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Steam-exploded biomass saccharification is predominately affected by lignocellulose porosity and largely enhanced by Tween-80 in *Miscanthus*

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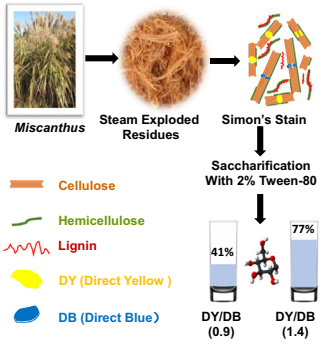
HIGHLIGHTS

- Diverse cell wall compositions in ten representative *Miscanthus* accessions.
- Largely varied biomass enzymatic saccharification in the stem-exploded (SE) residues.
- Supply with 2% Tween-80 led to much enhanced SE biomass enzymatic digestibility.
- Four wall polymer features negatively affected SE biomass saccharification.
- Lignocellulose porosity was the unique positive factor on SE enzymatic hydrolysis.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, total ten *Miscanthus* accessions exhibited diverse cell wall compositions, leading to largely varied hexoses yields at 17%–40% (% cellulose) released from direct enzymatic hydrolysis of steam-exploded (SE) residues. Further supplied with 2% Tween-80 into the enzymatic digestion, the Mis7 accession showed the higher hexose yield by 14.8-fold than that of raw material, whereas the Mis10 had the highest hexoses yield at 77% among ten *Miscanthus* accessions. Significantly, this study identified four wall polymer features that negatively affect biomass saccharification as $p < 0.05$ or 0.01 in the SE residues, including cellulose DP, Xyl and Ara of hemicellulose, and S-monomer of lignin. Based on Simons' stain, the SE porosity (defined by DY/DB) was examined to be the unique positive factor on biomass enzymatic digestion. Hence, this study provides the potential strategy to enhance biomass saccharification using optimal biomass process technology and related genetic breeding in *Miscanthus* and beyond.

1. Introduction

Lignocellulose has been considered as a major biomass resource for biofuels and chemicals. In principle, lignocellulose conversion involves in three major steps: physical and chemical pretreatments to disrupt plant cell wall; enzymatic hydrolysis to release soluble

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sugars; and yeast fermentation to produce ethanol (Wu et al., 2013). Because lignocellulose recalcitrance basically determines an unacceptably costly biomass process (Xie and Peng, 2011), it is important to identify the major factors of plant cell walls on biomass saccharification.

Plant cell walls are majorly composed of three wall polymers including cellulose, hemicellulose and lignin. It has been characterized that three wall polymer features basically decide biomass recalcitrance in plants. Cellulose crystalline index (CrI) and degree of polymerization (DP) are the major features that negatively affect biomass enzymatic saccharification under chemical pretreatments (Jia et al., 2014; Li et al., 2015, 2014c; Huang et al., 2015; Zhang et al., 2013), whereas arabinose substitution degree of hemicellulose has been reported as positive factor on biomass enzymatic digestibility by reducing cellulose CrI (Wu et al., 2013; Li et al., 2015). Furthermore, recent reports indicate that lignin plays dual effects on biomass saccharification, probably due to three monolignol proportions distinctive in different biomass materials (Li et al., 2014a,b; Studer et al., 2011).

Plant biomass is featured with a porous medium (Chen and Qiu, 2010; Duan et al., 2012). It has been proposed that there is a relation between lignocellulose enzymatic hydrolysis and pore size in the biomass residues (Tanaka et al., 1988; Divne et al., 1994). However, much remains unknown about different pore size impacts on biomass enzymatic hydrolysis, particularly in the steam-exploded biomass residues. Although steam explosion pretreatment largely reduces biomass particle size and changes biomass porous structures (Alvira et al., 2010; Kumar et al., 2009; Zhao and Chen, 2013), little is yet reported about effects of the lignocellulose composition and features on biomass porosity in plant species.

Simons' Stain (SS) is originally established to evaluate mechanical damage of beaten pulp fibers (Simons, 1950) and then used to detect wood fiber structure (Moore, 1953; Joutsimo and Robertsen, 2005). Hence, the SS method becomes a relatively easy assay based on competitive adsorption of two direct dyes in an aqueous environment, and it can provide useful information about the "overall" accessible surface area of a porous lignocellulosic substrate. More recently, the SS method has been applied to compare the relative accessibility of lignocellulosic substrates to cellulase (Esteghlalian et al., 2001; Chandra, 2008; Chandra et al., 2009, 2015, 2016; Meng et al., 2013, 2015). In principle, the blue dye molecules (diameter ~1 nm) can enter smaller pores, whereas the orange dye molecules (diameter ~5–36 nm) allow to penetrate the larger pores. Thus, the proportion of orange and blue dyes absorbed to the substrate can relatively indicate the distribution of small and large pores in a porous substrate (Esteghlalian et al., 2001; Chandra, 2008; Chandra et al., 2009, 2015, 2016; Meng et al., 2013, 2015).

