Preparation of Cellulose Nanofibers from Bamboo Using Microwave Liquefaction

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PREPARATION OF CELLULOSE NANOFIBERS FROM BAMBOO USING MICROWAVE LIQUEFACTION

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ABSTRACT

Cellulose nanofibers isolated from renewable lignocellulosic biomass are considered highly promising fillers in preparing sustainable composite materials. Although previous technologies on the production of cellulose nanofibers were encouraging, drawbacks such as chemical reagent, high energy consumption, time-consumption, and equipment degradation have limited these techniques for practical applications. In this work, bamboo particles were subjected to microwave liquefaction process and the liquefied bamboo residues were characterized to have a better understanding of the liquefaction behaviors of bamboo. Then, the microwave liquefaction process was optimized for the production of cellulose raw materials and the isolation of cellulose nanofibers. The lignin fraction fractionated from the microwave liquefaction process was also characterized for use in bio-based materials.

The overall results revealed that high conversion yield of bamboo to liquid could be archived in mild microwave liquefaction reaction conditions. Lignin and hemicellulose in bamboo could easily undergo decomposition during liquefaction, while cellulose was the main resistance to the liquefaction process. The chemical and morphology analysis results revealed that the liquefied bamboo residues retained fiber structure and cellulose. Bleaching and acid hydrolysis were proved to be effective in purifying the residues for pure white cellulose fibers by removing carboxyl groups and lignin fragments. Long nanofibrils were generated by subjecting the pure cellulose fibers to high-intensity ultrasonic treatment. Good quality fibers with high holocellulose content were successfully produced by removing lignin and extractives from bamboo when the microwave liquefaction temperature was below 120°C. The relative lignin and extractives contents of the liquefied residues from the reaction at 120 °C, 9min were
as low as 0.65 and 0.49 %, respectively. Cellulose nanofibers with diameters in the range of 2-30 nm were successfully extracted from the cellulose materials with a subsequent chemical treatment as a purification process and ultrasonication as a nanofibrillation process. The main functionality of the microwave liquefaction process on the nanofiber preparation process was efficiently converting bamboo bundles into micro-sized fibers by almost completely removing lignins and extractives. The isolated cellulose nanofibers have potential application for the fabrication of thermally stable composites because of their high thermal stability. Lignins recovered from the microwave liquefaction system showed high purity and retained their natural structures. The lignin samples were completely soluble in ethanol/water, DMSO, THF, 1, 4-dioxane, and 1mol/L NaOH solution. Polylactic acid (PLA)-lignin composites were successfully fabricated, and the lignin component in the PLA-lignin blends significantly improved the UV light barrier properties of the composites. The utilization of the lignin fraction should enhance the economic value of the microwave liquefaction system on the integrated utilization of bamboo.
CHAPTER 1. INTRODUCTION

1.1. BACKGROUND

1.1.1. Cellulose Nanofibers Preparation Technologies

Cellulose nanofibers have been considered to be one of the most potentially viable renewable materials and have been widely used for the preparation of nanostructured biocomposites [1-3], tough hydrogels [4], membranes [5], and transparent nanopaper or film [6-7], owing to its high crystalline structure with strong mechanical performance and outstanding thermal stability [8]. With the growing demand for sustainable green materials, isolation of cellulose nanofibers has been extensively investigated [9-10] and prepared from a variety of lignocellulosic biomass [11-20]. For the isolation of cellulose nanofibers, various pretreatment methods have been used to remove non-cellulosic materials in plants [8, 21-28], and different nanofibrillation technologies have been employed to generate high shear forces to separate the fibrils from the purified cellulose fibers [29-32].

Although cellulose nanofibers have been successfully isolated from these aforementioned technologies, and the results were encouraging, drawbacks still exist such as chemical reagent cost, high energy consumption, time-consumption, and equipment degradation. These limited techniques for practical applications. Advanced techniques for production of cellulose nanofibers with low cost, low environmental impact, and time efficiency are still required.

1.1.2. Utilization of Bamboo Processing Residues

Currently, bamboo has been used in the preparation of high-value added products, such as panel, parquets, furniture, and structural composites, because of its rapid growth rate, availability, renewable nature, high productivity, short maturity cycles, and multiple uses.
However, in the manufacturing of bamboo-based materials, the epidermis and wax layer of bamboo usually are split off due to the poor wettability or penetration of these portions for subsequent processing, which resulted in resources waste. Therefore, the efficient utilization of these bamboo processing residues is a great need. Moreover, bamboo was found to be composed of cellulose, hemicellulose, and lignin, and the relative content for the three components was about 40-50%, 20-30%, and 20-30%, respectively. These compounds are chemically known to have a potential for the production of platform chemicals via liquefaction. Furthermore, the higher cellulose content and lower lignin content in bamboo may make it more suitable for the production of cellulose nanofibers.

1.1.3. Application of Microwave Energy on Liquefaction

Lignocellulosic biomass composed of cellulose, hemicellulose, and lignin is a valuable and worldwide accessible bioresource which can provide alternative chemicals via proper conversion processes. Recent achievements in biomass thermochemical conversion techniques have stimulated great interests in the integrated utilization of lignocellulosic biomass for the production of hydroxyl-rich biopolyols. Thermochemical methods such as pyrolysis and liquefaction have great potential to produce biofuels and valuable bio-chemicals [33-34]. Studies have shown that liquefaction provides an efficient pathway to convert solid biomass into liquid products [35-36]. The application of microwave heating in lignocellulosic biomass liquefaction was first used in wood [37], and was used in the liquefaction of various lignocellulosic biomasses [38-40]. The reason for the efficient conversion of biomass by microwave liquefaction is that microwave irradiation can directly couple microwave energy with the molecules that are present in the reaction mixture with dipolar polarization and ionic
conduction. The main advantage of microwave over conventional heating sources is that the irradiation penetrates and simultaneously heats the materials at the molecular level and therefore reduces reaction times from hours to minutes [41-44].

Solvents with high tan δ values have better beat absorption rates and are heated more efficiently by microwave irradiation. Glycerol is among the high tan δ value of common solvents [38]. Therefore, liquefaction using microwave energy as the heating source and glycerol as a solvent has great potential to reduce reaction time and advance the commercialization of this process. Moreover, glycerol is presently the main byproduct of the oleo chemicals industry and can be as high as 10% of the total biodiesel production. With the increase in global biodiesel production, glycerol production has rapidly moved into oversupply, resulting in a crash in the price of crude glycerol. Thus, the commercial utilization of this compound has become a challenge. It is, therefore, desirable to find a novel, economical utilization method for crude glycerol to improve the economics of biodiesel production. Although methanol has a low boiling point, its combination with glycerol could significantly decrease the viscosity of glycerol, which will guarantee the solvent has a good mixture with the feedstocks. Meanwhile, the addition of methanol in glycerol has been proven to be effective in avoiding recondensation reactions of lignins in liquefaction, and the benefit of using methanol in liquefaction is that it can be easily recovered after liquefaction. Furthermore, in the decomposition of lignin, the methanol –OH group functions as the nucleophile and attacks electrophilic C adjacent to glycosidic bond in the liquefaction system, creating good leaving groups and a neutral hydroxyls group by cleaving the C-O bond.
Therefore, the liquefaction system employing glycerol and methanol as binary solvent and microwave energy may be a potential approach on biomass conversion and utilization regarding efficiency and environment.

1.1.4. Liquefaction Behaviors of Biomass Cell Wall Components

As for the liquefaction of wood residues in the binary of glycerol and ethylene glycerol, lignin and hemicellulose in the wood were significantly decomposed in the initial liquefaction stage (20-40 min) with the reaction temperature of 160°C, while the cellulose content in the wood increased [45]. Pan also observed that the lignin content in liquefied Chinese tallow wood residue significantly decreased as the liquefaction processes, resulting in enriched cellulose residues [46]. Previous research indicated that the three major components (cellulose, lignin, and hemicellulose) in lignocellulosic biomass exhibited different liquefaction rates in an organic solvent with acid as a catalyst, i.e., the decomposition of the components was in the order of lignin, hemicellulose, and cellulose. Based on this mechanism, Chen proposed a selective liquefaction process for the production of cellulose and biobased resins; the finding in his research showed that large amounts of hemicellulose and lignin could be liquefied at 100 °C in 30 min, and the retained cellulose had higher susceptibility for enzymatic attack. This approach offered a new approach for the utilization of lignocellulosic biomass in the bioethanol and biobased materials industries [47]. In order to extract cellulose for use in both bio-fuels and reinforcing materials, the combination of bleach and liquefaction processes was described in the research of Li et al. [48], in which hemicellulose was selectively liquefied to get cellulose.

From the previous research results, it was obvious that cellulosic fibers could be easily produced by subjecting the raw lignocellulosic biomass to a liquefaction process with reaction
conditions properly controlled. Therefore, in this study, microwave liquefaction was proposed to generate cellulose enriched residues from bamboo. This study attempts to achieve an efficient approach for the generation of cellulose nanofibers with the combination of microwave liquefaction and ultrasonic nanofibrillation.

1.2. OBJECTIVES

The objectives of this work are:

1) To understand the liquefaction behaviors of bamboo in glycerol/methanol binary solvent using microwave energy.

2) To characterize the microwave liquefied bamboo residues and extract cellulose nanofibers from the residues.

3) To optimize the process of the isolation of cellulose nanofibers using microwave liquefaction.

4) To have a brief understanding of the recovered lignin fraction and apply it to the preparation of bio-based films

1.3. ORGANIZATION OF DISSERTATION

Chapter 1 gives an overall introduction on microwave liquefaction, cellulose nanofibers, and bamboo utilization and the purpose of this research.

Chapter 2 presents the liquefaction behaviors of bamboo residues in glycerol/methanol binary solvent using microwave heating.

Chapter 3 presents the characterization of microwave liquefied bamboo residues and describes the isolation of cellulose nanofibers from the residues.

Chapter 4 presents the process optimization of the extraction of cellulose materials using
microwave liquefaction.

Chapter 5 describes the isolation of cellulose nanofibers from bamboo using microwave selective liquefaction as pretreatment process.

Chapter 6 introduces the recovery of the lignin fraction from microwave liquefaction and its potential utilization in the preparation of bio-based materials.

1.4. REFERENCES


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CHAPTER 2. LIQUEFACTION BEHAVIORS OF BAMBOO RESIDUES IN A GLYCEROL-BASED SOLVENT USING MICROWAVE ENERGY

2.1. INTRODUCTION

Liquefaction of biomass has long been of interest in the conversion of biomass waste into value-added polymeric products for the preparation of polyurethane films, polyurethane foams, phenolic resins, and liquid fuels [1-4]. As an important thermochemical method, liquefaction of forest and agricultural crop wastes have been carried out with various liquefaction solvents for different end products [5-9]. However, the most commonly explored solvents for liquefaction are phenol and polyalcohol. These solvents are effective, but require large quantities and are costly. Phenol is derived from petroleum and as such is generally not considered as an environmentally green chemical. A by-product of the transesterification process to manufacture bio-diesel is the production of glycerol. For each kilogram of biodiesel produced, about 0.1 kg of a crude glycerol accompanies [10]. Therefore, successful replacement of petroleum-based solvents, such as phenol by glycerol in a liquefaction process should have potential to improve the economics of the process.

Bamboo has been widely used for a variety of products such as flooring, furniture, panels, and paper. However, bamboo processing residues such as branches, epidermis, wax, and bamboo pulping by-products have been considered as a waste due to the limited processing technologies for these materials. Although bamboo residues have previously been used in liquefaction reactions to provide bio-based products [11-12], an economically viable bamboo waste conversion technology is yet to be realized because of the high cost of the liquefaction

process. Microwave energy has recently been applied in the liquefaction of lignocellulosic biomass [13-16]. Since microwave energy penetrates and produces a volumetrically distributed heat source, heat is generated throughout the material and leads to faster heating rate and improved kinetics as compared to conventional heating; the development of a liquefaction process combining the greater microwave heating efficiency and the by-product glycerol from the bio-diesel production to replace petroleum-based solvents is a promising method to enhance economic viability of the process and benefit agricultural producers, bio-based processing industries, and the environment. In this study, liquefaction of bamboo with a glycerol-based solvent using microwave energy was systematically investigated. The physical and chemical properties of the liquefied bamboo residues from different reaction conditions were comparatively analyzed. The objective of the study was to optimize the liquefaction reaction with glycerol as a major solvent component.

2.2. EXPERIMENTAL

2.2.1. Materials and Chemicals

Three-year-old bamboo (*Phyllostachys pubescens*) was harvested from the Kisatchie National Forest near Pineville, La. The bamboo culms were reduced to particles. The particles were screened to collect particle that passed through a 40-mesh sieve and retained on a 100-mesh sieve and then dried to a constant weight in an oven at 80°C. The dried particles were stored in polyethylene bags and used without further treatment. All acids, glycerol, and methanol used were of reagent grade and obtained from commercial sources.

2.2.2. Liquefaction Reaction

Liquefaction of bamboo was carried out in a Milestone (Shelton, CT) MEGA laboratory
microwave oven. A mixed glycerol and methanol ratio of 2/1 (w/w) was used as the solvent at a solvent to bamboo ratio of 4/1 (w/w). Sulfuric acid content of 1.75% of solvent weight was used as the catalyst. A typical reaction mixture consisting of 2 g of bamboo particles, 8 g of solvent, and 0.14 g of sulfuric acid was loaded in the Teflon vessels with a magnetic stirring bar. The Teflon vessels were then placed on the rotor tray inside the microwave cavity. The temperature was monitored using an ATC-400FO automatic fiber optic temperature control system. Based on monitored temperature, the output power was auto-adjusted during liquefaction.

In this chapter, the temperature was increased from room temperature to 150°C at a heating rate of 37.5°C min⁻¹ and then was kept constant for 3 min. unless otherwise noted. After a cooling period of 30 min. at the end of the reaction, the material was dissolved in 150 mL of methanol under constant stirring for 4 h. The liquefied solutions were then vacuum-filtered through Whatman No. 4 filter paper. The solid bamboo residue retained on the filter paper was oven-dried at 105°C and the conversion yield was calculated as follows:

\[
\text{Conversion yield (\%)} = \left(1 - \frac{\text{weight of residue}}{\text{weight of raw material}}\right) \times 100
\]

2.2.3. Scanning Electron Microscopy

The structure and the surface morphology of the liquefied bamboo residues were observed using scanning electron microscopy (SEM, JSM-6610). Test samples were coated with gold using a vacuum sputter coater before subjected to the SEM analysis.

2.2.4. Fourier Transform-Infrared Spectroscopy

The FT-IR analysis of the liquefied bamboo residues was performed by a Nicolet Nexus 670 spectrometer equipped with a Thermo Nicolet Golden Gate MKII Single Reflection ATR
accessory. A small amount of residue was applied directly on the diamond crystal. Data collection was performed with a 4 cm\(^{-1}\) spectral resolution and 32 scans were taken per sample.

### 2.2.5. Chemical Analysis

The Klason lignin and alcohol-toluene solubility of the liquefied bamboo residues were determined in accordance with ASTM D1105-96 (ASTM 1996) and D1106-96 (ASTM 1996) [17-18]. Each sample was performed in duplicate.

### 2.2.6. Thermogravimetry Analysis

TG/DTG analysis of the original bamboo and liquefied bamboo residues was conducted on a thermal analyzer, TGA Q50, to simultaneously obtain thermogravimetric data. About 2 mg of sample was used in the analysis of TG/DTG. Pyrolysis was terminated at 800\(^\circ\)C with a heating rate of 20\(^\circ\)C/min under a flow of 60mL/min of nitrogen gas.

### 2.3. RESULTS AND DISCUSSION

#### 2.3.1. Liquefaction Reaction Process

The effect of liquefaction temperature on the conversion yield at three reaction times is shown in Figure 2.1A. The conversion yield increased rapidly and consistently to the maximum as the liquefaction temperature increased; and thereafter, the conversion yield decreased slightly with further increase in the reaction temperature, indicating a possible curvilinear correlation between temperature and conversion yield.

It is generally recognized that liquefaction of lignocellulosic biomass in an organic solvent with an acid catalyst at high temperature is a complex solvolysis processes involving simultaneous reactions of chemical degradation/decomposition, de-polymerization, and re-condensation depending on the process parameters. Several previous studies on liquefaction
with conventional heating have shown that low residue content (i.e., increasing conversion yield) was mainly due to the decomposition of hemicellulose, lignin, and amorphous cellulose and the increasing residue content was attributed to the self-polymerization of the decomposed lignin compounds and/or condensation of lignin fragment with reagents [19-20]. Similar decomposition and condensation reactions are involved for liquefaction with microwave heating but with remarkable enhancement of the reaction kinetics for microwave energy as compared to conventional heating. A good curvilinear correlation was found between the liquefaction temperature and conversion yield with $R^2$ values of 0.988, 0.941, and 0.958, respectively for reaction times of 3, 7, and 11 min (Figure 1A). It is interesting to note that the values of the maximum conversion yield showed little difference, indicating that the different combinations of temperature and time contribute to almost the same maximum conversion yield. Nevertheless, at high operating temperature, liquefaction can lead to co-condensation reaction of the decomposed (depolymerized) compounds or lignin fragments which is mostly insoluble and these products are of little economic value unless they are subsequently upgraded. However, low temperature is a preferable operating parameter, but with the significant disadvantage of prolonging the reaction time for optimum conversion. Thus, considering reaction efficiency and cost, the reaction condition of 150°C/7 min seems to be promising.
The significant effect of reaction time on the conversion yield at three temperatures is
shown in Figure 2.1B. The results showed clearly the interactions between reaction time and temperature to affect the conversion yield. At a low temperature of 120°C, the conversion yield increased from 58.71% to 80.90% as reaction time increased from 3 to 13 min, indicating liquefaction was controlled mainly by a decomposition reaction; while under a high reaction temperature (180°C), the conversion yield showed a decreasing trend, indicating the maximum yield was attained rapidly before 3 min, and the decreasing yield indicated that liquefaction was controlled mainly by a re-condensation reaction.

