Renewable and Sustainable Energy Reviews 132 (2020) 110107

Contents lists available at ScienceDirect

Renewable and Sustainable Energy Reviews

journal homepage: http://www.elsevier.com/locate/rser

High yield and high concentration glucose production from corncob residues after tetrahydrofuran + H_2O co-solvent pretreatment and followed by enzymatic hydrolysis

Fengpei Yao [a], Fei Shen [b], Xue Wan [b], Changwei Hu [a,*]

[a] Key Laboratory of Green Chemistry and Technology, Ministry of Education, College of Chemistry, Sichuan University, Chengdu, Sichuan, 610064, PR China
[b] Institute of Ecological and Environmental Sciences, Sichuan Agricultural University, Chengdu, Sichuan, 611130, PR China

ARTICLE INFO

Keywords:
Corncob residues
Glucose
High solid loading
Response surface method
Pseudo lignin

ABSTRACT

To obtain high yield and high concentration glucose from corncob residues (CRs), tetrahydrofuran (THF) + H_2O co-solvent pretreatment was employed. The pretreatment parameters were firstly optimized by Box-Behnken Design of response surface method, and the maximum glucose yield (498.2 mg/g CRs) was obtained via enzymatic hydrolysis after pretreated under optimized conditions (53.7% THF concentration, 202.3 °C, 1.05 h). Then, the pretreated CRs under optimized conditions (optimized-CRs) were enzymatically hydrolyzed at high solid loading (15%–20%) and low enzyme input (5–8 FPU/g cellulose). The high concentration glucose production (128.6 mg/mL) was obtained from optimized-CRs after enzymatic hydrolysis at 20% solid loading with 10 FPU/g cellulose for 72 h, and then showed potential for reduction of enzyme input. Finally, the interactions between the 9 substrate-related factors and cellulose conversion were analyzed through correlation analysis, and found that Klason lignin remained in the cellulose-rich fractions and pseudo lignin condensed on the surface of the recovered substrate were the predominant inhibitors for enzymatic hydrolysis.

1. Introduction

Increasing energy demand, climate change and environment pollution have encouraged the development of renewable resources to replace fossil ones in the past few decades, such as sunlight, hydrogen and biomass [1–3]. Due to the plentiful, CO_2-neutral, and widely available characters, lignocellulosic biomass is one of the most promising renewable resources and has been widely used to produce various high value-added chemicals, biofuels and materials [4–7]. However, low conversion efficiency is one of the crucial obstacles for lignocellulosic biomass utilization, so finding an approach to gain high yield and concentration of target products can better deal with this challenge [8–10].

Corncob residues (CRs) is one kind of typically wasted lignocellulose derived from furfural industry, and the production of CRs in China is approximately 2.5×10^7 tons per year [11]. Besides, hemicellulose in corncob has been almost removed after furfural extraction process, and the CRs has high content of cellulose (45%–65%) [12,13], which indicates that CRs is a suitable feedstock for glucose-based product manufacture. Although the firmly link between cellulose, hemicellulose and lignin of corncob has been partially broken down by sulfuric acid during furfural extraction process, complex and recalcitrant structures still exist in CRs, which are the obstacles for components fractionation and polysaccharides utilization [3,14]. According to previous studies, the cellulose conversion of unpretreated CRs was 10%–50% after enzymatic hydrolysis for 72 h or 96 h [15,16]. The efficiency of the cellulose-glucose bioconversion could be increased by adding auxiliary, such as Gleditsia saponin (GS) and rhamnolipid, both were surfactants which could reduce the adsorption of the cellulase on the residual lignin and decrease the surface tension of saccharification system to prevent the inhibition [11,17]. On the other hand, pretreatment is an essential step for biomass conversion, which is responsible for breaking down the recalcitrant structures, reducing cellulose crystallinity, and increasing the accessibility of cellulose and biomass components fractionation performance [9,18–20], so many pretreatment methods have been applied on CRs utilization. Chemical pretreatment methods, such as the mixture of green liquor (GL, the main composition is listed in Table 1), hydrogen peroxide and ethylenediaminetetraacetic acid (EDTA) [15], alkaline hydrogen peroxide [21], formic acid followed by alkaline hydrogen peroxide [22], the mixture of GL, ethanol and anthraquinone (AQ) [23], potassium hydroxide [24], bisulfite [16], and sodium bisulfite [25], have been used to boost the subsequent enzymatic

* Corresponding author.
E-mail address: changweihu@scu.edu.cn (C. Hu).

https://doi.org/10.1016/j.rser.2020.110107
Received 21 February 2020; Received in revised form 1 July 2020; Accepted 14 July 2020
Available online 27 July 2020
1364-0321/

Nomenclature

CRs	Corncob residues
ASL	Acid-soluble lignin
RSM	Response surface method
ANOVA	Analysis of Variance
CA	Correlation analysis
CrI	Crystallinity index
optimized-CRs	The pretreated CRs under optimized conditions
CELF	Co-solvent-enhanced lignocellulosic fractionation
GS	Gleditsia saponin
AQ	Anthraquinone
THF	Tetrahydrofuran
KL	Klason lignin
EDTA	Ethylenediaminetetraacetic acid
BBD	Box-Behnken Experimental Design
GL	Green liquor

polysaccharides of biomass could be dramatically decreased [27,32]. Some previous works added acid catalyst (0.5% H_2SO_4) in THF + H_2O co-solvent system and gained enhanced lignocellulosic biomass pretreatment and fractionation performances [32], but it had some admitting flaws. For example, the use of acid catalyst increased the requirements of the reaction vessel, and it could reduce the carbohydrates during co-solvent pretreatment and resulted in lower cellulose recovery [18]. Previous work found that 89.8% of lignin in CRs can still be degraded by THF + H_2O co-solvent (7:3, *v/v*) pretreatment (200 °C, 1 h) without acid catalyst, and high yield of monophenols (24.3%) was gained by further reaction (300 °C, 8 h) without hydrogen addition [13]. Furthermore, the solid recovered after pretreatment with high content cellulose could be promising feedstock for saccharification to produce sugar or other biofuels.

Thus, in present study, the THF + H_2O co-solvent pretreatment was employed followed by enzymatic hydrolysis to obtain high yield sugar and high concentration sugar titer from CRs. Briefly, Box-Behnken experimental Design (BBD) of response surface method (RSM) was firstly employed to optimize the THF + H_2O co-solvent pretreatment process to gain the maximum glucose yield from pretreated CRs, and the

Table 1
Comparison of glucose yield of CRs pretreated by different methods.

Pretreatment method and condition	Main composition		Main composition changes		Enzymatic hydrolysis solid loading %	Enzymes	Enzyme loading	Enzymatic hydrolysis condition	Cellulose conversion (%)	Glucose yield (g/100 g DS)	Refs.
	Cellulose (%)	Lignin (%)	Cellulose recovery (%)	Lignin removal (%)							
0.6 g H_2O_2/g DS, 6 mL GL[a]/g DS, 80 °C, 3 h	45.9	45.3	93.9	52.7	2.5	Celluclast 1.5 L, Novozyme 188	18 FPU/g cellulose, 27 CBU/g cellulose	50 °C, pH = 4.8, 96 h	83.6	36.0	[15]
1% NaOH (*w/w*), 1% H_2O_2 (*v/v*), 120 °C, 1 h	35.1	43	96.2	29.7	2	Celluclast 1.5 L, Novozyme 188	10 FPU/g cellulose, 20 IU/g cellulose	50 °C, pH = 4.8, 72 h	80.3	27.1	[21]
1:1 ethanol/H_2O, 1.0 mL GL/g DS, 0.4% (*w/w*) anthraquinone/g DS, 140 °C, 1 h	45.9	44.9	96.5	42.7	2.5	Celluclast 1.5 L, Novozyme 188	18 FPU/g cellulose, 27 CBU/g cellulose	50 °C, pH = 4.8, 96 h	85.3	37.8	[23]
0.6 g $NaHSO_3$/g DS, 100 °C, 3 h	30.14	43.9	86.4	22.4	2.5	Celluclast 1.5 L, Novozyme 188	18 FPU/g cellulose, 27 CBU/g cellulose	50 °C, pH = 4.8, 72 h	90.0	23.4	[16]
53.7% THF + 46.3% H_2O (*v/v*), 202.3 °C, 1.04 h	62.7	22.1	88.2	71.9	2	CTec 2	10 FPU/g cellulose	50 °C, pH = 5.0, 72 h	90.1	49.8	This paper

[a] The main component of green liquor (GL) was sodium carbonate (75.2 g/L), making up 75% of the liquid and the sodium hydroxide content was 23.0 g/L.

saccharification and bioethanol production. However, the glucose yield and cellulose conversion of the CRs after pretreated by these methods were not high enough. So, finding a new pretreatment method that can efficiently utilize the cellulose of CRs is imperative.

Tetrahydrofuran (THF), which is a polar aprotic solvent, can be easily manufactured from furfural and is miscible with water over a wide range of ratios [26]. Due to its renewability, low boiling point, easy for recover, and efficient fractionation performance, THF has been widely used for lignocellulosic biomass conversion [27]. Although as one of the volatile organic solvents, the toxicity for human and environment of THF has raised some concerns [28], Fowles et al. [29] concluded that the THF showed low acute toxicity, no mutagenicity, and was not a sensitizer or irritant. Recently, a new pretreatment method based on THF called co-solvent-enhanced lignocellulosic fractionation (CELF) has been developed for cellulosic biofuels production [30,31]. By CELF pretreatment, biomass delignification and sugar polymer deconstruction can be improved, and enzyme costs for high glucose yield from

effects of parameters on the main composition changes, enzymatic hydrolysis and glucose yield were also studied. Then, the CRs pretreated by optimized conditions were enzymatically hydrolyzed at high solid loading (15%–20%) with 10 FPU/g cellulose to gain high concentration glucose production and at 15% solid loading with low enzyme input (5–8 FPU/g cellulose) to evaluate the potential for reduction of enzyme input. Finally, the interactions between the 9 substrate-related factors and cellulose conversion were analyzed through correlation analysis (CA) to find the predominant inhibition for enzymatic hydrolysis after THF + H_2O co-solvent pretreatment, since finding inhibitors of cellulose conversion was a significant step towards more efficient cellulose-glucose bioconversion in biorefinery.

