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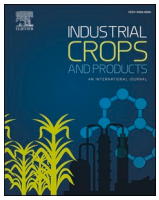

Integrating mild chemical pretreatments with endogenous protein supplement for complete biomass saccharification to maximize bioethanol production by enhancing cellulases adsorption in novel bioenergy *Amaranthus*

Meysam Madadi [a,b], Youmei Wang [a,c], Ran Zhang [a,b], Zhen Hu [a,b], Hairong Gao [a,b], Dan Zhan [b], Hua Yu [a,b], Qiaomei Yang [a,b], Yanting Wang [a,b], Yuanyuan Tu [a,b], Tao Xia [b,c], Liangcai Peng [a,b,1,*]

[a] *Biomass & Bioenergy Research Center, College of Plant Science & Technology, Huazhong Agricultural University, Wuhan 430070, China*
[b] *Laboratory of Biomass Engineering & Nanomaterial Application in Automobiles, College of Food Science & Chemical Engineering, Hubei University of Arts & Science, Xiangyang 441053, China*
[c] *College of Life Science & Technology, Huazhong Agricultural University, Wuhan 430070, China*

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ABSTRACT

Amaranthus is a fast-growing perennial plant containing large amounts of extractable green proteins and lignocellulose residues. However, it remains to explore optimal *Amaranthus* biomass process technology for biofuel production. In this study, we examined that mild alkali and acid pretreatments (1% NaOH, 2% H_2SO_4) were sufficient for near-complete biomass enzymatic saccharification, while 8% *Amaranthus* endogenous proteins were supplemented into the enzymatic hydrolyses. By performing classic yeast fermentation with all carbon sources from directly-extractable sugars and starch and lignocellulose enzymatic hydrolysis, this work achieved the bioethanol yield up to 23.5% (% dry matter). Notably, with respect to extremely high yield of *Amaranthus* straw, the maximum bioethanol yield was estimated at 14.46 t/ha/year, which was much higher than those of other major bioenergy crops as previously reported. Furthermore, this work proposed a hypothetic model to elucidate how the complete biomass saccharification was achieved to maximize bioethanol production at large scale, based on the effective wall polymer extraction and distinct lignocellulose feature modification for remarkably enhanced biomass porosity and cellulases adsorption. Hence, this work has demonstrated that *Amaranthus* could be applied as a novel and leading bioenergy crop, providing a powerful strategy for optimal biomass processing towards high bioethanol production.

1. Introduction

Plant cell wall represents an immense lignocellulose source for renewable biofuels and biomaterials (Robak and Balcerek, 2020; Sharma et al., 2020). To date, cellulosic ethanol has been considered as an excellent additive to gasoline for partial replacement of petrol fuels (Ragauskas et al., 2006; Sun et al., 2016). In principle, cellulosic ethanol production involves three conversion steps: biomass pretreatment to disrupt cell wall composition, sequential enzymatic saccharification of pretreated lignocellulose to release fermentable sugars, and final yeast fermentation to obtain bioethanol productivity (Liu et al., 2018; Raud et al., 2019). However, because of lignocellulose recalcitrance, biomass pretreatment basically requires severe conditions to enhance enzymatic hydrolysis and bioethanol production in the most bioenergy crops examined, which leads to an unacceptable high-priced bioethanol conversion along with secondary waste liberation (Ho et al., 2019; Madadi et al., 2017a, 2017b).

Over the past years, various physical and chemical pretreatments

* Corresponding author at: Biomass & Bioenergy Research Center, College of Plant Science & Technology, Huazhong Agricultural University, Wuhan 430070, China.
E-mail address: lpeng@mail.hzau.edu.cn (L. Peng).
[1] Web: http://bbrc.hzau.edu.cn.

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have been carried out to overcome lignocellulose recalcitrance by effectively extracting non-cellulosic polymers for improved biomass porosity and cellulose accessibility (Kim et al., 2016; Kumar et al., 2020). For example, alkali (NaOH) pretreatment can effectively extract lignin at high concentrations, whereas sulphuric acid is commonly employed to partially digest hemicellulose. Despite the H_2SO_4 and NaOH pretreatments under strong conditions are effective to diminish lignocellulose recalcitrance in various bioenergy crops (Ho et al., 2019; Wang et al., 2016), it needs to find out cost-effective biomass process technology by combining mild chemical pretreatments with desirable lignocellulose substrates for complete enzymatic saccharification.

Lignocellulose recalcitrance has been defined by accounting for cell wall composition (cellulose, hemicellulose, lignin), wall polymer feature, and wall network style (Wang et al., 2016). Among three major wall polymers, lignin deposition has been examined as a major barrier against cellulases accession and adsorption, whereas cellulose features (crystalline index/CrI and degree of polymerization/DP) are the major negative factors accountable for lignocellulose recalcitrance (Li et al., 2013, 2014; Xu et al., 2012). By contrast, biomass porosity and cellulose accessibility have been characterized to be the positive parameters that are directly accountable for biomass enzymatic saccharification (Alam et al., 2019; Lv et al., 2021; Sun et al., 2017). Hence, it has been considered to obtain the recalcitrance-reduced lignocellulose substrates by performing effective biomass pretreatment and genetic lignocellulose modification in bioenergy crops (Wang et al., 2016, 2021). Moreover, chemicals (Tween, PEG) and plant proteins have been applied as active surfactants to block lignin interaction with cellulases enzymes (Florencio et al., 2016; Jin et al., 2016; Madadi et al., 2021a). Nonetheless, it remains a challenge to explore integrated approaches for effective biomass pretreatment and efficient enzymatic hydrolysis towards high bioethanol production in desirable bioenergy crops.

Amaranthus plant is an industrial, perennial, and fast-growing C_4 grass with high dry biomass yield, distinct cell wall composition, and rich in proteins that can be considered as an ideal feedstock for biofuels purposes (Tucker, 1986; Madadi et al., 2021a; Viglasky et al., 2009). Using *Amaranthus* de-protein residues, this study determined large amounts of soluble sugars and starch, which should be of advantage for direct yeast fermentation into bioethanol. Notably, we performed mild chemical (NaOH, H_2SO_4) pretreatments with the de-protein straw under relatively low chemical concentrations and incubation temperatures, and then supplied a low dosage of endogenous *Amaranth* proteins into the enzymatic hydrolysis of pretreated residues, which resulted in an almost complete biomass enzymatic saccharification. By integrating all carbon sources from soluble sugars and starch and enzymatic hydrates, this work conducted a classic yeast fermentation to achieve the highest bioethanol production among all major bioenergy crops examined. Finally, we attempted to elucidate why the *Amaranthus* plant is a novel and leading bioenergy crop by assessments of its characteristic lignocellulose features.

2. Material and methods

2.1. Collection of biomass materials

Amaranthus straw (*Amaranthus retroflexus* L.) was kindly provided by China *Amaranthus* Ecological Technology Co., Ltd. All straw tissues were dried at 50 ℃ until constant weight and then ground through a 40-mesh screen and stored in a sealed dry container until further use.

2.2. Wall polymer extraction and detection

Lignocellulose fractionation was carried out to extract wall polymers as previously described (Peng et al., 2000). The soluble sugars, lipid, starch, and pectin were consecutively 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 residues were extracted with 4 M KOH and 1.0 mg/mL sodium borohydride for 1 h at 25 °C and the combined supernatants were used as KOH-extractable hemicelluloses fraction. The remaining pellet was specified for determination of hexoses as cellulose level by suspended with H_2SO_4 (67%, v/v) for 1 h at 25 °C (Fry, 2000). Total hemicelluloses were calculated by estimating total hexoses and pentoses of the KOH-extractable hemicellulose fraction, and the pentoses of the non-KOH extractable fraction (Dische et al., 1962). The hexoses and pentoses concentration was measured by a UV/VIS spectrometer (Shanghai MAPADA Instruments Co., Ltd. V-1100D) and absorbance reading at 660 and 620 nm, respectively. Total lignin (acid-soluble and insoluble lignin) was detected using the Laboratory Analytical Procedure of the National Renewable Energy Laboratory (Sluiter et al., 2008). All experimental analyses were carried out in triplicate.

2.3. Hemicellulose monosaccharides and lignin monomers determination

GC–MS (SHIMADZU GCMS-QP2010 Plus) was applied to determine monosaccharides of hemicellulose as previously described (Li et al., 2015). Trifluoroacetic acid (TFA) and *myo*-inositol were obtained from Aladdin Reagent Inc. *1*-Methylimidazole was purchased from Sigma-Aldrich Co. LLC. Acetic anhydride and acetic acid were obtained from Sinopharm Chemical Reagent Co., Ltd. HPLC (1525, Waters Corp., MA, USA) was applied to detect lignin monomers (H, G, S) by nitrobenzene oxidation method as described (Zhao et al., 2021).

2.4. Cellulose features (CrI and DP) measurement

Cellulose crystalline index (CrI) was measured with a Rigaku-D/MAX instrument (Ultima III, Japan) as previously described (Li et al., 2018). The CrI value was calculated subjectively to the following equation: $CrI = 100 \times (I_{200} - I_{am})/I_{200}$. The degree of polymerization (DP) of cellulose was measured by the viscosity method according to the following equation: $DP^{0.905} = 0.75[\eta]$ (Alam et al., 2019). All experiments were performed in triplicate at 25 ± 0.5 °C.

