Bioprocess Biosyst Eng (2016) 39:1539–1551
DOI 10.1007/s00449-016-1629-2

ORIGINAL PAPER

Improvement of radio frequency (RF) heating-assisted alkaline pretreatment on four categories of lignocellulosic biomass

Xiaofei Wang[1] · **Steven Taylor**[1] · **Yifen Wang**[1]

Received: 22 January 2016 / Accepted: 20 May 2016 / Published online: 4 June 2016

Abstract Pretreatment plays an important role in making the cellulose accessible for enzyme hydrolysis and subsequent conversion because it destroys more or less resistance and recalcitrance of biomass. Radio frequency (RF)-assisted dielectric heating was utilized in the alkaline pretreatment on agricultural residues (corn stover), herbaceous crops (switchgrass), hardwood (sweetgum) and softwood (loblolly pine). Pretreatment was performed at 90 °C with either RF or traditional water bath (WB) heating for 1 h after overnight soaking in NaOH solution (0.2 g NaOH/g Biomass). Pretreated materials were characterized by chemical compositional analysis, enzyme hydrolysis, scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR). The glucan yields of RF-heated four categories of hydrolysates were 89.6, 72.6, 21.7, and 9.9 %. Interestingly, RF heating raised glucan yield on switchgrass and sweetgum but not on corn stover or loblolly pine. The SEM images and FTIR spectra agreed with results of composition analysis and hydrolysis. GC–MS detected some compounds only from RF-heated switchgrass. These compounds were found by other researchers only in high-temperature (150–600 °C) and high-pressure pyrolysis processes.

Keywords Lignocellulosic biomass · Radio frequency · NaOH pretreatment · Enzymatic hydrolysis

Introduction

To reduce the dependence on foreign suppliers of petroleum, the United States is in need of alternative energy sources. Lignocellulosic biomass (LCB) is a potential and competitive source for bioenergy production. There are two main reasons: biomass is one of the few energy sources that can actually be utilized to produce several types of energy (motor fuel, electricity, heat); cellulosic biomass is renewable and commonly found [1]. LCB refers to plant biomass that is composed of cellulose, hemicelluloses, and lignin [2]. The structure of LCB can be described as a skeleton of cellulose chains embedded in a cross-linked matrix of hemicellulose surrounded by a crust of lignin, shown in Fig. 1. The amount of each component varies depending on the species of LCB. In general, hardwood has greater amount of cellulose meanwhile wheat straw and leaves have more hemicellulose, shown in Table 1. LCB can be grouped into four main categories: (1) agricultural residues (corn stover and straw, which can be used for production of second generation biofuels); (2) dedicated energy plants (switchgrass, eucalyptus, etc.); (3) wood biomass from hardwood and softwood trees; and (4) municipal paper waste (mainly municipal solid waste, packaging waste wood, household waste wood, market waste, food processing wastes, etc.). Pretreatment of LCB is required to alter the structure of biomass to be converted to simple fermentable sugars, including removing lignin and hemicellulose, reducing the crystalline structure of cellulose, as well as increasing the porosity of the materials. It can be roughly divided into several different categories: physical, chemical, physicochemical, biological and others [4]. However, none of these can be recognized as a "winner" since each pretreatment has its intrinsic advantages and disadvantages. Alkaline pretreatment was chosen

✉ Yifen Wang
wangyif@auburn.edu

[1] Department of Biosystems Engineering, Auburn University, 200 Tom E. Corley Building, Auburn, AL 36849-5417, USA

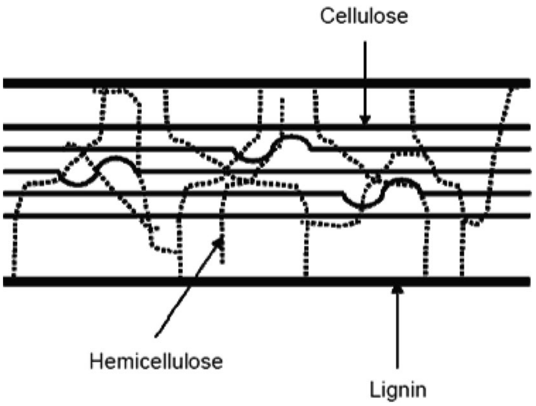


Fig. 1 General structure of lignocellulosic biomass *f* [*f* 546 (from 23, 28)]

Table 1 Different lignocellulosic biomass chemical compositions (% dry basis)

Lignocellulosic biomass	Cellulose	Hemicellulose	Lignin
Softwood	40–50	15–20	23–33
Loblolly pine	46.4	18.8	29.4
Spruce	43.8	19.8	28.8
Douglas fir	40	19.9	24.6
Hardwood	40–55	20–35	21–31
Eucalyptus	45	19.2	31.3
Polar	43.8	18.4	29.1
Sweetgum	40.8	30.7	25.4
Herbaceous grasses	25–40	35–50	10–30
Switchgrass	31.0	20.4	17.6
Agricultural residues	25–30	20–30	12–40
Corn stover	37.5	22.4	17.6
Rice straw	25–30	25–30	30–40
Nut shells	31.1	22.3	13.3

Source [11, 26]

in this study because the advantage of this pretreatment technology lies in the fact that it would create a washed clean substrate that is highly digestible and rich in cellulose and xylan. A relatively clean sugar stream could be obtained at reasonably high yield and economically relevant enzyme dose.

Radio frequency (RF) heating is a promising dielectric heating technology, and alternative method for conventional heating. Convection heating is based on superficial heat transfer, while dielectric heating transforms electromagnetic energy into heat that is volumetric and fast [5]. At the same time, the electromagnetic field could generate nonthermal effects, which can also accelerate the destruction of the crystallinity structure [6]. Depending on the wavelength, dielectric heating can be divided into microwave and RF [7]. Microwave-based heating has been studied in the pretreatment of various LCB [8]. However, compared to microwave heating, RF heating systems have higher electricity to electromagnetic power conversion efficiency, and a much deeper penetration of RF energy into a wide array of materials. To the best of our knowledge, there were only two papers regarding the alkaline pretreatment on biomass assisted by RF heating: Hu et al. [6] collaborated with our lab handling switchgrass and Iroba et al. [9] pretreated barley straw.

The primary focus of this work was to carry out systematic study on RF heating technology and judge its feasibility for downstream biofuel production. We compared RF heating and conventional heating methods with chemical pretreatment on four different feedstocks including agricultural residues (corn stover), herbaceous crops (switchgrass), hardwood (sweetgum) and softwood (loblolly pine). The resultant substrates were evaluated based on their chemical compositions and enzymatic hydrolysis yields and rates. We investigated the difference between RF heating and water bath (WB) heating on the residual xylan and lignin content, as well as on enzymatic digestibility. Physical characteristics were also measured and compared using scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR). Therefore, the objectives of this study were to explore the difference of physical and chemical characteristics of four different LCBs pretreated with alkaline assisted by RF heating and traditional heating.

Materials and methods

Raw materials

All samples were collected from recently harvested biomass. Briefly, corn stover, switchgrass (*Panicum virgatum*), sweetgum (*Liquidambar styraciflua*) and loblolly pine (*Pinus taeda*) were acquired from the Bioenergy and Bioproducts Center at Auburn University. The materials were air-dried and milled. All feedstocks were ground by Wiley mill (Thomas Scientific, Philadelphia, PA) for the same time period. The particles were reduced to an average size of 1.0 × 2.0 × 0.3 cm3 (L × W × H) by a Waring commercial blender (Dynamics Corporation of America, New Hartford, CT) as raw materials. The air-dried materials were collected in sealed plastic bags, and stored at room temperature. The main components of raw material were analyzed before pretreatment.