As the initial step of biomass process, it is essential to find out the cost-effective and environment-friendly pretreatments for largely enhancing sequential biomass enzymatic hydrolysis (Wang et al., 2016). Among those pretreatments, the steam explosion process offers several attractive features compared with other technologies including significantly lower environmental impact, less hazardous process chemicals, and greater potential for energy efficiency (Alvira et al., 2010). Steam explosion has been characterized as a desired pretreatment for hemicellulose and lignin extraction and cellulose exposure at high temperature and pressure. For instance, steam explosion pretreatment has been used for enhancing biomass digestibility in rapeseed straw (Wood et al., 2014) and cotton stalks (Huang et al., 2015). In addition, Tween-80 is a powerful surfactant for increasing biomass enzymatic digestion in reed (Jin et al., 2016).

Miscanthus has been considered as one of leading lignocellulose-rich bioenergy crops for biofuels and chemical production (Brosse et al., 2012). Despite various physical and chemical pretreatments are applied in biomass process of *Miscanthus* (Huang et al., 2012; Xu et al., 2012; Li et al., 2013, 2014a,b; Zhang et al., 2013; Si et al., 2015), steam explosion pretreatment has not been performed in *Miscanthus* samples. In this study, we selected ten representative *Miscanthus* accessions, and compared lignocellulose enzymatic saccharification of their steam-exploded (SE) residues. We also detected much enhanced biomass enzymatic digestibility from Tween-80 co-supplement, which is a cheap surfactant (6.4 USD per 500 mL). Notably, we examined porosity of total ten SE biomass residues using Direct Yellow 11 (DY11) and Direct Blue 15 (DB15) staining, and observed unique positive impact of the porosity (DY/DB ratio) on biomass enzymatic hydrolysis. Hence, this study could further sort out mechanisms that link lignocellulose major features, SE porosity and biomass saccharification in *Miscanthus* accessions.

2. Materials and methods

2.1. Plant samples

The 5/6-year-old *Miscanthus* accessions were grown in Hanchuan and Wuhan experimental fields, and the mature stalks were harvested, dried at 50 °C, ground into powder through 40 mesh screen and stored in sealed dry container until in use.

2.2. Plant wall polymer extraction

The plant cell wall fraction method was used to extract hemicelluloses and cellulose as described by Peng et al. (2000) and Wu et al. (2013). Total hemicellulose was calculated based on total hexoses and pentoses determined in the hemicellulose fraction, and total hexoses were measured as cellulose in the cellulose fraction. All experiments were carried out in biological triplicate.

2.3. Colorimetric assay of hexoses and pentoses

An UV–vis spectrometer (V-1100D, Shanghai MAPADA Instruments Co., Ltd. Shanghai, China) was used to determine the hexoses and pentoses. Hexoses were detected by the anthrone/H_2SO_4 method (Fry, 1988), and the pentoses were measured by the orcinol/HCl method (Dische, 1962). The standard curves for hexoses and pentoses assay were drawn by using D-glucose and D-xylose as standards (purchased from Sinopharm Chemical Reagent Co., Ltd.), respectively. Regarding the high pentose levels that affect the absorbance reading at 620 nm for hexoses assay, the deduction from pentoses reading at 660 nm was carried out for a final hexoses calculation. A series of xylose concentrations were used for plotting the standard curve referred for the deduction, verified by GC–MS analysis. All experiments were conducted in triplicate.

2.4. Total lignin and monolignol assay

Total lignin content was measured by the two-step acid hydrolysis method according to the Laboratory Analytical Procedure of the National Renewable Energy Laboratory (Sluiter et al., 2008). Monolignols were detected by HPLC according to Li et al. (2014a). H-, G- and S-monolignol were purchased from Sinopharm Chemical Reagent Co., Ltd. as standards during HPLC analysis. Kromat Universal C18 column (4.6 mm × 250 mm, 5 μm) was used for HPLC analysis with SHIMADZU LC-20A machine with a UV-detector at 280 nm. CH_3OH: H_2O: HAc (25:74:1, v/v/v) was used as mobile phase (flow rate: 1.1 mL/min), the injection volume was 20 μL. All experiments were carried out in technological triplicate.

2.5. Hemicelluloses monosaccharide determination by GC–MS

Trifluoroacetic acid (TFA) and *myo*-inositol were purchased from Aladdin Reagent Inc. Acetic anhydride and acetic acid were obtained from Sinopharm Chemical Reagent Co., Ltd. 1-Methylimidazole was purchased from Sigma–Aldrich Co. LLC. Monosaccharide standards including L-rhamnose (Rha), L-arabinose (Ara), L-fucose (Fuc), D-xylose (Xyl), D-galactose (Gal), D-glucose (Glc) and D-mannose (Man), were obtained from Sinopham Chemical Reagent Co., Ltd. The hemicellulose samples were run by GC–MS (SHIMADZU GCMSQP2010) as described by Xu et al. (2012). The mass spectrometer was operated in the EI mode with ionization energy of 70 eV. Mass spectra were acquired with full scans based on the temperature program from 50 to 500 m/z in 0.45 s. Calibration curves of all analytes routinely yielded correlation coefficients in 0.999 or better.

