Observation of Potential Contaminants in Processed Biomass Using Fourier Transform Infrared Spectroscopy

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Abstract: With rapidly increased interests in biomass, diverse chemical and biological processes have been applied for biomass utilization. Fourier transform infrared (FTIR) analysis has been used for characterizing different types of biomass and their products, including natural and processed biomass. During biomass treatments, some solvents and/or catalysts can be retained and contaminate biomass. In addition, contaminants can be generated by the decomposition of biomass components. Herein, we report FTIR analyses of a series of contaminants, such as various solvents, chemicals, enzymes, and possibly formed degradation by-products in the biomass conversion process along with poplar biomass. This information helps to prevent misunderstanding the FTIR analysis results of the processed biomass.

Keywords: poplar; FTIR; contaminants; by-products

1. Introduction

A proper understanding of biomass characteristics is important for the effective utilization of biomass. It not only provides natural properties of biomass but also tells the influences of the applied processes on the biomass structures. Characteristics of biomass have been investigated in different aspects, including physical, chemical, thermal, mineral, and surface properties. For a better understanding of biomass and its products, several different analytical approaches have been developed using diverse analytical techniques such as high-performance liquid chromatography (HPLC), gas chromatography (GC), gel permeation chromatography (GPC), nuclear magnetic resonance (NMR), time-of-flight secondary ion mass spectrometry (ToF-SIMS), X-ray, transmission electron microscopy (TEM), scanning electron microscopy (SEM), differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), and Fourier transform infrared (FTIR) for measuring carbohydrate contents, identification and quantity of products, molecular weights, structural information such as linkages and composition, spatial distribution of molecules and chemical structures on surface, crystallinity, morphological characteristics in nano- and micro-scales, thermal properties (melting point, glass transition temperature, etc.), functional groups and chemical bonds, and other important information of biomass and its products and by-products [1–9]. Among these methods,
FTIR spectroscopy is one of the most widely applied analytical methods to study the functional groups of biomass by measuring the absorption bands of samples [10]. It provides qualitative and semi-quantitative information for functional groups of biomass by determining the presence of fundamental molecular vibrations [11]. Its Fellgett and Jacquinot advantages allow for rapid and ready characterization compared to many other biomass analysis methods [12]. Moreover, it does not need any modification and/or deconstruction of biomass; therefore, original properties can be monitored as the sample is. Despite these advantages, the characterization of biomass using FTIR is still challenged by overlapping the bands from different biomass components and/or unexpected impurities from the applied catalysts and solvents. In particular, fingerprint regions are complicated to identify because of many series of absorptions. For fast and reliable analysis of the substances from different processing, detection, and identification of possible contaminants are very important.

Lignocellulosic biomass is a heterogeneous matrix. Due to the complicated composition and structural properties of biomass, single or multi-stage pretreatment/preprocessing is necessary for its utilization, isolation, and analyses. Various chemicals, such as organic solvents, acids, alkalis, and inorganic salt solutions have been applied for isolation, pretreatment, conversion, and other reactions on biomass [13–19]. Biological catalysts, such as enzymes, have also been used in many biomass conversion processes or characterization methods [20]. Besides, each biomass component could be decomposed and/or modified under severe process conditions [21]. The presence of these chemicals and by-products are considered as impurities and could potentially affect their characterization results; therefore, they should be completely removed after the processes. Unfortunately, these components are possibly retained on the surface of biomass after these preprocessing and cause misinterpretation of the targeted biomass structure by their overlapped FTIR spectra. Besides the misreading of the biomass properties, the detection of contaminants can be used to determine the necessity of biomass washing step. Although the IR assignments of many chemicals and solvents are available individually, their actual contaminations are not easily detected due to the spectra of biomass itself. In this study, poplar biomass was mixed with known chemicals and enzymes, which are potential contaminants, and their overlapped FTIR spectra in each sample were identified and discussed.

