

Article

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

Jingshun Zhuang ¹, Mi Li ², Yunqiao Pu ³, Arthur Jonas Ragauskas ^{2,3,4,*} and Chang Geun Yoo ^{1,3,*}

¹ Department of Chemical Engineering, State University of New York College of Environmental Science and Forestry, Syracuse, NY 13104, USA; jzhuan02@syr.edu

² Center for Renewable Carbon, Department of Forestry, Wildlife, and Fisheries, University of Tennessee Institute of Agriculture, Knoxville, TN 37996, USA; mli47@utk.edu

³ Center for Bioenergy Innovation, Biosciences Division, University of Tennessee-Oak Ridge National Laboratory Joint Institute for Biological Science, Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA; puy1@ornl.gov

⁴ Department of Chemical and Biomolecular Engineering, University of Tennessee, Knoxville, TN 37996 USA

* Correspondence: aragausk@utk.edu (A.J.R.); cyoo05@esf.edu (C.G.Y.)

Received: 2 June 2020; Accepted: 22 June 2020; Published: 24 June 2020

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, choline chloride, 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) and hydrogen bonding acceptor (HBA: choline chloride) at 80 °C prior to the FTIR analysis.

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 μ L 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	3367	O-H stretching	Cellulose, Hemicellulose, Lignin
2	2914	C-H stretching	Cellulose, Hemicellulose, Lignin
3	1745	C=O stretching	Hemicellulose, Lignin
4	1618	Aromatic skeletal vibration, C=O stretching, adsorbed O-H	Hemicellulose, Lignin
5	1508	C=C-C aromatic ring stretching and vibration	Lignin
6	1457	C-H deformation (in methyl and methylene)	Lignin
7	1424	Symmetric CH_2 bending vibration, symmetric stretching band of carboxyl group, C-H deformation	Cellulose, Hemicellulose, Lignin
8	1370	C-H bending, C-H stretching in CH_3	Cellulose, Hemicellulose, Lignin

9	1317	CH ₂ wagging, C-O stretching of C ₅ substituted aromatic units	Cellulose, Hemicellulose, Lignin
10	1235	C-O stretching of guaiacyl unit	Lignin
11	1160	C-O-C stretching	Cellulose, Hemicellulose
12	1108	Aromatic C-H in plane deformation	Lignin
13	1053	C-OH stretching vibration, C-O deformation	Cellulose, Hemicellulose, Lignin
14	1032	C-O stretching, aromatic C-H in plane deformation	Cellulose, Lignin
15	896	C-O-C stretching	Cellulose, hemicellulose
16	846	Aromatic C-H out of plane bending	Lignin