2.6. Cellulose crystalline index (CrI) detection

The X-ray diffraction method was used for cellulose crystalline index (CrI) assay as described by Zhang et al. (2013). The Rigaku-D/MAX instrument (Ultima III, Japan) was used, and the well-mixed powders of biomass samples were analyzed under plateau conditions. The CrI was estimated using the equation: $\mathrm{CrI} = 100 \times (I_{200} - I_{\mathrm{am}})/I_{200}$ (Segal et al., 1959); I_{200} is intensity of the 200 peak (I_{200}, $\theta = 22.5°$), which represents crystalline cellulose. I_{am} (I_{am}, $\theta = 18.5°$) is the intensity at the minimum between the 200 and 110 peaks, which corresponds to amorphous cellulose. The CrI method was detected with ±SD from 0.05 to 0.15 using five representative samples in triplicate.

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

The dry biomass powders (0.2–1 g) of *Miscanthus* stem samples were extracted with 4 M KOH containing 1.0 mg/mL sodium borohydride (10 mL) at 25 °C for 1 h, and then centrifuged (2810×*g*) for 5 min. The pellet was re-extracted with 4 M KOH for one more time, and washed with distilled water five times until pH at 7.0. The remaining pellet was further extracted with 10 mL 8% $NaClO_2$ (8 g $NaClO_2$ dissolving in 100 mL distilled water followed with 1.5 mL glacial acetic acid) at 25 °C for 72 h ($NaClO_2$ change every 12 h). After centrifugation, the pellet samples were washed with distilled water for five times until pH at 7.0, and dried with vacuum suction filtration. The extracted crude cellulose was measured using the viscosity method (Puri, 1984) with minor modification (Huang et al., 2015). All experiments were performed at 25 ± 0.5 °C using an Ubbelohde viscosity meter. All experiments were performed in biological triplicate.

2.8. Steam explosion pretreatment

The dried *Miscanthus* stem biomass samples were pretreated under steam explosion (2.5 MPa, 180 s) using Steam Explosion Reactor (QBS-200, Hebi Zhengdao Machine Factory, Hebi, China). All conditions were described by Huang et al. (2015). The steam-exploded (SE) *Miscanthus* residues were dried and ground into powders through 40 mesh screen, and used for further experiments as described below.

2.9. Direct enzymatic hydrolysis coupled with Tween-80

Biomass direct enzymatic hydrolysis was performed as described by Jin et al. (2016), with minor modification. The biomass samples (raw materials and SE residues) were washed 2–3 times with 10 mL distilled water until the supernatants at pH 7.0, and once more with 10 mL of mixed-cellulase reaction buffer (0.2 M acetic acid-sodium acetate, pH 4.8). The washed samples were added with mixed-cellulases (containing β-glucanase $\geq 5.96 \times 10^4$ U and cellulase ≥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 2% (v/v). As a control, the sample was only added with 6 mL of reaction buffer, without Tween-80, then shaken under 150 r/min at 50 °C for 48 h. The samples were centrifuged at 3000×*g* for 5 min. The supernatants were collected for determining total pentose and hexose yields released from enzymatic hydrolysis. All samples were carried out in biological triplicate.

2.10. Simons' stain for pore size measurement

The alternate Simons' Staining (SS) procedure was applied as described by Chandra (2008) and Meng et al. (2015) with minor modification in direct dyes as suggested by Yu et al. (1995). Direct Blue 1 (Pontamine Fast Sky Blue 6BX) was replaced with Direct Blue 15 (Phenamine Sky Blue A Conc) and Direct Orange 15 (Pontamine Fast Orange 6RN) was replaced with Direct Yellow 11 provided by Pylam Products Co. Inc., Garden City, NY. The fractionation of the yellow dye to remove the low molecular weight part was performed using 100 (molecular weight cut off) ultracentrifugation membrane according to the method described by Chen et al. (2012).

Biomass samples (~100 mg) with 1 mL phosphate buffered sodium solution (PBS, 0.30 M PO_4, 1.40 M NaCl, pH 6.8) were added to each of six 15 mL Corning polypropylene centrifuge tubes. The DY 11 solution (1% or 10 mg/mL) was added in a series of increasing volumes (0.25, 0.50, 0.75, 1.0, 1.5, 2.0 mL) to each tube containing sample and PBS. The DB 15 solution (1% or 10 mg/mL) was also added to each tube in the same manner, thus preparing a set of tubes with a 1:1 mixture of DY and DB dyes. The final volume of the dyes mixture in the tubes was made up to 10 mL with distilled water, and the tubes were incubated at 70 °C for 6 h with constant shaking at 200 r/min. The gradient concentration was used to measure the dye adsorption isotherm. After cooling at room temperature, the tubes were centrifuged at 8000×*g* for 5 min, and the absorbance of the supernatant was measured at 410.5 nm (DY) and 612.5 nm (DB) on UV-1100 spectrophotometer, respectively.

