Industrial Crops & Products 240 (2026) 122534

Contents lists available at ScienceDirect

Industrial Crops & Products

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

Biomass saccharification of bioenergy crops enhanced by selective soybean proteins for distinct cellulosic ethanol and lactic acid conversions with high Cd^{2+} adsorption

Ruilan Yang [a,1], Yingying Su [b,c,1], Jiamin Li [a], Yasi Zhou [a], Yixiang Wang [b], Siqin Tan [a], Jiale Liu [a], Yanting Wang [a], Dan Sun [a], Tao Xia [b,c], Heng Kang [a,b,*,2], Liangcai Peng [a,b,*,2]

[a] Key Laboratory of Fermentation Engineering (Ministry of Education), Cooperative Innovation Center of Industrial Fermentation (Ministry of Education & Hubei Province), Hubei Key Laboratory of Industrial Microbiology, Biomass & Bioenergy Research Centre, School of Life & Health Sciences, Hubei University of Technology, Wuhan 430068, China

[b] College of Plant Science & Technology, Huazhong Agricultural University, Wuhan 430070, China

[c] College of Life Science & Technology, Huazhong Agricultural University, Wuhan 430070, China

ARTICLE INFO

Keywords:
Lignocellulose
Non-catalytic proteins
Mixed-cellulases
Cellulosic ethanol
Lactic acid
Cadmium adsorption

ABSTRACT

As crop straws provide enormous lignocellulose resources convertible for renewable biofuels and valuable bio-products, an efficient biomass saccharification is thus considered as the critical step for biochemical conversion. Although plant proteins have been applied as active biosurfactants for blocking lignin interaction with lignocellulose-degradation enzymes, little is yet reported about their distinct roles from different plant sources in entire lignocellulose process. In this study, we examined that the co-supplies of soybean seed and leaf proteins could consistently enhance enzymatic saccharification of diverse lignocelluloses substrates from physical and chemical pretreatments with mature straws in three representative bioenergy crops (rice, corn, *Miscanthus*). By performing yeast fermentation with enzymatic hydrates, the seed protein samples showed the bioethanol yields increased by 25 %-38 %, whereas the leaf protein samples had the bioethanol ones reduced by 31 %-48 %. However, both seed and leaf protein supplies improved the *Lactobacillus paracasei* fermentations for lactic acid yields raised by 11 %-61 %. Furthermore, the lignocellulose residues retained from ethanol and lactic acid fermentations were ultimately characterized as active biosorbents for cadmium (Cd^{2+}) adsorption, but only leaf protein samples showed significantly increased adsorptive capacities. Notably, this study attempted to sort out the mechanisms about how the seed and leaf protein supplies could significantly enhance biomass enzymatic saccharifications for either consistently improved lactic acid conversions or contrastively affected bioethanol fermentations, thereby providing a green-like strategy selective for the optimal bioethanol and lactic acid production and heavy metal adsorption with zero-biomass release.

1. Introduction

Lignocellulosic biomass, the most abundant renewable carbon source on earth, possesses transformative potential for partially replacing fossil fuels via production of biofuels (Oshikata et al., 2024). Its utilization aligns with global decarbonization strategies, particularly through integrated biorefineries such as co-production of renewable bioethanol and valuable biochemical such as lactic acid (Pei et al., 2024; Yang et al., 2018). However, the inherent recalcitrance of lignocellulose, which is mainly determined by lignin content, cellulose crystallinity and hemicellulose crosslinking, imposes a critical limitation on biomass enzymatic saccharification and subsequent fermentation capacity

* Corresponding authors at: Key Laboratory of Fermentation Engineering (Ministry of Education), Cooperative Innovation Center of Industrial Fermentation (Ministry of Education & Hubei Province), Hubei Key Laboratory of Industrial Microbiology, Biomass & Bioenergy Research Centre, School of Life & Health Sciences, Hubei University of Technology, Wuhan 430068, China.

E-mail addresses: hkang@mail.hzau.edu.cn (H. Kang), lpeng@mail.hzau.edu.cn (L. Peng).

[1] The authors contributed equally.

[2] Web: http://bbrc.hbut.edu.cn.

https://doi.org/10.1016/j.indcrop.2025.122534
Received 18 October 2025; Received in revised form 7 December 2025; Accepted 16 December 2025
Available online 6 January 2026

(Wang et al., 2015). Physical and chemical pretreatments partially address such challenge by weakening the lignocellulose matrix and making it more susceptible to enzymatic attack, but they are substantially energetic costs and environment burdens (Kaur et al., 2023; Zhang et al., 2023b). This thus necessitates the development of sustainable additives that enhance bioconversion without intensifying process severity.

Given the green-like pretreatment is efficient and sustainable (Zhang et al., 2023b), it is principally required to minimize energy consumption and also to eliminate the use of harmful chemicals. The uses of additives such as non-ionic surfactants (Tween 20 and 80), polymers (polyethylene glycol, PEG 6000) and non-catalytic proteins (bovine serum albumin, BSA; soybean protein; *Amaranthus* protein; milk protein) have drastically increased the enzymatic hydrolyses of cellulose microfibrils into fermentable sugar (Brethauer et al., 2011; Eriksson et al., 2002; Florencio et al., 2016; Hiltunen et al., 2024; Kapu et al., 2012; Kristensen et al., 2007; Kumar and Wyman. 2009; Madadi et al., 2021; Yang and Wyman, 2006). These additives could interact with the lignin of pretreated lignocellulose as lignin blockers and detoxifiers, thus preventing the non-productive adsorption between cellulose and lignin or dissociating hemicellulose (Huang et al., 2022; Jin et al., 2016; Madadi et al., 2023). Among those surfactants, soybean protein stands out as a promising cost-effective selection, because it is abundant, easy to obtain and much cheaper than others (Klein-Marcuschamer et al., 2012). Despite the soybean protein has been recently examined for distinctively enhancing biomass enzymatic saccharification in pretreated bamboo, eucalyptus and pine (Luo et al., 2019), much is unknown about the roles of different soybean protein sources in biomass saccharification for bioethanol and biochemical conversions.

As lignin-rich residues are retained from biomass saccharification and biofuel conversion, they are considered to generate highly-valuable bioproducts with zero-biomass release into the environment (Xu et al., 2021). Particularly, desirable lignocellulose-derived substrates are directly employable as green and economical biosorbents for removals of heavy metals from pollutant water and other resource (Mei et al., 2023). Since cadmium (Cd) is one of major heavy metals contaminated in agriculture and industry locations worldwide (Rasheed et al., 2024; Xia et al., 2021), it remains to examine the lignin-rich residues from lactic acid fermentation for Cd adsorption.

As soybean seed protein is a major source for human food and animal feed, plant leaves offer a promising alternative for improving biomass enzymatic saccharification (Madadi et al., 2021). In this study, we respectively extracted the proteins from soybean seeds and leaves, and examined their optimal dosages supplements for enhancing enzymatic saccharification of distinct lignocellulose substrates in three major bioenergy crops (rice, corn, *Miscanthus*) after different pretreatments were conducted (Fig. S1). By performing yeast fermentation with enzymatic hydrates, we found a contrastive impact on sugar-ethanol conversion between the seed and leaf protein samples, and also detected their distinct enhancements of sugar-lactic acid conversions via *Lactobacillus paracasei* fermentation. Furthermore, we collected all lignocellulose residues from the ethanol and lactic acid fermentations to examine their divergent adsorption capacities with cadmium (Cd^{2+}) (Zhang et al., 2025). Therefore, this study is aimed to sort out how soybean seed and leaf proteins play distinct roles in biomass enzymatic saccharification, bioethanol and lactic acid fermentation productivity and heavy metal removal capacity, providing a green-like strategy selective for cascading optimal enhancements of lignocellulose degradation, cellulosic ethanol and lactic acid conversion, and biosorbent adsorption with zero-biomass release.

2. Materials and methods

2.1. Collection of biomass samples

The lignocellulose samples of three representative crops (rice/*Oryza sativa* L./NPB; corn/*Zea mays L.*/B73; *Miscanthus*/ *M. sacchariflorus*/ Msa01) were respectively collected from the Experimental Fields of Huazhong Agricultural University. All biomass samples were dried at 60℃, cut into small pieces, screened by a 40-mesh sieve and stored in a dry container until in use.

2.2. Soybean seed and leaf protein extraction

For soybean seed protein extraction, the seeds were dried and crushed through a 60-mesh sieve to obtain powder sample. At a solid-liquid ratio of 1:10, distilled water was added to the powder for dissolution, and the protein was extracted by the alkali precipitation and acid extraction method as previously described (Souza et al., 2016). For soybean leaf protein extraction, fresh leaves were homogenized in a 1:5 ratio with acetate buffer (0.2 mol/L Na-acetate, pH 4.8), centrifuged at 4000 *g* for 5 min to collect the supernatant, and the protein was extracted using the alcohol precipitation method as described (Yoshikawa et al., 2012).

2.3. Biomass pretreatments

For H_2SO_4 pretreatments, the well-mixed biomass powder samples of rice, corn and *Miscanthus* were respectively treated with 6 mL H_2SO_4 (1.0 %, w/v) at 120 ℃ for 20 min. After centrifugation at 3000 *g* for 5 min, all supernatants were collected for hexoses and pentose assay as previously described (Alam et al., 2019; Gu et al., 2021).

For NaOH pretreatments, the well-mixed biomass powder samples were respectively incubated with 6 mL NaOH at different concentrations (0.5 %, 1.0 %, 4 %; w/v) under 150 r/min shaken at 50℃ for 2 h. After centrifugation at 3000 *g* for 5 min, all supernatants were collected for hexoses and pentose assay as described (Hu et al., 2018; Zhang et al., 2013).

Liquid hot water (LHW) pretreatments: The well-mixed biomass powder samples were placed in a 25 mL reactor and supplemented with 2.4 mL distilled water. The LHW pretreatment processes were conducted at 200℃ for 16 min. After the reaction, all supernatants were collected for hexoses and pentose assay as described (Chen et al., 2022).

2.4. Biomass enzymatic hydrolysis co-supplied with soybean proteins

Pretreated biomass residues were incubated with 1.6 g/L mixed-cellulase enzymes (HSB) with final concentrations of cellulases at 10.60 FPU/g biomass and xylanase at 6.72 U/g biomass with 5 % (w/v) solid loading, while co-supplied with various concentration of soybean proteins (0 %, 8 %, 12 %, 16 %, 20 %; w/w) (Gao et al., 2021). The hydrolysis reactions were performed at 50℃ for 48 h under 150 rpm shaken. The released hexose and pentose were measured by anthrone/H_2SO_4 and orcinol/HCl methods, respectively as described (Zhang et al., 2023b). A standard curve for hexose assay was established using anhydrous glucose based on the equation:

$$\text{Hexose yield (\% cellulose)} = \frac{\text{hexose content (\%)}}{\text{cellulose content (\%)}} \times 100\%$$

Because the high pentoses level of the biomass sample can affect the absorbance reading at 620 nm for hexoses content, the deduction from pentoses reading at 660 nm was conducted for final calculation of hexoses level. All assays were accomplished under independent triplicate.

