Bioresource Technology 243 (2017) 319–326

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Mild chemical pretreatments are sufficient for complete saccharification of steam-exploded residues and high ethanol production in desirable wheat accessions

Zahoor [a,b,c], Yuanyuan Tu [a,b,c], Lingqiang Wang [a,b,c], Tao Xia [a,b,d], Dan Sun [a,c,e], Shiguang Zhou [a,b,c], Yanting Wang [a,b,c], Ying Li [a,b,c], Heping Zhang [a,b,c], Tong Zhang [a,b,c], Meysam Madadi [a,b,c], Liangcai Peng [a,b,c,*]

[a] Biomass and Bioenergy Research Centre, Huazhong Agricultural University, Wuhan 430070, China
[b] National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan 430070, China
[c] College of Plant Science and Technology, Huazhong Agricultural University, Wuhan 430070, China
[d] College of Life Science and Technology, Huazhong Agricultural University, Wuhan 430070, China
[e] School of Materials and Chemical Engineering, Hubei University of Technology, Wuhan 430068, China

HIGHLIGHTS

- Four wheat accessions are distinctive at wall polymer extraction by steam explosion.
- It performed a combined pretreatment of steam explosion with dilute acid or alkali.
- With 1% Tween-80, Talq90 and Talq16 accessions showed a complete enzymatic hydrolysis.
- Talq90 and Talq16 also exhibited the highest bioethanol yields at 18.5%–19.4%.
- Talq90 and Talq16 had improved polymer features in raw and steam-exploded residues.

GRAPHICAL ABSTRACT

ARTICLE INFO

Article history:
Received 12 May 2017
Received in revised form 19 June 2017

ABSTRACT

In this study, a combined pretreatment was performed in four wheat accessions using steam explosion followed with different concentrations of H_2SO_4 or NaOH, leading to increased hexoses yields by 3–6 folds from enzymatic hydrolysis. Further co-supplied with 1% Tween-80, Talq90 and Talq16 accessions

* Corresponding author at: Biomass and Bioenergy Research Centre, Huazhong Agricultural University, Wuhan 430070, China.
E-mail addresses: lpeng@mail.hzau.edu.cn, pengliangcai2007@sina.com (L. Peng).
URL: http://bbrc.hzau.edu.cn (L. Peng).

http://dx.doi.org/10.1016/j.biortech.2017.06.111

Accepted 20 June 2017
Available online 23 June 2017

Keywords:
Wheat
Lignocellulose features
Steam explosion
Biomass saccharification
Bioethanol

exhibited an almost complete enzymatic saccharification of steam-exploded (SE) residues after 0.5% H_2SO_4 or 1% NaOH pretreatment, with the highest bioethanol yields at 18.5%–19.4%, compared with previous reports about wheat bioethanol yields at 11%–17% obtained under relatively strong pretreatment conditions. Furthermore, chemical analysis indicated that much enhanced saccharification in Talq90 and Talq16 may be partially due to their relatively low cellulose CrI and DP values and high hemicellulose Ara and H-monomer levels in raw materials and SE residues. Hence, this study has not only demonstrated a mild pretreatment technology for a complete saccharification, but it has also obtained the high ethanol production in desirable wheat accessions.

1. Introduction

Crop lignocellulosic residues have been considered as an abundant renewable biomass resource for biofuels and other chemical products (Chen and Peng, 2013). For bioethanol production, biomass process consists of three main steps: typical physical and chemical pretreatment, effective cellulase enzymatic hydrolysis and rapid ethanol fermentation (Himmel et al., 2007). However, because lignocellulose recalcitrance basically decides an unacceptably costly biomass process (Xie and Peng, 2011; Lynd et al., 2008), optimal technology is required for high bioethanol productivity (Wang et al., 2016; Li et al., 2017).

Plant cell walls could provide enormous biomass resource including cellulose, hemicellulose, lignin and minor pectic polysaccharides. Lignocellulose recalcitrance is determined by plant cell wall structure and wall polymer features. For instance, crystalline index (CrI) and degree of polymerization (DP) of β-1, 4-glucans are two major cellulose features that negatively affect biomass enzymatic digestibility (Zhang et al., 2013; Jia et al., 2014; Li et al., 2015). In comparison, hemicelluloses could positively impact biomass enzymatic digestibility by reducing cellulose CrI (Xu et al., 2012; Li et al., 2013), and in particular, arabinose (Ara) level or substitution degree of xylan has been identified as the major factor on biomass enzymatic hydrolysis under various chemical pretreatments in wheat, rice and other grasses (Li et al., 2013). In addition, lignin plays a complicated role in biomass enzymatic digestions distinctive in different plant species (Davison et al., 2006; Studer et al., 2011; Xu et al., 2012; Jia et al., 2014).

Over the past years, many physical and chemical pretreatments have been applied for biomass process in different plant species. Among physical pretreatments, steam explosion is a relatively economical technology with less secondary environmental pollution, because it could largely reduce biomass particle size, and partially remove hemicelluloses and lignin. Recent report has also indicated that steam explosion could largely reduce cellulose DP in cotton stalks (Huang et al., 2015). However, for most biomass residues, an additional pretreatment after steam explosion is required for a complete enzymatic hydrolysis (Huang et al., 2015; Jin et al., 2016). Acid and alkali are the classical agents used for chemical pretreatments such as H_2SO_4 and NaOH. In comparison, alkali pretreatment mainly extracts entire wall polymers by disassociating with cellulose microfibrils, whereas acid pretreatment could digest wall polymers at high temperature (Xu et al., 2012; Li et al., 2013; Wu et al., 2013). Hence, it is important to find out the cost-effective pretreatment for enhancing biomass enzymatic hydrolysis (Wang et al., 2016).

Wheat is an important food crop over the world with enormous biomass residues for biofuels. Despite several physical and chemical pretreatments have been performed in wheat residues (Wu et al., 2013; Zahoor and Yuanyuan, 2014; Bensah et al., 2015; Jaisamut et al., 2016; Joelsson et al., 2016; Qiu et al., 2017), it remains to examine whether biofuel production could be further increased by improving biomass process technology. In this study, we performed a combined physical (steam explosion) and chemical (H_2SO_4 or NaOH) pretreatment in the selected four wheat accessions distinctive at cellulose levels. We then detected biomass enzymatic saccharification and ethanol production under 1% Tween-80 co-supply. Finally, we examined steam explosion specific extraction with non-cellulosic polymers and determined major wall polymer features in wheat accessions.

