Bioresource Technology 243 (2017) 957–965

Contents lists available at ScienceDirect

Bioresource Technology

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

Biomass saccharification is largely enhanced by altering wall polymer features and reducing silicon accumulation in rice cultivars harvested from nitrogen fertilizer supply

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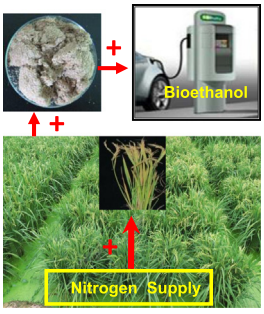
HIGHLIGHTS

- Rice samples were collected from field with seven nitrogen fertilizer supplies.
- Cellulose levels were increased in all samples from nitrogen fertilizer treatments.
- Silica levels were significantly reduced in all rice biomass samples.
- Cellulose DP and hemicellulosic Xyl/Ara were much decreased in biomass samples.
- Biomass saccharification were largely enhanced under various pretreatments.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 22 May 2017
Received in revised form 9 July 2017
Accepted 10 July 2017
Available online 12 July 2017

Keywords:
Rice
Nitrogen fertilizer
Wall polymer features
Biomass saccharification
Pretreatment

ABSTRACT

In this study, two rice cultivars were collected from experimental fields with seven nitrogen fertilizer treatments. All biomass samples contained significantly increased cellulose contents and reduced silica levels, with variable amounts of hemicellulose and lignin from different nitrogen treatments. Under chemical (NaOH, CaO, H_2SO_4) and physical (hot water) pretreatments, biomass samples exhibited much enhanced hexoses yields from enzymatic hydrolysis, with high bioethanol production from yeast fermentation. Notably, both degree of polymerization (DP) of cellulose and xylose/arabinose (Xyl/Ara) ratio of hemicellulose were reduced in biomass residues, whereas other wall polymer features (cellulose crystallinity and monolignol proportion) were variable. Integrative analysis indicated that cellulose DP, hemicellulosic Xyl/Ara and silica are the major factors that significantly affect cellulose crystallinity and biomass saccharification. Hence, this study has demonstrated that nitrogen fertilizer supply could largely enhance biomass saccharification in rice cultivars, mainly by reducing cellulose DP, hemicellulosic Xyl/Ara and silica in cell walls.

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http://dx.doi.org/10.1016/j.biortech.2017.07.057
0960-8524/

1. Introduction

Crop lignocellulose residues represent a renewable and abundant biomass resource for biofuels and other chemicals (Service, 2007). In terms of bioethanol production, biomass process principally involves in three major steps: physical and chemical pretreatments leading to wall polymer destruction, enzymatic digestion releasing soluble sugar and yeast fermentation producing bioethanol (Cardona and Sanchez, 2007). However, lignocellulose recalcitrance leads to an unacceptably costly biomass conversion, due to complicated structures and diverse biological functions of plant cell walls (Himmel et al., 2007). To reduce lignocellulose recalcitrance, genetic engineering and breeding are proposed as a promising solution by altering the major factors of plant cell walls that predominately affect biomass enzymatic digestibility in plants (Sun and Cheng, 2002; Cosgrove, 2005; Somerville et al., 2010; Xie and Peng, 2011; Margaritopoulou et al., 2016; Wang et al., 2016).

Plant cell walls are mainly composed of cellulose, hemicelluloses, lignin and pectic polysaccharides with minor structural proteins (Keegstra, 2010; Wang et al., 2016). Cellulose as the most abundant polymer, is composed of β-1, 4-glucan chains that form crystalline microfibrils by hydrogen bonds (Arioli et al., 1998). Cellulose crystallinity, defined by accounting for crystalline index (CrI) of lignocellulose from X-ray diffraction (XRD) pattern detection, has been reported as a key negative factor on biomass enzymatic hydrolysis in different plant species examined (Xu et al., 2012; Zhang et al., 2013; Wu et al., 2013; Jia et al., 2014). Meanwhile, the degree polymerization (DP) of cellulose is another factor on enzymatic hydrolysis (Huang et al., 2015; Li et al., 2015; 2017; Sun et al., 2017). Recently, it has indicated that cellulose crystallinity is positively correlated with its DP in *Miscanthus* (Zhang et al., 2013).

Hemicellulose is a class of heterogeneous polysaccharides, which positively affects biomass saccharification by reducing lignocellulose crystallinity (Scheller and Ulvskov, 2010; Li et al., 2013). In particular, arabinose (Ara) substitution degree of xylans (defined as reverse Xyl/Ara ratio), could positively affect lignocellulose digestibility upon various chemical pretreatments in *Miscanthus* and other grasses probably by Ara interaction with cellulose chains by hydrogen bonds (Li et al., 2013; Wu et al., 2013; Jia et al., 2014; Wang et al., 2016). By comparison, lignin is a stable water-proofing phenolic polymer consisting mainly of *p*-coumaryl alcohol (H), coniferyl alcohol (G), and sinapyl alcohol (S) (Sun et al., 2012). Due to its structural heterogeneity and diversity, lignin can greatly contribute to lignocellulosic recalcitrance (Chen and Dixon, 2007). However, recent results suggest that lignin should play dual roles in biomass enzymatic digestion, probably due to three monolignol proportions distinctive in different plant species (Li et al., 2014b,c; Si et al., 2015). In addition, silica is a cell wall component rich in cereals, and plays an active role in plant cell wall network formation. More recently, silica has been reported as the negative factor on biomass enzymatic hydrolysis in rice (Zhang et al., 2015).