Rapid liquefaction of bamboo with microwave energy under rather low reaction temperatures (i.e., 120°C) is one of the most interesting results of the study. The prevailing reaction time and temperature with conventional heating in wood liquefaction is 60 min at 150-160°C [21-22]. One possible explanation to the rapid liquefaction is the physical effect of the microwave treatment on lingo-cellulosic properties. Studies have shown that microwave pre-treatment can open water pathways in wood, dramatically increase permeability, and accelerate moisture migration in wood [23-24]. The physical changes in wood properties with microwave energy facilitate favorable conditions for the liquefaction solvents to react. Furthermore, the changes in wood properties combined with the faster heating rates through the volumetrically distributed heat of microwave energy enhance each other to create a heating system which is significantly superior to that of conventional heating. It should be noted that the faster heating rates and shorter process times with microwave energy, as compared to that of conventional heating, can provide enormous process development potential for economically viable liquefaction processes such as a continuous reactor which could greatly advance the commercialization of the process.
2.3.2. Microscopic Analysis of Residue

The SEM images of original bamboo and liquefied residues are shown in Figure 2.2. The original bamboo (Figure 2.2a) showed uneven-sized bundles with many small fragments on the rough surfaces. As can be seen in the image of the residue at 120°C, the large bundles were reduced into smaller fiber bundles with smooth surfaces indicating that the small fragments attached on the surface of the original bamboo were almost completely removed during the liquefaction reaction. Moreover, broken single fibers or parenchyma cells were also observed from the SEM image (Figure 2.2b) of the decomposed residue. It is apparent that the lignin component was removed, leaving the retained tissue cells in an untidy order.

Figure 2.2. SEM images of liquefied residues obtained from different conditions: a. raw material; b. 120°C, 3min; c. 120°C, 7min; d. 120°C, 13min; e. 150°C, 3min; f. 150°C, 7min; g. 150°C, 9min; h. 150°C, 13min; i. 180°C, 3min.
The significant temperature effect on liquefaction was most evident with reaction at 150°C. The SEM image at 3 min reaction time displayed similar individual fiber morphology to that at 120°C reacted for 13 min, which was with a reaction time of more than 4 times longer; indicating the significant effect of liquefaction temperature (Figure 2.2e). Furthermore, it is interesting to note, at a reaction time of 5 min., the fiber and parenchyma cells were broken and a large surface area was created and resulted in a relatively homogeneous texture (Figure 2.2f). As reaction time increased to 9 min, the liquefied residues turned into small spherical granule substances on the surface (app. 0.1-0.2μm in diameter) (Figure 2.2g); and finally, the granules fused and carbonized into a conglomerate mass as reaction time increased to 13 min (Figure 2.2h). It should be noted that at 180°C, the formation of carbonized conglomerate mass was the main substance produced early in the liquefaction (Figure 2.2i).

2.3.3. FT-IR Spectroscopy

The transmittance spectra of the residues from different liquefaction conditions are shown in Figure 2.3. Significant differences for the spectra were found between the original bamboo (spectra a) and liquefied residues (spectra b, c, d) (see Figure 2.3). A broad peak at around 3320 cm⁻¹ which was assigned to –OH groups and the peak at around 2880 cm⁻¹ related to methyl and methylene stretching were stronger in the original bamboo. The two peaks that presented in the spectra of all the bamboo components became weak or almost nonexistent as the liquefaction temperature increased, showing a global decomposition of the bamboo.
Figure 2.3. FTIR transmittances of (a) original bamboo and residues: (b) 95°C, 7min, (c) 120°C, 7min, (d) 140°C, 7min, (e) 180°C, 7min, (f) 150°C, 3 min, (g) 150°C, 5min, (h) 150°C, 9min. Other conditions: glycerol/methanol, 2/1; microwave power, 550W; sulfuric acid, 1.75%; solvent/bamboo, 4/1.

The absorbance peak at 1735 cm\(^{-1}\) assigned to C=O stretching vibration of the carboxyl
and acetyl groups in hemicellulose was weakened in the spectra of residues from 95 and 120°C, and then was reduced to a shoulder in the spectra of residue from 140°C. However, the peak was intensified in the spectra of residue at 180°C. It can be concluded that hemicellulose could be decomposed at lower temperature and then undergo repolymerization at higher temperature. The absorbance at 1596 cm\(^{-1}\) and 1506 cm\(^{-1}\) arising from the aromatic skeletal vibration, the absorbance at 1456 cm\(^{-1}\) assigned to C-H deformation combined with aromatic ring vibration, and the band at 1230 cm\(^{-1}\) corresponding to methoxyl groups of lignin were all shown to be strong peaks in the spectra of the original bamboo. These peaks were weakened in the spectra of residue at 95°C. As the temperature increased to 140°C, the absorbance bands at 1506, 1456 and 1230 cm\(^{-1}\) disappeared and the band at 1596 cm\(^{-1}\) became a small shoulder. It appears that the lignin was almost completely decomposed at 140°C within 7 min. However, with further increase in reaction temperature, these peaks intensified again; indicating that the decomposed compounds such as aromatic ketone groups interact with the aromatic hydroxyl group to form an aromatic network.

The bands for different functional groups of cellulose such as the methylene groups at 1420 and 1371 cm\(^{-1}\); CH\(_2\) rocking vibration at 1320 cm\(^{-1}\), and C-O band at 1162 cm\(^{-1}\) and 1108 cm\(^{-1}\) and CH deformation at 899 cm\(^{-1}\) were weakened in the spectra of residues from low liquefaction temperatures and then disappeared in the spectra of residue at 180°C. According to Akerholm [25], the band at around 1420 cm\(^{-1}\) is associated with the amount of crystalline structure of the cellulose. As shown in the spectra of residue from 180°C (Figure 3A), the band at 1420 cm\(^{-1}\) disappeared, indicating that cellulose, even the crystalline cellulose, could be liquefied with a glycerol/methanol co-solvent using microwave energy. This was consistent
with our previous results that microwave heating could provide favorable conditions for fast and complete liquefaction of bamboo. Though microwave irradiation could promote the decomposition of cellulose, it also caused lignin/hemicellulose recondensation. Hence, liquefaction conditions should be properly optimized for the specific functional applications.

The spectra of the liquefied residues as a function of reaction time are shown in Figure 3B. It was obvious that absorbance bands that characterized functional groups of hemicellulose and lignin have disappeared, leaving the weakened bands assigned to cellulose which is visible in the spectra of residue liquefied for 5 min at 150°C. This was in good agreement with the conversion yield result which showed that about 90% of the solid bamboo was converted into the liquid phase. By further prolonging the reaction time to 9 min, the retained cellulose was decomposed along with the recondensation of lignin or hemicellulose.

The disappearing bands assigned to the functional groups of cellulose and the intensive bands for those of the hemicellulose and lignin in the FT-IR spectra of the residues obtained from higher temperature/longer reaction time suggested that liquefaction under critical conditions induced by microwave heating was a complex reaction involving the lignin/hemicellulose repolymerization and cellulose decomposition reactions.

2.3.4. Chemical Analysis

The lignin content and the relative intensities of its typical bands for original bamboo and liquefied residues were determined. As shown in Figure 2.4, the Klason lignin content and alcohol-toluene extractive for original bamboo were 20.91% and 7.24%, respectively. After liquefaction, the lignin content was reduced to 11.54% and 1.85% for the residues from 95°C and 120°C, respectively. The negligible alcohol-toluene extractive content for the residues from
lower temperatures indicated that the alcohol-toluene soluble substances in bamboo such as waxes, fats, resins, and gums were transferred into liquid with the liquefaction process. The lignin content for the residues from 180°C was 51.1% and the alcohol-toluene extractive content was 40.3%, which was inconsistent with the average Klason lignin content and alcohol-toluene extractives for woody materials. Therefore, the classical chemical method was not suitable for the quantitative analysis of major components of the residues from multiple solvolysis reactions.

Figure 2.4. Effect of temperature on the lignin and alcohol-toluene extractive contents of liquefied residues. Other conditions: time, 7min; microwave power, 550W; sulfuric acid, 1.75%; solvent/bamboo, 4/1; the content was determined based on the mass of residues.

As listed in Table 2.1, the relative intensities of absorbance bands showed a decreasing trend as the liquefaction temperature increased from 95 to 140°C. It was interesting to note that the trend of relative intensities with the liquefaction temperatures was in accordance with that
for the lignin content as shown in Figure 4. It made sense that the intensities of bands assigned to the functional groups of lignin could be used to estimate the decomposed degree of lignin in bamboo. The absorbance intensities for the residues from 180°C are as strong as that in the original bamboo. The higher intensities were attributed to the functional groups of the insoluble intermediate compounds of lignin and not from lignin. Thus, the intensities could also be used to indicate the recondensation degree of lignin fragments in the liquefaction reactions.

Table 2.1. FT-IR absorbance bands assigned to functional groups of lignin retained in liquefied residues

<table>
<thead>
<tr>
<th>wavenumber/cm</th>
<th>assignment</th>
<th>control</th>
<th>95°C</th>
<th>120°C</th>
<th>140°C</th>
<th>180°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1596</td>
<td>aromatic</td>
<td>0.17</td>
<td>0.12</td>
<td>0.09</td>
<td>0.08</td>
<td>0.16</td>
</tr>
<tr>
<td>1506</td>
<td>skeleton</td>
<td>0.15</td>
<td>0.10</td>
<td>0.08</td>
<td>- a</td>
<td>0.15</td>
</tr>
<tr>
<td>1456</td>
<td>C-H stretching</td>
<td>0.18</td>
<td>0.14</td>
<td>0.10</td>
<td>-</td>
<td>0.19</td>
</tr>
<tr>
<td>1230</td>
<td>methoxyl groups</td>
<td>0.25</td>
<td>0.18</td>
<td>0.11</td>
<td>-</td>
<td>0.24</td>
</tr>
</tbody>
</table>

- a Indicates no peaks generated

2.3.5. Thermogravimetric Analysis

The TG and DTG curves of the original bamboo and residues are shown in Figure 2.5. The weight loss process for all the samples could be divided into three stages: the mild weight loss stage, the main pyrolysis stage, and the charring stage. As shown in Figure 2.5A, initial weight loss was found in the mild weight loss stage for the original bamboo and the residue at 140°C (curve a, b), while the TG curve for residues at 150°C/9min or 180°C/7min (curve d, e) showed no apparent weight loss in this stage.
Figure 2.5. TG (A) and DTG (B) curves for (a) original bamboo, decomposed residues (b) 140°C, 7min, (c) 150°C, 5min, recondensed residues (d) 150°C, 9min, (e) 180°C, 7min. Other conditions: glycerol/methanol, 2/1; microwave power, 550W; sulfuric acid, 1.75%.

The initial weight loss for the original bamboo was attributed to the evaporation of lower molecular weight compounds. The initial weight loss for the residue at 140°C may be attributed to the volatilization of retained fragments of decomposition from the major bamboo components with the liquefaction process; however, these fragments were resolved when liquefaction was conducted at 150°C as can be seen in Figure 2.5A (curve c). The TG curves
also showed that the weight loss percent of the residues at 140°C/7min, 150°C/5min was the highest in the main pyrolysis stage, and the pyrolysis char yield was the lowest. Compared to the original bamboo, the residue at 140°C/7min, and the residue at 150°C/5min, the residue at 180°C/7 showed wider pyrolysis temperature, less weight loss percent, and higher pyrolysis char. It is clear that this was correlated with acid-insoluble substances with polyphenolic structures such as an aromatic skeleton as indicated by the FT-IR spectra. The higher thermal stability of these structures contributed to its recondensed substances. This could be well evidenced by the findings of Niu\textsuperscript{26} who found that the thermal stability of the recondensed residue was mainly from the substances with higher molecular weight formed by polyphenolic structure compounds.

Significant differences were also found for the sharp peaks corresponding to the maximum degradation rate in the DTG curves between all the samples as shown in figure 2.5B. The DTG curves for the residues obtained under mild conditions (140°C/7min, 150°C/5min) showed higher degradation rate and lower maximum degradation rate temperature compared to those obtained from higher temperature/longer reaction time. The maximum degradation rate and temperature for the original bamboo were intermediate. These differences in degradation rate are largely related to the differences in chemical components between the original bamboo and residue samples. As for the three components of woody materials, hemicellulose was the easiest to be pyrolyzed, cellulose, then lignin in TG analysis.\textsuperscript{27} However, when lignocellulose was applied in a liquefied process using organic solvents, lignin was the most reactive component and easily underwent recondensation.\textsuperscript{28} Based on these reasons, the removal of lignin by liquefaction under mild reaction conditions contributed to the lower weight loss temperature
and higher weight loss rate for the residue. The recondensed substances from reactions of decomposed lignin fragments resulted in the lowest maximum weight loss rate and highest maximum weight loss rate temperature for the residues.

2.4. CONCLUSIONS

Conversion of bamboo at about 94.33% was achieved by liquefaction with glycerol/methanol using microwave energy. Liquefaction temperature and time had a mutual effect on the liquefaction process. The liquefaction was complex solvolysis chemical reactions under critical conditions according to the residue characteristics. The FT-IR spectra could be selected as a simple method to evaluate the liquefied degree of bamboo and its three main components. Liquefaction behaviors under different conditions were also distinguished by the SEM images.

2.5. REFERENCES


CHAPTER 3. EXTRACTION OF CELLULOSE NANOFIBERS FROM MICROWAVE LIQUEFIED BAMBOO RESIDUES

3.1. INTRODUCTION

Biomass is widely considered as an important feedstock because of its renewability, ease of degradation, and availability. According to Perlack and Stokes, the current biomass resource availability annually was about $1.37 \times 10^9$ dry tons from forestlands and croplands [1]. Recently, utilization of biomass for bio-energy or bio-chemicals has attracted great attention [2-6]. For the production of biopolyols or biobased polymers, pyrolysis and liquefaction are two common pathways. However, liquefaction has more potential in converting biomass into valuable products because of its mild reaction conditions compared to pyrolysis [7-9]. Liquefaction of biomass using organic solvents under conventional heating sources, such as oil has been carried out before, and the liquefied products have been also evaluated for the preparation of polyurethane foams and phenolic resins [10-13].

Usually, in the conventional liquefaction system, inefficient thermal conduction on the surface of the feedstocks results in ineffective energy utilization, and very fine feedstock grinding (smaller than 200 mesh) was required because fine particles increase overall heat transfer in a certain extent. However, this requires large amount of energy for size reduction, which in turn increases the whole energy consuming in the entire system. The application of microwave irradiation to wood liquefaction has been recently reported [14]. Results have shown that microwave-assisted liquefaction could convert fine grinding wood feedstock into

biopolyols with a high conversion yield (≥90%) in minutes [15-17]. This is mainly due to the fact that heating by microwave is direct and volumetric and thus results in efficient biomass conversion. Due to the benefits of microwave heating in wood liquefaction, various lignocellulosic such as wheat straw lignin [18], bamboo [19-20], sugarcane bagasse [21], and corn stover [22] have been subjected to microwave assisted liquefaction system for the production of biopolyols for alternatives of petroleum products. However, in all these studies only fine grinding feedstocks were used as raw materials, the attempts of using large particles has not been investigated. Thus, it is worth to examine that if microwave liquefaction could still efficiently liquefy large size feedstocks.

Even though microwave liquefaction has been recently considered as a promising approach for the conversion of renewable lignocellulosic biomass into liquid biopolyols, previous research mainly focused on the optimization of microwave liquefaction process and applications of the liquefied products on bio-based materials, while the liquefied residues were discarded as waste and explorations of its potential utilizations were ignored. As for the liquefied residues, Pan et al characterized the liquefied wood residues from different liquor ratios and cooking methods [23]. Zhang et al reported the effects of the reaction time, liquefaction temperature, acid concentration, and liquor ratio on the chemical properties of the liquefied wood residues [24]. Results in both of these research indicated that characterization of the liquefied residues could provide information for better understanding the liquefaction behaviors of lignocellulosic biomass. The research on utilizations of liquefied lignocellulosic biomass residues has not yet been reported.
The estimated annual yield of moso bamboo was about $1.8 \times 10^7$ tons and was greater than all other kinds of bamboo [25]. The integrated utilizations of moso bamboo via liquefaction may provide potential approach for the production of high value-added bio-products. Therefore, in this paper large particle size bamboo feedstocks were subjected to microwave liquefaction system; the liquid products were first evaluated for further applications. Then, the remaining residue was collected and its physicochemical properties including content, morphology, and chemical structures with respect to particle size and reaction conditions were comprehensively investigated. Finally, the residues were given to chemical treatment and ultrasonic nanofibrillation process for the extraction of nanofibrillated cellulosic fibers. The specific objective of this study is aimed to elucidate the microwave liquefied bamboo residue and exploit its potential use in the production of high value-added nanofibers. The results in this study will provide an efficient pathway in making full use of lignocellulosic biomass via microwave liquefaction and with subsequent process.

3.2. MATERIALS AND METHODS

3.2.1. Materials

Moso bamboo was harvested in Pineville, LA, USA. Bamboo culms were crushed into small particles using a Buffalo Hammer Laboratory Mill equipped with an 8 mm screen. Particles with size of 10-20, 20-40, 40-60, and 60-80 mesh were separated and collected by using an auto-screening machine (An RX-29 Ro-Tap). The particles were then dried to a constant weight in an oven at 105 °C. All acids, glycerol, and methanol used were of reagent grade and obtained from commercial sources.
3.2.2. Microwave Liquefaction

Liquefaction of bamboo was performed in a Milestone Ethos Ex Microwave Extraction System equipped with 100mL Teflon vessels. A mixture of 2 g feedstocks, 3 g methanol, 6 g glycerol, and 0.14 g sulfuric acid (98%) were loaded in a vessel. The vessels were put in the oven and irradiated with an output power of 550W. The temperature was increased from room temperature to the desired temperature and then was kept constant for 7 or 12 min. After the reaction, the vessels were cooled for approximately 30 min. At the end of the reaction, the material was dissolved in 150 mL of methanol under constant stirring for 4 h. The liquefied solutions were then vacuum-filtered through Whatman No. 4 filter paper. The solid bamboo residue retained on the filter paper was oven-dried at 105°C and the residue yield was the mass percentage of the residue to the original weight of bamboo.

3.3.3. Extraction of Nanofibrillated Cellulosic Fiber

The residue samples were initially bleached in acidified NaClO₂ solution (0.1% w/v) at 75°C for 1h. The bleaching process was to remove the phenolic compounds or molecules from lignin retained in the fibers as well as the carbonyl groups and carboxyl groups that may be introduced during the liquefaction process. The bleaching was carried out at a 100 mL/g solution to residues. The residue was filtered and washed with deionized water until its pH was neutral. Next, 1 g of the bleached samples was hydrolyzed in 20 mL sulfuric acid hydrolysis (2%, 25%, and 45%) at room temperature for 1h. Hydrolysis was terminated with 10 fold cold water. The diluted suspension was vacuum-filtered. The filtered residues were rinsed with deionized water followed by centrifugation (7500 rpm at 4°C for 15min). The process was repeated until the pH of the precipitate was constant. The acid hydrolysis was used to eliminate
traces of non-cellulosic impurities and partially hydrolyzed the amorphous cellulose. The chemically purified residue samples were soaked in deionized water (concentration 0.2wt %) and then subjected to ultrasonic fibrillation using an ultrasonic generator. The process was performed at a frequency of 25 kHz with an output power of 750 W for 25 min. The ultrasonic fibrillation was conducted in an ice bath, and the ice was maintained throughout the entire process. The sonicated suspension was centrifuged at 10000 rpm for 15 min. The precipitate was oven dried and weighed. The weight of the nanofibrillated cellulosic fibers was the difference between the acid hydrolyzed fiber and the precipitate which was mainly composed of micro sized and sub-micron sized cellulosic fibers. The yield of the nanofibrillated cellulosic fiber was defined as the percentage of its weight of that of the initially charged liquefied residues.

