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Luo, yiping, Fan, Jiajun orcid.org/0000-0003-3721-5745, Budarin, Vitaliy L. et al. (2 more authors) (2017) Microwave-assisted hydrothermal selective dissolution and utilisation of hemicellulose in Phyllostachys heterocycla cv. Pubescens. Green Chemistry. pp. 4889-4899. ISSN 1463-9270

https://doi.org/10.1039/c7gc02300f

Green Chemistry

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: Y. Luo, J. Fan, V. L. Budarin, C. Hu and J. Clark, *Green Chem.*, 2017, DOI: 10.1039/C7GC02300F.

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DOI: 10.1039/C7GC02300F

Journal Name

ARTICLE

Microwave-Assisted Hydrothermal Selective Dissolution and Utilisation of Hemicellulose in *Phyllostachys heterocycla cv. pubescens*

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

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A green process for the microwave-assisted hydrothermal selective dissolution and utilisation of hemicellulose in *Phyllostachys heterocycla cv. Pubescens* (shortened to *pubescens*) was developed. The process facilitated the efficient dissolution of hemicellulose at 200 °C, while obtaining hemicellulose-free residue that could be further used as starting materials within many industrial processes. A variety of analytical techniques (e.g., HPLC, FT-IR, SEM, Chemical titration, TG/TGA, Py-GC/MS, TG-IR, ^{13}C liquid NMR, 2D HSQC NMR, and ^{13}C CPMAS NMR analysis) were used for the analysis of the obtained liquid and solid products, which revealed that hemicellulose was completely extracted from *pubescens*. A solid residue left after this process consists of cellulose and lignin in a pure form and can be used for production of glucose and aromatic compounds. Interestingly, a new route to produce hemicellulose-based films that could potentially be used for food packaging was achieved. The developed approach opens avenue for a low-cost and sustainable bamboo-based biorefinery.

1. Introduction

To date the production of specialty chemicals from biomass has acquired a significant market share. Especially in the domain of highly oxygenated compounds whereby biomass as a starting point, poses significant advantages, which alleviate the utilisation of petroleum-derived chemicals. Furthermore the use of biomass as a raw material also allows obtaining directly highly functionalized polymers, most notably polysaccharides and lignin. Depending on the geographical location different biomass prevail. While wheat, miscanthus and spruce are commonly available in the Western world, the situation is markedly different for Asia. A highly abundant crop in Asia is bamboo, with approximately 7.6 million hectares of bamboo forests in China alone.[1] Of particular interest is also the fast-growing nature of bamboo, typically reaching its maximum height of 15-30 m in 2-4 months and full maturity within 3-8 years, allowing for a steady supply of the material.[2, 3] Bamboo finds widespread application in paper, textile and furniture industry.[4, 5] However within these industries, significant amounts of waste bamboo are generated for which applications are being sought. Alike other biomass, bamboo consists mainly of three components: cellulose, hemicellulose and lignin.[2, 5] To utilize this waste material, a sustainable method would involve the use of a bamboo-based refinery. Currently, many research focused on the simultaneous conversion of the three components in bamboo, obtaining a complex product mixture contained many kinds of carboxylic acids, furans, phenols and oligomers, which caused the difficulty in product separation and in the further use of the products.[6-8] Within this, methods need to be found to efficiently extract one of these components in bamboo. The recovery of a pure compound with reproducible properties holds a distinct advantage that can be used in applications without any further upgrading. The differences of structure and reactivity of hemicellulose, cellulose and lignin, mean that the efficient extraction of hemicellulose has been found possible, be it under acidic conditions.[9, 10] Hemicellulose is a versatile natural polymer which can be used in many applications e.g. chemicals, food, energy and polymeric materials.[11] Noteworthy is also its excellent biodegradability, biocompatibility and bioactivity, opening also medicinal applications.[2]

Multiple methods are applied for the removal of hemicellulose, such as steam pretreatment[12, 13], enzymatic hydrolysis[14-16], organic solvent[17-19], acid catalytic processes[20, 21] and other high pressure methods[22]. In recent years, the extraction and utilisation of hemicellulose from actual biomass also attracted much attention in order to use biomass to its fullest. Xiao *et al.* have extracted xylo-oligosaccharides with yield of 36.4% from bamboo by autohydrolysis

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†Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

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DOI: 10.1039/C7GC02300F

in stainless steel autoclave at 182 °C for 31 min.[23] Garrote *et al.* achieved the removal of most xylan in corncob using stainless steel parr reactors at 216 °C.[24] In our previous work, 36.8 wt% cellulose and 52.5 wt% lignin in *pubescens* were converted when more than 95 wt% hemicellulose was removed at 200 °C for 0.5 h using autoclave as reactor.[25] The selectivity to hemicellulose extraction needed to be improved from the viewpoint of using the three main components effectively, because traces of hemicellulose under cellulose hydrolysis conditions produce fermentation inhibitors like carboxylic acids and furfurals making the biomass difficult and in some cases impossible to ferment.[26] Pretreatment of the bamboo has been shown to be beneficial to the overall yields of extracted hemicellulose.[12] Furthermore, many of these approaches, including autohydrolysis (one of the more promising approaches) render soluble hemicellulose, either totally or in very significant quantity, in oligomeric form.[27] Therefore, the majority of investigations has been focused on the optimization of production of monosaccharides and xylo-oligosaccharides(XOS) from different types of biomasses such as Euccalyptus Globulus wood samples[28], poplar and pinewood[29] as well as bamboo[23]. These investigations has been carried out using conventional heating and require significant time and additional upgrading, leaving a distinct negative environmental footprint.[23-25, 30] In contrast, microwave technology is identified as an energy efficient method and has gained acceptance as a mild and controllable tool, allowing for simple and rapid processing.[31-34] It has been shown that the microwave heating considerably reduces the time of hemicellulose extraction, demonstrating substantial potential of microwave technology but research in this area has focused on the extraction of hemicellulose oligosaccharides.[34] However, aqueous oligosaccharides mixtures are of low value and there is no effective microbial species that can directly metabolize oligosaccharides to produce marketable products.[27] The hydrolysis based biorefinery still needs development towards high-value products manufacture to reach industrial commercialization. The cost of a polymer is usually higher than that of the oligosaccharide mixture and the market opportunity is larger. Xylan (one of the polymeric forms of hemicellulose) is used to achieve controlled drug release,[35] improve molecules bioavailability and bio distribution[36]. Therefore, separation of hemicellulose in a polymeric form could substantially improve the situation.