Rice is a staple food crop worldwide, and produces appropriately 800 million metric tons of biomass straws per year (Chen and Peng, 2013). To increase rice grain yield, nitrogen fertilizer, one of the most important nutrients essential for plant growth, has been extensively applied into the crop fields (Li et al., 2014a; Miao et al., 2011). Despite that nitrogen supply could largely enhance rice grain and biomass production (Peng et al., 2010; Deng et al., 2015: Vergara-diaz et al., 2016; Makino, 2011), much remains unknown about its impacts on biomass composition and features, as well as lignocellulose enzymatic digestibility. For instance, although N fertilizers have reportedly affected plant growth and biomass yield in bioenergy crops such as switchgrass, sweet sorghum, *Miscanthus* and corn (Sindelar et al., 2015a,b), little is yet reported about the N fertilizer impacts on the wall polymer features that significantly affect biomass enzymatic digestibility in plants. In this study, we collected biomass samples of two rice cultivars from the experimental fields supplied with seven types of nitrogen fertilizer treatments. Then, we examined major alterations of cell wall compositions and wall polymer features in the mature straw of rice cultivars. Based on comparative and correlative analyses, this study identified the main factors of plant cell walls that predominately determine biomass enzymatic saccharification and bioethanol production in two rice cultivars.

2. Materials and methods

2.1. N fertilizer treatments and sample collection

The N fertilizer supply were conducted in two rice cultivars (Yangliangyou 6-YLY6, Lvdao Q7-LDQ7) in the experimental fields in 2013–2014 at Wuxue County 9 (30°0′N, 115°44′E) of Hubei Province in China. The plot size was 6.5 m × 5.5 m and the treatments were arranged in a split plot design. Seven N fertilizer treatments and control (CN) were performed by feeding the urea (kg ha^{-1}) into the fields at three stages of rice growth (basic fertilizer, midtillering and panicle initiation) with three independent biological duplications. The seven N fertilizer treatments (I–VII) included: I (90-0-40), II (90-40-0), III (90-80-0), IV (90-0-80), V (90-80-40), VI (90-40-80) and VII (90-80-80), with other basic fertilizer. All the plots received 90 kg P_2O_5 ha^{-1} and 75 kg ZnSO ha^{-1} applied as basic fertilizers. 225 kg K_2O ha^{-1} was applied with split doses at 50% each at the basic and panicle initiation stages. Urea was incorporated into the soil for one day before seedlings were planted, and broadcasted into fields at tillering and panicle initiation stages. Control plants received no urea during the whole growth duration. At mature stage, rice plant samples were harvested from the experimental fields, twenty plants were collected from each replicate and stems of ten plants were used for cell wall analysis. The collected straws were dried at 50 °C in oven after inactivation at 105 °C for 20 min, and the dried biomass tissues were ground into powder through a 40-mesh screen and stored in a dry container until use.

2.2. Wall polymer extraction

The wall polymers were extracted as described by Peng et al. (2000) and Wu et al. (2013). The soluble sugars, lipid, starch and pectin in the rice samples were sequentially 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 was two parallels; one parallel residues was extracted with trifluoroacetic acid (TFA) for monosaccharides. The remaining one was extracted with 4 M KOH with 1.0 mg/mL sodium borohydride for 1 h at 25 °C for determination of free hexoses and pentoses as the KOH-extractable hemicelluloses. The remaining non-KOH-extractable residues was further extracted with H_2SO_4 (67%, v/v) for 1 h at 25 °C, and the supernatants were collected for determination of free hexoses and pentoses 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

Hexoses and pentoses were determined using an UV–VIS spectrometer (V-1100D, Shanghai MAPADA Instruments Co., Ltd. Shanghai, China) as previously described by Xu et al. (2012). Hex-

oses were measured by the anthrone/H_2SO_4 method (Fry, 1988), and pentoses were detected by the orcinol/HCl method (Dische, 1962). Considering the pentose can affect hexose readings at 620 nm, deduction of pentose was conducted at 660 nm and a calibration curve was established to correct for hexose values with pentose values. For cellulose assay, sample was dissolved in 67% H_2SO_4 and total hexoses were calculated by the anthrone/H_2SO_4 method. All experimental analyses were performed in biological triplicates.

2.4. Hemicellulose monosaccharides determination by GC–MS

The monosaccharide composition of hemicellulose was detected through GC–MS (SHIMADZU GCMS-QP2010 Plus) as described by Li et al. (2013).

2.5. Lignin and monolignol assay by HPLC

Total lignin was obtained by two-step acid hydrolysis method according to analytical method of the National Renewable Energy Laboratory. Acid-insoluble lignin was measured gravimetrically after correction for ash, and then acid-soluble lignin was detected using UV spectroscopy. Three monolignols were determined by HPLC as previously described by Wu et al. (2013).

2.6. Silica assay

Silica content was determined using the methods described by Zhu and Lin (1990) and Zhang et al. (2015). The well dried biomass stem tissues were ground through 80 mesh screens. The well grinded biomass powder was treated for 30 min in a mixture of NaClO and 2 M NaOH, and mixed with 1 M H_2SO_4, 5% ammonium molybdate, 5% oxalic acid and 0.5% ascorbic acid. The silicon was photometrically determined under reading at 810 nm. All experimental analyses were carried out in biological triplicate.