2.5. Yeast fermentation and ethanol measurement

Yeast (*Saccharomyces cerevisiae*) strain (Angel Yeast Co., Ltd., Yichang, China) was used in all ethanol fermentation reactions. The yeast powder was suspended in pH 4.8 phosphate buffer to achieve the final concentration of 0.5 g/L in all fermentation tubes, and the fermentation was performed at 37℃ for 48 h (Fan et al., 2022). After

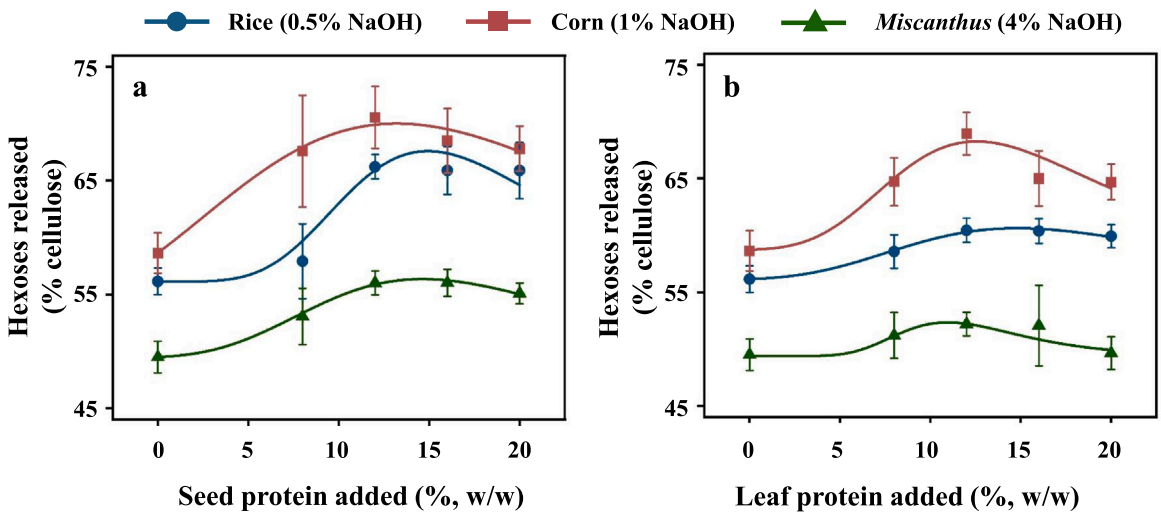

Fig. 1. Biomass saccharification enhanced by different dosages of soybean seed and leaf proteins supplements by accounting for hexoses yields (% cellulose) released from enzymatic hydrolyses of mature straws in rice, corn and *Miscanthus* after NaOH pretreatments at different concentrations. (a) Soybean seed protein supply; (b) Soybean leaf protein supply. Data as means (n = 3) and bar as standard deviation (SD).

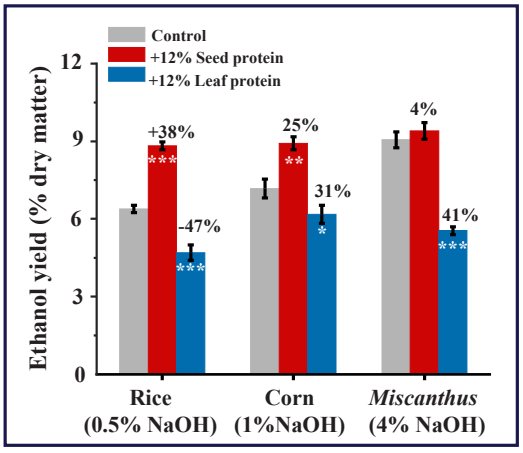

Fig. 2. Ethanol production by yeast fermentations using sugars released from enzymatic hydrolyses of mature straws in three crops co-supplied with 12 % soybean seed and leaf proteins. Data as means ± SD (n = 3); */**/***As significant difference between the protein-supplied sample and control (without protein supply) by student's *t*-test at $p < 0.05/0.01/0.01$ level; # As increased percentage in the protein-supplied sample relative to the control.

fermentation solution was distilled, the ethanol concentration was estimated by $K_2Cr_2O_7$ method under independent triplicate (Fan et al., 2022). Absolute ethanol (99.9 %) was used as the standard and the ethanol level was calculated according to the equation:

$$\text{Ethanol yield (\% dry matter)} = \frac{\text{actual ethanol mass (g)}}{\text{sample mass (g)}} \times 100\%$$

2.6. Lactic acid fermentation and lactate measurement

The enzymatic hydrates were sterile and adjusted to pH 5.5, and then inoculated with 10 % (v/v) *Lactobacillus paracasei* (provided by the Fermentation Engineering Laboratory, Huazhong Agricultural University) at 37℃ for 36 h. The lactic acid yield was accounted using the *P*-hydroxybiphenyl method at independent triplicate as previously described (Zhang et al., 2024). Standard lactic acid was used as the control and all assays were accomplished at independent triplicate.

2.7. Protein binding assay

Protein binding assay was conducted as previously described (Henshaw et al., 2004) with minor modification. About 0.036 g soybean protein was incubated with 0.3000 g commercial lignin in 6 mL 0.2 mol/L Na-acetate buffer (pH 4.8). After shaken at 50℃ for 5 min, the samples were centrifuged at 4000 *g* for 5 min to collect unbound protein in supernatant, and the SDS-PAGE separation was conducted as described (Madadi et al., 2021). All binding assays were accomplished at independent triplicate.

2.8. Contact angle analysis and Zate potentiometric measurement

Commercial lignin and soybean protein (12 %, w/v) were incubated at 50℃ for 15 min and dried to prepare the samples for water contact angle analysis using an automatic contact angle meter (100WDSA25, Life Technologies/ABI, Stockholm, USA) (Stadler et al., 2003). Each sample was examined three times, and the average contact angle value was calculated. The zeta potential of the adsorbed supernatant was determined by a dynamic light scattering (DLS) analyzer (Zetasizer Nano ZS, Malvern Instruments, Malvern, UK) equipped with laser Doppler microelectrophoresis (Luo et al., 2019). The samples were filtered by 0.45 μm syringe membranes (Millipore, Billerica, MA, USA) for analysis and deionized water was used for background correction. All measurements were performed under independent triplicate.

2.9. Fermentation of standard glucose co-supplied with soybean protein

Ethanol fermentation: 12 % (w/v) soybean protein was respectively added with 8 % and 32 % (w/v) glucose, and inoculated with yeast strain at 37℃ for 48 h. The OD_{600} values of yeast cells during a time-course growth (0, 4, 8, 16, 32, and 48 h) were determined at 600 nm, and the ethanol yield was estimated by $K_2Cr_2O_7$ method (Fan et al., 2022). All experiments were accomplished under independent triplicate.

Lactic acid fermentation: 12 % soybean protein was respectively added with 5 % and 10 % (w/v) glucose, and inoculated with *Lactobacillus paracasei* at 37℃ for 48 h. The OD_{600} values of yeast cells during a time-course growth (0, 4, 8, 16, 32, and 48 h) were determined at 600 nm, and the lactic acid was determined by *P*-hydroxybiphenyl method (Zhang et al., 2024). All assays were accomplished under independent triplicate.

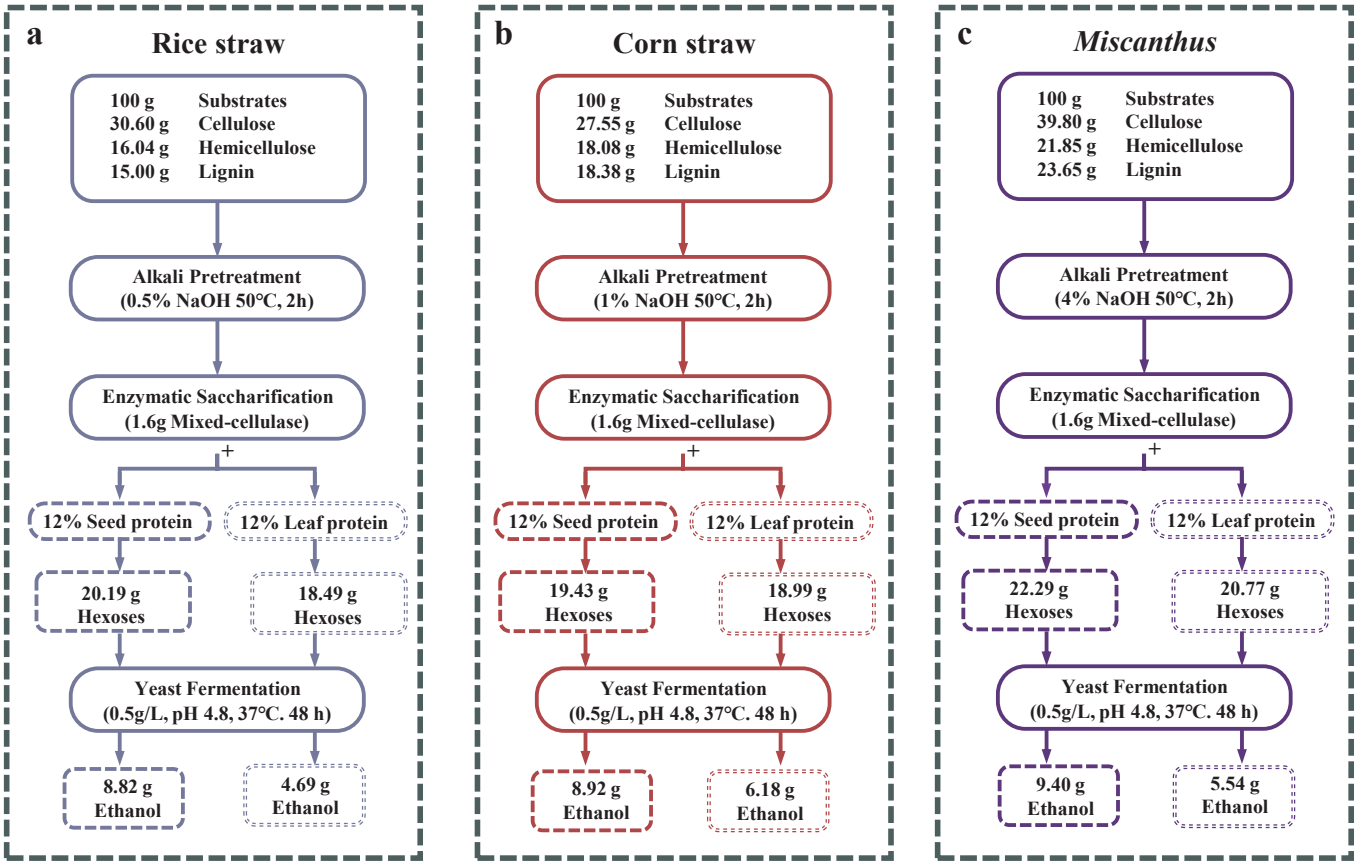

Fig. 3. Mass balance analyses of biomass processes in three crops for bioethanol production.

