic condition. In the reaction with OA, similarly to that with toluene, furfural, MF, and two phenolics (2-methoxy-4-propyl-phenol and 4-acetoxyacetophenone) were observed.
Table 4-5: Compounds in Organic Fraction Identified by GC-MS from Single-Step Reactions in Binary Solvent Systems at 150 °C for 2 h

<table>
<thead>
<tr>
<th>Organic solvent</th>
<th>Products from Reaction with OA</th>
<th>Products from Reaction with TMAH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toluene</td>
<td>Furfural and MF</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-methoxy-4-propyl-phenol</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1-(4-hydroxy-3-methoxyphenyl)-2-propanone</td>
<td></td>
</tr>
<tr>
<td>Butanol</td>
<td>Furfural</td>
<td>Dibutoxymethane*</td>
</tr>
<tr>
<td></td>
<td>Dibutyl ether*†</td>
<td>Butyl ester levulinic acid*</td>
</tr>
<tr>
<td></td>
<td>Butyl ester ethanedioic acid*†</td>
<td>1,2-di-2-furyl-1,2-ethanediol</td>
</tr>
<tr>
<td>MIBK</td>
<td>Ketones (C_7-C_10)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-methoxy-4-propyl-phenol</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4-acetoxyacetophenone</td>
<td></td>
</tr>
</tbody>
</table>

* BuOH was reacted to form these products. † Only BuOH or BuOH and OA were reacted.
Figure 4-6 shows the concentration of furfural and MF in the organic fractions. While the production of MF did not change significantly between the solvents toluene and MIBK, that of furfural increased by about 40%. This increase might be attributed to the higher conversion with MIBK (40.2%) than with toluene (32.7%). Still, this is presumably partial contribution since the difference of conversions is about 20%. Use of BuOH had a negative impact on the production of furfural, and the presence of furyl alcohol in the products indicates that the subsequent degradation of furfural undergo to a greater extent compared to the other two solvent cases. Overall, no correlation was found between the polarity of the solvent and the recovery of these furanic compounds.
4.3.6 Furfural and Methylfurfural from Two-Step Reactions

Two-step reactions of biomass in binary solvent system were conducted using toluene. Among four different two-step sequences, the reactions with TMAH, namely A-B, and B-B sequences, did not produce any furanic compounds or phenolics, as demonstrated in the single step with TMAH. The results of the other two sequences, B-A and A-A, are presented in Figure 4-7, together with that of the single-step reaction with OA.

A-A sequence did not show any products, which indicates the second step with OA did not convert any more pentose or rhamnose. This result is also reflected by the low conversion (9.9%). In B-A sequence, where the second step with OA was applied to the solid residue from

![Figure 4-7: Production of fufural and MF in the single- and two-step reactions, A, B-A, and A-A with toluene.](image-url)
the first step with TMAH, furfural was still formed as much as roughly 40% of that from the single OA step. This result interestingly shows that the residue after the first TMAH step still contains hydrolysable hemicellulose. Even though pentoses or pentose-derivatives such as furfural were not observed in any of the reactions with TMAH, it is generally considered that hemicellulose can readily hydrolyzed both in acidic and basic conditions. Therefore it was assumed that a substantial amount of hemicellulose was already converted at the first step with TMAH. Yet the result proved this is not true. OA treatment might be more effective for the conversion of hemicellulose than TMAH treatment. If such case is proved, it would be another interesting support for the concept of combinational use of acid and base. However, since determination of hemicellulose content in the solid and comprehensive quantification of various pentoses were difficult with current facilities in the laboratory, it remains as a recommendation for future work.

4.3.7 Reaction at Higher Temperature of 215 °C

Reactions with TMAH at 150 °C did not show any products in the organic phase at all. As mentioned in Section 4.3.4, despite its relatively high conversion of spruce and the evidence of lignin removal from the solid, it is likely that lignin was not fragmented into monophenols, but mostly large oligomeric compounds (polyphenols). TMAH treatment at this condition was shown to be useful in terms of an effort for fractionation of lignocellulose into carbohydrates and lignin. But for better utilization of lignin as chemical feedstock, it is desirable to break down further to obtain monophenols, which can be then converted into BTX or other valuable aromatic chemicals. In this respect, the single-step reactions with OA and TMAH at higher temperature of 215 °C were performed to reveal whether lignin fragmentation can be facilitated, and also whether it affects the products from carbohydrates.
GC-MS chromatograms of toluene fraction after the single-step reactions at 215 °C for 2 h with OA and TMAH are shown in Figure 4-8. From the reaction with OA, furfural, MF, and 2-methoxyphenol were produced. Furfural and MF are the same products as those at 150 °C. 2-methoxyphenol is the dealkylated form of two products at 150 °C, 2-methoxy-4-propylphenol and 1-(4-hydroxy-3-methoxyphenyl)-2-propanone (Figure 4-2). The reaction with TMAH produced three compounds: two phenols, 2-methoyphenol and 1,2-dimethoxybenzene, and a cyclic ketone, 2-ethyl-3-methoxy-2-cyclopentenone. It is demonstrated that increasing temperature of the reaction with TMAH to 215 °C enhances the fragmentation of lignin to produce monophenols.

Figure 4-8: GC-MS chromatograms of toluene fraction after the single-step reactions at 215 °C for 2 h with OA and TMAH.

Figure 4-9 presents formations of furfural, MF and phenols from the reactions with OA and TMAH at 150 and 215 °C. The relative peak areas of GC chromatograms were compared with an approximation of the same response factor among these compounds. In the reactions with OA, the amount of fufural decreased by more than half by increasing temperature from 150 to 215 °C, while those of MF and phenols did not change significantly. This should be attributed to further reaction of furfural such as condensation. This result is an indication of detrimental effect of higher temperatures on the products from carbohydrates.
On the other hand, in the case of TMAH, the production of phenols exhibited a strong dependence on the operating temperature. At 215 °C, the amount of phenols is even substantially greater than those from the reaction with OA. This supports the promise of basic condition for efficient depolymerization of lignin into monophenols.