2.7. Cellulose crystallinity detection

The X-ray diffraction method was used as previously described by Zhang et al. (2013) for detection of cellulose crystallinity (CrI) using the Rigaku-D/MAX instrument (Uitima III, Japan). The Standard error of the crystalline index CrI method was detected at ±0.05–0.15 using five representative samples in triplicate.

2.8. Determination of degree of polymerization (DP) of cellulose

The degree of polymerization of cellulose samples was determined using the viscosity method as described by Zahoor et al. (2017).

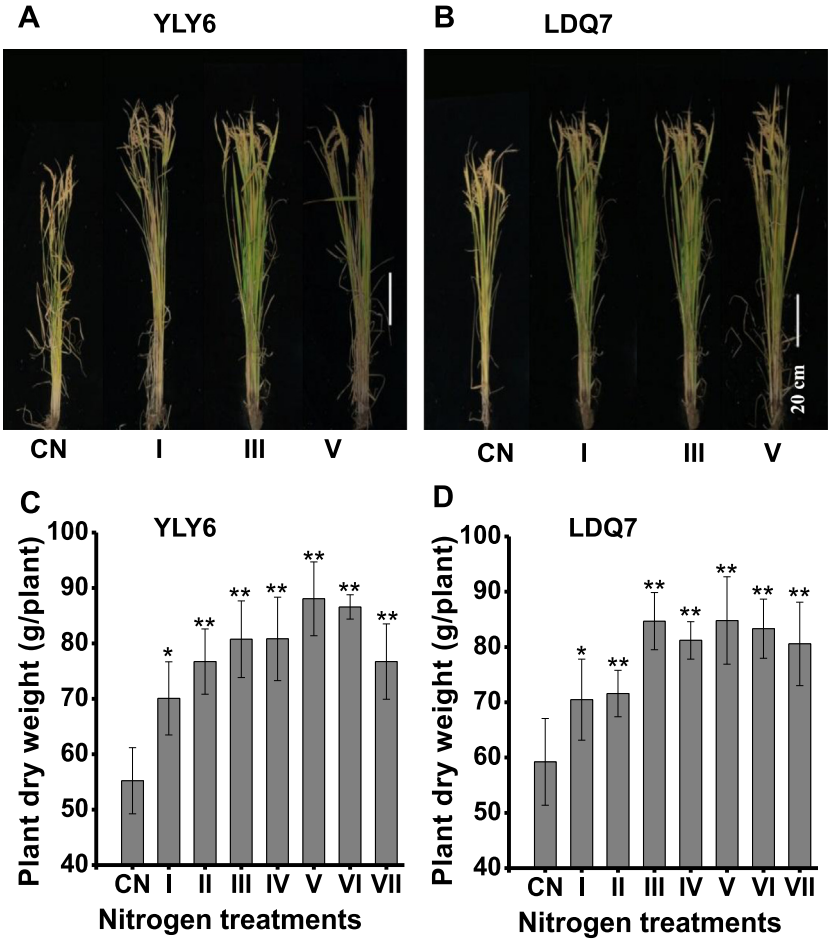

Fig. 1. Total biomass production in two rice cultivars under nitrogen (N) fertilizer supply. (A, B) Mature straws of two rice cultivars harvested from the fields with N fertilizer supplies; (C, D) Dry weight of total mature straws in two rice cultivars; * and ** As significant difference between control (CN) and seven N fertilizer treatments (I, II, III, IV, V, VI, VII) by *t*-test at $p < 0.05$ and 0.01; Bars indicated means ± SD (n = 3).

2.9. Biomass pretreatment

NaOH and H_2SO_4 pretreatments were described by Wu et al. (2013) and Zahoor et al. (2017). All experimental analyses were conducted in biological triplicates.

Lime (CaO) pretreatment was described by Pei et al. (2016). The well-dried biomass powder was added with 6 mL distill water and CaO at final concentrations (7.5% w/v). The sample tube with CaO solution was sealed and shaken at 50 °C, 150 rpm for 48 h. The supernatant was neutralized to pH 7.0 with H_2SO_4, then centrifuged. The remaining pellet was washed 3–6 times with 10mL distilled water until no detection of soluble sugar, stored at −20 °C for enzymatic hydrolysis. Samples with addition of 6 mL distilled water were shaken for 48 h at 50 °C as the control.

Hot water pretreatment: The well-dried biomass powder was added with 6 mL H_2O. The tube containing the sample was sealed and heated at 121 °C for 20, 60 and 90 min in autoclave (15 psi) after well mixing. The sample tubes were then shaken under 150 rpm for 2 h at 50 °C, and centrifuged at 3000*g* for 5 min. The pellet was washed 3–6 times with 10 mL distilled water, and stored at −20 °C for enzymatic hydrolysis. Samples with addition of 6 mL distilled water were shaken for 2 h at 50 °C as the control. All experimental analyses were conducted in biological triplicates.

2.10. Enzymatic hydrolysis

Enzymatic hydrolysis was performed as described by Wu et al. (2013) and Zahoor et al. (2017) using mixed-cellulases containing β-glucanase (≥2.98 × 104 U), cellulase (≥298 U) and xylanase (≥4.8 × 104 U) from Imperial Jade Bio-technology Co., Ltd. The samples were shaken at 150 rpm at 50 °C for 48 h during the enzymatic hydrolysis. For control, the samples with 6 mL of reaction buffer were shaken for 48 h at 50 °C. All experimental analyses were performed in biological triplicates.