Moisture, extractives, ash, cellulose (as glucan), hemicellulose (as xylan) and lignin (acid-insoluble lignin and acid-soluble lignin) in the raw substrates were analyzed according to NREL (National Renewable Energy Laboratory, USA) laboratory analytical procedures using the extractive-free samples. Chemical compositional analysis was determined on all of the four types of substrates using the NREL protocol.

NaOH pretreatment with RF heating

An RF heater (SO6B; Strayfield, Berkshire, England) was employed in this study, shown in Fig. 2. This RF heating system worked at a frequency of 27.12 MHz and maximum power output of 6 kW. The distance between the two electrodes was fixed at 8.5 cm.

Before pretreatment, the four feedstocks were soaked in NaOH solution (0.2 g NaOH/g Biomass) at a solid to liquid ratio of 1:10 in a 500-ml plastic container at room temperature overnight. During the RF heating process, four fiber-optic sensors (UMI, FISO Technologies, Quebec, Canada) were inserted to keep the system at 90 °C with ± 3 °C fluctuation for 60 min. When sample temperature reached 90 °C, the RF heater was paused for 0.5 min followed by another 1-min RF heating to keep the sample at 90 °C. This pause-heating pattern was repeated until the predetermined RF heating time was completed.

After pretreatment, the container was removed and cooled down at room temperature. The pretreated substrate was collected by filtration through a Whatman No.4 filter paper in a Buchner funnel, washed with warm deionized water for at least three times to neutralize the pH to 7.0 and stored at 4 °C. The wet pretreated substrate without drying was used for the chemical compositional analysis and following enzymatic hydrolysis.

NaOH pretreatment with conventional (WB) heating

For NaOH pretreatment with conventional heating, in this study WB heating was utilized. Similar to RF heating, the particles were soaked in NaOH solution overnight. Before treating the biomass, the WB was prepared to 90 °C and then the mixture was put into the 90 °C condition for 60 min. After pretreatment, the container was removed and cooled down at room temperature.

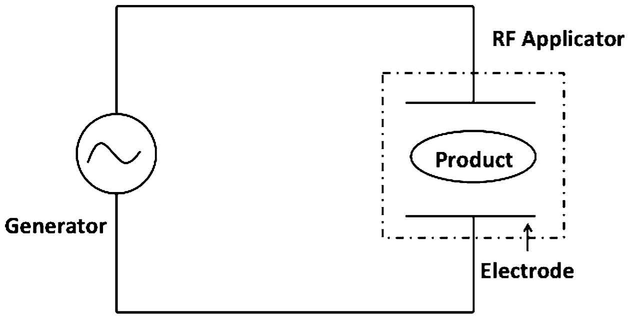


Fig. 2 RF heating system

Enzymatic hydrolysis

Commercial cellulase, Novozym 22C, was obtained from Novozymes (Franklinton, NC). The filter paper activity of Novozym 22C was 100 FPU/ml, and its β-glucosidase activity was 343 IU/ml. The enzyme loading used in enzymatic hydrolysis was 10 FPU/g glucan. Enzymatic hydrolysis was performed in 125 ml of 50 mM sodium citrate buffer (pH 4.8) at 2 % glucan (w/v) with commercial enzyme (Novozym 22C) and pretreated biomass. The hydrolysis reaction was incubated at 50 °C and 150 rpm for 72 h. The concentration of sugars (glucose and xylose) was determined using an Agilent 1260 Infinity Quaternary LC VL HPLC (Agilent Technologies, Santa Clara, CA) with refractive index detector (RID) as NREL protocol.

Scanning electron microscopy (SEM)

Scanning electron micrographs (SEMs) were obtained using a Zeiss DSM 940 scanning electron microscope. The images were taken at 20.00 kV. The samples were first dried under vacuum and gold-coated using a sputter coater (Pelco, model Sc-7) for 2 min and then imaged by SEM.

FTIR spectroscopy

FTIR spectra were collected using a PerkinElmer Spectrum model 100 (Perkin Elmer Co., Waltham, MA). This equipment utilized a single-reflectance ATR diamond with a repeatable vertical pressure between samples to ensure repeatability in spectra acquisition between samples. All measurements were carried out at room temperature. Background spectra were measured before every sample to eliminate the noises contributed by carbon dioxide in air, moisture and oxygen. Each spectrum was recorded over 32 scans, in the range between 4000 and 650 cm^{-1} with a resolution of 4 cm^{-1}.

GC–MS determination of pretreated switchgrass

The alkaline-pretreated switchgrass liquor assisted by radio frequency and water bath heating was tested for chemical compounds using GC–MS. An Agilent 6890 N GC equipped with an Agilent 5973 mass-selective detector (MSD) was employed along with a DB-1701 column (60 m × 0.25 mm, 0.25 μm film thickness). The oven was programmed to hold at 40 °C for 4 min, ramp at 3 °C/min to 250 °C, and then hold for 20 min. The injector temperature was 250 °C, and the injector split ratio was set at 30:1. The flow rate was 1 mL/min of the He carrier gas. The samples were prepared as 6 % solutions in methanol that were filtered through a 0.45 μm PTFE filter prior to injection.

Results and discussion

Characteristics of biomass before pretreatment

The biomass of corn stover, switchgrass, sweetgum and loblolly pine were used in this study to represent four types of biomass. Each type of the biomass was analyzed before pretreatment. The chemical composition of the four feedstocks is shown in Table 2. The untreated agricultural residue sample (corn stover) had 21.1 % lignin and 70.4 % carbohydrates while the raw dedicated plants sample (switchgrass) contained 21.4 % lignin and 67.3 % carbohydrates. For the untreated hardwood sample (sweetgum) there were 25.8 % lignin and 68.1 % carbohydrates while the untreated softwood sample (loblolly pine) had 28.7 % lignin and 60.0 % carbohydrates. Among all the biomass feedstocks, the major portion of the carbohydrates was glucan. Lignin, which contributes significantly to recalcitrance, was richest in the softwood (loblolly pine, 28.7 %), followed by hardwood (sweetgum, 21.6 %), and the least in herbaceous feedstocks (switchgrass, 21.4 %) and agricultural residues (corn stover, 21.1 %).

Characteristics of biomass after pretreatment

Contents of glucan (as for cellulose), xylan (as for hemicellulose) and lignin in these four feedstocks were used as direct (first level) indicators to determine an extent of efficiency of biomass during pretreatment.