2. Materials and Methods

2.1. Materials

Poplar was harvested in the Oak Ridge National Laboratory in 2008. Prior to the FTIR analysis, the sample was Wiley-milled and screened to 0.42 mm. Extractives were removed from the original poplar sample (~10 g) by toluene/ethanol Soxhlet extraction (2:1, v/v, 200 mL) for 8 h followed by 6 h of water extraction. All chemicals (acetone, ethanol, methanol, tetrahydrofuran, dioxane, toluene, glycerol, chloroform, pyridine, sulfuric acid, hydrochloric acid, phosphoric acid, acetic acid, sodium hydroxide, ammonium hydroxide, 1-butyl-3-methylimidazolium chloride, 1-benzyl-3-methylimidazolium chloride, chloride, hydroxymethylfurfural, and furfural) and enzymes (cellulase and β-glucosidase) used in this study were purchased from VWR, Sigma-Aldrich, or Fisher Scientific. Deep eutectic solvents (DESs) formed by combining hydrogen bonding donors (HBDs: urea, p-hydroxybenzoic acid, p-coumaric acid, hydroxymethylfurfural, and furfural) and enzymes (cellulase and β-glucosidase) used in this study were purchased from VWR, Sigma-Aldrich, or Fisher Scientific. Deep eutectic solvents (DESs) formed by combining hydrogen bonding donors (HBDs: urea, p-hydroxybenzoic acid, 4-hydroxybenzaldehyde, p-coumaric acid, hydroxymethylfurfural, and furfural) and enzymes (cellulase and β-glucosidase) used in this study were purchased from VWR, Sigma-Aldrich, or Fisher Scientific. Deep eutectic solvents (DESs) formed by combining hydrogen bonding donors (HBDs: urea, p-hydroxybenzoic acid, 4-hydroxybenzaldehyde, p-coumaric acid, hydroxymethylfurfural, and furfural) and enzymes (cellulase and β-glucosidase) used in this study were purchased from VWR, Sigma-Aldrich, or Fisher Scientific.

2.2. Isolation of Cellulose, Hemicellulose, and Lignin

Cellulose, hemicellulose, and lignin were isolated from the extractives-free poplar, as described in the previous studies [2,22]. In brief, the biomass was delignified using peracetic acid at 25 °C with 5% (wt/wt) solid loading for 24 h. The remaining solid, holocellulose, was air dried for 24 h. Two-step alkali extraction with 17.5% (wt/wt) and 8.75% (wt/wt) sodium hydroxide was conducted at 25 °C for 2 h in each step. The remaining solid fraction was called α-cellulose after being air dried, and the
liquid fraction was neutralized with anhydrous acetic acid and mixed with ethanol three times to precipitate hemicellulose.

Cellulolytic enzyme lignin (CEL) was separated from the poplar samples. The extractives-free poplar was ball-milled using Retsch PM 100 at 600 rpm for 2 h. The ball-milled sample was hydrolyzed at 50 °C with the CTec2 enzyme in acetate buffer solution (pH 4.8) for 48 h twice. The residual solid was extracted with 96% dioxane for 48 h. The dioxane-extracted fraction was recovered at 40 °C by rotary evaporation and freeze drying and used for further analysis.

2.3. Fourier Transform Infrared (FTIR) Analysis

To observe the FTIR spectra of contaminants from the spectra of biomass clearly, about 30–50 µL of contaminant was loaded to 0.3 g of extractives-free poplar in 20 mL glass vial and mixed by vortexing prior to the analysis. The prepared DESs were loaded to biomass and physically mixed using a glass rod due to their relatively high viscosity. FTIR analysis was conducted using the Spectrum One FTIR spectrometer (PerkinElmer, Wellesley, MA, USA) equipped with a universal attenuated total reflection (ATR) accessory. ATR-FTIR spectra between 4000 and 600 cm\(^{-1}\) were measured at a 4 cm\(^{-1}\) resolution and averaging 16 scans per sample.

3. Results and Discussion

3.1. Major Components of Biomass

Cellulose, hemicellulose, and lignin are three main components in lignocellulosic biomass. Table 1 and Figure 1 present the FTIR band assignments and spectra of poplar and its major components isolated from the same biomass. Prior to the analysis, other extractives in the poplar sample were removed by two-step extraction: 8 h toluene/ethanol Soxhlet-extraction followed by 6 h water extraction. Isolated cellulose, hemicellulose, and lignin were analyzed using FTIR and compared with the extractives-free poplar. The assignment of each band was identified according to the previous studies [23–31].