2.11. Yeast fermentation and bioethanol assay

The yeast fermentation was performed using Saccharomyces cerevisiae strain (purchased from Angel yeast Co., Ltd., Yichang, China) and ethanol assay as described by Li et al. (2014c) and Zahoor et al. (2017). The experiments were conducted in biological triplicate.

2.12. Statistical calculation

Statistical calculations were performed by superior Performance Software Systems (SPSS 17.0, Inc., Chicago, IL). Correlative analysis was conducted using Spearman's rank correlation analysis for all measured traits across the two-sided level of significance ($^{*}p < 0.05$, $^{**}p < 0.01$). The line graph, histogram, other variation and regression analysis are developed using Origin 8.5 software (Microcal Software, Northampton, MA) from the experimental data for the best fit curve. The measured aspects were calculated from the average values of original triplications.

3. Results and discussion

3.1. Increased biomass productivity in two rice cultivars from nitrogen fertilizer supply

In this study, seven distinct nitrogen (N) fertilizer treatments (I–VII) were conducted in two rice cultivars (Yangliangyou 6-YLY6 and Lvdao Q7-LDQ7) by supplying urea, a classic nitrogen fertilizer, into experimental fields in three stages of rice growth and development

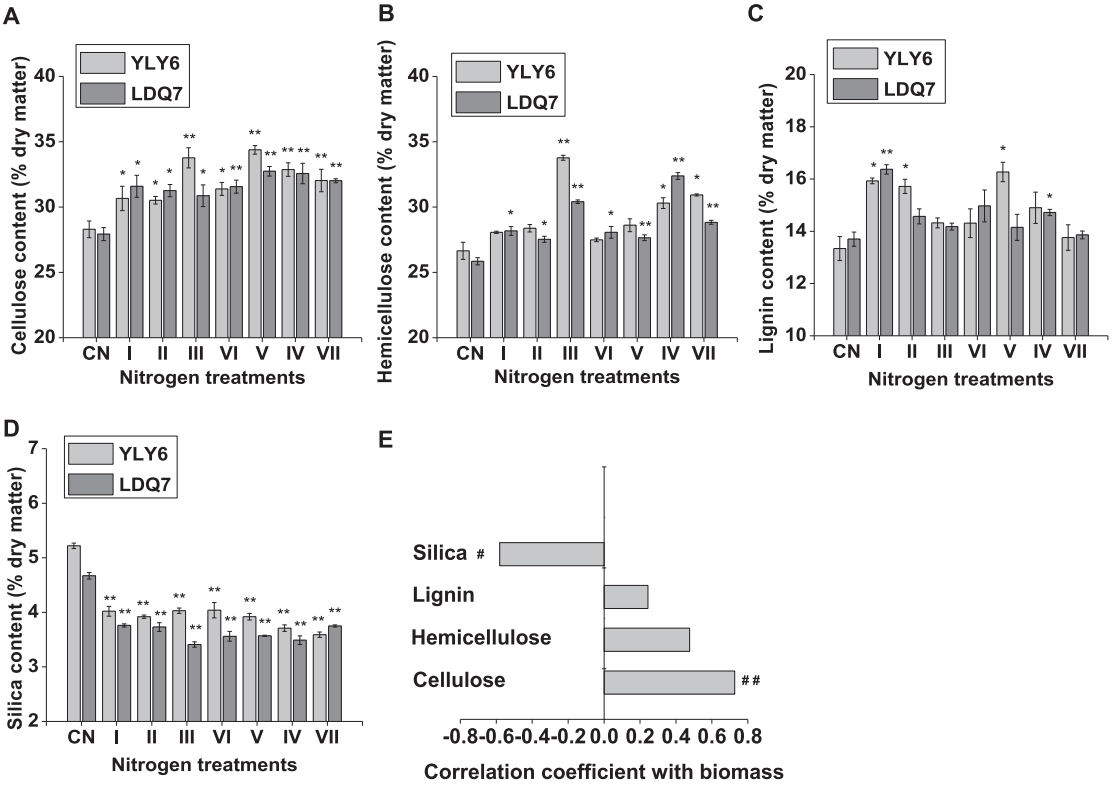

Fig. 2. Cell wall composition alterations in two rice cultivars under seven N fertilizer treatments. (A) Cellulose; (B) Hemicelluloses; (C) lignin; (D) Silica; (E) Correlation analysis between biomass levels and wall polymer contents (E), # and ## As significant correlation at $p < 0.05$ and 0.01 (n = 16); * and ** As significant difference between CN and seven N fertilizer treatments (I–VII) by *t*-test at $p < 0.05$ and 0.01; Bars indicated means ± SD (n = 3).

including basal fertilizer supply (before planting rice seedlings), mid-tillering and panicle initiation. As a result, all seven N fertilizer treatments could lead to much improved biomass/starch-related traits in two rice cultivars, compared with control (CN), including plant height, dry weight, tillers number, panicle weight and grain weight (Fig. 1A–D). In details, two rice cultivars, under N fertilizer supply, exhibited much increase in plant height from 4% to 12%, dry biomass by 19%–60%, dry spike (total grain yield) by 19%–54%, tillers number by 14%–48%, and grain weight by 3%–9% (Fig. 1C and D). In particular, correlative analysis showed that total dry biomass was positively correlated with tillers number and panicle weight at $p < 0.01$ level (n = 16), suggesting that N fertilizers should concurrently enhance both lignocellulose-based biomass and starch-derived grains in rice cultivars. Hence, the results were consistent with previous reports about N fertilizer enhancements to plant growth and biomass yields in bioenergy crops such as switchgrass, sweet sorghum, *Miscanthus* and corn (Sindelar et al., 2015a,b).