2. Materials and methods

2.1. Plant samples

Four wheat accessions were grown in Wuhan experimental field in 2014, and their mature straws were collected and dried at 50 °C. The dried samples were powdered by passing 40-mesh screen and storied in a dry container until use.

2.2. Plant wall polymer extraction

The wall polymers were extracted as previously described by Peng et al. (2000) and Wu et al. (2013) with minor modification. The soluble sugars, lipid, starch and pectin in the biomass samples were successively extracted by using potassium phosphate buffer (pH 7.0), chloroform–methanol (1:1, v/v), DMSO–water (9:1, v/v) and ammonium oxalate 0.5% (w/v). The remaining pellets were divided into two parallels; one parallel residues were extracted with trifluoroacetic acid (TFA) for monosaccharides determination. The second parallel residues were incubated with 4 M KOH (containing 1.0 mg/mL sodium borohydride) at 25 °C for 1 h, and after centrifugation, the supernatants were collected as KOH-extractable hemicelluloses. The remaining non-KOH-extractable residues were sequentially extracted with H_2SO_4 (67%, v/v) at 25 °C for 1 h, and the hexoses and pentoses of the supernatants were respectively detected as total cellulose and non-KOH-extractable hemicelluloses. All experimental analyses were carried out in biological triplicates.

2.3. Colorimetric assay of hexoses and pentoses

UV–VIS spectrometer (V-1100D, Shanghai MAPADA Instruments Co., Ltd. Shanghai, China) was used for determination of hexoses and pentoses as previously described by Wu et al. (2013). In terms of high pentose level impact on hexoses assay, the deduction from pentoses reading was carried out for a final hexoses calculation. All experiments were conducted in biological triplicate.

2.4. Total lignin and monolignol assay

Two-step acid hydrolysis method was used for detection of total lignin content, according to the Laboratory Analytical Procedure of the National Renewable Energy Laboratory (Sluiter et al., 2008).

Monolignols were measured by HPLC using nitrobenzene oxidation method as previously described by Li et al. (2014a).

2.5. Hemicelluloses monosaccharide determination by GC–MS

GC–MS (SHIMADZU GCMS-QP2010 Plus) was used for detection of monosaccharide composition of hemicellulose as previously described by Li et al. (2015). Trifluoroacetic acid (TFA) and *myo*-inositol were obtained from Aladdin Reagent Inc. *1*-Methylimidazole was purchased from Sigma–Aldrich Co. LLC. Acetic anhydride and acetic acid were obtained from Sinopharm Chemical Reagent Co., Ltd.

2.6. Detection of cellulose crystalline index (CrI)

For determination of cellulose crystalline index (CrI), X-ray diffraction method was conducted as previously described by Zhang et al. (2013) using the Rigaku-D/MAX instrument (Uitima III, Japan). Standard errors of the CrI values were measured at ±0.05 ∼ 0.15 using five representative samples in triplicate

2.7. Detection of degree of polymerization (DP) of cellulose

The dry powders of biomass samples (0.2–1 g) were extracted with 4 M KOH (containing sodium borohydride at 1.0 mg/mL) at 25 °C for 1 h. After centrifugation at 4000*g* for 5 min, the pellet was re-extracted with 4 M KOH, and washed five times with distilled water until pH at 7.0. The pellet was further extracted with 10 mL 8% $NaClO_2$ at 25 °C for 72 h (change $NaClO_2$ every 12 h). After centrifugation, the residues were washed five times with distilled water until pH at 7.0, and dried with vacuum suction filtration. The DP of crude cellulose sample was measured using the viscosity method (Puri, 1984) with minor modification (Huang et al., 2015). All experiments were performed in biological triplicate.

2.8. Steam explosion pretreatment

The well-dried wheat biomass samples were applied for steam explosion using Steam Explosion Reactor (QBS-200, Hebi Zhengdao Machine Factory, Hebi, China) as previously described by Huang et al. (2015). The steam-exploded (SE) wheat residues were dried and ground into powders through 40 mesh screen, and stored in a dry container until use.

2.9. Biomass pretreatment

NaOH pretreatment was performed as described by Wu et al. (2013). The well-mixed dry biomass samples were added with 6 mL NaOH at three concentrations (0.0%, 0.5%, 1%, 2% w/v). The sealed tube was shaken under 150 rpm at 50 °C for 2 h, and centrifuged at 4000*g* for 5 min for soluble sugar collection. As a control, 6 mL distilled water was added in the samples and shaken under 150 rpm at 50 °C for 2 h. All experimental analyses were carried out in biological triplicates.

H_2SO_4 pretreatment was described by Pei et al. (2016). The well-mixed dried biomass samples were added with 6 mL H_2SO_4 at three concentrations (0.0%, 0.5%, 1%, 2% v/v). The sealed sample tubes were heated at 121 °C for 20 min in autoclave (15 psi), shaken under 150 rpm at 50 °C for 2 h, and centrifuged at 4000*g* for 5 min. The pellet was washed with 10 mL distilled water, and stored it for sequential enzymatic hydrolysis. All experimental analyses were performed in biological triplicates.

2.10. Enzymatic hydrolysis

Enzymatic hydrolysis was determined as previously described by Wu et al. (2014) and Jin et al. (2016). The pretreated biomass residues were washed two times with distilled water and once with mixed-cellulases reaction buffer (0.2 M acetic acid-sodium acetate with pH 4.8). The samples were added with mixed-cellulases (containing β-glucanase $\geq 5.96 \times 10^4$ U and cellulase $\geq$ 596 U and xylanase $\geq 9.6 \times 10^4$ U, purchased from Imperial Jade Bio-technology Co., Ltd., China) and Tween-80, with the final enzyme concentration at 1.6 g/L and Tween-80 concentration at 1% (v/v). For enzymatic hydrolysis, the samples were shaken under 150 rpm at 50 °C for 48 h. After reaction, the samples were centrifuged at 4000*g* for 5 min, and supernatants were collected for total pentose and hexose yields assay. All samples were carried out in biological triplicate.

2.11. Yeast fermentation and ethanol assay

The yeast fermentation was conducted using *Saccharomyces cerevisiae* strain (purchased from Angel yeast Co., Ltd., Yichang, China) as previously described by Li et al. (2014a) and Jin et al. (2016). The experiments were performed with biological triplicate.