Table 1. Fourier transform infrared (FTIR) band assignments of poplar and its major components: cellulose, hemicellulose, and lignin [23–31].

| Wavenumber [cm\(^{-1}\)] | Assignment                                                                 | Components                          |
|--------------------------|----------------------------------------------------------------------------|-------------------------------------|
| 1                        | O-H stretching                                                             | Cellulose, Hemicellulose, Lignin    |
| 2                        | C-H stretching                                                             | Cellulose, Hemicellulose, Lignin    |
| 3                        | C=O stretching                                                             | Hemicellulose, Lignin               |
| 4                        | Aromatic skeletal vibration, C=O stretching, adsorbed O-H                  | Hemicellulose, Lignin               |
| 5                        | C=C-C aromatic ring stretching and vibration                               | Lignin                              |
| 6                        | C-H deformation (in methyl and methylene)                                  | Lignin                              |
| 7                        | Symmetric CH\(_2\) bending vibration, symmetric stretching band of carboxyl group, C-H deformation | Cellulose, Hemicellulose, Lignin    |
| 8                        | C-H bending, C-H stretching in CH\(_3\)                                   | Cellulose, Hemicellulose, Lignin    |
| 9                        | CH\(_2\) wagging, C-O stretching of C\(_5\) substituted aromatic units    | Cellulose, Hemicellulose, Lignin    |
| 10                       | C-O stretching of guaiacyl unit                                          | Lignin                              |
| 11                       | C-O-C stretching                                                          | Cellulose, Hemicellulose, Lignin    |
| 12                       | Aromatic C-H in plane deformation                                         | Lignin                              |
| 13                       | C-OH stretching vibration, C-O deformation                                 | Cellulose, Hemicellulose, Lignin    |
| 14                       | C-O stretching, aromatic C-H in plane deformation                         | Cellulose, Lignin                   |
| 15                       | C-O-C stretching                                                          | Cellulose, hemicellulose, Lignin    |
| 16                       | Aromatic C-H out of plane bending                                         | Lignin                              |
Figure 1. FTIR spectra of poplar and its major components: cellulose, hemicellulose, and lignin. (Note: The assignments of the numbered bands in the figure are described in Table 1).

The IR spectra of poplar and its components showed strong O-H stretching and C-H stretching absorptions at 3367 and 2914 cm\(^{-1}\), respectively. These two strong absorptions are because all three major components in biomass (cellulose, hemicellulose, and lignin) have hydroxy groups and many C-H bonds in their structures. The absorption at 1745 cm\(^{-1}\) was due to C=O stretching in hemicellulose and lignin. The absorption at 1618 cm\(^{-1}\) represented asymmetric stretching band of the carboxyl group of glucuronic acid in hemicellulose and C=O stretching in conjugated carbonyl of lignin. The band at 1650 cm\(^{-1}\) in the IR spectrum of cellulose was possibly caused by adsorbed H\(_2\)O. Higher absorption at 3367 cm\(^{-1}\) was also observed because of the moisture content in the biomass. In addition, the bands due to symmetric CH\(_2\) bending vibration in cellulose, carboxyl vibration in glucuronic acid with xylan, and C-H in plane deformation with aromatic ring stretching in lignin were observed at 1424 cm\(^{-1}\). The IR absorption bands at 1582 and 1508 cm\(^{-1}\) assigned to aromatic ring stretching and vibration (C=C-C) in lignin. The band at 1457 cm\(^{-1}\) was observed in lignin due to its C-H deformation in methyl and methylene. The C-H bending in cellulose, hemicellulose, and lignin (aliphatic C-H stretching in methyl and phenolic alcohol) was observed at 1370 cm\(^{-1}\). The CH\(_2\) wagging in cellulose and hemicellulose and the C-O stretching of C\(_5\) substituted aromatic units, such as syringyl and condensed guaiacyl units, were assigned at 1317 cm\(^{-1}\). Similarly, the C-O stretching of guaiacyl unit in lignin was assigned at 1235 cm\(^{-1}\). The bands at 1160 and 896 cm\(^{-1}\) arise from C-O-C stretching at the \(\beta\)-(1→4)-glycosidic linkages in cellulose and hemicellulose. The absorption at 1108 cm\(^{-1}\) was associated with aromatic C-H in plane deformation for the syringyl unit. The band at 1053 cm\(^{-1}\) was assigned to the C-OH stretching vibration of cellulose and hemicellulose. Moreover, this band was for C-O deformation in secondary alcohols and aliphatic ethers. The C-O stretching of cellulose and primary alcohols and C-H in plane deformation for guaiacyl unit exhibited at 1032 cm\(^{-1}\). Aromatic C-H out of plane bending in lignin was presented at 846 cm\(^{-1}\). Although several FTIR bands of different biomass components were overlapped, the IR spectra of samples still provide important clues, including changes of chemical composition, functionalization, and other transformation of biomass for understanding the applied biomass processing.