Figure 4-9: Relative peak areas of furfural, methylfurfural and phenolics from the reactions with OA and TMAH at 150 and 215 °C.

4.4 Conclusions

The effect of addition of organic solvents on hydrolytic conversion of lignocellulosic biomass (spruce) with oxalic acid (OA) or tetramethylammonium hydroxide (TMAH) was investigated. In the reaction with OA at 150 °C in binary solvent systems, hemicellulose and
cellulose in biomass are depolymerized to produce monomeric sugars such as glucose and xylose. GC-MS analysis of toluene-extract evidenced that those sugars further undergo subsequent reactions (e.g., dehydration) to form furfural and 5-methylfurfural in acidic conditions. At the same time, the presence of some phenols showed that the reaction with OA can also fragment lignin macromolecule. On the other hand, the reaction with TMAH did not generate observable phenols at 150 °C, although it is effectively converted lignin in biomass into soluble products as demonstrated in Chapter 2. Increasing temperature to 215 °C had a significant effect in the case of TMAH, and it could produce some dealkylated monophenols. The result suggests the dominance of highly polar oligomeric compounds over monomeric units in the liquid products from reactions at low temperature.

4.5 References


Chapter 5

Conclusions and Recommendation for Future Work

5.1 Conclusions

This present study demonstrated a promising concept of the sequential combination of organic acid and base as a novel approach for converting lignocellulosic biomass at moderate reaction conditions. The major conclusions generated from this work are as follows:

1. From the two-step reaction experiments, the enhancing effect by the combination of OA and TMAH treatments on conversion was proved for the sequence of A-B (the first reaction with acid and the second with base).

2. Different selectivity of C-O bond cleavage of hemicellulose, cellulose, and lignin between the reactions with oxalic acid (OA) and tetramethyammonium hydroxide (TMAH) was demonstrated: The cleavage of β-(1,4)-glycosidic bonds in polysaccharides is preferred under acidic conditions, while the cleavage β-O-4 aliphatic aryl ether bonds in lignin is preferred under basic conditions.

3. In A-B sequence, the enhancement of conversion of cellulose at the second step with TMAH was unexpectedly discovered. This enhancement was caused by the reduction of crystallinity of cellulose by the first reaction with OA. This finding was beyond the original hypotheses, and the present study demonstrated that pH condition of the treatments affects the reactivity of crystalline cellulose.

4. It was shown that some furans and phenols can be directly formed from the reactions in the binary solvent system. Influence of adding organic solvent on the products was revealed.
5.2 Recommendation for Future Work

The concept of combinational use of acidic and basic conditions for hydrolytic depolymerization of lignocellulose is proposed and investigated in the present study. The scientific questions that arose through this work and recommendation for future work are summarized below:

1. Deepen the fundamental understanding of the reason why acidic and basic conditions exhibit distinct selectivity of C-O bond cleavage in the reaction of biomass that was demonstrated in the present study. Studying simpler model systems in addition to the whole biomass should be able to assist obtaining further mechanistic information of hydrolysis of each of cellulose, hemicellulose, and lignin.

2. Identify the conditions or reagents for better selectivity of C-O bond cleavage. Neither of the reactions with OA or TMAH did exclusively convert cellulose, hemicellulose, or lignin. All of three components are subject to depolymerization under all of the studied conditions. Exploring the process variables further at each step will be useful. For example, it is worth studying lower temperature and shorter reaction time for acid treatment at the first step only to remove hemicellulose, followed by base treatment to remove lignin at lower temperature at the second step while minimizing the depolymerization of cellulose. In the context of biorefinery concept, designing step-wise reaction schemes more than two steps should be worthwhile.

3. Recovery of OA and TMAH remains to be an important issue in the study. Theoretically, majority of OA can be precipitated via crystallization by lowering the solution temperature close to 0 °C. In the present study, such attempt was not successful due to the decomposition of OA during the reaction. It is necessary to minimize such decomposition. In addition, use of larger dicarboxylic acid such as maleic acid might be
beneficial in terms of recovery. Recovery of TMAH is also problematic. Some studies of adsorptive separation of TMAH molecule from aqueous solution using mesoporous materials are found in literature, but the concentration is in the range of ppm. Thus a substantial dilution step is required first.

Appendix A

Reaction of Organosolv Lignin with Acid and Base

A.1. Introduction

Lignin, one of three main components of lignocellulose, is expected as an important renewable feedstock for fuels and chemicals.\textsuperscript{1,2} While much of recent biomass research have been made on carbohydrates, less attention seems to be paid to the conversion of lignin into value added chemicals. This is partly because much less is understood about the complex structure of lignin in nature, compared to that of polysaccharides. In Chapters 2 and 4, it was revealed that the reactions with oxalic acid (OA) and tetramethylammonium hydroxide (TMAH) depolymerize cellulose and hemicellulose to produce monomeric sugars and sugar derivatives, but lignin was converted mostly into oligomeric compounds. Some monophenols were observed from the reaction with OA at 150 °C and also that with TMAH at 215 °C. To obtain more knowledge on depolymerization of lignin, the reactions of a commercial isolated Organosolv lignin (OL) were investigated.

A.2 Experimental

Organosolv lignin was obtained from Sigma-Aldrich (Product # 371017) and used without further purification. In a typical reaction, solid sample (lignin, 0.5 g) and 10 wt% OA aqueous solution or 10 wt% TMAH aqueous solution (10 g) were added into a 25 mL batch micro-reactor. As organic solvent toluene (2.5g-5g) was also added to the system to extract organic compounds during the reactions. The reactor was then purged with nitrogen gas. The initial pressure was set atmospheric pressure at room temperature. Reactions were carried out in a
sand bath preheated at 150 °C for 2 h. After the reaction, the liquid and solid products/residues were separated by filtration. The solid residue was weighted after drying in a vacuum oven at 80 °C for more than 12 h. Conversion of biomass was calculated as loss of solid sample from the initial weight (0.5 g).