2.12. Statistical calculation of correlation coefficients

Superior Performance Software Systems software package (SPSS 17.0, Inc., Chicago, IL) was used for statistical analysis. Pairwise wise comparisons were performed between treated and control by Student's t-test. This analysis used the average values calculated from all original determinations values.

3. Results and discussion

3.1. Distinct wall polymer extraction of four wheat accessions by steam explosion

In this study, we determined cell wall compositions of four wheat accessions including cellulose, hemicelluloses and lignin (Table 1). As a result, four wheat accessions exhibited similar lignin and hemicelluloses levels, but they had largely varied cellulose contents from 28.93% to 40.82%. Under steam explosion pretreatment, hemicelluloses were largely extracted in four wheat accessions by 54%–59%, whereas lignin was less removed by 10%–14% (Table 1). Due to hemicellulose and lignin extraction, two wheat accessions (Talq90 & Talq16) showed relatively increased cellulose levels by 34% and 38% in the steam-exploded (SE) samples, consistent with previous reports in cotton stalk and other grasses (Huang et al., 2015; Jin et al., 2016; Sun et al., 2017). Notably, other two wheat accessions (Talq9 & Talq101) did not show significant increase at cellulose content, suggesting that cellulose of their samples should be partially removed from steam explosion. Despite of little increase of cellulose levels in Talq9 and Talq101, all four wheat accessions contained much higher cellulose contents in the SE residues at 40%–42%, compared with other SE samples examined in *Miscanthus* (19.7%–38.5%), reed (37%), corn (37%), and sweet sorghum (15–27%) (Zhang et al., 2013; Jin et al., 2016; Lu et al., 2010; Zhao et al., 2009).

3.2. Largely enhanced biomass saccharification under combined pretreatments

Biomass enzymatic saccharification (digestibility) is defined by measuring hexoses yield (% cellulose) released from enzymatic hydrolysis of pretreated biomass residues (Xu et al., 2012; Wu

Table 1
Cell wall compositions (% dry matter) in raw materials and SE residues of four wheat accessions.

Biomass sample		Cellulose		Hemicelluloses		Lignin	
TaLq90	Raw	28.93 ± 0.51**	37.78%[a]	30.18 ± 0.34**	−57.42%	23.40 ± 0.23*	−10.25%
	SE	39.86 ± 0.55		12.85 ± 0.17		21.00 ± 0.70	
TaLq16	Raw	30.44 ± 0.70**	33.93%	28.42 ± 0.37**	−54.29%	24.48 ± 0.37*	−12.29%
	SE	40.77 ± 0.80		12.99 ± 0.05		21.47 ± 0.77	
TaLq9	Raw	38.42 ± 0.22	7.41%	31.70 ± 0.49**	−57.91%	24.12 ± 0.51**	−14.26%
	SE	41.27 ± 0.95		13.34 ± 0.12		20.68 ± 0.67	
TaLq101	Raw	40.82 ± 0.80	1.81%	32.37 ± 0.13**	−58.63%	25.80 ± 0.51*	−11.04%
	SE	41.56 ± 0.74		13.39 ± 0.22		22.95 ± 0.15	

* or ** As significant difference between raw material (Raw) and steam-exploded (SE) residues in four wheat accessions by t-test at $p < 0.05$ or $p < 0.01$ (n = 3).
[a] Indicated the increased or decreased (−) percentage between Raw and SE values by subtraction of two values divided by Raw value; Data as mean ± SD (n = 3).

et al., 2013; Li et al., 2013; Zhang et al., 2013; Jin et al., 2016), which is directly accounting for cellulose digestion rate in biomass sample. In this study, we performed a combined pretreatment with steam explosion followed by acid (H_2SO_4) or alkali (NaOH) in four wheat accessions (Fig. 1). Without any pretreatment, four wheat accessions exhibited much low hexoses yields at 12%–26% (% cellulose) released from enzymatic hydrolysis of raw materials (Fig. 1A and B), but they had hexoses yields at 44%–52% from enzymatic digestion of steam-exploded (SE) residues (Fig. 1C and D), indicating a remarkably enhanced biomass saccharification from steam explosion. While the raw materials were pretreated with H_2SO_4 or NaOH at three concentrations (0.5%, 1%, 2%), four wheat accessions exhibited much enhanced hexoses yields by 2–3 folds, in particular from alkali pretreatments (Fig. 1A and B). Meanwhile, Talq90 and Talq16 accessions had higher hexoses yields than those of Talq9 and Talq101 accessions.

While the SE residues were pretreated with H_2SO_4 or NaOH, the Talq9 and Talq101 accessions were more enhanced than those of Talq90 and Talq16 accessions on biomass saccharification, leading to less variations of hexoses yields among four accessions (Fig. 1C and D). The acid pretreatments could more enhance enzymatic saccharification of SE residues than that of raw materials in four wheat accessions (Fig. 1A and C), consistent with previous reports in *Miscanthus*, corn, sweet sorghum and reed (Xu et al., 2012; Jia et al., 2014; Li et al., 2014c; Jin et al., 2016). By contrast, the alkali pretreatments led little enhancements in enzymatic saccharification of SE residues in Talq90 and Talq16, probably due to their characteristic cell wall structures and features. However, all