Here we set out to achieve the development of an environmentally friendly route to the efficient isolation of hemicellulose mostly in the polymeric form from *pubescens* using a microwave-assisted hydrothermal approach. According to our knowledge this is the first example of the application of microwave for bamboo hydrolysis. The investigation focuses on the extraction of the high molecular weight hemicellulose derived species. The full analysis of the water soluble and solid fractions obtained after microwave hydrothermal treatment has been carried out. This enabled us to achieve the efficient dissolution and utilisation of hemicellulose while keeping the cellulose and lignin largely intact, thus helpings to achieve the use of biomass to its fullest. The cellulose and lignin that remained in the solid residue are shown to be hemicellulose free and offering the potential of further use in separation processes. The microwave assisted auto-hydrolytic process relies on the action of water, without pre-treatment, or any acid nor other additives, to isolate the hemicellulose fraction of *pubescens*. Additionally, we show that this process can be easily adapted to the selective formation of small molecule products, such as xylose, furfural and acetic acid, adding robustness towards changing market demands. Furthermore, the preparation of hemicellulose-based film is achieved by directly using hemicellulose as a polymer, which demonstrates the potential utilisation of hemicellulose on an industrial scale.

2. Methods

2.1 Raw materials

Pubescens powder (80 meshes) were obtained from Anji, Zhejiang, China. The composition of the *pubescens* powder was 42.81 wt% cellulose; 20.58 wt% hemicellulose; 24.70 wt% lignin; 0.66 wt% ash; 0.62 wt% wax; 6.75 wt% moisture; 3.35 wt% water soluble and 0.53 wt% others. Xylan from beechwood (Sigma Aldrich), microcrystalline cellulose (Sigma Aldrich) and alkali kraft lignin (SERVA) were used without further purification.

2.2 Microwave processing of *pubescens*

Microwave-assisted hydrothermal conversion of *pubescens* was performed on a CEM "Discover" Explorer microwave reactor. The temperature of this device was measured by an IR probe requiring prior calibration. In a typical run, the sample (1 g) was mixed with 20 mL distilled water in a 35 mL microwave pressure vessel. Then, the reactor was sealed and heated to a range of temperatures between 140 and 216 °C for a desired time by using dynamic power of 150 W. The changes of pressure, temperature and power in the process were *in situ* monitored. The reaction vessel was pressurized due to vapour pressure of the solution at the reaction temperature achieved. After the desired reaction time, the reaction was stopped by performing air-flow cooling. After being cooled down to room temperature, the microwave reactor was opened, and a mixture of aqueous phase products and solid residue was collected. The mixture collected was filtered through a pre-weighed filter paper, and the solid residue obtained was dried at 105 °C overnight in an oven. The aqueous phase products obtained were further filtered using a 0.45 mm syringe filter prior to HPLC analysis. For the further depolymerization of hemicellulose derivatives to small molecular products, the aqueous phase fraction was loaded into the microwave reactor for a second run under different temperatures and holding times. The procedure for the second run was similar to the first run.

2.3 Pyrolysis behaviors of samples before and after microwave hydrothermal treatment

Thermogravimetric analysis (TG) of *pubescens* and the solid residues from microwave hydrothermal treatment were conducted on a Stanton Redcroft STA 625. Typically, 10 mg of sample was heated from 20 °C to 625 °C at a heating rate of 10 °C min^{-1} under a constant N_2 flow (60 mL min^{-1}). Py-GC/MS analysis of solid samples were performed using a CDS Pyroprobe 5250-T trapping pyrolysis autosampler attached to a GC/MS apparatus (Agilent Technologies).

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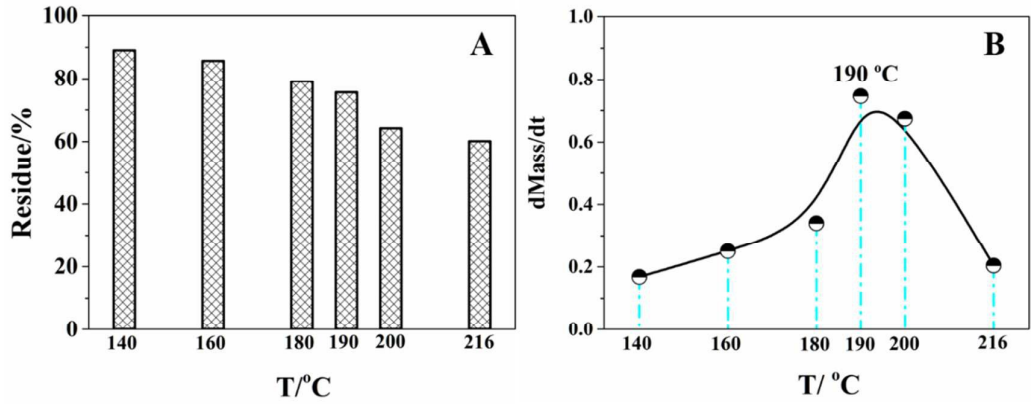

Figure 1 Microwave-assisted hydrothermal conversion of *pubescens* with holding time of 5 min:(A) The variation of residue content with microwave treatment at different temperatures; (B) The effect of temperature on the mass loss rate of *pubescens*

For each pyrolysis run, approximately 1 mg of sample was placed in a 20 mm quartz silica tube using quartz wool plugs and heated from room temperature to 600 °C for 10 min with a heating rate of 20 °C/ms. The GC-MS chromatograph was performed from 40 °C (2 min) to 300 °C at 10 °C/min and hold for 30 min. Helium was used as the carrier gas with constant flow rate of 1 mL/min and 1:50 split ratio. Simultaneous TG measurements coupling the FTIR spectrometer for the on-line analysis of volatile compounds formed during heating was carried out. The TG measurements were performed by a Netzsch STA 409 in a gas flow of 100 mL/min of nitrogen. The samples were heated from room temperature to 700 °C at the heating rate of 10 °C/min. FT-IR measurements were performed by a Bruker Equinox 55 spectrometer coupled to TG to measure the gaseous products. Spectra of the samples were collected in the range of 4000-550 cm^{-1} with a resolution of 2 cm^{-1} and 64 scans.

