



Use of metal chlorides during waste wheat straw autohydrolysis to overcome the self-buffering effect

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ABSTRACT

High ash content of waste wheat straw (WWS) is resistant to biorefinery autohydrolysis pretreatment due to its self-buffering effects. In this work, minor addition FeCl₃ and AlCl₃ were applied to overcome the self-buffering effects of WWS by cationic occupation of the negatively charged sites present on particulate ash's surface. The results showed that with the increasing concentrations (0–20 mM) of AlCl₃ and FeCl₃, the enzymatic efficiencies of autohydrolyzed WWS were enhanced from 49.7% to 62.1% and 66.6%, respectively. Acid buffer and cation exchange capacity of pretreated WWS were decreased by adding metal chlorides and the reducing results were mainly attributed to cation exchange. Meanwhile, a maximum monosaccharide production (185.3 mg/g-WWS) was achieved with 62.0 mg/g-WWS xylooligosaccharide by using 20 mM FeCl₃ during WWS autohydrolysis. The results demonstrated that the implications of FeCl₃ and AlCl₃ in WWS autohydrolysis were an effective strategy to enhance autohydrolysis efficiency by overcoming self-buffering effects.

1. Introduction

Development and implementation of low-emission and sustainable energy resources has been urgently demanded in the recent decade (Stern et al., 2016). One promising avenue is ethanol production from lignocellulose, which is recognized as a promising alternative to fossil fuel (Anđelković et al., 2017; Jiang et al., 2017). Agricultural and forestry wastes have been presented as potential raw materials for the production of ethanol due to their (current) waste-like nature and enrichment in structural polysaccharides. Regarding their waste-like nature, agricultural and forestry wastes are conventionally burned as fuel, which is a practice recognized for its contribution to air pollution (Chen and Xie, 2014). Because the cost of heavily-studied lignocellulosic feedstocks for ethanol production (wheat straw, corn stover and wood) is estimated as ~30–90 US\$ per ton, it is important to develop practices which produce ethanol from lower-cost starting materials (Geng et al., 2015; Lee et al., 2009).

Wheat straw is extensively used in the pulping industry, and it is estimated that about 2×10^5 – 4×10^5 tons of waste wheat straw (“WWS”) are annually produced by wheat straw pulp mills in China alone. WWS itself (consisting of wheat ears, leaves, straw scraps and a large amount of ash) is generated during the crude wheat straw screening operation used in the straw pulping industry. Compared with wheat straw, WWS is not utilizable for pulp production and is thus

regarded as waste. However, due to its low value, theoretical availability, and high abundance of polysaccharides (26.5% cellulose and 17.6% xylan), WWS is worthy of investigation as an attractive starting material for the fuel ethanol industry (Huang et al., 2018).

Our previous work on WWS identified that autohydrolysis was an appropriate low-cost and sustainable pretreatment for WWS, but certain concessions had to be made. First, the WWS required a pre-washing operation in order to remove unbound ash. It was found that this ash was causing an unwanted buffering effect on the autohydrolysis liquor, preventing the pH from reaching the values required to facilitate hemicellulose hydrolysis (required for pretreatment). To eliminate the buffering effect of the ash, a 500 times (v/w) water washing step prior to the pretreatment was adopted to remove the unbound ash of WWS effectively, and received significant enhancement of enzymatic hydrolysis efficiency in our previous work (Huang et al., 2016b). However, about 23.0% cellulose and 25.5% hemicellulose were lost after 100 times (v/w) water washing (Huang et al., 2017). Moreover, although water can be recycled, such a huge consumption of water is unpractical for industry. Therefore, it is urgent for researchers to find alternative methods to overcome the self-buffering effect of WWS.

Although it has been reported that implementations of metal chlorides (AlCl₃ and FeCl₃) during autohydrolysis pretreatment successfully facilitate decomposition of biomass which translates into remarkable enhancements to enzymatic hydrolysis efficiency (Kamireddy

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et al., 2013; Liu et al., 2009; Zhang et al., 2018), there have been no studies which apply these inorganic reagents into the system of autohydrolysis pretreatment of high ash WWS. We chose to investigate the effect of these reagents based on a knowledge of soil science, where ions such as Fe^{3+} & Al^{3+} show stronger cation exchange capacity than H^+ (Charlet and Tournassat, 2005). Thusly, if ash-rich WWS is autohydrolyzed in the presence Fe^{3+} or Al^{3+} , it may be possible for these cations to supersede the interactions problematically occurring between the H^+ generated during autohydrolysis and the ash from WWS. For this reason, AlCl_3 and FeCl_3 are hypothesized as being capable of eliminating the buffer system resulting from WWS' ash, resulting in monosaccharide gains during subsequent enzymatic hydrolysis. It is worthy to mention that excessive uses of metal chlorides may increase the cost of equipment maintenance due to the low pH values of the prehydrolysate. The aims of adding metal chlorides in autohydrolysis were to make WWS could be pretreated like prewashed WWS under a weakly acid medium (pH around 4). In that pH range, the corrosions of equipment were accessible. Meanwhile, metal chlorides were frequently used in chemical industry (Guisnet et al., 2011). Therefore, implementations of metal chlorides in WWS autohydrolysis showed potential prospects on enhancing the autohydrolysis efficiency by minimizing the self-buffering effects caused by problematic ash.

