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Production of ethanol, butanol, itaconic acid, 3-hydroxypropionic acid, polyhydroxyalkanoates, and lignin from lignocellulosic biomass

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The conversion of lignocellulosic biomass into biofuels, platform chemicals, and bioplastics offers a sustainable pathway for supply chain management. It reduces dependence on fossil resources and supports the development of a circular economy. To minimize water and chemical consumption during pretreatment, we proposed a one-pot CaO pretreatment method applied to corn stover (CS) and brewer's spent grain (BSG). This was followed by pH adjustment using either H_3PO_4 (P) or H_2SO_4 (S) and subsequent enzymatic hydrolysis. The hydrolysates (CS-P, CS-S, BSG-P, and BSG-S) were directly used for producing ethanol with *Saccharomyces cerevisiae*, *n*-butanol with *Clostridium tyrobutyricum*, 3-hydroxypropionic acid (3-HP) with *Issatchenkia orientalis*, itaconic acid (IA) with *Aspergillus terreus*, and polyhydroxyalkanoates (PHA) with *Haloferax mediterranei*. The results showed that the fermentation performance of the resulting hydrolysates is closely linked to the type of acid used for pH adjustment and the source of biomass. BSG hydrolysates outperformed CS in butanol (6.7 g L^{-1}), IA (8.0 g L^{-1}), and 3-HP (10.6 g L^{-1}) production, due to BSG's higher nitrogen content and more favorable C/N ratio, which supported microbial growth and acid biosynthesis. Conversely, CS hydrolysates excelled in ethanol (23 g L^{-1} for CS-P) and PHA (0.27 g g^{-1} substrate for CS-P) production, attributed to their higher sugar concentration. Moreover, the lignin-rich hydrolysis residues from CS and BSG had >90% and 80% SGH-type lignin, respectively. Interestingly, BSG lignins displayed substantially low M_w (551–841 g mol^{-1} *vs.* 4999–5142 g mol^{-1}) and $Đ_M$ (1.5–2.0 *vs.* 8.0–8.3) compared to CS lignins. This work demonstrates the feasibility of using one-pot CaO pretreatment to directly produce various value-added products through precision fermentation, thereby advancing a circular bioeconomy.

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1. This work introduces a one-pot CaO pretreatment with direct pH adjustment that eliminates washing and solid–liquid separation steps, significantly reducing water use, chemical inputs, and wastewater generation compared to conventional pretreatment methods.
2. The approach enables direct fermentation of hydrolysates into multiple value-added products (biofuels, platform chemicals, and bioplastics), aligning with circular economy principles.
3. Further research should focus on process intensification with high-solid loading and nutrient balancing, engineering robust microbial strains capable of co-utilizing glucose and xylose, integrating lignin valorization into functional materials, and conducting technoeconomic analysis and life-cycle assessment to quantify both economic viability and environmental benefits.

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1. Introduction

The production of biofuels and platform chemicals through microbial fermentation of renewable feedstocks, rather than chemical conversion of fossil sources, has gained increasing attention due to growing concerns over environmental sustainability.[1,2] Lignocellulosic biomass, such as corn stover (CS) and brewer's spent grain (BSG), holds significant interest due to its abundant amount of cellulose and hemicellulose.[3,4] These components can be enzymatically hydrolyzed into glucose and xylose, providing carbon sources for microorganisms. Over 80 million tons of CS are produced annually, but only a small portion is used for current agricultural purposes, while the majority remains on the field.[5,6] Similarly, BSG, a major byproduct of the brewing industry, constitutes nearly 85% of the total solid waste generated during beer production, and is typically disposed of as landfill waste or animal feeding.[7,8] Therefore, valorizing these underutilized resources not only mitigates environmental impacts but also contributes to the advancement of a circular bioeconomy.[9,10]