2.4 Characterization of solid samples

The FTIR spectra of solid samples were performed on a Bruker Vertex 70 spectrometer. The spectra of samples were recorded in attenuated total reflectance (ATR) mode, in the range of 4000-500 cm^{-1} with a resolution of 2 cm^{-1} and 32 scans. The ^{13}C CPMAS solid-state NMR experiments were carried out at room temperature with a BRUKER AVIII 400 HD instrument. A total of 800 scans were accumulated for each sample. The spin rate was 10000 Hz, and the relaxation delay was 5 s. Adamantane was used as reference for calibration of chemical shift. The SEM micrographs were collected by a JEOL JSM-7500F (acceleration voltage, 5 kV).

2.5 Analysis of liquid products

Sugars and furans in liquid products were quantitatively measured by a Hewlett Packard Series 1100 High Performance Liquid Chromatograph (HPLC). For the quantitative analysis of sugars, HPLC was run with an Alltech 3000 ELSD detector using a Luna NH_2 column. The temperature of the column oven and detector were 40 °C and 55 °C, respectively, and the mobile phase was H_2O/acetonitrile solution, at a flow rate of 1.0 mL/min. The concentration of furans in aqueous phase was determined by using HPLC equipped with C18 column and an UV detector. Acids in liquid products were quantitatively measured by Agilent 1260 HPLC fitted with Infinity II RI Detector using Agilent Hi-Plex H column (7.7 × 300 mm × 8 µm, p/n PL1170-6830). The mobile phase was 0.005 M H_2SO_4 with a flow rate of 0.4 mL/min and the column oven and detector were at 60 °C and 55 °C, respectively. The content of liquid products was quantified by an external standard method, and the yields of liquid products were based on the weight of *pubescens*. The ^{13}C NMR spectra were recorded on a Jeol ECX-500 spectrometer at 500 MHz using a relaxation delay of 30 s. The 2D HSQC NMR spectra of liquid fraction were qualitatively determined on a BRUKER ADVANCE 400 MHz spectrometer. The molecular weight distribution of liquid products was determined by GPC (Agilent 1260) equipped with a gel permeation chromatography column (Agilent PL aquagel-OH 20) and refractive index detector. The injection volume was 5 µL, and pure water was used as the mobile phase at a flow rate of 1.0 mL/min. Polysaccharides with molecular weight from 150 to 642000 Da were used as the standard for molecular weight calibration.

3. Results and discussion

3.1 Microwave-assisted hydrothermal selective conversion of hemicellulose in *pubescens*

Microwave-assisted hydrothermal treatment was evaluated as a method to extract the structural components of hemicellulose, cellulose and lignin from *pubescens*. In a first approach the amount of solid residue was recorded as a function of temperature. Due to the maximum pressure limit of 300 psi, the reaction temperature of the sample was restricted to 216 °C. It has been found that the

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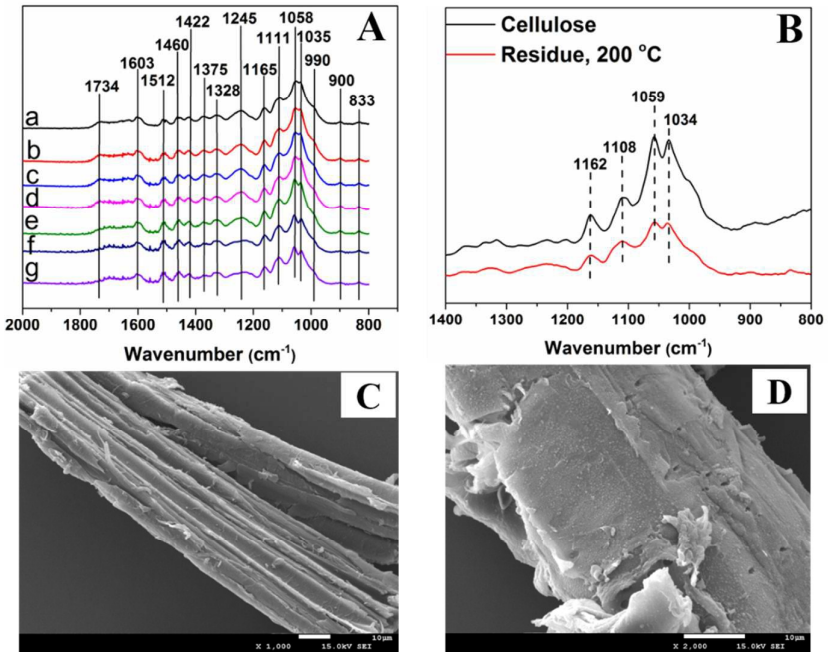

Figure 2 (A) FT-IR analysis results of solid samples with microwave treatment at different temperatures from 2000 to 800 cm^{-1} ((a) *pubescens*; (b) 140 °C; (c) 160 °C; (d) 180 °C; (e) 190 °C; (f) 200 °C; (g) 216 °C); (B) FTIR spectra of commercial cellulose and solid residue obtained after microwave hydrothermal treatment at 200 °C (Residue, 200 °C) from 1400 to 800 cm^{-1}; (C)SEM micrographs of solid residue obtained after microwave hydrothermal treatment at 200 °C; (D) The magnified view of SEM micrographs from C.

degree of hydrolysis could be significantly improved by prolonging holding time. For example, the conversion of *pubescens* at 180 °C can be increased from low conversion of only 13.7% without holding time to a high value of 28.4 % at 20 min (Figure S1). However, with 5 min holding time, 20.9% of *pubescens* was depolymerised. Taking into account the cost of energy used for 20 minutes microwaving and only a 7.5% of conversion difference, in comparison with 5 minutes, it was decided to use 5 minutes holding for further temperature investigations (see Figure 1A). It could be seen that the conversion of *pubescens* gradually increased with temperature, with maximum hydrolysate yield of 41% at 216°C. Interestingly, the mass derivative trace revealed a very pronounced increase in the hemicellulose-extractability from *pubescens* between 180 and 190 °C (see Figure 1B). Typically, within this temperature range the depolymerisation of amorphous cellulose is observed. [37-39]