The concentration of dyes in supernatant was calculated by solving the following two Eqs. (1) and (2) of Lambert-Beer law for binary solution simultaneously, and the amount of dye adsorbed onto the biomass was determined using the following adsorption Eq. (3):

$$A_{410.5\mathrm{nm}} = \varepsilon_{Y/410.5} L C_Y + \varepsilon_{B/410.5} L C_B \quad (1)$$

$$A_{612.5\mathrm{nm}} = \varepsilon_{Y/612.5} L C_Y + \varepsilon_{B/612.5} L C_B \quad (2)$$

$$\mathrm{Ae} = (\mathrm{Ci} - \mathrm{Ce}) \times \mathrm{V}/(\mathrm{M} \times 1000) \quad (3)$$

where A is the absorbance of the dye mixture at 410.5 or 612.5 nm, ε is the extinction coefficient of each dye at the respective wavelength, and L is the path length (1 cm cuvette width). The extinction coefficients were calculated by preparing standard curves of each dye and measuring the slope of their absorbance at 410.5 and 612.5 nm. The calculated values used in this study were $\varepsilon_{Y/410.5} = 31.83$, $\varepsilon_{B/410.5} = 3.418$, $\varepsilon_{Y/612.5} = 0.143$, $\varepsilon_{B/612.5} = 23.96$ L g^{-1} cm^{-1}. Ae is the amount of dye adsorbed onto the biomass at equilibrium (mg/g), Ci is the initial dye concentration added (mg/L), Ce is the dye concentration in solution at equilibrium (mg/L), M is the mass of biomass used (g) and V is the total volume of dye mixture (mL). All samples were carried out in technological triplicate.

2.11. Statistical calculation of correlation coefficients

Correlation coefficients were generated by performing spearman rank correlation analysis for all the measured traits across *Miscanthus* samples from different treatments (Xu et al., 2012; Li et al., 2013). The analysis used average values calculated from all original determinations values.

3. Results and discussion

3.1. Distinct wall polymer extraction from steam explosion pretreatment in ten Miscanthus accessions

In this study, we selected total ten representative *Miscanthus* accession samples and examined their cell wall compositions (cellulose, hemicellulose, lignin) in the raw materials (Table 1). As a comparison, cellulose contents of ten raw material samples varied from 26.64% to 41.45%, hemicellulose levels ranged from 22.89% to 32.11% and lignin contents were from 19.99% to 24.79%, indicating a diverse cell wall composition of ten *Miscanthus* accessions. Using our well-established steam explosion condition (Huang et al., 2015), the steam explosion pretreatment could largely extract hemicellulose by 52%–67% in all ten *Miscanthus* samples, whereas it led to the significant lignin removal by 16%–42% in eight samples (Table 1). As a consequence, six SE biomass samples showed significantly increased cellulose levels by 17%–71% as $p < 0.05$ or 0.01. Despite that steam explosion pretreatment has reportedly extracted hemicellulose and lignin, this study identified the exceptional *Miscanthus* accession samples without significant lignin extraction and cellulose increase. More importantly, Mis6 sample exhibited extremely high lignin extraction by 42% and Mis9 sample had much increased cellulose by 71%, quite different from previous reports in other biomass samples (Huang et al., 2015). Hence, the data indicated that total ten *Miscanthus* accession samples are distinctive for wall polymer extraction from steam explosion pretreatment, probably due to their diverse and characteristic cell wall compositions.

3.2. Varied biomass enzymatic saccharification of Miscanthus samples enhanced by Tween-80

Biomass enzymatic saccharification (or digestibility) has been defined by measuring the hexoses yield (% cellulose) released from enzymatic hydrolysis of the biomass residues (Xu et al., 2012; Jin et al., 2016). Without any pretreatment, total ten *Miscanthus* accessions exhibited much low hexoses yields at 5%–15% (% cellulose) from direct enzymatic hydrolyses of raw materials (Fig. 1A, Table S1). By comparison, ten SE samples exhibited hexoses yields from 17% to 40% (Fig. 1B), with increased ratios of hexoses yields from 2.3 to 7.0 relative to the raw materials (Table S1). Notably, supplied with 2% Tween-80 into enzymatic hydrolysis, the SE samples showed the hexoses yields from 41% to 77% (Fig. 1C), with ratios from 5.3 (Mis2) to 14.8 (Mis7) against the raw material (Table S1). Because the Mis10 accession had the highest hexoses yield released from enzymatic hydrolysis of SE residues coupled with Tween-80, it could be applied as the desired genetic material for *Miscanthus* bioenergy crop breeding. In addition, under supplements with higher concentrations of Tween-80, most *Miscanthus* samples did not show significantly increased hexoses yields (data not shown). On the other hand, large variations of hexoses yields among ten *Miscanthus* accessions should be due to their diverse cell wall compositions, in supporting for the previous assumption that lignocellulose feature basically determines biomass enzymatic saccharification (Wang et al., 2016; Zhu et al., 2008).

Table 1
Cell wall composition (% dry matter) of raw materials and steam-exploded residues of *Miscanthus* accessions.