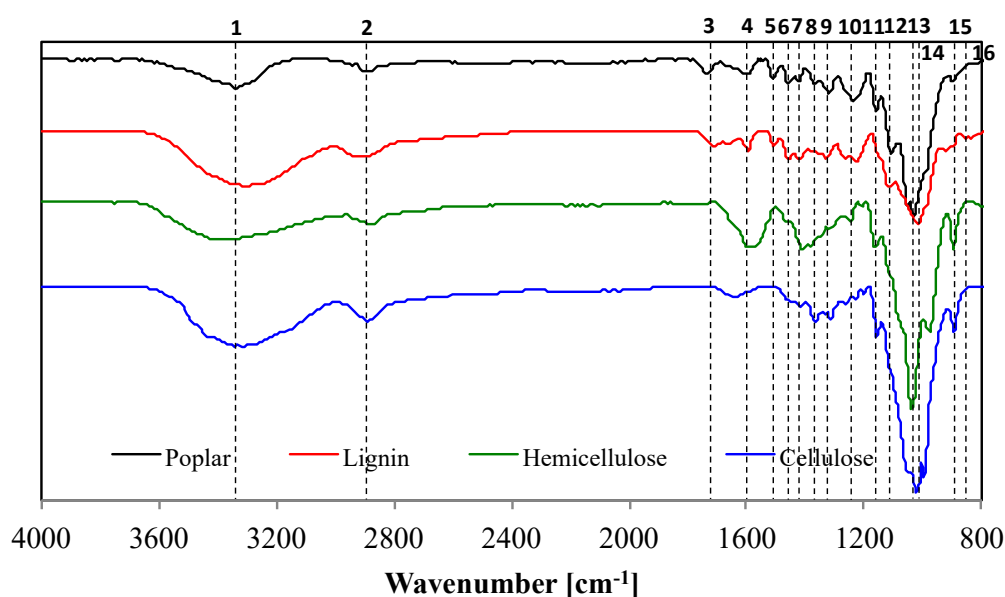


Figure 1. FTIR spectra of poplar and its major components: cellulose, hemicellulose, and lignin. (Note: The assignments of the numbered bands in the figure are described in Table 1).

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 H₂O. Higher absorption at 3367 cm^{-1} was also observed because of the moisture content in the biomass. In addition, the bands due to symmetric CH₂ 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 CH₂ wagging in cellulose and hemicellulose and the C-O stretching of C₅ 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 β -(1 $\rightarrow$ 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 stretching (symmetric), C-H bending (asymmetric), C-H bending (symmetric), O-H bending, CH_3 rocking, and C-O stretching, respectively [34,35,36]. 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 at 1485 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
Ethanol	3350	O-H stretching
	2980	C-H stretching
	1056	C-O stretching
	3352	O-H stretching
Methanol	2952	C-H asymmetric stretching
	2879	C-H symmetric stretching
	1465	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
Tetrahydrofuran	2875	C-H stretching
	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
Dioxane	1457	Symmetric CH ₂ deformation
	1322	CH ₂ wagging
	1255	CH ₂ twisting
	1119	C-O-C symmetric stretching
	1057	Ring trigonal deformation
	889	C-C stretching
	872	C-O-C stretching
	3341	O-H bending
Glycerol	2948	C-H stretching
	2897	C-H stretching
	1333	C-H deformation
	1239	C-H deformation
Chloroform	1034	C-O stretching
	923	O-H bending
	1220	C-H bending
	755	CCl ₃ stretching
Pyridine	3036	C-H stretching
	1583	C-C bonding
	1485	C-N stretching
	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 NH_3 content but N-H stretching of NH_4^+ 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
Hydrochloric acid	3370	O-H stretching
	2905	H-Cl stretching
	1660	O-H-O scissors bending
Phosphoric acid	3370	O-H stretching
	2905	P-OH bond
	1660	O-H-O scissors bending
	1161	P=O stretching
	1031	P=O stretching
Acetic acid	3351	O-H stretching
	2916	Symmetric CH_3 stretching
	1706	C=O stretching
	1427	CH_3 deformation
	1234	O-H bending
	1031	CH_3 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.

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 $-\text{NH}_2$ (asymmetric and symmetric), 1669 cm^{-1} for the bending vibration of $-\text{NH}_2$, 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 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 -OH group on the second carbon of the choline chloride [57–59].

3.5. Enzymes

Enzymes such as cellulase and β -glucosidase break polysaccharides in biomass to fermentable sugars. The bands at 3353 , 2942 , 2900 , 1642 , 1334 , 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 NH_2 scissoring, C-C stretching, C-N stretching, and C-O stretching, respectively [60–62]. β -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].