3.2. Commonly Used Pretreatment and Preprocessing Solvents

Table 2 and Figure S1 show the band assignment for common biomass processing solvents. Water is the most common solution in biomass analysis and the conversion processes. It also exists
in the air, and a certain amount can be accumulated in biomass during its storing and processing. The existence of water in biomass remarkably increased the bands at 3354 and 1653 cm\(^{-1}\) because of its O-H stretching and O-H-O scissors bending, respectively [32]. Acetone, ethanol, and methanol are common organic solvents for the diverse pre- and post-processing of biomass, and they are also produced from biomass [33]. Acetone contamination on poplar was observed at 3005, 2908, 1713, 1431, 1364, and 1222 cm\(^{-1}\) representing its CH\(_3\) degenerated stretching, CH\(_3\) symmetrical stretching, C=O stretching, CH\(_3\) degenerated deformation, CH\(_3\) symmetrical deforming, and C-C stretching, respectively [34]. A decrease in the bands at 3354 and 1653 cm\(^{-1}\) is possibly due to the displacement of water in biomass by acetone. The spectra of ethanol impurity were shown at 3350, 2980, and 1056 cm\(^{-1}\) for O-H stretching, C-H stretching, and C-O stretching, and those of methanol were at 3352, 2952, 2879, 1465, 1450, 1336, 1053 and 1026 cm\(^{-1}\) for O-H stretching, C-H stretching (asymmetric), C-H bending (asymmetric), C-H bending (symmetric), O-H bending, CH\(_3\) rocking, and C-O stretching, respectively [34–36]. Besides these chemicals, tetrahydrofuran (THF), dioxane, toluene, glycerol, pyridine, and chloroform are well-known solvents for diverse biomass pretreatment, isolation/purification, and analyses [1,3,19,37]. In addition, some chemicals, such as toluene, can be produced from biomass components [38]. The assignments of these impurities were assigned according to the previous studies. Contamination of poplar by THF appeared at 2977 and 2875 cm\(^{-1}\) for its C-H stretching, 1063 cm\(^{-1}\) for ring deformation, and 912 cm\(^{-1}\) for CH\(_2\) twisting [39]. The bands of dioxane were observed at 2960, 2890, 1457, 1322, 1255, 1119, 1057, 889 and 872 cm\(^{-1}\) to show its equatorial (higher frequency) C-H stretching, axial (lower frequency) C-H stretching, symmetric CH\(_2\) deformation, CH\(_2\) wagging, CH\(_2\) twisting, C-O-C symmetric stretching, ring trigonal deformation, C-C stretching, and C-O-C stretching, respectively [40]. The addition of toluene on poplar caused three bands including 3069 cm\(^{-1}\) for C-H stretching, 1497 cm\(^{-1}\) for C-C stretching, and 728 cm\(^{-1}\) for C-H out of plane bending [41]. Glycerol on poplar had the bands for O-H stretching at 3341 cm\(^{-1}\), C-H stretching at 2948 and 2897 cm\(^{-1}\), C-H deformation of secondary alcohol at 1333 and 1239 cm\(^{-1}\), C-O stretching of primary alcohol at 1034 cm\(^{-1}\), and O-H bending at 923 cm\(^{-1}\) [42,43]. Chloroform contaminants also showed at 1220 and 755 cm\(^{-1}\) for C-H bending and CCl\(_3\) stretching [34]. Pyridine contamination resulted in additional bands for C-H stretching at 3036 cm\(^{-1}\), C-C bonding at 1583 cm\(^{-1}\), C-N stretching at1485 cm\(^{-1}\), C-H in plane wagging at 1438 cm\(^{-1}\), symmetric C-H wagging at 1203 cm\(^{-1}\), C-H wagging at 1069 cm\(^{-1}\), C-C in plane wagging at 1032 cm\(^{-1}\), C-H out of plane bending at 750 and 693 cm\(^{-1}\) [44,45]. The intensities of the bands at 3353 and 1653 cm\(^{-1}\) decreased with the contaminants that do not contain OH groups such as acetone, THF, dioxane, toluene, chloroform, and pyridine due to the displacement of moisture in biomass by these solvents. On the other hand, the intensity increased with the contaminants having OH groups such as water, ethanol, methanol, and glycerol.