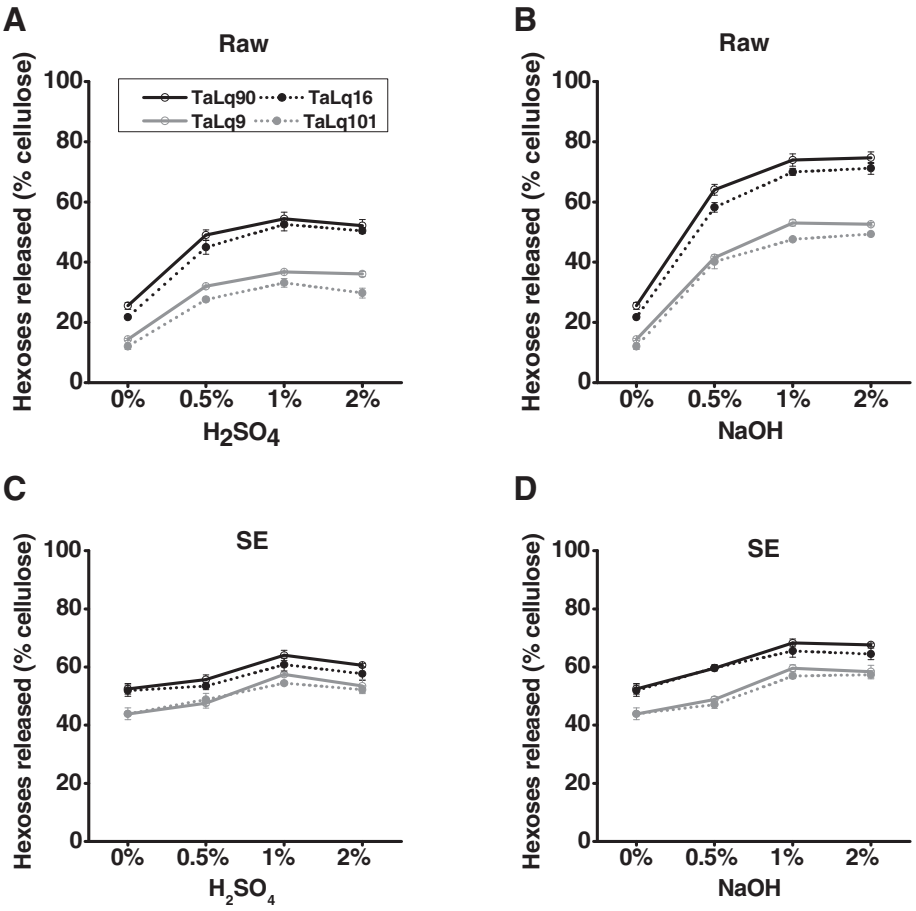

Fig. 1. Hexoses yields (% cellulose) released from enzymatic hydrolysis of raw materials and steam-exploded (SE) residues after pretreatments with three concentrations of H_2SO_4 and NaOH in four wheat accessions: (A, B) Raw materials; (C, D) SE residues; Bar as mean ± SD (n = 3).

acid and alkali pretreatments could not cause a complete enzymatic hydrolysis of either SE residues or raw materials in four what accessions.

3.3. Complete enzymatic digestibility with Tween-80

Tween-80 has been applied to enhance biomass enzymatic saccharification (Jin et al., 2016). In this study, Tween-80 was directly added into biomass enzymatic hydrolysis of raw materials or SE residues in four wheat accessions. Without any pretreatment, four wheat accessions exhibited little enhanced enzymatic hydrolysis of raw materials from 1% Tween-80 supply, with the hexoses yields at 15%–26% (Fig. 2A and B). While the raw materials were pretreated with three concentrations of H_2SO_4 or NaOH (0.5%, 1%, 2%), four accessions showed the hexoses yields at 31%–60% or 52%–90%, indicating a remarkably enhanced enzymatic saccharification from 1% Tween-80 supply. However, both Talq90 and Talq16 accessions remained much higher hexoses yields than those of Talq9 & Talq101 accessions from Tween-80 supply (Fig. 2A and B).

In terms of the SE residue saccharification, four wheat accessions showed the hexoses yields at 51%–64% from 1% Tween-80 supply (Fig. 2C and D). Notably, while the SE residues were pretreated with low concentrations (0.5%, 1%) of H_2SO_4 or NaOH, both Talq90 and Talq16 accessions had an almost complete biomass enzymatic hydrolysis from 1% Tween-80 supply, with the hexoses yields at 96%–100%, whereas the Talq9 & Talq101 accessions were also enhanced, with the highest hexoses yields at 89% under both acid and alkali pretreatments. Hence, the results indicated that Tween-80 is extremely effective for enhancing enzymatic hydrolysis of the SE residues under dilute acid and alkali pretreatments in all wheat accessions, in particular for Talq90 and Talq16 accessions.

3.4. Optimal bioethanol productivity from yeast fermentation

As the final step for bioethanol production, we performed yeast fermentation by using total soluble sugars released from both pretreatment and sequential enzymatic hydrolysis in four wheat accessions (Fig. 3). Without any pretreatment, four accessions exhibited much low ethanol yields at less than 4% (% dry matter) from raw materials (Fig. 3A), but they had the ethanol yields at 9.5–10% from SE residues, indicating that steam explosion could increase ethanol yields by 2-fold. While raw materials were pretreated with two concentrations (0.5%, 1%) of H_2SO_4 or NaOH, four wheat accessions showed an enhanced bioethanol productivity, with ethanol yields from 7% to 10%. By comparison, while the SE residues were pretreated with H_2SO_4 or NaOH, four wheat accessions had the ethanol yields at 11%–13% (Fig. 3B), consistent with their much enhanced enzymatic saccharification. Furthermore, due to remarkably enhanced enzymatic saccharification of SE residues from 1% Tween-80 supply, four wheat accessions exhibited ethanol yields at 15%–19% (Fig. 3C). In particular, the Talq90 had the highest ethanol yields at 19.4% and 18.5% from 0.5% H_2SO_4 and 1% NaOH pretreatments respectively, whereas the Talq16 had the ethanol yields at 18.5% and 17.5%. Hence, both Talq90 and Talq16 accessions showed high hexoses-ethanol conversion