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
	3435	NH_2 asymmetric stretching
	3340	NH_2 symmetric stretching
ChCl-Urea	1669	NH_2 bending vibration
	1597	OH bending vibration
	1474	CH_3 rocking
	1152	C-N stretching
	1062	CH_2 rocking
	961	Asymmetric stretching of COO
	790	C=O bonding
	3180	O-H stretching
	1681	C=O stretching
	1581	Asymmetric stretching of COO
ChCl-PHA	1282	C-O stretching vibration
	1082	C-O stretching
	953	C-N stretching
	861	CH_2 rocking vibrations
	838	Aromatic C-H out-of-plane bending
ChCl-PB	786	C-C stretching
	3122	The stretching vibration of the phenolic O-H

ChCl-PCA	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
	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
Cellulase	2942	C-H stretching (asymmetric)
	2900	C-H stretching (symmetric)
	1642	NH ₂ scissoring & C=N vibration
	1334	C-N stretching
	1036	C-N stretching
β-glucosidase	3351	N-H stretching
	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
Furfural	2859	C-H vibration of aldehyde group
	1671	C=O in conjugated carbonyl group
	1465	C=C stretching of furan ring
	1276/1021	C-O stretching vibration
	928/884/755	C-H bending out of plane peaks

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 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.

Author Contributions: C.G.Y. and A.J.R. conceived and designed the research. J.Z., C.G.Y., M.L., and Y.P. carried out the experiment. J.Z., M.L., and C.G.Y. wrote the manuscript. All the authors discussed data and revised the paper. All authors have given approval to the final version of the manuscript.

Acknowledgments: This manuscript has been authored by UT-Battelle, LLC under Contract No. DE-AC05-00OR22725 with the U.S. Department of Energy. This study was supported and performed as part of the BioEnergy Science Center (BESC) and Center for Bioenergy Innovation (CBI). The BESC and CBI are U.S. Department of Energy Bioenergy Research Centers supported by the Office of Biological and Environmental Research in the DOE Office of Science. The United States Government retains and the publisher, by accepting the article for publication, acknowledges that the United States Government retains a non-exclusive, paid-up, irrevocable, world-wide license to publish or reproduce the published form of this manuscript, or allow others to do so, for the United States Government purposes. The Department of Energy will provide public access to these results of federally sponsored research in accordance with the DOE Public Access Plan (<http://energy.gov/downloads/doe-public-access-plan>). The views and opinions of the authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof. Neither the United States Government nor any agency thereof, nor any of their employees, makes any warranty, expressed or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. The views and opinions of the authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof. Neither the United States Government nor any agency thereof, nor any of their employees, makes any warranty, expressed or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights.

Funding: This research received no external funding.

Conflicts of Interest: There are no conflicts to declare.

References

1. Pu, Y.; Cao, S.; Ragauskas, A.J. Application of quantitative ^{31}P NMR in biomass lignin and biofuel precursors characterization. *Energy Environ. Sci.* **2011**, *4*, 3154–3166.
2. Yoo, C.G.; Pu, Y.; Li, M.; Ragauskas, A.J. Elucidating Structural Characteristics of Biomass using Solution-State 2D NMR with a Mixture of Deuterated Dimethylsulfoxide and Hexamethylphosphoramide. *ChemSusChem* **2016**, *9*, 1090–1095.
3. Yoo, C.G.; Yang, Y.; Pu, Y.; Meng, X.; Muchero, W.; Yee, K.L.; Thompson, O.A.; Rodriguez, M.; Bali, G.; Engle, N.L. Insights of biomass recalcitrance in natural *Populus trichocarpa* variants for biomass conversion. *Green Chem.* **2017**, *19*, 5467–5478.
4. Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D.; Crocker, D. Determination of structural carbohydrates and lignin in biomass. *Lab. Anal. Proced.* **2010**, *1617*, 1–16.
5. Jung, S.; Foston, M.; Kalluri, U.C.; Tuskan, G.A.; Ragauskas, A.J. 3D chemical image using TOF-SIMS revealing the biopolymer component spatial and lateral distributions in biomass. *Angew. Chem.* **2012**, *124*, 12171–12174.
6. Tolbert, A.K.; Yoo, C.G.; Ragauskas, A.J. Understanding the Changes to Biomass Surface Characteristics after Ammonia and Organosolv Pretreatments by Using Time-of-Flight Secondary-Ion Mass Spectrometry (TOF-SIMS). *ChemPlusChem* **2017**, *82*, 686–690.
7. Sannigrahi, P.; Kim, D.H.; Jung, S.; Ragauskas, A. Pseudo-lignin and pretreatment chemistry. *Energy Environ. Sci.* **2011**, *4*, 1306–1310.
8. Figueira, M.; Volesky, B.; Mathieu, H. Instrumental analysis study of iron species biosorption by *Sargassum* biomass. *Environ. Sci. Technol.* **1999**, *33*, 1840–1846.
9. Kok, M.V.; Özgür, E. Thermal analysis and kinetics of biomass samples. *Fuel Process. Technol.* **2013**, *106*, 739–743.
10. Pu, Y.; Meng, X.; Yoo, C.G.; Li, M.; Ragauskas, A.J. Analytical methods for biomass characterization during pretreatment and bioconversion. In *Valorization of Lignocellulosic Biomass in a Biorefinery: From Logistics to Environmental and Performance Impact*; Kumar, R., Ed.; Nova Science Publishers: New York, NY, USA, 2016; pp. 37–78.
11. Acquah, G.E.; Via, B.K.; Fasina, O.O.; Eckhardt, L.G. Rapid quantitative analysis of forest biomass using fourier transform infrared spectroscopy and partial least squares regression. *J. Anal. Methods Chem.* **2016**, *2016*, 1839598.
12. Perkins, W. Fourier transform infrared spectroscopy. Part II. Advantages of FT-IR. *J. Chem. Educ.* **1987**, *64*, A269.