**Table 2.** FTIR band assignments of common biomass processing solvents on poplar [32–45].

| Contaminants | Wavenumber [cm\(^{-1}\)] | Assignments |
|--------------|--------------------------|-------------|
| Water        | 3354                     | O-H stretching |
|              | 1653                     | O-H-O scissors bending |
|              | 3005                     | CH\(_3\) stretching |
|              | 2908                     | CH\(_3\) stretching |
| Acetone      | 1713                     | C=O stretching |
|              | 1431                     | CH\(_3\) deforming |
|              | 1364                     | CH\(_3\) deforming |
|              | 1222                     | C-C stretching |
|              | 3350                     | O-H stretching |
| Ethanol      | 2980                     | C-H stretching |
|              | 1056                     | C-O stretching |
|              |                         |              |
Table 2. Cont.

| Contaminants    | Wavenumber [cm$^{-1}$] | Assignments                  |
|-----------------|------------------------|------------------------------|
| Methanol        | 3352                   | O-H stretching               |
|                 | 2952                   | C-H asymmetric stretching    |
|                 | 2879                   | C-H symmetric stretching     |
|                 | 1456                   | C-H asymmetric bending       |
|                 | 1450                   | C-H symmetric bending        |
|                 | 1336                   | O-H bending                  |
|                 | 1068                   | CH$_3$ rocking               |
|                 | 1026                   | C-O stretching               |
|                 | 2977                   | C-H stretching               |
|                 | 2875                   | C-H stretching               |
| Tetrahydrofuran | 1063                   | Ring deformation             |
|                 | 912                    | CH$_2$ twisting              |
|                 | 3069                   | C-H stretching               |
| Toluene         | 1497                   | C-C stretching               |
|                 | 728                    | C-H out of plane bending     |
|                 | 2960                   | C-H stretching               |
|                 | 2890                   | C-H stretching               |
|                 | 1457                   | Symmetric CH$_2$ deformation |
| Dioxane         | 1322                   | CH$_2$ bending               |
|                 | 1255                   | CH$_2$ twisting              |
|                 | 1119                   | C-O-C symmetric stretching   |
|                 | 1057                   | Ring trigonal deformation    |
| Glycerol        | 1333                   | C-H deformation             |
|                 | 1239                   | C-H deformation             |
|                 | 1034                   | C-O stretching              |
|                 | 923                    | O-H bending                  |
| Chloroform      | 1220                   | C-H bending                  |
|                 | 755                    | CH$_3$ stretching            |
|                 | 3036                   | C-H stretching               |
|                 | 1583                   | C-C bonding                 |
|                 | 1485                   | C-N stretching               |
| Pyridine        | 1438                   | C-H in plane wagging         |
|                 | 1203                   | Symmetric C-H wagging        |
|                 | 1069                   | C-H wagging                  |
|                 | 1032                   | C-C in plane wagging         |
|                 | 750/693                | C-H out of plane bending     |