Table 2 Chemical compositions of corn stover, switchgrass, sweetgum and loblolly pine before and after using RF and WB heating (% dry basis)

Biomass	Processes	Glucan	Xylan	Lignin
Corn stover	Untreated	44.9 ± 0.05	25.5 ± 0.67	21.1 ± 0.72
	WB pretreated	51.3 ± 0.39	25.8 ± 0.08	16.0 ± 0.78
	RF pretreated	52.1 ± 0.49	24.6 ± 0.12	15.8 ± 0.44
Switchgrass	Untreated	43.7 ± 0.23	23.6 ± 0.24	21.4 ± 0.79
	WB pretreated	58.4 ± 0.65	18.6 ± 0.35	11.2 ± 0.62
	RF pretreated	60.7 ± 0.55	16.6 ± 0.19	10.4 ± 0.14
Sweetgum	Untreated	47.8 ± 0.48	20.3 ± 0.28	25.8 ± 0.57
	WB pretreated	54.0 ± 0.12	16.1 ± 0.37	18.9 ± 0.27
	RF pretreated	56.6 ± 0.33	14.0 ± 0.54	18.2 ± 0.38
Loblolly pine	Untreated	52.1 ± 0.37	7.9 ± 0.38	28.7 ± 0.49
	WB pretreated	58.3 ± 0.15	5.6 ± 0.36	24.6 ± 0.05
	RF pretreated	57.3 ± 0.24	3.8 ± 0.29	24.8 ± 0.10

Percentages calculated from values on a dry weight basis. Data are averages of duplicates

Chemical compositions of biomass after NaOH pretreatment with WB heating

The chemical compositions of the four feedstocks after NaOH pretreatment with traditional WB heating are also shown in Table 2 for an easy comparison. Results showed that softwood (loblolly pine) had the least change (suggesting no significant carbohydrate hydrolysis), especially in terms of lignin losses, followed by hardwood (sweetgum), while the other two types of LCB (corn stover and switchgrass) experienced more "damage". Alvira et al. [10] reported that alkaline pretreatment is more effective on biomass with lignin content less than 26 %. Since the lignin content in untreated sweetgum is about 25.8 %, the glucan yield after enzyme hydrolysis was only around 20 %. This was in accordance with what we found in our study.

It is reported that NaOH is capable of removing lignin from lignocellulose of agricultural residues, herbaceous biomass, and hardwood, but not much from softwood [11]. The main mechanism of NaOH pretreatment on lignocellulosic biomass is to increase the porosity of biomass by means of delignification through breaking the ester bonds cross-linking lignin and xylan, and being accompanied with xylan solubilization. Among the three major components in LCB, hemicellulose was the most sensitive fraction to changes in pretreatment with its branched and somewhat irregular structure, while xylan was the most affected part among hemicellulose. In our experiment, lignin was partially solubilized, and degradation of the hemicellulose fraction occurred on switchgrass (herbaceous biomass) and corn stover (agricultural residues). It was in accordance with the above-mentioned observation that NaOH works better in removing lignin from agricultural residues, herbaceous biomass and hardwood than from softwood.

Lignin structures vary with different sources of lignocellulosic biomass, which means that NaOH pretreatment may work more efficiently on some sources of biomass containing a higher proportion of syringyl units (syringyl to guaiacyl) than others. Compared with softwood, the syringyl units in hardwood lignin are ~7–40 times higher, which makes hardwood more susceptible to alkaline pretreatment than softwood. Moreover, rice straw bagasse and some grasses have high levels of syringyl units (up to 65 %) in the lignin fraction, which resulted significant lignin removal following NaOH pretreatment [12]. It explains why the NaOH is less capable of removing lignin from softwood. The relationship between syringyl unit and lignin removal further confirms the order of extent of "damage" in our study: corn stover, switchgrass, sweetgum and loblolly pine.

Chemical compositions of biomass after NaOH pretreatment with RF heating

The chemical compositions of the four biomass feedstocks after NaOH pretreatment with RF heating are also shown in Table 2, which indicated that agricultural residues and dedicated energy plants were more easily to be damaged by alkaline, followed by hardwood samples, while softwood had the least changes. Compared with WB heating-assisted NaOH pretreatment, RF heating had the same order on the extent of "damage" among four types of biomass. According to Modenbach et al. [11], the final biomass composition was dependent on the type of substrates. In our study, the results indicated that the intrinsic properties of the biomass played a vital role in pretreatment process.

Comparisons of chemical compositions between RF and WB heating

The order of the effectiveness of pretreatment assisted by RF heating results (Sect. "Chemical compositions of biomass after NaOH pretreatment with RF heating") was exactly the same as that of WB heating in Sect. "Chemical compositions of biomass after NaOH pretreatment with WB heating". However, RF heating showed advantage on delignification and xylan solubilization on almost every type of feedstock with remarkable difference on switchgrass and sweetgum versus WB heating. Similar results have been reported previously [6]. They used RF-based dielectric heating in the NaOH pretreatment of switchgrass to enhance its enzymatic digestibility. Results showed a higher xylose yield than the conventional heating method. Moreover, RF heating used in this study utilized a temperature at less than 100 °C in an open container which saved a lot of energy and provided a safer working circumstance compared to high-temperature pretreatment methods.

It appears that a major factor in the difference of "damage" among the pretreated biomass materials was the heating mechanism. RF heating has been considered as an alternative to conventional (convection and conduction) heating method, such as hot air and infrared heating. Compared with conventional heating, in which heat is transferred from the heating medium to the product, RF generates a volumetric heat inside object as a result of the interaction between the RF waves and the ions or molecules of the object, and heat flows from inside to outside [13]. Both of RF and microwave heating methods, as dielectric heating techniques, produce rapid heat generation through direct interaction between an electromagnetic field and an object. If lignocellulose biomass is treated through dielectric heating, the more polar part would absorb more energy, and thus, a "hot pot" would be generated within nonhomogeneous materials [5]. A study by Hu and Wen [14] indicated that this special heating property results in an "explosion" effect among the particles and enhances the disruption of the recalcitrant structures of LCB. At the same time, the electromagnetic field created in the dielectric field could generate athermal effects, which can also accelerate the destruction of the crystallinity structure. Thus, mechanism of heating method mentioned above explained why pretreatment assisted by RF worked better on biomass than WB.

Enzyme hydrolysis of biomass after pretreatment

Besides direct (first level) parameters (lignin, glucan and xylan) to determine an extent of "damage" of biomass during pretreatment, another indirect (second level) important parameter to judge an extent of "damage" is glucan yield produced by enzyme hydrolysis.

Enzyme hydrolysis of biomass after NaOH pretreatment with WB heating

Enzymatic digestibility of all the four different LCB pretreated by NaOH and WB was examined, and results are shown in Fig. 3. The results showed that the different biomass had different enzymatic digestibility using the same pretreatment method. Among the four feedstocks, corn stover as a type of agricultural residue, had the highest enzymatic digestibility as the glucan yield could reach up to 89 %, followed by the herbaceous biomass switchgrass with a 51.8 % glucan yield, and then about 20 % for hardwood sweetgum, while softwood loblolly pine obtained less than 10 % of glucan yield. Zheng et al. [15] reported that structural and compositional features of lignocellulosic biomass form strong barriers to the biodegradation and the variability of these characteristics explains

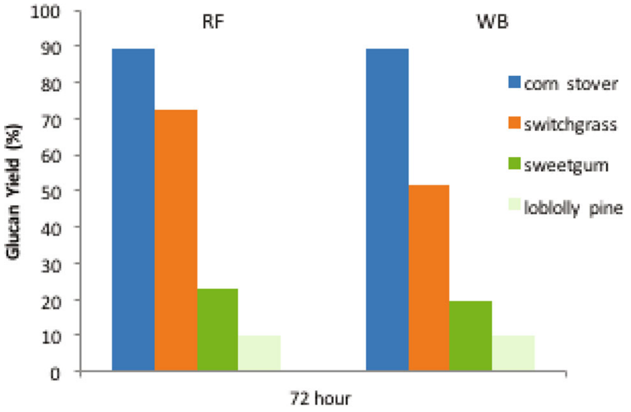


Fig. 3 Effect of RF and WB heating pretreatment on enzymatic digestibility at 72 hour of sodium hydroxide-pretreated corn stover, switchgrass, sweetgum and loblolly pine

the change of enzymatic digestibility among different sources of biomass. In our study, the glucan yield by enzyme hydrolysis of different biomass feedstocks was different even though the pretreatment was exactly the same (either NaOH with WB or NaOH with RF). It indicated that the enzymatic digestibility of biomass is substrate specific.