3.2. Altered cell wall compositions of biomass residues in two rice cultivars

With respect to the increase of dry biomass yields by N fertilizer supply, the cell wall compositions of biomass residues were determined in two rice cultivars (Fig. 2). Notably, all biomass samples exhibited largely enhanced cellulose levels ranged from 8% to 21%, but also had reduced silica contents by 24%–45% at $p < 0.05$ or 0.01 level (n = 3), compared with control (Fig. 2A and D). Meanwhile, two rice cultivars had variable hemicelluloses and lignin levels from different N fertilizer treatments (Fig. 2B and C). Further analysis indicated that total biomass (dry weight) exhibited a significant correlation either positively with cellulose level or negatively with silica content at $p < 0.05$ or 0.01 level, but correlation was not found with hemicellulose and lignin contents at $p > 0.05$ (Fig. 2E). Therefore, this study has indicated that the higher biomass yields should be majorly due to cellulose levels increased

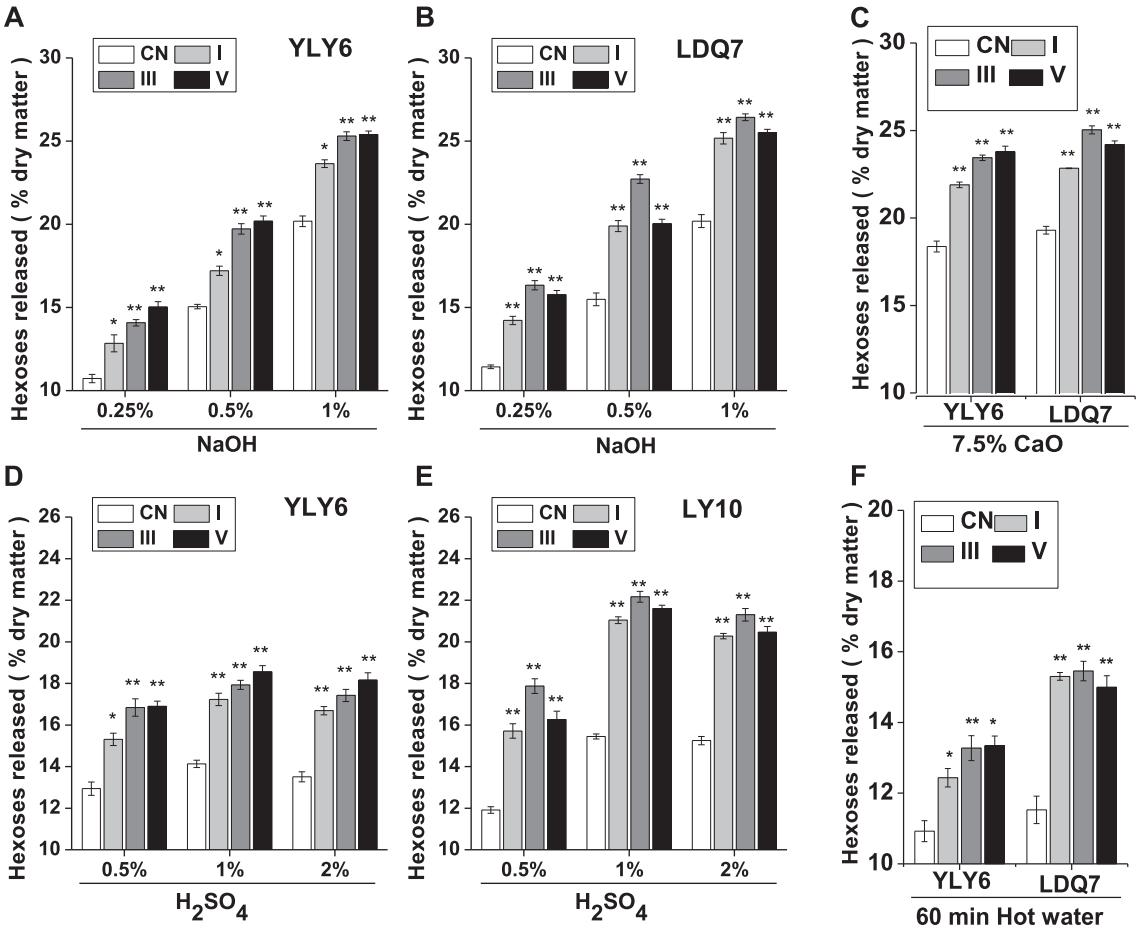

Fig. 3. Hexose yield (% dry matter) released from enzymatic hydrolysis after pretreatments with NaOH (A, B), CaO (C), H_2SO_4 (D, E), and hot water (F) in two rice cultivars under N fertilizer supply. * and ** As significant difference between CN and N fertilizer treatment (III) by *t*-test at $p < 0.05$ and 0.01; Bars indicated means ± SD (n = 3).