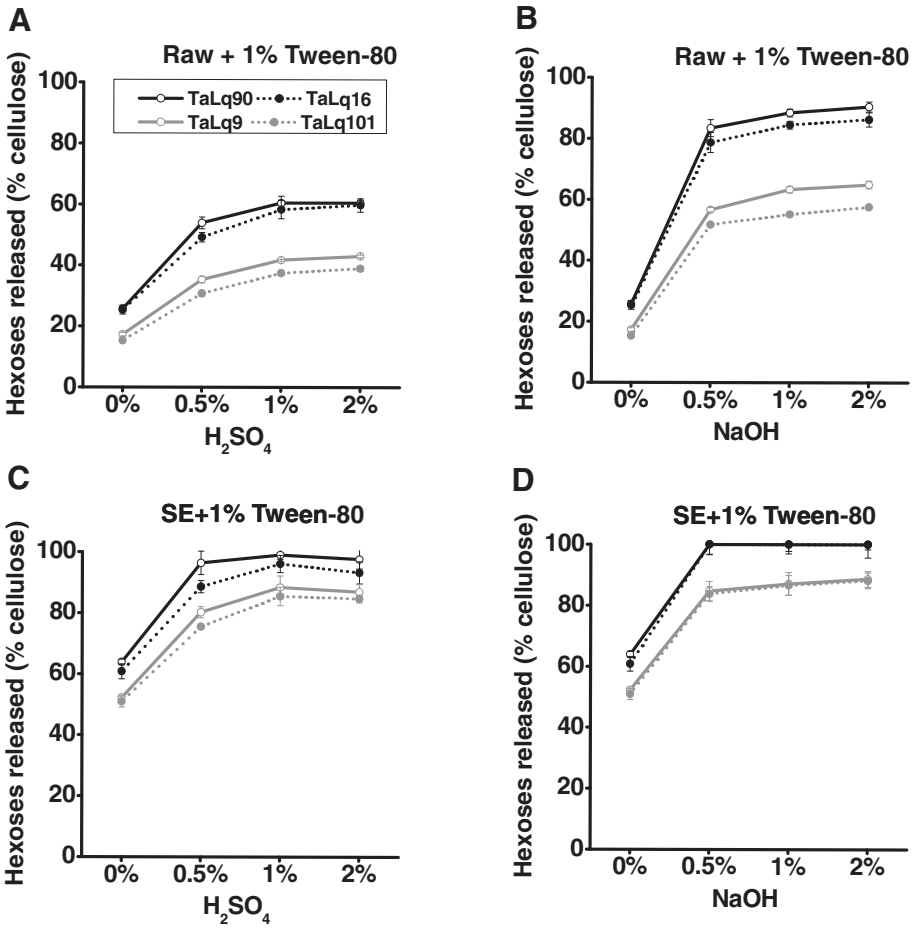

Fig. 2. Hexoses yields (% cellulose) released from enzymatic hydrolysis of raw materials and SE residues co-supplied with 1% Tween-80 after pretreatments with three concentrations of H_2SO_4 and NaOH in four wheat accessions: (A, B) Raw materials; (C, D) SE residues; Bar as mean ± SD (n = 3).

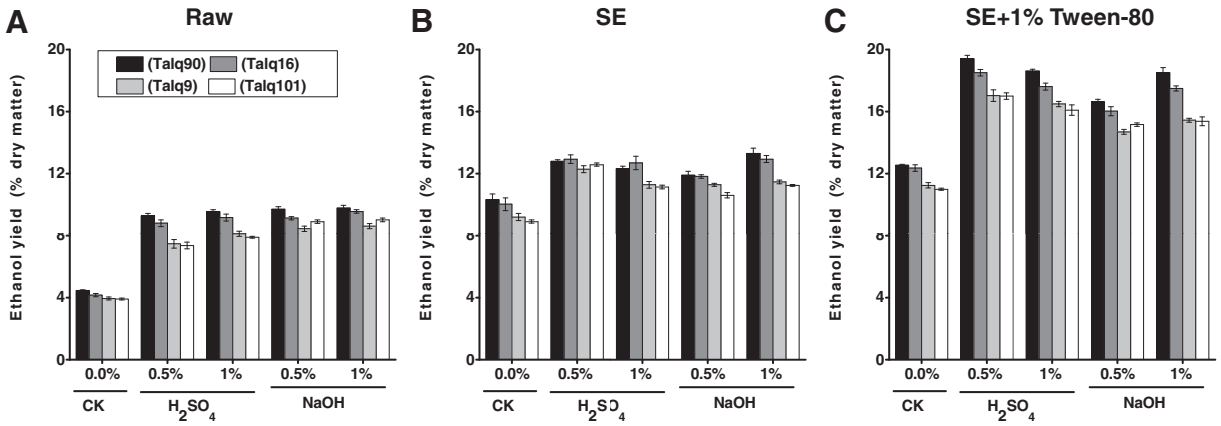

Fig. 3. Bioethanol production released from yeast fermentation using sugars obtained from H_2SO_4 and NaOH pretreatments and enzymatic hydrolysis in raw materials and SE residues of four wheat accessions: (A) Raw materials; (B) SE residues; (C) SE residues co-supplied with 1% Tween-80; Bar as mean ± SD (n = 3).

rates at 45%–48% from 0.5% H_2SO_4 and 1% NaOH pretreatments, close to the conversion rate (51%) in theory.

Recently, various physical (steam explosion, hydrothermal) and chemical (H_2SO_4, H_3PO_4, Na_2SO_3, H_2O_2) pretreatments have been applied in wheat biomass process, but those technologies could lead to the ethanol yields at 11%–17%, which are lower than those of two wheat accessions (Talq90 and Talq16) reported in this study (Table 2). Despite of higher ethanol yields from Talq90 and Talq16 biomass process, this study performed relatively mild pretreatments (low temperature, short incubation time) compared with the previous reports (Ko et al., 2009; Lindedam et al., 2012; Wi et al., 2013; Zhu et al., 2015; Qiu et al., 2017). In addition, many pretreatment technologies have also been used for bioethanol productivity in rice, leading to ethanol yields at 12%–19% (Table 2). Although one technology could lead to the ethanol yield at 19% in rice, it had used extreme pretreatment conditions including sulfuric acid at 100 °C for 120 min followed by sulfomethylation at 160 °C for 300 min. Therefore, this study has yet found an economical and environment-friendly technology for the highest bioethanol production in desire wheat accessions.

3.5. Mechanism of distinct enzymatic saccharification among wheat accessions

It has been recently characterized that lignocellulose features could distinctively affect biomass enzymatic saccharification under physical and chemical pretreatments in wheat and other grass plants (Alvira et al., 2010; Wu et al., 2013; Li et al., 2014b). To understand why two wheat accessions (Talq90 and Talq16) showed complete biomass saccharification and high bioethanol production, we compared major wall polymer features in raw materials and SE residues of four wheat accessions (Fig. 4). As a result, the Talq90 and Talq16 accessions exhibited relatively lower values of cellulose CrI and DP than those of the Talq9 andTalq101 accessions in both raw materials and SE residues (Fig. 4A and B). As cellulose CrI and DP are the key negative factors on biomass enzymatic saccharification under various pretreatments (Zhang et al., 2013; Li et al., 2017), the relatively low CrI and DP values of Talq90 and Talq16 were thus consistent with their much enhanced hexoses yields and bioethanol productions. Furthermore, because Ara level and Ara substitution degree (reverse Xyl/Ara ratio) of hemicelluloses are the positive factors on biomass digestibility (Li et al., 2013), the higher Ara levels or relatively lower Xyl/Ara ratios of the Talq90 and Talq16 should be another factor accounting for their enhanced biomass digestibility, compared with the Talq9 and Talq101 accessions (Fig. 4C and D). In addition, As H-monomer has been examined to positively affect biomass enzymatic hydrolysis in rice and wheat (Wu et al., 2013), the Talq90 and Talq16 with relatively higher H-monomer levels, in particular on SE residues, should be additional factor on biomass saccharification (Fig. 4E).