Sun and Cheng [1] reported that lignin interferes with hydrolysis by blocking access of cellulases to cellulose and by irreversibly binding hydrolytic enzymes. This explains the descending order of the glucan yield obtained from corn stove, switchgrass and sweetgum to loblolly pine, because the lignin content was exactly in the reversed order, from low to high, in these four untreated biomass, shown in Table 1.

Enzyme hydrolysis of biomass after NaOH pretreatment with RF heating

The results of enzymatic digestibility of all the four different LCB pretreated by NaOH and RF heating are shown in Fig. 4. Among the four feedstocks, corn stover showed the highest enzymatic digestibility while a glucan yield could reach 89 %, followed by switchgrass with a 72 % glucan yield, and then about 20 % for hardwood sample sweetgum, while softwood loblolly pine showed only 10 % of glucan yield. The order of glucan yield here was exactly the same as that of the four biomass pretreated in Sect. “Enzyme hydrolysis of biomass after NaOH pretreatment with WB heating”, no matter what kind of heating method was used. This happened to be the same as the results of chemical compositions in part 3.2, which provides additional evidence of the intrinsic properties of substrates.

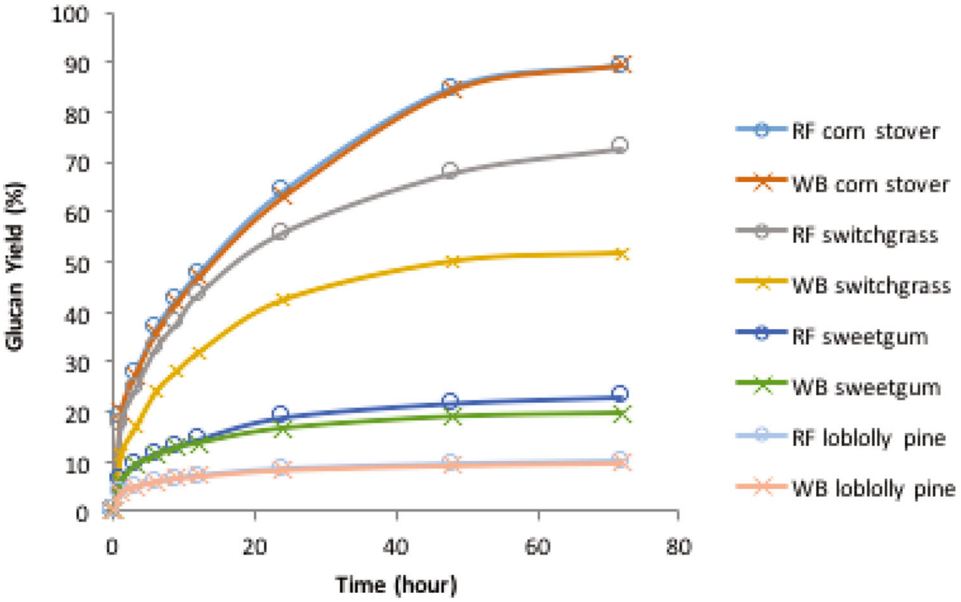
Comparisons of enzyme hydrolysis between RF and WB heating

The order of the effectiveness of pretreatment assisted by RF heating results was the same as that of in Sect. “Enzyme hydrolysis of biomass after NaOH pretreatment with WB heating” using WB heating. However, RF showed superiority on the final glucan yield after enzyme hydrolysis on almost every type of feedstock with greatest difference on switchgrass (herbaceous grass) and sweetgum (hardwood).

During this research, both of the RF heating and conventional heating methods experienced a similar glucan yield of 89 % from corn stover after enzyme hydrolysis shown in Fig. 4. Chen et al. [16] investigated the effect of pretreatment parameters on enzymatic hydrolysis of corn stover and concluded that the NaOH loading based on total solids (g NaOH/g biomass) is the most dominant variable for enzymatic digestibility since glucan conversion during hydrolysis was positively correlated with NaOH loading. Here in our study, the NaOH loading was 0.2 g NaOH/g biomass for both RF-assisted heating and traditional heating methods. Corn stover is easy to be pretreated due to its structure and compositions. We believed that at this loading of NaOH, almost the largest part of the lignin could be removed at the temperature of 90 °C no matter what heating method was adopted, which was in accordance with Chen’s conclusion. Therefore, at this loading of NaOH pretreated on corn stover, both of these heating methods are sufficient to breakdown the substrates.

Figure 4 shows that in the hydrolysis stage, NaOH-pretreated switchgrass with RF heating released 72.6 % glucan, about 20 % higher than that released from

Fig. 4 Effect of RF and WB heating on enzymatic hydrolysis sugar yield on corn stover, switchgrass, sweetgum, and loblolly pine



conventionally heated switchgrass (51.8 %). According to Hu et al. [6], there was an improvement on alkaline pretreatment of switchgrass with RF heating method compared to traditional heating, which was in accordance with our results. Moreover, Hu et al. and co-workers studied microwave-assisted alkaline pretreatment on switchgrass and found that microwave heating method resulted in higher sugar yields than conventional heating. They pretreated the switchgrass by microwave or traditional heating after soaking samples in different concentrations of NaOH solutions. With alkaline loading of 0.1 g/g biomass, they obtained the highest yield of 90 % of maximum potential sugars. According to all above studies, we concluded that dielectric-assisted (both microwave and RF) heating on alkaline pretreatment is an efficient method to improve the enzymatic digestibility of switchgrass. Since the only difference between these two pretreatment methods was the heating method, RF versus conventional heating, we believe that there was a unique "strength" in the RF heating techniques compared to normal superficial heat transfer. Dielectric heating methods transform electromagnetic energy into heat between RF electromagnetic field and the object being heated. When switchgrass was being heated through RF, the more polar parts would absorb more energy and create a hot spot. This special heating resulted in an enhanced disruption of the recalcitrant structures of switchgrass, as well as accelerated in the destruction of the crystallinity structure. Moreover, there is an increasing attention paid to ionic liquid among the techniques of pretreatment of LCB. Recent researchers proposed that the ionic liquid such as [C$_4$mim] Cl could effectively break the extensive network of intra- and intermolecular hydrogen bonds in cellulose, thus allowing cellulose dissolution in the ionic liquid [17]. Pinkert et al. [18] suggested that cations in ionic liquids also play a role in solvation of cellulose. Knowing about these technologies and mechanism of ionic liquid pretreatment of LCB, we believe that there may be similarities between RF heating technique and ionic liquids reactions, but the reactions of ionic liquid were chemically based while the RF utilized the physical "ionized" electromagnetic field. However, more researches about the mechanism of RF heating should be conducted to explain this phenomenon.

For the sweetgum, the glucan yield after enzymatic hydrolysis through RF heating pretreatment was 21.7 % while the glucan yield was 19.6 % using traditional heating method, indicating that NaOH pretreatment assisted by RF heating was more effective than that of WB heating. The same argument on switchgrass could be applied here for sweetgum.

The enzymatic digestibility of the loblolly pine under different heating conditions is summarized in Fig. 4, which showed no remarkable difference between RF and WB. In Sect. "Characteristics of biomass after pretreatment", the major reasons why loblolly pine was very difficult to be pretreated using NaOH assisted by either RF or WB heating were explained, although RF heating has an advantage on switchgrass and sweetgum compared with WB heating. In addition, loblolly pine has longer fibers than hardwood. Because of the long fiber, they have a compact structure that strongly resists on the pretreatment and biodegradation. Zheng et al. [15] reported that alkaline pretreatment was more effective on hardwoods, herbaceous crops, and agricultural residues at the same pretreatment conditions because of generally higher lignin content of the softwood. Therefore, we believe that different types of LCB should be exposed to different pretreatment techniques to enhance their own chemistry and enzyme accessibility and digestibility for the subsequent processing. The enzymatic digestibility of biomass is both substrate and pretreatment method specific.