Lignocellulosic biomass, characterized by its tightly bound polysaccharides-lignin matrix, exhibits significant recalcitrance to the accessibility of enzymes, rendering it challenging for enzymatic and microbial degradation. Therefore, pretreatment is a crucial step to unlock the complex and expose structural carbohydrates to enzymes.[11,12] Numerous pretreatment strategies have been developed to break down this matrix.[13,14] These methods typically involve solid–liquid separation and extensive post-washing to eliminate lignin, derivatives, and residual chemicals. Nevertheless, the liquid fractions, which typically contain lignin, soluble sugars, and chemicals, are discarded along with the washing solvents, resulting in large amounts of wastewater.[15] To address this issue, several strategies for eliminating the post-pretreatment washing step have been investigated. For example, Zhao *et al.* integrated acetic acid and NaOH pretreatments of 10% (w/v) industrial hemp in parallel without post-washing steps and achieved a sugar concentration of 42.9 g L^{-1}.[16] However, the resulting hydrolysate was detrimental to *Saccharomyces cerevisiae* for ethanol production. Moreover, they pretreated industrial hemp with H_2SO_4 or NaOH at initial solid loadings of 10 and 20% (w/v) and conducted separate hydrolysis and fermentation after pH adjustment with NaOH or H_2SO_4, respectively.[17] Both cases showed high sugar concentrations and yields, but only the hydrolysate from NaOH pretreatment followed by pH adjustment with H_2SO_4 at 10% solid loading was efficiently fermented to ethanol.[17] It was hypothesized that the toxic effects of H_2SO_4-pretreated hydrolysate and NaOH-pretreated hydrolysate on yeast cells are primarily attributed to furans and Na_2SO_4 salt, respectively.[15] Replacing NaOH with CaO for biomass pretreatment may result in a ready-to-ferment hydrolysate. This has already been partially demonstrated in a previous study, where the hydrolysate obtained from CaO pretreatment and subsequent pH adjustment with H_3PO_4 was fermented efficiently to ethanol.[18] Therefore, it is worthwhile to investigate whether this resulting hydrolysate is toxic to other microorganisms for the fermentation of other valuable products.

Recent advances in engineering microorganisms have improved the bioconversion of biomass into biofuels and platform chemicals. For ethanol production, considerable efforts have focused on overcoming the limited co-fermentation capacity of yeast strains for glucose and xylose by developing native xylose transporters using bioinformatics-guided mutagenesis and applying adaptive evolution to improve inhibitor tolerance.[19] Butanol production has been enhanced by engineered *Clostridium tyrobutyricum* and *Escherichia coli* strains that exhibit high metabolic flux towards butyryl-CoA and achieve a titer of about 25 g L^{-1} from biomass hydrolysates by improving NADH availability and suppressing byproduct formation *via* metabolic pathway regulation.[20,21] For 3-hydroxypropionic acid (3-HP) production, *Issatchenkia orientalis* has been developed to utilize glucose and xylose through metabolic pathways, such as the malonyl-CoA or glycerol routes for 3-HP production.[22,23] Itaconic acid (IA) is still primarily produced by *Aspergillus terreus*.[24] In addition, polyhydroxyalkanoates (PHA) production has made progress using *Haloferax mediterranei*, which produces PHBV copolyesters directly from simple carbohydrates without costly precursors, offering a sustainable alternative to petroleum plastics.[25,26] These developments highlight the potential of customized microbial systems.

In this study, we propose a green CaO pretreatment method for CS and BSG with subsequent pH adjustment using either H_3PO_4 (P) or H_2SO_4 (S) without requiring solid–liquid separation or post-washing steps. The objective is to demonstrate the fermentation potential of multiple value-added bioproducts—ethanol (with *S. cerevisiae*), *n*-butanol (with *C. tyrobutyricum*), 3-hydroxypropionic acid (3-HP, with *I. orientalis*), itaconic acid (IA, *with A. terreus*), and polyhydroxyalkanoates (PHAs, with *H. mediterranei*)—directly from four resulting hydrolysates (CS-P, CS-S, BSG-P, and BSG-S). In addition, the chemical composition and structural features of the resulting lignin-rich residues were analyzed to explore their potential applications. These investigations provide critical insights into the feasibility of producing diverse and valuable bioproducts from lignocellulosic biomass while reducing water and chemical consumption.

2. Results and discussion

2.1 Ethanol fermentation with *S. cerevisiae*

Fig. 1 shows the time course of ethanol fermentation from BSG and CS hydrolysates using an engineered *S. cerevisiae* strain. CS-P showed a higher ethanol concentration of 23.52 g L^{-1} than CS-S (21.01 g L^{-1}) (Fig. 1A and B), while BSG-P (13.18 g L^{-1}) and BSG-S (12.71 g L^{-1}) exhibited a similar ethanol concentration (Fig. 1C and D) as glucose was completely consumed after 72 h. The difference in CS hydrolysates can be attributed to two factors. Firstly, CS-P has slightly higher sugar concentrations than CS-S, and more xylose was consumed. Secondly, the lower solubility of $Ca_3(PO_4)_2$ compared to $CaSO_4$ results in a lower free Ca^{2+} concentration in the hydrolysate, which has a lesser impact on yeast cell