In the present paper we focus on the effects of metal chloride (AlCl_3 and FeCl_3) on facilitating autohydrolysis pretreatment efficiency of WWS. The influence of autohydrolysis pretreatment with or without adding metal chloride on the glucan recovery, xylan and lignin removal of the pretreated WWS, and the sequential enzymatic hydrolysis efficiency were studied. The acid buffer capacity changes and cation exchange capacity of pretreated WWS were investigated to show the collapse extent of buffer system and the reducing extents. Cation exchanges during the pretreatment process were estimated by the results obtained by inductively coupled plasma-optical emission spectrometer (ICP-OES).

2. Materials and methods

2.1. Materials and reagents

WWS used in this work was provided by a straw pulp mill (Shandong Province, China). Cellulase (Cellic® CTec2) was kindly provided by Novozymes North America (Franklinton, NC, USA), with filter paper activity of 250.0 FPU/mL and cellulase activity of 2731 U/mL (Ghose, 1987). All other reagents used in this work were analytical grade.

2.2. Autohydrolysis pretreatment

Autohydrolysis pretreatment of WWS was performed in 1.25 L vessels heated by a laboratory oil bath. 50 g (on a dry weight basis) of raw WWS were loaded into reactors and soaked with different concentrations (0–20 mM) of AlCl_3 , FeCl_3 , with a final solid to liquid ratio of 1:10 (w/v). The pre-washed WWS produced by 500 times (v/w) water washing was prepared prior to the pretreatment according to our previous work (Huang et al., 2016b) and serves as a control group to be pretreated. For pretreatment, the reactor temperature was raised from 60 °C to 180 °C at a heating rate of 2 °C/min, and maintained at 180 °C for 40 min (Narron et al., 2017; Wu et al., 2018). After pretreatment, reactors were rapidly cooled via submersion into a cold water bath. Pretreated residues were separated from the liquid fraction (prehydrolysate) using cloth bag filtration. After filtration, the pretreated residues were washed by deionized water until neutral to remove residual metal ions and then stored in sealed plastic bags at 4 °C. Prehydrolysate was analyzed for pH, and concentrations of inhibitors, monosaccharides, and oligosaccharides (mostly xylo-oligosaccharides, “XOS”). The scheme of metal chlorides assisted WWS autohydrolysis were shown in Fig. 1.

2.3. Cation exchange capacity and acid buffering capacity of pretreated WWS

The cation exchange capacity (CEC) of pretreated material was determined through a method modified from the literature (Hendershot and Duquette, 1986). About 1 g of each sample was milled, and then added to a 30 mL BaCl_2 solution to exchange the cations present in ash. Samples were then centrifuged (5000 rpm for 10 min), and the supernatant was discarded. This operation was repeated three times. Next, 50 mL of deionized water was then added to remove Cl^- anions from the solution. This operation was also repeated once more. Resultant samples were then mixed with 25 mL of 0.1 M H_2SO_4 solution to precipitate the adsorbed Ba^{2+} as BaSO_4 . The mixture was then filtered and the residual acid was collected from the filtrate for titration with 0.1 M NaOH solution. The CEC was calculated from the amount of H^+ adsorbed by the sample during the precipitation of BaSO_4 .

The acid buffering capacity (ABC) of pretreated WWS samples were determined using a procedure reported in literature (Lloyd and Wyman, 2005). For each sample, 10 g (on a dry weight basis) pretreated WWS was put into a polytetrafluoroethylene crucible and burned in Muffle furnace at 575 °C for 12 h. After combustion and cooling to room temperature, the remainder ash was dried to constant weight for 4 h at 105 °C. Next, the dried ash was mixed with 50 mL deionized water in a beaker. The ash & water mixture was then titrated with 0.1 M H_2SO_4 solution at a flow rate of 0.05 mL/min using a multi-water quality meter (MM-60R, TOADKK, Tokyo, Japan). The terminal pH value was set at 3.0, and the volume of titration was recorded. ABC was calculated from said results.