Physical and chemical pretreatments have been well demonstrated to effectively extract non-cellulosic polymers for cellulase enzyme accessible to cellulose digestion. As described above, however, lignocellulose features distinctively affect biomass enzymatic digestibility under various pretreatments by maintaining a native cellulose microfiber structure for an effective enzymatic digestion and creating more places for enzyme loading (Xu et al., 2012;

Table 2
Comparison of bioethanol yields from biomass process in wheat and rice.

Plant species	Pretreatment	Ethanol production (% dry matter)	Reference
Wheat	Steam explosion + 0.5% sulfuric acid (120 °C, 20 min)	12.8%	This study
	Steam explosion + 1% sodium hydroxide (50 °C, 120 min)	13.3%	
	Steam explosion + 1% sodium hydroxide + 1% Tween-80	18.5%	
	Steam explosion + 0.5% sulfuric acid + 1% Tween-80	19.4%	
Wheat	Combined H_3PO_4 85% and H_2O_2 30% (40.2 °C, 174 min)	11%	Qiu et al. (2017)
	Hydrothermal pretreatment (195 °C, 10 min)	15%	Bensah et al. (2015)
	Raw material soaked 1% acetic acid (60 min) + steam explosion	16%	Joelsson et al. (2016)
	Combined 1% H_2SO_4 and 2.4% Na_2SO_3 (180 °C, 30 min)	17%	Jaisamut et al. (2016)
Rice	Aqueous- ammonia(69 °C, 600 min)	12%	Ko et al. (2009)
	Torrefaction (220 °C, 40 min)	15%	Sheikh et al., 2013
	Popping pretreatment (220 °C, 360 min)	17%	Wi et al. (2013)
	AFEX (140 °C, 30 min)	17%	Zhong et al. (2009)
	Sulfuric acid (100 °C, 120 min) + sulfomethylation (160 °C, 300 min)	19%	Zhu et al. (2015)

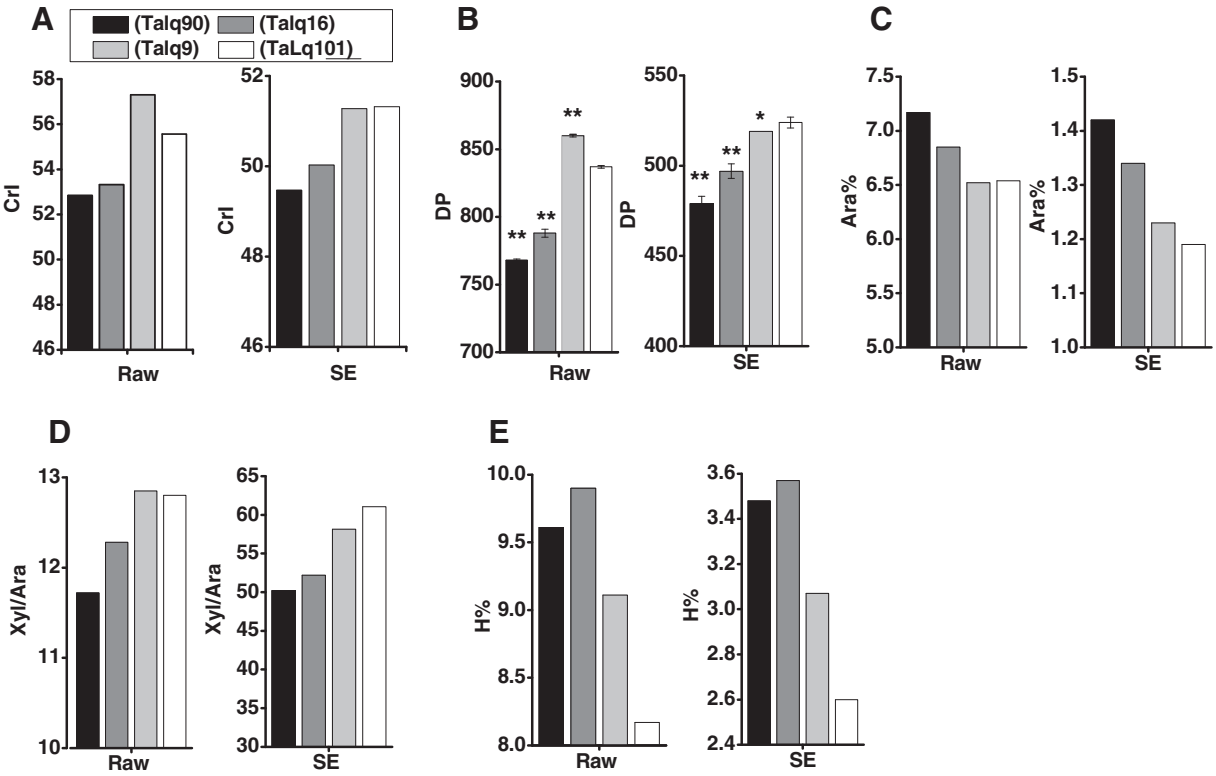

Fig. 4. Detection of wall polymer features in raw materials and SE residues of four wheat accessions: (A) Crystalline index (CrI) of cellulose; (B) Degree of polymerization of cellulose; (C) Arabinose proportion of hemicellulose; (D) Ratio of xylose and arabinose; (E) H-monomer proportion of lignin; * or ** as significant difference between wheat accession against TaLq101 accession by t-test at $p < 0.05$ or $p < 0.01$ (n = 3).

Zhang et al., 2013; Jia et al., 2014; Wang et al., 2016). In this work, two wheat accessions (Talq90, Talq16), probably due to their relatively lower cellulose CrI and DP and higher hemicellulose Ara and H-monomer, have exhibited more enhanced enzymatic saccharification in either raw materials or SE residues after dilute acid or alkali pretreatments, compared with the Talq9 and Talq101 accessions. Hence, this study has indicated that optimal technology could largely enhance wheat biomass enzymatic saccharification, but the desirable wheat accessions with improved lignocellulose features could further allow a complete biomass enzymatic hydrolysis for the highest ethanol production under mild pretreatments.