3.3.4. Characteristics Analysis

Scanning Electron Microscopy (SEM) was conducted using a NeoScope (JCM-5000) scanning electron microscope to characterize the structure of the residues. All samples were attached onto the SEM stubs using carbon tabs and then coated with gold using a sputter coater (EMITECH K550X). The dimensions of liquefied residue were measured by using a Leica laboratory micro system. The samples were pressed from two slides glass after blending with a 1:1 w/w solution of glycerol and methanol. 300 particles were randomly chosen to analyze.

Fourier transform infrared spectroscopy (FT-IR) analysis of the samples was performed by a Nicolet Nexus 670 spectrometer equipped with a Thermo Nicolet Golden Gate MKII Single Reflection ATR accessory. A small amount of residue was applied directly on the diamond crystal. Data collection was performed with a 4 cm⁻¹ spectral resolution and 32 scans.
were taken per sample. Lignin content of bamboo raw material and the liquefied residue samples were determined in accordance to Laboratory Analytical Procedure (LAP, Issue Date: 4/25/2014) [26].

Nanofibrillated cellulosic fibers was confirmed by Transmission electron microscopy (TEM). The concentration of aqueous cellulose nanofibers suspensions was diluted from 0.2% to 0.01% (w/w). A droplet of the diluted suspension was deposited on the surface of carbon-coated copper grids. As for contrast in TEM, the cellulose nanofibers were negatively stained in a 2% (wt) solution of uranyl acetate. The morphology of cellulose nanofiber was observed using a transmission electron microscope (TEM, JEOL 100CX, JEOL, Inc., Peabody, MA, USA) with an accelerating voltage of 80Kv. The diameter of the cellulose nanofibers were calculated by measuring one hundred fibers randomly selected from several TEM images.

3.3. RESULTS AND DISCUSSION

3.3.1. Microwave Liquefaction of Bamboo

The residue content with respect to particle size and different reaction conditions are shown in Figure 3.1. With reaction conditions of 120°C/10-15 min, the residue yield decreased with decreasing particle size. As for particle size, it is generally considered that the smaller the size or volume of the particle, the larger the surface area of the particle. From the results in this study, particles of smaller size could be more easily attacked or accessed by chemicals resulting in a uniform distribution of feeding materials in the dispersion. Meanwhile, microwave energy penetrates and produces a volumetrically distributed heat source; heat is generated throughout the material and leads to faster heating rates [17]. Therefore, the uniform distribution of chemical reagents in smaller particles combined with the uniformly distributed heat induced
by microwave energy enhanced the decomposition of bamboo, which resulted in relative low residue content.

![Graph showing residue content with respect to bamboo particle size under various liquefaction conditions](image)

**Figure 3.1.** Residue content with respect to bamboo particle size under various liquefaction conditions

For comparison, the residue content from 120°C/15min was lower than that from 120°C/10min, revealing that prolonging the reaction time could accelerate the liquefaction extent of bamboo. With the reaction temperature of 140°C, the residue content with respect to particle size exhibited a different pattern compared to that at 120°C; i.e., the residue content first decreased and then stayed stable, indicating that re-condensation occurred in the liquefaction of bamboo with a smaller particle size (proved by the higher lignin content as discussed latter). This result demonstrated that smaller particles could not only enhance the decomposition with mild reaction conditions (120 °C), but could also contribute to the re-polymerization by a critical liquefaction process (liquefaction under higher temperature with longer reaction time).
Figure 3.2. GC-MS chromatograms and chemical structures of compositions of the biopolyols from microwave liquefied bamboo particles (60-80 mesh) at 140°C within 15min.

Chemical compositions of the biopolyols from the liquefaction conditions of 140 °C, 15min, 60-80 mesh were analyzed using GC-MS. The GC-MS chromatogram and structures of the identified components in the biopolyols is presented in Figure 3.2. The majority of compounds decomposed from bamboo were comprised of five carbon sugars (alpha.-Ribopyranoside, methyl; beta.-D-Ribopyranoside, methyl), six carbon sugars [D-Allose; alpha.-D-Glucopyranoside, methyl; methyl (methyl 4-O-methyl-. alpha.-d-mannopyranoside) urinate], and phenolic compounds [3- (2, 3, 4 -Trimethoxyphenyl) propionic acid; 2-Propenoic acid, 3-(4-hydroxyphenyl)-, methyl ester; Benzaldehyde, 2-hydroxy-4-methoxy--; methyl biphenyl-4-carboxylate]. The presence of C₅ and C₆ sugars derived from hemicellulose and cellulose indicated the degradation of carbohydrates, and the aromatics in the biopolyols
evidenced the decomposition of the lignin. Since the sugar derivatives processed 2-5 hydroxyl groups, the biopolyols in this study could be used as potential chemicals for the preparation of polyurethane foams because of its large amount of hydrogen bonds. Further analysis and potential utilizations of the biopolyols will be evaluated in other study.

3.3.2. Morphology Analysis

The microstructures of the liquefied bamboo residues were characterized by SEM, and the SEM images are presented in Figure 3.3. The images showed that the liquefied bamboo residues exhibited micro sized shape and comprised of fibers and parenchymas. Residues mainly consisted of long fibers and fiber bundles were obtained from liquefaction of 120°C/20-40mesh/10min (Figure 3.3a), while the residues with short fibers and flat cell fragments were observed on the image for samples from 120°C /60-80/mesh/10min (Figure 3.3c). This result indicated that bamboo tissues were much more easily susceptible to decomposition as the particle size decreased with microwave liquefaction. It is known that the middle lamella possesses the most lignin, and then the cell wall itself [27]. Since single fibers and parenchymas were shown in the residues from reactions of 120°C, it could be concluded that lignins in the middle lamella should be all dissolved and resulting in the dissociation of bamboo fiber bundles.

Figure 3.3 also illustrates the effect of temperature on the morphology of the residues. For comparison, residues from conditions of 140°C/10-20mesh/10min were found to have much less complete fibers than those from 120°C/10-20mesh/10min. Most of the residues from 140°C were broken cells (fibers and parenchymas) (Figure 3.3 d-e). It is apparent that the cell wall components have been decomposed under 140°C. As for the cell wall, its dominate component is microfibrils (aggregated by cellulose molecules) embedded in matrix polymers such as lignin
and hemicellulose [28]. The damage of the cell wall indicated that lignin and hemicellulose wrapped around the microfibrils has been almost completely decomposed and the exposure of the microfibrils to the chemical reagents resulted in the depolymerization of celluloses. The changes in microstructures of residues with respect to reaction conditions may also provide explanation that the liquefaction of bamboo components was in the order of lignin, hemicellulose, and then cellulose. Significant differences in residue morphology between samples from 140°C/10min (Figure 3.3d) and 140°C/15min (Figure 3.3e) were also found, revealing that at 140 °C reaction time could significantly affect the microstructures of the residues. It was interesting to note that small spherical granule substances were observed on the surface of residues from 140°C/60-80mesh/15min (Figure 3.3f). The observed granules were ascribed to recondensed lignin fragments [29]. This result revealed that microwave liquefaction of bamboo particles under 140 °C was complicated involving decomposition and recondensation.
In order to further clarify the changes in morphology of the liquefied bamboo residues during microwave liquefaction, dimensions including length, diameter, and aspect ratio of the residues were measured. The dimensions of the residues with respect to particle size from different liquefaction temperatures (120°C/140°C) are shown in Table 3.1. The result in Table 3.1 showed that liquefaction temperature has a significant effect on the length of the residue, i.e., the length of residue was significantly reduced (56.75%) when the reaction temperature increased from 120°C to 140°C. However, no significant changes in residue diameter were

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Particle Size</th>
<th>Time</th>
<th>Length Reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>120°C</td>
<td>10-20mesh</td>
<td>10min</td>
<td></td>
</tr>
<tr>
<td>140°C</td>
<td>10-20mesh</td>
<td>15min</td>
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</tbody>
</table>

Figure 3.3. SEM images of liquefied residues obtained from different reaction conditions: (a) 120°C, 10-20mesh, 10min; (b) 120°C, 20-40mesh, 10min; (c) 120°C, 60-80mesh, 10min; (d) 140°C, 10-20mesh, 10min; (e) 140°C, 10-20mesh, 15min; (f) 140°C, 60-80mesh, 10min. Other conditions: glycerol/methanol, 2/1; microwave power, 550W; sulfuric acid, 1.75%; solvent/bamboo, 4.5/1.
observed with an increase in the reaction temperature. This result allowed the statement that liquefaction of bamboo using microwave energy primarily caused the reduction of bamboo particles along the longitudinal direction. This finding is consistent with the fact that no ray cells/units exist in the radial direction of bamboo, and the reagents primarily penetrated in the longitudinal plane, and thus reduced the fiber length. Furthermore, with the reaction processing, the aspect ratio of the residue dramatically decreased as the reaction temperature increased from 120°C to 140°C. Since there was no significant reduction in diameter, the reduction in length highly contributed the decrease of the aspect ratio.

The results in Table 1 also demonstrate the effect of particle size on particle dimensional change after liquefaction. As shown in Table 3.1, at 120°C, the reduction in length slightly increased as the particle size increased, revealing that bamboo with a larger particle size could be also efficiently liquefied or dissolved. This may be due to the uniform heating by microwave energy throughout the material, and particle size had no significant effect on the liquefaction efficiency based on residue dimensions. Particle size did not affect the variation in the residue diameter since no apparent variability was observed with decreasing particle size. According to the ANOVA analysis, no significant difference in diameter was found among the residues from the liquefaction of bamboo with different sizes. At 120°C, the increment in aspect ratio showed a decreasing trend as the particle size decreased. With the temperature ascending to 140°C, a relative high aspect ratio can be seen in the particle size of 10-20mesh and then the aspect ratio decreased to 14-18 as the particle size decreased (20-40mesh to 60-80mesh), indicating that bamboo fiber was thoroughly degraded.
Table 3.1. Changes in dimensions of the liquefied residues under various conditions

<table>
<thead>
<tr>
<th>Samples</th>
<th>Length</th>
<th>Diameter</th>
<th>Aspect ratio</th>
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<td></td>
<td>Before</td>
<td>After</td>
<td>Reduction (%)</td>
</tr>
<tr>
<td></td>
<td>liquefaction</td>
<td>liquefaction</td>
<td></td>
</tr>
<tr>
<td>120 10 min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10-20 mesh</td>
<td>7164</td>
<td>1063</td>
<td>85.16</td>
</tr>
<tr>
<td>20-40 mesh</td>
<td>7166</td>
<td>1015</td>
<td>85.84</td>
</tr>
<tr>
<td>40-60 mesh</td>
<td>3449</td>
<td>679</td>
<td>80.31</td>
</tr>
<tr>
<td>60-80 mesh</td>
<td>1008</td>
<td>385</td>
<td>61.81</td>
</tr>
<tr>
<td>120 15 min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10-20 mesh</td>
<td>7164</td>
<td>880</td>
<td>87.72</td>
</tr>
<tr>
<td>20-40 mesh</td>
<td>7166</td>
<td>954</td>
<td>86.69</td>
</tr>
<tr>
<td>40-60 mesh</td>
<td>3449</td>
<td>657</td>
<td>80.95</td>
</tr>
<tr>
<td>60-80 mesh</td>
<td>1008</td>
<td>378</td>
<td>62.50</td>
</tr>
<tr>
<td>140 10 min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10-20 mesh</td>
<td>7164</td>
<td>594</td>
<td>91.71</td>
</tr>
<tr>
<td>20-40 mesh</td>
<td>7166</td>
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<td>93.59</td>
</tr>
<tr>
<td>40-60 mesh</td>
<td>3449</td>
<td>238</td>
<td>93.10</td>
</tr>
<tr>
<td>60-80 mesh</td>
<td>1008</td>
<td>281</td>
<td>72.12</td>
</tr>
<tr>
<td>140 15 min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10-20 mesh</td>
<td>7164</td>
<td>580</td>
<td>91.90</td>
</tr>
<tr>
<td>20-40 mesh</td>
<td>7166</td>
<td>159</td>
<td>97.78</td>
</tr>
<tr>
<td>40-60 mesh</td>
<td>3449</td>
<td>152</td>
<td>95.59</td>
</tr>
<tr>
<td>60-80 mesh</td>
<td>1008</td>
<td>137</td>
<td>86.31</td>
</tr>
</tbody>
</table>
3.3.3. Chemical Analysis

FTIR spectra were used to character the chemical structure of both bamboo and liquefied residues. As shown in Figure 3.4, the -OH stretching vibration at 3309 cm\(^{-1}\) and the methyl and methylene stretching vibration at 2881 cm\(^{-1}\) in these spectra are nearly the same. The peak at 1728 cm\(^{-1}\) which was assigned to C=O stretching vibration of the carboxyl and acetyl groups in hemicellulose had a weak tendency after liquefaction. It was almost reduced to a shoulder in the spectra of residues from 120°C/60-80 mesh/15min and 140°C/10-20mesh/15min, indicating that hemicellulose could be easily decomposed during the liquefaction process, while at reaction conditions of 140°C/60-80mesh/15min, intensive peak was displayed. This may be due to the re-condensation of the decomposed hemicellulose derivatives in acid glycerol.

The strong absorptions at 1596cm\(^{-1}\), 1506 cm\(^{-1}\), and 1456 cm\(^{-1}\) in Figure 3.4a and Figure 3.4d are characteristic peaks for the aromatic skeleton vibration of bamboo. Residues from 140°C/10min/10-20 mesh showed the lowest intensities at 1596cm\(^{-1}\) and 1506cm\(^{-1}\). The peaks at 1456 cm\(^{-1}\) and 1230 cm\(^{-1}\) were related to C-H deformation combined with aromatic ring vibration and the methoxyl groups of lignin. The two groups have more intense peaks in original bamboo compared to the liquefied residues. However, the absorptions at 1596cm\(^{-1}\)and 1456 cm\(^{-1}\) in the spectrum of residues from 140°C/15min/60-80 mesh intensified again, this was due to the interactions of the decomposed lignin components forming an aromatic network. This result was consistent with the higher residue yield under the liquefaction conditions and was further evidenced by the higher lignin content in the residues as discussed later. The bands at 1316 cm\(^{-1}\), 1103cm\(^{-1}\), and 895 cm\(^{-1}\) were all attributed to cellulose which was assigned to O-H in plane bending and CH2 wagging, and glucose ring asymmetric valence vibration, respectively. The predominant peak at 1020 cm\(^{-1}\) was assigned to the C-OH bending model. The insignificant changes in these cellulose bands among the spectra of bamboo and liquefied
residues indicate that cellulose was the main resistance to the liquefaction system despite the variety in particle size and reaction conditions.

From the morphology and the chemical structure analysis results, the liquefied residues mainly exhibited a fiber structure with remaining cellulose. Since the liquefied residues showed a brown color, we predicted that unliquefied lignin or recondensed lignin derivatives were existed in the residues because lignins are responsible for the brown color of the liquefied residues. Therefore, the lignin content of the liquefied residues was determined, and the results are shown in Figure 3.5.

![Figure 3.4. FT-IR transmittances of (a) bamboo material of 10-20mesh; (b) 120°C, 10-20mesh, 15min; (c) 140°C, 10-20mesh, 15min; (d) bamboo material of 60-80mesh, 15min; (e) 120°C, 60-80mesh, 15min; (c) 140°C, 60-80 mesh, 15min. Other conditions: glycerol/methanol, 2/1; microwave power, 550W; sulfuric acid, 1.75%; solvent/bamboo, 4.5/1.](image)

The lignin content for original bamboo particles was 27.31%. The lignin content slightly decreased with decreasing particle size under reaction conditions of 120°C/10min and 120°C/15min, indicating that the reduction in particle size could accelerate the decomposition of lignin. The lignin content for the residue from 120°C/15min/60-80mesh was the lowest
(4.9%). This result was concord with the morphology of the residues, which were composed of well displayed long fibers (Figure 3.3 a-c). For the liquefaction at 140°C, the lignin content for the residues from liquefaction of 10-20 mesh with 10 min was the lowest. The lignin content displayed an increase trend with decreasing particle size under reaction conditions of 140°C/10min and 140°C/15min. This result may be attributed to the self-depolymerization and/or recondensation of decomposed lignin obtained at the initial solvolysis process, and the recondensed lignin fragments, usually with large molecular weight, could not be redissolved, and thus resulted in higher lignin content in the residues [30]. As for the liquefaction of 10-20 mesh samples, in the initial step, reactions mainly occurred on the outer surface of the particles. With the reagents slowly permeating though the whole particles, the fluid of the reagents combined with the uniform heat source promoted the decomposition of the cell wall components, mainly lignin. Therefore, it can be concluded that liquefaction of large particles with microwave energy resulted in the dissociation of tissue elements by dissolving lignin along with the reagent permeating process. Since particles with smaller sizes could be more easily accessed by chemicals, the degradation of cell wall components occurred immediately as the microwave radiation was introduced into the liquefaction system, which contributed to solvolysis of the overall particles. Then re-polymerization of the already decomposed lignin compounds took place, resulting in higher lignin content for the residue. This coincides with the aggregated lignin granules that were observed on the surface of residues from 140°C/60-80mesh/15min.
3.3.4. Extraction of Nanofibrillated Cellulosic Fiber

Based on the aforementioned analysis, the microwave liquefied bamboo residues were mainly composed of microfibers attaching with non-cellulosic materials, indicating that the residues had potential in the extraction of cellulose nanofibers. In order to isolate nanofibrillated cellulosic fibers from the liquefied residues, residue samples from liquefaction of 120°C/60-80 mesh/15min and 140°C/60-80/15min were used and treated with chemical treatment prior to nanofibrillation process. The steps involved during the process and visual changes of the samples in each step are illustrated in Figure 3.6. Dark brown color of the residues was changed to pure white after bleaching. The influence of sulfuric acid concentration used in the acid hydrolysis process and the ultrasonic treatment time on the yield of nanofibrillated cellulosic fiber were elucidated.
Table 3.2 presents the effect of sulfuric acid concentration on the yield of chemically purified cellulose and nanofibrillated cellulosic fiber. As the sulfuric acid concentration increased from 2% to 45%, the yield of purified cellulose decreased, while that of the nanofibrillated cellulosic fiber increased. Since the sulfuric acid mainly worked on the removal of non-cellulosic materials and hydrolyzed the amorphous cellulose, the higher of its concentration, the more non-cellulose substances it eliminates and the higher the crystal cellulose remains, therefore it could be easily understood that with increasing acid concentration, the yield of nanofibrillated cellulosic fiber increased. For comparison, residue samples from liquefaction at 140°C yielded less purified cellulose and more nanofibrillated cellulosic fibers than those from 120°C, respectively. At 140°C, the cellulose in bamboo started to decompose as evidenced by the damaged cell walls shown in the SEM images because cellulose was the dominate component of the cell wall. Meanwhile, recondensation of lignin also took place at 140°C. The degradation of the cellulose and recondensation of lignin both
contributed to the relative low purity of cellulose in the residues leading to the low yield of the purified cellulose. The higher yield of nanofibrillated cellulose from residues of 140 °C may be due to that the cellulose structure was disrupted during the liquefaction and with chemical purification and ultrasonic treatments crystal nano fibrils could be easily split off resulting high nanofibrillated cellulosic fiber yield.