2.4. Element analysis

Quantification of metal cations was performed using a procedure modified from the literature (Souza et al., 2018). For quantification in solids samples, solid ash was obtained through incineration of pretreated WWS at 575 °C for 12 h. Next, ~0.01 g ash or 1 mL liquid prehydrolysate liquid (liquid samples) were inserted into a reaction vessel. Following sample addition, 5 mL HNO_3 (65% m m⁻¹) and 1 mL H_2O_2 (30% m m⁻¹) was added into the reaction vessel for analyzing metal cations. However, for detecting Si contents, 2.5 mL HF (48% m m⁻¹), 3 mL HNO_3 (65% m m⁻¹), 1 mL HCl (12 M) and 1.25 g granular boric acid was loaded into the reaction vessel (Bernas, 1968). A closed-vessel microwave system (MARS6, CEM, USA) was then used to acid digest the materials. Digestion was conducted at 165 °C for 5 min, followed by a ramp up to 185 °C for 20 min. After digestion, acid removal was performed at 160 °C for 1 h to evaporate nitric acid and resulting in ~1 mL of residual liquids following digestion. The remaining liquid was then mixed with de-ionized water to the constant volume 30 mL for cation measurement. Analytical blanks (deionized water) were also subjected to the same digestion procedure. After sample preparation, Fe, Al, Ca, Mg, K and Na concentrations were determined by ICP-OES (Optima 8000, Perkin-Elmer).

2.5. Enzymatic hydrolysis

Enzymatic hydrolysis of WWS samples was performed in 0.05 M citrate buffer (pH 4.8) at 50 °C with a substrate loading of 5% (w/v), with the reaction slurry agitated using a thermostat shaker at 150 rpm. The enzyme loading during all hydrolysis experiments was maintained at 25 FPU/g-glucan. Samples were withdrawn during the course of hydrolysis at 2, 4, 6, 12, 24, 48 and 72 h. The obtained enzymatic hydrolysates were centrifuged at 10000 rpm for 10 min to remove residual suspended solids. As compared to autohydrolyzed WWS, no residual metal cations (Al and Fe) were found in the enzymatic hydrolysate of metal chloride pretreated residues. To quantify monosaccharides in enzymatic hydrolysate, the supernatants were diluted and subsequently filtered through a 0.22 μm syringe filter for HPLC (high performance

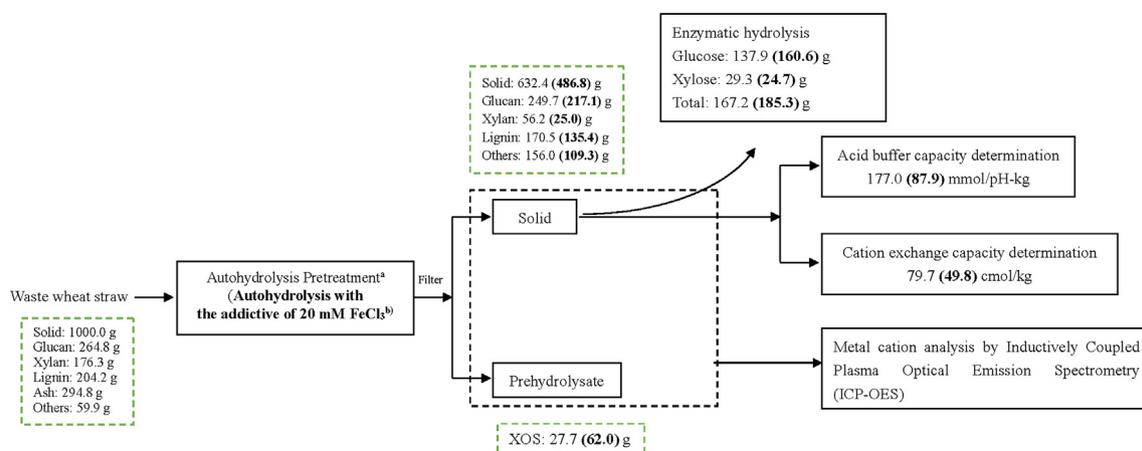


Fig. 1. The scheme and sequence of WWS autohydrolysis with or without the additives of metal chloride. ^aAutohydrolysis without metal chloride. ^bAutohydrolysis with the addition of 20 mM FeCl₃ and the results were showed in brackets.

Table 1

The pH values after pretreatment, fermentation inhibitors and monosaccharides concentrations in the prehydrolyzate at different pretreated conditions.