SEM analysis on untreated and pretreated biomass

Since there was a portion of xylan and lignin removed by alkaline pretreatment, it was important to examine the physical changes in the biomass [19]. For this purpose, we conducted SEM pictures on untreated and pretreated biomass samples. The surface morphology and microstructure of all four feedstocks before and after pretreatment were studied and are shown in Fig. 5. To obtain more information about microstructure of these samples, the SEM micrographs were obtained at a magnification of 1000×. For the untreated samples, the complete and compact lignocellulosic structure showed rigid and highly ordered fiber cells (Fig. 5a–d-1). After undergoing the pretreatment, a certain proportion of hemicelluloses and lignin has been removed, resulting in disruption of the biomass network structure.

For the corn stover pretreated with RF and WB heating, both of the structures have been damaged. The microfibrils were separated from the original connected structure (shown in untreated samples) and fully exposed to the air, even some fragments had flaked off from the biomass surface, shown in Fig. 5a-2, a-3. Moreover, similar results have reported previously that there were significant morphological changes on corn stover by aqueous ammonia [20]. The differences shown by these SEM images were in accordance with chemical analysis after enzyme hydrolysis in Sect. "Enzyme hydrolysis of biomass after pretreatment" previously.

For the WB-pretreated switchgrass, the structure was damaged to some extent and some cracks were seen on the surface (Fig. 5b-3). However, the RF-pretreated switchgrass was affected in a significant way. As a result, much more debris was obtained and the disruption of the lignocellulosic structure became more pronounced (Fig. 5b-2). Results turned out the same as previous enzyme hydrolysis.

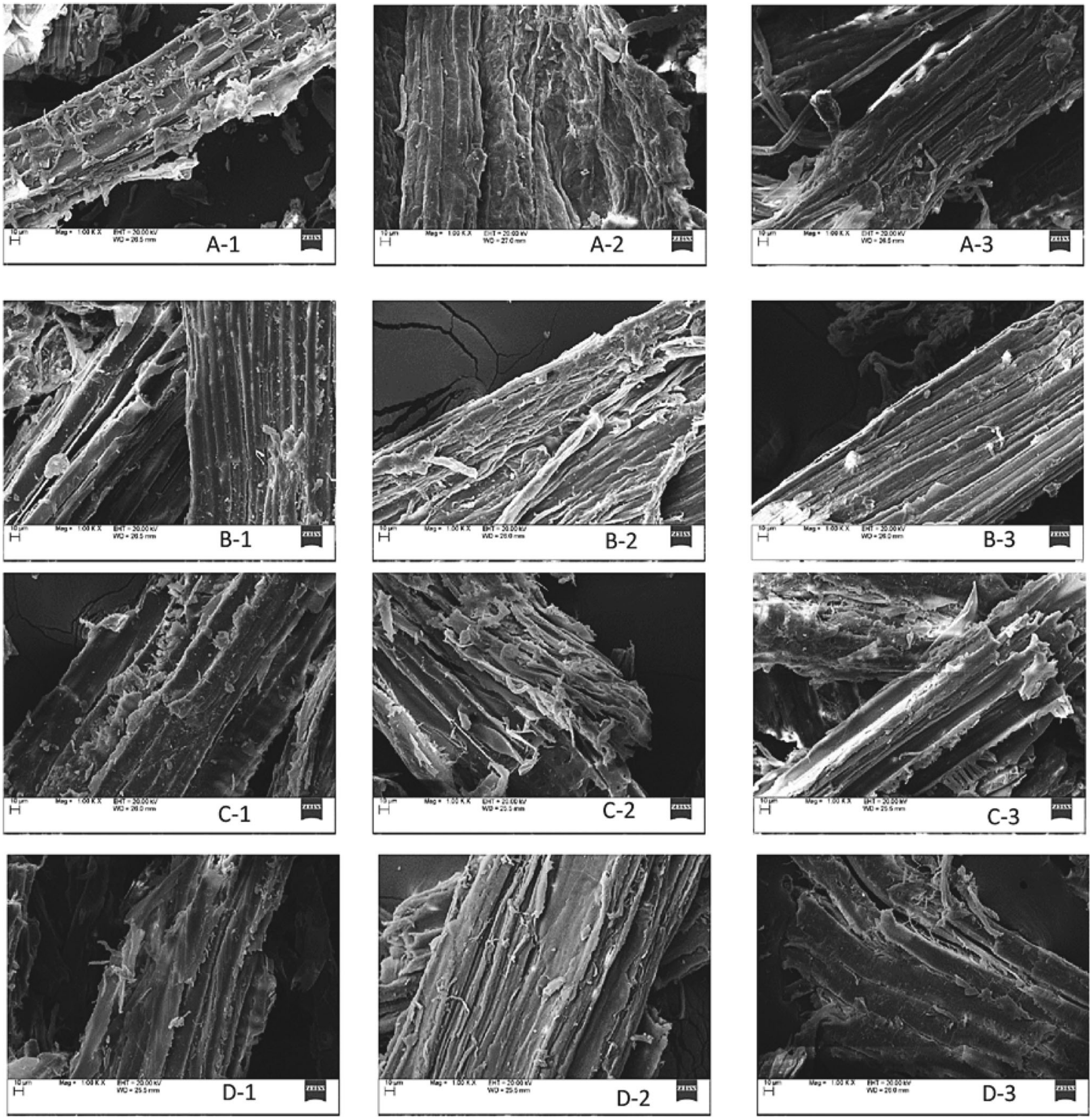


Fig. 5 Scanning electron micrographs of untreated, RF and WB heating biomass samples. A B C D stands for corn stover, switchgrass, sweetgum and loblolly pine. 1, 2, and 3 means untreated, RF and WB heating pretreatment

For the sweetgum as a kind of hardwood, results turned out the same as the chemical analysis and similar with the switchgrass. Some morphological changes occurred after pretreatment. The untreated sample exhibited highly ordered structure, while both pretreatment methods reduced fiber length and disrupted the structure.

For the loblolly pine samples, both of the pretreated samples showed limited changes similar to the results of enzyme hydrolysis with light “damage”. The order fibrils could still be seen (Fig. 5d-2 and 3). However, we found that pretreated biomass appeared to be softer to touch than the untreated ones through hand touch of the material.