Table 3.2. Yield of chemically purified cellulose fibers and nanofibrillated cellulose from microwave liquefied bamboo residues. Ultrasonic time: 25min

<table>
<thead>
<tr>
<th>Sample</th>
<th>Yield of chemically purified cellulose (%)</th>
<th>Yield of nanofibrillated cellulose (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>120°C/2.0%</td>
<td>85.60</td>
<td>10.75</td>
</tr>
<tr>
<td>120°C/25%</td>
<td>77.78</td>
<td>31.96</td>
</tr>
<tr>
<td>120°C/45%</td>
<td>69.59</td>
<td>37.18</td>
</tr>
<tr>
<td>140°C/2.0%</td>
<td>78.07</td>
<td>37.17</td>
</tr>
<tr>
<td>140°C/25%</td>
<td>67.09</td>
<td>50.20</td>
</tr>
<tr>
<td>140°C/45%</td>
<td>65.61</td>
<td>52.02</td>
</tr>
</tbody>
</table>

The effect of high-intensity ultrasonic time on the nanofibrillated cellulosic fiber yield is shown in Figure 3.7. It can be seen that the yield showed significant increase with increasing the ultrasonic time. This result indicated that ultrasonic time had obvious impact on nanofibrillated cellulosic fiber yield. This was because the use of ultrasonic treatment can break the hydrogen bonds and disintegrate micro fibers into nanofibrils. Though, the nanofibrillated cellulosic fiber yield isolated from different liquefied bamboo residues all showed increasing trend with respect to ultrasonic time, the yield of nanofibrillated cellulosic fibers generated from residues from liquefaction of 140 °C was always higher than those from 120 °C. This may be explained by the differences in the characteristics of the two kinds of residues.
Figure 3.7. Effect of ultrasonic time on yield of nanofibrillated cellulose from microwave liquefied bamboo residues

Transmission electron microscope (TEM) image of the nanofibrillated cellulosic fibers produced from microwave liquefied bamboo residues is shown in Figure 3.8. The TEM images demonstrate the presence of nanofibrils. The nanofibrils had a length of 550 nm or longer and ranged roughly around 4-18 nm in diameter. Figure 3.8 also illustrates that liquefaction conditions, acid concentration, and ultrasonic time had no influence on the morphology of the produced nanofibrillated cellulosic fibers. In terms of nanofiber dimensions, the resulted nanofibrillated cellulosic fibers had comparable properties to those isolated from raw lignocellulosic biomass as reported in other studies [31-33]. The results indicated that nanofibrillated cellulosic fibers generated from microwave liquefied bamboo residues had potential in the fabrication of nanofiber reinforced composite materials.
Biopolyols comprising of C₅ and C₆ sugars and phenolic compounds were produced by microwave liquefaction of bamboo despite the large particle size. Feedstocks with smaller particles could accelerate the liquefaction with low temperature (120°C), while lead to lignin recondensation under high temperature (140°C). The chemical and morphology analysis results revealed the liquefied bamboo residues retained fiber structure and remaining cellulose. Bleaching and acid hydrolysis were proved to be efficient process in purifying the residues for pure white cellulose fibers. The chemically purified cellulose fibers from liquefied residues were reduced to nanofibrillated cellulosic fibers by given to high-intensity ultrasonic treatment.
Characteristics of liquefied samples, aid hydrolysis, and ultrasonic time were impact factors on the yield of nanofibrillated cellulosic fibers. The generated nanofibrillated cellulosic fibers were in the range of long nanofibrils which was suitable for use in reinforcing composites.

3.5. REFERENCES


CHAPTER 4. PROCESS OPTIMIZATION ON THE PRODUCTION OF CELLULOSE MATERIALS USING MICROWAVE LIQUEFACTION

4.1. INTRODUCTION

Global concerns regarding fossil fuels depletion and associated adverse environmental impacts has encouraged research in renewable biofuels [1]. Moreover, the demand for environmentally friendly products has renewed interest in finding new bio-based materials that are biodegradable and environmentally compatible [2]. Lignocellulosic biomass represents a renewable, abundant, and inexpensive source of raw materials for the chemical industry to develop biofuels, renewable chemicals, and biomaterials. Conversion of abundant lignocellulosic biomass to ethanol as a transportation fuel provides an important opportunity to improve energy security, rural development, and environmental stewardship.

Cellulosic fibers isolated from lignocellulosic biomass can be used as reinforcing elements in polymeric matrices and are considered highly promising fillers in preparing sustainable composite materials. During the bioconversion of lignocellulosic biomass to biofuels via enzymatic hydrolysis the carbohydrate component needs to be exposed to enzymatic action, and the lignin in the biomass acts as a barrier and hinders the accessibility of enzymatic molecules to the cellulose [3]. Thus, lignin removal is necessary. In addition, to generate natural cellulose enriched fibers or microfibrillated cellulose (MFC), wood should be treated by various chemical processes to remove lignin and other substances, before it is used as a raw material for the production of MFC [4]. Moreover, in the manufacturing of wood plastic composites (WPC) by Ou et al [5-6], the removal of lignin and extractives in wood flour could increase tensile modulus. Therefore, large scale production of holocellulose fibers is necessary for paper pulping, bioconversion, MFCs, and high performance bio-based

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composites industries, and thus an efficient pretreatment process of lignocellulosic biomass is required.

In recent decades, a number of different pretreatments processes, such as acidic/alkaline pretreatment [7], wet oxidative [8], liquid hot water [9], steam explosion [10], and ultrasound [1] have been applied in the treatment of different lignocellulosic biomass. However, to achieve industrial scale production, these pretreatments techniques will need to become more efficient, inexpensive, and environmentally-friendly.

Glycerol, a high-boiling-point solvent, is presently the main byproduct of the oleochemicals industry and can be as high as 10% of the total biodiesel production [8]. With the increase in global bio-diesel production, glycerol production has rapidly moved into oversupply resulting in a crashing in the price for crude glycerol. Thus, the commercial utilization of this compound has become a challenge. It is, therefore, desirable to find a novel, economical utilization method for crude glycerol to improve the economics of biodiesel production. Recent advancements in techniques for treatment of woody materials using organic solvents have stimulated research on the use of glycerol pulping as a potential utilization approach. Several studies have been conducted on the pretreatment and delignification of biomass using glycerol [11]. Although study of lignocellulosic biomass pretreatment using glycerol for enzymatic hydrolysis or renewable chemicals has shown encouraging results, an economically viable technology is yet to be realized because of the high cost and time-consuming nature of the biomass pretreatment process.

Compared to conventional heating, microwave energy penetrates and produces a volumetrically distributed heat source; heat is generated throughout the material and leads to faster heating rates and improved kinetics. Therefore, the development of a reaction process combining the greater microwave heating efficiency and the by-product glycerol from the biodiesel production to replace petroleum-based solvents is a promising method to enhance
economic viability of the process and benefit agricultural producers, bio-based processing industries, and the environment. According to the potential benefits of the microwave heat, we developed a new microwave-assisted liquefaction system using glycerol as the main solvent [12-15]. In the investigation on the liquefaction behavior of bamboo, lignin in bamboo could be easily decomposed in glycerol with sulfuric acid as catalyst using microwave energy under mild reaction conditions. Moreover, the addition of methanol in glycerol has been proven to be effective in avoiding recondensation reactions of lignin in liquefaction of bamboo, and the benefit of using methanol in liquefaction is that they can be easily recovered after liquefaction. Moreover, in the decomposition of lignin, the methanol –OH group functions as the nucleophile and attacks electrophilic C adjacent to the glycosidic bond in the liquefaction system, creating good leaving groups and a neutral hydroxyl group, by cleaving the C–O bond [12].

From this point of view, we proposed that this microwave-assisted liquefaction system could be also applied in biomass main components separation if the reaction conditions are properly controlled. Accordingly, in this study, microwave-assisted selective liquefaction was introduced for the production of holocellulose fibers to enhance the paper pulping, bioethanol, and biomaterials industry. The aim of the research was to achieve a rapid process for the production of holocellulose fibers. The specific objective of this study was to evaluate the reaction temperature and time on physicochemical properties of the extracted holocellulose fibers.

4.2. MATERIALS AND METHODS

4.2.1. Materials

Three-year-old bamboo culms (Phyllostachys pubescens) were harvested from the Kisatchie National Forest, Pineville, La, USA. The bamboo culms were reduced to particles using a Thomas Wiley Laboratory mill. The particles were screened to collect particles that passed through a 20-mesh sieve and then retained on a 40-mesh sieve and then dried to a
constant weight in an oven maintained at 80 °C. The dried particles were stored in polyethylene bags and used without further treatment. All acids, glycerol, and methanol used were of reagent grade and obtained from commercial sources.

4.2.2. Microwave-assisted Selective Liquefaction

Selective liquefaction of bamboo was carried out in a Milestone MEGA laboratory microwave oven. Mixed glycerol and methanol at ratio of 2/1 (w/w) was used as the solvent at a solvent to bamboo ratio of 4/1 (w/w). Sulfuric acid (1.75% of solvent weight) was used as the catalyst. A typical reaction mixture consisting of 2 g of bamboo particles, 8 g of solvent, and 0.14 g of sulfuric acid were loaded in 100 mL Teflon vessels with a magnetic stirring bar. The Teflon vessels were then placed on the rotor tray inside the microwave cavity. The temperature was monitored using an ATC-400FO automatic fiber optic temperature control system. Based on monitored temperature, the output power was auto-adjusted during liquefaction. In this study, the temperature was increased from room temperature to 120°C and then was kept constant for 4 min, unless otherwise noted. At the end of the reaction, the vessels were taken from the microwave cavity and cooled for 15 min.

Afterwards, the resulting reaction mixtures were dissolved in methanol and then vacuum-filtered through Whatman No. 4 filter paper. The solid retained on the filter paper, which was designated as holocellulose fibers in this study, was washed with methanol and oven-dried at 105°C. The yield is the mass percentage of the residue solid to the original bamboo, and was calculated as follows:

\[
\text{Yield} \text{ (%)} = \frac{\text{weight of solid residue}}{\text{weight of raw material}} \times 100
\]  

(1)

4.2.3. Preparation of Jeffrey’s Solution Fiber

Complete bamboo fiber was obtained using Jeffrey’s solution in order to have a comparison with the fiber obtained by microwave liquefaction. The Jeffrey’s solution fiber was
used as a control because it kept the original structure of the fiber and was usually used for fiber morphology analysis [16]. Bamboo samples were softened in hot water for 3 hours, then bamboo splints were macerated in a binary solution of chromic acid and nitric acid (10% chromic acid: 10% nitric acid mixtures=1:1). The mixture was maintained at 50 °C for 1 hour. Thereafter, the macerated splints were washed carefully with distilled water. The splint fibers were air-dried and then collected for further usage.

4.2.4. Chemical Component Analysis

The holocellulose, Klason lignin, and alcohol-toluene extractive of the samples were determined according to ASTM standards D1104-56, D1106-96, and D1110-96, respectively [17-19]. Each test was conducted in duplicate and the average value was reported.

4.2.5. FTIR Spectra

The FT-IR analysis was performed by a Nicolet Nexus 670 spectrometer equipped with a Thermo Nicolet Golden Gate MKII Single Reflection ATR accessory. Data collection was performed with a 4 cm⁻¹ spectral resolution and 32 scans were taken per sample.

4.2.6. X-ray Diffraction Analysis

Crystallinity of the original bamboo and solid residue samples were analyzed using wide angle X-ray diffraction (Bruker D 5000). The data were generated by a diffractometer with Cu Kα radiation (λ=1.54 Å) at 40 kV and 30 mA over the angular range 2θ=5-40° and a step time of 2.0s. A focusing powder diffraction method was applied. The crystallinity index (CrI) is determined using the following equation:

\[
CrI \ (\%) = \frac{I_{002} - I_{am}}{I_{002}} \times 100
\]

(2)

Where \(I_{002}\) is the intensity of the diffraction from the (200) plane at 2θ=22.1 °, and \(I_{am}\) is the intensity for amorphous material taken at 2θ=18 ° [20].
4.2.7. Morphology Analysis

The surface morphology of the bamboo particles and solid residue samples were observed using scanning electron microscopy (SEM, JCM-5000). Test samples were coated with gold using a vacuum sputter coater before subjected to the SEM analysis.

4.2.8. Thermal Stability Analysis

TG/DTG analysis was conducted with a thermal analyzer, TGA (Q50), to simultaneously obtain thermos gravimetric data. About 2 mg of sample was analyzed by the thermal analyzer. Pyrolysis was terminated at 800°C with a heating rate of 20°C/min under a flow of 60mL/min of nitrogen gas.

4.2.9. Gas Chromatograph-Mass Spectrometer (GC-MS)

The general profile for the liquefied liquor was obtained using electron ionization mass spectrometry (EI-MS). Analysis of the product was conducted on a mass spectrometer (Agilent 5975C VL MSD), and the products were separated into their components using a gas chromatograph (Agilent 7890A) equipped with a fused capillary column (DP-5, L=30 m, i.d. 0.32 mm, film thickness 0.25μm) with 5% phenyl and 95% dimethylpolysiloxane as the stationary phase. The carrier gas was helium at a flow rate of 1.8mL/min. Condition for analysis: injection mode was split at split rate 35; the column was held at 50°C for 2min and then heated to 250°C at the rate of 10°C/min, while the injector temperature was 250°C. The identification of the product components was confirmed using total ion chromatograms as well as fragmentation patterns.

4.2.10. Statistical Analysis

Statistical analysis was carried out using SAS (version 9.1, SAS Institute, Cary, NC). Analysis of variance (ANOVA) was performed to determine significant difference (α=0.05) among the different samples.
4.3. RESULTS AND DISCUSSION

4.3.1. Microwave-assisted Selective Liquefaction

The yield of solid residue with respect to reaction temperature and time is presented in Table 1. As shown in Table 1, the yield dramatically decreased from 81.4 to 39.65% as the reaction temperature increased from 75 to 120°C, indicating that the increase in temperature combined with the microwave heating showed significant ability to enhance the decomposition of bamboo substances. In the research on selective liquefaction of wheat straw in phenol using conventional heating, the reaction conditions for obtaining about 40% of liquefied wheat straw was 125°C, 60 min with a sulfuric acid loading of 3% [21]. For comparison, the introduction of microwave energy in selective liquefaction of bamboo allowed the temperature to be lower and shortened the reaction time as well as reduced sulfuric acid usage. This may be because microwave irradiation intensified the heat and mass transfer during the reactions. The highly efficient liquefaction of this process may also be due to the fact that extractives and lignins in bamboo could be easily liquefied under lower reaction temperatures with microwave irradiation.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Yield (%)</th>
<th>Holocellulose (%)</th>
<th>Lignin (%)</th>
<th>Extractives (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100±0.00</td>
<td>63.08±1.03</td>
<td>20.91±0.24</td>
<td>7.24±0.29</td>
</tr>
<tr>
<td>75°C/7min</td>
<td>81.44±1.55</td>
<td>65.79±1.32</td>
<td>14.60±0.28</td>
<td>4.75±0.89</td>
</tr>
<tr>
<td>90°C/7min</td>
<td>65.75±1.77</td>
<td>71.07±1.29</td>
<td>11.31±0.48</td>
<td>2.60±0.13</td>
</tr>
<tr>
<td>105°C/7min</td>
<td>53.52±1.90</td>
<td>81.28±1.35</td>
<td>4.87±0.53</td>
<td>2.13±0.21</td>
</tr>
<tr>
<td>120°C/7min</td>
<td>42.44±2.17</td>
<td>93.00±1.73</td>
<td>1.66±0.15</td>
<td>0.59±0.04</td>
</tr>
<tr>
<td>3min/120°C</td>
<td>59.35±1.95</td>
<td>78.34±1.14</td>
<td>7.44±0.61</td>
<td>2.26±0.19</td>
</tr>
<tr>
<td>5min/120°C</td>
<td>45.95±1.06</td>
<td>86.64±1.33</td>
<td>3.82±0.05</td>
<td>1.07±0.03</td>
</tr>
<tr>
<td>9min/120°C</td>
<td>37.85±1.33</td>
<td>94.37±0.48</td>
<td>0.65±0.27</td>
<td>0.49±0.05</td>
</tr>
</tbody>
</table>

A selective liquefaction of bamboo using conventional liquefaction was conducted in a Parr reactor in order to have a rough comparison in energy demands with microwave selective liquefaction. The reaction temperature and time for yielding similar solid residue yield to
microwave liquefaction process of 120 °C and 9 min were 120 °C and 35 min for conventional liquefaction. A referenced method was used to estimate the energy requirement for the conventional liquefaction [22]. The heating power of the Parr reactor was 1500 W at the maximum temperature of 400 °C, the power required to maintain temperature of 120 °C was determined to be 450 W (120/400 multiplying 1500). The energy requirement was calculated as the power multiplied by time and determined to be 9.45×10^5 J. Since microwave power was automatically adjusted based on continuous feedback from the process sensors to allow the reaction to follow the desired temperature profile, by taking the derivative of the power curve, the average microwave power during the whole reaction process was determined to be 230 W. For the microwave selective liquefaction, the energy determined to be 1.24 ×10^5 J. It noteworthy that energy demanded in microwave selective liquefaction was much less than that required by conventional heating which indicates that microwave selective liquefaction also has energy advantage.