13. Di Fidio, N.; Raspolli Galletti, A.M.; Fulignati, S.; Licursi, D.; Liuzzi, F.; De Bari, I.; Antonetti, C. Multi-Step Exploitation of Raw *Arundo donax* L. for the Selective Synthesis of Second-Generation Sugars by Chemical and Biological Route. *Catalysts* **2020**, *10*, 79.
14. Licursi, D.; Antonetti, C.; Fulignati, S.; Corsini, A.; Boschi, N.; Galletti, A.M.R. Smart valorization of waste biomass: Exhausted lemon peels, coffee silverskins and paper wastes for the production of levulinic acid. *Chem. Eng. Trans.* **2018**, *65*, 637.
15. Lara-Serrano, M.; Morales-de-laRosa, S.; Campos-Martín, J.M.; Fierro, J.L.G. Fractionation of Lignocellulosic Biomass by Selective Precipitation from Ionic Liquid Dissolution. *Appl. Sci.* **2019**, *9*, 1862.
16. Shen, X.J.; Wen, J.L.; Mei, Q.Q.; Chen, X.; Sun, D.; Yuan, T.Q.; Sun, R.C. Facile fractionation of lignocelluloses by biomass-derived deep eutectic solvent (DES) pretreatment for cellulose enzymatic hydrolysis and lignin valorization. *Green Chem.* **2019**, *21*, 275–283.
17. Agbor, V.B.; Cicek, N.; Sparling, R.; Berlin, A.; Levin, D.B. Biomass pretreatment: Fundamentals toward application. *Biotechnol. Adv.* **2011**, *29*, 675–685.
18. Yoo, C.G.; Pu, Y.; Ragauskas, A.J. Ionic liquids: Promising green solvents for lignocellulosic biomass utilization. *Curr. Opin. Green Sustain. Chem.* **2017**, *5*, 5–11.
19. Nguyen, T.Y.; Cai, C.M.; Osman, O.; Kumar, R.; Wyman, C.E. CELF pretreatment of corn stover boosts ethanol titers and yields from high solids SSF with low enzyme loadings. *Green Chem.* **2016**, *18*, 1581–1589.
20. Yang, B.; Dai, Z.; Ding, S.-Y.; Wyman, C.E. Enzymatic hydrolysis of cellulosic biomass. *Biofuels* **2011**, *2*, 421–449.
21. Rasmussen, H.; Sørensen, H.R.; Meyer, A.S. Formation of degradation compounds from lignocellulosic biomass in the biorefinery: Sugar reaction mechanisms. *Carbohydr. Res.* **2014**, *385*, 45–57.
22. Li, M.; Pu, Y.; Yoo, C.G.; Gjersing, E.; Decker, S.R.; Doeppke, C.; Shollenberger, T.; Tschaplinski, T.J.; Engle, N.L.; Sykes, R.W. Study of traits and recalcitrance reduction of field-grown COMT down-regulated switchgrass. *Biotechnol. Biofuels* **2017**, *10*, 12.
23. Faix, O. Fourier transform infrared spectroscopy. In *Methods in Lignin Chemistry*; Lin, S.Y., Dence, C.W., Eds.; Springer: Berlin/Heidelberg, Germany, 1992; pp. 83–109.