2. Materials and methods

2.1. Materials

The corncob residues sample gifted from Shandong Futaste Investment Co., Ltd (Shandong Province in China) was mechanically grinded into powder between 40 and 80 meshes. The main composition of CRs was determined by the modified National Renewable Energy Laboratory (NREL) general procedure proposed by Karimi and Taherzadeh [33], which included 62.7 ± 0.56% of cellulose (represented by glucan content), 22.1 ± 0.48% of Klason lignin (KL) and 3.1 ± 0.15% of acid-soluble lignin (ASL), 11.3 ± 0.94% of ethanol extractives, 2.2 ± 0.11% of ash and 3.5 ± 0.13% of unknown others. The hemicellulose (represented by xylan) in CRs has been almost removed (3.6 ± 0.57%) after furfural industrial process, so it would not take into account in this paper.

2.2. THF + H_2O co-solvent pretreatment

The THF + H_2O co-solvent pretreatments of CRs were carried out in a 600 mL Parr® autoclave reactor (Parr Instruments Co., Moline, IL) equipped with a double blade stirrer rotated at 400 rpm. In each pretreatment run, 5.0 g CRs (dry basis) and 100 mL THF + H_2O mixture were firstly put into the reactor. Then, the air in the reactor was replaced by N_2 (6 mL/min) for 1.0 min, and then kept the initial pressure at 2.0 MPa. The reaction time was started to be recorded until the temperature reached the designed value. After pretreatment, tap water was used to cool down the reactor until the temperature was 30 °C, and then the reactor was washed by 100 mL THF + H_2O mixture being the same as the co-solvent used. Afterwards, the pretreated slurry was filtrated to get the cellulose-rich fractions and the liquid. As for the filtration, degraded lignin (lignin-derived oligomers) dissolved in THF can be used for further reaction without catalysis at higher temperature and longer time or with catalysis at lower temperature to obtain monophenols based on previous study [13,34]. Finally, distilled water was used to wash the cellulose-rich fractions to remove the residual THF and other byproducts derived during pretreatment until the residual THF in waste water could not be detected by HPLC. Then, the recovered solids were stored in sealed bag in the −18 °C freezer for further main composition analysis and structural characterization as well as enzymatic saccharification. Besides, the THF lefted in filtration and waste water can be combined and then recovered by low temperature distillation [30].

2.3. RSM experimental design

BBD of RSM was employed to optimize the THF + H_2O co-solvent pretreatment parameters to gain maximum glucose yield from pretreated CRs. Design Expert software (version 10.0.7) was applied to design the experiments and to analyze the results. The influence of pretreatment parameters on the cellulose recovery, lignin removal, cellulose conversion and glucose yield were studied and used as the responses. The parameters (variables) were (A) THF/H_2O ratio (represented by THF concentration), (B) pretreatment time, and (C) pretreatment temperature. Their ranges (levels), which were chosen based on previous work [13,26,31], were 40%–80%, 0.5–1.5 h and 180–220 °C (see Table S1), respectively. 15 THF + H_2O co-solvent pretreatment runs, including 3 central points, were carried out according to the experimental design matrix. The effects of variables on the responses were analyzed by Analysis of Variance (ANOVA), and the models were analyzed by p-values, F-values, the coefficient determination (R^2), and lack of fit. The optimized pretreatment conditions were verified to obtain maximum glucose yield from pretreated CRs to check the validity.

2.4. Enzymatic hydrolysis

Cellic® CTec2, the cellulase employed for cellulose-glucose bioconversion, was gifted by Novozymes Beijing branch, in China. The cellulase activity of Cellic® CTec2 was 114 FPU/mL, which was quantified by filter paper activity (FPA) test method with the 1×6^{-cm} strip of Whatman No. 1 filter paper as the standard substrate [35], and the protein concentration of the enzyme cocktail was 228.7 mg/mL [36]. Firstly, the pretreated CRs and acetate buffer (0.05 M, pH = 5.0) were put into the 30 mL flasks, and then 60 μL tetracycline hydrochloride solution (100 mg/L) was added into the system to restrain the microorganisms that might consume the produced sugar. Afterwards, the flasks were screwed, and then the samples were enzymatically hydrolyzed at 50 °C and 200 rpm with 12.0 mL working volume. The supernatants (about 500 μL) were sampled during enzymatic hydrolysis, and then heated at 100 °C for 5 min and centrifuged at 10,000 rpm and 4 °C for 5 min. Finally, the samples were stored in the −18 °C freezer for further sugar analysis.

2.5. Main composition and sugar analysis

The main composition of the raw CRs and the pretreated CRs was analyzed by the modified NREL general procedure for the determination of cellulose, hemicellulose, lignin, extractives and ash in biomass [33]. Briefly, the samples were hydrolyzed in 72% sulfuric acid solution (*w/w*) for 2.0 h at room temperature, and then autoclaved in 4% sulfuric acid solution (*w/w*) at 121 °C for 1.0 h. Afterwards, the samples were filtrated to get the solid residue (KL) and hydrolysate. The KL was washed by deionized water until pH = 7.0 and then dried at 105 °C, and its content was calculated by weight. ASL and glucose in hydrolysate were measured by an UV spectrophotometer (λ = 205 nm) and a high-performance liquid chromatograph (HPLC) equipped with refractive index (RI) detector, respectively. Aminex HPX-87H Column (Bio--Rad Laboratories) was employed to determine the sugar with mobile phase (0.05 M H_2SO_4, 0.6 mL/min), and the obtained glucose content was used for calculating the glucan content, which represented the content of cellulose. The designed temperatures of column and detector were 50 °C and 35 °C, respectively. The produced glucose after enzymatic hydrolysis was analyzed by the same method, and the cellulose conversion and glucose yield were calculated by Eq. (1) and Eq. (2), respectively.

$$Cellulose\ conversion\ (\%) = \frac{Produced\ glucose}{Cellulose\ in\ sample \times 1.1} \times 100\% \quad (1)$$

$$Glucose\ yield\ (mg/g) = \frac{Produced\ glucose\ (mg)}{Unpretreated\ CRs\ (g)} \quad (2)$$

2.6. Characterization of the cellulose-rich fractions

The changes of functional groups in the cellulose-rich fractions gained after different pretreatment runs were analyzed by Fourier transform infrared spectrometer (FT-IR) (Nicolet iS10, Thermo Scientific, USA). The crystallinity of unpretreated and pretreated CRs were analyzed by X-ray diffraction (XRD), and the crystallinity index (CrI) were calculated by Refs. [37].

X-ray photoelectron spectroscopy (XPS) (KRATOS Axis Ultra DLD, Japan, equipped with Al-Kα X-ray radiation) was used to determine the amount of oxygen and carbon, O/C ratio and their distributions on the surface of pretreated CRs. The C1s peak of contaminated carbon was set to 285.0 eV to calibrate the energy scale, and chemical shifts of C1s were deconvoluted by XPSPEAK41 software into 4 categories [38,39], including C_1 (C–C/C–H), C_2 (-C-O/-C-OH), C_3 (C═O/O–C–C) and C_4 (O–C═O), and the locations of the corresponding peaks were 284.9 ± 0.1 eV (C_1), 286.7 ± 0.1 eV (C_2), 287.9 ± 0.1 eV (C_3) and 289.0 ± 0.1 eV (C_4).

Table 2
Box-Behnken design (BBD) of pretreatment process parameters affecting cellulose recovery, lignin removal, cellulose conversion and glucose yield.

Std	Run	Variables			Responses			
		THF concentration (%)	Time (h)	Temperature (°C)	Cellulose recovery (%)	Lignin removal (%)	Cellulose conversion (%)	Glucose yield (mg/g)
13	1	60	1.0	200	87.4	66.1	87.7	475.8
11	2	60	0.5	220	68.2	83.4	97.5	424.8
10	3	60	1.5	180	94.1	64.2	71.0	412.6
1	4	40	0.5	200	93.0	0.8	51.6	297.6
9	5	60	0.5	180	99.3	48.8	42.8	263.8
4	6	80	1.5	200	64.1	67.9	30.2	120.0
14	7	60	1.0	200	88.0	71.1	80.1	456.3
8	8	80	1.0	220	26.8	60.4	6.3	9.4
5	9	40	1.0	180	94.1	0.8	40.8	238.2
7	10	40	1.0	220	88.5	16.0	70.6	387.8
3	11	40	1.5	200	86.7	15.9	72.4	389.3
12	12	60	1.5	220	45.8	77.8	98.9	276.1
2	13	80	0.5	200	80.8	64.2	30.5	153.0
6	14	80	1.0	180	94.1	61.9	32.9	192.0
15	15	60	1.0	200	90.1	73.9	87.3	487.7

2.7. Statistical analysis

The determination for each sample was repeated in duplicate, and all of the results were the average of each run with error bars and standard deviation. The correlations between the 9 substrate-related factors obtained from different pretreatment runs and cellulose conversion were analyzed through correlation analysis (CA) by SPSS software (IBM SPSS Statistics V21.0).