2.5. Protein detection

Amaranth proteins (AP) were extracted with liquid nitrogen and protein level was detected by a Nitrogen Analyzer (SpectraMax i3x Multi-Mode Detection Platform, USA) as previously described (Madadi et al., 2021a).

2.6. Lignocellulose observation and characterization

Lignocellulose morphology was observed using SEM (SEM JSM-IT300, Akishima, Tokyo, Japan) as previously described (Sun et al., 2020). X-ray photoelectron spectroscopy (XPS) and Fourier transform infrared (FTIR) methods were respectively applied to detect chemical linkages of lignocellulose samples by using facilities (XPS: NEXUS 470, Thermo Fisher Scientific, Waltham, MA, USA; FTIR: Thermo Fisher Scientific, Waltham, USA) as previously described (Wu et al., 2019a, 2019b; Xu et al., 2021).

2.7. Measurement of biomass porosity and cellulose accessibility

Biomass porosity and cellulose accessibility were measured by Simons staining (Alam et al., 2020). Cellulose accessibility was detected by Congo red staining method as described (Deng et al., 2020). About 100 mg biomass powders were added into dye solution at a series of concentrations (0.5, 1.0, 2.0, 3.0, 4.0, 5.0 g/L) in 0.3 M phosphate buffer and incubated at 60 °C for 24 h. After centrifugation at 8000 ×*g*, the supernatant absorbance was read at 498 nm. All measurements were conducted in triplicate.

2.8. Chemical pretreatment and enzymatic hydrolysis

Sodium hydroxide (NaOH) and sulfuric acid (H_2SO_4) pretreatments were performed at a series of concentrations with 5% biomass loading as previously described (Wu et al., 2019a, 2019b; Zahoor et al., 2017). The raw material was loaded with distilled water and shaken for 2 h at 50 °C as a control sample. The well-mixed de-protein biomass was added with 6 mL NaOH at different concentrations (0.5%, 1%, 1.5%, 2%, 4% w/v) and shaken for 2 h at 50 °C under 150 rpm. H_2SO_4 pretreatment: A total of 6 mL of H_2SO_4 at various concentrations (0.5%, 1%, 1.5%, 2%, 4% v/v) was added with well-dried de-protein biomass in 15 mL plastic centrifuge tubes and heated at 121 °C for 20 min in an autoclave (0.15 MPa). After the tubes were cooled down, the sample was shaken for 2 h at 50 °C under 150 rpm. After centrifugation at 3000 *g* for 5 min, the supernatant was collected for estimation of sugars (pentose and hexose) yield.

The washed pretreated residues with 5% biomass loading were incubated with 6 mL (1.6 g L-1, w/v) of mixed-cellulases (containing cellulases at 10.60 FPU g^{-1} biomass and xylanase at 6.72 U g^{-1} biomass from Imperial Jade Biotechnology Co, Ltd) co-supplied with 8% *Amaranth* proteins (AP) and 1% Tween-80. The sample was shaken at 50 °C and 150 rpm for 48 h and then centrifuged at 3000 ×*g* for 5 min. The supernatants were collected to measure hexoses and pentoses yields according to Eq. (1) (Gao et al., 2021; Madadi et al., 2021a)

$$Hexoses\ \ yield\ \ (\%) = Hexoses\ \ released\ \ (g) \times 100/Cellulose\ \ content\ \ (g) \quad (1)$$

Where hexoses released (g) from residues after 48 h of hydrolysis; cellulose content (g) of de-proteins biomass. All experiments were carried out in triplicates.

2.9. Yeast fermentation and ethanol assay

The fermentation process was carried out using *Saccharomyces cerevisiae* (Angel yeast Co., Ltd., Yichang, China) strain (final concentration of 0.5 g/L,) with total hexoses obtained from both pretreatment and sequential enzymatic hydrolysis and incubation at 37 °C for 48 h as previously described (Liu et al., 2021). The 5% $K_2Cr_2O_7$ method was used to measure ethanol yield as described (Gao et al., 2021). The distillation was carried out at 100 °C for 10 min, and then 1 mL of the produced ethanol sample and 2 mL of $K_2Cr_2O_7$ were placed into glass tubes and heated for 10 min in a water bath. After cooling at room temperature, the distilled water was added to make the volume up to 10 mL. The ethanol absorbance was read at 600 nm by a UV/VIS spectrometer. Absolute ethanol (99.9%) was utilized as standard. All

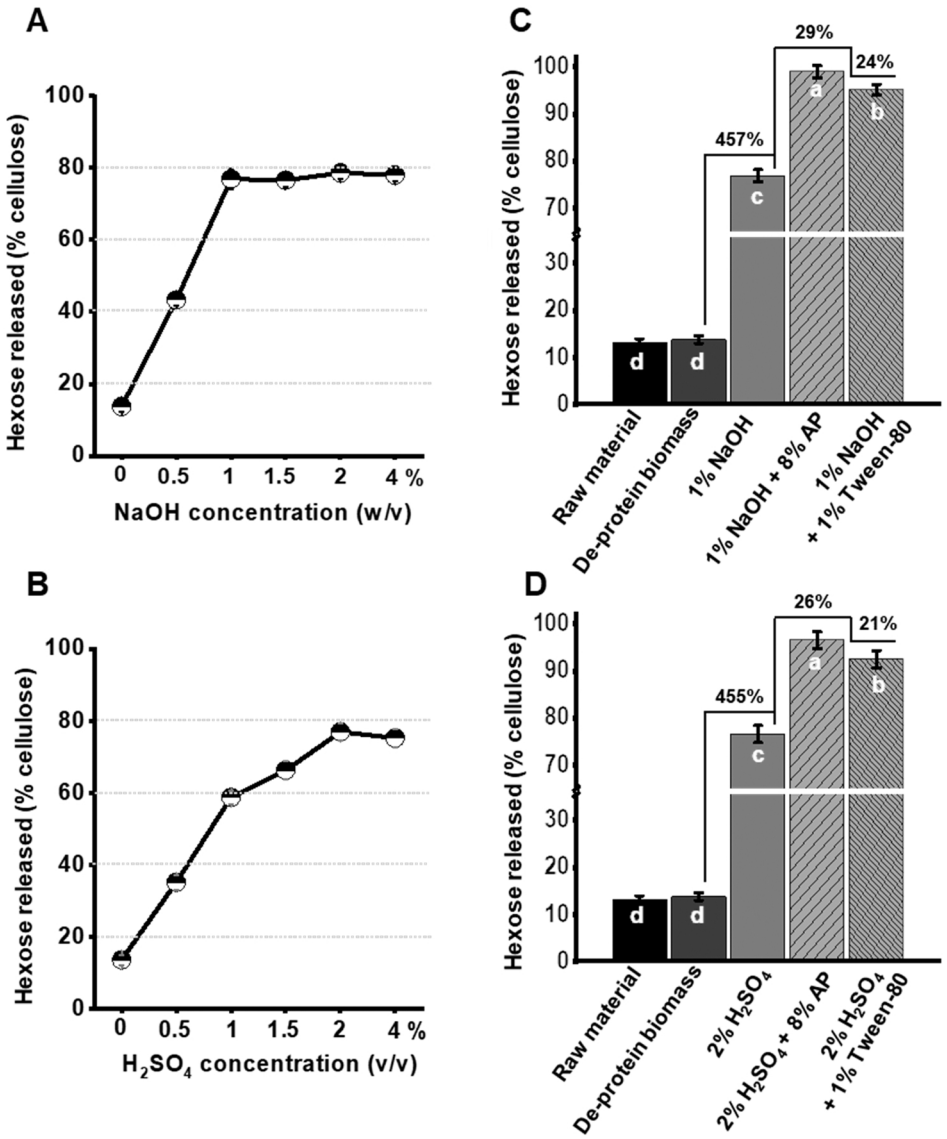

Fig. 1. Biomass enzymatic hydrolysis upon alkali and acid pretreatments in *Amaranthus* straw. (**A, B**) Hexose yields (% cellulose) released from enzymatic hydrolyses after NaOH and H_2SO_4 pretreatments at various concentrations; (**C, D**) Hexose yields (% cellulose) released from enzymatic hydrolyses under optimal chemical pretreatments supplemented with 8% *Amaranth* proteins (AP) or 1% Tween-80; The letters above bars as significant difference by LSD test ($P < 0.05$). Data as means ± SD (n = 3).

experiments were performed in triplicates.

3. Results and discussion

3.1. Combined chemical pretreatments with endogenous proteins supply for complete enzymatic saccharification in Amaranth straw

Using our previously-established approaches (Wu et al., 2019b; Zahoor et al., 2017), this study initially carried out chemical pretreatments with NaOH and H_2SO_4 at a series of concentrations, and determined hexoses yields (% cellulose) released from enzymatic hydrolyses of pretreated lignocellulose residues or total sugars yields (% dry matter) released from both pretreatments and enzymatic hydrolyses of mature *Amaranthus* straws (Figs. 1 and S1). Incubated with 1% NaOH and 2% H_2SO_4, the *Amaranth* straw samples showed the hexoses yields of 76.89% and 76.59% (% cellulose) and total sugars yields of 46.81% and 43.82% (% dry matter), respectively (Figs. 1A and B; S1A and B). However, both hexoses and total sugars yields started to be reducing while further incubated with higher concentrations of NaOH and H_2SO_4 chemicals, suggesting that both optimal alkali and acid pretreatments were not effective enough for a complete biomass hydrolysis of *Amaranth* straws.