4. Conclusion

Under a combined pretreatment of steam explosion followed with different concentrations of H_2SO_4 or NaOH, four wheat accessions were distinctively enhanced for biomass saccharification, with increased hexoses yields by 3–6 folds. Further supplied with 1% Tween-80, Talq90 and Talq16 accessions exhibited an almost complete enzymatic hydrolysis under 0.5% H_2SO_4 or 1% NaOH, leading to the highest bioethanol yields at 18.5%–19.4%, probably due to their relatively low cellulose CrI and DP and high hemicellulose Ara and H-monomer in both raw materials and SE residues. This study has hence indicated an optimal biomass process technology for desirable wheat accessions.

Acknowledgements

This work was supported in part by grants from the National Key Research and Development Program (2016YFD0800804), the Fundamental Research Funds for the Central Universities of China (2662015PY018 and 2662015PY009), and the 111 Project of Ministry of Education of China and State Administration of Foreign Experts Affairs, China (B08032).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2017.06.111.

References

Alvira, P., Tomas-Pejo, E., Ballesteros, M., Negro, M.J., 2010. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: a review. Bioresour. Technol. 101, 4851–4861.

Bensah, E.C., Kadar, Z., Mensah, M.Y., 2015. Ethanol production from hydrothermally-treated biomass from West Africa. BioResources 10, 6522–6537.

Chen P., Peng L., 2013. In: Sun, J., Ding, S.Y., Peterson, J.D. (ed.) Biological conversion of biomass for fuels and chemicals: exploration from natural utilization system, first ed. Royal Society of Chemistry, Oxfordshire, pp. 83–113 (Ch. 6).

Davison, B.H., Drescher, S.R., Tuskan, G.A., Davis, M.F., Nghiem, N.P., 2006. Variation of S/G ratio and lignin content in a Populus family influences the release of xylose by dilute acid hydrolysis. Appl. Biochem. Biotechnol. 129–132, 427–435.

Himmel, M.E., Ding, S.Y., Johnson, D.K., Adney, W.S., Nimlos, M.R., Brady, J.W., Foust, T.D., 2007. Biomass recalcitrance: engineering plants and enzymes for biofuels production. Science 315, 804–807.

Huang, Y., Wei, X., Zhou, S., Liu, M., Tu, Y., Li, A., Chen, P., Wang, Y., Zhang, X., Tai, H., Peng, L., Xia, T., 2015. Steam explosion distinctively enhances biomass enzymatic saccharification of cotton stalks by largely reducing cellulose polymerization degree in *G. barbadense* and *G. hirsutum*. Bioresour. Technol. 181, 224–230.

Jaisamut, K., Paulova, L., Patakova, P., Kotucova, S., Rychtera, M., 2016. Effect of sodium sulfite on acid pretreatment of wheat straw with respect to its final conversion to ethanol. Biomass Bioenergy 95, 1–7.

Jia, J., Yu, B., Wu, L., Wang, H., Wu, Z., Li, M., Huang, P., Feng, S., Chen, P., Zheng, Y., Peng, L., 2014. Biomass enzymatic saccharification is determined by the non-koh-extractable wall polymer features that predominately affect cellulose crystallinity in corn. PLoS One 9 (9), e108449.

Jin, W., Chen, L., Hu, M., Sun, D., Li, A., Li, Y., Hu, Z., Zhou, S., Tu, Y., Xia, T., Wang, Y., Xie, G., Li, Y., Bai, B., Peng, L., 2016. Tween-80 is effective for enhancing steam-exploded biomass enzymatic saccharification and ethanol production by specifically lessening cellulase absorption with lignin in common reed. Appl. Energy 175, 82–90.

Joelsson, E., Dienes, D., Kovacs, K., Galbe, M., Wallberg, O., 2016. Combined production of biogas and ethanol at high solids loading from wheat straw impregnated with acetic acid: experimental study and techno-economic evaluation. Sustain. Chem. Process. 4, 14.

Ko, J.K., Bak, J.S., Jung, M.W., Lee, H.J., Choi, I.G., Kim, T.H., Kim, K.H., 2009. Ethanol production from rice straw using optimized aqueous-ammonia soaking pretreatment and simultaneous saccharification and fermentation processes. Bioresour. Technol. 100, 4374–4380.

Li, F., Ren, S., Zhang, W., Xu, Z., Xie, G., Chen, Y., Tu, Y., Li, Q., Zhou, S., Li, Y., Tu, F., Liu, L., Wang, Y., Jiang, J., Qin, J., Li, S., Li, Q., Jing, H.C., Zhou, F., Gutterson, N., Peng, L., 2013. Arabinose substitution degree in xylan positively affects lignocellulose enzymatic digestibility after various NaOH/ H_2SO_4 pretreatments in *Miscanthus*. Bioresour. Technol. 130, 629–637.

Li, M., Si, S., Hao, B., Zha, Y., Wan, C., Hong, S., Kang, Y., Jia, J., Zhang, J., Li, M., Zhao, C., Tu, Y., Zhou, S., Peng, L., 2014a. (a). Mild alkali-pretreatment effectively extracts guaiacyl-rich lignin for high lignocellulose digestibility coupled with largely diminishing yeast fermentation inhibitors in *Miscanthus*. Bioresour. Technol. 169, 447–456.

Li, Z., Zhao, C., Zha, Y., Wan, C., Si, S., Liu, F., Zhang, R., Li, F., Yu, B., Yi, Z., Xu, N., Peng, L., Li, Q., 2014b. (b). The minor wall-networks between monolignols and interlinked-phenolics predominantly affect biomass enzymatic digestibility in *Miscanthus*. PLoS One 9 (8), e105115.

Li, M., Feng, S., Wu, L., Li, Y., Fan, C., Zhang, R., et al., 2014c. Sugar-rich sweet sorghum is distinctively affected by wall polymer features for biomass digestibility and ethanol fermentation in bagasse. Bioresour. Technol. 167, 14–23.

Li, F.C., Zhang, M.L., Guo, K., Hu, Z., Zhang, R., Feng, Y.Q., et al., 2015. High-level hemicellulosic arabinose predominately affects lignocellulose crystallinity for genetically enhancing both plant lodging resistance and biomass enzymatic digestibility in rice mutants. Plant Biotechnol. J. 13 (4), 514–525.