Cell wall composition	Biomass sample	*Miscanthus* accession									
		Mis1	Mis2	Mis3	Mis4	Mis5	Mis6	Mis7	Mis8	Mis9	Mis10
Cellulose	Raw	34.32 ± 0.96	35.57 ± 1.59	41.45 ± 1.07	38.94 ± 1.70	35.47 ± 0.52	38.33 ± 0.73	37.29 ± 0.21	27.07 ± 1.50	26.64 ± 0.60	35.18 ± 1.17
	SE	38.71 ± 2.65	45.35 ± 0.65**	43.06 ± 1.83	45.42 ± 0.33*	40.22 ± 1.23	41.32 ± 0.47	43.58 ± 0.35**	43.75 ± 2.77**	45.64 ± 1.24**	42.38 ± 0.87**
			27.65%[@]		16.64%			16.87%	61.62%	71.32%	20.47%
Hemicellulose	Raw	30.65 ± 0.81	26.69 ± 0.77	31.97 ± 0.48	24.78 ± 0.76	32.11 ± 0.59	27.21 ± 0.80	27.70 ± 1.13	29.90 ± 0.02	24.77 ± 0.30	22.89 ± 0.88
	SE	14.44 ± 0.80**	12.65 ± 0.28**	13.36 ± 0.26**	11.47 ± 0.26**	11.70 ± 0.22**	10.36 ± 0.06**	10.84 ± 0.13**	9.87 ± 0.19**	10.31 ± 0.25**	11.08 ± 0.24**
		−52.89%	−52.60%	−58.21%	−53.71%	−63.56%	−61.93%	−60.87%	−66.99%	−58.38%	−51.59%
Lignin	Raw	24.79 ± 0.30	24.14 ± 0.25	24.21 ± 1.43	24.13 ± 0.23	23.44 ± 0.62	22.53 ± 0.35	23.31 ± 0.21	21.60 ± 0.53	19.99 ± 0.60	20.46 ± 0.36
	SE	20.73 ± 0.34**	18.45 ± 0.73**	17.41 ± 0.42**	16.45 ± 0.58**	19.01 ± 0.09**	15.86 ± 0.44**	17.17 ± 0.93**	16.21 ± 1.16**	17.62 ± 1.18	17.16 ± 0.83
		−16.38%	−23.57%	−28.09%	−31.83%	−18.90%	−42.06%	−26.34%	−24.95%		

[@] Percentage of the increased or decreased level between raw material and steam-exploded (SE) biomass residue: subtraction of two values divided by raw material. The data as mean ± SD (n = 3). * and ** indicated significant difference between raw and SE by *t*-test at $p < 0.05$ and 0.01 (n = 3).

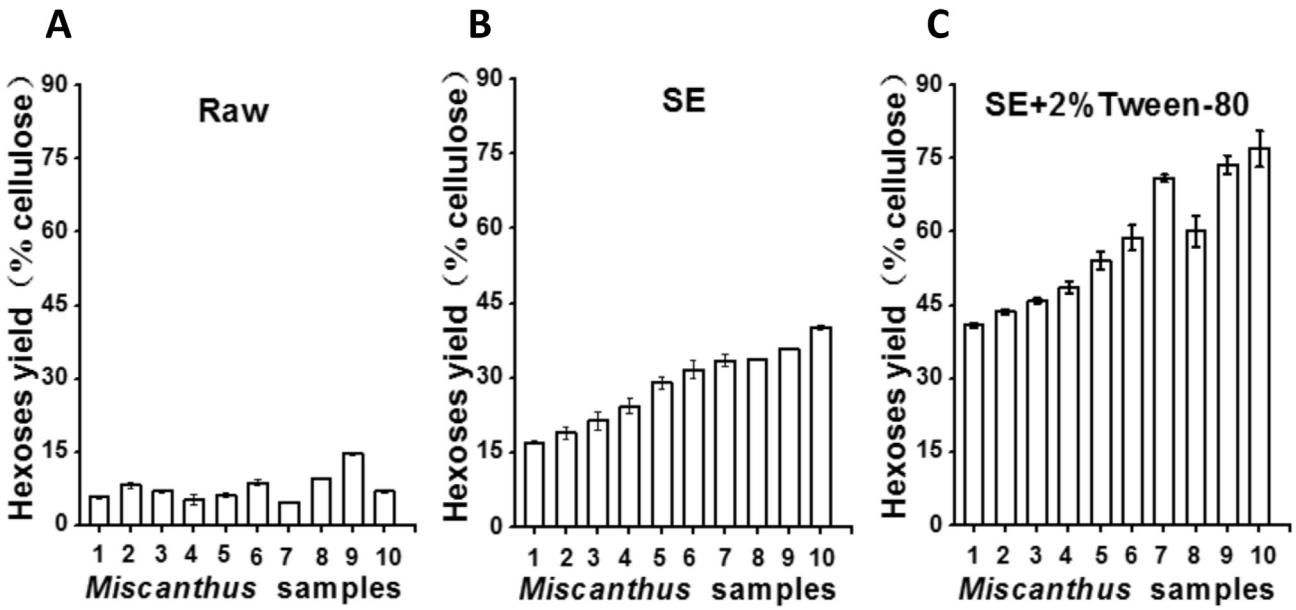

Fig. 1. Hexoses yields (% cellulose) released from direct biomass enzymatic hydrolysis of ten *Miscanthus* accession samples: (A) raw material, (B) steam-exploded (SE) residues and (C) SE residues co-supplied with 2% Tween-80; the bar as mean ± SD (n = 3).