Table 1
Bioethanol yields from yeast fermentation using sugars from pretreatments and enzymatic hydrolyses.

Cultivar	N fertilizer	7.5% CaO pretreatment		1% H_2SO_4 pretreatment	
		% dry matter	g/plant	% dry matter	g/plant
YLY6	CN	10.82 ± 0.23	2.75 ± 0.06	10.49 ± 0.17	2.67 ± 0.04
	V	13.11 ± 0.21**	5.79 ± 0.09**	11.82 ± 0.22**	5.21 ± 0.10**
LDQ7	CN	10.10 ± 0.22	3.11 ± 0.07	9.56 ± 0.11	2.94 ± 0.03
	V	12.32 ± 0.31**	5.24 ± 0.13**	11.46 ± 0.13**	4.88 ± 0.05**

** Indicated significant difference between control (CN) and the N fertilizer treatment (V) by *t*-test at $p < 0.01$ (n = 3).

from N fertilizer supplies, leading to relatively reduced silica levels in two rice cultivars.

3.3. Enhanced biomass enzymatic saccharification and ethanol production

Biomass enzymatic saccharification (digestibility) has been measured by calculating the hexoses yield (% dry matter or% cellulose) released from cellulases hydrolysis of physical and chemical pretreated biomass residues (Xu et al., 2012; Wu et al., 2013). In this work, four pretreatments were performed in two rice cultivars using three chemicals (NaOH, CaO, and H_2SO_4) and hot water (Fig. 3). Compared with control, two rice cultivars under all seven N fertilizer treatments, exhibited much enhanced hexoses yields (% dry matter) ranged from 18% to 45% after pretreated with NaOH at three concentrations (0.25%, 0.5%, 1%) or with 7.5% CaO (Fig. 3A–C). Similarly, an increased biomass enzymatic saccharification was measured by 12%–36% in two rice cultivars after H_2SO_4 pretreatments at three concentrations (Fig. 3D and E). However, both rice cultivars showed relatively higher hexoses yields from alkali pretreatments than that of acid pretreatments, consistent with previous reports in rice and other plants (Lee et al., 2012; Wu et al., 2013; Li et al., 2015). In addition, all seven N fertilizer treatments could also lead to higher hexose yields than those of control in two rice cultivars by 11%–44% under hot water pretreatments at different incubation times (Fig. 3F). As cellulose level was predominately increased in total biomass (dry matter) from N fertilizer treatments, we also calculated the hexoses yields based on the percentage of cellulose in two rice cultivars. Notably, both rice cultivars under seven N fertilizer treatments, also exhibited significantly higher hexoses yields (% cellulose) than that of control, which confirmed the enhanced biomass enzymatic digestibility from N fertilizer supply in rice.

Furthermore, classic yeast fermentation was performed for bioethanol production using total soluble sugars released from two mild pretreatments (7.5% CaO; 1% H_2SO_4) and sequential enzymatic hydrolyses in representative samples (Table 1). The N fertilizer supply could lead to much higher bioethanol yields than that of control (CN) by 65–110% in two rice cultivars, consistent with the previous reports a in wheat and rice (Murozuka et al., 2014; Dhiman et al., 2015; Baldwin et al., 2017).

3.4. Altered wall polymer features in two rice cultivars

To understand the N fertilizer enhancements on biomass enzymatic saccharification, we determined three major wall polymer features including cellulose CrI and DP, monosaccharide composition of hemicellulose and three monomer constitution of lignin in two rice cultivars. Despite of the increase in cellulose levels, seven N fertilizer treatments could lead to a significant reduction in DP values of either crystalline cellulose or crude cellulose materials in two rice cultivars, compared with control (Fig. 4A and B), but their cellulose CrI values are much variable among different N fertilizer treatments. In terms of hemicellulosic monosaccharide compositions, two rice cultivars showed an increased Ara proportion (%) or reduced Xyl/Ara ratio in all seven N fertilizer treatments (Fig. 4C and D), but other monosaccharides are variable in different N treatments. In addition, three monolignols (G, S, H) proportions are also variable in two rice cultivar among seven N fertilizer treatments. Because wall polymer features largely affect biomass digestibility in different plant species examined (Wu et al., 2013; Jia et al., 2014; Li et al., 2015; Pei et al., 2016), these data also suggested that the altered major wall polymer features should be the