3. Results and discussions

3.1. Optimization of THF + H_2O co-solvent pretreatment on CRs to obtain high glucose yield

3.1.1. Optimization of the pretreatment parameters by RSM

The 15 runs of THF + H_2O co-solvent pretreatment on CRs were carried out and the pretreated CRs were enzymatically hydrolyzed at 2% solid loading (*w/v*, dry pretreated substrate) with 10 FPU/g cellulose (20 mg protein/g cellulose) enzyme input. The corresponding pretreatment performances of different runs were presented in Table 2, including cellulose recovery, lignin removal, cellulose conversion and glucose yield. The experimental data were used to develop four equations to relate the variables (coded) and responses, as shown in Eqs. (3)–(6).

$$\textit{Cellulose recovery} = +88.49 - 12.06A - 6.32B - 19.04C - 2.58AB - 15.45AC - 4.33BC - 4.18A^2 - 3.19B^2 - 8.45C^2 \quad (3)$$

$$\textit{Lignin removal} = +70.36 + 27.60A + 3.58B + 7.73C - 2.86AB - 4.16AC - 5.25BC - 33.48A^2 + 0.30B^2 - 2.12C^2 \quad (4)$$

$$\textit{Cellulose conversion} = +85.04 - 16.94A + 6.25B + 10.73C - 5.30AB - 14.10AC - 6.69BC - 39.38A^2 + 0.51B^2 - 8.01C^2 \quad (5)$$

$$\textit{Glucose yield} = +473.27 - 104.82A + 7.33B - 1.04C - 31.18AB - 83.05AC - 74.38BC - 185.38A^2 - 47.90B^2 - 81.03C^2 \quad (6)$$

The validities of the developed models were assessed by p-value, F-value, lack of fit, and R^2 attained from the ANOVA. The p-value was the indicator that could assess the significance of the individual parameters, quadratic terms and developed models, and the lower p-value ($p < 0.05$) proved the higher statistical significance [40]. The F-value was an indicator that represented the effects of the individual parameters and quadratic terms on the responses, and the larger the F-value, the higher the effect [41]. From Table 3, the p-values of the 4 models were <0.001 and the F-values of these models were >6, which indicated that the developed models were significant. Besides, the adequacy of the models to fit the experimental data can be determined by the lack of fit test in the ANOVA [40,41]. If the p-value of the lack of fit was >0.05, the lack of fit was insignificant relative to pure error [42]. The R^2 value could show the strength of the model in predicting the responses, and the R^2 value closer to 1 indicated the better prediction [41]. As illustrated in Table 3, all of the lack of fit was not significant, which suggested that the obtained experimental values were in good agreement with these models, and the R^2 of these models >0.92 displayed a high correlation between predicted and experimentally obtained values [42]. Above all, all the indicators proved that the developed models were efficient to predict the maximum glucose yield.

3.1.2. The influence of pretreatment parameters on the responses

As presented in Table 3, the impacts of variables on the responses and their interactions with the responses were analyzed by the ANOVA. The significance and the influence of the individual variables and their quadratic terms were assessed by p-values and F-values, respectively. The lower p-value ($p < 0.05$) and higher F-values indicated stronger relative influence [42,43]. Besides, the 3D surface plots of the influence of variables on glucose yield were given in Fig. S1.

During hydrothermal pretreatment, cellulose was sensitive to temperature and organic solvent concentration, and the pretreatment time was less important according to previous work [42]. However, the data obtained in this study indicated that the cellulose recoveries were ranged from 26.8% to 99.3%, and all of the variables had strong effects on cellulose recovery ($p < 0.05$) (Table 3), including THF concentration, temperature and time. Among them, temperature had the highest F-value (141.8) than other factors, which showed that temperature had the most significant influence on cellulose recovery. This result was the same as previous work that lower pretreatment temperature was benefit for cellulose preservation [44]. As for cellulose bioconversion, only high cellulose recovery alone was not enough, lignin removal was also an important index. However, temperature was less important for lignin removal than THF concentration, due to the fact that the THF concentration had the highest F-value (152.4) and the lowest p-value ($p < 0.0001$). H_2O and THF in co-solvent system showed synergistic effects on the breakage of intermolecular linkages between cellulose and lignin and the degradation of them [27,45]: (1) H_2O preferentially occupied the activated sites of intermolecular linkages (mainly hydrogen bond and ether bond) between cellulose and lignin, and then cleaved these linkages to enhance lignin removal; (2) THF, which was more densely distributed close to lignin than H_2O, limited the formation of hydrogen

Table 3
ANOVA analysis for the responses.

Sources	Cellulose recovery			Lignin removal			Cellulose conversion			Glucose yield		
	F Value	p-value Prob > F	Significance	F Value	p-value Prob > F	Significance	F Value	p-value Prob > F	Significance	F Value	p-value Prob > F	Significance
Model	31.3	0.0007	significant	30.7	0.0007	significant	6.9	0.0007	Significant	118.1	<0.0001	significant
A-THF Concentration	56.9	0.0006		152.4	<0.0001		13.6	0.0006		323.8	<0.0001	
B-Time	15.6	0.0108		2.6	0.1705		1.8	0.0108		1.6	0.2637	
C-Temperature	141.8	<0.0001		12.0	0.0181		5.5	<0.0001		0.0	0.8651	
AB	1.3	0.3049		0.8	0.4080		0.7	0.3049		14.3	0.0128	
AC	46.7	0.0010		1.7	0.2450		4.7	0.0010		101.6	0.0002	
BC	3.7	0.1135		2.8	0.1577		1.1	0.1135		81.5	0.0003	
A^2	3.2	0.1360		103.5	0.0002		33.9	0.1360		467.4	<0.0001	
B^2	1.8	0.2332		0.0	0.9304		0.0	0.2332		31.2	0.0025	
C^2	12.9	0.0157		0.4	0.5478		1.4	0.0157		89.3	0.0002	
Lack of Fit	17.0	0.0562	not significant	3.6	0.2259	not significant	14.7	0.0562	not significant	1.1	0.4995	not significant
	$R^2 = 0.9826$			$R^2 = 0.9822$			$R^2 = 0.9255$			$R^2 = 0.9953$		

Table 4
Experimental and predicted values of pretreatment performances under optimized conditions.

Variables	Experimental values	Predicted values
Cellulose recovery (%)	88.2 ± 0.14	89.6
Lignin removal (%)	71.9 ± 2.16	69.7
Cellulose conversion (%)	90.1 ± 0.50	88.7
Glucose yield (mg/g)	498.2 ± 2.21	490.1

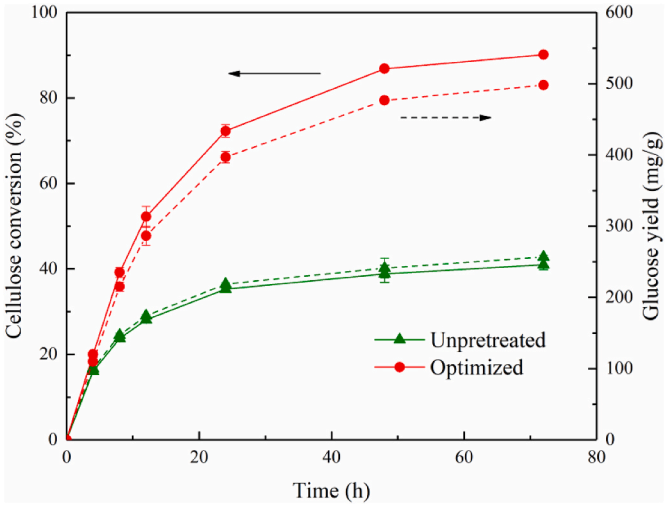

Fig. 1. Cellulose conversion and glucose yield of unpretreated CRs and optimized-CRs.

bonds between water molecule and lignin, restricted the self-aggregation of lignin derivatives, and was favorable for the cleavage of the aryl methoxy carbon bond and β-*O*-4 bond of lignin to solubilize the degraded lignin fragments; (3) the hydrogen bonds formed between THF and H_2O could facilitate H_2O to attack the linkages between cellulose and lignin, and change the structure of lignin. Besides, because H_2O had higher hydrogen bond acceptor ability than THF, it played an important role to disturb the intramolecular and intermolecular hydrogen bonds of cellulose [13]. So, this was why the THF concentration not only controlled lignin removal, but also had significant influence on cellulose recovery ($p < 0.01$).

After different runs of THF + H_2O co-solvent pretreatment on CRs, the cellulose conversion and the glucose yield ranged from 6.3% to 98.9% and 9.4 mg/g CRs to 487.7 mg/g CRs, respectively. According to previous work [46], the extents of cellulose hydrolysis decreased, rather than increased with increasing severity of liquid hot water pretreatment. During pretreatment, the condensation reactions of lignin molecules and the generation of pseudo lignin occurred, so that the content of KL increased after pretreatment [47]. Besides, the more severe the pretreatment conditions, the more pseudo lignin was generated [48]. To obtain efficient enzymatic hydrolysis and high glucose yield, high cellulose recovery and adequate lignin removal were both needed [36]. So, this was why the lowest cellulose conversion (6.3%) and lowest glucose yield (9.4 mg/g CRs) were gained after pretreated at the most severe conditions (80% THF concentration, 220 °C, 1 h). From Fig. S1 and Table 3, the THF concentration was the significant variable to control the cellulose conversion and the glucose yield (both $p < 0.01$). So, the THF concentration was the crucial parameter needed to be optimized. On the other hand, temperature played an important role in the efficiency of cellulose conversion ($p < 0.05$). This result was attributed to the fact that the elevated temperature could help to cleave the chemical bonds in lignin and the bonds between lignin and cellulose to deconstruct the complex structures, but it also made the lignin and other byproducts condensed on the surface of substrate to decrease cellulose