3.3. Acids and Alkalis

Sulfuric acid, hydrochloric acid, acetic acid, phosphoric acid, ammonium hydroxide, and sodium hydroxide on the poplar sample were observed. As Table 3 and Figure S2 present, most acids on poplar, including sulfuric acid, hydrochloric acid, and phosphoric acid, commonly had the bands at 3370 and 1660 cm$^{-1}$ to represent O-H bonding and O-H-O scissors bending, respectively, because of water content. The contamination bands from sulfuric acid in the literature at 1362 and 750 cm$^{-1}$ for S=O (1362 cm$^{-1}$) and S-O stretching (750 cm$^{-1}$) were not clearly appeared in this study [46]. A relatively low concentration of sulfuric acid (4%) could be the reason for weak intensities of the contaminant. Hydrochloric acid showed H-Cl stretching at 2942 cm$^{-1}$, while phosphoric acid had a P-OH bond and P=O stretching at 2904 and 1161 cm$^{-1}$, respectively [34,47,48]. Acetic acid bands appeared at 3351, 2916, 1706, 1427, 1234, and 1031 cm$^{-1}$ to indicate its O-H stretching, symmetric CH$_3$ stretching, C=O stretching, CH$_3$ deformation, O-H bending and CH$_3$ rocking, respectively [34]. Sodium hydroxide
had the bands caused by water at 3360 and 1660 cm⁻¹, but there were no other clear contamination bands observed. Similarly, ammonium hydroxide had the IR bands at 3350 and 1660 cm⁻¹ from both water and NH₃ content but N-H stretching of NH₄⁺ also appeared at 2914 cm⁻¹. Previous study also said that adsorption of ammonia increased the overall polarity and resulted in the absorbance of several bands (e.g., 1115 and 1036 cm⁻¹ in this study) not from the N-H vibrations [49].

Table 3. FTIR band assignments of acids and alkalis contaminants on poplar [34,46–49].

| Contaminants                  | Wavenumber [cm⁻¹] | Assignments                      |
|------------------------------|-------------------|----------------------------------|
| Sulfuric acid                | 3370              | O-H stretching                   |
|                              | 1660              | O-H-O scissors bending           |
|                              | 1362              | S=O stretching                   |
|                              | 750               | S-O stretching                   |
|                              | 3370              | O-H stretching                   |
| Hydrochloric acid            | 2905              | H-Cl stretching                  |
|                              | 1660              | O-H-O scissors bending           |
|                              | 3370              | O-H stretching                   |
|                              | 2905              | P-OH bond                        |
| Phosphoric acid              | 1660              | O-H-O scissors bending           |
|                              | 1161              | P=O stretching                   |
|                              | 1031              | P=O stretching                   |
|                              | 3351              | O-H stretching                   |
|                              | 2916              | Symmetric CH₃ stretching         |
|                              | 1706              | C=O stretching                   |
| Acetic acid                  | 1427              | CH₃ deformation                  |
|                              | 1234              | O-H bending                      |
|                              | 1031              | CH₃ rocking                      |
| Ammonium hydroxide           | 3350              | N-H stretching & O-H stretching  |
|                              | 2914              | N-H stretching                   |
|                              | 1660              | O-H-O scissors bending           |

3.4. Ionic Liquids

Besides the aforementioned chemicals, FTIR spectra and the band assignments of ionic liquids, enzymes, and biomass-derived chemicals on poplar are presented in Table 4 and Figures S3–S5. The bands from 1-butyl-3-methylimidazolium chloride contaminant were observed at 3341, 1658, and 1604 cm⁻¹ representing the formation of quaternary amine salt formation with chlorine, C=C stretching, and C=N stretching, respectively. However, the band at 835 cm⁻¹ representing C-N stretching vibration was not clearly observed [50]. The bands from 1-benzyl-3-methylimidazolium chloride were 2961, 1574, 765, and 633 cm⁻¹ from C-H stretching, C-C stretching of ring vibration, and C-N/C-Cl in-plane bending, respectively [51]. Moreover, two bands at 1383 and 1176 cm⁻¹ were observed; however, further study is needed to identify them.

Table 4. FTIR band assignments of ionic liquids, enzymes, and biomass-derived chemicals on poplar [49–65].

| Contaminants                                      | Wavenumber [cm⁻¹] | Assignments                           |
|--------------------------------------------------|-------------------|---------------------------------------|
| 1-Butyl-3-methylimidazolium chloride             | 3341              | Quaternary amine salt formation       |
|                                                  | 1658              | C=C stretching                        |
|                                                  | 1604              | C=N stretching                        |
|                                                  | 2961              | C-H stretching                        |
| 1-Benzyl-3-methylimidazolium chloride            | 1574              | C-C stretching ring vibration         |
|                                                  | 633               | C-N/C-Cl in-plane bending             |
Table 4. Cont.