FTIR analysis on untreated and pretreated biomass

Infrared spectroscopy is frequently used to investigate structure of materials and the chemical changes in lignocellulosic materials [21]. FTIR spectra for untreated and

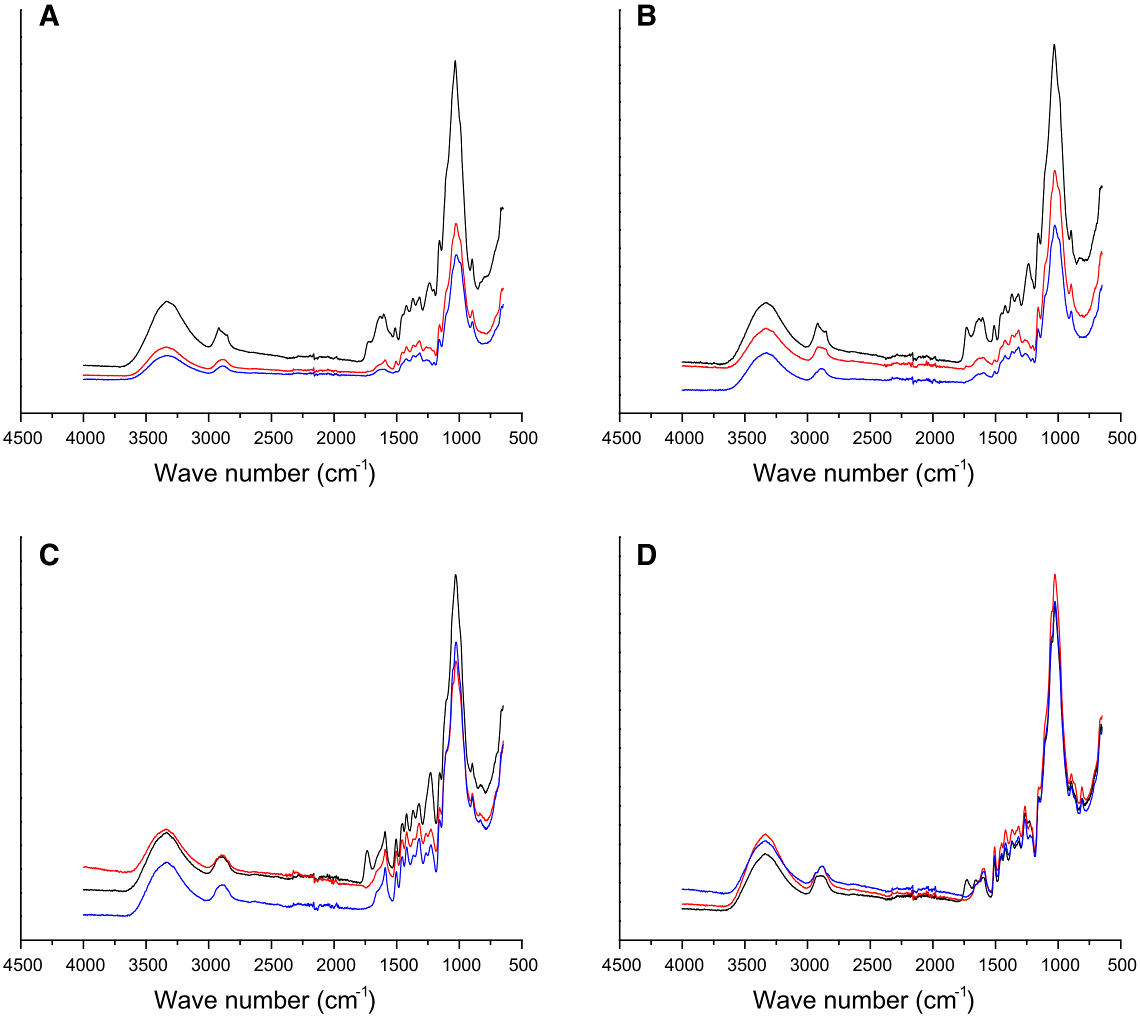


Fig. 6 FTIR spectra of various biomass samples. **a**, **b**, **c**, and **d** stand for corn stover, switchgrass, sweetgum and loblolly pine. *Black*, *blue* and *red lines* represent untreated, RF and WB heating treated methods (color figure online)

pretreated biomass samples are presented in Fig. 6. For the FTIR spectrum, the vibrational frequencies of different peaks assign different functional group in biomass. The peaks around 3348 cm^{-1}, and 2900 cm^{-1} are attributed to OH stretching and C-H stretching, respectively [22, 23]. The reduction in the peaks around 1745 cm^{-1} is attributed to hemicellulose acetyl and uronic ester groups or linkages in lignin and/or ester hemicellulose ferulic and p-coumaric acid carboxylic groups [3]. There is also a change in the intensity of the peak around 1610 cm^{-1}, which is characteristic of amide linkages [22]. The peaks around 1595, 1508 and 1458 cm^{-1} are from aromatic skeletal vibrations in lignin [23]. Peaks at 1428 and 1378 cm^{-1} are the bands of cellulose and hemicellulose, respectively. Other important peaks represent different meanings shown in Table 3.

The effect of different pretreatment processes on four feedstocks was determined in terms of relative change in absorbance at specific band positions. The relative changes were calculated by the following equation:

$$\%\ \text{Relative change} = 100 \times \frac{\text{Intensity of untreated biomsss} - \text{Intensity of pretreated biomass}}{\text{Intensity of untreated biomass}}$$

Table 3 shows the relative percentage change in intensities of various bands between untreated biomass and differently pretreated biomass. Values of relative change for most of the absorption bands were positive for almost all situations, which demonstrated that there was efficient removal of the biomass components to different extent in various pretreatments. According to Singh [24], the positive changes indicated a reduction of the particular component attributed to that band. For the corn stover in Table 3, both of the RF and the WB heating-assisted alkaline pretreatment produced the greatest degree of change at almost every band positions

Table 3 Characterization of pretreated biomass by FTIR spectroscopy in terms of relative change in intensities

Band position (cm^{-1})	Assignment[b]	Corn stover		Switchgrass		Sweetgum		Loblolly pine	
		RF	WB	RF	WB	RF	WB	RF	WB
3348	O–H stretching (indicates rupture of cellulose hydrogen bonds)	63.6	53.5	54.6	28.1	36.1	−5.3	−15.4	−23.0
2900	C–H stretching (related to rupture of methyl/methylene group of cellulose)	63.7	51.6	61.5	29.1	47.0	−3.0	−13.2	−13.8
1745	Carbonyl bonds (associated with lignin side chain removal)	71.8	60.9	78.0	51.9	86.7	54.1	20.9	26.6
1720	Carboxylic acids or ester groups	75.6	66.7	79.2	56.5	84.7	47.9	25.8	31.0
1595	Aromatic ring stretch (associated to lignin removal)	75.8	62.6	70.3	49.9	44.0	22.2	−9.0	−14.2
1508	C = C stretching of aromatic ring in lignin	74.3	57.7	65.9	49.6	46.6	17.9	−6.9	−18.2
1458	C–H bending of methyl and methylene groups	67.8	51.9	59.6	41.2	35.9	17.2	−3.2	−15.0
1428	Band of cellulose	65.9	52.3	56.1	37.9	36.3	20.1	−4.2	−17.7
1378	Band of hemicellulose	66.1	54.7	58.2	41.6	43.5	26.7	1.0	−12.4
1260	Ester absorbance (related to removal of uronic acid)	72.0	58.5	64.1	52.5	47.7	31.3	5.4	−4.2
1245	C–O adsorption (resulting from acetyl groups cleavage)	74.6	62.3	68.8	58.0	53.0	39.4	12.6	3.9
1238	Hemicellulose–lignin linkage	75.8	62.8	70.5	60.1	52.3	40.1	13.8	5.5
1059	C–O stretch	61.5	51.6	52.2	36.6	21.0	25.4	−2.5	−10.9
1098	Heavy atom (CC and CO) stretching	66.0	55.2	56.3	42.4	23.1	23.9	−2.0	−11.0
900	Remove of amorphous cellulose	49.5	40.8	49.0	33.0	22.4	19.8	2.9	−4.8
1098/900[a]	Amorphous to crystalline cellulose ratio	1.2	1.3	1.2	1.2	1.4	1.4	1.3	1.4

% Relative change = 100 × (intensity of untreated biomass − intensity of pretreated biomass)/(intensity of untreated biomass); where positive number indicates reduction