24. Sim, S.F.; Mohamed, M.; Lu, N.A.L.M.I.; Sarman, N.S.P.; Samsudin, S.N.S. Computer-assisted analysis of fourier transform infrared (FTIR) spectra for characterization of various treated and untreated agriculture biomass. *BioResources* **2012**, *7*, 5367–5380.
25. Pandey, K. A study of chemical structure of soft and hardwood and wood polymers by FTIR spectroscopy. *J. Appl. Polym. Sci.* **1999**, *71*, 1969–1975.
26. Ciolacu, D.; Ciolacu, F.; Popa, V.I. Amorphous cellulose—Structure and characterization. *Cell. Chem. Technol.* **2011**, *45*, 13.
27. Yang, H.; Yan, R.; Chen, H.; Lee, D.H.; Zheng, C. Characteristics of hemicellulose, cellulose and lignin pyrolysis. *Fuel* **2007**, *86*, 1781–1788.
28. Le, D.M.; Nielsen, A.D.; Sørensen, H.R.; Meyer, A.S. Characterisation of Authentic Lignin Biorefinery Samples by Fourier Transform Infrared Spectroscopy and Determination of the Chemical Formula for Lignin. *Bioenergy Res.* **2017**, *10*, 1025–1035.
29. Liu, C.F.; Xu, F.; Sun, J.X.; Ren, J.L.; Curling, S.; Sun, R.C.; Fowler, P.; Baird, M.S. Physicochemical characterization of cellulose from perennial ryegrass leaves (*Lolium perenne*). *Carbohydr. Res.* **2006**, *341*, 2677–2687.
30. Xu, F.; Sun, J.-X.; Sun, R.; Fowler, P.; Baird, M.S. Comparative study of organosolv lignins from wheat straw. *Ind. Crop. Prod.* **2006**, *23*, 180–193.
31. Sills, D.L.; Gossett, J.M. Using FTIR to predict saccharification from enzymatic hydrolysis of alkali-pretreated biomasses. *Biotechnol. Bioeng.* **2012**, *109*, 353–362.
32. Mojet, B.L.; Ebbesen, S.D.; Lefferts, L. Light at the interface: The potential of attenuated total reflection infrared spectroscopy for understanding heterogeneous catalysis in water. *Chem. Soc. Rev.* **2010**, *39*, 4643–4655.
33. Zhang, K.; Pei, Z.; Wang, D. Organic solvent pretreatment of lignocellulosic biomass for biofuels and biochemicals: A review. *Bioresour. Technol.* **2016**, *199*, 21–33.
34. The Virtual Planetary Laboratory Molecular Database. Available online: <http://vpl.astro.washington.edu/spectra/allmoleculeslist.htm> (accessed on 8 May 2020).
35. Plyler, E.K. Infrared Spectra of Methanol, Ethanol, and *n*-Propanol. *J. Res. Natl. Bur. Stand.* **1952**, *48*, 281–286.