Table 1
Comparison of hexoses and ethanol yields achieved from soybean protein supply and other additives.

Additives	Biomass	Increasing rate of hexose yield (%)	Increasing rate of ethanol yield (%)	Ref.
Seed proteins	**Rice**	**18 %[a]**	**38 %[b]**	**This study**
Cationic kraft lignin	Corn	12 %	-	(Xu et al. 2023)
Sophorolipid	Sugarcane bagasse	18 %	-	(Xu et al. 2021)
Peptone	Rice	14 %	-	(Hu et al. 2015)
Soy skim	Corn	-	20 %	(Sekhon et al. 2015)
Bovine serum albumin	Rice	-	14 %	(Wang et al. 2014)
Surfactant	Wheat	14 %	-	(Cui et al. 2011)
Tween-80	Green coconut	-	34 %	(Ribeiro et al. 2024)
Silwet L-77	*Miscanthus*	24 %	-	(Sun et al. 2020)

[a]As increased rates of hexoses yield (% cellulose) and [b] ethanol yield (% dry matter) compared to the control (without additives); - As unavailable data.

2.10. Cd^{2+} adsorption assay

The lignocellulose residues were obtained as biosorbents samples from pretreatment, enzymatic hydrolysis and fermentation after distilled water washing and drying at 50°C. Batch adsorption experiments of $Cd(NO_3)_2$ were performed for 4.0 h at room temperature with 150 rpm shaking as previously described (Xu et al., 2021). After reaction, the residual Cd^{2+} levels was determined by flame atomic adsorption spectrophotometer (FAAS HITACHI Z-2000, Japan) (Zhang et al., 2023c). All experiments were accomplished at independent triplicate. The amount of adsorption at equilibrium qe (mg/g) and the percentage removal efficiency (%R) were calculated as previously described (Vo et al., 2020).

Table 2
Estimation of total ethanol yields enhanced by soybean seed proteins supply at large scale.

Protein supply	Protein weight (kg/ha)	Ethanol yield (kg)		
		Rice	Corn	*Miscanthus*
Control	0	37.12	41.72	52.72
+Seed protein	1084.48	797.10 (24.5 fold)#	806.14 (19.3 fold)	849.51 (16.1 fold)

Soybean seed yield at 2711.2 kg/ha/year and seed protein content at 40 % (FAO. 2023. Production: Soya bean, total. FAOSTAT Database. Accessed 15 July 2024; Kataria et al., 2019); #Enhanced fold relative to the control (without protein supply).

2.11. Amino acids assays of soybean proteins

Amino acid compositions of soybean seed and leaf proteins were determined by Biochrom 30 + Amino Acid Analyzer (UK) as described (Andini et al., 2013), and Fourier transform infrared (FTIR) spectroscopy was applied to detect chemical bands of soybean proteins as described (Tintor et al., 2024).

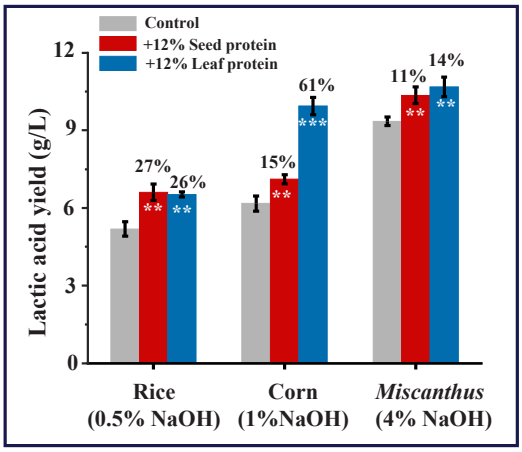

Fig. 4. Lactic acid production by *Lactobacillus paracasei* fermentations using sugars released from enzymatic hydrolyses of mature straws in three crops co-supplied with 12 % soybean seed and leaf proteins. Data as means ± SD (n = 3); * and **As significant difference between the protein-supplied sample and control (without protein supply) by student's *t*-test at $p < 0.05$ and 0.01 levels; # As increased percentage in the protein-supplied sample relative to the control.

2.12. Characterization of crop straws and lignocellulose residues

Wall polysaccharides levels of crop straws were determined by performing fractionation of cell walls and total lignin contents were measured by the two-step acid hydrolysis method according to the Laboratory Analytical Procedure of the National Renewable Energy Laboratory as previously described (Jin et al., 2016). Both anthrone/H_2SO_4 and orcinol/HCl methods were used to measure total hemicelluloses levels, whereas the anthrone/H_2SO_4 was applied to determine cellulose content. The lignocellulose residues for Cd^{2+} adsorption were characterized by Brunauer-Emmett-teller (BET), scanning electron microscopy (SEM) and Fourier transform infrared (FTIR) spectroscopy as previously described (Ramrakhiani et al., 2017; Zhang et al., 2025).

2.13. Statistical calculation

Statistical analyses were performed using SPSS 27.0 (IBM Corp., Chicago, IL). Spearman's rank correlation analysis (two-tailed, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$) was accomplished to assess relationships between variables. Variation and regression analyses were conducted in Origin 2021 (Origin Lab, Northampton, MA) to generate best-fit curves based on experimental data. All analyses were performed

Table 3
Estimation of total lactic acid yields enhanced by soybean proteins supply at large scales.

Protein supply	Protein weight (kg/ha)	Lactic acid yield (kg)		
		Rice	Corn	*Miscanthus*
Control	0	33.64	39.99	60.60
+Leaf protein	69.82	84.71 (2.5 fold)	167.92 (4.2 fold)	174.32 (2.9 fold)
+Seed protein	1084.48	1337.53 (39.8 fold)#	1796.63 (44.9 fold)	2608.19 (43.0 fold)

Soybean leave fresh weight at 6.82 g/plant/year and total leave fresh weight at 375,000 kg/ha/year for leave protein at 2557.5 kg/ha/year and leave protein content at 2.73 % (Feng et al., 2019; Mandal et al., 2009); Soybean seed yield at 2711.2 kg/ha/year and seed protein content at 40 % (FAO. 2023. Production: Soya bean, total. FAOSTAT Database. Accessed 15 July 2024; Kataria et al., 2019); #Enhanced fold relative to the control (without protein supply).

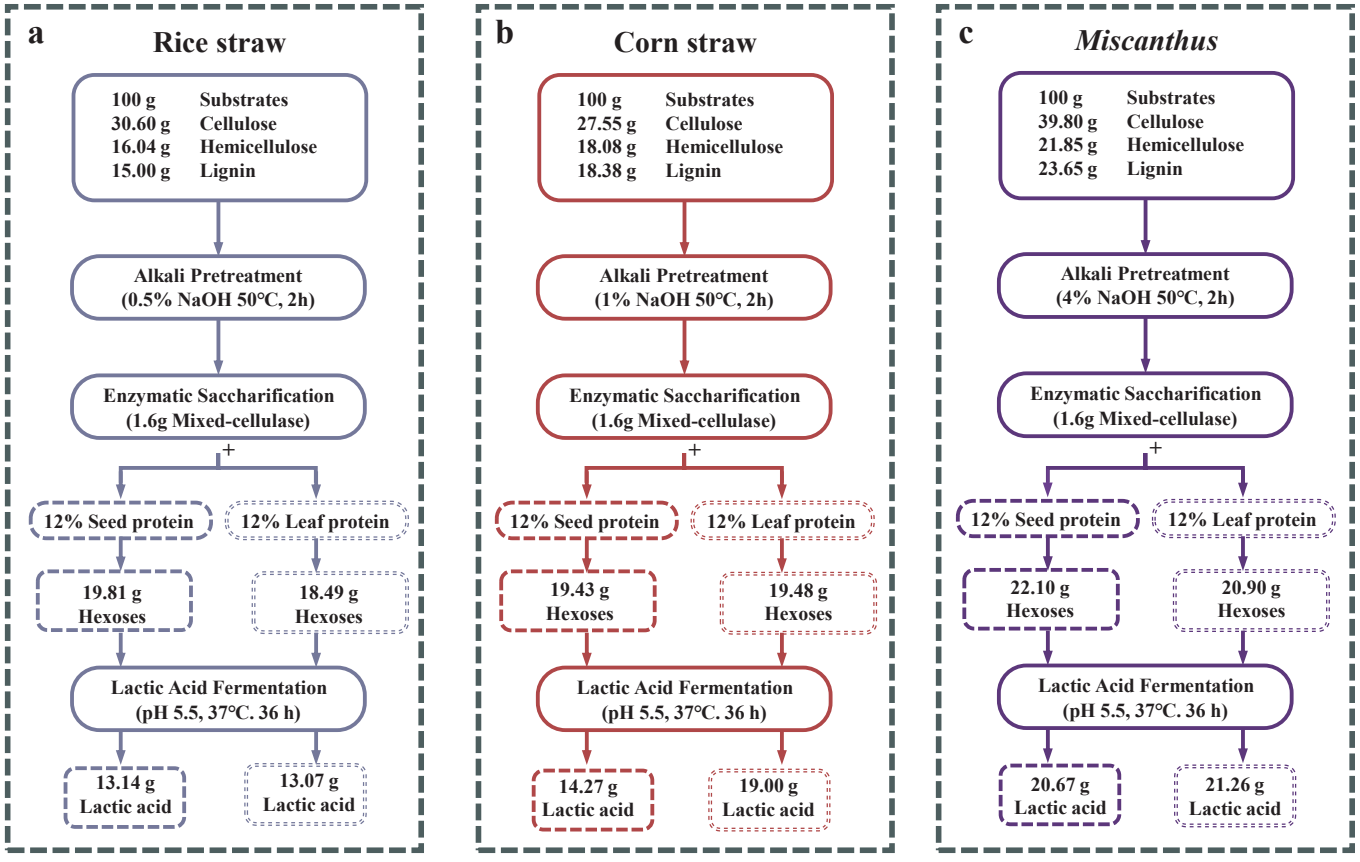

Fig. 5. Mass balance analyses of biomass processes in three crops for lactic acid production.