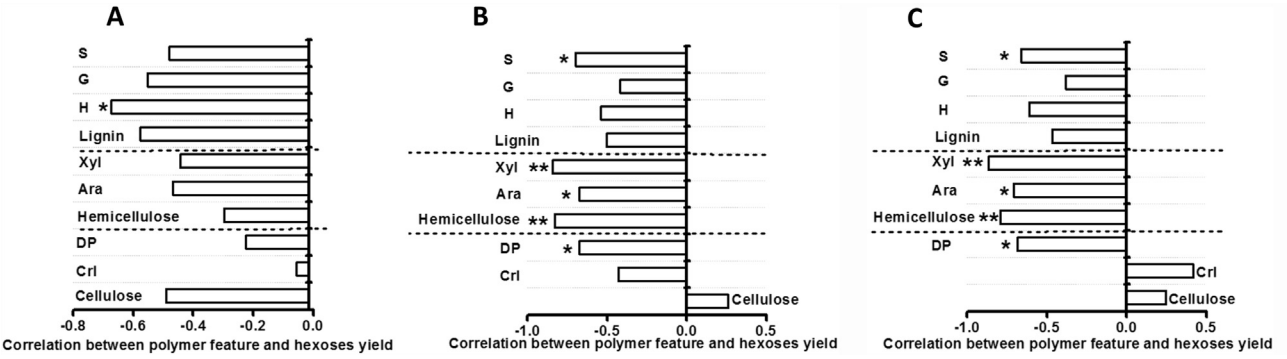

Fig. 2. Correlation analysis between wall polymer features and hexoses yields released from direct biomass enzymatic hydrolysis in ten *Miscanthus* accession samples: (A) raw material, (B) SE residues and (C) SE residues co-supplied with 2% Tween-80; * and ** indicated significant correlation as $p < 0.05$ and 0.01 (n = 10).

3.3. Negative impacts of lignocellulose features on biomass enzymatic saccharification

To understand large variations of biomass saccharification in the SE biomass residues, we determined three major wall polymer features of the ten *Miscanthus* accessions, including cellulose CrI and DP, major monosaccharides (Xyl, Ara) of hemicellulose, and three monomers of lignin (Table S2). As a comparison, ten *Miscanthus* accessions exhibited distinct wall polymer features in both raw materials and SE residues, consistent with their diverse cell wall compositions and biomass enzymatic saccharification.

Correlation analysis has been applied to examine wall polymer feature impacts on biomass digestibility (Pei et al., 2016; Wei et al., 2016; Li et al., 2015). In this study, we performed correlation analyses between three major wall polymer features and hexoses yields released from enzymatic hydrolysis of ten *Miscanthus* samples (Fig. 2). Except H-monomer, all other wall polymer features did not show any significant correlation with biomass saccharification in the raw materials (Fig. 2A). By comparison, four wall polymer features exhibited significantly negative impacts on biomass enzymatic digestibility in the SE biomass residues as $p < 0.05$ or 0.01 including cellulose DP, Xyl and Ara levels, and S-monomer content (Fig. 2B), different from the previous reports in physical (hot water) and chemical (acid, alkali) pretreated biomass residues (Si et al., 2015; Li et al., 2014a; Jin et al., 2016). Notably, those four wall polymer features remained negative effects on the SE enzymatic digestion supplied with 2% Tween-80 (Fig. 2C), consistent with the assumption that the wall polymer features predominately affect biomass saccharification (Wang et al., 2016). The results also suggest that the steam explosion pretreatment could distinctly alter lignocellulose features compared to other physical and chemical pretreatments.

3.4. Positive effect of lignocellulose porosity on biomass enzymatic digestibility

In terms of the four wall polymer features all showing negative impacts on biomass saccharification described above, we attempted to find out any positive factors by measuring biomass porosity of the SE residues in ten *Miscanthus* accessions (Table S3). In this work, Simon's stain (SS) method was used to evaluate the accessibility of a substrate by applying a two-color differential stain: the DB15 stain for smaller-size pores and the DY11 dye for relatively large-size pores. The maximum dye adsorption capacity of *Miscanthus* biomass was measured by using non-linear regression of Langmuir isotherm model in Excel SOLVER