Substrate	Additives	Concentration (mM)	pH after the pretreatment	Fermentation inhibitors (g/L)				Monosaccharides concentration (g/L)	
				Formic acid	Acetic acid	Furfural	HMF	Glucose	Xylose
Raw WWS	/	/	5.7	0.3	0.8	0.1	0.0	0.0	0.8
	AlCl ₃	1	5.4	0.2	0.7	0.1	0.0	0.0	0.5
	AlCl ₃	5	5.0	0.2	0.7	0.4	0.0	0.0	0.8
	AlCl ₃	10	4.8	0.0	0.8	0.6	0.1	0.4	1.2
	AlCl ₃	20	4.0	0.1	0.5	0.6	0.1	0.4	1.0
	FeCl ₃	1	5.6	0.2	0.8	0.1	0.0	0.2	0.6
	FeCl ₃	5	5.2	0.2	0.9	0.4	0.0	0.3	1.0
	FeCl ₃	10	4.9	0.3	0.8	0.4	0.1	0.2	0.9
	FeCl ₃	10	4.9	0.3	0.8	0.4	0.1	0.2	0.9
	FeCl ₃	20	4.5	0.3	0.9	0.5	0.1	0.5	1.0
Pre-washed WWS	/	/	3.8	0.2	0.7	0.7	0.0	0.2	1.5

liquid chromatography) analysis (HPLC details in Section 2.6).

2.6. Analytical methods and calculations

All experiments were carried out in duplicate and the means of the duplicate analysis was reported in each figure and Table. The moisture content of the material was determined by infrared moisture meter (FD-720), which is produced by KETT. Chemical compositions (including structural polysaccharides, lignin and ash) of all samples were measured using the National Renewable Energy Laboratory (NREL) standard method (Sluiter et al., 2010). To measure oligosaccharides in prehydrolyzate solution, 4 mL prehydrolyzate was hydrolyzed using 4% (w/w) H₂SO₄ at 121 °C for 1 h (Xiao et al., 2013). The XOS concentration was calculated from the increase in xylose concentration after 4% (w/w) H₂SO₄ hydrolysis.

Analysis of monosaccharides and inhibitors was performed using HPLC (Agilent 1260 series, Agilent Technologies, Santa Clara, CA, USA). Mobile phase used was 0.05 M H₂SO₄ at a flow rate of 0.6 mL/min with a refractive index detector. Sample separation took place on a Coregel-87H column (Transgenomic Technologies, New Haven, CT, USA), with the column temperature held at 55 °C.

Recovery yields of carbohydrates, degree of delignification and enzymatic hydrolysis efficiency were calculated according to the following equations:

$$\text{Glucan recovery yield (\%)} = \frac{\text{glucan in pretreated WWS residue (g)}}{\text{glucan in the raw WWS (g)}} \times 100\%$$

$$\text{Xylan removal yield (\%)} = 1 - \frac{\text{xylan in pretreated WWS residue (g)}}{\text{xylan in the raw WWS (g)}} \times 100\%$$

$$\text{Delignification (\%)} = 1 - \frac{\text{lignin in pretreated WWS residue (g)}}{\text{lignin in the raw WWS (g)}} \times 100\%$$

$$\text{Enzymatic efficiency (\%)} = \frac{\text{glucose in enzymatic hydrolyzate (g)}}{\text{initial glucose in substrate (g)}} \times 100\%$$

3. Results and discussion

3.1. Prehydrolyzate composition and pH as a function of pretreatment conditions

pH value is an important parameter related to the efficiency of autohydrolysis pretreatment, where the extent of beneficial biomass degradation is related to the level of acidity that can be generated by degraded biomass components (Pedersen and Meyer, 2010). Prior to discussion, it is first important to establish the labeling scheme used for referencing the different samples and modes of autohydrolysis. Resulting solids from raw WWS with AlCl₃ and FeCl₃ are termed as AlCl₃-WWS and FeCl₃-WWS, respectively. As shown in Table 1, following autohydrolysis, prehydrolysis pH values decreased compared to liquor generated by water-only autohydrolysis. increasing concentrations (0–20 mM) of AlCl₃ and FeCl₃ resulted in pH values after autohydrolysis which decreased from 5.7 to 4.0 and 4.5, respectively. Obviously, a weak acid medium was successfully formed and it could be inferred that the loss of strong buffering capacity of WWS was achieved by adding metal chlorides. Meanwhile, for pre-washed WWS autohydrolysis, the pH values of the prehydrolyzate were 3.8. It can be imagined that while involving metal chlorides in WWS autohydrolysis, raw WWS could be pretreated like prewashed WWS. Moreover, due to the formation of

weak acid medium involved in autohydrolysis, the pehydrolyzate composition were also changed. The concentration of fermentation inhibitors and carbohydrates in the pretreated liquor were also the important evaluation index of successful pretreatment (Jönsson et al., 2013).