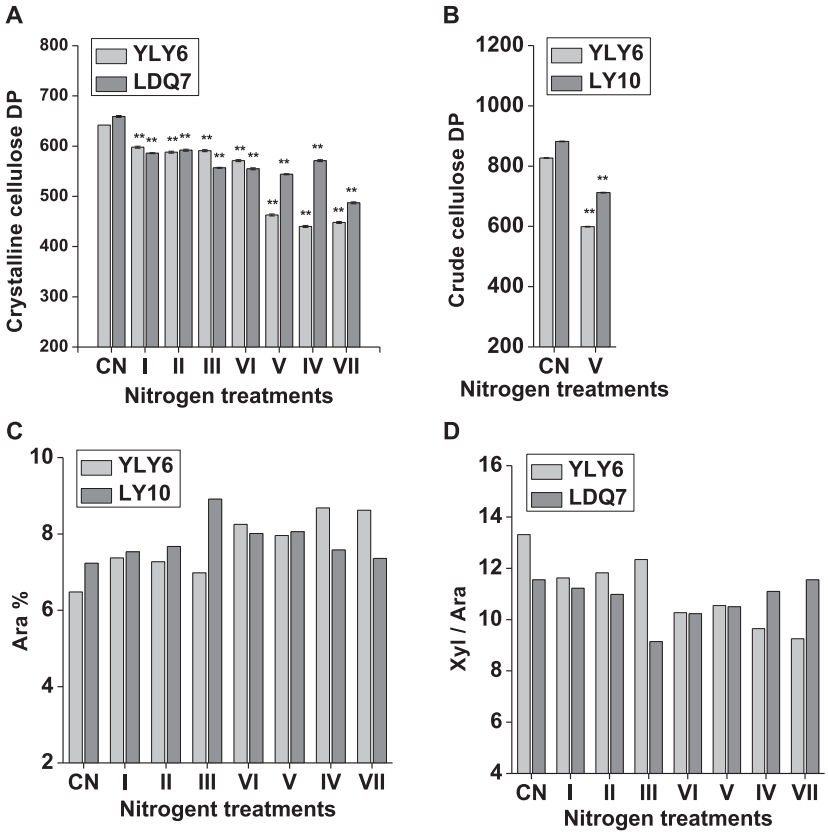

Fig. 4. Wall polymer feature alterations in two rice cultivars under seven N fertilizer treatments. (A) Crystalline cellulose DP; (B) Crude cellulose DP; (C) Hemicellulosic Ara; (D) Hemicellulosic Xyl/Ara; ** As significant difference between CN and seven N fertilizer treatments (I–VII) by *t*-test at $p < 0.01$ (n = 3).

important factors on hexoses yields released from enzymatic hydrolysis in two rice cultivars from N fertilizer supply.

3.5. Major wall polymer features affecting biomass digestibility

A correlation analysis has been well applied to account for wall polymer impacts on biomass enzymatic saccharification in rice and other plant species (Li et al., 2013; Wang et al., 2014, 2015). Despite it has been reported that three major wall polymer features distinctively affect biomass digestibility in rice (Wu et al., 2014; Zhang et al., 2015; Li et al., 2015), the correlative analysis indicated that only cellulose CrI values had significant effects on hexoses yields released from enzymatic hydrolysis at $p < 0.05$ or 0.01 level (n = 16) from all chemical (NaOH, CaO, H_2SO_4) and physical (hot water) pretreatments performed in two rice cultivars under sever N fertilizer treatments (Fig. 5A), consistent with the previous findings that cellulose CrI is the key negative factor on biomass enzymatic digestibility in all plant species examined (Xu et al., 2012; Wu et al., 2013; Jia et al., 2014; Li et al., 2015; Pei et al., 2016). By comparison, the monosaccharide proportions of hemicellulose could exhibit significant correlation with hexoses yields under partial pretreatments, whereas three monomers of lignin did not show any correlation, different from recent report about monolignol positive effects in rice mutants (Li et al., 2015). Furthermore, two major non-cellulosic polymers (hemicellulose, lignin) levels did not exhibit any significant impact on biomass saccharification, probably due to the N fertilizer supply that predominately increased cellulose-based biomass without much alteration on non-cellulosic polymer levels and features. Notably, the silica levels could negatively affect the hexoses yields at $p < 0.05$ or

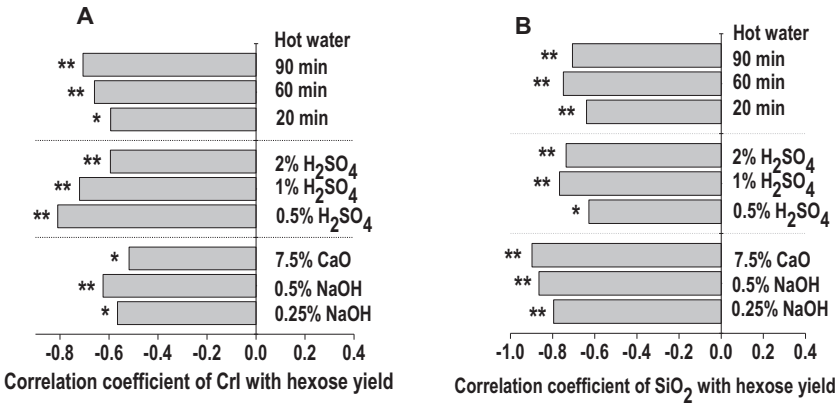

Fig. 5. Correlation analyses between wall polymer features and hexose yield released from enzymatic hydrolysis after various pretreatments in two rice cultivars under N fertilizer treatments. (A) Cellulose CrI; (B) Wall silica; * and ** As significant correlations at $p < 0.05$ and 0.01 (n = 16), respectively.