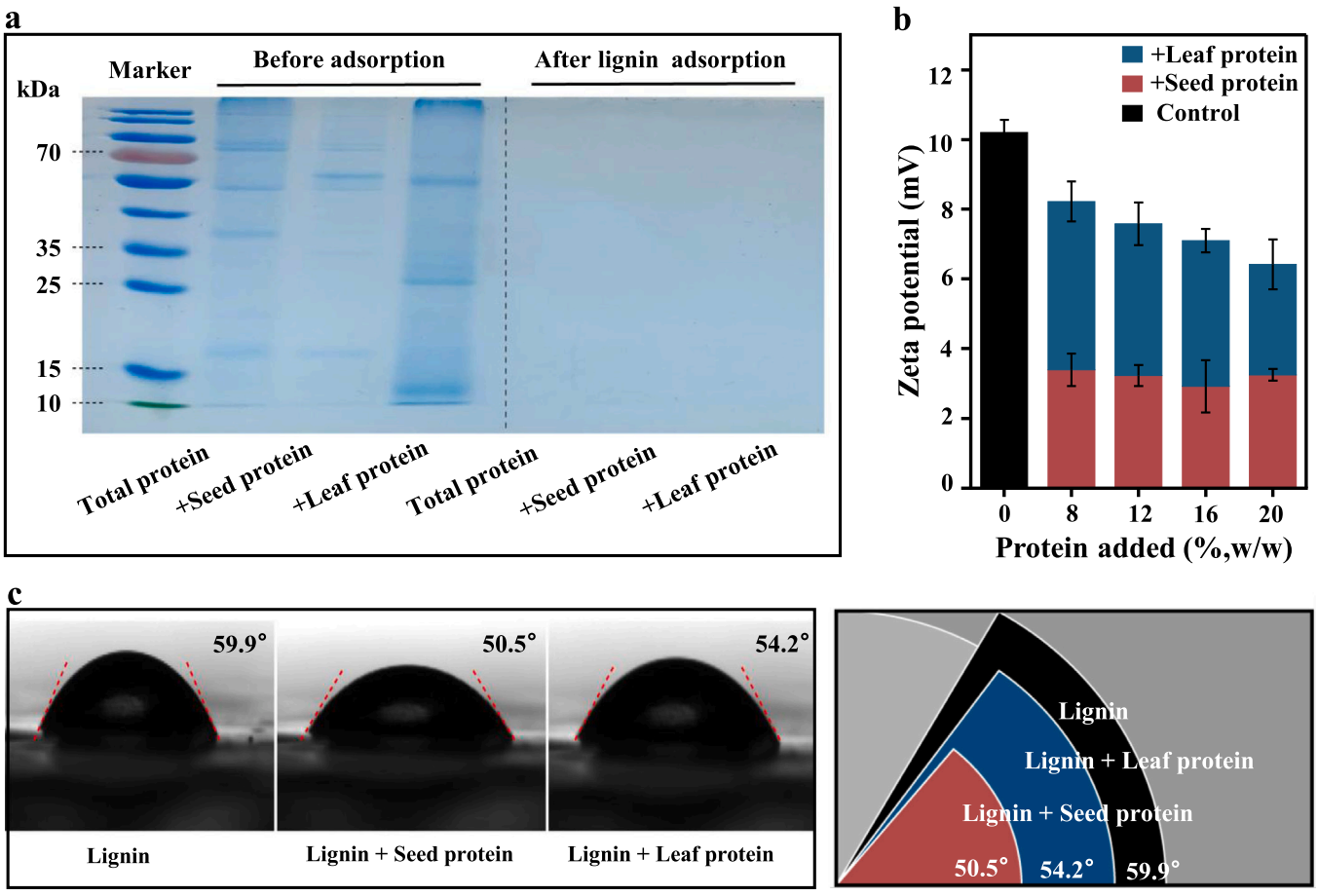

Fig. 6. Characterization of soybean proteins as biosurfactant for lignin blocking. (a) SDS-PAGE detection of lignin and protein adsorption; (b) Zeta potential reduced from lignin interaction with soybean proteins relative to the control (without protein), Data as means ± SD (n = 3); (c) Contact angles reduced from lignin interaction with soybean protein; Total protein from commercial soybean protein.

Table 4
Amino acids compositions of soybean seed and leaf proteins.

Categories of amino acids	Seed protein	Leaf protein
Essential amino acid		
Thr	**25.23(3.93 %)**	**13.64 (5.25 %)**#
Val	**26.64 (4.15 %)**	**15.12 (5.82 %)**
Met	9.00 (1.40 %)	3.91 (1.50 %)
Ile	27.31 (4.25 %)	12.78 (4.92 %)
Leu	47.37 (7.38 %)	20.74 (7.98 %)
Phe	34.59 (5.39 %)	15.13 (5.82 %)
Lys	39.66 (6.18 %)	16.50 (6.35 %)
Nonessential amino acid		
Asp	73.91 (11.51 %)	29.56 (11.37 %)
Ser	34.30 (5.34 %)	16.31 (6.27 %)
Glu	**137.82 (21.46 %)**	**38.58 (14.84 %)**
Gly	**26.27 (4.09 %)**	**13.36 (5.14 %)**
Ala	**25.73 (4.01 %)**	**13.10 (5.04 %)**
Cys-Cys	**9.72 (1.51 %)**	**7.12 (2.74 %)**
Tyr	**26.59 (4.14 %)**	**13.95 (5.37 %)**
His	16.12 (2.51 %)	5.14 (1.98 %)
Arg	**51.09 (7.96 %)**	**14.11 (5.43 %)**
Pro	30.77 (4.79 %)	10.88 (4.19 %)

Highlighting the amino acid levels of leaf and seed proteins are different at > 1 %.

using mean values derived from replicate measurements for each trait pair.

3. Results and discussion

3.1. Optimal soybean protein supply for remarkably enhanced biomass saccharification

To evaluate soybean seed and leaf proteins roles in biomass enzymatic saccharification, this study performed our previously-established alkali pretreatments with mature straws of three bioenergy crops (rice, corn, *Miscanthus*) distinctive at lignocellulose compositions and total extractable carbohydrates levels (Table S1) (Alam et al., 2019; Madadi et al., 2021). The rice and corn straws contained relatively high extractable-carbohydrates, suggesting that partial pectin and hemicellulose should be initially extracted from lignocellulose fractionation procedure, whereas *Miscanthus* straw contained high lignocellulose from lignin-rich stem tissue. As three crop straws provided diverse lignocellulose substrates, they should be favour for investigating two types of soybean proteins impacts on biomass enzymatic saccharification. We then measured the hexoses yields (% cellulose) released from enzymatic hydrolyses of pretreated lignocelluloses using the commercial mixed-cellulases co-supplied with different dosages of soybean proteins (Fig. 1). As a result, all six samples showed the highest hexoses yields from either 12 % seed protein supply or 12 % leaf protein, suggesting a similar enhancement role from seed and leaf proteins. As a comparison, three bioenergy crops showed different hexoses yields from both seed and leaf protein supplies, being accountable for their distinct lignocellulose recalcitrant properties as previously examined (Alam et al., 2019; Zhang et al., 2023a, 2023b).

To further test soybean protein enhancement role, this study

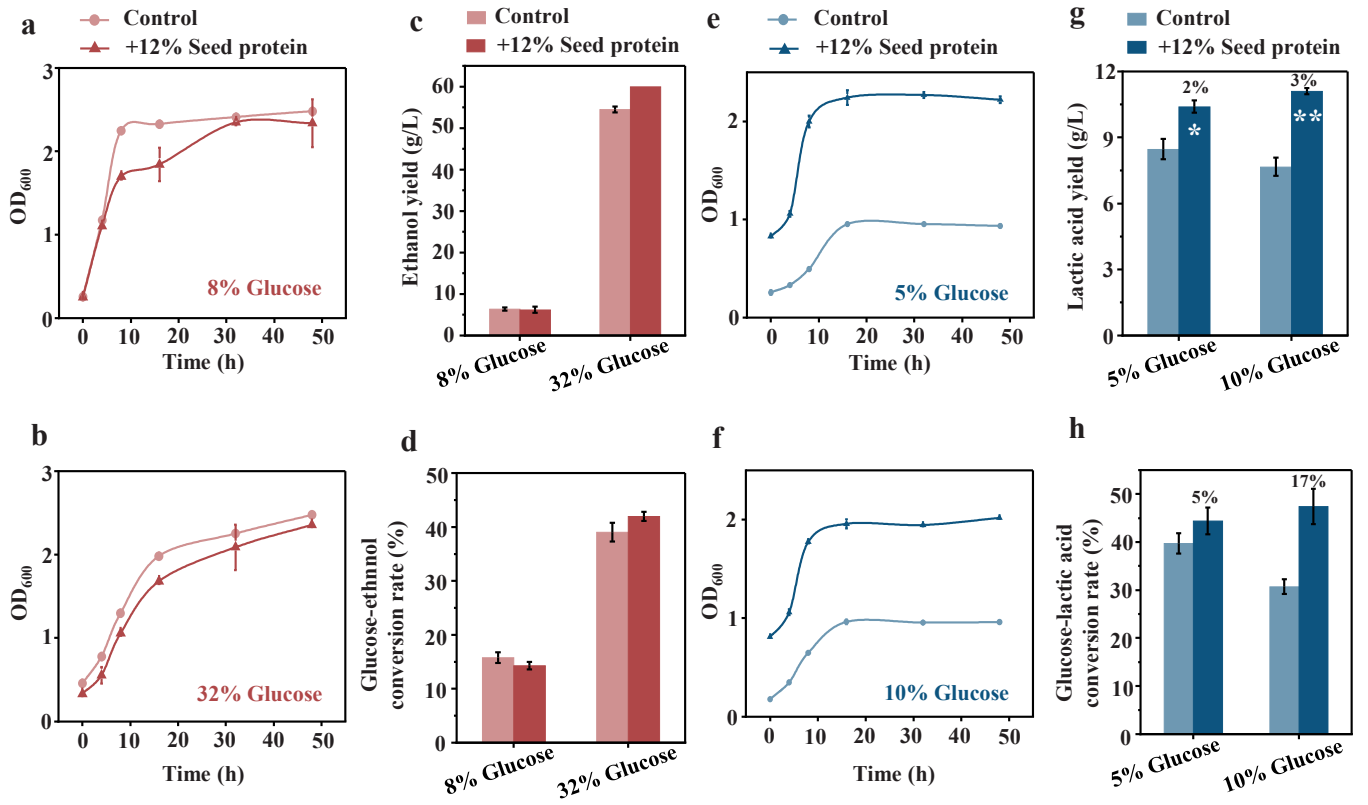

Fig. 7. Characterization of ethanol and lactic acid fermentations using standard glucose co-supplied 12 % soybean seed protein. (a, b) Yeast cell growths with 8 % and 32 % glucose, control without seed protein; (c, d) Ethanol yield and conversion rate; (e, f) *Lactobacillus paracasei* cell growths with 5 % and 10 % glucose; (g, h) Lactic acid yield and conversion rate; Data as means ± SD (n = 3); * and ** As significant difference between the protein-supplied sample and control (without protein supply) by student's *t*-test at $p < 0.05$ and 0.01 level; # As increased percentage in the protein-supplied sample relative to the control.

employed the optimal dosages of seed and leaf proteins (12 %) into the enzymatic hydrolyses of diverse lignocellulose substrates after three different pretreatments (acid, alkali and liquid hot water) were conducted in three bioenergy crops. Although the three pretreatments could distinctively extract hemicellulose and lignin for producing diverse pretreated-lignocellulose substrates as previously reported (Sun et al., 2020; Xu et al., 2012; Zhang et al., 2013), both seed and leaf proteins supplies could significantly increase the hexoses yields by 12 %-40 % compared to the controls (without protein supply) at $p < 0.05$ and 0.01 level (n = 3), except the 0.5 % NaOH pretreated-lignocellulose of *Miscanthus* supplied with leaf protein (Fig. S2). In addition, the corn straw had the highest hexoses yields, whereas the *Miscanthus* stalk remained the lowest hexoses yields, reconfirming a distinct recalcitrant property in three bioenergy crops. Meanwhile, soybean seed and leaf protein significantly affected the hexoses yields, suggesting its potential as a viable alternative to conventional chemical active agents.