As shown in Table 1, the concentrations of formic acid, acetic acid, furfural and HMF were 0.3, 0.8, 0.1 and 0.0 g/L in the prehydrolyzate of autohydrolyzed WWS, respectively. Addition of 20 mM AlCl_3 and 20 mM FeCl_3 led to an increase in furfural generation (from 0.1 to 0.6 and 0.5 g/L) as well as HMF (from 0.0 to 0.1 and 0.1 g/L). This effect can be attributed to the additional acidity delivered by the metal chlorides which facilitated further carbohydrate dehydration (Peng et al., 2010). When increasing concentration (0 ~ 20 mM) of AlCl_3 and FeCl_3 applied to raw WWS autohydrolysis, increasing concentrations of glucose (0–0.5 g/L) and xylose (0.8–1.2 g/L) were quantifiable in prehydrolyzate. From the pH values in the prehydrolyzate, the reason might be attributed to the more acidic condition of metal chloride involved WWS autohydrolysis, even at the presence of high free ash.

Xylo-oligosaccharide (XOS) has been considered as an important valuable by-product in autohydrolyzate (Zhuang et al., 2016). As shown in Fig. 2, with the increasing concentration from 0 to 20 mM FeCl_3 in autohydrolysis, XOS concentrations increased from 2.8 to 6.2 g/L, correspondingly. The results indicated that the addition of FeCl_3 facilitates the production of XOS in WWS autohydrolysis. Huang et al. (2016a) revealed that autohydrolysis could gain 6.3 g XOS (based on 100 g dry solid) from hardwood at 180 °C for 40 min. The results (6.2 g) are quite similar to their work (6.3 g). Therefore, it can be inferred that WWS could gain reasonable XOS production like normal biomass autohydrolysis by adding 20 mM FeCl_3 in WWS autohydrolysis. However, the XOS concentration was decreased from 4.8 to 3.3 g/L with the increasing concentration (1–20 mM) of AlCl_3 . Meanwhile, the xylose and furfural concentrations increased from 0.8 to 1.0 g/L and from 0.4 to 0.6 g/L. This suggests that XOS is more susceptible to hydrolysis into xylose and subsequently furfural in the presence of AlCl_3 and the acidity it delivers (Dong et al., 2009; Mamman et al., 2008). The results showed that compared with AlCl_3 , the additions of FeCl_3 in WWS autohydrolysis are more suitable for receiving XOS productions.

3.2. Effects of adding AlCl_3 and FeCl_3 in autohydrolysis on glucan recovery yield, xylan removal yield and delignification

Autohydrolysis of wheat straw has been shown to remove up to 80%

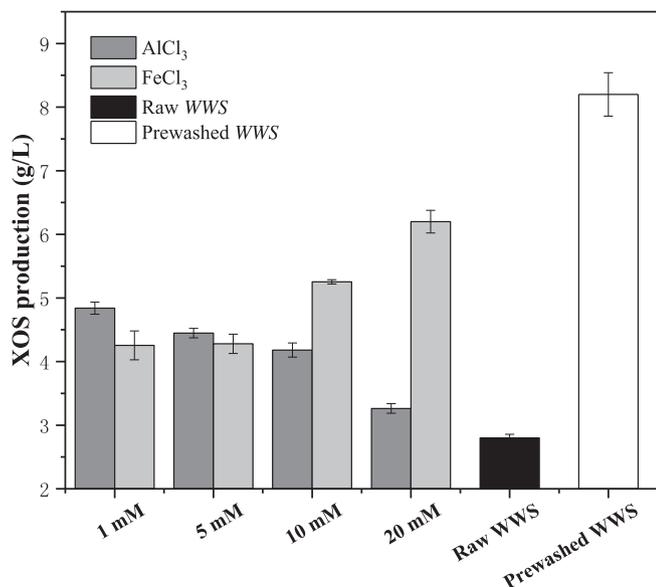


Fig. 2. XOS productions in prehydrolyzate at different pretreated conditions.

of hemicellulose in the step of pretreatment (Pérez et al., 2008). As we have established, xylan removal by autohydrolysis upon raw WWS is hindered by the presence of buffering ash. As shown in Table 2, only 68.1% of xylan was removed at the tested autohydrolysis conditions. Introducing AlCl_3 or FeCl_3 in the autohydrolysis process resulted in an increase in xylan removal. Specifically, xylan removal was 87.6% and 85.8% for autohydrolysis with 20 mM AlCl_3 -WWS and 20 mM FeCl_3 -WWS, respectively. Interestingly, these values were quite close to the xylan removal (88.7%) recorded from autohydrolysis of pre-washed WWS. Thusly, the additions of metal chlorides in WWS autohydrolysis remains potential prospects on replacing prewashed WWS autohydrolysis which also could receive remarkable xylan removal yield in WWS autohydrolysis in this respect.