GC-MS analysis of toluene fraction of the liquid product was carried out using a Shimadzu GC-17A gas chromatograph (Column: Rxi-5 ms, 30 m × 0.25 mm i.d. × 0.25 μm film thickness) coupled with a Shimadzu QP-5000 mass spectrometer. Identification was conducted according to the installed matching software according to the National Institute of Standards and Technology (NIST) standard library.

A.3 Results and Discussion

A.3.1 Conversions

Conversions of Organosolv lignin (OL) are compared with those of biomass (Chapter 2) in Table A-1. Reactions in 10% H₂SO₄ and 10% NaOH were also performed for comparisons. As shown in the table, conversions are lower for OL than biomass in acid. Use of stronger acid that has lower pKa did not improve the conversion. This result suggests that OL is resistant to acid treatment. Figure A-1 shows the pictures of OL before and after the reaction with OA. Char-like hard solids comprised of aggregates were formed. Presumably condensation and polymerization predominantly occur in acidic conditions. The apparent conversions of OL in base are greater than 95%, but these values are not meaningful as they are, because of very high solubility of OL at high pH. In other words, OL easily dissolved in basic solution at room temperatures. This is examined by a simple solubility test of OL, which is summarized in Table A-2. More than 95 wt% of OL was dissolved in 10% TMAH solution, and about 91 wt% was re-precipitated by lowering the pH to around 5.
Table A-1: Conversions of Organosolv Lignin (OL) and Biomass by Single-Step Reactions at 150 °C for 2 h

<table>
<thead>
<tr>
<th>Acid or Base</th>
<th>Lignin (%)</th>
<th>Biomass (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10% H₂SO₄</td>
<td>22.4</td>
<td>35.1</td>
</tr>
<tr>
<td>10% OA</td>
<td>23.1</td>
<td>32.7</td>
</tr>
<tr>
<td>10% NaOH</td>
<td>97.4*</td>
<td>23.5</td>
</tr>
<tr>
<td>10% TMAH</td>
<td>99.1*</td>
<td>46.2</td>
</tr>
</tbody>
</table>

*Should not be directly compared with others due to very high solubility of lignin sample in basic solution

Figure A-1: Pictures of Organosolv Lignin (OL) before and after the reaction with 10% OA.

Table A-2: Solubility Test of OL

<table>
<thead>
<tr>
<th>Solution</th>
<th>pH</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toluene</td>
<td>-</td>
<td>92.9</td>
</tr>
<tr>
<td>10% TMAH</td>
<td>&gt;14</td>
<td>3.4</td>
</tr>
<tr>
<td>10% TMAH</td>
<td>~ 5 (Neutrized after 30 min)</td>
<td>91.1</td>
</tr>
<tr>
<td>10% OA</td>
<td>&lt; 1</td>
<td>97.4</td>
</tr>
</tbody>
</table>

0.3 g of OL is mixed with 10 g of sol. and stirred for 30 min at RT. Filtered and recover the solid.
A.3.2 Phenols from Organosolv Lignin

GC-MS chromatograms of organic phase after the reactions with OA and TMAH are presented in Figure A-2. Compounds identified are shown in Figure A-3, and their relative amounts are shown in Table A-3. Similarly to biomass reactions (Chapter 4), some monophenolic compounds were found in the reaction with OA. They are (1) vanillin, (2) 4-hydroxy-3,5-deimethoxy-benzaldehyde, and (3) 3,5-diethoxy-4-hydroxyphenylacetic acid. Structural differences of these compared to those from biomass are the presence of aldehyde group for (1) and (2), and two methoxy groups for (2) and (3). In the chromatograms, there are 4-5 more noticeable peaks, but the match with library was not satisfactory to identify the exact structure. Most of suggestions from the library are oligomeric phenols with molar weight greater than 300.

It is worth noting that the low conversion of OL by the reaction with OA is presumably due to condensation reactions of lignin and lignin fragments. Such condensation reactions occurs in acidic conditions between the side chain benzylic carbons, and often interfere the cleavage of ether bonds in lignin. Therefore, it will be critical to minimize this competing condensation to facilitate the depolymerization.

On the other hand, the reaction with TMAH did not produce any monophenolic compounds. Only two peaks were observed possibly from oligomeric phenols, but the match was not sufficient to identify the structure. Despite the high solubility of OL in 10% TMAH solution, the reaction did not effectively fragment the lignin into small molecules. These are consistent with the results of biomass reactions in Chapter 4. Thus reaction at higher temperature is studied and discussed in the next section.
Figure A-2: GC-MS chromatograms of toluene fraction after the reactions of OL with 10% OA and 10% TMAH at 150 °C for 2 h.

Figure A-3: Identified phenols in the products from the reaction of OL with 10% OA.

Table A-3: Relative Amount of Identified Compounds in Toluene Fraction from Reactions of OL at 150 °C

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Rxn with 10%</th>
<th>Rxn with 10%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OA</td>
<td>TMAH</td>
</tr>
<tr>
<td>(1) Vanilin</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>(2) Benzaldehyde, 4-hydroxy-3,5-deimethoxy-</td>
<td>4.2</td>
<td>-</td>
</tr>
<tr>
<td>(3) 3,5-diemthoxy-4-hydroxyphenylacetic acid</td>
<td>1.5</td>
<td>-</td>
</tr>
</tbody>
</table>

*relative to (1)
A.3.3 Reactions at Higher Temperature of 215 °C

Figure A-4 presents GC-MS chromatograms of toluene fraction after the reactions of OL with OA and TMAH at temperatures of 150 and 215 °C. In the case of OA, most of the peaks from monomeric and oligomeric phenols disappeared at 215 °C, except only one peak of 2,6-dimethoxyphenol. This result indicates elevating temperature accelerated condensation reactions and is detrimental for fragmentation of lignin.