As *Amaranth* proteins can act as active biosurfactants for enhancing biomass enzymatic saccharification in diverse lignocellulose residues examined (Madadi et al., 2021a), we extracted all soluble *Amaranth* proteins from the *Amaranth* straw and examined that both raw straw and de-protein straw samples were of similar hexoses yields and total sugars yields from direct enzymatic hydrolysis without any pretreatment to be performed (Figs. 1C and D; S1C and D), suggesting the *Amaranth* proteins extraction could not significantly alter lignocellulose features of *Amaranth* straw. Nevertheless, while 8% *Amaranth* extractable proteins were supplied into the enzymatic hydrolyses after 1% NaOH and 2% H_2SO_4 pretreatments with *Amaranth* de-protein straws, the de-protein samples respectively showed the hexoses yields of 99% and 97%, indicating a near-complete biomass enzymatic saccharification achieved from combined mild chemical pretreatments with native *Amaranth* proteins supplement. Such enhancement suggests that residual lignin in pretreated residues might be well blocked with AP, resulting in more released cellulase enzymes for effective lignocellulose enzymatic hydrolyses (Madadi et al., 2021a). Notably, the *Amaranth* proteins supply

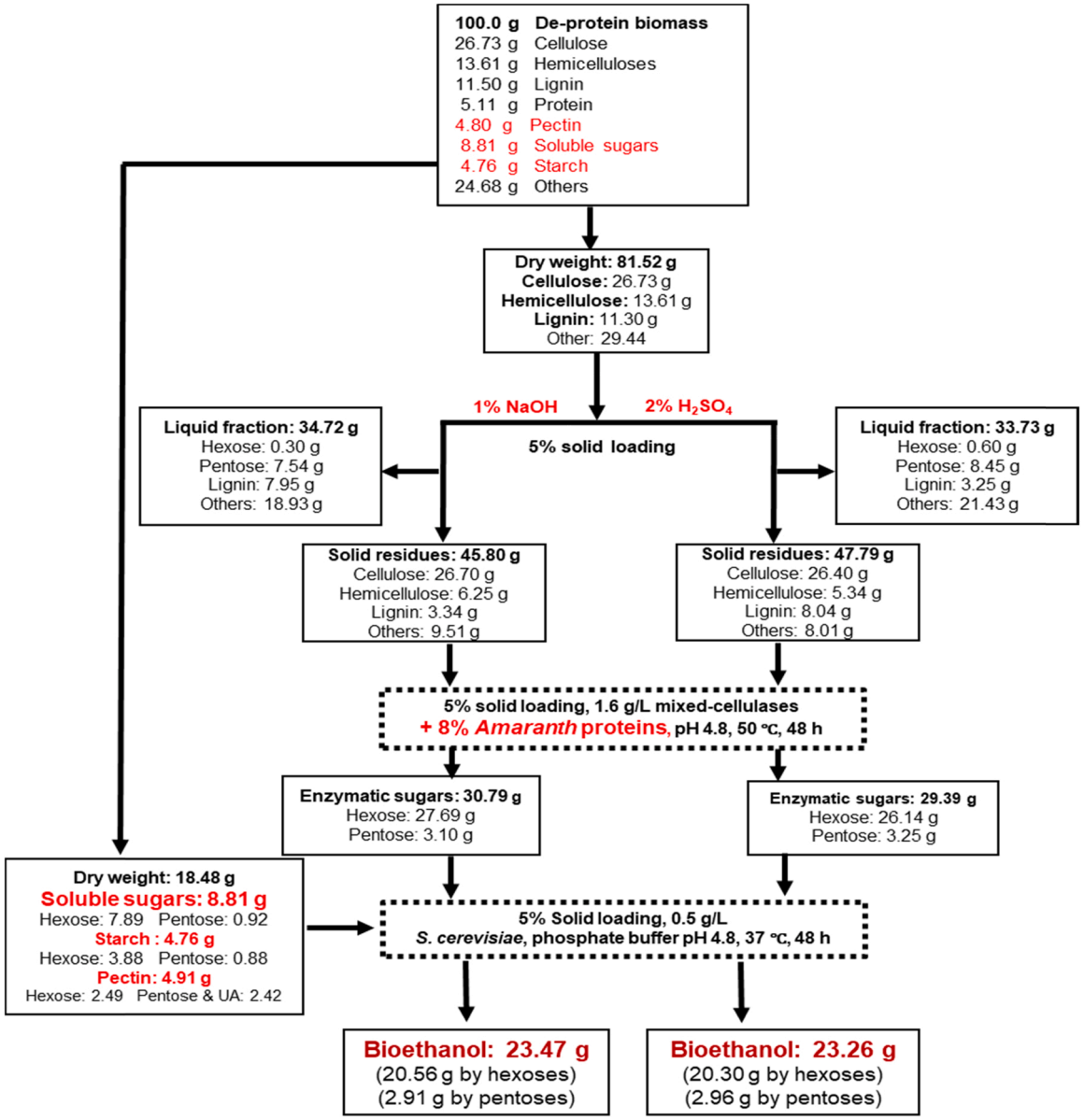

Fig. 2. Mass balance analysis of *Amaranthus* biomass process for bioethanol production upon mild NaOH and H_2SO_4 pretreatments.

could well cause significantly higher hexoses yields than those of the 1% Tween-80 at $P < 0.05$ level (n = 3), which has been well characterized as an efficient chemical surfactant for boosting enzymatic hydrolysis rate (Deng et al., 2020; Jin et al., 2016; Zahoor et al., 2017). In addition, this study examined that total hexoses released from enzymatic hydrolysis contain 79% and 76% glucose (% total) with small amounts of mannose and galactose (21% and 24%) after mild alkali and acid pretreatments (Table S1). However, while 8% *Amaranth* proteins were supplemented, the glucose covered 98.6% and 96.8% of total hexoses, confirming that the native proteins supply could significantly enhance cellulose enzymatic digestion for near-complete biomass enzymatic saccharification in *Amaranth* straw.

3.2. Integrated bioethanol yields using total hexoses from soluble sugars, starch, and enzymatic saccharification in Amaranth plant

As the *Amaranth* straw could be efficiently digested into fermentable sugars under combined chemical pretreatments (1% NaOH and 2% H_2SO_4) with *Amaranth* proteins supply as described above, this work conducted a classic yeast fermentation with all carbon sources from soluble sugars, starch, and enzymatic saccharification of de-protein lignocellulose by presenting a full biomass balance analysis (Fig. 2). Based on 100 g de-protein *Amaranth* straw biomass, 27.69 g and 26.14 g hexoses were obtained from the enzymatic hydrolyses of 1% NaOH and 2% H_2SO_4 pretreated residues along with 3.10 g and 3.25 g pentoses achieved, respectively. Meanwhile, 8.81 g soluble sugars, 4.76 g starch, and 4.91 g pectin were directly extractable from 100 g de-protein straw. Hence, about 20 g ethanol yield could be obtained by yeast fermentation with all hexoses, but it could be theoretically raised up to 23 g while engineered yeast strain would be applied for co-fermentation of pentoses (xylose) as previously reported (Rodrussamee et al., 2018; Valinhas et al., 2018).

Because desirable *Amaranthus* plant could provide large amount of straw lignocellulose residue (Madadi et al., 2021a; Viglasky et al., 2009), this study further compared bioethanol productivity with other major bioenergy crops (Table 1). Except for sweet sorghum and corn straw, *Amaranthus* straw accumulated much higher directly-fermentable hexoses, and *Amaranthus* plant had the highest biomass yield at 54 t/ha/year among all crops presented in this study. Notably, *Amaranthus* straw could produce 14.46 ton of bioethanol per hectare per year (t/ha/year) by estimating all available hexoses and pentoses, being much higher than other major bioenergy crops with bioethanol yields ranged from 1.73 to 7.89 t/ha/year. In addition, the pretreated *Amaranthus* straws showed relatively higher sugar-ethanol conversion rates, compared to the most bioenergy crops examined, which may be due to relatively lower toxic compounds generated from mild chemical pretreatments conducted in this study. Taken together, this study has indicated that the *Amaranthus* plant should be a novel and desirable bioenergy crop for bioethanol productivity at large scale.

3.3. Effective wall polymers extraction and wall network alteration upon mild chemical pretreatments

To test how the mild chemical pretreatments could enhance biomass enzymatic saccharification to large degree in the *Amaranthus* straw, this study examined wall polymers extractions (Table 2). Although about 66% of proteins were extractable from *Amaranthus* raw straw, the de-protein biomass was of very similar cell wall composition, which confirmed that the *Amaranth* proteins extraction could not significantly alter the lignocellulose feature. Provided that both 1% NaOH and 2% H_2SO_4 pretreatments could extract 75% and 73% proteins and 89% and 90% pectin from the *Amaranth* de-protein biomass, the alkali pretreatment was much more effective for lignin removal by 71% and the acid pretreatment led to relatively more hemicellulose extraction by 60%,

Table 1
Estimation of bioethanol yields (t/ha/year) in *Amaranthus* straw and other bioenergy crops.