Delignification of AlCl_3 -WWS and FeCl_3 -WWS increased from 16.5% to 26.8% and 33.7% (respectively) when increasing concentration of metal chlorides from 0 to 20 mM. This observation could be explained by the additional acidity delivered by the reagents, where the higher concentration of H^+ can facilitate additional decomposition of β -O-4 bonds (Yokoyama, 2014). However, the delignification of prewashed WWS was only 26.6%, while the delignification of 20 mM FeCl_3 -WWS was 33.7%. It is also possible that the metal cations become involved in reaction kinetics through lignin-metal complexation (Wang et al., 2016). A recent report demonstrated a similar finding, where addition of AlCl_3 to hydrothermal pretreatment resulted in unique changes to a hardwood lignin's chemical structure (Shen et al., 2016). The residual lignin in pretreated biomass has been considered as a barrier for improving its enzymatic hydrolysis efficiency (Geng et al., 2018). Therefore, it can be imagined that the enzymatic hydrolysis efficiency of pretreated WWS could be enhanced by such remarkable delignification in sequential enzymatic hydrolysis.

3.3. Enzymatic hydrolysis efficiency of pretreated WWS

To evaluate the effects of AlCl_3 and FeCl_3 on WWS sugar generation, pretreated residues were next subjected to enzymatic saccharification. Fig. 3 displays that enzymatic hydrolysis efficiency of AlCl_3 -WWS and FeCl_3 -WWS was found to be greater than that of autohydrolyzed-WWS. For example, the 72 h enzymatic hydrolysis efficiency of 20 mM AlCl_3 -WWS and 20 mM FeCl_3 -WWS was 62.1% and 66.6%, while the autohydrolyzed-WWS was only 49.7%. It has been reported that “blocking effect” of xylan was one of the major barriers which limited the enhancement of enzymatic efficiency (Hu et al., 2011). As evidence of this effect, xylan removal yields of autohydrolyzed WWS, 20 mM AlCl_3 -WWS and 20 mM FeCl_3 -WWS were 68.1%, 87.6% and 85.8%, respectively. Therefore, it can be inferred that the one of the factors for the enhancement of enzymatic efficiency of AlCl_3 -WWS and FeCl_3 -WWS was achieved by removing greater amounts of xylan in the step of pretreatment. In WWS autohydrolysis, the extent of xylan removal was restricted by the ash buffering effects and the buffering effects were effectively minimized by adding metal chlorides. Meanwhile, the 72 h enzymatic efficiency of pre-washed WWS was 59.9%, while the enzymatic efficiency of 20 mM AlCl_3 -WWS and 20 mM FeCl_3 -WWS was 62.1% and 66.6%. Therefore, implications of metal chlorides in WWS autohydrolysis seemed more efficient to gain higher enzymatic efficiency from autohydrolyzed WWS than prewashed WWS. However, it is still worth mentioning that the residual metal cation involved in pretreated solids might also exert negative effects on enzymatic hydrolysis. Liu et al. 2010 reported that iron-lignin complexation exerts inhibitory effects on the enzymatic hydrolysis of autohydrolyzed biomass. However, as shown in Fig. 3, no significant inhibitory effects on the enzymatic hydrolysis of metal chlorides pretreated WWS were found.

3.4. Cation exchange capacity and acid buffer capacity of pretreated WWS

As we have established, the high acid buffer capacity (ABC) and cation exchange capacity (CEC) in raw WWS is attributed to the

Table 2
Effects of metal chloride on the chemical compositions of pretreated WWS.

Substrate	Additives	Concentration (mM)	Glucan recovery yield (%)	Xylan removal yield (%)	Delignification (%)
Raw WWS	/	/	94.3	68.1	16.5
	AlCl ₃	1	93.4	74.7	17.2
	AlCl ₃	5	90.3	78.4	19.6
	AlCl ₃	10	86.1	85.6	20.0
	AlCl ₃	20	83.6	87.6	26.8
	FeCl ₃	1	93.8	75.6	19.5
	FeCl ₃	5	87.5	81.2	23.9
	FeCl ₃	10	84.5	83.5	24.1
	FeCl ₃	20	82.0	85.8	33.7
	Pre-washed WWS	/	/	82.3	88.4