![GC-MS chromatograms](image)

Figure A-4: GC-MS chromatograms of toluene fraction after the reactions of OL with 10% OA and 10% TMAH at temperatures of 150 and 215 °C for 2 h.

In reactions of biomass with TMAH, no phenols were observed at 150 °C, but increasing temperature to 215 °C resulted in formation of two monoaromatic compounds, 2-methoxyphenol and 1,2-dimethoxybenzene. In this study of OL, a similar trend was shown. At 215 °C, two monoaromatic compounds, 1,2-dimethoxybenzene and 1,2,3-trimethoxybenzene were produced. TMAH has been reported to effectively cleave β-O-4 aliphatic aryl ether bonds, and the reported
temperature condition is around 250 °C.\textsuperscript{4,5} This indicates that fragmenting lignin by TMAH reaction into monoaromatic structure requires higher temperature than 150 °C.

\textbf{A.4 Conclusions}

Conversion of Organosolv lignin (OL) was studied using 10% oxalic acid (OA) and 10% tetramethylammonium hydroxide (TMAH) solutions at two temperatures of 150 and 215 °C. The reaction with OA produced some monomeric and oligomeric phenols, which evidenced the cleavage of aryl ether bonds in lignin, but at the same time the competing condensation reaction occurred resulting in a low conversion (23%). This condensation became even more dominant at elevated temperature of 215 °C. In TMAH case, no monomeric compounds was found at 150 °C, and some monoaromatic compounds were formed at 215 °C. It is revealed that conversion of lignin under basic conditions into monomeric fragments requires high temperature such as 215 °C.

\textbf{A.5 References}


Appendix B

Detailed Procedures of Analytical Derivatization for Analysis of Carbohydrates

B.1 Introduction

For analysis of monosaccharides and oligosaccharides in a solution, column chromatography is the most powerful analytical technique. Separation of these carbohydrates is based on their different adsorption characteristics in the column. Such characteristics are the function of partition coefficient, polarities, and sizes of the molecules. Three common chromatographic techniques for analysis of carbohydrates are Thin layer chromatography (TLC), Gas chromatography (GC) and High Performance Liquid chromatography (HPLC). HPLC is currently the most popular choice for carbohydrate chemists; It can measure rapidly and sensitively. GC, on the other hand, requires the sample to be volatile, and thus derivatization is usually necessary. These techniques are often used in conjunction with NMR or mass spectrometry to provide sufficient information on chemical structure for identification.

After testing the HPLC instrument in EMS Energy Institute for analysis of carbohydrates with standard sugar solutions, it is confirmed that the installed detector could not detect any carbohydrates. Therefore, in the present thesis study, the main analysis of carbohydrates in the liquid samples from the reaction of biomass and cellulose was performed using GC with analytical derivatization. In the following, the exact procedures and considerations of these analytical methods are described.
B.2 Procedures for Analysis of Carbohydrates

First, the applicability of the High performance liquid chromatography available in the EMS Energy Institute, Water 600E, for analysis of carbohydrates was examined. The column installed was Pinnacle II PAH (250 x 4.6 mm, particle size: 5 mm, spherical, pore size: 110 Å, pH range: 2.5 ~ 10, temperature limit: 80 °C, alkyl-based stationary phases C18). The detector was Photodiode Array Detector (Water 996 PDA detector) and operated at the wavelength of 270 nm. As mobile phase 0.7% acetic acid:methanol (60:40 v/v) was used at the flow rate of 0.5 mL/min. The standard sample injected was a mixture of phenol, catechol, xylose, glucose, mannose, arabinose, galactose, and mannose with concentration of 0.67 mg/mL for each compound.

As for GC analysis, two derivatization methods were tested based on the reports found in literature. One is based on formation of trimethylsilyl (TMS) ether, and the other is based on formation of oxime-trimethylsilyl (oxime-TMS) ether. Figure B-1 shows the schematic illustrations of these transformations in the case of glucose.

Figure B-1: Derivatization of glucose via (a) formation of trimethylsilyl (TMS) ether and (b) formation of oxime-trimethylsilyl ether.
The detailed procedures for derivatization TMS formation method are as follows:

1. Prepare several standard solutions of glucose and cellobiose (6-140 µg/mL).
2. Take a 15 mL aliquot of the standard solution into a round-bottom flask.
3. Concentrate the solution to ~1.5 mL using a rotary evaporator.
4. Open the flask and further evaporate to the complete dryness under the stream of N₂.
5. Add 250 µL of BSTFA (N,O-bis(trimethylsilyl) trifluoroacetamide) containing 1%TMCS (trimethylchlorosilane) and 100 µL of pyridine.
6. Place the flask in a water bath at 70 °C for 3 h. (TMS formation)
7. Evaporate the derivatized extracts to dryness with N₂.
8. Redissolve in 250 µL of hexane for GC injection.

The following is the procedures for derivatization oxime-TMS formation method, based upon the method by Rojas-Escudero⁷:

1. Prepare several standard solutions of glucose and cellobiose (6-140 µg/mL).
2. Take a 15 mL aliquot of the standard solution into a round-bottom flask.
3. Concentrate the solution to ~1.5 mL using a rotary evaporator.
4. Open the flask and further evaporate to the complete dryness under the stream of N₂.
5. Add 1mL of hydroxylamine hydrochloride and 50 mg of aniline
6. Place the flask in a water bath at 60 °C for 10 minutes. (Oxime formation)
7. Add 250 µL of BSTFA containing 1%TMCS.
8. Leave at ambient temperature for 10 minutes. (Oxime-TMS formation)
9. Add 1 ml of hexane, and sonicate for 10 minutes (extraction)
10. Collect the upper phase of hexane for GC injection.
For both of two derivatization methods, a 1 μl of the final hexane extract was injected into GC-FID (Varian CP-3800 with VF-5ms column: 30 m × 0.25 mm capillary column, 0.25 mm thickness). The column oven temperature was set as follows: initial temperature 160 °C, held for 1 min, and increased to 172 °C at a rate of 2 °C/min, to 210 °C at a rate of 10 °C/min, to 320 °C at a rate of 30 °C, and then held for 2 min. Temperatures of injector and detector were 250 and 320 °C, respectively. The flow rate of carrier gas was set at 2.7 mL/min.