| Contaminants       | Wavenumber [cm\(^{-1}\)] | Assignments                        |
|--------------------|---------------------------|------------------------------------|
|                    |                           | NH\(_2\) asymmetric stretching     |
|                    |                           | NH\(_2\) symmetric stretching      |
| ChCl-Urea          | 3435                      | OH bending vibration                |
|                    | 3340                      | C-N rocking                        |
|                    | 1669                      | CH\(_3\) rocking                   |
|                    | 1597                      | CH\(_2\) rocking                   |
|                    | 1474                      | C-N stretching                     |
|                    | 1152                      | C-O stretching vibration           |
|                    | 1062                      | C-O stretching vibration           |
|                    | 961                       | Asymmetric stretching of COO       |
|                    | 790                       | C=O bonding                        |
|                    | 3180                      | O-H stretching                     |
|                    | 1681                      | C=O stretching                     |
|                    | 1581                      | Asymmetric stretching of COO       |
| ChCl–PHA            | 3180                      | O-H stretching                     |
|                    | 1681                      | C=O stretching                     |
|                    | 1581                      | Asymmetric stretching of COO       |
|                    | 1282                      | C-O stretching vibration           |
|                    | 953                       | C-N stretching                     |
|                    | 861                       | CH\(_2\) rocking                   |
|                    | 838                       | Aromatic C-H out-of-plane bending  |
|                    | 786                       | C-C stretching                     |
|                    | 3122                      | The stretching vibration of the phenolic O-H |
| ChCl–PB            | 1667                      | The stretching vibration of carbonyl group |
|                    | 1272                      | Methylene                          |
|                    | 1030                      | C-H binding                        |
|                    | 3126                      | Bending vibration of –NH\(_2\)     |
|                    | 1675                      | C=O stretch of carboxylic acid     |
| ChCl–PCA           | 1606                      | C=C stretching                     |
|                    | 1160                      | C-OH stretching                    |
|                    | 848                       | C-H stretching                     |
|                    | 771                       | Stretching of the -OH group        |
|                    | 3353                      | N-H stretching & O-H stretching    |
|                    | 2942                      | C-H stretching (asymmetric)        |
| Cellulase          | 2900                      | C-H stretching (symmetric)         |
|                    | 1642                      | NH\(_2\) scissoring & C=N vibration|
|                    | 1334                      | C-N stretching                     |
|                    | 1036                      | C-N stretching                     |
|                    | 3351                      | N-H stretching                     |
| β-glucosidase      | 1646                      | N-H bonding & C=O stretching       |
|                    | 1432                      | N-H stretching                     |
|                    | 620                       | N-H out of plane bending           |
| HMF                | 3364                      | O-H stretching                     |
|                    | 1661                      | Carbonyl stretching                |
|                    | 1561                      | C=C stretching (furan ring)        |
|                    | 3134                      | C-H stretching                     |
|                    | 2859                      | C-H vibration of aldehyde group    |
|                    | 1671                      | C=O in conjugated carbonyl group    |
| Furfural           | 1465                      | C=C stretching of furan ring       |
|                    | 1276/1021                 | C-O stretching vibration           |
|                    | 928/884/755               | C-H bending out of plane peaks     |