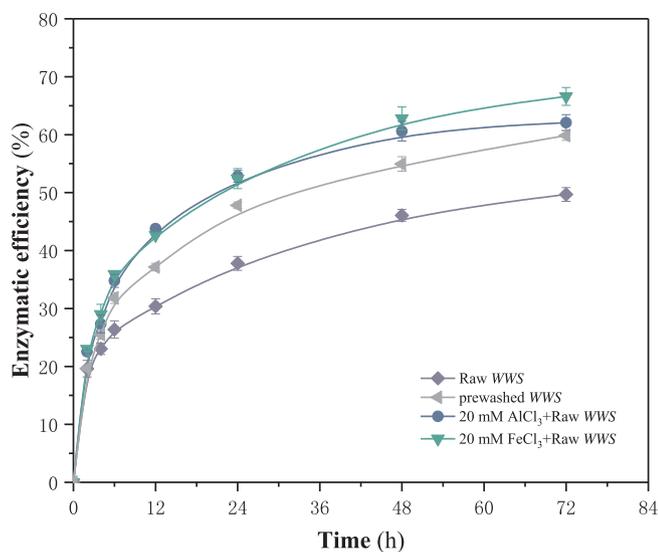


Fig. 3. Enzymatic hydrolysis of pretreated WWS at different pretreated conditions for 72 h.

Table 3
Acid buffer capacity and cation exchange capacity of autohydrolyzed WWS with or without adding metal chlorides.

Substrate	Additives	Concentration (mM)	ABC ^a (mmol/pH·kg)	CEC (cmol/kg)
Raw WWS	/	/	177.0	79.7
	AlCl ₃	1	174.1	59.1
	AlCl ₃	5	161.3	55.5
	AlCl ₃	10	158.2	46.8
	AlCl ₃	20	103.4	45.6
	FeCl ₃	1	144.5	65.9
	FeCl ₃	5	108.2	60.4
	FeCl ₃	10	96.8	57.1
	FeCl ₃	20	87.9	49.8
	Pre-washed WWS	/	/	49.3

^a Acid buffer capacity in the pH range of 6 to 3.

existence of a significant amount of free ash (Huang et al., 2016b). As shown in Table 3, a strong buffering was witnessed for autohydrolyzed WWS, with 177.0 mmol/pH·kg in the pH range 3 to 6 and the CEC value of autohydrolyzed WWS was 79.7 cmol/kg. However, when increasing concentration of AlCl₃ and FeCl₃ from 0 to 20 mM, the CEC of AlCl₃-WWS and FeCl₃-WWS was decreased from 79.7 to 45.6 and 49.8 cmol/kg, respectively. ABC of AlCl₃-WWS and FeCl₃-WWS also decreased from 177.0 to 103.4 and 87.9 mmol/pH·kg, correspondingly. It is conventionally noted that a CEC value of 5 cmol/kg represents the boundary between high and low ABC samples (Takahashi et al., 2001). However, the CEC values of AlCl₃ and FeCl₃ catalyzed WWS were far

higher than 5 cmol/kg. Interestingly, CEC value of pre-washed WWS was only 4.3 cmol/kg. These results are mainly attributed to the effects which AlCl₃ and FeCl₃ have on autohydrolysis, which are quite different from prewashing alone. Most logically, the additional cations delivered by the metal chlorides are shifting the CEC values upwards. In our work, due to the pH range (7–4) involved in autohydrolysis, the reason of WWS strong acid buffer capacity of raw WWS was mainly attributed to cation exchange (Van Breemen et al., 1982). The lowered CEC and ABC values indicated that the addition of FeCl₃ and AlCl₃ changes the cation contents of ash, effectively silencing the buffering capacity of WWS ash. To further investigate, metal cation quantities were measured.

3.5. Elements analysis by ICP-OES to estimate cation exchange extent

Elements (Fe, Al, Ca, Mg, K, Na and Si) involved in soil or plants could be effectively determined by ICP-OES and this method presents much advantages such as excellent reproducibility, quick determination and high accuracy (Souza et al., 2018). The surface of soil ash generally have net negative charge and the negatively charged sites were occupied by Al³⁺, Ca²⁺, Mg²⁺, K⁺, Na⁺ and H⁺ (Chute and Quirk, 1967). Due to the ion preference of soil ash, the original absorbed cations (Ca²⁺, Mg²⁺, K⁺ and Na⁺) in soil ash would be exchanged by H⁺ and enter into the prehydrolyzate (Coleman, 1983). As shown in Table 4, the prehydrolyzate from autohydrolysis of raw WWS had a base cation concentration of 3.09 g/L, while the base cation concentration in the prehydrolyzate of raw WWS autohydrolysis was only 0.79 g/L. The significant amount of released H⁺ with low base cation concentrations in prehydrolyzate indicated that the cation exchange behavior was almost eliminated by prewashing prior to the pretreatment. Moreover, it is well known that Si is the main element in soil ash and the main form of existence was silica dioxide. SiO₂ will not disassociate and produce Si ion in our metal chlorides assisted autohydrolysis. As an evidence, nearly no changes of Si contents (from 23.12% to 23.83%) in the ash of pretreated residues were observed. Meanwhile, no Si signals (0.00 g/L) were found in the prehydrolysate. The results suggested that Si components in the ash were stable in these pretreated conditions.