B.3 Results of HPLC and GC-FID with Two Derivatization Methods

HPLC chromatogram of the standard mixed solution of phenol, catechol, xylose, glucose, mannose, arabinose, galactose, and mannose is shown in Figure B-2. Only two peaks are observed at the retention times of 10.5 and 17 min. These are identified as phenol and catechol, respectively, according to the results of the solution of the single compound.

Figure B-2: HPLC chromatogram of the standard mixed solution of phenol, catechol, xylose, glucose, mannose, arabinose, galactose, and mannose.
No peaks of sugars, on the other hand, are confirmed. This is due to the fact that sugars do not have absorption in the region of UV applied (270 nm), while phenolic compounds have sufficient absorption. It appears that Refractive Index (RI) detector is more effective for HPLC analysis of carbohydrates. In the present study, GC with derivatization is chosen because of the availability of instrument in the facility.

Figures B-3 and B-4 illustrate the calibrations of glucose and cellobiose by GC-FID with TMS formation method, respectively. Figures B-5 and B-6 shows those with oxime-TMS formation method. Coefficient of determination $R^2$ was estimated for each of these four calibrations, and it was clearly shown that the oxime-TMS formation method is superior to the TMS formation method. Based on this result of comparison, the oxime-TMS formation was applied for the present study as a means of carbohydrate analysis. In the analysis of liquid products from the reactions of biomass and cellulose, as described in Chapter 2 and 3, an internal standard, salicin, was added to the sample prior to the analysis to further enhance the accuracy of the quantification.
Figure B-3: Calibration for glucose by GC-FID with TMS formation method.

Figure B-4: Calibration for cellobiose by GC-FID with TMS formation method.
Figure B-5: Calibration for glucose by GC-FID with oxime-TMS formation method.

Figure B-6: Calibration for cellobiose by GC-FID with oxime-TMS formation method.
B.4 References

1. Restek website, information of Pinnacle® II PAH:
   http://www.restek.com/restek/prod/7645.asp


Appendix C

Issue of Corrosion of the Reactor during the Reaction with Acid

C.1 Introduction

Corrosion of an engineered material is the process of disintegration of its constituent atoms by chemical reactions. In chemical industry, especially when homogeneous strong acid or base is used, corrosion of reactors or other equipment often becomes an issue. This is behind the motivation for extensive research on development of solid acid or base that could replace conventional mineral acids or alkali. In the present study, the reactions with 10% oxalic acid (OA) and/or 10% tetramethylammonium hydroxide (TMAH) were performed in a stainless-steel (A182-F316) batch reactor. F316 steel contains metal elements such as Mn, Cr, Mo, Cu, Ni, and Co, and it is known to have better corrosion resistance than another common steel type of F304. Yet, corrosion resistance is reduced as the solution temperature is increased. In Chapter 3, XRD study of cellulose solid residue after acid- or base- treatments suggested the corrosion of the reactor in the reaction with OA. Further discussion on this issue is made as follows.

C.2 Evidence of Corrosion: XRD, SEM-EDS Analysis

As shown in Figure 3-6, XRD patterns of the sample after OA treatment exhibited some peaks at $2\theta = 17.5^\circ$, $17.9^\circ$, $28.7^\circ$, and $33.5^\circ$, which are identified as iron oxalate dihydrate, Fe(C$_2$O$_4$)$\cdot$2H$_2$O (PDF number: 97-016-1344; monoclinic system.). This compound is found in nature and has the mineral name of humboldtine. The four peaks are from (-2, 0, 2), (2, 0, 0), (-4, 0, 2), and (-1, 1, 3) planes, respectively. The chemical structure of humboldtine is shown in
Figure C-1. This compound is not magnetic, thus it should not affect the NMR analysis; if a magnetic substance is present it would interfere with the detection of carbon signals.

Humboldtine were also observed acid-treated samples after two-step reactions of cellulose. Figure C-2 presents XRD patterns of cellulose samples after A, A-A, and A-B treatments. Peaks of humboldtine grew after A-A treatment, supporting that it is characteristic to the OA treatment. Oxalic acid was used in the study, and it is reasonable that corrosion of steel forms an oxalate. On the other hand, most of such peaks disappeared in case of the sample after A-B treatment, indicating such oxalate has been converted into some other composition during the reaction under basic condition. A new peak at $2\theta = 36^\circ$ was not well identified from search in the library of patterns.
Figure C-2: XRD patterns of raw cellulose and samples after A, A-A, and A-B treatments. Red dots show the peaks for Fe(C₂O₄)•2H₂O.

Figure C-3 shows XRD patterns of raw cellulose and samples after B, B-B, and B-A treatments. In contrast to XRD patterns of samples after A and A-A treatments, no peaks of iron species are found in patterns of samples after base treatment. Only the sample after B-A treatment shows the same peaks of Fe(C₂O₄)•2H₂O. The results indicates the corrosion of the reactor is not significant in case of TMAH treatment. Additionally, XRD pattern of sample after OA treatment is compared with the original OA reagent, which is shown in Figure C-4. It is clear that the peaks are not in well match with oxalic acid dihydrate, and confirmed the absence of OA as a residue in the solid sample.
Figure C-3: XRD patterns of raw cellulose and samples after B, B-B, and B-A treatments.
To further confirm the presence of iron in the solid samples of cellulose, scanning electron microscopic images were taken using a Hitachi S-3500N instrument, and chemical composition was analyzed using the equipped Energy Dispersive X-Ray Spectroscopy. Samples were covered with a thin layer of gold using a vacuum sputter-golder for better conductivity of the samples. Among the cellulose samples analyzed, it was revealed that those after OA treatment contain iron. They are not uniformly dispersed; some primary particle does not contain any iron while some particle does up to 30 atom%. Figure C-2 shows a representative result of mapping for carbon and iron atoms from the cellulose sample after A-B sequence. Mapping was conducted across the line in the center of the image, and it was clearly shown that iron atoms (green) are localized to some certain particles, while carbon atoms (red) are present homogeneously across the area. On the other hand, no iron was observed from the samples after the single-step TMAH
treatment, indicating that corrosion in 10% TMAH was not problematic under the present condition. This was consistent with the XRD study, in which no humboldtine was confirmed in the sample after TMAH treatment.