[a] The ratio of the intensity at two band positions

[b] *Source* [11]

presented, and these two methods showed no big difference. This indicated that they successfully disrupted the corn stover structure, which was in agreement with the chemical analysis after enzymatic hydrolysis in Sect. "Chemical compositions of biomass after NaOH pretreatment with RF heating". It also conformed to what Kumar [25] did using lime to pretreat corn stover. However, for the switchgrass, RF heating-assisted alkaline pretreatment showed better results for disrupting the methyl and methylene portions of cellulose (at 2,900 cm^{-1}), as well as more cleavage of lignin side chains (1745 cm^{-1}) in comparison with WB heating-assisted pretreatment. This phenomenon was also in accordance with the results from chemical analysis using HPLC in this study. As for the sweetgum, the RF heating had a better performance in disrupting the hydrogen bonds, methyl and methylene portions of cellulose, as well as linkages in lignin, compared to WB heating. Things happened to be the same as in chemical hydrolysis. As for the loblolly pine, some of the relative change values were negative, which indicated that these two pretreatment methods did not efficiently remove biomass components. The loblolly pine was more recalcitrant than other types of LCB [26]. FTIR showed agreement with results from chemical analysis and SEM, and provided rapid indications of the effect of pretreatment method through spectrum chart. Moreover, it is economical and more convenient to carry out.

Different GC–MS results of pretreated switchgrass

Compounds were detected differently for the alkaline-pretreated switchgrass heated by radio frequency and traditional water bath. There were several compounds including hydroxyl-acetaldehyde, propanoic acid, 2-methoxy-4-vinyl phenol, 2-methoxy phenol and 1,2-benzenediol (catechols) found in switchgrass with radio frequency heating, while no detection in traditional water bath heating. Mullen and Boateng [27] conducted research on the chemical compositions of fast pyrolysis bio-oils from switchgrass and identified a total of 62 chemical compounds in the liquids including all of these five compounds detected in our experiment. Moreover, Wild et al. [28] and Fivga [29] presented a more detailed overview of important value-added chemicals that can be obtained from each of the main biomass fractions via pyrolysis. They found that acetic acid could be generated through hemicellulose degradation at the temperature range of 150–300 °C, and hydroxyl-acetaldehyde could be created through cellulose degradation at 200–400 °C, while 2-methoxy phenols and catechols could be formed through lignin degradation around 150–600 °C. Tumuluru et al. [30] and Fang [31] reported that lignin pyrolysis products include liquid (methanol, acetone, acetaldehyde, phenol, guaiacol, eugenol and other multi-substituted phenols, etc.), solid residues, gas and solid residues. Fang demonstrated that grass lignin produced p-vinylphenol as its major pyrolysis compound. Klinke [32] utilized alkaline wet oxidation pretreatment on wheat straw and found the main phenol monomers were 2-methoxy-4-vinyl phenol. However, we found the five compounds in pretreated switchgrass assisted by radio frequency heating. Therefore, we could make a conclusion that radio frequency heating had a similar effect on pretreated switchgrass as that of pyrolysis and wet oxidation at high temperature, which we could further say our conjectures about selective overheating and an explosion inside the heated object happened during radio frequency heating. RF heating might have equivalent effects on switchgrass similar to effects of high temperature and high pressure in pyrolysis process.

Conjectures on the better performance of radio frequency

For the switchgrass and sweetgum, the radio frequency worked better compared to traditional heating method. Up to now, there was no research published about why this phenomenon happened. However, research about the mechanism of microwave heating on biomass pretreatment, another kind of dielectric heating, was studied and some possible reasons were stated. Here are some of our assumptions based on the mechanism of dielectric heating that made RF work better to conventional heating. One is the selectively overheating of ion and or polar liquids. Convection/conduction heating is based on superficial heat transfer, while dielectric heating utilizes the ability of some compounds to transform electromagnetic energy into heat through a direct interaction between RF electromagnetic field and the object being heated. Energy transmission is produced by dielectric losses, and the magnitude of heating depends on the dielectric properties of the molecules [33]. These characteristics mean that absorption of the radiation and heating may be performed selectively. Recently, ionic liquids have demonstrated great promise as efficient solvents for biomass dissolution with easy recovery of cellulose upon anti-solvent addition. Ionic liquids have been shown to be very effective in solubilizing crystalline cellulose and enhancing the rates of subsequent saccharification [24]. For radio frequency heating, it may have some similar reaction mechanism with ionic liquid because of selective heating of ion liquids, which needs further exploration before implementation. Moreover, Zhang and Zhao [34] reported a variant of pretreatment methods with ionic liquids by the microwave-assisted process. During the process, microwave irradiation at an appropriate power significantly reduced the reaction time and showed a better solubilization of the biomass [35]. We hypothesized that there may be “explosion” effect among the particles, and improves the disruption of the recalcitrant structures of lignocellulosic biomass. The other reason could be non-thermal effect caused by the radio frequency heating. The agitation and mobility of molecular probably reduce the activation energy among the reaction [33]. It is reported that during the thermal decomposition of sodium bicarbonate, the activation energy of the reaction is reduced by microwave radiation [36]. Even though the mechanism is not well understood, the dielectric heating induces rapid rotation of the polarized dipoles and ionized ions in the molecules, which generates heat due to the friction and increases the probability of contact between molecules and atoms simultaneously, thus enhancing the reaction rate and reducing the activation energy. However, RF heating did not have remarkable advantage over WB heating on loblolly pine and corn stover. Because loblloy pine contains very low proportion of syringyl units, even alkaline pretreatment assisted with RF heating was not able to destroy much resistance and recalcitrance of its structure. On the contrary, since corn stover has very high proportion of syringyl units, alkaline pretreatment in this study was sufficient enough to breakdown the structure no matter assisted by either RF or WB heating. This suggests that the mechanism of heating-assisted alkaline pretreatment is syringyl unit dependent. In light of the differences in the physicochemical properties of LCB after radio frequency heating and traditional water bath heating, further research is needed to better understand the influence of biomass structure and composition during pretreatment process.

Conclusions

RF- and WB-assisted alkaline pretreatments were compared on four different feedstocks. For corn stover, both of the RF and WB heating received similar level of disruption. For switchgrass and sweetgum, the RF heating method showed advantage over WB heating on both chemical compositions and glucan yields after enzyme hydrolysis. However, loblolly pine was not disrupted to a large extent using NaOH assisted by either RF or WB heating. Therefore, the chemical composition and enzymatic digestibility of biomass is both substrate and pretreatment method specific. Moreover, several compounds found in RF-heated switchgrass were normally detected in pyrolysis bio-liquid.