3.2. *Contrastive impact on bioethanol conversion from seed and leaf protein supply*

As a consequence, this study performed classic yeast fermentation as our previously established to achieve bioethanol production using all hexoses released from enzymatic hydrolyses in three bioenergy crops co-supplied with seed and leaf proteins. As a result, the three crop samples with seed protein supplies respectively showed the bioethanol yields at 8.83 %, 8.92 %, and 9.40 %, and particularly, the bioethanol yields of rice and corn samples were significantly higher than those of the control samples (without protein supply) by 38 % and 25 % at $p < 0.001$ and 0.01 level (n = 3) (Fig. 2), which were accountable for their enhanced biomass saccharification examined. By contrast, the leaf protein supplies caused significantly reduced bioethanol yields by 31 %-47 % in three crop samples compared to the control ones, suggesting that the lead protein solution should contain much more toxic compounds that inhibit yeast fermentation (Jönsson and Martín, 2016). These inhibitors are primarily derived from weak aliphatic acids, furanaldehydes, phenolic and aromatic compounds from leaf protein extraction, which have been examined with toxicity to microbial fermentation (Jönsson and Martín, 2016; Suckling et al., 2017). On the other hands, the bioethanol conversion enhanced with seed protein supplies could be explained by its binding ability with toxic compounds (such as furanaldehydes and phenolic) in the hydrolysates through hydrophobic interactions and hydrogen bonding, thereby reducing the toxicity of the hydrolysates (Ebrahimi et al., 2025; Le Bourvellec and Renard, 2012). Based on the preceding ethanol fermentation data, the scaled mass balance assay further revealed high hexose-ethanol conversion rates from the seed protein supply, whereas much reduced conversion rates were examined in the leaf protein samples (Fig. 3).

Notably, this study found that the seed protein supply could even more increase hexose and ethanol yields compared to the other biosurfactants as previously reported (Table 1). For instance, in the study by Xu et al. (2023), the enzymatic hydrolysis efficiency of corn stalks increased by 11.62 % at a solid content of 10 % (w/v) after 24 h with 2 g/L JLQKL$_{50}$. By comparison, the addition of 12 % soybean seed protein in this study improved the enzymatic hydrolysis efficiency of rice straw by 18 %. Similarly, (Kataria et al., 2019), reported a 20 % increase in ethanol yield with the addition of soy skin, whereas this study observed a substantially greater increase of 38 % with soybean seed protein. Compared with other reported biosurfactants, the addition of soybean seed protein resulted in a higher ethanol yield, suggesting its significant potential for application. Further compared with chemical surfactants (Tween-80, SilwetL-77), the soybean proteins showed relatively less enhancement of biomass saccharification, but had higher

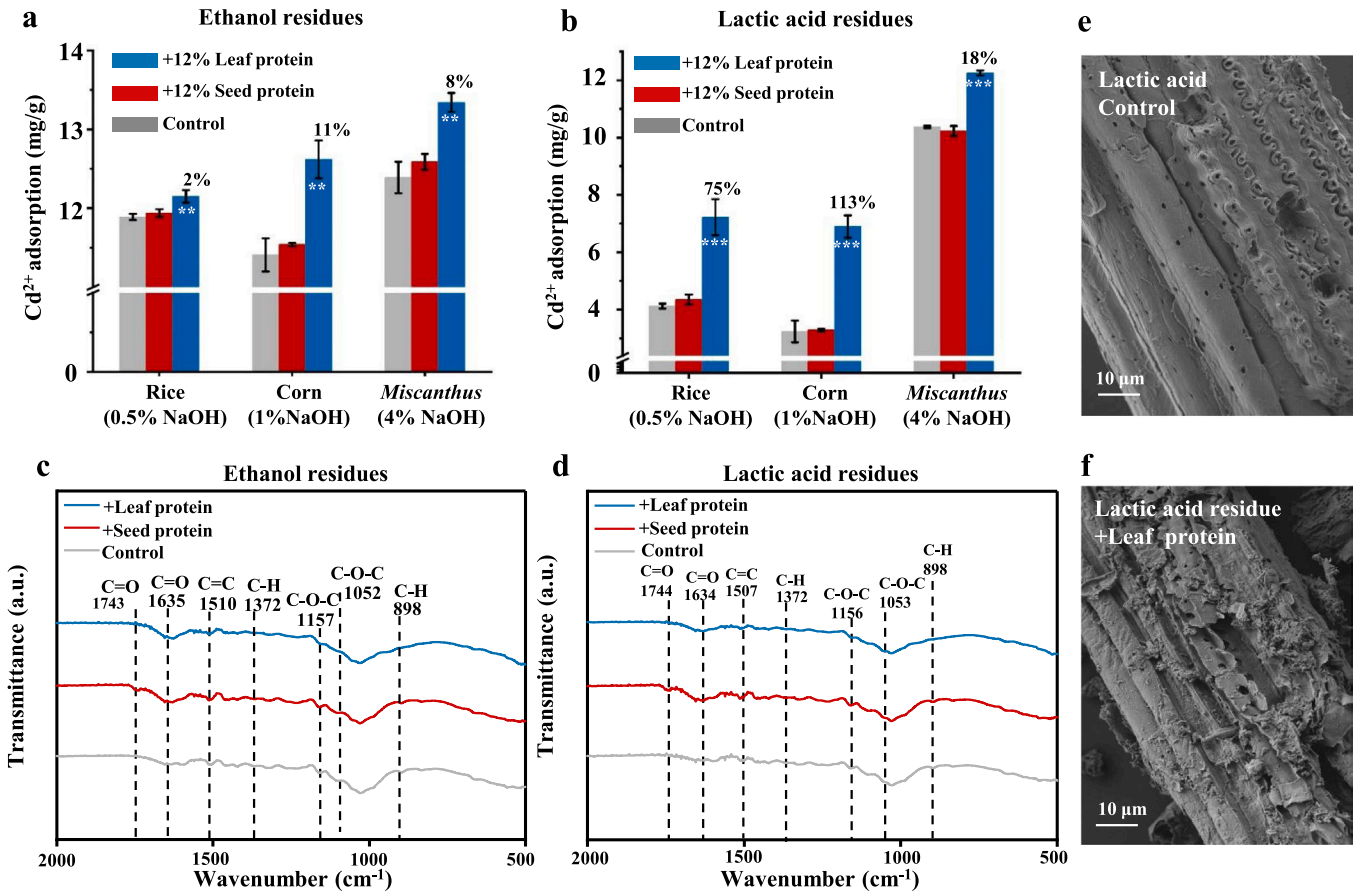

Fig. 8. Characterization of the biosorbents obtained from ethanol and lactic acid fermentation residues for Cd^{2+} adsorption. (a) Cd^{2+} adsorption capacity with the ethanol-fermentation residues; (b) Cd^{2+} adsorption capacity with the lactic acid-fermentation residues; (c, d) FTIR profiling of biosorbents; (e, f) SEM observations of residues surfaces of lactic acid fermentation; Data as means ± SD (n = 3); */**/***As significant difference between the protein-supplied sample and control (without protein supply) by student's *t*-test at $p < 0.05/0.01/0.01$ level; # As increased percentage in the protein-supplied sample relative to the control.

ethanol conversion, suggesting that seed protein supply should cause less inhibitory compounds of yeast fermentation (Lu et al., 2025; Sun et al., 2020). For bioeconomic benefit, this study also calculated the total seed protein yields collected from soybean cultivation per year (Kataria et al., 2019), which enabled the bioethanol yields to be increased by 16–25 folds from biomass processes of three bioenergy crops on large scales, compared to the controls without seed protein supplies (Table 2). Thus, the soybean seed protein supply caused a cascading enhancement of biomass saccharification and bioethanol productivity. In addition, as soybean seed protein can be extracted from the byproduct of soybean oil production, the seed protein supply should have economic benefits in biomass saccharification and biofuel production.

3.3. Consistently improved lactic acid conversion from soybean protein supply

By employing the hexoses released from enzymatic hydrolyses of three bioenergy crops co-supplied with seed and leaf proteins, this study conducted lactic acid fermentation by *Lactobacillus paracasei* (Fig. 4). Consistently, both seed and leaf protein supplies caused significantly higher lactic acid yields than those of the controls by 11 %-61 % in three bioenergy crops at $p < 0.01$ and 0.001 levels (n = 3). Notably, the leaf protein supply could even result in relatively higher lactic acid yields than the seed protein supply, in particular for corn process, which was quite different from the yeast fermentation showing a reduced ethanol production from the leaf protein supply examined above. Among three crop samples supplied with leaf proteins, the *Miscanthus* sample yielded the highest lactic acid concentration at 10.68 g/L, being consistent with its highest bioethanol yield with seed protein supply. The scaled mass balance analyses further confirmed a relatively higher hexoses-lactic acid conversion rate from leaf protein supply from the corn and *Miscanthus* processes (Fig. 5). As soybean leaves are available from diverse sources, the leaf protein supply should be of economic benefit from biomass saccharification and lactic acid fermentation. Furthermore, this study estimated that total lactic acid yields could be raised by 2.5–4.2 folds by leaf protein supply from soybean growth per year per hectare (Feng et al., 2019; Mandal et al., 2009), whereas the seed protein supply on large scale enabled the lactic acid yields to be increased by 40–45 folds (Table 3). Hence, both seed and leaf proteins supplies should be of specifically advantages for enhancing lactic acid production, compared to the ethanol conversion as examined above.