This raises an important question in that obviously the depolymerisation of isolated biopolymers, such as cellulose, occurs at markedly different temperatures than observed in actual biomass. To further understand this observation, the FTIR spectra of *pubescens* feedstock and solid residues obtained after microwave hydrothermal treatment at different temperatures are presented in Figure 2A. The FT-IR absorption peaks at 1734 and 1245 cm^{-1} can be assigned to carboxylic acid functional group (the major component of xylan). [40, 41] These two peaks gradually decreased with increasing temperature before they disappeared after microwave hydrothermal treatment at 200 °C. Furthermore, the peaks at 900 and 990 cm^{-1}, assigned to β-glycosidic linkages between xylose units in the hemicellulose,[42, 43] gradually decreased and nearly disappeared at above 200 °C . All these data prove that hemicellulose in *pubescens* was almost fully extracted at 200 °C. It is interesting to note that there was almost no change in the characteristic peaks assigned to cellulose at ν= 1375, 1328, 1165, 1058 and 1035 cm^{-1}, showing that cellulose was not significantly affected by microwave radiation. [44, 45] The cellulose stability was also confirmed by the clear appearance of characteristic peak in cellulose fingerprint area (Figure 2B). For lignin, the peaks at 1603, 1512, 1460, 1422, 1111 and 833 cm^{-1} showed no obvious change with increasing temperature, [46] which showed the lignin remained in the solid residues after the cleavage of intermolecular linkages between hemicellulose and lignin. ^{13}C CPMAS solid-state NMR results also confirmed that the efficient extraction of hemicellulose in *pubescens* was achieved, while the dominant structure of cellulose and lignin in *pubescens* was not affected significantly, and the solid residue was enriched in cellulose and lignin (Figure S2). In order to study the influence of microwave hydrothermal treatment on morphological structure of *pubescens*, SEM analysis of *pubescens* samples before and after treatment were carried out (Figure 2 and Figure S3). Compared to *pubescens* feedstock (Figure S3(A)), the cellulose bundle was still intact even

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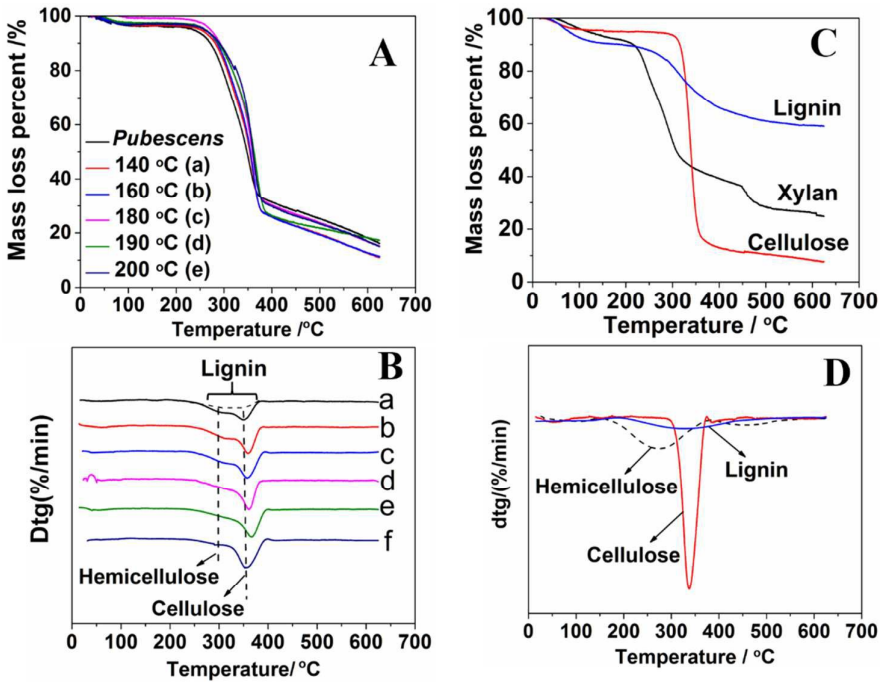

Figure 3 TG analysis results of pure components, *pubescens* and solid residues: (A) The TG of *pubescens* and solid residues obtained with microwave hydrothermal treatment at different temperature; (B) The DTG corresponding to A. (C) The TG of three pure components; (D) The DTG corresponding to C.

after microwave hydrothermal treatment at 200 °C (Figure 2C and Figure 2D). These results suggested that the cellulose and lignin retained in solid residue after the extraction of hemicellulose in *pubescens* at 200 °C, opening the potential of using these materials. For example, there is also a possibility of using enzymatic digestion of the cellulose residue to make sugars, while the residual lignin could be tranformed to value-added chemicals such as phenols.[47-49] Thus, we can envisage sustainable conversion process for the utilisation of *pubescens* to its fullest.