Sample	Dry biomass yield (t/ha/year)[a]	Pretreatment	Hexoses		Sugar-ethanol conversion (%)	Ethanol yield by hexoses (% DM)	Ethanol yield by hexoses (t/ha/year)	Pentoses (% DM)	Ethanol yield by pentoses[d] (t/ha/year)	Total bioethanol (t/ha/year)	Ref.
			Soluble sugars, starch & pectin (% DM)[b]	Cellulose (% DM)							
***Amaranthus* straw**	**54**	**1% NaOH + 8% AP[c]**	**14.26**	**26.70**	**98**	**20.56**	**11.10**	**17.83**	**3.36**	**14.46**	**This study**
		2% H_2SO_4 + 8% AP	**14.26**	**26.14**	**96**	**20.30**	**10.96**	**17.83**	**3.36**	**14.32**	
Sweet sorghum	20	12.5% CaO	15	39	68	24	5.76	25.42	2.13	7.89	(Kim et al., 2012)
Miscanthus straw	24	4% NaOH + 1% Tween-80	ND	44.4	98	19	4.56	29.10	2.44	7	(Alam et al., 2019)
Corn straw	21	LHW (200 ℃, 20 min)	24	17	95	19	3.99	23.72	1.74	5.73	(Wu et al., 2019a, 2019b)
Poplar stem	14	0.15 M Oxalic acid (150 ℃, 45 min)	ND	22	73	8	1.12	21.08	1.03	2.15	(Lv et al., 2021)
Wheat straw	10	Microwave (300 W, 15 min) + 0.2 M H_2SO_4	ND	25	85	11	1.10	28.42	0.994	2.094	(Mikulski and Grzegorz, 2020)
Rapeseed stalk	6	Steam explosion + 5% CaO	ND	43	93	21	1.26	23.99	0.503	1.733	(Deng et al., 2020)
Rice straw	7	1% NaOH + 1% Tween-80	ND	48	100	21	1.47	33.22	0.813	2.28	(Li et al., 2018)

[a] As ton/per hectare /per year
[b] Dry matter
[c] *Amaranth* proteins
[d] According to the average xylose-ethanol conversation rate of 35% (Rodrussamee et al., 2018; Valinhas et al., 2018)

Table 2
Wall polymers and proteins levels (% dry matter) in *Amaranthus* straw, de-protein biomass, and lignocellulose residues after alkali and acid pretreatments.

Pretreatment	Solid recovery rate (%)	Cellulose		Hemicelluloses		Lignin		Pectin		Protein	
Raw material	100	26.99 ± 0.70		13.79 ± 0.12		11.29 ± 0.37		4.91 ± 0.32		14.88 ± 0.54	
De-protein biomass	89.33	26.73 ± 0.63		13.61 ± 0.08		11.50 ± 0.78		4.80 ± 0.12		5.11 ± 0.23	
1% NaOH	51.73	38.09 ± 2.37**	42%[a]	6.25 ± 0.04**	-54%	3.34 ± 0.70**	-71%	0.51 ± 0.03**	-89%	1.23 ± 0.18**	-75%
2% H_2SO_4	55.82	34.67 ± 1.15*	30%	5.34 ± 0.12**	-60%	8.04 ± 1.37*	-30%	0.44 ± 0.05**	-90%	1.33 ± 0.12**	-73%

* & * * As significant difference between de-protein biomass and pretreated biomass by t-test at $P < 0.05$ and 0.01 ($n = 3$); [a]*Percentage was estimated by subtraction between de-protein biomass and pretreated biomass value divided by de-protein value. Data as mean ± SD ($n = 3$).*

consistent with the previous findings about distinct wall polymer extractions between alkali and acid pretreatments (Alam et al., 2019; Cheng et al., 2019; Li et al., 2015). However, compared to other major bioenergy crops (Lv et al., 2021; Xu et al., 2012), two mild alkali and acid pretreatments could more efficiently extract hemicellulose and lignin, resulting in cellulose levels being relatively raised by 42% and 30% in the pretreated residues.

With respect to effective extractions of wall polymers from mild chemical pretreatments, we applied XPS to test potential alterations of wall polymers interlinkages in the pretreated residues (Fig. 3). As it has been characterized that the C_1 peak (C═C, C-H) is originated from lignin and the C_2 (C-O or C-O-C) and C_3 (O-C-O or C═O) peaks correspond to wall polysaccharides including cellulose and hemicellulose (Chu et al., 2021a, 2021b), the decreasing amount of C_1 should be ascribed to lignin extraction during demethylation, aromatic ring-opening and side-chain cleavage, whereas the increasing amounts of C_2 and C_3 should be accountable for raised cellulose levels (Fig. 3A, Table S2). Moreover, $O_{1\,s}$ spectra were sorted out into three components, which were appointed to O_1: O-C═O and Ar-O-Ar (~531.3 eV), O_2: C-O-, C═O, C-O-C, and O-C═O (~532.4 eV). Because the phenolic oxygen (PhOH) was ascribed to O_3 with a binding energy of ~533.3 eV, it was apparent that both mild chemical pretreatments could decrease the PhOH group (i.e. lower O_3 peak) at the surfaces, which should attribute to the reduced cleavage of the β-O-4 aryl ether linkages (Fig. 3B).

Furthermore, the FTIR profiling of pretreated residues exhibited relatively reduced peaks of 834, 1247, 1383, 1512, 1627, and 1735 cm^{-1} assigned to C-H, C-O-C, C-H_2, C═C, and C═O associated with lignin and polysaccharide interlinkages (Fig. 4, Table S3), which was consistent with the XPS findings. Therefore, both XPS and FTIR analyses have confirmed effective wall polymer extractions from mild alkali and acid pretreatments performed in this study.

3.4. Significantly improved lignocellulose features upon mild chemical pretreatments

Since wall polymers extraction could distinctively improve lignocellulose recalcitrance for enhanced biomass enzymatic digestibility (Alam et al., 2019; Liu et al., 2021; Madadi et al., 2021b), we examined three major wall polymers features by means of the previously established approaches (Fig. 5). Compared to the *Amaranth* de-protein biomass, this study detected remarkably reduced cellulose CrI and DP values by 44–26% and 48–39% in the lignocellulose residues after 1%

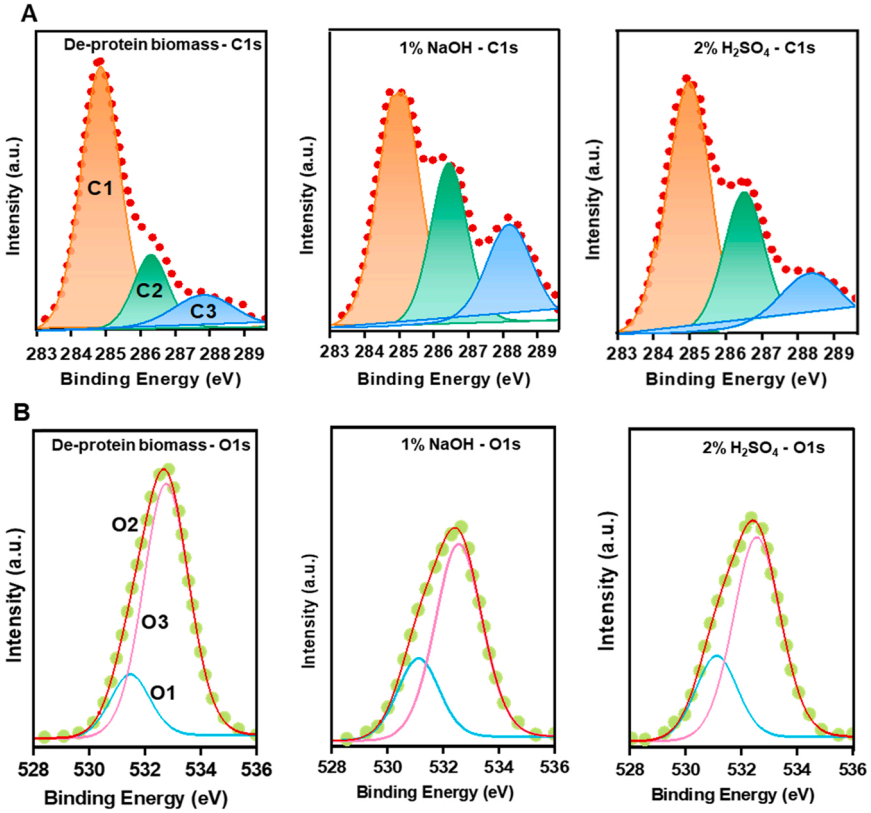

Fig. 3. High-resolution XPS for carbon (C1s) and oxygen (O1s) analyses in the de-protein biomass and chemical-pretreated residues of *Amaranthus* straw.