3.4. Mechanism of cascading enhancements for biomass saccharification and conversion

As plant proteins have been characterized as the biosurfactants that interact with lignin to maintain cellulases unblocked for enhancing biomass enzymatic saccharification (Madadi et al., 2021), this study performed a classic adsorption assay between soybean proteins and standard lignin substrates *in vitro* (Fig. 6). Based on SDS-PAGE profiling, all proteins of three samples (12 % seed protein, 12 % leaf protein, commercial total soybean proteins) could be almost precipitated down from lignin adsorption, retaining non-detectable proteins in the supernatants (Fig. 6a). By incubating different dosages of soybean protein (8 %-20 %) with lignin substrates, we examined drastically reduced zeta potential values relative to the control without protein supply (Fig. 6b),

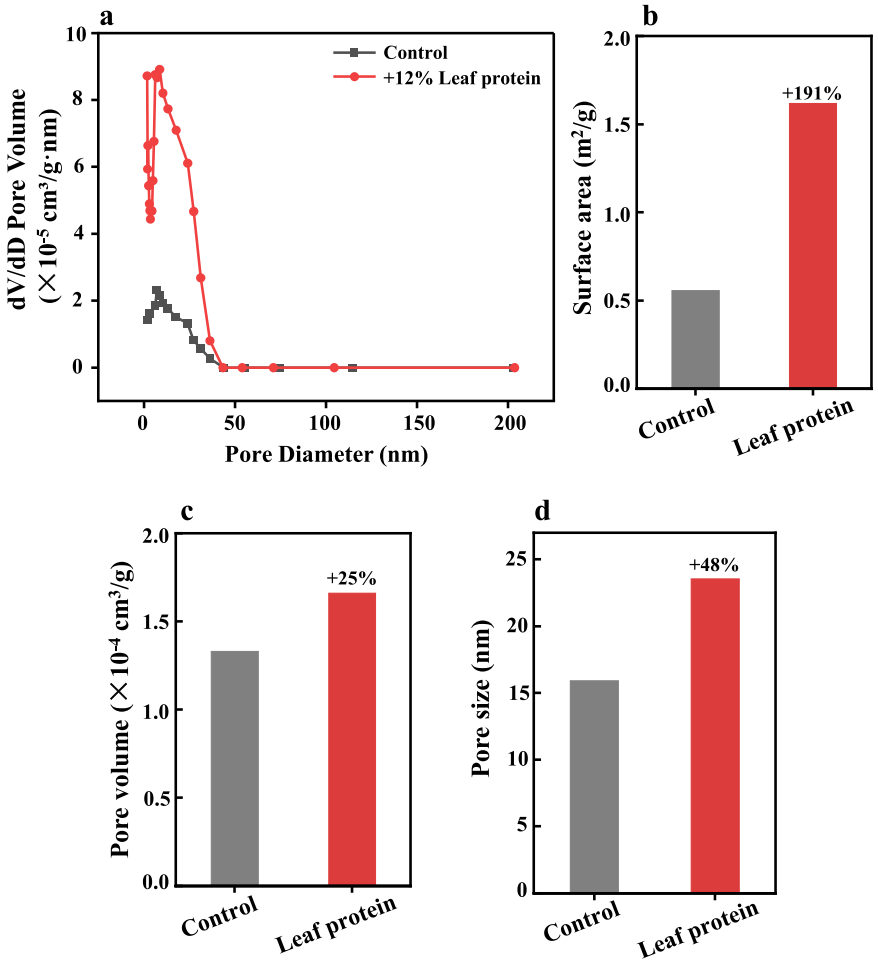

Fig. 9. Brunauer-Emmett-Teller (BET) analyses of two biosorbents samples obtained from ethanol and lactic acid fermentation residues. (a) Barret-Joyner-Halenda (BJH) adsorption micropore distribution; (b) BET specific surface area; (c) Total pore volume; (d) Average pore size.

indicating an active interaction between soybean protein and lignin substrate.

However, the seed protein samples had much lower zeta potential values than those of the leaf protein samples, suggesting that the lignin substrate should be much more blocked with the seed protein. These findings were validated by reduced contact angles from lignin interaction with soybean protein, and the seed protein supply remained lower contact angle (50.5°) than the leaf protein supply did (54.2°) (Fig. 6c), suggesting that the seed protein was of more effective interaction with lignin to be accountable for its more enhanced biomass saccharification compared to the leaf protein.

To understand seed and leaf protein interactions with lignin substrates, this study determined their amino acid compositions (Table 4) and also observed their FTIR profiling (Fig. S3; Table S2). As a result, the seed protein contained relatively higher proportions of charge amin acids (Glu, Arg) and lower proportions of hydrophobic amino acids (Gly, Ala) than those of the leaf protein, and different FTIR peaks were observed in the two sources of proteins, which should be the major cause accounting for the seed and leaf proteins interactions with lignin substrates to some different degrees examined (Dong et al., 1990; Ganim et al., 2008; Stani et al., 2020). In addition, as seed protein was rich at charge amino acids, it may facilitate its interactions with toxic compounds to reduce inhibition of yeast fermentation as described above.

Meanwhile, this study detected that both seed and leaf protein supplies could not specifically affect cellulose enzymatic hydrolyses (Fig. S4). By incubating the standard glucose substrates at optimal and sufficient dosages, this study found that the optimal seed protein supply (12 %) could not significantly affect yeast cell growths and ethanol fermentation, but it remarkably enhanced *Lactobacillus paracasei* cell growths by 2-fold for significantly increased lactic acid yields at $p < 0.05$ and 0.01 levels (n = 3) accounting for glucose-lactic acid conversion rates raised by 5 % and 17 % relative to the controls (without protein supply), respectively (Fig. 7), suggesting that the soybean protein may involve in *Lactobacillus paracasei* biochemical metabolism for enhanced cell growth. However, it remains to explore how soybean protein could improve *Lactobacillus paracasei* growth for high lactic acid fermentation in the future. Taken all together, the soybean protein could not only enhance biomass enzymatic saccharification by specifically blocking lignin, but it should also improve *Lactobacillus paracasei* growth for cascading improvement of lactic acid fermentation.

3.5. Ultimate improvement of Cd^{2+} adsorption capacity from fermentation residues

As a green-like biomass process, this study collected all biomass residues retained from ethanol and lactic acid fermentations as conducted above, and then directly detected their adsorptive capacities with Cd^{2+} by using previously-established methods (Madadi et al., 2021; Ramrakhiani et al., 2017; Zhang et al., 2025, 2023c). As a result, the control residues (without protein supply) and the seed protein samples were of similar Cd^{2+} adsorption capacities from both ethanol and lactic acid fermentations (Fig. 8a, b). As a comparison, the leaf protein samples showed significantly increased Cd^{2+} adsorptions at $p < 0.01$ and 0.001 levels (n = 3) with increased rates of 2 %-11 % from ethanol

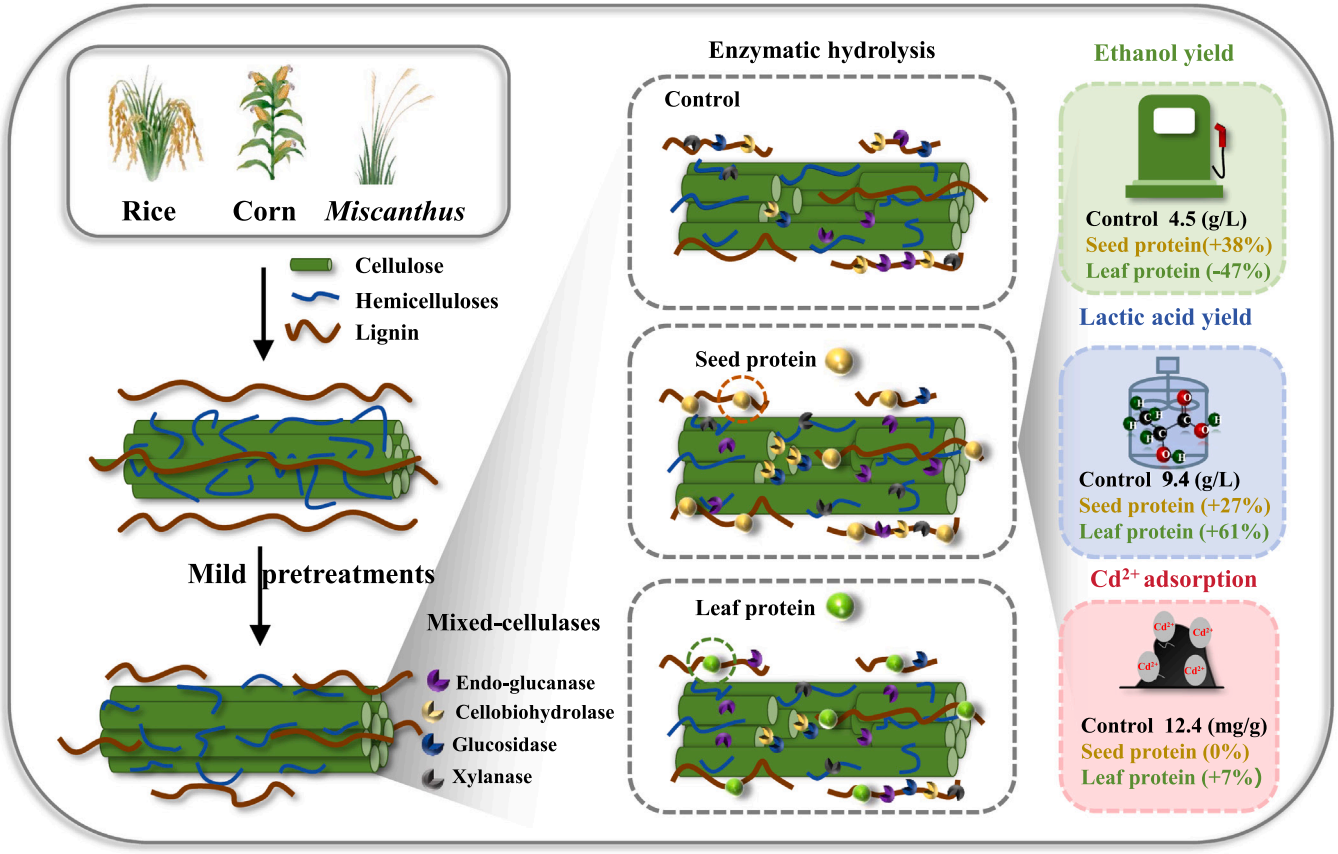

Fig. 10. A mechanism model highlighting how seed and leaf proteins distinctively improve enzymatic saccharification of pretreated lignocelluloses in representative bioenergy crops by effective interaction with lignin, which enables either dual impacts on bioethanol fermentation or specific increase of lactic acid production coupled with the only residues retained from leaf samples as active biosorbents for high Cd^{2+} adsorption.

fermentation and 8 %-113 % from lactic acid fermentation.

To understand why the leaf protein samples were of significantly higher Cd^{2+} adsorptive capacities, we presented the FTIR profiling among six residues samples examined, and the leaf protein samples exhibited more altered peaks characteristic for the functional chemical groups that may associate with Cd^{2+} adsorption such as C-O-C, C-H, C═C, C═O (Fig. 8c, d) (Baum et al., 2017; Ong et al., 2020; Sun et al., 2015; Wang et al., 2015; Zhou et al., 2017). Meanwhile, the leaf protein samples displayed much rougher surfaces than those of the controls from both ethanol and lactic acid fermentations (Fig. 8e, f; Fig. S5). Because the rough faces are favour for Cd^{2+} loading and interaction, it should explain why the resides of leaf protein sample was of high Cd^{2+} adsorption.