In addition to the FTIR results, the thermal gravimetric analysis of original *pubescens* and solid residues were performed (see figure 3A). To identify dTG peaks (Figure 3B) within the samples, the TG analysis of biomass structural components were also carried out (Figure 3C and 3D). It could be seen that hemicellulose is the most reactive among all components.[50] The onset temperature was at 165 °C with the temperature for the maximum mass loss rate being 275 °C, and there was still about 25 wt% solid residues left after pyrolysis at 625 °C. Among the three components, lignin was the most difficult one to decompose. It decomposed slowly in the whole range of temperature, and more than 60 wt% solid residues were left from lignin pyrolysis even at 625 °C. Compared with hemicellulose and lignin, cellulose exhibited the maximum mass loss. The temperature range of mass loss was very narrow and the maximum mass loss rate was observed at 338 °C. The three main components in biomass showed different pyrolysis behaviours, ascribing to the fact that the physical and chemical properties were quite different. The different pyrolysis behaviours of the three main components in biomass can give a clue for a better understanding of biomass thermal chemical conversion. The derivative thermogravimetric (DTG) curves of actual biomass generally showed one broad peak with a shoulder at the low temperature side.[50-52] This shoulder was attributed to hemicellulose decomposition, and the main peak corresponds to cellulose decomposition, while the slow further decomposition was caused by the gradual breakdown of lignin.[52] Meng *et al.* reported that hemicellulose pyrolysis peaked at 220-315 °C and the maximum mass loss rate was normally at around 250-300 °C. Cellulose pyrolysis focused at 300-400 °C and the maximum mass loss rate normally at around 350 °C.[53] Lignin pyrolysed almost from 300 to 500 °C.[54] Therefore, according to the literature and the above thermogravimetric results of pure components, the DTG curves of *pubescens* can be fitted into three peaks at 301, 353 and 325 °C, corresponding to the temperature for the maximum mass loss rate of hemicellulose, cellulose and lignin fraction in *pubescens* (Figure S4). In the DTG curves of *pubescens* as shown in Figure 3B, the small shoulder peak at 305 °C can be referred to the maximum mass loss rate for hemicellulose components. The maximum mass loss rate for cellulose components occurred at 350 °C, while lignin components exhibited a wide temperature range of mass loss rate from 175 °C to 385 °C. The temperature for the maximum mass loss rate in the three main components from *pubescens* was different from that in the

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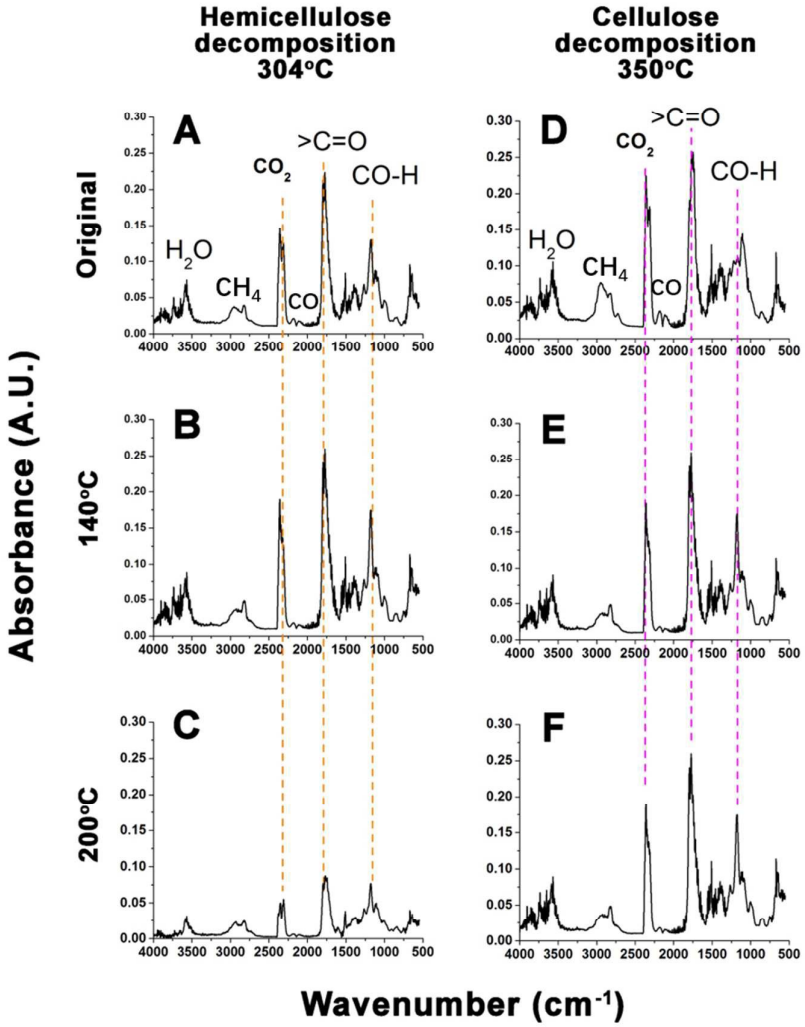

Figure 4 FT-IR spectra of evolved gases and volatile compounds from *pubescens* and solid residues pyrolysis represented decomposition of hemicellulose at 304 °C: A) original; B) after MW hydrolysed at 140 °C; C) after MW hydrolysed at 200 °C; and decomposition of cellulose at 350 °C: A) original; B) after MW hydrolysed at 140 °C; C) after MW hydrolysed at 200 °C. (The resonance assignment of FT-IR spectra were according to literature.[53])

pure components. The intermolecular chemical bonds existed among the three main components in biomass may result in more difficulty to decompose the three main components than the pure components. As shown in Figure 3B, it was clear that the contribution of hemicellulose diminished with increasing temperature of the microwave hydrothermal treatment, while cellulose and lignin content stay almost retained. The hemicellulose component in solid residues almost completely disappeared with microwave treatment
at 200 °C, indicating that hemicellulose was completely removed during microwave hydrothermal process. It was found that pure hemicellulose, cellulose and lignin were not pyrolysed completely during thermogravimetric analysis, especially lignin, with more than 60% lignin remaining in solid residues (Figure 3C). Besides, about 20% *pubescens* or solid residues were also left after pyrolysis during thermogravimetric analysis (Figure 3A). Therefore, the quantitative analysis of the three components in solid samples through their relative peak area in DTG curves only referred to the pyrolysed fraction during thermogravimetric analysis. Using the relative peak area of the three peaks assigned to hemicellulose, cellulose and lignin, the component analysis results of the residue after microwave treatment under different temperature could be obtained. So the removal ratio of hemicellulose can be calculated. As shown in Table 1, 49.6 % hemicellulose, 34.4 % cellulose and 15.8 % lignin were

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Table 1 The relative peak area of the three peaks in solid samples given in Figure S4

Samples	Relative peak area /%			Cellulose/lignin ratio[c]
	Peak 1[a]	Peak 2[a]	Peak 3[a]	
Pubescens	49.6	34.4	15.8	2.2
140 °C[b]	42.4	41.1	16.5	2.5
160 °C[b]	33.4	48.4	18.3	2.6
180 °C[b]	27.8	51.4	20.8	2.5
190 °C[b]	25.4	53.5	21.1	2.5
200 °C[b]	2.4	67.2	30.4	2.2

[a] Peak1, Peak2 and Peak 3 were assigned to hemicellulose, cellulose and lignin in *pubescens* respectively.