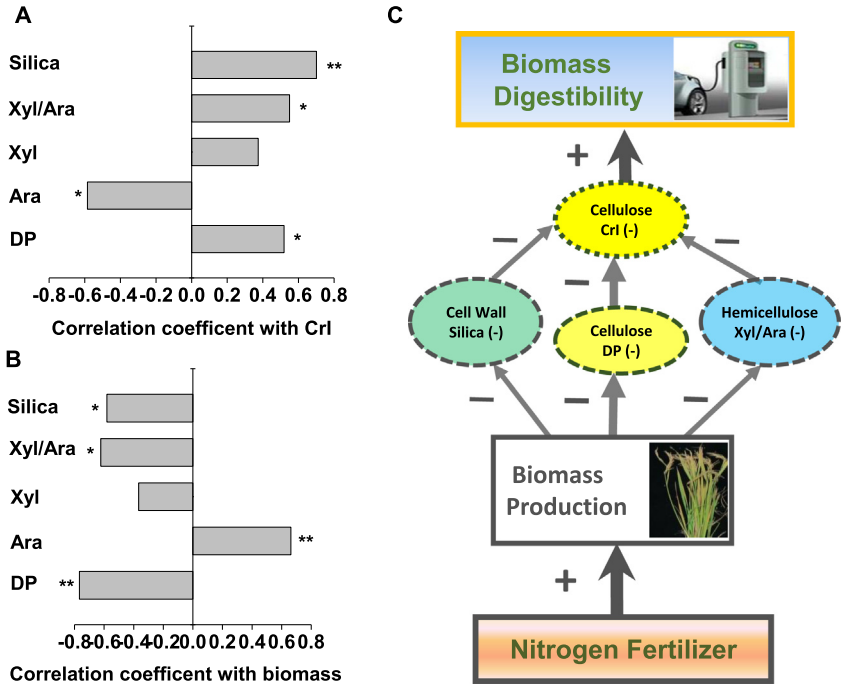

Fig. 6. A mechanism about the major wall polymer features impacts on biomass enzymatic saccharification in rice under N fertilizer supply. (A) Correlation analysis between cellulose CrI and other wall polymer features; (B) Correlation analysis between biomass yield and wall polymer features, * and ** As significant correlation coefficient values at $p < 0.05$ and 0.01 (n = 16), respectively; (C) A model about N fertilizer enhancements on biomass yield and enzymatic digestibility by altering wall polymer features; +/− As increased/reduced effect, respectively; (−) As a negative wall factor on biomass enzymatic saccharification.

0.01 level from almost all pretreatments performed, except 1% NaOH pretreatment (Fig. 5B). Such exception may be due to the 1% NaOH pretreatment capable for an effective extraction of silica, leading to a high enzymatic hydrolysis of lignocellulose. On the other hand, as the silica plays a role in cell wall network construction and mechanical strength maintaining (Zhang et al., 2015), it is understandable about silica negative effects on biomass enzymatic digestibility. Hence, either cellulose CrI or silica level could significantly play a negative role in lignocellulose enzymatic digestibility in rice from N fertilizer supply.

3.6. Mechanism of nitrogen fertilizer enhancements on biomass saccharification

To understand cellulose CrI and silica negative effects on biomass enzymatic digestibility, a correlative analysis was performed among wall polymer features. As a result, cellulose CrI value exhibited significant correlations either positively with cellulose DP value, Xyl/Ara ratio and silica level or negatively with four monosaccharide proportions of hemicellulose at $p < 0.05$ or 0.01 level in two rice cultivars under seven N fertilizer treatments (Fig. 6A). Similarly, positive correlations were also found between silica and cellulose DP or Xyl/Ara. In addition, three monolignols did not show any significant correlation with cellulose CrI or silica.

As both biomass level and biomass digestibility were largely enhanced in two rice cultivars by N fertilizer supply (Figs. 1–3), correlation analysis was further conducted between biomass level and wall polymer features. Despite of non-correlation with cellulose CrI, the biomass level exhibited significantly negative effects on cellulose DP value and Xyl/Ara (or positive on Ara) in two rice cultivars under N fertilizer treatments (Fig. 6B), as well on silica level as described above (Fig. 2D). In addition, the biomass levels were also correlated with H and S proportions.

Taken all together, a hypothesis model could be thus proposed towards understanding the mechanism of nitrogen fertilizer enhancements on biomass enzymatic saccharification (Fig.6C). First, seven N fertilizer treatments could consistently increase biomass productions in two rice cultivars (Fig. 1); Second, the N fertilizer treatments largely reduced cellulose DP value, hemicellulosic Xyl/Ara ratio and silica level (Figs. 2 and 4); Third, the cellulose CrI was negatively affected by the reduced cellulose DP, Xyl/Ara and silica. Finally, the decreased cellulose CrI, as a key negative factor, could be accounting for the much enhanced biomass enzymatic digestibility under various physical and chemical pretreatments in two rice cultivars from all seven N fertilizer treatments. In other words, the model has highlighted that the three negative wall factors (cellulose DP, Xyl/Ara, silica) could positively affect biomass enzymatic saccharification by reducing cellulose CrI in two rice cultivars under seven N fertilizer treatments. In addition, since N fertilizer could distinctively alter wall polymer features and reduce silica accumulation, it remains interesting to explore whether it also affects plant resistances to lodging and biotic stress in rice and other crops in the future.

4. Conclusions

Two rice cultivars had much increased lignocellulose-based biomass production from seven N fertilizer supplies into experimental fields. All biomass samples also exhibited largely enhanced biomass digestibility and bioethanol production under various physical and chemical pretreatments, probably due to reduced cellulose DP value, hemicellulosic Xyl/Ara ratio and silica levels in two rice cultivars from N fertilizer treatments. Correlation analysis further indicated that both cellulose DP and hemicellulosic Xyl/Ara could negatively affect cellulose crystallinity for enhancing biomass enzymatic saccharification.

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 the 111 Project of Ministry of Education of China and State Administration of Foreign Experts Affairs (B08032) – China.

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.2017.07.057.

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