Previous studies on complete liquefaction with microwave heating have shown that microwave heating could not only enhance the decomposition of bamboo components at low temperature, but could also accelerate the self-polymerization of lignin components and or condensation of lignin fragments with reagents at high temperature [14-15]. Thus, in order to obtain a proper yield of solid residue with a high carbohydrate component, as well as avoiding lignin recondensation, the preferred reaction temperature was 120 °C. Table 1 also shows the effect of reaction time on the solid yield at a reaction temperature of 120 °C. As the reaction time increased, the yield significantly decreased from 59.35 to 37.85% from 3 to 9 min.

4.3.2. Chemical Analysis

The chemical components of the bamboo and solid residue samples are presented in Table 4.1. As shown in Table 4.1, the Klason lignin and alcohol-toluene extractive contents in the solid residue samples rapidly decreased from 14.60 to 1.66% and 4.75 to 0.59%, respectively,
as the reaction temperature increased from 75 to 120°C. The holocellulose content increased from 65.79 to 93.00% at the same time. In terms of the influence of time on the main chemical components of the solid residues, the lignin content and alcohol-toluene extractive decreased as the liquefaction time increased. The holocellulose content increased from 74.17 to 94.28% as the time increased from 3 to 9min at the reaction temperature of 120°C. The obtained samples from the liquefaction of 120°C/9min exhibited the highest holocellulose content (94.28%) and the lowest lignin (0.65%) and alcohol-toluene extractives (0.49%). This result revealed that microwave-assisted selective liquefaction process could efficiently remove non-carbohydrate substances and result in maximum holocellulose content.

Figure 4.1. Relationship between solid residue yield and holocellulose, klason lignin, extractives content in the solid residue from different microwave selective liquefaction conditions.

Fig 4.1 presents the relationship between solid residue yield and the content of the main chemical components. The lignin and extractive content decreased with the decrease in solid
residue yield, while holocellulose content increased. A good linear correlation was found between chemical components and solid residue yield with $R^2$ values of 0.9713, 0.9769, and 0.9738 for holocellulose, lignin, and extractives, respectively. This result revealed the reduction in solid residue yield was mainly attributed to the weight loss of lignin and extractives. The degradation of lignin and the removal of extractives enrich the residue, and thereafter result in the (relatively) high holocellulose content in the solid samples.

![GC-MS chromatograms and chemical composition of the liquid products from microwave selective liquefaction process at 120 °C for 9min.](image)

Figure 4.2. GC-MS chromatograms and chemical composition of the liquid products from microwave selective liquefaction process at 120 °C for 9min.

The analysis of the liquid products was also conducted using GC-MS, and the GC-MS chromatogram is presented in Fig 4.2. It was found that the majority of compounds were comprised of unreacted glycerol, glycerol derivatives such as 1, 2-Propanediol, 3-methoxy- and Oxirane, (methoxymethyl)-, five carbon sugars (D-Ribopyranoside, methyl), six carbon sugars (D-Glucopyranoside, methyl), and phenolic compounds [2-Propenoic acid, 3-(4-hydroxyphenyl)-, methyl ester]. Except for the glycerol and glycerol derivatives in the liquid,
the presence of C5 sugars derived from hemicellulose indicated the cleaving of hemicellulosic ethers. Since the ethers act as linkages between cellulose and lignin, the decomposition of such linkages enhanced the release of the lignin from cellulose, and the aromatics in the liquid further evidenced the decomposition of the lignin during the liquefaction process.

4.3.3. FTIR Spectroscopy

The transmittance spectra of the fibers from different reaction temperatures are shown in Figure 4.3a. The ester bonds (C=O) at 1735 cm\(^{-1}\) were weakened in the spectrum of the solid residue compared to that of bamboo particles, indicating the cleavage of the linkages between ferulic acid or p-coumaric acid or (p-) hydroxycinnamic acids and lignin in the fibers [10]. The dissociating of the key ester linkages between lignin and carbohydrates promoted the dissolution of lignin into the solvents. This result was concord with the results of GC-MS analysis. The absorbance at 1596 cm\(^{-1}\) and 1506 cm\(^{-1}\) arising from the aromatic skeletal vibration, the absorbance at 1456 cm\(^{-1}\) assigned to C-H deformation combined with aromatic ring vibration, and the band at 1230 cm\(^{-1}\) corresponding to methoxyl groups of lignin were all shown to be strong peaks in the spectra of the bamboo particles. These peaks were weakened in the spectra of the solid residue samples at 75, 90, and 105°C. As the temperature increased to 120°C, the absorbance bands at 1596, 1456, and 1230 cm\(^{-1}\) disappeared and the band at 1506 cm\(^{-1}\) became a small shoulder. This result indicates that lignin functional groups such as aromatic rings were almost completely dissociated and dissolved with only a small amount of associated lignin retained on the solid residue (indicated by the band at 1506 cm\(^{-1}\)) , which was consistent with the wet chemistry analyses as discussed above.
The spectra of the solid residue as a function of reaction time are shown in Figure 4.3b. By prolonging the reaction time, the absorbance bands assigned to lignin decreased (3-7min). By further prolonging the reaction time to 9 min, the absorbance bands that characterized functional groups of lignin disappeared on the spectrum of the solid residue, leaving visible intensity bands assigned to carbohydrates. This was in good agreement with the holocellulose content for the solid residues, which was more than 94% comprised of holocellulose. The bands
for different functional groups of carbohydrates such as the methylene groups at 1420 and 1371 cm\(^{-1}\), C-O band at 1162 cm\(^{-1}\) and 1108 cm\(^{-1}\), and CH deformation at 899 cm\(^{-1}\) became much more intensive in the spectra of the solid residues from different liquefaction conditions, which also gave evidence of the high holocellulose content in the solid residues (88-94.28\%).

The ratio of relative intensities of three characteristic absorbance bands of holocellulose (1370, 1160, and 899 cm\(^{-1}\)) to the two characteristic absorption ones of lignin (1506 and 1230 cm\(^{-1}\)) were calculated to further clarify the chemical changes during the liquefaction process. As shown in Fig. 4, the ratio increased by prolonging the reaction time revealing the decrease in lignin content and increase in holocellulose content. It was interesting to find that good linear correlations existed between the ratio of band intensities of the holocellulose/lignin (\(I_{899}/I_{1506}\), \(I_{1160}/I_{1506}\), \(I_{1370}/I_{1506}\), \(I_{899}/I_{1230}\), \(I_{1160}/I_{1230}\), and \(I_{1370}/I_{1230}\)) and reaction time (\(R^2 = 0.91-0.99\))
Figure 4.4. Relationship between FTIR intensity ratio of solid residues and microwave selective liquefaction time.

4.3.4. Crystallinity Analysis

Figure 4.5A shows the wide angle X-ray-diffraction pattern of the original bamboo and the solid residue from 120 °C and 9min. Both the XRD patterns displayed peaks at \(2\theta = 14.9^\circ\) and 22.1 indicating the typical cellulose I crystalline structure. The calculated crystallinity index (CrI) values were 52.3 for the original bamboo [20].
Figure 4.5. Typical X-ray diffraction spectra of original bamboo, solid residue (A) and effect of reaction time on the crystallinity value of solid residue from microwave liquefaction process at 120°C (B).

After liquefaction, the CrI value was significantly increased. With increasing reaction time, the CrI value displayed increasing trend and reached a maximum value for the solid residue from 120°C, 9min (Figure 4.5B). This significant increase of crystallinity in solid residue with comparison to the original bamboo was undoubtedly attributed to the efficient dissolution and removal of non-cellulosic materials such as lignin and extractives from the amorphous regions.
and enrichment in cellulose content in solid residue during microwave liquefaction. This was in good agreement with the chemical results and FTIR analysis as shown in Table 1 and Figure 4.3, respectively. This result further confirmed the efficient removal of non-crystalline components and that microwave selective liquefaction could be used for pre-purification of lignocellulosic biomass for cellulose fiber production.

4.3.5. Thermal Stability Analysis

Figure 4.6 presents the thermal degradation curves of original bamboo particles and solid residue from 120°C/9min. As shown in Figure 4.6, the samples showed a more significant weight loss below 200°C. A slight shoulder was observed on the TG curve of bamboo particles but was absent on the TG curve for the solid residue. This may be attributed to the removal of extractives and degradation of a portion of hemicellulose during the microwave liquefaction process, which was also indicated by the weakened absorbance band at 1735 cm\(^{-1}\) assigned to hemicellulose on the FTIR spectrum of holocellulose fibers. The onset temperature, \(T_o\), is considered as the temperature at which the sample loses 5% of its weight. The \(T_o\) value for the bamboo particles was particularly lower than that for the solid residues (Table 4.2). It has been reported that extractives could promote ignition of woody materials at lower temperature [23]. Thus, in this study, the removal of extractives during the liquefaction process contributed to the higher initial weight loss temperature, and the extractive component in the bamboo particles accelerated the degradation process of the other components resulting in a lower initial weight loss temperature. Prominent peaks corresponding to the maximum decomposition rate were observed on both the bamboo particles and the solid residues. The DTG peak temperature for the bamboo particles was about 20°C higher than that for the solid residue (320.96 and 338.23 °C for bamboo particle and solid residue, respectively). As for the three components of woody materials, hemicellulose was the easiest to be pyrolyzed followed by cellulose then lignin based on TG analysis [24]. Consequently, the lower DTG peak temperature for the solid residue was
mainly due to the removal of lignin by the microwave liquefaction process. The obtained result in this study was also in agreement with the results of Chen et al who reported that liquefaction could significantly decrease the maximum degradation rate temperature (temperature at DTG peak) [25]. The differences in thermal stability between the bamboo particles and liquefied bamboo solid gave evidence to the efficient removal of lignin and extractives by this liquefaction process.

Table 4.2. Thermal degradation temperatures ($T_0$, $T_1$, $T_2$) for the original bamboo particles and solid residue from 120°C/9min

<table>
<thead>
<tr>
<th>Sample</th>
<th>$T_0$ (°C)</th>
<th>$T_1$ (°C)</th>
<th>$T_2$ (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bamboo particles</td>
<td>220.21</td>
<td>280</td>
<td>320.96</td>
</tr>
<tr>
<td>Solid residue</td>
<td>288.61</td>
<td>-a</td>
<td>338.23</td>
</tr>
</tbody>
</table>

Note: *No shoulder was observed*
Figure 4.6. TG and DTG curves of bamboo particles and solid residues from microwave selective liquefaction process at 120°C for 9min.

4.3.6. SEM Images

The SEM images of bamboo particles and the solid residue samples from 120°C within 9min are shown in Figure 4.7. The bamboo particles (Figure 4.7a) showed large bundles with many small fragments and rough surfaces. As can be seen in Figure 4.7b, microwave-assisted selective liquefaction led to split fasciculus with smooth surfaces, indicating that lignin
components in the middle lamella were completely removed, resulting in the collapse of the compact structure of bamboo. Figure 4.7c shows small cracks 1-2μm width on the exterior surface. Jeffrey’s solution fibers (Figure 4.7d) were prepared in order to verify the specific character of the fibers obtained in this study, and no cracks were observed. The Jeffrey’s solution fibers were used as a control because fibers obtained by this method retained their original structure and showed little damage. This comparative observation indicated that fibers isolated from the microwave liquefaction process may be more susceptible to chemical access or enzyme attack. One possible explanation for the cracks on the fibers from liquefaction is the physical effect of the microwave treatment on lignocellulosic materials properties. Studies have shown that microwave pre-treatment can open pathways in wood, dramatically increase permeability, and accelerate moisture migration in wood [26-27]. Thus, the cracks observed may be created by the solvent absorption behaviors induced by microwave energy heating during the liquefaction process. Moreover, the physical changes in wood properties with microwave energy facilitate favorable conditions for the solvents to react. Accordingly, the changes in wood properties resulting in the uniform distribution of solvents along with the uniform temperature distribution induced by microwave energy heating also enhanced the bamboo chemical component degradation.

The dimensions of the original bamboo and liquefied solid residues from 120°C, 9min were also evaluated and the results are listed in Table 4.3. Significant difference (P<0.01) in the dimensional size between the bamboo particles and the solid residues was observed. The average length and width for the original bamboo particles and the solid residues were 2312.01 and 891.19, 567.67 and 17.02 μm, respectively. The solid residues had a high aspect ratio compared to that of the original bamboo particles (4.08 and 65.08 for the bamboo and the solid residues, respectively). The dimension and morphology results of the solid residues revealed that the solid residues were micro sized fibers and were comparable to that of the original single
fibers in terms of morphology. This result indicated that the solid residues in this study may have potential in fabrication of microfiber reinforced composites [28].

Figure 4.7. SEM images of (a) bamboo particles, (b) split fasciculus, (c) single fiber from microwave selective liquefaction at 120°C for 9min, and (d) Jeffrey’s solution fiber.

Table 4.3. Length, width, and aspect ratio of original bamboo particles and solid residue from 120°C/9min

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dimension</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Length (µm)</td>
<td>Width (µm)</td>
<td>Aspect ratio</td>
<td></td>
</tr>
<tr>
<td>Bamboo particle</td>
<td>2312.01±594.99</td>
<td>567.67±162.36</td>
<td>4.08±1.77</td>
<td></td>
</tr>
<tr>
<td>Solid residue</td>
<td>891.19±256.43</td>
<td>17.02±6.93</td>
<td>65.08±10.44</td>
<td></td>
</tr>
</tbody>
</table>

4.4. CONCLUSIONS

The proposed microwave-assisted selective liquefaction process could efficiently remove lignin and extractives from bamboo and produce good quality fibers with high holocellulose content. The holocellulose, lignin, and extractives contents of the solid residues from the
reaction at 120 °C, 9min were 94.28, 0.65, and 0.49 %, respectively. The FTIR results revealed that aromatic rings of lignin could be completely dissociated with this liquefaction process. The improvement in crystallinity for the solid residue confirmed the efficient removal of non-cellulosic materials by microwave selective liquefaction. TG/DTG analysis gave further evidence to the liquefaction of bamboo since the removal of lignin could significantly decrease the thermal stability of bamboo. The SEM images of the solid residue provide further information to better understand the effect of microwave energy heating on the liquefaction process.

4.5. REFERENCES


[23] Poletto, M., Zattera, A.J., and Santana, R.M.C. Structural differences between wood


CHAPTER 5. ISOLATION OF CELLULOSE NANOFIBERS FROM BAMBOO USING MICROWAVE LIQUEFACTION AS PRETREATMENT PROCESS

5.1. INTRODUCTION

Renewable and biodegradable lignocellulosic biomass is of great interest because of depleting fossil fuel reserves and increasing public concern for environmental stewardship. Cellulose is the main component of lignocellulosic biomass and has received increasing attention due to its wide existence, excellent performance, biocompatibility, low density, thermal stability, and environmental benefits [1]. Recently, cellulose has been considered as a promising resource for reinforcing polymer matrixes for the preparation of green sustainable materials and bioethanol production. Regardless of its potential applications in emerging bio-based materials and bioenergy industries, cellulose consists of a linear homopolysaccharide composed of β-D-glucopyranose units linked together by β-1-4- linkages [2]. The cellulose molecules are aggregated into elemental fibrils with diameter ranges of 2-5 nm by intermolecular forces and hydrogen bonds [3]. Therefore, from its unique biological structure, it is reasonable that various nanometer sized single fibers that are also referred to as cellulose nanocrystals, whiskers, nanowhiskers, cellulose nanofibrils, microfibrillated cellulose, or nanofibers have been isolated [4-8].

With the growing demand for sustainable green materials, isolation of cellulose nanofibers has been extensively investigated [2, 9]. Nanofibers have been widely used for the preparation of nanostructured biocomposites [10-12], tough hydrogels [13], membranes [14], and transparent nanopaper or film owing to its high crystalline with strong mechanical performance and outstanding thermal stability [15-17]. Over the past decades, a large number of

lignocellulosic biomass has been explored for the generation of cellulose nanofibers [18]. The common raw materials used for the production of cellulose nanofibers are wood [19], bamboo [20], wheat straw [21], Phormium tenax [22], banana peels [8, 23], orange peel waste [3], oil palm empty fruit bunch [24], de-pectinated sugar beet pulp [25].

Usually, pretreatment and nanofibrillation are essential processes for the isolation of cellulose nanofibers from plant fibers. The pretreatment process such as acid or alkali treatment [26-27], enzymatic pretreatment [17, 28], ionic liquids [29], TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl) mediated oxidation [30-32], and steam explosion treatment [33] were first used to remove non-cellulosic materials in plants, and then nanofibrillation technologies such as grinders [34-35], high-pressure homogenizers [4], and ultrasonic method [36] were used to generate high shear forces to separate the fibrils from the purified cellulose fibers.

Though cellulose nanofibers have been successfully isolated from these aforementioned technologies and the results were encouraging, drawbacks still exist such as chemical regent cost, high energy consumption, time-consuming, and equipment degradation has limited these techniques for practical applications. Advanced techniques for production of cellulose nanofibers with low cost, environmentally friendly, and time efficiency are still required.

As an efficient method, liquefaction has been applied in the conversion of solid woody materials into soluble liquid products, and the liquid products have shown great potential as alternatives for petroleum to produce value-added bio-based products [37]. In recent years, microwave energy has been applied in the liquefaction of lignocellulosic biomass to enhance the biomaterials industry [39-40]. Compared to conventional liquefaction, microwave-assisted liquefaction has advantages such as time efficiency, low chemicals and energy consumption, and economically viability. The products from this technique also showed comparable properties compared to the commercialized ones. As for the liquefaction of wood residues in
the binary of glycerol and ethylene glycerol, lignin and hemicellulose in the wood were significantly decomposed in the initial liquefaction stage (20-40 min) with the reaction temperature of 160°C, while the cellulose content in the wood increased [41]. Pan also observed that the lignin content in the liquefied Chinese tallow wood residue significantly decreased as the liquefaction processes resulting in enriched cellulose residues [42]. The lignin content in the microwave liquefied bamboo residues also showed significantly decrease as the reaction temperature increased from 75°C to 120°C [38]. Therefore, it could be concluded that in the reactions of both conventional and microwave-assisted liquefaction of lignocellulosic biomass in an organic solvent with acid as a catalyst, the decomposition of the three major components (cellulose, lignin, and hemicellulose) in lignocellulosic biomass was in the order of lignin, hemicellulose, and cellulose.