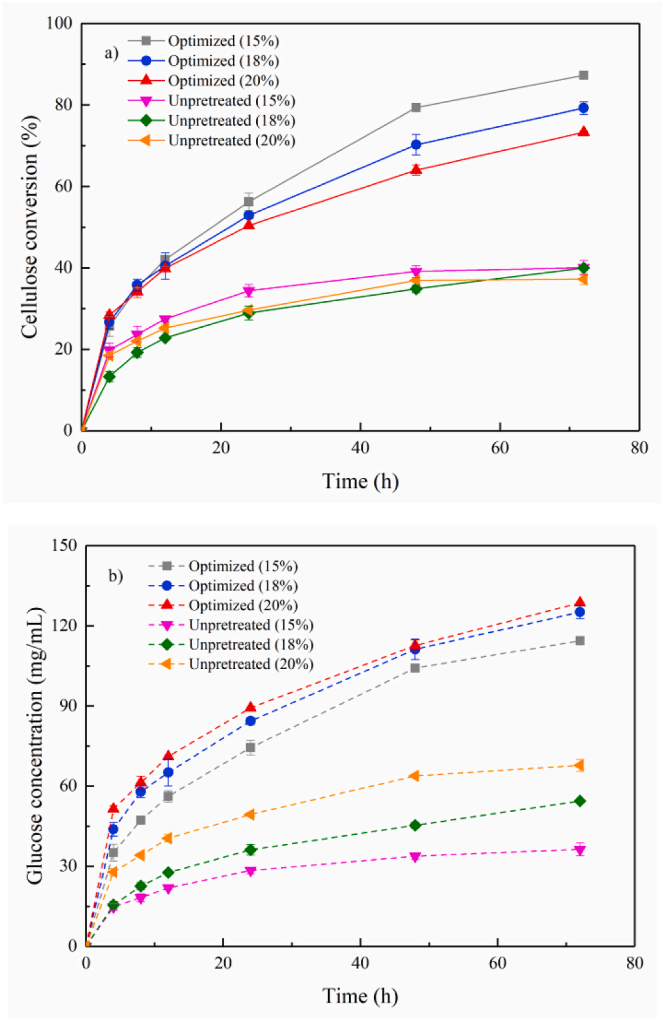

Fig. 2. Enzymatic hydrolysis of unpretreated CRs and optimized-CRs with different high solid loading (enzyme input was fixed at 10 FPU/g cellulose). a) Cellulose conversion of CRs; b) Glucose concentration of hydrolysate.

conversion [48]. However, the temperature and time were unimportant for the glucose yield (both $p > 0.05$). Because the effects of individual factor on the responses were not linear, it was necessary to analyze the quadratic effect of them. Interestingly, all of the quadratic terms, including AB, AC, BC, A^2, B^2, C^2, had stronger influence than the individual variables except for THF concentration.

3.1.3. Validation of the developed RSM model for maximum glucose yield

The validation of the model for maximum glucose yield was performed under the optimized conditions, that is, CRs pretreated by THF + H_2O co-solvent with the THF concentration of 53.7% at 202.3 °C for 1.05 h (optimized-CRs). The experimental data and predicted values of pretreatment performances under optimized conditions were illustrated in Table 4. Besides, the comparison of cellulose conversion and glucose yield of unpretreated CRs and optimized-CRs was also investigated (Fig. 1). The cellulose conversion and glucose yield of unpretreated CRs were 41.0% and 256.8 mg/g CRs after 72 h, which meant that the polysaccharides were locked in the recalcitrant structure that needed a pretreatment step before saccharification. After pretreatment under the optimized conditions, the glucose yield and cellulose conversion were boost to 498.2 mg/g CRs and 90.1%, respectively. The experimental glucose yield was close to the model prediction value of 490.1 mg/g CRs and the difference of the experimental and predicted values was only 0.01, which was enough to justify the validity of the developed model [42].

Recently, to enhance the cellulose digestion, many kinds of pretreatment methods have been employed on CRs, and the main composition changes of CRs after pretreatment, the cellulose conversion, and the glucose yield after enzymatic hydrolysis, were listed in Table 1. The maximum glucose yield after many kinds of pretreatment methods were ranged from 234.3 mg/g CRs to 377.8 mg/g CRs, and the relatively highest yield of glucose (498.2 mg/g CRs) was achieved in the present work. In contrast to these previous studies, although the cellulose recovery was 88.2% in this study, the lignin removal (71.9%) and cellulose conversion (90.1%) were the most efficient among them. The residual lignin was a notorious inhibitor for enzymatic saccharification [49], so the higher cellulose conversion and glucose yield could be attributed to the 71.9% of lignin removal after pretreatment.

3.2. Enzymatic hydrolysis with high solid loading and low enzyme loading

For the conversion of cellulose to produce glucose from lignocellulosic biomass, the high yield of sugar was important, while obtaining high concentration of glucose was also important [50]. Cost-effective enzymatic saccharification and fermentation of pretreated lignocellulosic biomass at an industrial scale would necessitate the use of high solid loading (≥15%, *w/w*) [50]. Enzymatic hydrolysis at high solid loading potentially offered many advantages, which could help to obtain high concentration sugar production and decrease the capital costs [11]. Besides, enzyme input was the predominant costs in cellulose-sugar bioconversion, thus high enzyme input for the hydrolysis should be avoided [31]. So, the unpretreated CRs and optimized-CRs were firstly enzymatically hydrolyzed at high solid loading (15%–20%) (*w/v*, dry pretreated substrate) with 10 FPU/g cellulose enzyme input to gain high concentration glucose production. Then, the unpretreated CRs and optimized-CRs were hydrolyzed with low enzyme input (5–8 FPU/g cellulose) at 15% solid loading to evaluate the potential for reducing the use of enzymes to save process costs.

3.2.1. To obtain high concentration glucose titer

The production of high concentration glucose titer from feedstock was crucial in terms of process operation and economics of bio-based product industry, so it was necessary to have high solid loading in saccharification [50]. From Fig. 2a, the cellulose conversion of 20% solid loading (28.4%) was higher than that of 18% (26.7%) and 15% (25.7%) at the early stage of cellulose bioconversion (<4 h). This result could be attributed to the fact that higher solid loading contained more amorphous cellulose, and the amorphous region was often hydrolyzed preferentially during cellulose utilization [31,51]. However, with the prolonging of hydrolysis time, the hydrolysis efficiency of lower solid loading was higher. The cellulose conversion of 15% solid loading was 87.3% after 72 h, and then the cellulose conversion substantially dropped to 73.3% when the solid loading was increased to 20%. Too high substrate loading resulted in the opposite trend in the efficiency of hydrolysis, because high-solids slurry had challenging rheological characteristics, such as low mass and heat transfer and very high viscosities [50]. Besides, inhibitory compounds for cellulose conversion, such as residual lignin, produced sugars and organic acids accumulated in the substrate, decreased the enzymatic activity of the system especially at high solid loading [9,50,52]. On the other hand, the glucose concentration of 20% solid loading was higher than that of 18% and 15% during the whole enzymatic hydrolysis reaction. After enzymatically hydrolyzed for 72 h, the obtained glucose concentration was 128.6 mg/mL at 20%, 125.1 mg/mL at 18%, and 114.4 mg/mL at 15% (Fig. 2b). Assuming ethanol yields of 0.45 g/g glucose [53], the sugar hydrolysate with concentration of 128.6 mg/mL could yield 57.9 g/L ethanol. According to previous studies [31,54], the ethanol titer concentration > 50 g/L could decrease the downstream operating costs. So, it was feasible and efficient to hydrolyze the THF + H_2O co-solvent pretreated CRs at 20% solid loading, and achieved a satisfying

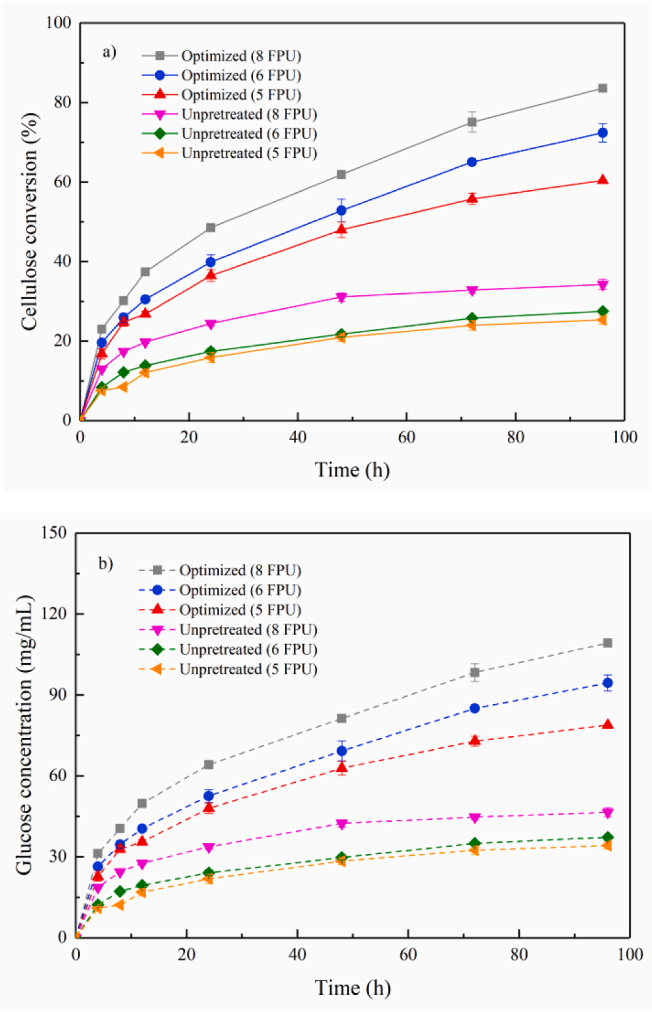

Fig. 3. Enzymatic hydrolysis of unpretreated CRs and optimized-CRs with low enzyme input (solid loading was fixed at 15%). a) Cellulose conversion of CRs; b) Glucose concentration of hydrolysate.

cellulose-glucose conversion. Besides, when compared with the cellulose conversion of unpretreated CRs at 20%, the glucose concentration of optimized-CRs was 1.9 times higher than that of the unpretreated CRs (67.8 mg/mL) (Fig. 2b).