[b] Solid residues obtained with microwave hydrothermal treatment at different temperature.

[c] The cellulose/lignin ratio is calculated by the relative peak area of peak 2 divided by that of peak 3.

contained in *pubescens*. For solid residues obtained with microwave hydrothermal treatment under different temperature, the temperature for the maximum mass loss rate of hemicellulose gradually moved to lower temperature as shown in Figure S4, with increasing temperature of microwave treatment from 140 to 200 °C. This could be a result of microwave assisted hemicellulose-water interaction. The relative content of hemicellulose in solid residue gradually decreased from 49.6% to 2.4% (Table 1), suggesting that more than 95% hemicellulose was extracted at 200 °C. While the relative content of cellulose and lignin gradually increased with increasing microwave hydrothermal temperature. However, the cellulose to lignin ratio was stable at all the temperatures studied. This is further evidence that the microwave activation of *pubescens* at temperature below 200 °C involves activation of the hemicellulose only.

Based on the DTG curves in Figure 3B, the FTIR spectra of evolved gases and volatile compounds from *pubescens* feedstock and solid residues (obtained with microwave hydrothermal treatment at 140 °C and 200 °C) pyrolysis in the first stage and second stage of maximum mass loss are shown in Figure 4. In the FTIR spectra of *pubescens* feedstock pyrolysis at 304 °C, corresponding to first stage of DTG curves, it showed that the main typical gaseous products were CO_2, H_2O, alcohols, acids and aldehydes. CO and CH_4 were also found in the IR spectra, but it was not obvious. According to the thermogravimetric analysis results, these gaseous products may be from hemicellulose and lignin pyrolysis. While in the FTIR spectra of *pubescens* feedstock pyrolysis at 350 °C, corresponding to second stage of DTG curves, it was found that the intensity of peaks assigned to CH_4 and CO_2 increased. This was mainly due to the pyrolysis of cellulose and lignin. The IR spectra of gaseous products from the pyrolysis of the solid residues (obtained after microwave hydrothermal treatment at 140 °C) at around 304 °C and 350 °C, respectively looked similar to those from *pubescens* feedstock pyrolysis (Figure 4B and 4E). Once again, this suggested that the pyrolysis behaviours of *pubescens* were not significantly affected after microwave hydrothermal treatment at lower temperatures of 140 °C. However, as shown in Figure 4C, it was obviously observed that the intensity of evolved gases and volatile compounds significantly decreased at 304 °C, while the intensity of all the evolved gases and volatile compounds increased at 350 °C that was similar to that in the IR spectra of *pubescens* feedstock pyrolysis at 350 °C. In the first pyrolysis stage (around 300 °C), the evolved gases and volatile compounds were mainly from hemicellulose and small amount of lignin. While those compounds produced in the second pyrolysis stage (around 350 °C) were mainly from cellulose and small amount of lignin.

Another powerful tool for the *in situ* characterization of plant constituents is Py-GC/MS. [46] Zhou *et al.* appiled Py-GC/MS to check the structure of lignin. [55] The identification and relative peak area of the compounds released after Py-GC/MS of *pubescens* and residues obtained with microwave hydrothermal treatment at 200 °C are shown in Table S2. Relative peak areas were calculated for pyrolysis products from phenylpropanoid compounds (including guaiacyl (G) and syringyl-type (S) phenols), and the total areas of the peaks were normalized to 100%. For the *pubescens* raw material, the S/G ratio was 2.05, while the S/G ratio were 2.00 for residues with microwave treatment at 200 °C. It was evident that the S-units of lignin degraded faster than the G-units, which was consistent with Araya *et.al* 's work.[56] This might be ascribed to the fact that S units have more reactive -OCH_3 groups than G units.[57] Because the S/G ratio of residue (get with microwave treatment at 200 °C) was similar to that of *pubescens* feedstocks (2.05), it indicated that the dominant structure of lignin in *pubescens* was not affected significantly after microwave hydrothermal treatment at 200 °C.

The microwave-assisted conversion of other types of biomass, such as softwood and wheat straw were also carried out. It can be seen from Figures S5 and S6 that the mass- derivative trace revealed a very pronounced increase in the hemicellulose-extractability from softwood between 160 and 180 °C, and from wheat straw between 180 and 190 °C. As shown in Figure S7, the amount of hemicellulose in softwood or wheat straw diminished with increasing temperature of the microwave hydrothermal treatment, while the quantities of cellulose and lignin almost retained. The hemicellulose component in softwood almost completely disappeared with microwave treatment at 180 °C, while that in wheat straw completely disappeared with microwave treatment at 190 °C. These results suggested that hemicellulose in softwood and wheat straw can also be efficiently extracted at 180 and 190 °C, while keeping most cellulose and lignin relatively retained. In *pubescens* a higher temperature is required (200 °C), demonstrating unusual thermal stability of hemicellulose in this plant, accessing the hemicellulose in *pubescens* is especially difficult.