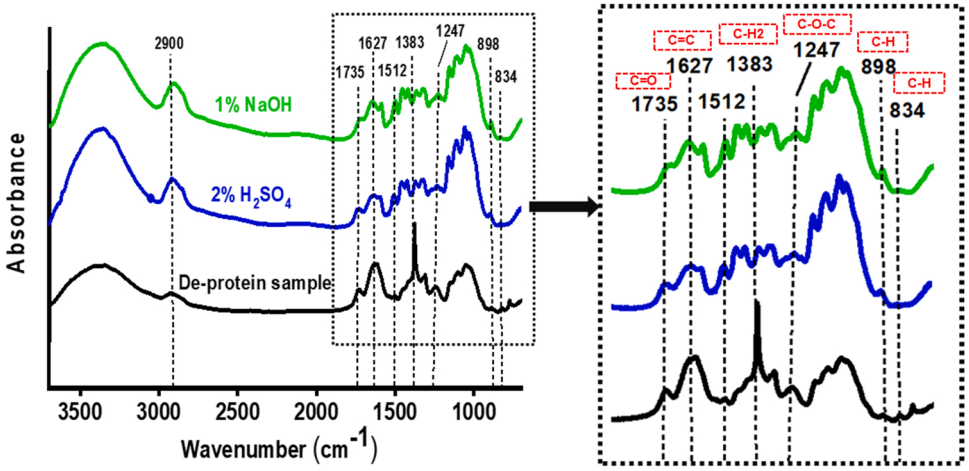

Fig. 4. FTIR spectroscopic profiling for the de-protein biomass and chemical-pretreated residues of *Amaranthus* straw. Dot squares highlighted the peak alteration as clarified in Table S3.

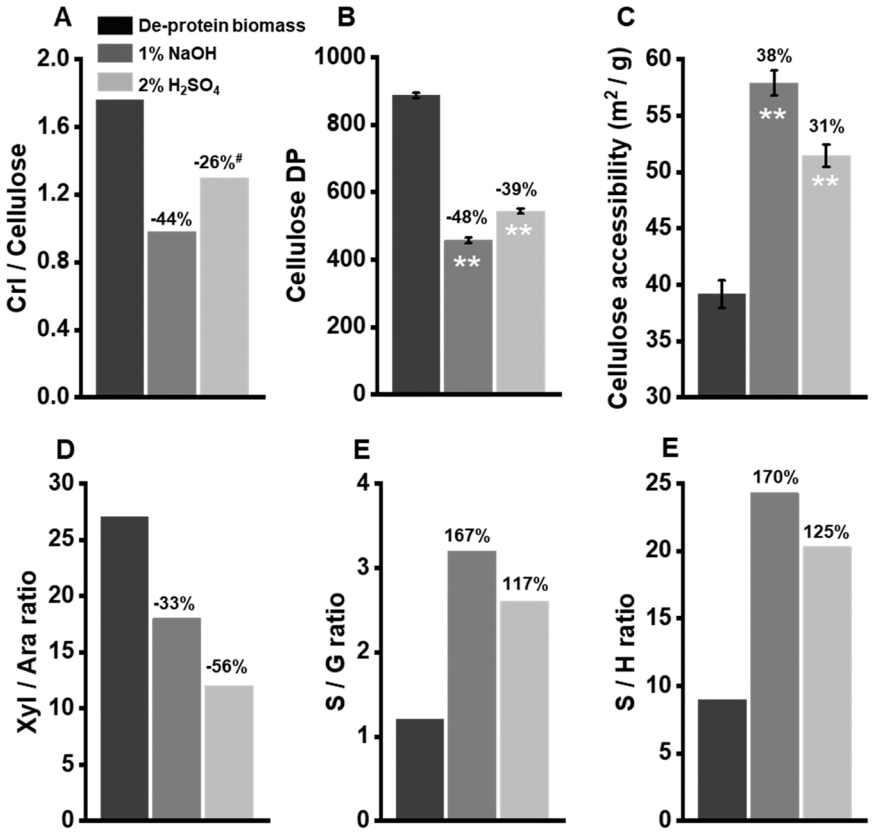

Fig. 5. Characterization of lignocellulose features in de-protein biomass and chemical-pretreated residues of *Amaranthus* straw. (**A, B, C**) Cellulose CrI, DP and accessibility; (**D**) Hemicellulose Xyl/Ara ratio; (E, F) Lignin S/G and S/H ratios; * *As significant difference between de-protein biomass and pretreated biomass by t-test at $P < 0.01$; #Calculated by subtraction between de-protein biomass and pretreated biomass values divided by de-protein value. Data as mean ± SD (n = 3).

NaOH and 2% H_2SO_4 pretreatments (Fig. 5A and B), which are two major cellulose features that are negatively accounting for lignocellulose recalcitrance (Gao et al., 2021; Wu et al., 2019a, 2019b). As a consequence, we measured significantly raised cellulose accessibility by 38–31% in the pretreated residues relative to the raw straw (Fig. 5C), which should be the positive parameter directly accountable for enhanced biomass enzymatic saccharification examined in this study.

Moreover, this work detected the monosaccharide content of hemicellulose and monomer proportion of lignin (Tables S4 and S5), which are accountable for two wall polymers' features (Wang et al., 2016). Among seven monosaccharides of hemicellulose examined, two chemical-pretreated residues showed much-reduced xylose (Xyl) proportions (76%, 62%) than that of the de-protein raw straw (85%) with relatively raised arabinose (Ara) ones, which led to much lower Xyl/Ara ratios in the pretreated residues relative to the raw straw (Fig. 5D). As the Xyl/Ara has been well examined as a negative factor on biomass enzymatic saccharification in the most grass crops examined (Alam et al., 2019; Li et al., 2013; Sun et al., 2020; Xu et al., 2012), the remarkably reduced Xyl/Ara ratio should be another factor accounting for much-improved lignocellulose recalcitrance from both mild alkali

and acid pretreatments performed in this study. In terms of three monomers (S, G, H) proportion of lignin, two chemical-pretreated residues were of relatively higher S-monomer and lower G- and H-monomers than those of the raw straw, leading to the S/G and S/H ratios raised by 2–3 folds (Fig. 5E and F), which suggested that the mild alkali and acid pretreatments should be effective to extract the G- and H-rich lignin from the *Amaranth* de-protein straw. It was also consistent with the previous findings that three lignin monomers play dual roles in biomass enzymatic hydrolyses (Li et al., 2014; Yoo et al., 2018).

3.5. Enhanced biomass porosity and cellulases adsorption

With respect to the recalcitrance-improved lignocellulose residues obtained from two mild chemical pretreatments, this study further detected biomass porosity by performing a classic Simons staining (Fig. 6). Compared to the *Amaranth* de-protein straw, the biomass residues obtained from 1% NaOH and 2% H_2SO_4 pretreatments respectively exhibited significantly increased yellow dye (DY) values by 57% and 49% at $P < 0.01$ levels (n = 3). Despite three samples showed small difference of blue dye (BY) values, the pretreated residues remained significantly higher total dye (DY+DB) values than those of the raw straw by 43% and 37% (Fig. 6A), indicating a remarkably enhanced biomass porosity from two mild chemical pretreatments. As DY and DB are respectively accounting for large and small pores of biomass residues (Alam et al., 2019; Deng et al., 2020), we further calculated much higher DY/DB ratios in the pretreated residues (Fig. 6B), which confirmed the previous findings that the large pore (DY) is more effective for cellulases enzymes adsorption and loading. As a further comparison, the alkali pretreatment could lead to relatively higher biomass porosity than that of the acid pretreatment including DY, total dye, and DY/DB ratio, consistent with their distinct lignin extractions described above. Meanwhile, we observed biomass morphology under SEM (Fig. 6C). As a comparison with the raw straw, two biomass residues obtained from mild alkali and acid pretreatments obviously displayed much rougher surfaces with more pores, consistent with significantly raised biomass porosity and cellulose accessibility described above.

To further test the biomass porosity enhanced from mild chemical pretreatments, we measured the adsorption capacity of mixed-cellulases enzymes with lignocellulose residues during enzymatic hydrolyses (Fig. 7). Compared to the de-protein raw straw, the lignocellulose

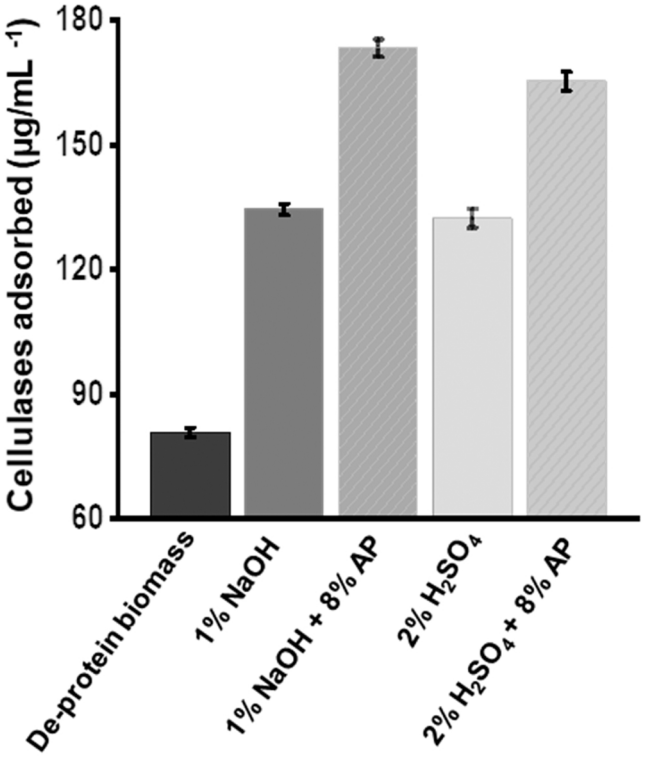

Fig. 7. Detection of cellulases enzymes adsorption in the de-protein biomass and chemical-pretreated residues of *Amaranthus* straw supplemented with 8% *Amaranth* proteins (AP).