3.2.2. Potential for reduction of enzyme input

Enzyme input was also one of the most critical parameters for enzymatic hydrolysis [44]. High enzyme input was in favor of the polysaccharides utilization since more enzymes were available for breakdown cellulose into glucose, that is, reducing enzyme input could make the cellulose conversion to be low, especially at high solid loading [50]. However, it saved process costs. As expected, after enzymatically hydrolyzed for 72 h, the cellulose conversion (glucose concentration) dramatically decreased from 75.1% (98.3 mg/mL) to 55.8% (72.9 mg/mL) as enzyme input reduced from 8 FPU/g cellulose (16 mg protein/g cellulose) to 5 FPU/g cellulose (10 mg protein/g cellulose) (Fig. 3a and b). Since low enzyme input was difficult for enzyme to diffuse or absorb onto the substrate, prolonging the time could help to increase cellulose conversion [2]. After hydrolysis time was prolonged to 96 h, the cellulose conversion (glucose concentration) of 8 FPU/g cellulose and 5 FPU/g cellulose increased to 83.6% (109.3 mg/mL) and 60.4% (78.8 mg/mL), and it might increase further with the prolongation of time. Comparing with the unpretreated CRs hydrolyzed with 5 FPU/g cellulose, the cellulose conversion of optimized-CRs was 2.4 times higher than that of the unpretreated CRs (25.3%). Obviously, the current results on glucose production were competitive with some references, which meant the optimized-CRs could be hydrolyzed with low enzyme input. However, it needed to admit that reducing enzyme input indeed decreased the cellulose conversion in this study.

3.3. Analysis of enzymatic hydrolysis inhibitors

Efficient and rapid sugar generation was essential for lignocellulosic biomass valorization [5]. Based on the discussion in 3.2 part, although the comparable efficiency of cellulose conversion could be gained at high solid loading (15%) with low enzyme input (5–8 FPU/g cellulose), enzyme inactivation indeed happened. Many substrate-related factors could be obstacles for enzymatic hydrolysis, such as the innate recalcitrance of lignocellulose, cellulose crystallinity, lignin and cellulose contents and their distribution in recovered cellulose-rich fractions after pretreatment [18]. So, determining the interactions between these factors and cellulose conversion, and their effects, could help to develop strategies to enhance biomass digestibility [51]. The statistical analysis employed for enzymatic hydrolysis inhibitors has been developed for many years. Therefore, the interactions between the 9 cellulose and residual lignin related factors obtained after different pretreatment runs and cellulose conversion were analyzed through CA by SPSS (Fig. 4 and Table S2), which included cellulose content, CrI of cellulose-rich fractions, CrI/cellulose values, lignin removal, KL, ASL and surface composition (O/C, amount of C_1 (C–C/C–H) and C_2 (-C-O/-C-OH)). Besides, the metrix of correlation coefficients among all factors were illustrated in Fig. 5.

3.3.1. Cellulose and CrI

The cellulose of biomass consisted of amorphous region cellulose (low molecular order), crystallinity region cellulose (high crystalline order) and a small amount of intermediate order cellulose [33]. Its crystallinity was regarded as one of the most important factors for sugar production, and the content of amorphous and crystalline region could be changed during pretreatment to affect the followed cellulose utilization [55,56]. So, the relationship between the cellulose conversion and the cellulose content, the CrI of pretreated CRs, and the CrI/cellulose value obtained from different THF + H_2O co-solvent pretreatment runs needed to be investigated.

As illustrated in Table 5 and Fig. S2, the cellulose content varied from 50.1% to 78.7%, and FT-IR spectra showed that the intensity of the characteristic peaks of cellulose did not change significantly after different pretreatment runs, including bands at 1374 cm^{-1} (aliphatic C–H), 1166 cm^{-1} and 897 cm^{-1} (C–*O*–C of β-1,4-glycosidic), 1113 cm^{-1} (glucose ring C–*O*–C) [57]. However, the cellulose content showed indistinct correlation with cellulose conversion ($r = 0.446$, $p > 0.05$) (see Fig. 4 and Table S2). The CrI of pretreated CRs was quantified by XRD, which ranged from 80.8% to 93.3%, and also presented unclear relationship with cellulose conversion ($r = 0.320$, $p > 0.05$). In fact, the obvious correlation between CrI and cellulose conversion occurred only in pure cellulose and not commonly in real pretreated biomass [51]. Therefore, a parameter (CrI/cellulose value) was adopted to associate the CrI and cellulose content, to analyze the actual changes in cellulose crystallinity, and to analyze the correlation between the cellulose degree of polymerization and cellulose conversion [58]. Interestingly, CrI/-cellulose values varied from 1.12 to 1.81, and were negatively correlated with cellulose content ($r = -0.903$, $p < 0.01$), which meant that the more cellulose preserved in substrate, the lower CrI/cellulose value. This phenomenon could be ascribed to the fact that stronger pretreatment condition could degrade more amorphous cellulose, which left the crystalline region [18]. Disappointedly, the correlation between CrI/-cellulose value and cellulose conversion was still indistinct ($r = -0.410$, $p > 0.05$). Previous work found that the Cellic® CTec2 was the kind of bifunctional enzymes, which could both utilize amorphous and crystalline cellulose [55]. At the beginning of enzymatic hydrolysis, the amorphous cellulose preferentially hydrolyzed, and presenting linear

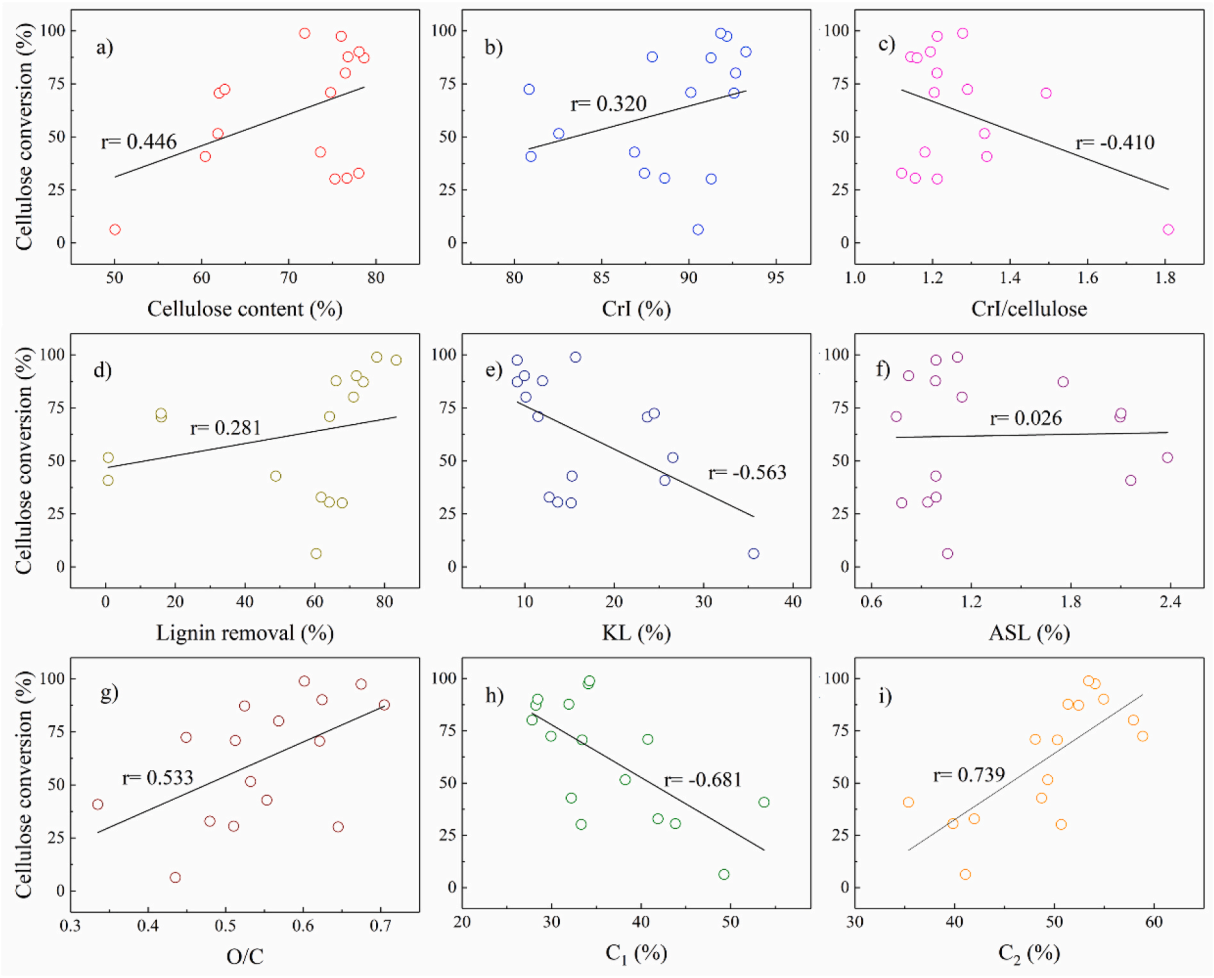

Fig. 4. Correlation analysis between the 9 substrate-related factors and cellulose conversion.

reaction rate [51]. After that, although the conversion rate was slower than before, the crystalline region could also be digested by Cellic® CTec2. So, with the extension of hydrolysis time, the difference between them became smaller. Moreover, in other cases, no relationship between crystallinity and cellulose digestion has been found, because other factors, such as residual lignin, could affect the correlation [33]. So, the CrI of pretreated CRs and CrI/cellulose value had no correlation with cellulose conversion. It indicated that the inhibition of crystallinity of cellulose for cellulose conversion might be less important than other factors in this study.

3.3.2. *Residual lignin*

Lignin, as a major component of lignocellulose, was a notorious inhibitor for enzymatic hydrolysis [49]. Although pretreatment could reduce lignin content to improve saccharification efficiency, a certain part of lignin still remained in the recovered cellulose-rich fractions (residual lignin), and this kind of lignin impeded cellulase to utilize cellulose by physical steric hindrance, nonproductive adsorption (nonspecific adsorption) and small molecule inhibition [49,59,60]. Therefore, the relationships between residual lignin and its related factors and cellulose conversion after THF + H_2O co-solvent pretreatment should be investigated further.