Incorporating FeCl₃ and AlCl₃ into autohydrolysis of WWS was hypothesized to enable avoidance of the H⁺ buffering by ash witnessed during autohydrolysis of raw WWS. As shown in Table 4, the Al and Fe content in the ash of 20 mM AlCl₃-WWS and 20 mM FeCl₃-WWS was 4.55 and 5.36 wt%, while the autohydrolyzed WWS was 1.06 and 0.91 wt%. The results showed that the added metal cations were absorbed by WWS and the adding metal cations would change the cation composition of ash which would further influence the buffering effects of ash. With the introduction of Al³⁺ and Fe³⁺ to ash, decreased CEC (from 79.7 to 45.6 and 49.8 cmol/kg) and ABC (from 177.0 to 103.4 and 87.9 mmol/pH·kg) was achieved for both AlCl₃-WWS and FeCl₃-WWS, which resulted in an increase in the amount of catalytic H⁺ released for biomass deconstruction during autohydrolysis. 87.6% and 85.8% of xylan removal were achieved for 20 mM AlCl₃-WWS and

Table 4
Elements analysis of solids and prehydrolysate of autohydrolyzed WWS with or without adding metal chlorides.

Substrate	Additives	Concentration (mM)	Elements composition in ash (wt, %)							Base cation ^a (wt, %)
			Si	Al	Fe	Ca	Mg	K	Na	
Raw WWS	/	/	23.83	1.06	0.91	5.15	0.42	0.21	0.12	5.89
	AlCl ₃	20	23.66	4.55	1.39	4.27	0.63	0.71	0.12	5.74
	FeCl ₃	20	23.12	1.31	5.36	4.24	0.63	0.55	0.11	5.52
Pre-washed WWS	/	/	28.90	0.01	0.03	0.24	0.02	0.01	0.01	0.28
Prehydrolysate			Cation concentration (g/L)							Base cation ^a (g/L)
Raw WWS	/	/	0.00	0.00	0.01	0.81	0.22	1.93	0.12	3.09
	AlCl ₃	20	0.00	0.01	0.04	1.14	0.21	1.56	0.08	3.00
	FeCl ₃	20	0.00	0.00	0.29	1.21	0.26	1.80	0.12	3.39
Pre-washed WWS	/	/	0.00	0.00	0.00	0.57	0.12	0.06	0.04	0.79

^a Base cation was calculated by the sums of Ca, Mg, K and Na contents.

20 mM FeCl₃-WWS. This additional level of xylan removal translated to improved enzymatic hydrolysis efficiencies, from 49.7% to 62.1% and 66.6% with the increasing addition (0–20 mM) of AlCl₃ and FeCl₃, respectively. Moreover, based on the enzymatic hydrolysis results (62.1% and 66.6%), Fe³⁺ can be described as more effective than Al³⁺. The addition of 20 mM FeCl₃ lead to 3.39 g/L base cations stripping from ash, which then entered into prehydrolysate. This value was higher for prehydrolysate of 20 mM AlCl₃-WWS, 3.09 g/L. Meanwhile, the base cations content (5.52 wt%) in the ash of 20 mM FeCl₃-WWS were lower than that of 20 mM AlCl₃-WWS (5.74 wt%). It has been considered that base cation contents in soil were a major reason for it showing acid buffer capacity (Houle et al., 2006). The results were in accordance with the ABC of 20 mM FeCl₃-WWS (87.9 mmol/pH·kg) and 20 mM AlCl₃-WWS (103.4 mmol/pH·kg). Overall, for Al³⁺ and Fe³⁺ inclusion into WWS autohydrolysis resulted in destruction of the ash-delivered buffer system. This was achieved mechanistically through a cation exchange effect between the applied reagents and the problematic ash.

4. Conclusion

Metal chlorides addition to autohydrolysis of WWS demonstrated the ability to reduce the pestering buffering capacity of WWS. Results showed that FeCl₃ exerts higher catalytic efficiency as compared to AlCl₃. Enzymatic hydrolysis of FeCl₃ pretreated material resulted in maximum monosaccharide production of 185.3 mg/g-WWS, alongside 6.2 g/L XOS. The implications of Al³⁺ and Fe³⁺ in WWS autohydrolysis lead to base cations being stripped from the ash through cation exchange. It can be acknowledged that minor additions of FeCl₃ and AlCl₃ could facilitate the autohydrolysis efficiency of high ash content WWS, resulting in this material being more viable for biorefinery applications.

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