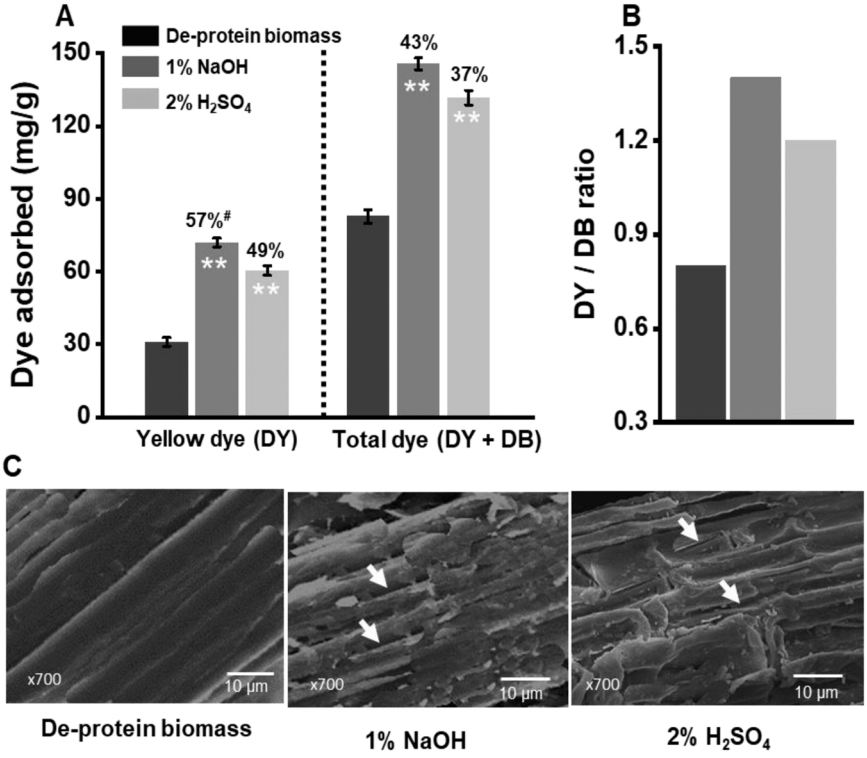

Fig. 6. Biomass porosity and morphology in the de-protein biomass and chemical-pretreated residues of *Amaranthus* straw. (**A**) Biomass porosity by Simons stain accounting for yellow dye (DY) and blue dye (DB) as large and small pores, respectively; (**B**) DY/DB ratio; (**C**) Scanning electron microscopic (SEM) observation of lignocellulose residues. * *As a significant difference between de-protein biomass and pretreated biomass by t-test at $P < 0.01$; #Calculated by subtraction between de-protein biomass and pretreated biomass values divided by de-protein value. Data as mean ± SD (n = 3).

residues obtained from 1% NaOH and 2% H_2SO_4 pretreatments showed much higher cellulases adsorption values (133 μg/mL^{-1}) with raised rates by more than 1.66 folds. However, while 8% *Amaranth* proteins were supplied into the enzymatic hydrolysis, the two chemical-pretreated residues respectively had cellulases adsorption capacity at 173 μg/mL^{-1} and 165 μg/mL^{-1}, which confirmed the previous findings that the *Amaranth* proteins could act as active biosurfactant to block cellulases interaction with lignin and other toxic compounds (Madadi et al., 2021a). Hence, this study has demonstrated that the cellulases adsorption was accountable by both biomass porosity and *Amaranth* proteins supply, which should cause an integrated role for enhanced biomass enzymatic saccharification examined above.

3.6. *Distinct mechanisms of enhanced biomass enzymatic saccharification and integrated bioethanol production in novel bioenergy Amaranth crop*

Based on all data obtained in this study, we proposed a hypothetic model to interpret why near-complete biomass enzymatic saccharification was achieved to maximize bioethanol production in *Amaranthus* straw (Fig. 8). The model illustrates that mild alkali and acid (1% NaOH, 2% H_2SO_4) pretreatments could extract large amounts of hemicellulose and lignin along with almost all pectin removal from the de-protein straw, which caused a significant modification of lignocellulose features such as cellulose CrI and DP, hemicellulose Xyl/Ara and lignin S/G and S/H ratios. These could largely reduce lignocellulose recalcitrance for remarkably improved biomass porosity and cellulose accessibility enabled to enhance cellulases adsorption. Meanwhile, co-supplement of *Amaranthus* proteins at low dosage could lead to almost complete biomass enzymatic saccharification by further enhancing cellulases adsorption. By integrating directly extractable sugars and starch with lignocellulose enzymatic hydrolysates, the model thus highlights the maximum bioethanol production achieved in the *Amaranthus* straw that is of the highest biomass yield among all major bioenergy crops examined, indicating that *Amaranthus* should be a novel and desirable bioenergy crop.

4. Conclusions

By performing mild alkali and acid pretreatments (1% NaOH, 2% H_2SO_4) followed with 8% endogenous proteins supplement into enzymatic hydrolysis, this study examined near-complete enzymatic saccharification with hexose yields of 99% and 97% (% cellulose) in the *Amaranthus* de-protein straw. The mild chemical pretreatments could effectively extract wall polymers and significantly improve lignocellulose features such as cellulose DP and CrI, hemicellulosic Xyl/Ara, and lignin S/G and S/H ratios, which led to remarkably enhanced biomass porosity and cellulose accessibility for high cellulase adsorption with pretreated lignocellulose substrates. In terms of high yield of *Amaranthus* biomass straw, this study estimated that the maximum bioethanol yield (14.46 t/ha/year) could be achieved by yeast fermentation with all carbon sources from directly extractable sugars and starch and complete enzymatic hydrolysis of pretreated lignocellulose substrates. Therefore, this study has not only demonstrated an effective biomass process technology for high bioethanol productivity, but it has also indicated that *Amaranthus* could be considered as one of the leading bioenergy crop candidates in the future.

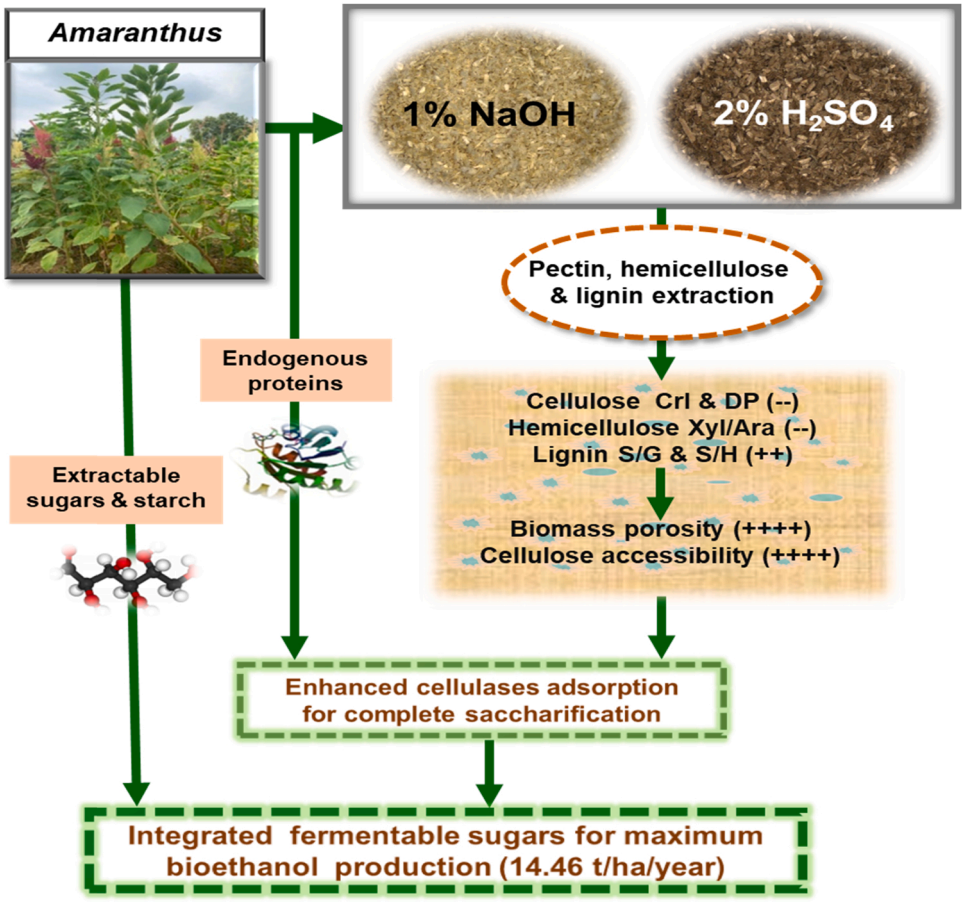

Fig. 8. A hypothetic model to interpret why near-complete biomass saccharification was achieved to maximize bioethanol production in bioenergy *Amaranthus* by connecting all major findings in this study. (-) and (+) as negative and positive factors accountable for biomass enzymatic saccharification.