Figure C-2: SEM image of cellulose sample after A-B treatment sequence and EDS mapping result across the line in the center. Green line: Iron; Red line: Carbon.

C.3 Reference

Appendix D

Supplemental Information

D.1 Thermogravimetric Analysis (TGA)

D.1.1 Experimental

Proximate analysis of spruce sample was conducted using a thermogravimetric analyzer (TGA), TA Instruments SDT Q600. As the analytical procedure, ASTM D5142-09 (Standard Test Methods for Proximate Analysis of the Analysis Sample of Coal and Coke) was adopted. The typical analysis is carried out as follows: About 10 mg of solid sample was placed onto the pan; Under a pure N\textsubscript{2} flow with a flow rate of 100 cm\textsuperscript{3}/min, the sample was first heated from room temperature to 107 °C at a rate of 10 °C/min and kept for 2 hrs (stage 1); And then it was further heated to 950 °C at a rate of 50 °C/min and kept for 7 min (stage 2); The sample was then cooled down to 750 °C, the gas was switched to O\textsubscript{2} flow, and the temperature was kept at 750 °C for 2 hrs (stage 3). The weight loss at stage 1-3 corresponds to moisture, volatile matter, fixed carbon, respectively. The final residue is considered as ash.

Decomposition of oxalic acid was also examined by TGA. Basic procedure and conditions are same as above except the temperature profile: The sample was heated from room temperature to 500 °C at a rate of 5 °C/min and kept for 30 min.
D.1.2 Results

According to ASTM D5142-09, the proximate analysis of spruce sample was conducted. From the result of TGA analysis in Figure D-1, the composition can be estimated as shown in Table D-1.

![TGA analysis for biomass (spruce)](image)

Figure D-1: TGA analysis for biomass (spruce).

<table>
<thead>
<tr>
<th>Moisture</th>
<th>Volatile matter</th>
<th>Fixed Carbon</th>
<th>Ash</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.6%</td>
<td>77.5%</td>
<td>13.8%</td>
<td>3.2%</td>
</tr>
</tbody>
</table>

Table D-1: Proximate Analysis of Biomass (Spruce)
Figure D-2 presents TGA result for OA dihydrate. Initial loss of weight is due to the removal of water. The decomposition of OA starts at as low as 120 °C, and almost half of OA (35% of initial dihydrate form) is decomposed at around 180 °C. This confirms the observation of OA decomposition in the reactions of biomass at 150 °C as suggested in Chapter 2. Since other types of organic acids should also have a similar thermal stability, lower reaction temperatures should be considered in the future work in order to achieve a good recovery of the acid.
D.2 Elemental Analysis

D.2.1 Experimental

Elemental analysis (C, H, and N) was performed by LECO Tru Spec CHN analyzer. In a typical procedure, about 50 mg of sample was encapsulated in a tin foil capsule. Then the sample was loaded to the chamber and kept held until being dropped into the furnace. The sample was rapidly combusted at 950 °C in the oxygen rich environment. The products of combustion of C, H, and N elements were CO$_2$, H$_2$O, and NO$_x$. Amounts of CO$_2$ and H$_2$O were measured by infrared detectors, while NO$_x$ was reduced to N$_2$ over copper and determined by a thermal conductivity detector (TCD).

D.2.2 Results

CHN elemental compositions of solid samples are summarized in Table D-2. As for raw materials of spruce, cellulose and organosolv lignin, carbon content increased in the order of cellulose (44.3%) < spruce (49.9%) < lignin (69.8%). This reflects the difference of chemical structure and C/H ratio between polysaccharides and lignin. Lignin has an aromatic structure and higher C/H ratio, resulting in higher carbon content than polysaccharides such as cellulose. A biomass sample, spruce, is a lignocellulose that is comprised of both polysaccharides and lignin. Therefore, the carbon content of biomass reasonably is in between the other two.

For estimation of carbon content in liquid products, precipitates were recovered from the solution after the reactions with OA and TMAH by evaporation the water, and then the CHN analysis of these precipitates were performed (Entries: “Sp_OA_from liquid” and “Sp_TMAH_from liquid”). Higher carbon content (60.5%) of the precipitate from TMAH solution confirms higher content of lignin fragments in the solution compared to OA case.
<table>
<thead>
<tr>
<th>Sample ID</th>
<th>C</th>
<th>H</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spruce</td>
<td>49.9%</td>
<td>6.3%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Sp_Water</td>
<td>51.0%</td>
<td>6.3%</td>
<td>0.1%</td>
</tr>
<tr>
<td>Sp_OA_treated</td>
<td>54.0%</td>
<td>5.7%</td>
<td>0.1%</td>
</tr>
<tr>
<td>Sp_TMAH_treated</td>
<td>45.1%</td>
<td>6.3%</td>
<td>0.2%</td>
</tr>
<tr>
<td>Sp_OA_from liquid*</td>
<td>45.6%</td>
<td>4.1%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Sp_TMAH_from liquid*</td>
<td>60.5%</td>
<td>7.4%</td>
<td>2.3%</td>
</tr>
<tr>
<td>Sp_AA</td>
<td>55.1%</td>
<td>5.7%</td>
<td>0.1%</td>
</tr>
<tr>
<td>Sp_BB</td>
<td>45.5%</td>
<td>6.2%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Sp_AB</td>
<td>56.8%</td>
<td>6.1%</td>
<td>0.5%</td>
</tr>
<tr>
<td>Sp_BA</td>
<td>47.9%</td>
<td>6.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Cellulose</td>
<td>44.3%</td>
<td>6.3%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Cellulose_OA</td>
<td>41.1%</td>
<td>5.9%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Cellulose_TMAH</td>
<td>43.4%</td>
<td>6.3%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Cellulose_AA</td>
<td>36.2%</td>
<td>5.3%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Cellulose_BB</td>
<td>43.1%</td>
<td>6.3%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Cellulose_AB</td>
<td>41.1%</td>
<td>6.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Cellulose_BA</td>
<td>39.6%</td>
<td>5.7%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Organosolv lignin</td>
<td>69.8%</td>
<td>5.9%</td>
<td>0.0%</td>
</tr>
</tbody>
</table>
Table **D-3**: Dry-basis Elemental Composition of Biomass (Spruce)