The bands of choline chloride-urea, which is a well-known DES, were at 3435 and 3340 cm\(^{-1}\), which ascribed to the stretching of –NH\(_2\) (asymmetric and symmetric), 1669 cm\(^{-1}\) for the bending vibration of –NH\(_2\), 1597 cm\(^{-1}\) for bending vibration of -OH possibly due to the existence of water, 1474 cm\(^{-1}\) for CH\(_3\) rocking, 1152 cm\(^{-1}\) for asymmetric C-N stretching, 1062 cm\(^{-1}\) for CH\(_2\) rocking, 961 cm\(^{-1}\) for asymmetric stretching of COO from choline structure and 790 cm\(^{-1}\) from C=O bonding [52,53]. Three lignin-based DESs, choline chloride–p-hydroxybenzoic acid (PHA), choline chloride–4-hydroxybenzaldehyde (PB), and choline chloride–p-coumaric acid (PCA), were mixed with poplar sample to observe the possible contamination bands. The bands of choline chloride–PHA were observed at 3180 cm\(^{-1}\) for O-H stretching, 1681 cm\(^{-1}\) for C=O stretching, 1581 cm\(^{-1}\) for the asymmetric stretch of COO vibrations, 1282 cm\(^{-1}\) for C-O stretching vibration, 1082 cm\(^{-1}\) for C-O stretching, 953 cm\(^{-1}\) for C-N stretching, 861 cm\(^{-1}\) for CH\(_2\) rocking vibrations, 838 cm\(^{-1}\) for aromatic C-H vibrations, and 786 cm\(^{-1}\) for C-C stretching.
out-of-plane bending, 786 cm\(^{-1}\) for C–C stretching [54,55]. The bands from choline chloride–PB were observed at 3122 cm\(^{-1}\) for the stretching vibration of the phenolic O-H group exhibiting intermolecular hydrogen bonding, 1667 cm\(^{-1}\) for the stretching vibration of carbonyl group, 1272 cm\(^{-1}\) for the methylene, 1030 cm\(^{-1}\) for C-H binding vibration [56]. The bands from choline chloride–PCA DES were observed at 3126 cm\(^{-1}\), 1675 cm\(^{-1}\), 1606 cm\(^{-1}\), 1160 cm\(^{-1}\), 848 cm\(^{-1}\) from bending vibration of \(\text{NH}_2\), C=O stretch of carboxylic acid, C=C stretching, C-OH stretching, C-H stretching and 771 cm\(^{-1}\) from stretching of the \(-\text{OH}\) group on the second carbon of the choline chloride [57–59].

3.5. Enzymes

Enzymes such as cellulase and \(\beta\)-glucosidase break polysaccharides in biomass to fermentable sugars. The bands at 3353, 2942, 2900, 1642, and 1036 cm\(^{-1}\) were observed from cellulase (Table 4 and Figure S4). The bands at 3353, 2942, and 2900 cm\(^{-1}\) were from N-H/O-H stretching and the C-H stretching (asymmetric and symmetric) of cellulase. The bands at 1642, 1432, 1334, and 1036 cm\(^{-1}\) were possibly from \(\text{NH}_2\) scissoring, C-C stretching, C-N stretching, and C-O stretching, respectively [60–62]. \(\beta\)-glucosidase also showed similar bands at 3351, 1646, 1432, and 620 cm\(^{-1}\), which represented N-H stretching, N-H bonding and C=O stretching, N-H bending, and N-H out of plane bending, respectively [63].

3.6. Biomass-Derived Chemicals

Biomass can be contaminated by its decomposed fractions. For instance, furan-based chemicals such as furfural and hydroxymethylfurfural can be produced through the dehydration of hexoses and pentoses in biomass. As Figure S5 presents, HMF contamination showed at 3364, 1661, and 1561 cm\(^{-1}\) from O-H stretching, C=O stretching (carbonyl), and C=C stretching of furan ring, respectively [64]. Furfural also showed bands at 3134 cm\(^{-1}\) from C-H stretching of furan ring, at 2859 cm\(^{-1}\) from the C-H vibration of aldehyde group, 1671 cm\(^{-1}\) from C=O in the conjugated carbonyl group, 1465 cm\(^{-1}\) from C=C stretching of furan ring, 1276 and 1021 cm\(^{-1}\) from C-O stretching vibration, 928, 884, and 755 cm\(^{-1}\) from C-H bending out of plane peaks [65,66].

4. Conclusions

The identification of contaminants on the biomass surface after preprocessing is important to avoid the unwanted misleading of analysis data. This study investigated and discussed diagnostic FTIR bands from 26 potential chemicals, including organic solvents, acids and alkalis, ionic liquids, enzymes, and biomass-derived components through diverse biomass preprocessing. The observation of these contaminants will improve the FTIR analysis with diverse biomass and bioproducts in the biorefinery.

**Supplementary Materials:** The following are available online at http://www.mdpi.com/2076-3417/10/12/4345/s1, Figure S1: FTIR spectra of preprocessing solvent contaminants on poplar, Figure S2: FTIR spectra of preprocessing acid and alkaline contaminants on poplar, Figure S3: FTIR spectra of ionic liquid contaminants on poplar, Figure S4: FTIR spectra of enzyme contaminants on poplar, Figure S5: FTIR spectra of biomass-derived chemical contaminants on poplar.

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