Li, F., Xie, G., Huang, J., Zhang, R., Li, Y., Wang, Y., Li, A., Li, X., Xia, T., Qu, C., Hu, F., Ragauskas, A.J., Peng, L., 2017. OsCESA9 conserved-site mutation leads to largely enhanced plant lodging resistance and biomass enzymatic saccharification by reducing cellulose DP and crystallinity in rice. Plant Biotech. J., 1–12

Lindedam, J., Andersen, S.B., DeMartini, J., Bruun, S., Jorgensen, H., Felby, C., Magid, J., Yang, B., Wyman, C.E., 2012. Cultivar variation and selection potential relevant to the production of cellulosic ethanol from wheat straw. Biomass Bioenergy 37, 221–228.

Lu, Y., Wang, Y., Xu, G., Chu, J., Zhuang, Y., Zhang, S., 2010. Influence of high solid concentration on enzymatic hydrolysis and fermentation of steam-exploded corn stover biomass. Appl. Biochem. Biotechnol. 160, 360–369.

Lynd, L.R., Laser, M.S., Brandsby, D., Dale, B.E., Davison, B., Hamilton, R., Himmel, M., Keller, M., McMillan, J.D., Sheehan, J., Wyman, C.E., 2008. How biotech can transform biofuels. Nat. Biotechnol. 26, 169–172.

Pei, Y., Li, Y., Zhang, Y., Yu, C., Fu, T., Zou, J., et al., 2016. G-lignin and hemicellulosic monosaccharides distinctively affect biomass digestibility in rapeseed. Bioresour. Technol. 203, 325–333.

Peng, L., Hocart, C.H., Redmond, J.W., Williamson, R.E., 2000. Fractionation of carbohydrates in Arabidopsis root cell walls shows that three radial swelling loci are specifically involved in cellulose production. Planta 211, 406–414.

Puri, V.P., 1984. Effect of crystallinity and degree of polymerization of cellulose on enzymatic saccharification. Biotechnol. Bioeng. 26, 1219–1222.

Qiu, J., Ma, L., Shen, F., Yang, G., Zhang, Y., Deng, S., Zhang, J., Zeng, Y., Hu, Y., 2017. Pretreating wheat straw by phosphoric acid plus hydrogen peroxide for enzymatic saccharification and ethanol production at high solid loading. Bioresour. Technol. 238, 174–181.

Sheikh, M.M.I., Kim, C.H., Park, H.J., Kim, S.H., Kim, G.C., Lee, J.Y., Sim, S.W., Kim, J.W., 2013. Effect of torrefaction for the pretreatment of rice straw for ethanol production. J. Sci. Food Agric. 93, 3198–3204.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Crocker, D., 2008. Determination of structural carbohydrates and lignin in biomass. Tech. Rep. NREL/TP-510-42618, NREL, Golden Co.

Studer, M.H., DeMartini, J.D., Davis, M.F., Sykes, R.W., Davison, B., et al., 2011. Lignin content in natural Populus variants affects sugar release. Proc. Natl. Acad. Sci. U. S.A. 108, 6300–6305.

Sun, D., Alam, A., Tu, Y., Zhou, S., Wang, Y., Xia, T., Huang, J., Li, Y., Zahoor, Wei, X., Hao, B., Peng, L., 2017. Steam-exploded biomass saccharification is predominately affected by lignocellulose porosity and largely enhanced by Tween-80 in Miscanthus. Bioresour. Technol. 239, 74–81.

Wang, Y., Fan, C., Hu, H., Li, Y., Sun, D., Wang, Y., et al., 2016. Genetic modification of plant cell walls to enhance biomass yield and biofuel production in bioenergy crops. Biotechnol. Adv. 34, 997–1017.

Wi, S.G., Choi, I.S., Kim, K.H., Kim, H.M., Bae, H.-J., 2013. Bioethanol production from rice straw by popping pretreatment. Biotechnol. Biofuels 6, 166.

Wu, Z.L., Zhang, M.L., Wang, L.Q., Tu, Y.Y., Zhang, J., Xie, G.S., Zhou, W.H., Li, F.C., Guo, K., Li, Q., Gao, C.B., Peng, L.C., 2013. Biomass digestibility is predominantly affected by three factors of wall polymer features distinctive in wheat accessions and rice mutants. Biotechnol. Biofuels 6, 183.

Wu, Z., Hao, H., Zahoor, Tu., et al., 2014. Diverse cell wall composition and varied biomass digestibility in wheat straw for bioenergy feedstock. Biomass Bioenergy 70, 347–355.

Xie, G., Peng, L., 2011. Genetic engineering of energy crops: a Strategy for biofuel production in China. J. Integr. Plant Biol. 53, 143–150.

Xu, N., Zhang, W., Ren, S., Liu, F., Zhao, C., Liao, H., Xu, Z., Huang, J., Li, Q., Tu, Y., Yu, B., Wang, Y., Jiang, J., Qin, J., Peng, L., 2012. Hemicelluloses negatively affect lignocellulose crystallinity for high biomass digestibility under NaOH and H_2SO_4 pretreatments in *Miscanthus*. Biotechnol. Biofuels 5, 58.

Zahoor, Tu, Yuanyuan, 2014. Pretreatments to enhance the digestibility of wheat straw. Intern. J. Ren. Sust. Energy 3 (1), 26–34.

Zhang, W., Yi, Z., Huang, J., Li, F., Hao, B., Li, M., Hong, S., Lv, Y., Sun, W., Ragauskas, A., Hu, F., Peng, J., Peng, L., 2013. Three lignocellulose features that distinctively affect biomass enzymatic digestibility under NaOH and H_2SO_4 pretreatments in *Miscanthus*. Bioresour. Technol. 130, 30–37.

Zhao, Y.L., Dolat, A., Steinberger, Y., Wang, X., Osman, A., Xie, G.H., 2009. Biomass yield and changes in chemical composition of sweet sorghum cultivars grown for biofuel. Field Crop Res. 111, 55–64.

Zhong, C., Lau, M.W., Balan, V., Dale, B.E., Yuan, Y.J., 2009. Optimization of enzymatic hydrolysis and ethanol fermentation from AFEX-treated rice straw. Appl. Microbiol. Biotechnol. 84, 667–676.

Zhu, S., Huang, W., Huang, W., Wang, K., Chen, Q., Wu, Y., 2015. Pretreatment of rice straw for ethanol production by a two-step process using dilute sulfuric acid and sulfomethylation reagent. Appl. Energy 154, 190–196.