Notably, this study conducted Brunauer-Emmett-Teller (BET) assay, and the leaf protein sample showed remarkably improved porosity than the control sample (Fig. 9a). As a comparison, the leaf protein sample had the surface area increased by 191 %, with the pore volume and size raised by 25 % and 48 %, respectively (Fig. 9b-d), which should provide the direct evidence to support for the upgraded Cd^{2+} adsorption capacity examined in the leaf protein sample.

As the BET and FTIR data are accountable for improved physical and chemical interactions with Cd^{2+} in the leaf samples compared to the seed protein and control samples, it remains to explore Cd^{2+} adsorption isotherm and kinetic models in the future. More importantly, despite the residues of leaf protein sample acted as desirable biosorbent for Cd^{2+} adsorption, advanced chemical modification could further improve its performance and function, which is not only effective for high heavy metal interactions, but may also be active for other chemical adsorptions as environmental remediation.

4. Conclusions

In this study, soybean seed and leaf proteins supplies could consistently enhance biomass enzymatic saccharification of diverse pretreated lignocelluloses substrates by effectively blocking lignin interaction with lignocellulose-degradation enzymes (Fig. 10). The seed protein supply consequently increased yeast fermentation for high cellulosic ethanol production, whereas the leaf protein supply inhibited bioethanol conversion. However, both seed and leaf protein supplies significantly improve *Lactobacillus paracasei* fermentations for high lactic acid production. Given all lignocellulose residues retained from fermentations acted as active biosorbents for Cd^{2+} adsorption, the residues from leaf protein supply sample remained significantly higher Cd^{2+} adsorptive capacity. Notably, this study attempted to sort out why the seed and leaf proteins could distinctively affect either cellulosic ethanol and lactic acid conversions or Cd^{2+} adsorption, providing a green-like strategy selective for cascading enhancements of lignocellulose saccharification, bioethanol and lactic acid conversion and heavy metal adsorption with zero-biomass release.

CRediT authorship contribution statement

Heng Kang: Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Dan Sun:** Validation, Supervision. **Xiao Tao:** Validation, Supervision. **Yixiang Wang:** Methodology, Investigation, Formal analysis. **Jiale Liu:** Methodology, Investigation, Formal analysis. **Siqin Tan:** Methodology, Investigation, Formal analysis. **Yanting Wang:** Validation, Supervision. **Liangcai Peng:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Ruilan Yang:** Writing – original draft, Methodology,

Investigation, Formal analysis. **Yingying Su:** Writing – review & editing, Investigation, Formal analysis. **Jiamin Li:** Methodology, Investigation, Formal analysis. **Yasi Zhou:** Methodology, Investigation, Formal analysis.

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.

Acknowledgements

This work was in part supported by the National Natural Science Foundation of China (32470273, 32170268), and the Initiative Grant of Hubei University of Technology for High-level Talents (GCC20230001).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.indcrop.2025.122534.

Data availability

Data will be made available on request.

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 (1), 99. https://doi.org/10.1186/s13068-019-1437-4.

Andini, R., Yoshida, S., Ohsawa, R., 2013. Variation in protein content and amino acids in the leaves of grain, vegetable and weedy types of Amaranths. Agron. J. 3 (2), 391–403. https://doi.org/10.3390/agronomy3020391.

Baum, A., Dominiak, M., Vidal-Melgosa, S., Willats, W.G.T., Søndergaard, K.M., Hansen, P.W., Meyer, A.S., Mikkelsen, J.D., 2017. Prediction of pectin yield and quality by FTIR and carbohydrate microarray analysis. Food Bioprocess Tech. 10 (1), 143–154. https://doi.org/10.1007/s11947-016-1802-2.

Brethauer, S., Studer, M.H., Yang, B., Wyman, C.E., 2011. The effect of bovine serum albumin on batch and continuous enzymatic cellulose hydrolysis mixed by stirring or shaking. Bioresour. Technol. 102 (10), 6295–6298. https://doi.org/10.1016/j.biortech.2011.02.016.

Chen, W.-H., Nižetić, S., Sirohi, R., Huang, Z., Luque, R., M.Papadopoulos, A., Sakthivel, R., Phuong Nguyen, X., Tuan Hoang, A., 2022. Liquid hot water as sustainable biomass pretreatment technique for bioenergy production: A review. Bioresour. Technol. 344, 126207. https://doi.org/10.1016/j.biortech.2021.126207.

Dong, A., Huang, P., Caughey, W.S., 1990. Protein secondary structures in water from second-derivative amide I infrared spectra. Biochemistry 29 (13), 3303–3308. https://doi.org/10.1021/bi00465a022.

Ebrahimi, P., Lante, A., Grossmann, L., 2025. Protein-polyphenol complexation vs. conjugation: A review on mechanisms, functional differences, and antioxidant-emulsifier roles. Food Hydrocoll. 169, 111590. https://doi.org/10.1016/j.foodhyd.2025.111590.

Eriksson, T., Börjesson, J., Tjerneld, F., 2002. Mechanism of surfactant effect in enzymatic hydrolysis of lignocellulose. Enzym. Micro Technol. 31 (3), 353–364. https://doi.org/10.1016/S0141-0229(02)00134-5.

Fan, C., Zhang, W., Guo, Y., Sun, K., Wang, L., Luo, K., 2022. Overexpression of PtoMYB115 improves lignocellulose recalcitrance to enhance biomass digestibility and bioethanol yield by specifically regulating lignin biosynthesis in transgenic poplar. Biotechnol. Biofuels Bioprod. 15 (1), 119. https://doi.org/10.1186/s13068-022-02218-7.

Feng, L., Raza, M.A., Li, Z., Chen, Y., Khalid, M.H.B., Du, J., Liu, W., Wu, X., Song, C., Yu, L., Zhang, Z., Yuan, S., Yang, W., Yang, F., 2019. The influence of light intensity and leaf movement on photosynthesis characteristics and carbon balance of soybean. Front Plant Sci. 9, 1952. https://doi.org/10.3389/fpls.2018.01952.

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.

Ganim, Z., Chung, H.S., Smith, A.W., DeFlores, L.P., Jones, K.C., Tokmakoff, A., 2008. Amide I Two-Dimensional Infrared Spectroscopy of Proteins. Acc. Chem. Res 41 (3), 432–441. https://doi.org/10.1021/ar700188n.

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.

Gu, Y., Guo, J., Nawaz, A., ul Haq, I., Zhou, X., Xu, Y., 2021. Comprehensive investigation of multiples factors in sulfuric acid pretreatment on the enzymatic hydrolysis of waste straw cellulose. Bioresour. Technol. 340, 125740. https://doi.org/10.1016/j.biortech.2021.125740.

Henshaw, J.L., Bolam, D.N., Pires, V.M.R., Czjzek, M., Henrissat, B., Ferreira, L.M.A., Fontes, C.M.G.A., Gilbert, H.J., 2004. The family 6 carbohydrate binding module CmCBM6-2 contains two ligand-binding sites with distinct specificities*. J. Biol. Chem. 279 (20), 21552–21559. https://doi.org/10.1074/jbc.M401620200.

Hiltunen, S., Sapkota, J., Ioannou, E., Haddad Momeni, M., Master, E., Ristolainen, M., 2024. Comparative assessment of chemical and biochemical approaches for the activation of lignocellulosic materials and emerging opportunities for expansin-related proteins. Cellulose 31 (1), 147–168. https://doi.org/10.1007/s10570-023-05637-3.

Hu, M., Yu, H., Li, Y., Li, A., Cai, Q., Liu, P., Tu, Y., Wang, Y., Hu, R., Hao, B., Peng, L., Xia, T., 2018. Distinct polymer extraction and cellulose DP reduction for complete cellulose hydrolysis under mild chemical pretreatments in sugarcane. Carbohydr. Polym. 202, 434–443. https://doi.org/10.1016/j.carbpol.2018.08.039.

Huang, C., Wenqian, L., Zheng, Y., Zhao, X., Ragauskas, A., Meng, X., 2022. Evaluating the mechanism of milk protein as an efficient lignin blocker for boosting the enzymatic hydrolysis of lignocellulosic substrates. Green. Chem. 24. https://doi.org/10.1039/D2GC01160C.

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

Jönsson, L.J., Martín, C., 2016. Pretreatment of lignocellulose: Formation of inhibitory by-products and strategies for minimizing their effects. Bioresour. Technol. 199, 103–112. https://doi.org/10.1016/j.biortech.2015.10.009.

Kapu, N.U.S., Manning, M., Hurley, T.B., Voigt, J., Cosgrove, D.J., Romaine, C.P., 2012. Surfactant-assisted pretreatment and enzymatic hydrolysis of spent mushroom compost for the production of sugars. Bioresour. Technol. 114, 399–405. https://doi.org/10.1016/j.biortech.2012.02.139.

Kataria, S., Baghel, L., Jain, M., Guruprasad, K.N., 2019. Magnetopriming regulates antioxidant defense system in soybean against salt stress. Biocatal. Agric. Biotechnol. 18, 101090. https://doi.org/10.1016/j.bcab.2019.101090.

Kaur, P., Bohidar, H.B., Pfeffer, F.M., Williams, R., Agrawal, R., 2023. A comparative assessment of biomass pretreatment methods for the sustainable industrial upscaling of rice straw into cellulose. Cellulose 30 (7), 4247–4261. https://doi.org/10.1007/s10570-023-05161-4.

Klein-Marcuschamer, D., Oleskowicz-Popiel, P., Simmons, B.A., Blanch, H.W., 2012. The challenge of enzyme cost in the production of lignocellulosic biofuels. Biotechnol. Bioeng. 109 (4), 1083–1087. https://doi.org/10.1002/bit.24370.

Kristensen, J.B., Börjesson, J., Bruun, M.H., Tjerneld, F., Jørgensen, H., 2007. Use of surface active additives in enzymatic hydrolysis of wheat straw lignocellulose. Enzym. Micro Technol. 40 (4), 888–895. https://doi.org/10.1016/j.enzmictec.2006.07.014.

Kumar, R., Wyman, C.E., 2009. Effect of additives on the digestibility of corn stover solids following pretreatment by leading technologies. Biotechnol. Bioeng. 102 (6), 1544–1557. https://doi.org/10.1002/bit.22203.

Le Bourvellec, C., Renard, C.M., 2012. Interactions between polyphenols and macromolecules: quantification methods and mechanisms. Crit. Rev. Food Sci. Nutr. 52 (3), 213–248. https://doi.org/10.1080/10408398.2010.499808.

Lu, K., Fan, Z., Liao, Y., Wu, X., Liu, H., Jin, M., Wang, H., Han, S., Zhang, Y., Sun, F., 2025. Enhancing the enzymatic hydrolysis efficiency of wheat straw pulp residue by prewashing and ferric chloride assisted hydrothermal pretreatment. Ind. Crops Prod. 223, 120238. https://doi.org/10.1016/j.indcrop.2024.120238.