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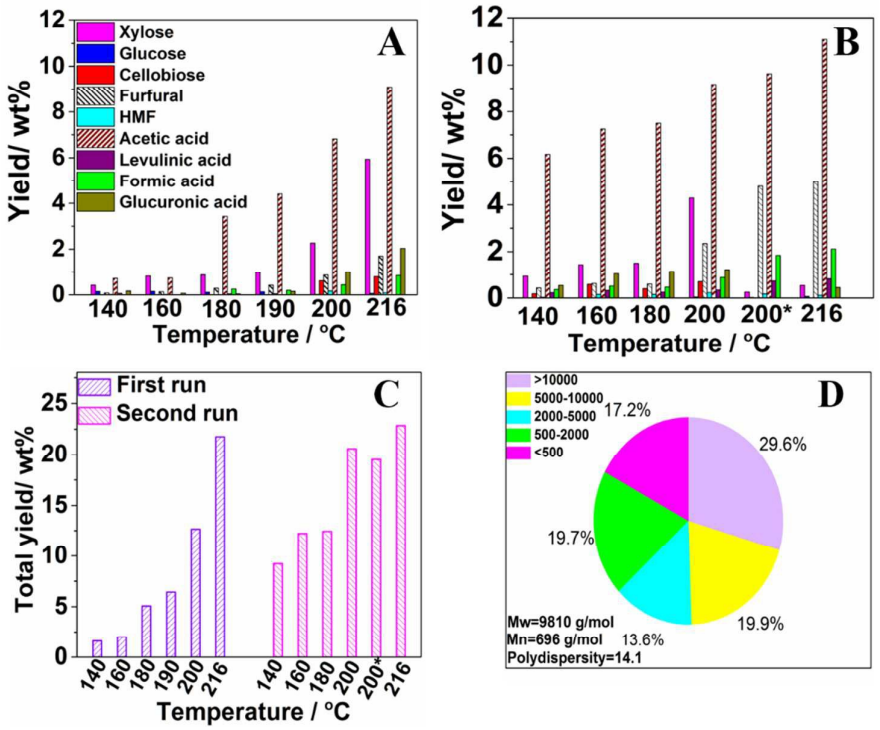

Figure 5 (A) The influence of temperature on the distribution of the main small molecular products for 5 min in the first run; (B) The yield of the main small molecular products by the further reaction of hydrolysate obtained at 200 °C for 5 min at different temperature for 10 min in the second run (*The further reaction was carried out at 200 °C for 10 min); (C) The total yield of small molecular products in the first run(Corresponding to A) and second run (corresponding to B). (D) GPC results of the hydrolysate obtained with microwave hydrothermal treatment at 200 °C for 5 min. The yield of small molecular products was based on the mass weight of *pubescens* feedstock (wt%).

3.2 The application of the extracted hemicellulose from *pubescens*

3.2.1 Further depolymerization of extracted hemicellulose to small molecular products

Results of HPLC analysis of the hydrolysates obtained with microwave hydrothermal treatment under different temperatures showed that the major small molecular products were xylose and acetic acid (the first run, see Figure 5A). It was observed that xylose and acetic acid yield increased gradually with increasing temperature. Acetic acid mainly came from hydrolysis of acetyl groups in O-acetyl- 4-O-methylglucuronoxylan in hemicellulose.[58] The dominance of xylose and acetic acid, suggested that at this temperature microwaves and H_2O mainly interacted with hemicellulose. The yield of furfural gradually increased with increasing temperature from 140 °C to 216 °C, but its yield was quite low. It was also found that higher temperatures increased the yield of some small molecular products (cellobiose, HMF, formic acid, glucuronic acid, etc). A small amount of levoglucosan, rhamnose, sucrose and lactic acid was also detected, but not shown for brevity. As shown in Figure 5C, the total yield of small molecular products gradually increased with increasing temperature, but the total yield only up to 12.7 wt% at 200 °C (mass loss of *pubescens* was 36 wt%). GPC was used to analyse the molecular weight distribution of hydrolysate obtained at 200 °C (Figure 5D). The results showed that M*w* was 9810 g mol^{-1} and M*n* was 696 g mol^{-1}, which confirmed the formation of oligomers with polydispersity of 14.09.

The molecular weights of oligomers in the hydrolysate was mostly greater than 10000 g mol^{-1} (29.67%). The hydrolysate obtained with microwave hydrothermal treatment at 200 °C was also investigated by $^{1}H/^{13}C$ NMR. As shown in Table 2 and Figure S8, strong signals at 101.71, 76.39, 73.70, 72.74, and 63.01 ppm for hydrolysate (200 °C for 5 min) were observed by ^{13}C NMR analysis, which respectively corresponded to C-1, C-2, C-3, C-4 and C-5 of the (1→4)-linked β-D-xylp units.[59] The NMR data obtained for a reference ^{13}C spectrum of birch xylan (Figure S9 and Table 2) showed similar signals at 101.65, 76.32, 73.63, 72.68, 62.94 ppm. The signals assigned to lignin fraction in aromatic region (103-161 ppm) and aliphatic region (50-103 ppm) were very weak (Figure S9). The characterization of the dissolved hydrolysate with microwave treatment at 200 °C by the 2D HSQC analysis was also carried out (Figure S10). This further confirmed that the efficient extraction of hemicellulose with microwave treatment at 200 °C was achieved.

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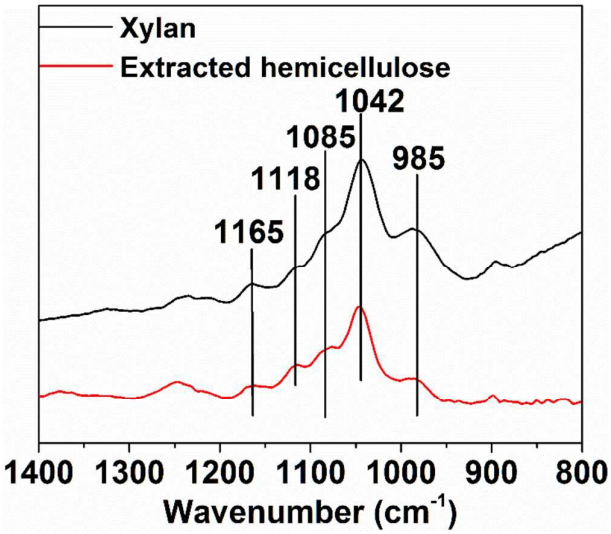

Figure 6 FT-IR results of different samples of commercial xylan and hydrolysate obtained with microwave hydrothermal treatment at 200 °C (extracted hemicellulose) from 800 to 1400 cm^{-1}.