CRediT authorship contribution statement

Meysam Madadi: Conceptualization, Investigation, Methodology, Formal analysis, Writing – original draft. **Youmei Wang**: Methodology, Formal analysis, Validation. **Ran Zhang**: Methodology, Formal analysis. **Zhen Hu**: Methodology, Formal analysis. **Hairong Gao:** Formal analysis, Methodology. **Dan Zhan:** Methodology, Investigation. **Hua Yu:** Methodology, Formal analysis, Validation. **Qiaomei Yang:** Investigation, Methodology. **Yanting Wang**: Validation, Project administration. **Yuanyuan Tu**: Co-Supervision, Methodology. **Tao Xia**: Editing, Validation. **Liangcai Peng**: Conceptualization, Writing – original draft, Writing – review & editing, Supervision, Funding acquisition.

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 this paper.

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Declaration of Competing Interest

The authors have no conflicts of interest to declare.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.indcrop.2021.114471.

References

Alam, A., Zhang, R., Liu, P., Huang, J., Wang, Y., Hu, Z., Madadi, M., Sun, D., Hu, R., Ragauskas, A.J., Tu, Y., Peng, L., 2019. A finalized determinant for complete lignocellulose enzymatic saccharification potential to maximize bioethanol production in bioenergy Miscanthus. Biotechnol. Biofuels 12, 99. https://doi.org/10.1186/s13068-019-1437-4.

Alam, A., Wang, Youmei, Liu, F., Kang, H., Tang, S. wen, Wang, Yanting, Cai, Q., Wang, H., Peng, H., Li, Q., Zeng, Y., Tu, Y., Xia, T., Peng, L., 2020. Modeling of optimal green liquor pretreatment for enhanced biomass saccharification and delignification by distinct alteration of wall polymer features and biomass porosity in Miscanthus. Renew. Energy 159, 1128–1138. https://doi.org/10.1016/j.renene.2020.06.013.

Cheng, L., Wang, L., Wei, L., Wu, Y., Alam, A., Xu, C., Wang, Y., Tu, Y., Peng, L., Xia, T., 2019. Combined mild chemical pretreatments for complete cadmium release and cellulosic ethanol co-production distinctive in wheat mutant straw. Green Chem. 21, 3693–3700. https://doi.org/10.1039/c9gc00686a.

Chu, Q., Tong, W., Wu, S., Jin, Y., Hu, J., Song, K., 2021a. Modification of lignin by various additives to mitigate lignin inhibition for improved enzymatic digestibility of dilute acid pretreated hardwood. Renew. Energy 177, 992–1000. https://doi.org/10.1016/j.renene.2021.06.048.

Chu, Q., Tong, W., Wu, S., Jin, Y., Hu, J., Song, K., 2021b. Eco-friendly additives in acidic pretreatment to boost enzymatic saccharification of hardwood for sustainable biorefinery applications. Green Chem. 23, 4074–4086. https://doi.org/10.1039/d1gc00738f.

Deng, J., Zhu, X., Chen, P., He, B., Tang, S., Zhao, W., Li, X., Zhang, R., Lv, Z., Kang, H., Yu, L., Peng, L., 2020. Mechanism of lignocellulose modification and enzyme disadsorption for complete biomass saccharification to maximize bioethanol yield in rapeseed stalks. Sustain. Energy Fuels 4, 607–618. https://doi.org/10.1039/C9SE00906J.

Dische, Z., Pallavicini, C., Kavasaki, H., Smirnow, N., Cizek, L.J., Chien, S., 1962. Influence of the nature of the secretory stimulus on the composition of the carbohydrate moiety of glycoproteins of the submaxillary saliva. Arch. Biochem. Biophys. 97, 459–469. https://doi.org/10.1016/0003-9861(62)90108-X.

Florencio, C., Badino, A.C., Farinas, C.S., 2016. Soybean protein as a cost-effective lignin-blocking additive for the saccharification of sugarcane bagasse. Bioresour. Technol. 221, 172–180. https://doi.org/10.1016/j.biortech.2016.09.039.

Fry, S., 2000. The Growing Plant Cell Wall: Chemical And Metabolic Analysis. Longman, London, p. 3.

Gao, H., Wang, Y., Yang, Q., Peng, H., Li, Y., Zhan, D., Wei, H., Lu, H., Bakr, M.M.A., EI-Sheekh, M.M., Qi, Z., Peng, L., Lin, X., 2021. Combined steam explosion and optimized green-liquor pretreatments are effective for complete saccharification to maximize bioethanol production by reducing lignocellulose recalcitrance in one-year-old bamboo. Renew. Energy 175, 1069–1079. https://doi.org/10.1016/j.renene.2021.05.016.

Ho, M.C., Ong, V.Z., Wu, T.Y., 2019. Potential use of alkaline hydrogen peroxide in lignocellulosic biomass pretreatment and valorization – a review. Renew. Sustain. Energy Rev. 112, 75–86. https://doi.org/10.1016/j.rser.2019.04.082.

Jin, W., Chen, L., Hu, M., Sun, D., Li, A., Li, Ying, Hu, Z., Zhou, S., Tu, Y., Xia, T., Wang, Y., Xie, G., Li, Yanbin, Bai, B., Peng, L., 2016. Tween-80 is effective for enhancing steam-exploded biomass enzymatic saccharification and ethanol production by specifically lessening cellulase absorption with lignin in common reed. Appl. Energy 175, 82–90. https://doi.org/10.1016/j.apenergy.2016.04.104.

Kim, J.S., Lee, Y.Y., Kim, T.H., 2016. A review on alkaline pretreatment technology for bioconversion of lignocellulosic biomass. Bioresour. Technol. https://doi.org/10.1016/j.biortech.2015.08.085.

Kim, M., Han, K.J., Jeong, Y., Day, D.F., 2012. Utilization of whole sweet sorghum containing juice, leaves, and bagasse for bio-ethanol production. Food Sci. Biotechnol. 21, 1075–1080. https://doi.org/10.1007/s10068-012-0139-5.

Kumar, B., Bhardwaj, N., Agrawal, K., Chaturvedi, V., Verma, P., 2020. Current perspective on pretreatment technologies using lignocellulosic biomass: an emerging biorefinery concept. Fuel Process. Technol. 199, 106244 https://doi.org/10.1016/j.fuproc.2019.106244.

Li, F., Ren, S., Zhang, W., Xu, Z., Xie, G., Chen, Y., Tu, Y., Li, Qing, Zhou, S., Li, Y., Tu, F., Liu, L., Wang, Y., Jiang, J., Qin, J., Li, S., Li, Qiwei, Jing, H.C., Zhou, F., Gutterson, N., Peng, L., 2013. Arabinose substitution degree in xylan positively affects lignocellulose enzymatic digestibility after various NaOH/H2SO4 pretreatments in Miscanthus. Bioresour. Technol. 130, 629–637. https://doi.org/10.1016/j.biortech.2012.12.107.

Li, F., Zhang, M., Guo, K., Hu, Z., Zhang, R., Feng, Y., Yi, X., Zou, W., Wang, L., Wu, C., Tian, J., Lu, T., Xie, G., Peng, L., 2015. High-level hemicellulosic arabinose predominately affects lignocellulose crystallinity for genetically enhancing both plant lodging resistance and biomass enzymatic digestibility in rice mutants. Plant Biotechnol. J. 13, 514–525. https://doi.org/10.1111/pbi.12276.

Li, Ming, Si, S., Hao, B., Zha, Y., Wan, C., Hong, S., Kang, Y., Jia, J., Zhang, J., Li, Meng, Zhao, C., Tu, Y., Zhou, S., Peng, L., 2014. Mild alkali-pretreatment effectively extracts guaiacyl-rich lignin for high lignocellulose digestibility coupled with largely diminishing yeast fermentation inhibitors in Miscanthus. Bioresour. Technol. 169, 447–454. https://doi.org/10.1016/j.biortech.2014.07.017.

Li, Y., Liu, P., Huang, J., Zhang, R., Hu, Z., Feng, S., Wang, Y., Wang, L., Xia, T., Peng, L., 2018. Mild chemical pretreatments are sufficient for bioethanol production in transgenic rice straws overproducing glucosidase. Green Chem. https://doi.org/10.1039/C8GC00694F.

Liu, C., Xiao, Y., Xia, X., Zhao, X., Peng, L., Srinophakun, P., 2018. Cellulosic ethanol production: progress, challenges and strategies for solutions. Biotechnol. Adv. 37, 491–504. https://doi.org/10.1016/j.biotechadv.2019.03.002.

Liu, F., Li, J., Yu, H., Li, Y., Wang, Y., Gao, H., Peng, H., Hu, Z., Wang, H., Zhang, G., Tu, Y., Peng, L., 2021. Optimizing two green-like biomass pretreatments for maximum bioethanol production using banana pseudostem by effectively enhancing cellulose depolymerization and accessibility. Sustain. Energy Fuels 5, 3467–3478. https://doi.org/10.1039/d1se00613d.

Lv, Z., Liu, F., Zhang, Y., Tu, Y., Chen, P., Peng, L., 2021. Ecologically adaptable Populus simonii is specific for recalcitrance-reduced lignocellulose and largely enhanced enzymatic saccharification among woody plants. GCB Bioenergy 13, 348–360. https://doi.org/10.1111/gcbb.12764.

Madadi, M., Peng, C., Aqleem, A., 2017a. Advances in genetic manipulation of lignocellulose to reduce biomass recalcitrance and enhance biofuel production in bioenergy crops. J. Plant Biochem. Physiol. 05, 1–15. https://doi.org/10.4172/2329-9029.1000182.