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>H</th>
<th>N</th>
<th>S</th>
<th>O</th>
<th>Ash</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>52.8%</td>
<td>6.0%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>38.1%</td>
<td>3.2%</td>
</tr>
</tbody>
</table>

Table **D-4**: Mass Balance of Reactions with OA and TMAH (Weight basis)

<table>
<thead>
<tr>
<th></th>
<th>OA</th>
<th>TMAH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Input</td>
<td>Output</td>
</tr>
<tr>
<td>Solid</td>
<td>4.8</td>
<td>3.2</td>
</tr>
<tr>
<td>Liquid</td>
<td>95.2</td>
<td>85.7</td>
</tr>
<tr>
<td>Gas</td>
<td>0.0</td>
<td>2.9</td>
</tr>
<tr>
<td>Total</td>
<td>100.0</td>
<td>91.8</td>
</tr>
</tbody>
</table>
D.3 Infrared Spectroscopy Measurement for Biomass Samples

D.3.1 Experimental

FTIR spectra were obtained using a Nicolet 470 FT-IR spectrometer (Thermo Electron Corp.) with 200 scans/sample collected for the range of 4000-650 cm$^{-1}$ at the ambient temperature under N$_2$ atmosphere. Three samples, biomass (raw spruce), biomass sample after acid treatment, and that after base treatment, were measured.

D.3.2 Results

Figure D-3 presents FTIR spectra of biomass samples after acid and base treatment at 150 °C for 2 h. According to some literature$^{1,2}$, assignment of the major infrared absorption bands observed in lignocellulosic biomass in the spectra is summarized in Table D-5.
Figure D-3: DRIFT spectra of biomass samples after acid and base treatment.
In every sample, a broad O-H stretching mode is found at 3600-3100 cm\(^{-1}\), but slightly shaper for the acid treated sample. Other significant peaks are observed in the range of 1800-900 cm\(^{-1}\). As shown in Table D-5, many absorption bands can be assigned to the chemical bonds in both polysaccharides and lignin, making it difficult to distinguish and see significant differences. The acid treated sample showed a slightly higher peak at 1510 cm\(^{-1}\), which is only characteristic to aromatic structure. This might indicate that the presence of greater amount of lignin in the sample after acid treatment than that after base treatment, as already supported in Chapter 2 from Py-GC-MS and Solid-NMR study. In addition, it is worth mentioning that the sample after acid treatment gave the greater absorbance compared to the other two over the spectrum, which might suggest the acid treatment increased the density of the sample.

Table D-5: Assignment of Infrared Absorption Bands in Lignocellulosic Biomass

<table>
<thead>
<tr>
<th>Band (cm(^{-1}))</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>3600-3100</td>
<td>O-H stretching</td>
</tr>
<tr>
<td>2880-2850</td>
<td>CH(_2) symmetric stretching</td>
</tr>
<tr>
<td>1630</td>
<td>Adsorbed H(_2)O</td>
</tr>
<tr>
<td>1510</td>
<td>-</td>
</tr>
<tr>
<td>1460</td>
<td>-</td>
</tr>
<tr>
<td>1423</td>
<td>CH(_2) symmetric bend</td>
</tr>
<tr>
<td>1367</td>
<td>C-H deformations</td>
</tr>
<tr>
<td>1269</td>
<td>-</td>
</tr>
<tr>
<td>1155, 1065</td>
<td>C-O and C-C stretching and CH(_2) rocking</td>
</tr>
<tr>
<td>1026</td>
<td>C-O and C-C stretching and CH(_2) rocking</td>
</tr>
<tr>
<td>894</td>
<td>C1 group frequency</td>
</tr>
</tbody>
</table>

In every sample, a broad O-H stretching mode is found at 3600-3100 cm\(^{-1}\), but slightly shaper for the acid treated sample. Other significant peaks are observed in the range of 1800-900 cm\(^{-1}\). As shown in Table D-5, many absorption bands can be assigned to the chemical bonds in both polysaccharides and lignin, making it difficult to distinguish and see significant differences. The acid treated sample showed a slightly higher peak at 1510 cm\(^{-1}\), which is only characteristic to aromatic structure. This might indicate that the presence of greater amount of lignin in the sample after acid treatment than that after base treatment, as already supported in Chapter 2 from Py-GC-MS and Solid-NMR study. In addition, it is worth mentioning that the sample after acid treatment gave the greater absorbance compared to the other two over the spectrum, which might suggest the acid treatment increased the density of the sample.
D.4 Considerations on Experimental Errors

D.4.1 Evaluations of Experimental Errors

To obtain meaningful results from raw data, all experiments were repeated three times or more and the average of obtained values was presented as the result of conversion. Mathematical definitions for the sample mean and standard error are:

Sample mean (average): \( \bar{x} \equiv \frac{1}{N} \sum_{i=1}^{N} x_i \), where \( N \) is the number of observations.