Acknowledgments

This study was supported by the Chinese government scholarship offered by the China Scholarship Council. The authors would like to thank Auburn University Center for Bioenergy and Bioproducts for preparing biomass.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

References

1. Sun Y, Cheng J (2002) Hydrolysis of lignocellulosic materials for ethanol production: a review. Bioresour Technol 83:1–11
2. Wyman CE, Dale BE, Elander RT, Holtzapple M, Ladisch MR, Lee YY (2005) Coordinated development of leading biomass pretreatment technologies. Bioresour Technol 96:1959–1966. doi:10.1016/j.biortech.2005.01.010
3. Kumar P, Barrett DM, Delwiche MJ, Stroeve P (2009) Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. Ind Eng Chem Res 48:3713–3729. doi:10.1021/ie801542g
4. Zhu JY, Pan X, Zalesny RS (2010) Pretreatment of woody biomass for biofuel production: energy efficiency, technologies, and recalcitrance. Appl Microbiol Biotechnol 87:847–857. doi:10.1007/s00253-010-2654-8
5. Liu S, McDonald T, Wang Y (2010) Producing biodiesel from high free fatty acids waste cooking oil assisted by radio frequency heating. Fuel 89:2735–2740. doi:10.1016/j.fuel.2010.03.011
6. Hu Z, Wang Y, Wen Z (2008) Alkali (NaOH) pretreatment of switchgrass by radio frequency-based dielectric heating. Appl Biochem Biotechnol 148:71–81. doi:10.1007/s12010-007-8083-1
7. Wang Y, Wig TD, Tang J, Hallberg LM (2003) Dielectric properties of foods relevant to RF and microwave pasteurization and sterilization. J Food Eng 57:257–268. doi:10.1016/S0260-8774(02)00306-0
8. Zhu S, Wu Y, Yu Z, Chen Q, Wu G, Yu F et al (2006) Microwave-assisted alkali pre-treatment of wheat straw and its enzymatic hydrolysis. Biosyst Eng 94:437–442. doi:10.1016/j.biosystemseng.2006.04.002
9. Iroba KL, Tabil LG, Dumonceaux T, Baik OD (2013) Effect of alkaline pretreatment on chemical composition of lignocellulosic biomass using radio frequency heating. Biosyst Eng 116:385–398. doi:10.1016/j.biosystemseng.2013.09.004
10. Alvira P, Tomás-Pejó E, Ballesteros M, Negro MJ (2010) Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: a review. Bioresour Technol 101:4851–4861. doi:10.1016/j.biortech.2009.11.093
11. Modenbach A (2013) Sodium hydroxide pretreatment of corn stover and subsequent enzymatic hydrolysis: An investigation of yields, kinetic modeling and glucose recovery. Dissertation, University of Kentucky
12. Cabrera E, Muñoz MJ, Martín R, Caro I, Curbelo C, Díaz AB (2014) Alkaline and alkaline peroxide pretreatments at mild temperature to enhance enzymatic hydrolysis of rice hulls and straw. Bioresour Technol 167:1–7. doi:10.1016/j.biortech.2014.05.103
13. Marra F, Lyng J, Romano V, McKenna B (2007) Radio-frequency heating of foodstuff: solution and validation of a mathematical model. J Food Eng 79:998–1006. doi:10.1016/j.jfoodeng.2006.03.031
14. Hu Z, Wen Z (2008) Enhancing enzymatic digestibility of switchgrass by microwave-assisted alkali pretreatment. Biochem Eng J 38:369–378. doi:10.1016/j.bej.2007.08.001
15. Zheng Y, Pan Z, Zhang R (2009) Overview of biomass pretreatment for cellulosic ethanol production. Int J Agric Biol Eng 2:51–68. doi:10.3965/j.issn.1934-6344.2009.03.051-068
16. Chen Y (2013) Stevens Ma, Zhu Y, Holmes J, Xu H. Understanding of alkaline pretreatment parameters for corn stover enzymatic saccharification. Biotechnol Biofuels 6:8. doi:10.1186/1754-6834-6-8
17. Cheng G, Varanasi P, Li C, Liu H, Melnichenko YB, Simmons BA et al (2011) Transition of cellulose crystalline structure and surface morphology of biomass as a function of ionic liquid pretreatment and its relation to enzymatic hydrolysis. Biomacromolecules 12:933–41. doi:10.1021/bm101240z
18. Pinkert A, Marsh KN, Pang S, Staiger MP (2009) Ionic liquids and their interaction with cellulose. Chem Rev 109:6712–6728. doi:10.1021/cr9001947
19. Li Q, Gao Y, Wang H, Li B, Liu C, Yu G et al (2012) Comparison of different alkali-based pretreatments of corn stover for improving enzymatic saccharification. Bioresour Technol 125:193–199. doi:10.1016/j.biortech.2012.08.095
20. Kim TH, Kim JS, Sunwoo C, Lee Y (2003) Pretreatment of corn stover by aqueous ammonia. Bioresour Technol 90:39–47. doi:10.1016/S0960-8524(03)00097-X
21. Via BK, Adhikari S, Taylor S (2013) Bioresource Technology Modeling for proximate analysis and heating value of torrefied biomass with vibration spectroscopy. Bioresour Technol 133:1–8. doi:10.1016/j.biortech.2013.01.108
22. Chundawat SPS, Venkatesh B, Dale BE (2007) Effect of Particle Size Based Separation of Milled Corn Stover on AFEX Pretreatment and Enzymatic Digestibility. Biotechnol Bioeng 96:219–231. doi:10.1002/bit
23. Sun XF, Xu F, Sun RC, Fowler P, Baird MS (2005) Characteristics of degraded cellulose obtained from steam-exploded wheat straw. Carbohydr Res 340:97–106. doi:10.1016/j.carres.2004.10.022
24. Singh S, Simmons BA, Vogel KP (2009) Visualization of biomass solubilization and cellulose regeneration during ionic liquid pretreatment of switchgrass. Biotechnol Bioeng 104:68–75. doi:10.1002/bit.22386
25. Kumar R, Mago G, Balan V, Wyman CE (2009) Physical and chemical characterizations of corn stover and poplar solids resulting from leading pretreatment technologies. Bioresour Technol 100:3948–3962. doi:10.1016/j.biortech.2009.01.075
26. Zhu JY, Pan XJ (2010) Woody biomass pretreatment for cellulosic ethanol production: technology and energy consumption evaluation. Bioresour Technol 101:4992–5002. doi:10.1016/j.biortech.2009.11.007
27. Mullen CA, Boateng AA (2008) Chemical composition of bio-oils produced by fast pyrolysis of two energy crops. Energy and Fuels 22:2104–9. doi:10.1021/ef700776w
28. De Wild P, Reith H, Heeres E (2011) Biomass pyrolysis for chemicals. vol. 2. doi:10.4155/bfs.10.88
29. Fivga A (2012) Comparison of the effect of pre-treatment and catalysts on liquid quality from fast pyrolysis of biomass. Dissertation, Aston University
30. Tumuluru JS, Sokhansanj S, Wright CT, Kremer T (2012) GC Analysis of volatiles and other products from biomass torrefaction process. In: Mohd MA (ed) Advanced gas chromatography–progress in agricultural, biomedical and industrial applications. InTech. doi:10.5772/33488
31. Fang Z, Smith Jr RL, Qi X (2014) Production of biofuels and chemicals with microwave. vol. 3. Springer
32. Klinke HB, Ahring BK, Schmidt AS, Thomsen AB (2002) Characterization of degradation products from alkaline wet

oxidation of wheat straw. Bioresour Technol 82:15–26. doi:10.1016/S0960-8524(01)00152-3

33. De la Hoz A, Díaz-Ortiz A, Moreno A (2005) Microwaves in organic synthesis. Thermal and non-thermal microwave effects. Chem Soc Rev 34:164–178. doi:10.1039/b411438h
34. Zhang Z, Zhao ZK (2009) Solid acid and microwave-assisted hydrolysis of cellulose in ionic liquid. Carbohydr Res 344:2069–2072. doi:10.1016/j.carres.2009.07.011
35. Zhu S, Wu Y, Chen Q, Yu Z, Wang C, Jin S et al (2006) Dissolution of cellulose with ionic liquids and its application: a mini-review. Green Chem 8:325. doi:10.1039/b601395c
36. Shibata C, Kashima T, Ohuchi K (1996) Nonthermal influence of microwave power on chemical reactions. Japanese J Appl Physics, Part 1 Regul Pap Short Notes Rev Pap 35:316–9. doi:10.1143/JJAP.35.316