Previous studies have proved that lignin removal was strongly positively correlated with cellulose bioconversion [61]. However, the results in this study showed some differences. After different runs of THF + H_2O co-solvent pretreatment, the lignin removal ranged from 0.8% to 83.4% (Table 2). Although lignin removal enhanced cellulose digestion when compared with unpretreated CRs, the lignin removal showed implicit correlation with cellulose conversion after pretreatment ($r = 0.281$, $p > 0.05$). This indicated that the lignin removal of substrate might help cellulose bioconversion to some extent. Besides, the ASL, as one of the inhibitors for enzymatic hydrolysis [49], varied from 0.75% to 2.38% after different pretreatment runs (Table 5), also exhibited no obvious relation with cellulose conversion ($r = 0.026$, $p > 0.05$). Interestingly, the KL ranged from 9.2% to 35.6% and presented negative correlation with cellulose conversion ($r = -0.563$, $p < 0.05$) (see Fig. 4 and Table S1). KL was the predominant lignin in recovered cellulose-rich fractions, and it could limit enzymes or cellulases accessibility by physical steric hindrance [62]. Therefore, it was natural that KL exerted a pronounced adverse effect on the enzymatically saccharification. Besides, previous study found that the residual lignin on the surface of substrate was more likely related to cellulose conversion than the bulk lignin content [63]. So, it was imperative to investigate the surface compositions and lignin coverage of pretreated CRs and their relations with cellulose conversion.

Herein, the surface carbon (C1s) and oxygen (O1s) of pretreated CRs were firstly determined by XPS, and then the surface-lignin coverage was identified by the O/C ratio. The theoretical O/C ratios of lignin and cellulose were 0.33 and 0.83, respectively [48,51], which indicated that a higher O/C ratio meant the lignin was less distributed than the cellulose on the surface. The surface O/C ratio of THF + H_2O co-solvent pretreated CRs ranged from 0.34 to 0.70, and it exhibited negative correlation with KL ($r = -0.539$, $p < 0.05$) and positive correlation with cellulose conversion ($r = 0.533$, $p < 0.05$) (see Fig. 4 and Table S2). During enzymatic hydrolysis, the cellulose on the surface could be initially hydrolyzed, and then the biomass porosity increased, which

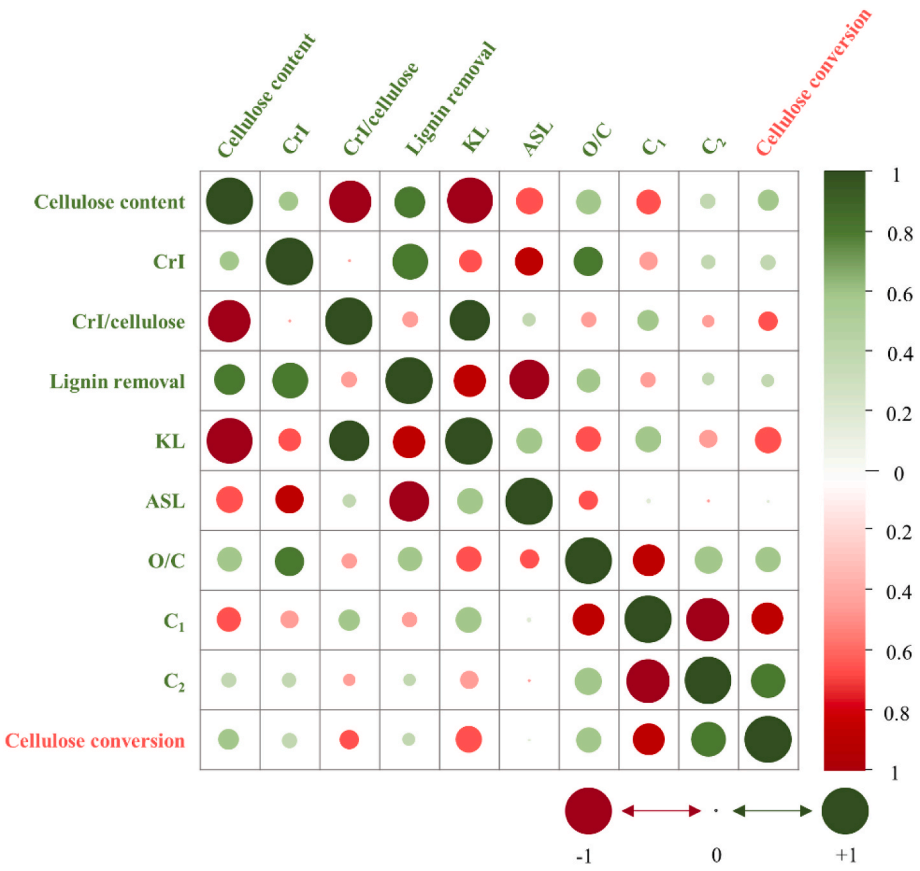

Fig. 5. Metrix of correlation coefficients among all factors. Larger greener (redder) plots display stronger positive (negative) correlations.

Table 5
Cellulose and lignin content and CrI of unpretreated and pretreated CRs.

Sample	Cellulose content (%)	CrI (%)	CrI/cellulose values	KL (%)	ASL (%)
Unpretreated	62.7	79.7	1.27	22.1	3.1
Run 1	76.8	87.9	1.14	12.0	1.0
Run 2	76.0	92.2	1.21	9.2	1.0
Run 3	74.8	90.1	1.20	11.5	0.7
Run 4	61.9	82.5	1.33	26.6	2.4
Run 5	73.6	86.9	1.18	15.3	1.0
Run 6	75.3	91.3	1.21	15.2	0.8
Run 7	76.5	92.7	1.21	10.1	1.1
Run 8	50.1	90.5	1.81	35.6	1.1
Run 9	60.4	80.9	1.34	25.7	2.2
Run 10	62.0	92.6	1.49	23.7	2.1
Run 11	62.6	80.8	1.29	24.5	2.1
Run 12	71.8	91.8	1.28	15.7	1.1
Run 13	76.7	88.6	1.16	13.7	0.9
Run 14	78.0	87.4	1.12	12.7	1.0
Run 15	78.7	91.3	1.16	9.2	1.8
Optimized	78.1	93.3	1.19	10.0	0.8

Table 6
C_{1s} and O_{1s} peaks and relative concentration of different bonds on the surface determined by XPS.

Sample	O/C ratio	C1% (C–C/ C–H) 284.8 ± 0.1 eV	C2% (C–O) 286.4 ± 0.1 eV	C3% (C═O/ O–C–O) 287.0 ± 0.1 eV	C4% (O–C═O) 288.5 ± 0.1 eV
Run 1	0.70	31.9	51.4	13.0	3.7
Run 2	0.67	34.1	54.1	6.7	5.1
Run 3	0.51	40.8	48.1	5.0	6.2
Run 4	0.53	38.2	49.3	8.5	3.9
Run 5	0.55	32.2	48.7	14.6	4.5
Run 6	0.65	33.3	50.7	13.4	2.6
Run 7	0.57	27.8	57.9	10.2	4.1
Run 8	0.44	49.3	41.1	1.1	8.5
Run 9	0.34	53.8	35.4	1.5	9.4
Run 10	0.62	33.4	50.3	12.6	3.7
Run 11	0.45	29.9	58.9	7.2	4.0
Run 12	0.60	34.2	53.4	8.3	4.0
Run 13	0.51	43.8	39.8	9.1	7.2
Run 14	0.48	41.9	42.0	8.6	7.6
Run 15	0.52	28.2	52.4	17.3	2.1
Optimized	0.62	28.4	54.9	14.7	1.9

could further boost the hydrolysis of the rest of cellulose. These results suggested that more cellulose and less lignin on the surface could result in efficient cellulose digestion. For confirmation, XPS C1s was deconvoluted into 4 categories [38,39], including C_1, C_2, C_3 and C_4 (see Table 6 and Fig. S3), and the C_1 was primarily assigned to C–C or C–H in lignin, and the C_2 came mainly from C–O in cellulose [39]. As expected, the O/C ratio showed positive correlation with C_2 ($r = 0.595$, $p < 0.05$) and strongly negative correlation with C_1 ($r = -0.680$, $p < 0.01$) (see Fig. 4 and Table S2), and C_2 was strongly positively correlated with cellulose conversion ($r = 0.739$, $p < 0.01$) (Fig. 4), which proved that more cellulose on the surface increased O/C ratio and enhanced cellulose utilization.

During the hydrothermal pretreatment, pseudo lignin, which was composed of lignin and sugar degraded derivatives, gradually generated on the surface of lignocellulosic fiber [38,47]. Pseudo lignin located on the substrate surface had strong inhibition for enzymatic hydrolysis due to its readily nonproductive association with cellulases [63,64]. Based on previous works [38,48], the distinct increase of C_1 was observed with the generation of pseudo lignin, and the KL increased in substrate along with more pseudo lignin formed. Notably, in this study, C_1 presented

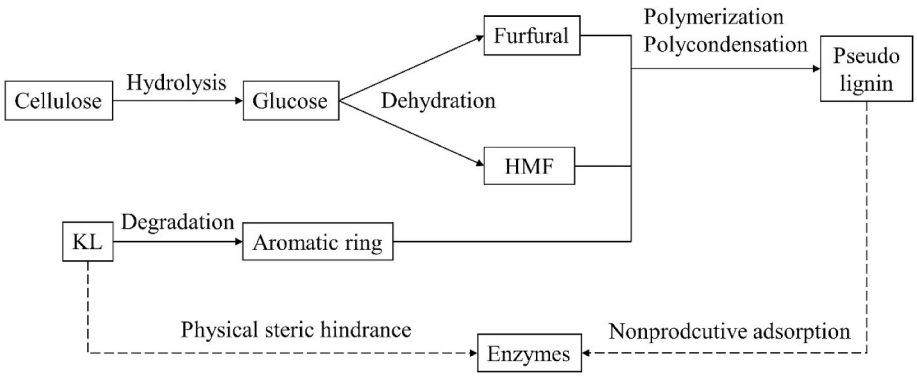

Fig. 6. Hypothesized reaction pathways for enzymes activity decreases.

positive correlation with KL ($r = 0.555$, $p < 0.05$) and extremely negative correlation with cellulose conversion ($r = -0.681$, $p < 0.01$) (see Fig. 4 and Table S2). So, the more KL could result in an increase of pseudo lignin on the surface and a corresponding decrease of cellulose conversion. Furthermore, the negative correlation between C_1 and cellulose content ($r = -0.528$, $p < 0.05$) indicated that cellulose decrease could cause more C_1 generated on the surface. Cellulose was sensitive when temperature was elevated above 180 °C, and its degradation aggravated the generation of by-products, such as furfural and HMF [38]. During solvothermal pretreatment, these compounds could be further converted to other ring compounds, which were the key intermediates that could combined with degraded lignin to form pseudo lignin [48,63]. Unfortunately, all of THF + H_2O co-solvent pretreatment temperature was higher than 180 °C. Besides, FT-IR spectra showed that the intensity of the characteristic peaks of pseudo lignin increased as temperature elevated (see Fig. S2), including bands at 1515 cm^{-1} and 1605 cm^{-1} (aromatic C═C) and 1709 cm^{-1} (C═O) [38,48]. Pseudo lignin was prone to bind cellulases due to its electrostatic, hydrophobic and hydrogen-bond interactions with enzymes by phenolic hydroxyl, carboxylic, and aromatic structures [48,49]. So, pseudo lignin was probably another crucial inhibitor for cellulose conversion in this study.