Standard deviation: \( s_N \equiv \sqrt{\frac{1}{N} \sum_{i=1}^{N} (x_i - \bar{x})^2} \)

Standard error of the mean \( SE_{\bar{x}} \equiv \frac{s_N}{\sqrt{N}} \)

As a typical example, some of the raw conversion data of cellulose for A-B and B-A sequences with 10% OA and 10% TMAH are summarized in Table D-5.

Table D-6: Some Raw Data of Conversions of Cellulose by Two-Step Reactions

<table>
<thead>
<tr>
<th>Run</th>
<th>Conv. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A-B 2nd-step (overall)</td>
</tr>
<tr>
<td>1</td>
<td>63.6 (74.2)</td>
</tr>
<tr>
<td>2</td>
<td>61.5 (72.7)</td>
</tr>
<tr>
<td>3</td>
<td>60.6 (72.1)</td>
</tr>
<tr>
<td>Average</td>
<td>61.9 (73.0)</td>
</tr>
</tbody>
</table>
The variation is within 1-3 wt%, and the general trend is consistent for each run: A-B > B-A. Accordingly, it is concluded that there is a meaningful difference between the two treatment sequences in this case. As for other experiments, the standard errors were less than 3%.

**D.4.2 Evaluations of the Coefficient of Determination**

For calibration purpose, as described in Appendix B, the coefficient of determination $R^2$ is used to evaluate the goodness of the fit of a statistical model. The coefficient ranges between 0 to 1, and it presents the accuracy of the prediction. Higher the $R^2$ is, the better the model fits the data. The coefficient of determination is defined as follows:

$$R^2 \equiv 1 - \frac{SS_{err}}{SS_{tot}}$$

where

$$SS_{err} \equiv \sum_i (x_i - f_i)^2$$

where $f$ is modeled (predicted) value;

$$SS_{tot} \equiv \sum_i (x_i - \bar{x}_i)^2$$

where $\bar{x}_i$ is the sample mean (average).

**D.5 Estimation of pH from Known Value of pKa**

When preparing solutions, measured pH value was confirmed by checking with a calculated value. Derivation of pH from known value of pKa is shown below:

$$pK_a = -\log \frac{[A^-][H^+]}{[HA]}$$

$$K_a = \frac{[A^-][H^+]}{[HA]}$$

$$HA \leftrightarrow A^- + H^+$$
\[ C_A = [HA] + [A^-] \]

\[ [A^-] \approx [H^+] \]

\[ K_\alpha = \frac{[H^+]^2}{C_A - [H^+]} \]

\[ [H^+]^2 + K_\alpha [H^+] - K_\alpha C_A = 0 \]

\[ [H^+] = \frac{-K_\alpha + \sqrt{K_\alpha^2 + 4K_\alpha C_A}}{2} \]

**D.6 Difference in Colors of Liquid and Solid Samples after Reactions**

Figure **D-4** shows the photos taken for the liquid products from single-step reactions of biomass at 150 °C for 2 h. A clear distinction in color is observed between the product after reactions with water, acid (10% OA) and base (10% TMAH). The liquid before the reaction is colorless (photo not shown). Blank reactions without biomass at the same conditions did not show any color change. Therefore, the color change is the evidence for different chemical characteristics of hydrolysis of biomass with water, acid, and base.

![Products in aq. sol.](image)

**Figure D-4:** Pictures of liquid products from reactions of biomass at 150 °C for 2 h.
Pictures of spruce sample before any chemical treatment (milled into powder) and residual solid samples after two-step reactions at 150 °C for 2 h are shown in Figures D-4 and D-6. In Figure D-6, the samples are collected from several runs of reactions with different number of repetitions; therefore the quantity of the mass in the picture does not represent any significant meanings. Compared with the original spruce sample, the samples after the acid treatment have a slightly darker color, while that after the base treatment does not show much difference. Nevertheless, after A-B sequence, the sample became even darker although the second treatment was by base. The cause of this particular change in color is still unknown, and requires further attentions in the future work. In addition, when a magnet is attached to a vial containing these samples, only the sample after A-B showed some magnetic property. This might be the source of interference in Solid-NMR, where no significant carbon signal was detected. There is a possibility that the iron impurities, which might come in to the sample from the corrosion of the reactor during the first reaction with acid as discussed in Appendix C, further reacted in the second step with base, generating other types of impurities than iron oxalate. This possibility need to be carefully examined and is a suggestion for a future study.

Figure D-5: Pictures of spruce sample (soft wood biomass).
D.7 Additional XRD Measurements

Additional XRD measurements were performed on PANalytical Xpert Pro MPD theta-theta diffractometer (Source: Copper Long Fine Focus 60kV, 2.2 kW). Figure D-7 shows XRD patterns of biomass sample before and after single-step reactions at 150 °C. Compared to patterns by Scintag Pad V powder diffractometer (Figures 3-8, C-2, C-3, and C-4), clearer patterns with less noise were obtained. In a future study, this new diffractometer is suggested for XRD measurements.

Figure D-6: Pictures of biomass samples after two-step reactions at 150 °C for 2 h.
Figure D-7: XRD patterns of biomass sample before and after single-step reactions at 150 °C.

D.8 References


VITA

Yu Noda

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  - Ph. D. in Energy & Mineral Engineering with Option in Fuel Science; Minor in Chemical Engineering
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  - B.S. in Chemical Engineering
    The University of Tokyo, Japan (Thesis advisor: Dr. Kazunari Domen) *Mar. 2006*

- **Publications**

- **Conference Presentations**

- **Awards**
  - Anne C. Wilson Graduate Student Research Award (2007)
  - EME Outstanding Graduate Teaching Assistants Award (2008)
  - EMS Centennial Research Travel Award (2010)
  - The Prize for Excellence in JFTC Essay Competition 2010 (Japan Foreign Trade Council, Inc.)
  - Charles B. Darrow Award in Fuel Science (2011)
  - EMS Energy Institute Student Service Award (2011)
  - First Prize of Student Research Poster Competition at 2012 Penn State Bioenergy Symposium