36. Conklin, Jr, A.; Goldcamp, M.J.; Barrett, J. Determination of ethanol in gasoline by FT-IR spectroscopy. *J. Chem. Educ.* **2014**, *91*, 889–891.
37. Hu, S.; Li, Y. Two-step sequential liquefaction of lignocellulosic biomass by crude glycerol for the production of polyols and polyurethane foams. *Bioresour. Technol.* **2014**, *161*, 410–415.
38. Elfadly, A.; Zeid, I.; Yehia, F.; Abouelela, M.; Rabie, A. Production of aromatic hydrocarbons from catalytic pyrolysis of lignin over acid-activated bentonite clay. *Fuel Process. Technol.* **2017**, *163*, 1–7.
39. Dwivedi, A.; Baboo, V.; Bajpai, A. Fukui Function Analysis and Optical, Electronic, and Vibrational Properties of Tetrahydrofuran and Its Derivatives: A Complete Quantum Chemical Study. *J. Theor. Chem.* **2015**, *2015*, 345234.
40. Borowski, P.; Gac, W.; Pulay, P.; Woliński, K. The vibrational spectrum of 1, 4-dioxane in aqueous solution—theory and experiment. *New J. Chem.* **2016**, *40*, 7663–7670.
41. IR Spectroscopy Tutorial. Available online: <https://orgchemboulder.com/Spectroscopy/irtutor/tutorial.shtml> (accessed on 8 May 2020).
42. Wen Yee, T.; Tin Sin, L.; Rahman, W.; Samad, A. Properties and interactions of poly (vinyl alcohol)-sago pith waste biocomposites. *J. Compos. Mater.* **2011**, *45*, 2199–2209.
43. Danish, M.; Mumtaz, M.W.; Fakhar, M.; Rashid, U. Response surface methodology based optimized purification of the residual glycerol from biodiesel production process. *Chiang Mai J. Sci.* **2015**, *43*, 1570–1582.
44. Swoboda, A.; Kunze, G. Infrared study of pyridine adsorbed on montmorillonite surfaces. *Clay Clay Miner.* **1964**, *13*, 277.
45. Testa, A.C. Molecular Vibrations of Pyridine. Available online: <http://facpub.stjohns.edu/~testaa/anim27vib.html> (accessed on 6 May 2020).
46. Segneanu, A.E.; Gozescu, I.; Dabici, A.; Sfirloaga, P.; Szabadai, Z. Organic compounds FT-IR spectroscopy. In *Macro To Nano Spectroscopy*; Uddin, J., Ed.; InTech: Rijeka, Croatia, 2012; pp. 145–164.
47. Eisazadeh, A.; Kassim, K.A.; Nur, H. Physicochemical characteristics of phosphoric acid stabilized bentonite. *Electron. J. Geotech. Eng.* **2010**, *15*, 327–335.
48. Arai, Y.; Sparks, D.L. ATR-FTIR spectroscopic investigation on phosphate adsorption mechanisms at the ferrihydrite–water interface. *J. Colloid Interface Sci.* **2001**, *241*, 317–326.
49. Valentin, R.; Horga, R.; Bonelli, B.; Garrone, E.; Renzo, F.D.; Quignard, F. FTIR spectroscopy of NH₃ on acidic and ionotropic alginate aerogels. *Biomacromolecules* **2006**, *7*, 877–882.
50. Dharaskar, S.A.; Varma, M.N.; Shende, D.Z.; Yoo, C.K.; Wasewar, K.L. Synthesis, characterization and application of 1-butyl-3 methylimidazolium chloride as green material for extractive desulfurization of liquid fuel. *Sci. World J.* **2013**, *2013*, 395274.
51. Seethalakshmi, K.; Jasmine Vasantha Rani, E.; Padmavathy, R. Study of vibrational spectra and solvation number of non-aqueous solutions of 1-benzyl-3-dimethylimidazolium chloride through ultrasonic technique. *Int. J. Recent Sci. Res.* **2015**, *6*, 2347–2349.
52. Yue, D.; Jia, Y.; Yao, Y.; Sun, J.; Jing, Y. Structure and electrochemical behavior of ionic liquid analogue based on choline chloride and urea. *Electrochim. Acta* **2012**, *65*, 30–36.
53. Du, C.; Zhao, B.; Chen, X.-B.; Biribilis, N.; Yang, H. Effect of water presence on choline chloride-2urea ionic liquid and coating platings from the hydrated ionic liquid. *Sci. Rep.* **2016**, *6*, 29225.
54. Dega-Szafran, Z.; Dutkiewicz, G.; Kosturkiewicz, Z.; Szafran, M. Crystal structure and spectroscopic properties of the complex of trigonelline hydrate with *p*-hydroxybenzoic acid. *J. Mol. Struct.* **2011**, *985*, 219–226.
55. Sun, R.; Tomkinson, J.; Bolton, J. Separation and characterization of lignins from the black liquor of oil palm trunk fiber pulping. *Sep. Sci. Technol.* **1999**, *34*, 3045–3058.
56. Shareef, B.A.; Waheed, I.F.; Jalaot, K.K. Preparation and Analytical Properties of 4-Hydroxybenzaldehyde, Biuret and Formaldehyde Terpolymer Resin. *Orient. J. Chem.* **2014**, *29*, 1391–1397.
57. Kaur, J.; Katopo, L.; Hung, A.; Ashton, J.; Kasapis, S. Combined spectroscopic, molecular docking and quantum mechanics study of β -casein and *p*-coumaric acid interactions following thermal treatment. *Food Chem.* **2018**, *252*, 163–170.
58. Moosavinejad, S.M.; Madhoushi, M.; Vakili, M.; Rasouli, D. Evaluation of degradation in chemical compounds of wood in historical buildings using FT-IR and FT-Raman vibrational spectroscopy. *Maderas-Cienc. Tecnol.* **2019**, *21*, doi:10.4067/S0718-221X2019005000310.