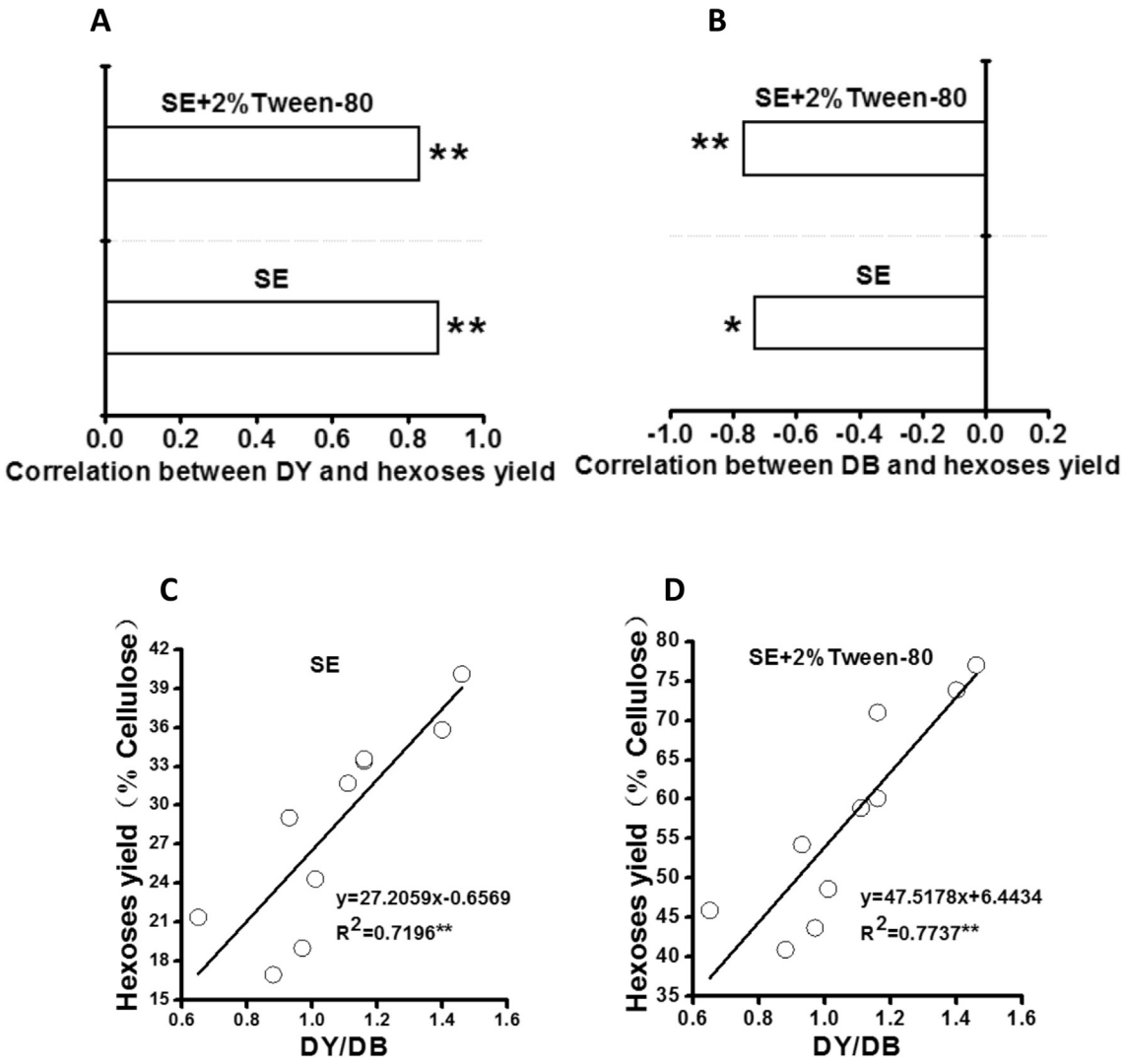

Fig. 3. Correlation analysis between lignocellulose porosity and biomass enzymatic saccharification in the SE residues of ten *Miscanthus* accession samples: correlation coefficients among DY (A) or DB (B) or DY/DB (C, D) and hexoses yields (% cellulose) released from enzymatic hydrolysis with or without 2% Tween-80; * and ** indicated significant correlation as $p < 0.05$ and 0.01 (n = 10).

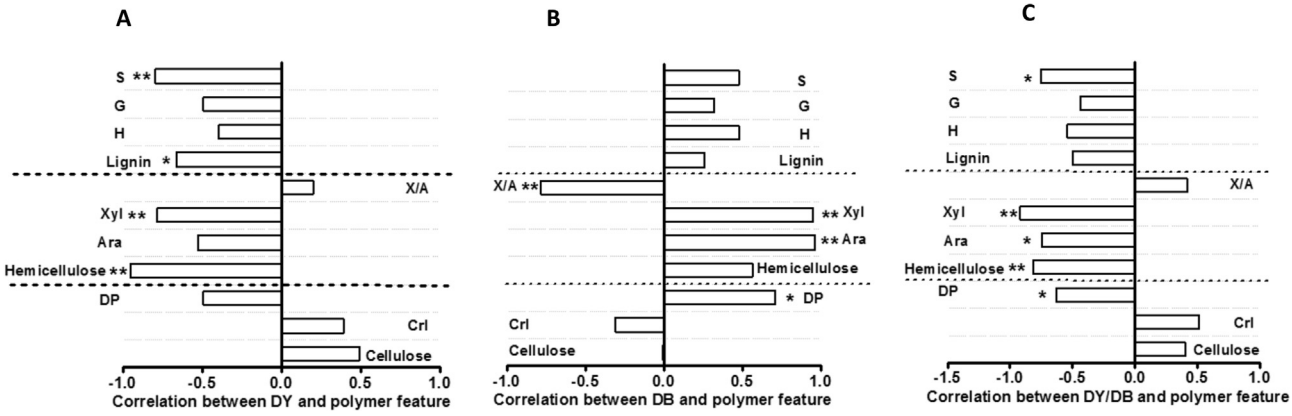

Fig. 4. Correlation analysis between wall polymer features and lignocellulose porosity in the SE residues of ten *Miscanthus* accession samples: correlation coefficients among DY (A) or DB (B) or DY/DB (C) and wall polymer features; * and ** indicated significant correlation as $p < 0.05$ and 0.01 (n = 10).

function as described by Brown (2001). The ratio of adsorbed DY and DB (DY/DB) was further calculated to estimate the porosity accounting for cellulase enzyme accessible ability and relative loading dosage (Meng et al., 2013). As a result, ten *Miscanthus* accessions had largely varied DY or DB values in the SE residues, leading to the DY/DB ratios ranged from 0.65 to 1.46 (Table S3).