Based on this mechanism, Chen proposed a selective liquefaction process for the production of cellulose and biobased resins, the finding in his research showed that large amount of hemicellulose and lignin could be liquefied at 100°C in 30 min, and the retained cellulose had higher susceptibility for enzymatic attack. This approach offered a new approach for the utilizations of lignocellulosic biomass in the bioethanol and biobased materials industries [43]. In order to extract cellulose for use in both bio-fuels and reinforcing materials, the combination of bleach and liquefaction processes was described in the research of Li et al [44], in which hemicellulose was selectively liquefied to get cellulose. From the previous research results, it was obvious that cellulosic fibers could be easily produced by subjecting the raw lignocellulosic biomass to a liquefaction process with reactions conditions properly controlled.

Therefore, in this study microwave liquefaction was proposed to generate cellulose enriched residues from bamboo. Thereafter, the cellulose enriched residues were chemically purified with low charging of chemicals. Cellulose nanofibers were isolated by ultrasonic
nanofibrillation of the chemically purified cellulose fibers. Morphology, crystallinity, and thermal stability of the isolated nanofibers were determined by employing scanning electron microscopy, X-ray diffraction, and thermogravimetry, respectively. This study attempts to achieve an efficient approach for the generation of cellulose nanofibers with the combination of microwave liquefaction and ultrasonic nanofibrillation.

5.2. MATERIALS AND METHODS

The major procedures for isolation of nanofiber in this study included reduction of bamboo, microwave liquefaction, anti-solvent fractionation, chemical treatments, and ultrasonication. Fig. 1 depicts the flow chart of the procedures.

Figure 5.1. Procedures for the isolation of cellulose nanofibers in this study

5.2.1. Materials

Three-year-old moso bamboo culms (*Phyllostachys pubescens*) were harvested from the Kisatchie National Forest, Pineville, La, USA. The moso bamboo was selected because the annual yield of moso bamboo was greater than all other kinds of bamboo (about $1.8 \times 10^7$ tons) and the integrated utilizations of moso bamboo via liquefaction may provide potential approach
for the production of high-value added bio-products [45]. The bamboo culms were reduced to particles using a Thomas Wiley Laboratory Mill (Model 4) equipped with a 2 mm screen. The particles (length: 0.05-6.23 mm, diameter: 0.01-1.1 mm) were screened to collect particles that passed through a 20-mesh sieve and then retained on a 40-mesh sieve. The average length and diameter of the particles of 20-40 mesh were 5.06 mm and 0.567 mm, respectively. The weight percentage of particles between 20 mesh and 40 mesh was 25.20%, and that for particles in the range of 10-20 mesh, 40-60 mesh, 60-80 mesh, 80-100 mesh, and >100 mesh were 0.22%, 44.42%, 10.08%, 5.86%, and 14.22%, respectively. The particles were dried to a constant weight in an oven maintained at 80 °C. The dried particles were stored in polyethylene bags and used without further treatment. All chemicals including acids, glycerol, and methanol were of reagent grade and obtained from commercial sources.

5.2.2. Microwave Liquefaction

Microwave liquefaction of bamboo was carried out in a Milestone laboratory microwave oven (Ethos EX, 1200 W maximum microwave power, 100 bar maximum operating pressure, ASM-400 magnetic stirrer for homogenous mixing of samples, 300 °C maximum operating temperature, fiber-optic temperature sensor) equipped with 100mL sealed Teflon reaction vessels that are actually a system of components that consist of a vessel that contains the sample, a vessel cover, a safety shield, a vent indicator ring, and a pressure adapter plate. The Teflon vessels used in this study have excellent properties with regard to temperature, acid, and pressure resistance, which was suitable for the chemicals used in this research. The application of this vessel system also enables that the microwave system could provide a closed, clean environment, prevent loss of volatile species, minimize the use of expensive acids, and safely release the over-pressures in the vessel.

Mixed glycerol and methanol at a ratio of 2/1 (w/w) was used as the solvent at a solvent to bamboo ratio of 4/1 (w/w). Sulfuric acid (1.75% of solvent weight) of concentration of 98%
(wt) was used as the catalyst because sulfuric acid could provide strong acid sites during the liquefaction reaction. The highly reactive protons in sulfuric acid are able to activate the oxygen atoms in the glycosidic bonds. The reaction mixture consisting of 2 g of bamboo particles, 8 g of solvent, and 0.14 g of sulfuric acid were loaded in 100 mL Teflon vessels with a magnetic stirring bar. The Teflon vessels were then placed on the rotor tray inside the microwave cavity. The temperature was increased from room temperature to 120°C in 3 min and then was kept constant for a desired time. The temperature was monitored using an ATC-400FO automatic fiber optic temperature control system by putting the sensor into a reference vessel. As for the reaction setup in this study, microwave power was automatically adjusted in the range of 0-550 W based on continuous feedback from the process sensors to allow the reaction to follow the desired temperature profile. At the end of the reaction, the vessels were removed from the microwave cavity and cooled for 15 min. Afterwards, the resulting reaction mixtures were dissolved in methanol and then vacuum-filtered through Whatman No. 4 filter paper. The solid retained on the filter paper was washed with methanol and oven-dried at 105°C. The yield is the mass percentage of the residue solid to the original bamboo.

5.2.3. Chemical Treatments

The residues from the microwave liquefaction process were further chemically purified. The residues were first bleached in acidified NaClO2 solution (0.1% w/v) at 75 °C for 1h. The bleaching process was to remove the phenolic compounds or molecules from lignin retained in the residues after microwave liquefaction. The residue was filtered and washed with deionized water until its pH was neutral. Next, the beached residues were treated with NaOH solution (0.4%) at 75 °C for 30 min to partially remove hemicellulose. Thereafter, the residual was filtered and rinsed with deionized water until the pulp was neutral.

5.2.4. Ultrasonic Nanofibrillation

The chemically purified residues were soaked in deionized water (concentration 0.2 wt %)
and then subjected to ultrasonic fibrillation using an ultrasonic generator equipped with a 1.5 cm cylindrical probe. The process was performed at a frequency of 25 kHz with an output power of 750 W for 30 min. The ultrasonic fibrillation was conducted in an ice bath, and the ice was maintained throughout the entire process.

5.2.5. Characterization

5.2.5.1. Cellulose content

The purity of the fibers from each stage was characterized by determination of α-cellulose content in accordance with ASTM D 1102-84. The residual lignin content was determined by the Klason lignin method (ASTM D 1106-96). A referenced method was used to determine the hemicellulose content [41].

5.2.5.2. Scanning electron microscope

The surface morphology of the bamboo particles and fiber samples were observed using scanning electron microscopy (SEM, JCM-5000). Test samples were coated with gold using a vacuum sputter coater before subjected to the SEM analysis.

5.2.5.3. Transmission electron microscope

The concentration of aqueous cellulose nanofibers suspensions was diluted from 0.2% to 0.01% (w/w). A droplet of the diluted suspension was deposited on the surface of carbon-coated copper grids. As for contrast in TEM, the cellulose nanofibers were negatively stained in a 2% (wt) solution of uranyl acetate. The morphology of cellulose nanofiber was observed using a transmission electron microscope (TEM, JEOL 100CX, JEOL, Inc., Peabody, MA, USA) with an accelerating voltage of 80Kv. The diameter of the cellulose nanofibers were calculated by measuring one hundred fibers randomly selected from several TEM images.

5.2.5.4. Fourier transform infrared spectroscopy

The FT-IR analysis was performed by a Nicolet Nexus 670 spectrometer equipped with a Thermo Nicolet Golden Gate MKII Single Reflection ATR accessory. Data collection was
performed with a 4 cm⁻¹ spectral resolution and 32 scans were taken per sample.

5.2.5.5. X-ray diffraction

Crystallinity of the original bamboo and fiber samples were analyzed using wide angle X-ray diffraction (Bruker D 5000). The data were generated by a diffractometer with Cu Kα radiation (λ=1.54 Å) at 40 kV and 30 mA over the angular range 2θ=5-40° and a step time of 2.0s. A focusing powder diffraction method was applied. The crystallinity index (CrI) was determined using the following equation:

\[
CrI (\%) = \frac{I_{002} - I_{am}}{I_{002}} \times 100
\]  

(1)

Where \(I_{002}\) is the intensity of the diffraction from the (200) plane at 2θ=22.1 °, and \(I_{am}\) is the intensity for amorphous material taken at 2θ=18 ° [46].

5.2.5.6. Thermogravimetric analysis

TG/DTG analysis was conducted with a thermal analyzer, TGA (Q50), to simultaneously obtain thermogravimetric data. About 2 mg of sample was analyzed by the thermal analyzer. Pyrolysis was terminated at 800°C with a heating rate of 20°C/min under a flow of 60mL/min of nitrogen gas.

5.3. RESULTS AND DISCUSSION

5.3.1. Liquefaction and Preparation of Cellulose Fibers

Cellulose fibers were isolated by the combination of a microwave liquefaction process combined with bleaching and alkali treatment prior to subjecting to ultrasonic nanofibrillation. Table 1 illustrates the yield and chemical compositions of the residues after the microwave liquefaction and chemical treatment stages. The residue yield decreased with increasing reaction time. The residual lignin content in the liquefied residues showed a significant decrease with respect to reaction time, while the cellulose content significantly increased. Table 1 also indicated that the liquefaction of bamboo could also partially eliminate the hemicellulose.
However, the hemicellulose content first increased and then decreased with increasing liquefaction time. The increase in hemicellulose content in the initial reaction stage was attributed to the significant weight loss of lignin, and thereafter enriched the residue resulting in higher (relatively) cellulose and hemicellulose content, and the decrease in hemicellulose as the liquefaction processing was mainly due to the degradation of hemicellulose itself. The decrease in residue yield induced by prolonging the reaction time is mainly due to the efficient removal of non-cellulosic components such as lignin and hemicellulose from bamboo, and thus resulting in the increase in cellulose content. The residues obtained from the liquefaction at 120°C/7min had a cellulose content of 70.74%, which was about 170% of that for the original bamboo, and the residual lignin content was as low as 1.66%.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Residue yield (%)</th>
<th>Cellulose (%)</th>
<th>Hemicellulose (%)</th>
<th>Klason lignin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original bamboo</td>
<td>100.00±0.00</td>
<td>41.72±2.37</td>
<td>22.86±2.19</td>
<td>20.91±0.24</td>
</tr>
<tr>
<td>120°C/3min</td>
<td>60.95±3.42</td>
<td>52.85±3.16</td>
<td>25.49±3.06</td>
<td>7.44±0.61</td>
</tr>
<tr>
<td>120°C/5min</td>
<td>45.99±1.87</td>
<td>66.74±2.09</td>
<td>19.90±1.58</td>
<td>3.82±0.05</td>
</tr>
<tr>
<td>120°C/7min</td>
<td>42.28±2.66</td>
<td>70.74±1.78</td>
<td>18.85±1.74</td>
<td>1.66±0.15</td>
</tr>
<tr>
<td>Bleaching</td>
<td>37.51±1.47</td>
<td>75.30±1.31</td>
<td>18.53±1.43</td>
<td>0.29±0.02</td>
</tr>
<tr>
<td>Alkali treatment</td>
<td>34.17±1.66</td>
<td>83.67±2.69</td>
<td>13.97±1.67</td>
<td>0.13±0.06</td>
</tr>
</tbody>
</table>

However, the residues with high cellulose content from the liquefaction process at 120°C/7min showed a light brownish color (Figure 5.1), indicating that a small amount of lignin was retained in the residue as confirmed by the above mentioned chemical analysis results. To further purify the fibers from the liquefaction process at 120°C/7min, chemical treatments were conducted in sodium chlorite and alkali solution (0.4%). After chemical treatment, the residues became pure white, and the cellulose content increased to 83.67%, and the lignin content was as low as 0.13%. The chemically purified residues obtained in this study showed comparable cellulose content as that reported in Chen’s report [21], i.e., α-cellulose content for chemically purified bamboo fiber was 84.4%. Moreover the time required for such
purity of cellulose fibers was mere 1/8 of that in Chen’s study. Chemically purified cellulose as reported in Chen’s study was obtained from a process involving dewaxing, bleaching, alkali treatment in 16 h. Furthermore, after liquefaction, the chemical usage for further purifying residues was much lower than that used in previously reported methods [5], in which dewaxed samples were bleached three time in 1.4% acidified NaClO₂ followed by alkali treatment with 5% KOH for 4h.

5.3.2. Morphological Observation

SEM images of the raw bamboo particles and samples from microwave liquefaction and chemical treatments are presented in Figure 5.2. The original bamboo showed large fiber bundles and intact structures. Some small fragments were attached on the uneven surfaces (Figure 5.2a and Figure 5.2b). The diameter of the raw bamboo sample was 567 ± 149 μm. The morphological structure of the residues from microwave liquefaction differed significantly from that of the original bamboo. After 7 min of reaction time, the fiber bundles were separated into individual micro-sized fibers with a diameter of 17±6.9 μm. The separation of fasciculus induced by microwave liquefaction is mainly attributed to the removal of lignin in the middle lamella, resulting in the collapse of the compact bamboo structure. It was obvious that the microwave liquefaction was efficient in defibrillation of bamboo by dissociating and dissolving the cementing components. As can be seen in Figure 5.2d, small granules (1-2μm) were found on the surface of the residues. These granules were ascribed to lignin substrates.
Figure 5.2. SEM images of original bamboo (a, b), residue from microwave liquefaction of 120°C/7min (c, d), residue from the bleaching of liquefied residue (e, f), and residue from alkali treatment of bleached samples (g, h)
Similar granules were also observed on the residues from microwave liquefaction of bamboo for biopolyols, and the granules were confirmed as lignin substrates by FTIR analysis [38-39]. This result indicated that a small amount of lignin was still retained on the residues, which was consistent with results from the wet chemistry analysis as discussed above.

After bleaching due to the complete elimination of lignin on the fiber surface and in the fiber cell wall, the micro-sized fibers were opened as evidenced by fissures (Figure 5.2e). Meanwhile the fiber surface became rough, and microfibrils bundles (0.5-1.0 μm) that were composed of nano-sized fibrils were obviously visible and began to peel off from the micro-sized fibers (Figure 5.2f). Compared to the fibers from the bleached residues, fibers from the alkali treated residues became irregular and were much rougher because of the partial removal of hemicellulose (Figure 5.2g). Curled and flat fiber cells with many fibril bundles attached were observed in the SEM images of the alkali treated residues (Figure 5.2g). From the SEM images of the alkali treated fibers, it seemed that the nano-sized fibril bundles attached to each other without any non-cellulosic cements filling in the space (Figure 4.2h). The ultrasonic treatments resulted in the breakdown of the hydroxyl bonds and nano-sized fibril bundles and individual nano fibrils were released as evident from the TEM images.
Figure 5.3. TEM images of (a) individual/elemental fibril, (b) nanofiber network structure, (c) opening of fibril bundle, (d) aggregated bundle, and (e) diameter distribution.
The obtained cellulose nanofiber aqueous suspension was diluted and characterized by TEM. The TEM images confirmed the presence of individual nanofibers (Figure 5.3a). As shown in Figure 5.3b, individual long fiber-like nano fibrils were entangled together forming a web-like structure. An interconnected network of nanofibers could provide great reinforcing capability for composite applications [47]; therefore, the nanofibers obtained in this study are of particular interest for future research in this regard. It is interesting to note that an opening of a fibril bundle (19 nm) is clearly shown in Figure 5.3c. The diameters of the split single fibrils in the opening position were 2-5 nm, which was in the diameter range of single elemental fibrils in higher plants [48]. This result demonstrated that the nanofibillation force introduced by the ultrasonicator was strong enough to defibrillate the cellulose fibers which were obtained by microwave liquefaction and chemical treatment to the elemental fibril level. This was because ultrasonic treatment can break the hydrogen bonds and disintegrate microfibers into nanofibrils. Figure 5.3d presents the aggregate of individual fibrils. It can be seen that individual fibrils associated with one another and formed a large aggregated bundle with a diameter of about 20 nm.

The TEM images were further processed with analysis software (Image J) to determine the diameter. The diameter of the nanofibers was in the range of 2-30 nm. For comparison, the nanofibers obtained in this study had a similar diameter to the cellulose nanofibers isolated from bamboo in other studies; the diameter for nanofibers prepared by using mechanochemical and chemical-ultrasonic processes were 15-30 nm and 10-40nm, respectively [21, 49]. Figure 5.3e presents the diameter distribution of the nanofibers. About 70% of fibers have a diameter within 2-10 nm, 22% of the fibers had a diameter within 12-20nm, and only 9% of the fibers had a diameter greater than 20 nm. The TEM images and the diameter distribution results revealed that the aqueous suspension contained nanometer scale fibers comprised of a large number of elemental fibrils, some fibril bundles, and a small amount of aggregated bundles.
There was no precipitate formed in the nanofiber aqueous suspension after set up for 24h, indicating that the nanofiber samples were still stable after set up for 24h (Figure 5.1). This may be attributed to the strong fibrillation forces induced by high-intensity ultrasonication. The introduction of sulphate groups during the liquefaction process that was confirmed by the FTIR spectra as discussed later may also contribute to the stability of the nanofiber suspensions since the sulphate groups could provide negative electrostatic layer on the surface of the nanofibers resulting in the homogeneous and stable aqueous suspensions (Bondeson & Oksman, 2007).

5.3.3. FTIR Spectroscopy Analysis

The FTIR spectra of the original bamboo, microwave liquefied residue, bleached residue, alkali treated residue, and cellulose nanofiber are presented in Figure 5.4. The absorbance peak at 3330 cm\(^{-1}\) represents the stretching vibration of OH and the intensity peak at 2890 cm\(^{-1}\) was attributed to the asymmetric stretching vibration of CH\(_2\) in cellulose, hemicellulose, and lignin were found in all spectra.

![Figure 5.4. FTIR spectra of (a) original bamboo, (b) microwave liquefied residue, (c) bleached residue, (d) alkali treated residue, and (e) cellulose nanofiber](image)

The spectrum of the original bamboo showed a significant difference from that of the
microwave liquefied residue. The peaks at 1735 cm\(^{-1}\) (attributed to the acetyl and uronic ester groups or the ester linkage of the carboxylic group of ferulic and \(p\)-coumaric acid of hemicellulose), 1596 cm\(^{-1}\) and 1506 cm\(^{-1}\) (arising from the aromatic skeletal vibration), 1456 cm\(^{-1}\) (assigned to C-H deformation combined with aromatic ring vibration), and 1230 cm\(^{-1}\) (corresponding to methoxyl groups of lignin) were all shown to be strong peaks in the spectra of the original bamboo. The peak at 1735 cm\(^{-1}\) was weakened in the spectrum of microwave liquefied residue, indicating the cleavage of the linkages between ferulic acid or \(p\)-coumaric acid or \((p-)\) hydroxycinnamic acids and lignin in the fibers [50]. The dissociation of the key ester linkages between lignin and carbohydrates promoted the dissolution of lignin into the solvents. The absorbance bands at 1596 and 1456 cm\(^{-1}\) disappeared and the bands at 1506 and 1230 cm\(^{-1}\) became small shoulders. This result indicates that lignin functional groups such as aromatic rings were almost completely dissociated and dissolved with only a small amount of associated lignin retained on the residues. The retained lignin was confirmed by the aforementioned SEM images, in which lignin residues were observed on the exterior surface of the fibers from the residues. The absorbance band at 1203 cm\(^{-1}\) attributing to S=O vibration appeared in the spectra except for that of the original bamboo [51], revealing that sulphate groups were introduced during the microwave liquefaction process since sulfuric acid was used as the catalyst.