Table 2 ^{13}C NMR chemical shifts in ppm of different samples

Samples	Major ^{13}C NMR peaks (ppm)				
Hydrolysate[a]	101.71	76.39	73.70	72.74	63.01
Birch xylan	101.65	76.32	73.63	72.68	62.94
Reference[b]	101.6	76.2	73.5	72.6	62.9

[a] Hydrolysate obtained with microwave hydrothermal treatment at 200 °C. [b] Assignations in the reference for β-D-xylose unit in the hemicellulose polymer. [57]

Additionally, the FT-IR C-O stretching region of the hydrolysate at 200 °C was found to be the same as one obtained from commercial xylan (Figure 6). This also suggested the extracted hemicellulose at 200 °C in the first run was mainly in the form of β-D-xylose oligomers.

For the further depolymerisation of extracted hemicellulose to obtain small molecular products, the hydrolysate obtained at 200 °C was loaded into the microwave for a second run under different conditions. A significant influence of secondary reactions during the microwave assisted hydrolysis could be proved by HPLC results of microwave treatment of hydrolysate at different temperatures and times (Figure 5B). With increasing temperature from 140 °C to 200 °C for 10 min, acetic acid yield gradually increased to 11.1 wt%. With the holding times of 10 min at 200 °C in the second run, the yield of xylose was doubled compared with the results of hydrolysate at 200 °C in the first run. However, after that it was converted to furfural. Prolonging the holding time from 10 min to 20 min at 200 °C for the further reaction of hydrolysate obtained at 200 °C, the yield of xylose significantly decreased, while the yield of furfural increased from 2.3% to 4.8 wt%. When further reacting at 216 °C for 10 min, the maximum furfural and acetic acid yield of 5.0 and 11.1 wt% were obtained,

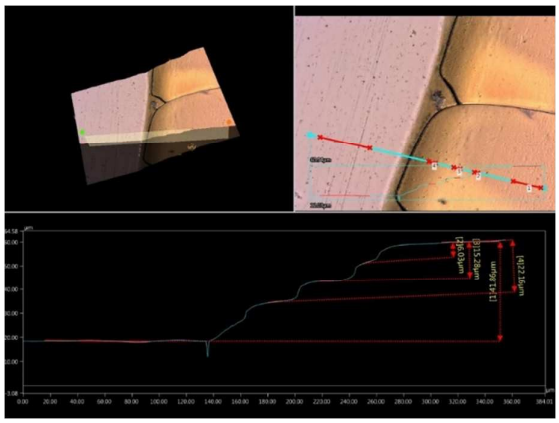

Figure 7 The picture of film prepared from extracted hemicellulose

respectively. If all the furfural and acetic acid were from the conversion of hemicellulose, the total yield of furfural and acetic acid would be 78.2 % based on the weight of hemicellulose in *pubescens*.

Ren *et al.* achieved a relatively high yield of furfural (9.0%, based on the dry weight of corncob) under the microwave-assisted hydrothermal treatment with 1% $SnCl_4$ catalyst. [60] Here, 5.0 wt% furfural (based on the dry weight of *pubescens*) was obtained, less than that of Ren *et al.*, but the process is more environmental friendly, without the need of acid or other additives. The maximum total yield of small molecular products was 22.9 wt% in the second run, which was doubled compared with the results of hydrolysate at 200 °C in the first run (Figure 5C). If the yield of liquid products obtained was based on the converted weight of *pubescens*, the selectivety to small molecular products would be 63.6%. Therefore, the extracted hemicellulose could be further reacted to produce small molecular products.

3.2.2 Direct utilisation of extracted hemicellulose to make film

Hemicellulose has received increasing interests as an alternative to petroleum based polymers for packaging applications because of its abundance, renewability and biodegradability. As early as 1949, Smart and Whistler reported the formation of film from hemicellulose acetates. [61] We attempted to prepare films directly from extracted hemicellulose (obtained after *pubescens* being treated at 200 °C for 5 min with microwave) by evaporating H_2O very gently at ~40 °C, and found a film with thickness of 40 μm could be made (Figure 7). However, hemicellulose-based film is water sensitive, so the application of hemicellulose-based film in the packing area is challenging and need to be modified to meet the requirements for packaging materials. This could require the addition of plasticizer or cross-linking agent to improve the tensile strength and the oxygen and moisture barrier properties. [62-64] The hydroxyl groups of hemicellulose can be esterified or etherified improving the moisture barrier properties.[65] The hemicellulose-based film also can be modified by surface coating and enzymes to improve the film properties. [66-67] Considering the length and focus of the present manuscript, we have not discussed this issue yet.

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Currently, more than 41% petroleum based plastic products were used for packing film. Because the petroleum based film was difficult to decompose, so the use of petroleum based film inevitably result in environmental pollution. The preparation of renewable hemicellulose based films demonstrates the utilisation of selectively extracted hemicellulose on a potential industrial scale.

3.Conclusions

The first reported microwave-assisted auto-hydrolysis of *pubescens* was an effective route for the extraction of hemicellulose. A high dissolution of hemicellulose (more than 95%) was achieved at 200 ^{o}C, leaving the other two components, cellulose and lignin, almost unchanged and in a form that could be used to produce renewable fuels and commodity chemicals making a bamboo-based bio-refinery more profitable. This process is green and energy efficient, making it highly favourable in terms of sustainable chemistry. The full analysis of the three main components in *pubescens* was done, and gave detailed information about the structure of dissolved hemicellulose, cellulose and lignin, which helped the use of all the three main components of biomass to its fullest. It has been demonstrated that hemicellulose was extracted in unusually high molecular weight form. This provided an interesting new route to hemicellulose-based films by direct utilisation of the extracted hemicellulose: this could potentially be used for food packaging. Additionally, the extracted hemicellulose can be further depolymerised to produce small molecular products, and the selectivity to small molecular products reached 63% based on the converted weight of *pubescens*.

Acknowledgements

This work is financially supported by National Basic Research Program of China (973 Program, No.2013CB228103), EPSRC for research grant no. EP/k014773/1 and the Industrial Biotechnology Catalyst (Innovate UK, BBSRC, EPSRC) to support the translation, development and commercialisation of innovative industrial Biotechnology processes (EP/N013522/1). The authors would like to thank members of the Green Chemistry Centre of Excellence for their input and useful discussions. Y. P. Luo acknowledges support from China Scholarship Council (CSC No. 201506240162).

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