Luo, X., Liu, J., Zheng, P., Li, M., Zhou, Y., Huang, L., Chen, L., 2019. Promoting enzymatic hydrolysis of lignocellulosic biomass by inexpensive soy protein. Biotechnol. Biofuels 12. https://doi.org/10.1186/s13068-019-1387-x.

Madadi, M., Wang, Y., Xu, C., Liu, P., Wang, Y., Xia, T., Tu, Y., Lin, X., Song, B., Yang, X., Zhu, W., Duanmu, D., Tang, S.-w, Peng, L., 2021. 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., Song, G., Gupta, V.K., Aghbashloh, M., Sun, C., Sun, F., Tabatabaei, M., 2023. Non-catalytic proteins as promising detoxifiers in lignocellulosic biomass pretreatment: unveiling the mechanism for enhanced enzymatic hydrolysis. Green. Chem. 25 (18), 7141–7156. https://doi.org/10.1039/D3GC01718D.

Mandal, K.G., Hati, K.M., Misra, A.K., 2009. Biomass yield and energy analysis of soybean production in relation to fertilizer-NPK and organic manure. Biomass.. Bioenergy 33 (12), 1670–1679. https://doi.org/10.1016/j.biombioe.2009.08.010.

Mei, M., Du, P., Li, W., Xu, L., Wang, T., Liu, J., Chen, S., Li, J., 2023. Amino-functionalization of lignocellulosic biopolymer to be used as a green and sustainable adsorbent for anionic contaminant removal. Int. J. Biol. Macromol. 227, 1271–1281. https://doi.org/10.1016/j.ijbiomac.2022.11.322.

Ong, H.C., Chen, W.-H., Singh, Y., Gan, Y.Y., Chen, C.-Y., Show, P.L., 2020. A state-of-the-art review on thermochemical conversion of biomass for biofuel production: A TG-FTIR approach. Energy Convers. Manag. 209, 112634. https://doi.org/10.1016/j.enconman.2020.112634.

Oshikata, M.S.K., Blas, N.S., Silva, B. d L., Fukamizu, D.I., da Silva, D.R.B., Gauto, L.P., Cruz, A.J.G., Morandim-Giannetti, A. d A., Pratto, B., 2024. Cotton waste upcycling:

biofuel and cellulose derivatives production. Cellulose 31 (11), 6693–6704. https://doi.org/10.1007/s10570-024-06012-6.

Pei, X., Fan, M., Zhang, H., Xie, J., 2024. Assessment for industrial production of poplar ethanol after analysis of influencing factors and predicted yield. Cellulose 31 (18), 10801–10829. https://doi.org/10.1007/s10570-024-06236-6.

Ramrakhiani, L., Halder, A., Majumder, A., Mandal, A.K., Majumdar, S., Ghosh, S., 2017. Industrial waste derived biosorbent for toxic metal remediation: Mechanism studies and spent biosorbent management. Chem. Eng. J. 308, 1048–1064. https://doi.org/10.1016/j.cej.2016.09.145.

Rasheed, A., He, P., Long, Z., Gillani, S., Wang, Z., Morsy, K., Hashem, M., Jie, Y., 2024. Cadmium (Cd) tolerance and phytoremediation potential in fiber crops: research updates and future breeding efforts. Agron. J. 14, 2713. https://doi.org/10.3390/agronomy14112713.

Souza, D. d, Sbardelotto, A.F., Ziegler, D.R., Marczak, L.D.F., Tessaro, I.C., 2016. Characterization of rice starch and protein obtained by a fast alkaline extraction method. Food Chem. 191, 36–44. https://doi.org/10.1016/j.foodchem.2015.03.032.

Stadler, H., Mondon, M., Ziegler, C., 2003. Protein adsorption on surfaces: dynamic contact-angle (DCA) and quartz-crystal microbalance (QCM) measurements. Anal. Bioanal. Chem. 375 (1), 53–61. https://doi.org/10.1007/s00216-002-1664-5.

Stani, C., Vaccari, L., Mitri, E., Birarda, G., 2020. FTIR investigation of the secondary structure of type I collagen: New insight into the amide III band. Spectrochim. ACTAA 229, 118006. https://doi.org/10.1016/j.saa.2019.118006.

Suckling, I.D., Jack, M.W., Lloyd, J.A., Murton, K.D., Newman, R.H., Stuthridge, T.R., Torr, K.M., Vaidya, A.A., 2017. A mild thermomechanical process for the enzymatic conversion of radiata pine into fermentable sugars and lignin. Biotechnol. Biofuels 10 (1), 61. https://doi.org/10.1186/s13068-017-0748-6.

Sun, D., Yang, Q., Wang, Y., Gao, H., He, M., Lin, X., Lu, J., Wang, Y., 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. Crop Prod. 153, 112559. https://doi.org/10.1016/j.indcrop.2020.112559.

Sun, S.L., Sun, S.N., Wen, J.L., Zhang, X.M., Peng, F., Sun, R.C., 2015. Assessment of integrated process based on hydrothermal and alkaline treatments for enzymatic saccharification of sweet sorghum stems. Bioresour. Technol. 175, 473–479. https://doi.org/10.1016/j.biortech.2014.10.111.

Tintor, Đ., Ninković, K., Milošević, J., Polović, N.Đ., 2024. Gaining insight into protein structure via ATR-FTIR spectroscopy. Vib. Spectrosc. 134, 103726. https://doi.org/10.1016/j.vibspec.2024.103726.

Vo, T.S., Vo, H., Hossain, M.M., Jeong, H.M., Kim, K., 2020. Heavy metal removal applications using adsorptive membranes. Nano Converg. 7 (1), 36. https://doi.org/10.1186/s40580-020-00245-4.

Wang, H., Kobayashi, S., Mochidzuki, K., 2015. Effect of non-enzymatic proteins on enzymatic hydrolysis and simultaneous saccharification and fermentation of different lignocellulosic materials. Bioresour. Technol. 190, 373–380. https://doi.org/10.1016/j.biortech.2015.04.112.

Xia, X., Wu, S., Zhou, Z., Wang, G., 2021. Microbial Cd(II) and Cr(VI) resistance mechanisms and application in bioremediation. J. Hazard. Mater. 401, 123685. https://doi.org/10.1016/j.jhazmat.2020.123685.

Xu, C., Xia, T., Wang, J., Yu, L., Wu, L., Zhang, Y., Liu, P., Chen, P., Feng, S., Peng, L., 2021. Selectively Desirable Rapeseed and Corn Stalks Distinctive for Low-Cost Bioethanol Production and High-Active Biosorbents. Waste Biomass.. Valoriz. 12 (2), 795–805. https://doi.org/10.1007/s12649-020-01026-0.

Xu, J., Li, H., Alam, M.A., Muhammad, G., Lv, Y., Zhao, A., Zhang, S., Xiong, W., 2023. Employing cationic kraft lignin as additive to enhance enzymatic hydrolysis of corn stalk. Polymers 15 (9), 1991. https://doi.org/10.3390/polym15091991.

Xu, N., Zhang, W., Ren, S., Liu, F., Zhao, C., Liao, H., Xu, Z., Huang, J., Li, Q., Tu, Y., Yu, B., Wang, Y., Jiang, J., Qin, J., Peng, L., 2012. Hemicelluloses negatively affect lignocellulose crystallinity for high biomass digestibility under NaOH and H_2SO_4 pretreatments in *Miscanthus*. Biotechnol. Biofuels 5 (1), 58. https://doi.org/10.1186/1754-6834-5-58.

Yang, B., Wyman, C.E., 2006. BSA treatment to enhance enzymatic hydrolysis of cellulose in lignin containing substrates. Biotechnol. Bioeng. 94 (4), 611–617. https://doi.org/10.1002/bit.20750.

Yang, Y., Tilman, D., Lehman, C., Trost, J.J., 2018. Sustainable intensification of high-diversity biomass production for optimal biofuel benefits. Nat. Sustain 1 (11), 686–692. https://doi.org/10.1038/s41893-018-0166-1.

Yoshikawa, H., Hirano, A., Arakawa, T., Shiraki, K., 2012. Mechanistic insights into protein precipitation by alcohol. Int J. Biol. Macromol. 50 (3), 865–871. https://doi.org/10.1016/j.ijbiomac.2011.11.005.

Zhang, A., Hu, Z., Hu, X., Zhao, T., Wang, Y., Li, Y., Wang, Y., Peng, L., Ren, X., 2023a. Large-scale screening of diverse barely lignocelluloses for simultaneously upgrading biomass enzymatic saccharification and plant lodging resistance coupled with near-infrared spectroscopic assay. Ind. Crop Prod. 194, 116324. https://doi.org/10.1016/j.indcrop.2023.116324.

Zhang, H., Wang, Y., Peng, H., He, B., Li, Y., Wang, H., Hu, Z., Yu, H., Wang, Y., Zhou, M., Peng, L., Wang, M., 2025. Distinct lignocelluloses of plant evolution are optimally selective for complete biomass saccharification and upgrading Cd^{2+}/Pb^{2+} and dye adsorption via desired biosorbent assembly. Bioresour. Technol. 417, 131856. https://doi.org/10.1016/j.biortech.2024.131856.

Zhang, R., Gao, H., Wang, Y., He, B., Lu, J., Zhu, W., Peng, L., Wang, Y., 2023b. Challenges and perspectives of green-like lignocellulose pretreatments selectable for low-cost biofuels and high-value bioproduction. Bioresour. Technol. 369, 128315. https://doi.org/10.1016/j.biortech.2022.128315.

Zhang, W., Yi, Z., Huang, J., Li, F., Hao, B., Li, M., Hong, S., Lv, Y., Sun, W., Ragauskas, A., Hu, F., Peng, J., Peng, L., 2013. Three lignocellulose features that distinctively affect biomass enzymatic digestibility under NaOH and H_2SO_4 pretreatments in *Miscanthus*. Bioresour. Technol. 130, 30–37. https://doi.org/10.1016/j.biortech.2012.12.029.

Zhang, X., Xue, W., Zhang, C., Wang, C., Huang, Y., Wang, Y., Peng, L., Liu, Z., 2023c. Cadmium pollution leads to selectivity loss of glutamate receptor channels for permeation of Ca^{2+}/Mn^{2+}/Fe^{2+}/Zn^{2+} over Cd^{2+} in rice plant. J. Hazard Mater. 452, 131342. https://doi.org/10.1016/j.jhazmat.2023.131342.

Zhang, X., Hu, X., Li, C., Shen, R., Wang, L., Liu, G., Chen, C., 2024. Comparative study of methods for the determination of lactic acid content in fermentation broth. Chem. Eng. J. 51 (3), 34–39. https://doi.org/10.13543/j.bhxbzr.2024.03.003.

Zhou, X., Li, Q., Zhang, Y., Gu, Y., 2017. Effect of hydrothermal pretreatment on *Miscanthus* anaerobic digestion. Bioresour. Technol. 224, 721–726. https://doi.org/10.1016/j.biortech.2016.10.085.