Notably, the Mis10 sample had the highest DY/DB ratio (1.46), consistent with its highest biomass saccharification among ten *Miscanthus* accessions.

To detect SE porosity impact on biomass saccharification, we also performed a correlation analysis (Fig. 3). As a comparison, the DY values of SE samples exhibited significantly positive correlation with the hexoses yields released either from direct enzymatic hydrolysis or co-supplied with 2% Tween-80 (Fig. 3A), whereas the DB values had the negative impacts as $p < 0.05$ or 0.01 in the ten *Miscanthus* accessions (Fig. 3B). As a consequence, the DY/DB ratio showed significantly positive impact on the hexoses yields as $p < 0.01$, with high R^2 values at 0.72 and 0.77 (Fig. 3C and D). Hence, despite that the four wall polymer features negatively affect SE biomass saccharification as described above, the DY or DY/DB should be the unique positive factor for enhancing biomass enzymatic digestibility in the SE residues of *Miscanthus* accessions.

3.5. Mechanisms that link wall polymer feature, lignocellulose porosity and biomass saccharification

To understand the positive impacts of lignocellulose porosity on biomass saccharification, correlation analysis was conducted among wall polymer features and lignocellulose porosity in ten *Miscanthus* accessions (Fig. 4). As a result, only two wall polymer features (Xyl and S-monomer) exhibited significant negative correlation with DY values in the SE residues as $p < 0.01$ (Fig. 4A), indicating that DY should not much associate with the other two wall polymer features (cellulose DP and Ara) that significantly affect biomass saccharification (Fig. 2B). It also indicated that hemicellulose and lignin levels negatively affect DY values in the SE residues of ten *Miscanthus* accessions. Hence, the amounts of large-size pore (DY) should be majorly decided by the Xyl and S-monomer levels in the SE residues. By comparison, the DB values showed a positive correlation with three wall polymer features (cellulose DP, Xyl, Ara) as $p < 0.05$ or 0.01 (Fig. 4B), suggesting that S-monomer should not much affect the amounts of small-size pore (DB) in the SE residues. Notably, the DY/DB ratio exhibited significantly correlations with all four wall polymer features that negatively affect biomass enzymatic digestibility with/without Tween-80 (Figs. 4C; 2B and C). Because the DY/DB is negatively correlated with the four polymer features, it should be the unique parameter that could completely reflect positive impacts of lignocellulose porosity on the direct enzymatic saccharification in SE residues of *Miscanthus* accessions. Taken all together, a model was proposed to interpret how the SE porosity plays unique enhancement role in biomass enzymatic digestions by reducing the four wall polymer features (Fig. 5). It also provides the potential strategy for either genetic engineering of *Miscanthus* as bioenergy crop or optimal technology for biomass process in *Miscanthus* accessions and beyond.

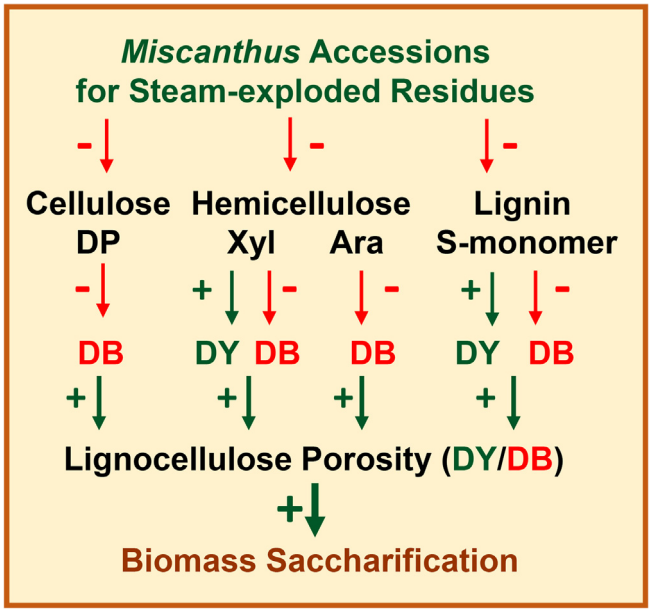

Fig. 5. A hypothesis model links wall polymer features and lignocellulose porosity in the SE residues of *Miscanthus* accessions: "+" and "−" Indicated for increasing and reducing polymer features, lignocellulose porosity, and biomass saccharification, which could be applied in genetic breeding or biomass process in bioenergy *Miscanthus* crops.

4. Conclusion

Among ten representative *Miscanthus* accessions, steam explosion pretreatment led to much enhanced hexoses yields by 5.3–14.8 folds from enzymatic hydrolysis with 2% Tween-80 supplement, and in particular, the Mis10 accession had the highest hexoses yield at 77% (% cellulose). Correlation analysis demonstrated that four wall polymer features negative affected biomass saccharification in the SE residues of ten *Miscanthus* accessions, but the SE porosity defined as DY/DB was the unique positive factor on biomass digestion. It has thus suggested the potential strategy to increase biomass porosity for high biomass saccharification by reducing those four wall polymer features in *Miscanthus*.

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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.2017.04.114.

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