Above all, the probable mechanism of inhibition for cellulose conversion in this study were that: (1) degraded lignin reacted with sugar decomposition intermediates that formed pseudo lignin deposited on the surface of substrate during pretreatment; (2) the pseudo lignin through cellulases nonproductive adsorption combined with KL remained in substrate through physical steric hindrance to make less enzymes available for action on cellulose (Fig. 6). According to previous study [36,62], KL could be removed by kinds of pretreatment methods. As for nonproductive adsorption, some approaches have been studied [11,49], such as adding surfactants to decrease the surface tension of saccharification system or block agents to initially bind with residual lignin. Of course, how to remove KL and eliminate the cellulases nonproductive adsorption by pseudo lignin and to improve cellulose conversion at high solid loading after THF + H_2O co-solvent pretreatment would be performed further. Besides, the physiochemical structures of residual lignin also have significant influences on the lignin-enzyme interactions during enzymatic hydrolysis, including S/G ratio, aliphatic hydroxyl groups, phenolic hydroxyl groups, carboxylic acid groups, etc. [47,55,56], which also need to be investigated further.

4. Conclusions

The THF + H_2O co-solvent pretreatment on CRs was optimized by BBD of RSM, and the maximum glucose yield was 498.2 mg/g CRs obtained after pretreated under optimized conditions (53.7% THF concentration, 202.3 °C, 1.05 h) and enzymatic hydrolysis. From optimized-CRs enzymatic hydrolysis, the high glucose concentration titer (128.6 mg/mL) was obtained, and then showed the potential for reducing enzyme input. Bulk and surface compositions and lignin coverage of pretreated CRs were determined by XPS, and found that KL remained in the cellulose-rich fractions and pseudo lignin formed and condensed on the surface of the recovered substrate might be responsible for the enzyme activity decreases.

Credit author statement

Fengpei Yao, Investigation, Methodology, Data curation, Writing - original draft, etc. Fei Shen, Methodology, Resources, etc. Xue Wan, Investigation, Methodology, etc. Changwei Hu; Project administration, Funding acquisition, Supervision, Resources, Writing - review & editing, etc.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in paper.

Acknowledgment

This work was supported by the National Key R&D Program of China (2018YFB1501404), the 111 program (B17030) and Fundamental Research Funds for the Central Universities. We thank Yunfei Tian of analytical & testing center of Sichuan University for XPS experiments.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rser.2020.110107.

References

[1] Liu B, Rajagopal D. Life-cycle energy and climate benefits of energy recovery from wastes and biomass residues in the United States. Nature Energy 2019;4(8):700–8.
[2] Straub CT, Khatibi PA, Wang JP, Conway JM, Williams-Rhaesa AM, Peszlen IM, Chiang VL, Adams MWW, Kelly RM. Quantitative fermentation of unpretreated transgenic poplar by Caldicellulosiruptor bescii. Nat Commun 2019;10(1):3548.
[3] Van den Bosch S, Schutyser W, Vanholme R, Driessen T, Koelewijn SF, Renders T, De Meester B, Huijgen WJJ, Dehaen W, Courtin CM, Lagrain B, Boerjan W, Sels BF. Reductive lignocellulose fractionation into soluble lignin-derived phenolic monomers and dimers and processable carbohydrate pulps. Energy Environ Sci 2015;8(6):1748–63.
[4] Zabed HM, Akter S, Yun J, Zhang G, Awad FN, Qi X, Sahu JN. Recent advances in biological pretreatment of microalgae and lignocellulosic biomass for biofuel production. Renew Sustain Energy Rev 2019;105:105–28.
[5] Schutyser W, Renders T, Van den Bosch S, Koelewijn SF, Beckham GT, Sels BF. Chemicals from lignin: an interplay of lignocellulose fractionation, depolymerisation, and upgrading. Chem Soc Rev 2018;47(3):852–908.
[6] Zabed H, Sahu JN, Boyce AN, Faruq G. Fuel ethanol production from lignocellulosic biomass: an overview on feedstocks and technological approaches. Renew Sustain Energy Rev 2016;66:751–74.