All of the four characteristic absorbance bands of lignin (1596, 1506, 1456, 1230 cm\(^{-1}\)) were absent in the spectrum of the bleached residues (Fig. 4, spectrum c), suggesting the complete removal of lignin from the residue. The peak at 1735 cm\(^{-1}\), attributed to hemicellulose, disappeared in the spectrum of the alkali treated residue. This disappearance indicated that the removal of hemicellulose was achieved by the diluted sodium hydroxide solution (Fig. 4, spectrum d). No significant difference was found between the spectra of the alkali treated residue and the nanofibers indicated that the ultrasonic nanofibrillation process did not change
the chemical structures of the fibers.

5.3.4. Crystallinity Analysis

Figure 5.5 shows the WXRD spectra of the original bamboo, microwave liquefied residue, bleached residue, alkali treated residue, and cellulose nanofibers. All of the XRD patterns displayed peaks at $2\theta = 14.9^\circ, 16.1^\circ, 22.1^\circ, \text{ and } 34.5^\circ$, corresponding to the (1 1 0), (1 1 0), (2 0 0), and (0 0 4) crystallographic planes, respectively [46]. These peaks were typical signatures of cellulose I crystalline structure, which was performed by repeating $\beta-(1\rightarrow4) - D$-glucopyranose units, and building blocks of parallel glucan chains [52].

The calculated crystallinity index (CrI) values were 52.3 and 70.6% for the original bamboo and microwave liquefied residue, respectively. The bamboo crystallinity was improved by 135% after liquefying for 7 min. This significant increase ($p<0.05$) of crystallinity in the microwave liquefied residues with comparison to the original bamboo was attributed to the efficient dissolution and removal of non-cellulosic materials such as lignin and extractives from the amorphous regions and enrichment in cellulose content in the liquefied residues during microwave liquefaction. This was in good agreement with the chemical analyses results (Table 5.1). This result also indicated that microwave liquefaction could be used as an efficient pre-purification process for the isolation of cellulose fibers.

The crystallinity was further increased to 72.5% by the application of a bleaching treatment. This was mainly due to the complete elimination of retained lignin in the liquefied residues. The alkali treatment also improved the crystallinity of the bleached residue to 74.2% by partially removing the hemicellulose. However, the crystallinity of the nanofibers was found to be lower than that for the alkali treated residue; the CrI for the nanofiber was 67.4%. This was likely due to the breakdown of the hydrogen bonds of the cellulose fibers during the ultrasonic nanofibrillation process, revealing that the ultrasonication treatment caused damage to the crystalline domain of the nanofibers. This result was in accordance with a previous study in
which Lu found that ultrasonication treatment could damage the crystalline region of cellulose resulting in a decrease in the crystallinity index [49]. The decrease in the crystallinity for nanofibers from cellulose pulp was also reported when a high pressure homogenizer was applied for nanofibrillation [1].

![Figure 5.5. X-ray diffraction spectra of (a) original bamboo, (b) microwave liquefied residue, (c) bleached residue, (d) alkali treated residue, and (e) cellulose nanofiber](image)

5.3.5. Thermal Stability

The TG and DTG curves of the original bamboo and samples from each stage are illustrated in Figure 5.6. Three different weight loss processes were observed in the TG curve for the original bamboo. The initial weight loss was found in the temperature range of 50-200 °C due to the evaporation and removal of bound water and loss of extractives existing in bamboo [53]. A dramatic weight loss was shown in the temperature range of 220-450°C which was attributed to the thermal depolymerization of carbohydrates and lignin. The small weight loss in the temperature range of 450-650 °C contributed to the degradation of lignin residues from the second stage tar and char. On the DTG curve of the original bamboo, a small shoulder at 260°C corresponding to the decomposition of hemicellulose was found. The peaks on the
DTG curves were the maximum degradation rate temperature ($T_{max}$) corresponding to the thermal decomposition of cellulose and the $T_{max}$ for the original bamboo was 333°C.

The $T_{max}$ for the microwave liquefied residue was 25°C lower than that for the original bamboo. The reason may be that, the compact bamboo structure was dissociated by microwave liquefaction; the obtained residues had a larger surface area comprised of small hemicellulose and lignin fragments that could be easily decomposed at lower temperature [54]. These substances may initiate more active sites and accelerate the decomposition of the residues. Another explanation may be the introduction of sulphate groups into the residues during the liquefaction process, which was confirmed by the FTIR spectra as shown in Fig.4 and discussed earlier. The sulfate groups worked as a dehydration catalyst and decreased the activation energy of cellulose chain degradation [55]. The bleached residue and alkali treated residue showed a higher $T_{max}$ than the original bamboo. This was largely due to the complete elimination of lignin and partial removal of hemicellulose. The highest $T_{max}$ was found for nanofiber, 374°C. Meanwhile, the lowest char yield was obtained for the nanofiber, which was due to the removal of non-cellulosic components in the nanofibers. Similar results were found in the isolation of nanofibrils from the Helicteres isora plant [33]. The thermogravimetric analysis revealed the nanofiber obtained in this study had high thermal stability.
Figure 5.6. TGA and DTG curves of (a) original bamboo, (b) microwave liquefied residue, (c) bleached residue, (d) alkali treated residue, and (e) cellulose nanofiber
**5.3.6 Formation Mechanism**

A formation mechanism of cellulose nanofiber from bamboo was proposed based on the aforementioned results and analyses (Figure 5.7). At the first step, the ester linkages between the carbohydrates and lignin were dissociated and lignin in the middle lamella was depolymerized by microwave liquefaction, which resulted in the dissociation of the intact raw bamboo into micro-sized residues. The microwave liquefied residues had considerable accessibility for subsequent chemical purification. Then, bleaching and alkali treatment were applied to completely eliminate the lignin and partially remove the hemicellulose in the residues, respectively. After alkali treatment, the cellulose fibers showed an irregular and rough morphology allowing it more accessible for physical force. Finally, ultrasonic radiation was used to break the hydrogen bonds. Therefore, element nano fibrils and nano fibril bundles were obtained. The microwave liquefaction process serves a prominent role in the purification and defibrillation of raw bamboo during the whole process since it enhanced the efficiency for
subsequent operations by decreasing the chemical usage and time requirement.

5.4. CONCLUSIONS

The present work showed that cellulose nanofibers could be successfully extracted from bamboo with microwave liquefaction and chemical treatment as a purification process and ultrasonication as a nanofibrillation process. Bamboo bundles were converted into micro-sized fibers with high cellulose content by microwave liquefaction prior to chemical treatments. For the extraction of purified cellulose fibers, bleaching and alkali treatment were employed. However, the amount of chemical reagents and time required was 1/8 of that of traditional methods. Due to the complete elimination of lignin and partial removal of hemicellulose, the chemically purified residues took on an irregular and rough morphology and fibril bundles began to split off from the fiber framework. The presence of cellulose nanofibers were confirmed by TEM images, and the diameters of the nanofibers were in the range of 2-30 nm. FTIR and XRD spectra revealed that the combination of microwave liquefaction and chemical treatment was an efficient approach for removing non-cellulosic materials in bamboo for cellulose nanofibers production purpose. Ultrasonic irradiation resulted in a reduction in crystallinity of cellulose nanofibers. The isolated cellulose nanofibers have potential application for the fabrication of thermally stable composites because of their high thermal stability. Based on these results, this new proposed method could be developed into a practical technique for the generation of cellulose nanofibers.

5.5. REFERENCES


[43] Chen, H.Z., Zhang, Y.C., & Xie, S.P. Selective liquefaction of wheat straw in phenol and


CHAPTER 6. PHYSICO-CHEMICAL CHARACTERIZATION OF THE
RECOVERED LIGNINS FROM THE MICROWAVE LIQUEFACTION
FOR USE IN BIOBASED MATERIALS

6.1. INTRODUCTION

Alcohol-based fuels such as bioethanol derived from biomass have attracted the attention of researchers worldwide due to the continuing depletion of fossil fuels. However, to facilitate large-scale utilization it is imperative to develop an economic and cost-effective process for commercial implementation of biofuels [1]. Lignocellulosic biomass, primarily composed of cellulose, hemicellulose, and lignin, has been considered the most suitable for biofuels and green chemical production. Therefore, use of substrates such as lignocellulosic biomass in the form of agricultural and forest residues is a viable solution to boost the economic viability of biofuels. However, the most costly component of alcoholic biofuels synthesis is the pretreatment of the biomass prior to fermentation. The maximum amount of sugar monomers (mainly cellulose and hemicellulose) from raw biomass is necessary for efficient and economic alcoholic biofuels production [2]. Lignin, which fills the space between cellulose and hemicelluloses and cross-links with hemicellulosic polysaccharides, acts as a barrier for the accessibility of chemicals and enzymes to cellulose [3]. It is, therefore, necessary to extract lignin. Various pretreatments processes of lignocellulosic biomass, such as acidic/alkaline pretreatment [4], wet oxidative [5], steam explosion [6], ultrasound-assisted [7], and mechanical forces [8] have been applied in the fractionation of different lignocellulosic biomass to fulfill the demand for lignocellulosic bioethanol.

However, lignin from the biorefinery industries is currently an underutilized waste product because lignin is often relegated to the low value of combustion or sold as a natural component

for animal feeds [9]. About 225 million tons of lignin generation is expected from the cellulosic alcohol industry in the United States in the near future, and only 2% is being used for value added applications [10]. Therefore, new processes are needed that generate value-added products from lignin to sustain the lignocellulosic bioethanol industry. Several studies have explored the use of lignin as an inexpensive filler/additive component to modify thermoplastic material properties such as hydrophobicity, stiffness, and mechanical properties [11-17]. Liquefied/depolymerized lignin usually with high reactivity and low molecular weight has been applied in the fractionation of green polyurethane foams and other biobased products [18-19]. With all these attempts to expand the industrial applications of lignin in thermoplastic industries, PLA is mainly used as the plastic matrix because of its biodegradable character. In addition, PLA also plays a predominant role as a sustainable alternative to petrochemical-derived products. Recently, PLA has been widely studied for use in packaging materials [20-21]. However, the use of PLA in the packaging industry is limited to the production of high value products such as films and rigid thermoforms because of its high cost.

In our recent studies, lignin samples were recovered from a microwave-assisted delignification system. The objective of this study is to understand the recovered lignin fraction and its potential applications in biobased materials. Therefore, the physico-chemical characteristics of the lignins were evaluated. PLA-lignin composites were prepared and the mechanical properties, thermal stability, and optical transmission of the obtained materials were determined. The results of this study will provide preliminary information to facilitate the commercialization of lignin by-products from biorefinery industries.

6.2. MATERIALS AND METHODS

6.2.1. Materials

Moso bamboo, Chinese tallow tree wood, pine wood, and switchgrass were chosen as the experimental feedstocks. All materials were harvested from the Kisatchie National Forest,
Pineville, Louisiana, USA. Each sample was reduced to particles using a Thomas Wiley Laboratory mill. The particles were screened to collect particles that passed through a 20-mesh sieve and then retained on a 40-mesh sieve and then dried to a constant weight in an oven maintained at 80°C. The dried particles were stored in polyethylene bags and used without further treatment. The density and melt flow index of the PLA matrix was 1.24g/cm³, and 20g/10min at 190°C/2.16kg, respectively. All chloroform, glycerol, methanol, and acid used were of reagent grade and obtained from commercial sources. Westvaco and Reed lignins were purchased and used as industrial lignin samples.

**6.2.2. Microwave-assisted Liquefaction and Lignin Recovery**

Liquefaction of lignocellulosic biomass was carried out in a Milestone MEGA laboratory microwave oven at an output power of 550W using a modified solvolysis liquefaction process. Mixture of glycerol and methanol (glycerol/methanol: 2/1 (w/w)) was used as the solvent, and sulfuric acid was used as the catalyst. A typical reaction mixture consisting of 2 g of lignocellulosic particles, 8 g of solvent, and 0.14 g of sulfuric acid were loaded in Teflon vessels with a magnetic stirring bar. The Teflon vessels were then placed on a rotor tray inside the microwave cavity. The temperature was monitored using an ATC-400FO automatic fiber optic temperature control system. Based on the monitored temperature, the output power was auto-adjusted during liquefaction. In this study, the temperature was increased from room temperature to 120°C at a heating rate of 25°C min⁻¹ and was then kept constant for 4 min. After a cooling period of 30 min at the end of reaction, the resulting reaction mixtures were then vacuum-filtered through Whatman No.4 filter paper.

The filtrated liquid was evaporated at 45°C under vacuum to remove methanol, then distilled water (10/1, w/w) was added to the obtained liquid. The mixture was stirred thoroughly with a glass rod. Afterwards, the mixture was centrifuged at 5000rpm for 10min. The precipitates were dried at 30°C for 12h. The recovery yield is the mass percentage of the
precipitates to the original weight of lignocellulosic biomass.

6.2.3. Preparation of PLA-lignin Composites

PLA and lignin samples were vacuum dried at 60 °C for 24h before the preparation of PLA-lignin composites. Recovered bamboo lignin samples (25mg) were stirred in 5mL of 1, 4-dioxane for 20 min. Thereafter, the lignin solution was blended with PLA (500mg) in 10 mL chloroform for 2h. Then, the mixture was vacuum dried at 60 °C prior to use. PLA-lignin composites were prepared by a hot-press (Carver Laboratory Press, Model C) process. The dried mixture was first kept at 180 °C for 20s without pressure, then 0.125 ton for 15s, and then 0.5 ton for 90s.

6.2.4. Wet Chemistry Analysis

The Klason lignin and ash content of the samples were determined according to ASTM standards D1106-96 and D1102-84, respectively [22-23].

6.2.5. FTIR Spectra

The FT-IR analysis was performed by a Nicolet Nexus 670 spectrometer equipped with a Thermo Nicolet Golden Gate MKII Single Reflection ATR accessory. Data collection was performed with a 4 cm\(^{-1}\) spectral resolution and 32 scans were taken per sample.

6.2.6. Thermogravimetric Analysis

TG/DTG analysis was conducted with a thermal analyzer, TGA (Q50), to simultaneously obtain thermogravimetric data. About 2 mg of sample was analyzed by the thermal analyzer. Pyrolysis was terminated at 800°C with a heating rate of 20°C/min under a flow of 60mL/min of nitrogen gas.

6.2.7. Mechanical Testing

Mechanical properties of the PLA-lignin composites were characterized by the tensile strength, and the specimen used for the experiments were sample strips of 50mm×5mm×0.1mm. The tensile tests were performed using an Instron testing machine (Instron 4465), with a load
of 5 KN at a crosshead speed of 5mm/min. Five replicates for each type of composite were tested and the average values are presented.

6.2.8. Optical Transmittance

The transmittance of neat PLA and PLA-lignin films 0.03 mm in thickness were measured using a HP UV-vis spectrometer (UV-HP 8453). The samples were scanned from 190-800nm.

6.3. RESULTS AND DISCUSSION

6.3.1. Recovery Yield

The recovery lignin yield and its main composition were determined and presented in Table 6.1. The recovery lignin yield was 17.62, 23.04, 20.78, and 18.75% for bamboo, Chinese tallow tree wood, switchgrass, and pine wood, respectively; while the original klason lignin content in bamboo, Chinese tallow tree wood, switchgrass, and pine wood was 20.91, 19.13, 20.61, and 30.96%, respectively. No significant difference in the lignin recovery yield and the original klason lignin content was found except for pine wood. All the recovered lignin samples showed high klason lignin content (>75%). Recovered lignin samples except switchgrass had low ash content (0.01-0.06%).

<table>
<thead>
<tr>
<th>Biomass type</th>
<th>Recovery yield (%)</th>
<th>Klason lignin (%)</th>
<th>Ash content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bamboo</td>
<td>17.62</td>
<td>75.66</td>
<td>0.031</td>
</tr>
<tr>
<td>Chinese Tallow tree</td>
<td>23.04</td>
<td>75.63</td>
<td>0.057</td>
</tr>
<tr>
<td>Switchgrass</td>
<td>20.78</td>
<td>77.56</td>
<td>0.091</td>
</tr>
<tr>
<td>pine wood</td>
<td>18.75</td>
<td>75.44</td>
<td>0.010</td>
</tr>
</tbody>
</table>

6.3.2. Chemical Structure

The FTIR analysis was used for in-depth elucidation of structural features of the recovered lignin, and the IR spectra of the recovered lignin as well as industrial lignin sample (Westvaco lignin) are depicted in Figure 6.1. The assignments of absorption bands corresponding to different functional groups of lignin are presented in Table 6.2 [24-25]. The bands at around 3389 cm⁻¹ are assigned to O-H stretching; and were weak bands in all the lignin samples. The
bands at around 2938 and 2847 cm⁻¹ are assigned to CH stretch in CH₂ and CH₃ groups. Typical lignin absorption bands, such as aromatic ring vibration of phenyl-propane (1601-1594 cm⁻¹), aromatic ring vibration C=C of the benzene ring (1506-1501 cm⁻¹), C-H deformation combined with aromatic ring vibration (1459-1451 cm⁻¹) were significantly visible in all lignin spectra. This confirmed that the core structure of the lignin was still retained after the delignification process.