59. Asare, S. Synthesis, Characterization and Molecular Dynamic Simulations of Aqueous Choline Chloride Deep Eutectic Solvents. Ph.D. Thesis, South Dakota State University, Brookings, SD, USA, 2018.
60. Bohara, R.A.; Thorat, N.D.; Pawar, S.H. Immobilization of cellulase on functionalized cobalt ferrite nanoparticles. *Korean J. Chem. Eng.* **2016**, *33*, 216–222.
61. Zhang, D.; Hegab, H.E.; Lvov, Y.; Snow, L.D.; Palmer, J. Immobilization of cellulase on a silica gel substrate modified using a 3-APTES self-assembled monolayer. *SpringerPlus* **2016**, *5*, 48.
62. Swarnalatha, V.; Ester, R.A.; Dhamodharan, R. Immobilization of α -amylase on gum acacia stabilized magnetite nanoparticles, an easily recoverable and reusable support. *J. Mol. Catal. B-Enzym.* **2013**, *96*, 6–13.
63. Bai, H.; Wang, H.; Sun, J.; Irfan, M.; Han, M.; Huang, Y.; Han, X.; Yang, Q. Production, purification and characterization of novel beta glucosidase from newly isolated *Penicillium simplicissimum* H-11 in submerged fermentation. *EXCLI J.* **2013**, *12*, 528.
64. Tsilomelekis, G.; Josephson, T.R.; Nikolakis, V.; Caratzoulas, S. Origin of 5-hydroxymethylfurfural stability in water/dimethyl sulfoxide mixtures. *ChemSusChem* **2014**, *7*, 117–126.
65. Garba, N.A.; Muduru, I.; Sokoto, M.A.; Dangoggo, S.M. Production of liquid hydrocarbons from millet husk via catalytic hydrodeoxygenation in NIO/AL₂O₃ catalysts. In *WIT Transactions on Ecology and the Environment*; Syngellakis, S., Magaril, E., Eds.; WIT PRESS: Billerica, MA, USA, 2018; pp. 125–130.
66. Allen, G.; Bernstein, H.J. Internal rotation: VIII. The infrared and raman spectra of furfural. *Can. J. Chem.* **1955**, *33*, 1055–1061.



© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).