[7] Nie S, Zhang C, Zhang Q, Zhang K, Zhang Y, Tao P, Wang S. Enzymatic and cold alkaline pretreatments of sugarcane bagasse pulp to produce cellulose nanofibrils using a mechanical method. Ind Crop Prod 2018;124:435–41.
[8] Ho MC, Ong VZ, Wu TY. Potential use of alkaline hydrogen peroxide in lignocellulosic biomass pretreatment and valorization – a review. Renew Sustain Energy Rev 2019;112:75–86.
[9] Biswal AK, Atmodjo MA, Li M, Baxter HL, Yoo CG, Pu Y, Lee YC, Mazarei M, Black IM, Zhang JY, Ramanna H, Bray AL, King ZR, LaFayette PR, Pattathil S, Donohoe BS, Mohanty SS, Ryno D, Yee K, Thompson OA, Rodriguez Jr M, Dumitrache A, Natzke J, Winkeler K, Collins C, Yang X, Tan L, Sykes RW, Gjersing EL, Ziebell A, Turner GB, Decker SR, Hahn MG, Davison BH, Udvardi MK, Mielenz JR, Davis MF, Nelson RS, Parrott WA, Ragauskas AJ, Neal Stewart Jr C, Mohnen D. Sugar release and growth of biofuel crops are improved by downregulation of pectin biosynthesis. Nat Biotechnol 2018;36(3):249–57.
[10] Luo Y, Li D, Li R, Li Z, Hu C, Liu X. Roles of water and aluminum sulfate for selective dissolution and utilization of hemicellulose to develop sustainable corn stover-based biorefinery. Renew Sustain Energy Rev 2020;122:109724.
[11] Zheng T, Lei F, Li P, Liu S, Jiang J. Stimulatory effects of rhamnolipid on corncob residues ethanol production via high-solids simultaneous saccharification and fermentation. Fuel 2019;257:116091.
[12] Liu W, Wu R, Wang B, Hu Y, Hou Q, Zhang P, Wu R. Comparative study on different pretreatment on enzymatic hydrolysis of corncob residues. Bioresour Technol 2019;295:122244.
[13] Jiang Z, He T, Li J, Hu C. Selective conversion of lignin in corncob residue to monophenols with high yield and selectivity. Green Chem 2014;16(9):4257–65.
[14] Petridis L, Smith JC. Molecular-level driving forces in lignocellulosic biomass deconstruction for bioenergy. Nature Rev Chem 2018;2(11):382–9.
[15] Yu HL, Tang Y, Xing Y, Zhu LW, Jiang JX. Improvement of the enzymatic hydrolysis of furfural residues by pretreatment with combined green liquor and hydrogen peroxide. Bioresour Technol 2013;147:29–36.
[16] Xing Y, Bu L, Sun D, Liu Z, Liu S, Jiang J. High glucose recovery from direct enzymatic hydrolysis of bisulfite-pretreatment on non-detoxified furfural residues. Bioresour Technol 2015;193:401–7.
[17] Xing Y, Ji L, Liu Z-p, Zhang W-m, Jiang J-x. Effects of Gleditsia saponin on high-solids enzymatic hydrolysis of furfural residues. Ind Crop Prod 2015;64:209–14.
[18] Solarte-Toro JC, Romero-García JM, Martínez-Patiño JC, Ruiz-Ramos E, Castro-Galiano E, Cardona-Alzate CA. Acid pretreatment of lignocellulosic biomass for energy vectors production: a review focused on operational conditions and techno-economic assessment for bioethanol production. Renew Sustain Energy Rev 2019;107:587–601.
[19] Tian S-Q, Zhao R-Y, Chen Z-C. Review of the pretreatment and bioconversion of lignocellulosic biomass from wheat straw materials. Renew Sustain Energy Rev 2018;91:483–9.
[20] Lin X, Wu Z, Zhang C, Liu S, Nie S. Enzymatic pulping of lignocellulosic biomass. Ind Crop Prod 2018;120:16–24.
[21] Wang K, Yang H, Chen Q, Sun RC. Influence of delignification efficiency with alkaline peroxide on the digestibility of furfural residues for bioethanol production. Bioresour Technol 2013;146:208–14.
[22] Chen C-Z, Li M-F, Wu Y-Y, Sun R-C. Integration of ambient formic acid process and alkaline hydrogen peroxide post-treatment of furfural residue to enhance enzymatic hydrolysis. Ind Eng Chem 2014;53(33):12935–42.
[23] Yu H, Xing Y, Lei F, Liu Z, Liu Z, Jiang J. Improvement of the enzymatic hydrolysis of furfural residues by pretreatment with combined green liquor and ethanol organosolv. Bioresour Technol 2014;167:46–52.
[24] Chi X, Liu C, Bi Y-H, Yu G, Zhang Y, Wang Z, Li B, Cui Q. A clean and effective potassium hydroxide pretreatment of corncob residue for the enhancement of enzymatic hydrolysis at high solids loading. RSC Adv 2019;9(20):11558–66.
[25] Chen H, Jiang L, Cheng Y, Lu J, Lv Y, Yan J, Wang H. Improving enzymatic hydrolysis efficiency of corncob residue through sodium sulfite pretreatment. Appl Microbiol Biotechnol 2019;103(18):7795–804.
[26] Cai CM, Zhang T, Kumar R, Wyman CE. THF co-solvent enhances hydrocarbon fuel precursor yields from lignocellulosic biomass. Green Chem 2013;15(11):31–40.
[27] Mostofian B, Cai CM, Smith MD, Petridis L, Cheng X, Wyman CE, Smith JC. Local phase separation of Co-solvents enhances pretreatment of biomass for bioenergy applications. J A Chem Soc 2016;138(34):10869–78.
[28] Fenner-Crisp PA, Mayes ME, David RM. Assessing the human carcinogenic potential of tetrahydrofuran: I. Mode of action and human relevance analysis of the male rat kidney tumor. Regul Toxicol Pharmacol 2011;60(1):20–39.
[29] Fowles J, Boatman R, Bootman J, Lewis C, Morgott D, Rushton E, van Rooij J, Banton M. A review of the toxicological and environmental hazards and risks of tetrahydrofuran. Crit Rev Toxicol 2013;43(10):811–28.
[30] Smith MD, Mostofian B, Cheng X, Petridis L, Cai CM, Wyman CE, Smith JC. Cosolvent pretreatment in cellulosic biofuel production: effect of tetrahydrofuran-water on lignin structure and dynamics. Green Chem 2016;18(5):1268–77.
[31] Nguyen TY, Cai CM, Osman O, Kumar R, Wyman CE. CELF pretreatment of corn stover boosts ethanol titers and yields from high solids SSF with low enzyme loadings. Green Chem 2016;18(6):1581–9.
[32] Nguyen TY, Cai CM, Kumar R, Wyman CE. Co-solvent pretreatment reduces costly enzyme requirements for high sugar and ethanol yields from lignocellulosic biomass. ChemSusChem 2015;8(10):1716–25.
[33] Karimi K, Taherzadeh MJ. A critical review of analytical methods in pretreatment of lignocelluloses: composition, imaging, and crystallinity. Bioresour Technol 2016;200:1008–18.
[34] Fang Q, Jiang Z, Guo K, Liu X, Li Z, Li G, Hu C. Low temperature catalytic conversion of oligomers derived from lignin in pubescens on Pd/NbOPO4. Appl Catal B Environ 2020;263:118325.
[35] Cai C, Bao Y, Zhan X, Lin X, Lou H, Pang Y, Qian Y, Qiu X. Recovering cellulase and increasing glucose yield during lignocellulosic hydrolysis using lignin-MPEG with a sensitive pH response. Green Chem 2019;21(5):1141–51.
[36] Yao F, Tian D, Shen F, Hu J, Zeng Y, Yang G, Zhang Y, Deng S, Zhang J. Recycling solvent system in phosphoric acid plus hydrogen peroxide pretreatment towards a more sustainable lignocellulose biorefinery for bioethanol. Bioresour Technol 2019;275:19–26.
[37] Gong W, Liu C, Mu X, Du H, Lv D, Li B, Han S. Hydrogen peroxide-assisted sodium carbonate pretreatment for the enhancement of enzymatic saccharification of corn stover. ACS Sustainable Chem Eng 2015;3(12):3477–85.
[38] Ma X, Yang X, Zheng X, Chen L, Huang L, Cao S, Akinosho H. Toward a further understanding of hydrothermally pretreated holocellulose and isolated pseudo lignin. Cellulose 2015;22(3):1687–96.
[39] Wu K, Ying W, Shi Z, Yang H, Zheng Z, Zhang J, Yang J. Fenton reaction-oxidized bamboo lignin surface and structural modification to reduce nonproductive cellulase binding and improve enzyme digestion of cellulose. ACS Sustainable Chem Eng 2018;6(3):3853–61.
[40] Chohan NA, Aruwajoye GS, Sewsynker-Sukai Y, Gueguim Kana EB. Valorisation of potato peel wastes for bioethanol production using simultaneous saccharification and fermentation: process optimization and kinetic assessment. Renew Energy 2020;146:1031–40.
[41] Malakar B, Das D, Mohanty K. Optimization of glucose yield from potato and sweet lime peel waste through different pre-treatment techniques along with enzyme assisted hydrolysis towards liquid biofuel. Renew Energy 2020;145:2723–32.
[42] Tsegaye B, Balomajumder C, Roy P. Optimization of microwave and NaOH pretreatments of wheat straw for enhancing biofuel yield. Energy Convers Manag 2019;186:82–92.
[43] Milano J, Ong HC, Masjuki HH, Silitonga AS, Chen W-H, Kusumo F, Dharma S, Sebayang AH. Optimization of biodiesel production by microwave irradiation-assisted transesterification for waste cooking oil-Calophyllum inophyllum oil via response surface methodology. Energy Convers Manag 2018;158:400–15.
[44] Kim DH, Park HM, Jung YH, Sukyai P, Kim KH. Pretreatment and enzymatic saccharification of oak at high solids loadings to obtain high titers and high yields of sugars. Bioresour Technol 2019;284:391–7.
[45] Zhang H, Liu X, Li J, Jiang Z, Hu C. Performances of several solvents on the cleavage of inter- and intramolecular linkages of lignin in corncob residue. ChemSusChem 2018;11(9):1494–504.
[46] Ko JK, Ximenes E, Kim Y, Ladisch MR. Adsorption of enzyme onto lignins of liquid hot water pretreated hardwoods. Biotechnol Bioeng 2015;112(3):447–56.
[47] He J, Huang C, Lai C, Huang C, Li X, Yong Q. Elucidation of structure-inhibition relationship of monosaccharides derived pseudo-lignin in enzymatic hydrolysis. Ind Crop Prod 2018;113:368–75.
[48] Shinde SD, Meng X, Kumar R, Ragauskas AJ. Recent advances in understanding the pseudo-lignin formation in a lignocellulosic biorefinery. Green Chem 2018;20(10):2192–205.
[49] Dos Santos AC, Ximenes E, Kim Y, Ladisch MR. Lignin-enzyme interactions in the hydrolysis of lignocellulosic biomass. Trends Biotechnol 2019;37(5):518–31.
[50] Chen X, Kuhn E, Jennings EW, Nelson R, Tao L, Zhang M, Tucker MP. DMR (deacetylation and mechanical refining) processing of corn stover achieves high monomeric sugar concentrations (230 g L^{-1}) during enzymatic hydrolysis and high ethanol concentrations (> 10% v/v) during fermentation without hydrolysate purification or concentration. Energy Environ Sci 2016;9(4):1237–45.
[51] Chai L, Liu M, Yan X, Cheng X, Zhang T, Si M, Min X, Shi Y. Elucidating the interactive impacts of substrate-related properties on lignocellulosic biomass digestibility: a sequential analysis. ACS Sustainable Chem Eng 2018;6(5):6783–91.
[52] Kalyani DC, Zamanzadeh M, Müller G, Horn SJ. Biofuel production from birch wood by combining high solid loading simultaneous saccharification and fermentation and anaerobic digestion. Appl Energy 2017;193:210–9.
[53] Chen Z, Jacoby WA, Wan C. Ternary deep eutectic solvents for effective biomass deconstruction at high solids and low enzyme loadings. Bioresour Technol 2019;279:281–6.
[54] Zhao Y, Damgaard A, Christensen TH. Bioethanol from corn stover – a review and technical assessment of alternative biotechnologies. Prog Energy Combust Sci 2018;67:275–91.
[55] Zhang X, Qu T, Mosier NS, Han L, Xiao W. Cellulose modification by recyclable swelling solvents. Biotechnol Biofuels 2018;11(1):191.
[56] Tao P, Zhang Y, Wu Z, Liao X, Nie S. Enzymatic pretreatment for cellulose nanofibrils isolation from bagasse pulp: transition of cellulose crystal structure. Carbohydr Polym 2019;214:1–7.
[57] Jiang Z, Zhang H, He T, Lv X, Yi J, Li J, Hu C. Understanding the cleavage of inter- and intramolecular linkages in corncob residue for utilization of lignin to produce monophenols. Green Chem 2016;18(14):4109–15.
[58] Pihlajaniemi V, Sipponen MH, Liimatainen H, Sirviö JA, Nyyssölä A, Laakso S. Weighing the factors behind enzymatic hydrolyzability of pretreated lignocellulose. Green Chem 2016;18(5):1295–305.
[59] Li X, Li M, Pu Y, Ragauskas AJ, Klett AS, Thies M, Zheng Y. Inhibitory effects of lignin on enzymatic hydrolysis: the role of lignin chemistry and molecular weight. Renew Energy 2018;123:664–74.
[60] Donohoe BS, Resch MG. Mechanisms employed by cellulase systems to gain access through the complex architecture of lignocellulosic substrates. Curr Opin Chem Biol 2015;29:100–7.
[61] Lin X, Wu L, Huang S, Qin Y, Qiu X, Lou H. Effect of lignin-based amphiphilic polymers on the cellulase adsorption and enzymatic hydrolysis kinetics of cellulose. Carbohydr Polym 2019;207(1):52–8.
[62] Akinosho H, Dumitrache A, Natzke J, Muchero W, Jawdy SS, Tuskan GA, Brown SD, Ragauskas AJ. Effects of biomass accessibility and Klason lignin

contents during consolidated bioprocessing in Populus trichocarpa. ACS Sustainable Chem Eng 2017;5(6):5075–81.
[63] Nitsos CK, Lazaridis PA, Mach-Aigner A, Matis KA, Triantafyllidis KS. Enhancing lignocellulosic biomass hydrolysis by hydrothermal pretreatment, extraction of surface lignin, wet milling and production of cellulolytic enzymes. ChemSusChem 2019;12(6):1179–95.
[64] Hu F, Jung S, Ragauskas A. Impact of pseudolignin versus dilute acid-pretreated lignin on enzymatic hydrolysis of cellulose. ACS Sustainable Chem Eng 2012;1(1):62–5.