Madadi, M., Tu, Y., Abbas, A., 2017b. Present status on enzymatic hydrolysis of lignocellulosic biomass for bioethanol production. In: Electronic Journal of Biology. John Wiley & Sons, Inc., Hoboken, NJ, USA, pp. 85–95. https://doi.org/10.1002/9781119117322.ch4.

Madadi, M., Wang, Youmei, Xu, C., Liu, P., Wang, Yanting, Xia, T., Tu, Y., Lin, X., Song, B., Yang, X., Zhu, W., Duanmu, D., Tang, S. wen, Peng, L., 2021a. Using Amaranthus green proteins as universal biosurfactant and biosorbent for effective enzymatic degradation of diverse lignocellulose residues and efficient multiple trace metals remediation of farming lands. J. Hazard. Mater. 406, 124727 https://doi.org/10.1016/j.jhazmat.2020.124727.

Madadi, M., Zhao, K., Wang, Youmei, Wang, Yanting, Tang, S. wen, Xia, T., Jin, N., Xu, Z., Li, G., Qi, Z., Peng, L., Xiong, Z., 2021b. Modified lignocellulose and rich starch for complete saccharification to maximize bioethanol in distinct polyploidy potato straw. Carbohydr. Polym. 265, 118070 https://doi.org/10.1016/j.carbpol.2021.118070.

Mikulski, D., Grzegorz, K., 2020. Microwave-assisted dilute acid pretreatment in bioethanol production from wheat and rye stillages. Biomass Bioenergy 136. https://doi.org/10.1016/j.biombioe.2020.105528.

Peng, L., Hocart, C.H., Redmond, J.W., Williamson, R.E., 2000. Fractionation of carbohydrates in Arabidopsis root cell walls shows that three radial swelling loci are specifically involved in cellulose production. Planta 211, 406–414. https://doi.org/10.1007/s004250000301.

Ragauskas, A.J., Williams, C.K., Davison, B.H., Britovsek, G., Cairney, J., Eckert Jr., C.A., Hallett, W.J.F., Leak, J.P., Liotta, D.J., Mielenz, C.L., Murphy, J.R., Templer, R.,

Tschaplinski, T, R., 2006. The path forward for biofuels. Science 311, 484–489 (80–.).
Raud, M., Kikas, T., Sippula, O., Shurpali, N.J., 2019. Potentials and challenges in lignocellulosic biofuel production technology. Renew. Sustain. Energy Rev. 111, 44–56. https://doi.org/10.1016/j.rser.2019.05.020.
Robak, K., Balcerek, M., 2020. Current state-of-the-art in ethanol production from lignocellulosic feedstocks. Microbiol. Res. 240, 126534 https://doi.org/10.1016/j.micres.2020.126534.
Rodrussamee, N., Sattayawat, P., Yamada, M., 2018. Highly efficient conversion of xylose to ethanol without glucose repression by newly isolated thermotolerant Spathaspora passalidarum CMUWF1-2. BMC Microbiol 18, 73. https://doi.org/10.1186/s12866-018-1218-4.
Sharma, B., Larroche, C., Dussap, C.G., 2020. Comprehensive assessment of 2G bioethanol production. Bioresour. Technol. 313, 123630 https://doi.org/10.1016/j.biortech.2020.123630.
Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Slui, J., Templeton, D., Crocker, D., 2008. Determination of structural carbohydrates and lignin in biomass. Lab. Anal. Proced. 1617, 1–16.
Sun, D., Alam, A., Tu, Y., Zhou, S., Wang, Y., Xia, T., Huang, J., Li, Y., Zahoor, Wei, X., Hao, B., Peng, L., 2017. Steam-exploded biomass saccharification is predominately affected by lignocellulose porosity and largely enhanced by Tween-80 in Miscanthus. Bioresour. Technol. 239, 74–81. https://doi.org/10.1016/j.biortech.2017.04.114.
Sun, D., Yang, Q., Wang, Yanting, Gao, H., He, M., Lin, X., Lu, J., Wang, Youmei, Kang, H., Alam, A., Tu, Y., Xia, T., Peng, L., 2020. Distinct mechanisms of enzymatic saccharification and bioethanol conversion enhancement by three surfactants under steam explosion and mild chemical pretreatments in bioenergy Miscanthus. Ind. Crops Prod. 153, 112559 https://doi.org/10.1016/j.indcrop.2020.112559.
Sun, J., Murthy Konda, N.V.S.N., Shi, J., Parthasarathi, R., Dutta, T., Xu, F., Scown, C.D., Simmons, B.A., Singh, S., 2016. CO_2 enabled process integration for the production of cellulosic ethanol using bionic liquids. Energy Environ. Sci. 9, 2822–2834. https://doi.org/10.1039/c6ee00913a.
Tucker, Jonathan, 1986. Amaranth: The Once and Future Crop, 36. Oxford Univ. Press, pp. 9–13.
Valinhas, R.V., Pantoja, L.A., Maia, A.C.F., Miguel, M.G.C.P., Vanzela, A.P.F.C., Nelson, D.L., Santos, A.S., 2018. Xylose fermentation to ethanol by new Galactomyces geotrichum and Candida akabanensis strains. PeerJ 4, 1–16. https://doi.org/10.7717/peerj.4673.
Viglasky, J., Andrejack, I., Huska, J., Suchomel, J., 2009. Amaranth (Amarantus L.) is a potential source of raw material for biofuels production. Agron. Res. 7, 865–873.
Wang, Yanting, Fan, C., Hu, H., Li, Y., Sun, D., Wang, Youmei, Peng, L., 2016. Genetic modification of plant cell walls to enhance biomass yield and biofuel production in bioenergy crops. Biotechnol. Adv. 34, 997–1017. https://doi.org/10.1016/j.biotechadv.2016.06.001.
Wang, Youmei, Liu, P., Zhang, G., Yang, Q., Lu, J., Xia, T., Peng, L., Wang, Yanting, 2021. Cascading of engineered bioenergy plants and fungi sustainable for low-cost bioethanol and high-value biomaterials under green-like biomass processing. Renew. Sustain. Energy Rev. 137, 110586 https://doi.org/10.1016/j.rser.2020.110586.
Wu, L., Feng, S., Deng, J., Yu, B., Wang, Y., He, B., Peng, H., Li, Q., Hu, R., Peng, L., 2019a. Altered carbon assimilation and cellulose accessibility to maximize bioethanol yield under low-cost biomass processing in corn brittle stalk. Green. Chem. 21, 4388–4399. https://doi.org/10.1039/c9gc01237k.
Wu, Y., Wang, M., Yu, L., Tang, S., Xia, T., Kang, H., Xu, C., Gao, H., Madadi, M., Alam, A., Cheng, L., Peng, L., 2019b. A mechanism for efficient cadmium phytoremediation and high bioethanol production by combined mild chemical pretreatments with desirable rapeseed stalks. Sci. Total Environ. 708, 135096 https://doi.org/10.1016/j.scitotenv.2019.135096.
Xu, C., Zhu, J., Yu, Haizhong, Yu, Hua, Yang, Y., Fu, Q., Zhan, D., Wang, Y., Wang, H., Zhang, Y., Li, T., El-Sheekh, M.M., Peng, L., Xia, T., 2021. Recyclable cascading of arsenic phytoremediation and lead removal coupled with high bioethanol production using desirable rice straws. Biochem. Eng. J. 168, 107950 https://doi.org/10.1016/j.bej.2021.107950.
Xu, N., Zhang, W., Ren, S., Liu, F., Zhao, C., Liao, H., Xu, Z., Huang, J., Li, Q., Tu, Y., Yu, B., Wang, Y., Jiang, J., Qin, J., Peng, L., 2012. Hemicelluloses negatively affect lignocellulose crystallinity for high biomass digestibility under NaOH and H2SO4 pretreatments in Miscanthus. Biotechnol. Biofuels 5, 58. https://doi.org/10.1186/1754-6834-5-58.
Yoo, C.G., Dumitrache, A., Muchero, W., Natzke, J., Akinosho, H., Li, M., Sykes, R.W., Brown, S.D., Davison, B., Tuskan, G.A., Pu, Y., Ragauskas, A.J., 2018. Significance of lignin S/G ratio in biomass recalcitrance of populus trichocarpa variants for bioethanol production. ACS Sustain. Chem. Eng. 6, 2162–2168. https://doi.org/10.1021/acssuschemeng.7b03586.
Zahoor, Tu, Wang, Y., Xia, L., Sun, T., Zhou, D., Wang, S., Li, Y., Zhang, Y., Zhang, H., Madadi, T., Peng, L, M., 2017. Mild chemical pretreatments are sufficient for complete saccharification of steam-exploded residues and high ethanol production in desirable wheat accessions. Bioresour. Technol. 243, 319–326. https://doi.org/10.1016/j.biortech.2017.06.111.
Zhao, K., Jin, N., Madadi, M., Wang, Youmei, Wu, L., Xu, Z., Wang, J., Dong, J., Tang, S., Wang, Yanting, Peng, L., Xiong, Z., 2021. Incomplete genome doubling enables to consistently enhance plant growth for maximum biomass production by altering multiple transcript co-expression networks in potato. Theor. Appl. Genet. https://doi.org/10.1007/s00122-021-03976-y.