![FTIR spectra of lignin samples](image)

Figure 6.1. FTIR spectra of lignin samples recovered from delignified (a) bamboo, (b) Chinese tallow tree, (c) switchgrass, (d) pine wood, and (e) Westvaco lignin

However, significant differences in bands attributed to lignin functional groups were also observed among lignin samples recovered from different lignocellulosic biomass types. The bands at 1371 cm⁻¹, which is assigned to O-H deformation in phenols were apparent in the spectra of the Westvaco lignin and lignin recovered from pine wood, while that was almost invisible in the lignin spectra of bamboo, Chinese tallow tree wood, and switchgrass. Lignin structure bands at 1325cm⁻¹ assigned to S ring breathing and G ring substituted in position 5
and 1116-1113 cm\(^{-1}\) assigned to aromatic C-H in plane deformations (S units) were found in the lignin spectra of bamboo, Chinese tallow tree wood, and switchgrass; while, the former band was absent and the latter shifted to 1143 cm\(^{-1}\) in the lignin spectra of pine wood and Westvaco. Such shifts of this band toward to higher wavenumbers usually contributed to the increase in S units [26]. Meanwhile, bands at 912 and 833 cm\(^{-1}\) contributed to aromatic C-H out-of-plane deformation, specific to hardwood lignin, were also absent in the lignin spectra of pine wood and Westvaco. C-O stretching in G lignins appeared at bands of 1266 cm\(^{-1}\) in the pine wood lignin spectrum confirmed pine wood possessed high fraction of G units.

Comparing the spectra between pine wood and Westvaco lignin, it was found that the two spectra were almost identical; suggesting the structures of the recovered pine wood lignin was close to that of Westvaco lignin. The FTIR results clearly showed that Chinese tallow tree wood and pine wood lignins still contain traces of hardwood and softwood lignin functional groups, respectively.

6.3.3. Thermal Behavior

Thermogravimetric (TGA) and differential thermal analysis (DTG) of recovered lignin samples from different lignocellulosic biomass are described in Figure 6.2. Thermal decomposition profiles of commercially obtained lignin sample (Westvaco lignin) were also tested as a comparison. As shown in Figure 6.2, the Westvaco lignin was thermally decomposed over a wide temperature (130-800\(^{\circ}\) C) with two maximum decomposition rates. Accordingly, the lignin structure is very complex because it is mainly composed of aromatic rings with C\(_{\alpha}\) - C\(_{\beta}\) - C\(_{\gamma}\) side chains and has various oxygen functional groups [27]. These structural characteristics result in an extremely wide temperature range of decomposition. Two thermal events were identified during the degradation of the Westvaco lignin: in the first step (240-420 \(^{\circ}\) C), volatiles such as, CO\(_2\), H\(_2\)O, and CO were primarily released by breaking the side chains of carboxyl groups, hydroxyl groups, and aldehyde groups, respectively; and in the second one
(450-800°C), the primarily released substances were CH₄ (generated by breaking the methoxy group-OCH₃-), CO₂, and CO [26]. The DTG curves of Westvaco lignin showed wide and flat peaks with a gently sloping baseline. This is different for the sharp DTG peaks of recovered lignins with a flat tailing section at higher temperatures. It was evident that the structural groups of the recovered lignin differed significantly from the Westvaco lignin.

Table 6.2. Band assignments in FTIR

<table>
<thead>
<tr>
<th>Wavenumber (cm⁻¹)</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>3330-3389</td>
<td>O-H stretching (rupture of cellulose hydrogen bonds)</td>
</tr>
<tr>
<td>2926-2938/2834-2847</td>
<td>C-H stretch in methyl and methylene groups</td>
</tr>
<tr>
<td>1741-1719</td>
<td>C=O stretching due to ester linkage between carbohydrate and lignin</td>
</tr>
<tr>
<td>1601-1594</td>
<td>Aromatic ring vibration of phenyl-propane (C₀)</td>
</tr>
<tr>
<td>1506-1501</td>
<td>Aromatic skeletal vibration C=C of the benzene ring</td>
</tr>
<tr>
<td>1458-1451</td>
<td>C-H deformation combined with aromatic ring vibration</td>
</tr>
<tr>
<td>1430-1419</td>
<td>Aromatic skeletal vibrations associated with C-H in plane deformation in cellulose</td>
</tr>
<tr>
<td>1371</td>
<td>Aliphatic C-H symmetric deformation in methyl + O-H deformation in phenols</td>
</tr>
<tr>
<td>1320-1326</td>
<td>S ring breathing + G ring substituted in position 5</td>
</tr>
<tr>
<td>1266-1272</td>
<td>G ring breathing and C-O stretching</td>
</tr>
<tr>
<td>1213-1218</td>
<td>C-O stretching in phenols and ethers</td>
</tr>
<tr>
<td>1114/1143</td>
<td>Aromatic C-H in plane deformations (S units)</td>
</tr>
<tr>
<td>1026-1030</td>
<td>Aromatic C-H in-plane deformations in G units + C-O deformations in primary alcohols</td>
</tr>
<tr>
<td>909-918</td>
<td>Aromatic C-H out-of-plane deformation (only in GS lignin type)</td>
</tr>
<tr>
<td>830/809</td>
<td>Aromatic C-H out-of-plane in position 2 and 6 from S unit</td>
</tr>
</tbody>
</table>
Variability in the weight loss occurred at the initial stages (180-300 °C), which was mainly due to the decomposition of side chains connected to the multiple inter-units, were found among different recovered lignin samples. For the decomposition of recovered pine wood lignin, it started at relative low temperature (180 °C) with a flat peak, while decomposition of the Chinese tallow tree wood and switchgrass lignin samples occurred at 230-280 °C, with small peaks at 249 and 274 °C, respectively. The result indicated that more substances with lower molecular weight in pine wood lignin were degraded during this decomposition stage. This may be explained by the results of FTIR analysis, as discussed above. The breaking of hydroxyl groups specific to pine wood lignin that were indicated by the band at 1371 cm⁻¹
released more H₂O, which resulted in a larger initial weight loss at relative low temperature. Interestingly, no apparent weight loss was observed at this stage for recovered bamboo lignin. This may be due to the absence or few of side chain groups in recovered bamboo lignins.

Comparing the maximum decomposition rate temperature (T_{max}) as observed in the TGA curves among the recovered lignin samples, the pine wood lignin had higher T_{max} than that for the other three lignins, indicating that the recovered pine wood lignin was thermally stable. This finding was in good agreement with the result that the coniferous lignin was more thermally stable than deciduous lignin because T_{max} for pine wood lignin obtained in this study was about 10 °C higher than that for Chinese tallow tree wood lignin [28]. This result also clarified lignin recovered from the microwave-assisted delignification process retained their natural origin structure, which is consistent with the FTIR analysis results.

6.3.4. Solubility

The insolubility of the pulping industrial lignins in common solvents has severely inhibited efforts to produce value-added platform chemicals due to their poor molecular interactions with other reagents. In order to evaluate the solubility of the recovered lignins, samples were tested in several organic solvents (acetone, ethanol/water (7/3), THF, DMSO, 1, 4-dioxane). The results showed that all the recovered lignin samples were completely soluble in acetone, ethanol/water, THF, DMSO, and 1, 4-dioxane at an initial test concentration of 1mg/mL. Also, the recovered lignins were completely soluble in 1 mol/L solution of NaOH. For comparative purpose, the solubility of recovered bamboo lignin and two commercial lignin samples (Westvaco and Reed lignin) were further tested. As shown in Figure 6.3, by adding lignin in the solvents to 5mg/mL, the bamboo lignin could be completely soluble; while the
Westvaco lignin was partially soluble and the Reed lignin was completely insoluble. These results suggested that the recovered lignin should have better reaction activity because a homogeneous phase can be formed in many reactions by solvents.

![Figure 6.3. Photograph of recovered bamboo lignin in (a) acetone, (b) THF, and (c) 1, 4-dioxane. Note: MCW lignin: Bamboo lignin samples](image)

6.3.5. Preparation and Characteristics of PLA-lignin Composites

Neat PLA composite and PLA-lignin composites with different lignin contents (1, 5, and 10% wt) were prepared, and the tensile properties are reported in Table 3. The tensile strength, tensile modulus, and strain of the neat PLA composite were 40.38±6.64 MPa, 2.56±0.30 GPa, and 2.58±0.49 %, respectively. The addition of lignin into PLA contributed to increase in tensile properties. With 1% wt of lignin, the increments in tensile properties for the composites are
32.17, 12.89, and 10.07% for tensile strength, tensile modulus, and strain, respectively. Similar results were obtained by Chung, et al, who reported the incorporation of modified lignin-g-PLA copolymers into PLA could result in a modest increase in tensile strength and strain, while the unmodified lignin addition decreased the tensile strength [16]. Other studies argued the addition of commercial lignin to polymer matrix decreased the mechanical properties of composites, while the addition of acetylated commercial lignin or organosolv lignin contributed to improved mechanical properties [15, 17]. Surface modification, mainly via acetylation of lignocellulosic components (cellulose fibers or lignin) could increase their dispersion and affinity with biopolymers, and the good interfacial adhesion between the modified lignocellulosic components and polymer matrix attributed to the higher mechanical properties of biobased materials [15, 29]. Based on these results, it can be easily understood that the higher tensile properties of PLA-lignin composites obtained in this experiment compared to neat PLA composites is due to the well dispersion of lignin and its interfacial adhesion with PLA because of its good solubility as discussed above.

<table>
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<tr>
<th>Composition</th>
<th>Tensile strength (MPa)</th>
<th>Tensile strain (%)</th>
<th>Tensile modulus (GPa)</th>
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<tr>
<td>PLA</td>
<td>40.38±6.64</td>
<td>2.56±0.30</td>
<td>2.58±0.49</td>
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<tr>
<td>PLA/1%lignin</td>
<td>53.37±7.84</td>
<td>2.89±0.38</td>
<td>2.84±0.59</td>
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<tr>
<td>PLA/5%lignin</td>
<td>48.38±8.92</td>
<td>2.81±0.73</td>
<td>3.04±0.29</td>
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<tr>
<td>PLA/10%lignin</td>
<td>49.59±6.55</td>
<td>2.50±0.43</td>
<td>3.16±0.03</td>
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</tbody>
</table>

TG and DTG curves of neat PLA and PLA-lignin composites are shown in Fig. 4. The initial decomposition temperature corresponding to 5% weight loss ($T_{5\%}$), maximum degradation rate temperature ($T_{\text{max}}$), and char yield are presented in Table 4. The $T_{5\%}$ of the neat PLA composite was particularly high with comparison to PLA-lignin composites. This is
mainly due to the decomposition of lignin substances with low molecular weight since the decomposition of lignin started at low temperature (180-300°C) and covered a wide temperature range as discussed above. $T_{\text{max}}$ for the PLA-lignin composites are a little bit higher than that for the neat PLA composite, however, no significant difference was found. It was reported that the good compatibility of lignin with a PLA matrix resulted in a good retention of the thermal properties [11]. From this point of view, the good miscibility of the recovered lignin with PLA is the reason that the $T_{\text{max}}$ is not obviously affected by the addition of lignin. Char yield showed a decreasing with the addition of lignin, which was contrasted with the results in another study [14]. This result may be due to the character differences among various lignin sources.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$T_{5%}$ (°C)</th>
<th>$T_{\text{max}}$ (°C)</th>
<th>Char yield (%)</th>
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<tr>
<td>PLA</td>
<td>317.15</td>
<td>361.78</td>
<td>3.42</td>
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<td>295.57</td>
<td>362.39</td>
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<td>306.45</td>
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<td>0.07</td>
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<td>PLA-10%lignin</td>
<td>286.04</td>
<td>366.89</td>
<td>1.04</td>
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Table 6.4. Temperature and char residue characteristics of PLA-lignin composites
Figure 6.4. TG and DTG curves of neat PLA composites (PLA) and PLA-lignin composites (PLA/1L, PLA/5L, and PLA/10L for composite with 1, 5, and 10 % bamboo lignin content, respectively)

The transmissions of visible and ultraviolet light are important parameters in designing the right packaging materials to preserve and protect light sensitive foods/products. Despite the fact that PLA films had better UV light barrier properties than LDPE, it is slightly worse than those of PET which does not transmit both UV-C (100-280 nm) and UV-B (280-315 nm) [30]. As shown in Fig. 5, PLA showed no UV light transmission in the lower range of UV-C (100-230 nm), while the UV light is transmitted in the higher range of UV-C (230-280 nm) and nearly all the UV-B (280-315 nm) and UV-A (315-400 nm) light passed through the PLA films. The addition of a small amount of the recovered lignin (0.5% wt) in PLA resulted in a significant improvement in light barrier properties, i.e., about 80% of UV-C (230-280 nm) and 40% of UV-B light (280-315 nm) were blocked by the PLA-lignin films. By adding 1% wt of
lignin in PLA, the PLA-lignin film blocked nearly all the UV-C and UV-B and 75-80% of UV-A light. The result revealed that the recovered lignin could be used as an efficient additive in PLA to block UV light transmission.

![Figure 6.5. UV-vis spectra for PLA and PLA-lignin composites (PLA/0.5L and PLA/1L for composite with 0.5 and 1 % bamboo lignin content, respectively)](image)

**6.4. CONCLUSIONS**

Lignins recovered from microwave-assisted liquefied lignocellulosic biomass system showed high purity and retained their natural structures. Lignin samples from delignified pine wood exhibited the highest thermal stability. All the lignin samples could be completely soluble in ethanol/water, DMSO, THF, 1, 4-dioxane, and 1mol/L NaOH solution. The PLA-lignin composites had higher tensile properties than that for neat PLA composite. The addition of lignin into PLA had no significant influence on the thermal degradation property of the
composites. Lignin component in the PLA-lignin blends significantly improved the UV light barrier properties of the composites.

6.5. REFERENCES


CHAPTER 7. CONCLUSIONS AND FURTHER WORK

7.1. OVERALL CONCLUSIONS

Bamboo particles were liquefied in a glycerol/methanol binary solvent system using microwave energy. The liquefied bamboo was thoroughly characterized and purified for the isolation of cellulose nanofibers. The overall conclusions of this work are listed below:

(1) Conversion of bamboo at about 94.33% could be achieved by liquefaction with glycerol/methanol using microwave energy with temperature of 150°C. The liquefaction temperature and time had a mutual effect on the liquefaction process. The liquefaction was complex solvolysis chemical reactions under critical conditions according to the residue characteristics, i.e., the recondensation of lignin and hemicellulose took place as well as cellulose decomposition. The FT-IR spectra could be selected as a simple method to evaluate the liquefied degree of bamboo and its three main components. The SEM images of the residues from different conditions showed significant differences revealing the variance in liquefaction behaviors with respect to conditions.

(2) Bamboo feedstocks with large particle size (20-40 mesh) were successfully converted into biopolyols comprising of C₅ and C₆ sugars and phenolic compounds by microwave liquefaction. Using feedstocks with smaller particles, liquefaction yields increased dramatically at fairly low temperature (120°C), but higher temperature (140°C) led to lignin recondensation. The chemical and morphology analysis results revealed the liquefied bamboo residues retained fiber structure and remaining cellulose. Bleaching and acid hydrolysis proved to be efficient in purifying the residues for pure white cellulose fibers. The chemically purified cellulose fibers from liquefied residues were reduced to nanofibrillated cellulosic fibers by subjecting them to high-intensity ultrasonic treatment. The generated nanofibrillated cellulosic
fibers were in the range of long nanofibrils, which was suitable for use in reinforcing composites.

(3) Microwave liquefaction at 120 °C proved to be an efficient approach in the elimination of lignin and extractives from bamboo, which could be used to produce cellulosic materials with high purity in a considerable yield. The optimized process for the production of cellulose enriched fibers was 120 °C/9min, with which the cellulose content was as high as 70%. For the extraction of purified cellulose fibers, bleaching and alkali treatment were employed. After bleaching and alkali treatment, the cellulose content reached 85%.

(4) By converting into micro-sized fibers with high cellulose content using microwave liquefaction and the complete elimination of lignin and partial removal of hemicellulose with subsequent chemical purification, the cellulose fibers took on an irregular and rough morphology, and fibril bundles began to split off from the fiber framework. FTIR and XRD spectra revealed that the combination of microwave liquefaction and chemical treatment was an efficient approach for removing non-cellulosic materials in bamboo for cellulose nanofibers production purpose. The isolated cellulose nanofibers have potential application in the fabrication of thermally stable composites because of their high thermal stability.

(5) The physico-chemical properties of the recovered lignin from microwave liquefaction of bamboo showed that the samples had a high Klason lignin content (>75%) and retained their natural structure. Thermogravimetric analysis (TGA) revealed that the recovered lignin exhibited a different thermal decomposition pattern from that of commercial lignins. The lignin samples had good solubility in common organic solvents (acetone, 1, 4-dioxane, THF, DMSO, and ethanol/water) and 1mol/L NaOH solution. PLA-lignin composites were successfully
fabricated by adding lignin into the polylactic acid (PLA) matrix. The addition of lignin resulted in improvement in tensile properties of PLA-lignin composites. PLA films with low lignin content had good UV light resistant properties, indicating that the recovered lignin has potential in packaging of light sensitive products.

7.2. FURTHER WORK

Microwave liquefaction of bamboo was proven to be an efficient and environmentally friendly method to produce cellulose nanofibers in this work. Possible further work is still needed regarding several aspects:

(1) For the characterization of the liquefied residues from various liquefaction conditions, this work mainly focused on tracing cellulose structure and morphology. The non-cellulosic components that contributed to the brown color were not identified. Research on the chemical composition identification and the determination methods should be performed in further work.

(2) Exploration should be conducted on low environmental impact methods to purify the cellulosic materials from the liquefied fraction and the application of other nanofibrillation process such as high pressure homogenizer.

(3) All the research results in this work were collected using a laboratory microwave reactor. A pilot scale experiment on the microwave liquefaction of bamboo and the production of cellulose nanofibers should be conducted.

(4) Tests need to be conducted to determine whether these cellulose nanofibers are actually suitable for use in nanofiber reinforced materials.

(5) The chemical compositions of the liquid products were identified using GC-MS and FTIR, while properties such as hydroxyls groups, acid number, and viscosity were not
determined. The physical properties, as well as the potential applications of the liquid products, should be investigated in the future.

(6) The glycerol used in this research were reagent grade, liquefaction of bamboo in crude glycerol from bio-diesel should be investigated to test the feasibility of crude glycerol on microwave liquefaction.
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Jiulong Xie was born in Henna Province, China. He studied wood science in College of Forestry at Sichuan Agricultural University, Chengdu, and obtained his BS in engineering in 2011. He got a Master degree in Forestry from Sichuan Agricultural University with Dr. Jinqiu Qi as his advisor in 2014. The thesis on his master program concentrated on the characterization of moso bamboo and its integrated utilizations. During the period of August 2012 – August 2013, Jiulong Xie studied wood chemistry in School of Renewable Natural Resources at Louisiana State University as a visiting student. Since 2015, he has been a PhD student in School of Renewable Natural Resources at Louisiana State University with Dr. Cornelis F de Hoop as advisor. His research for the doctoral program focuses on the preparation of cellulose nanofibers from bamboo using microwave liquefaction. He anticipates graduating with PhD degree in May 2019.