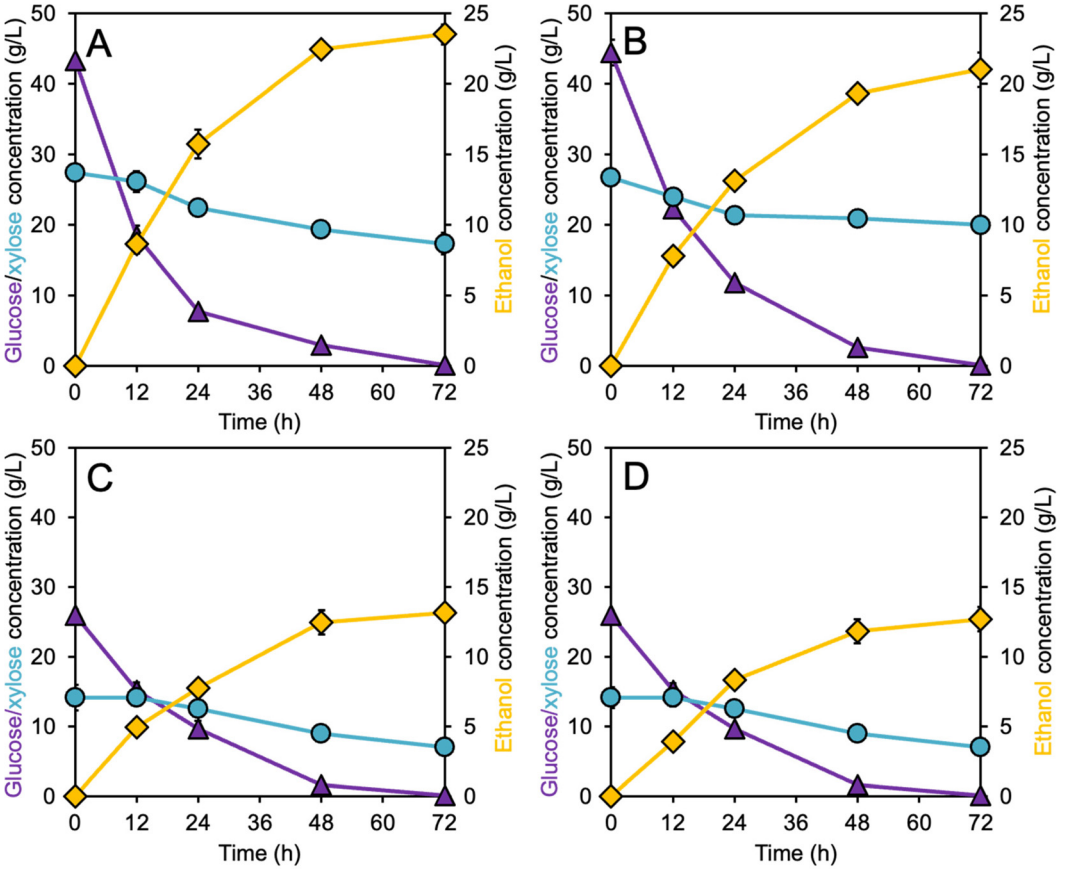

Fig. 1 Ethanol fermentation of four hydrolysates (A: CS-P; B: CS-S; C: BSG-P; and D: BSG-S) by an engineered *S. cerevisiae* strain.

growth.[27] BSG is rich in proteins, phenolic compounds, and Maillard reaction products. These will undergo chemical transformations when subjected to high-temperature pretreatment, releasing nitrogenous and other compounds.[28] Thus, the similar ethanol concentration observed in BSG-P and BSG-S might be attributed to the nutrient-rich environments present in the hydrolysates, which effectively mitigated the impact of Ca^{2+} ions and other fermentation stressors.[7,29] The engineered *S. cerevisiae* in this study demonstrated an inefficient capacity to digest xylose (namely, glucose repression effect),[30] with residual concentrations ranging from 17.29 to 19.95 g L^{-1} for CS hydrolysates and 7.06 to 7.34 g L^{-1} for BSG hydrolysates (Fig. 1). To address this issue, a strategy that combines simultaneous saccharification and fermentation with a pre-saccharification step would be highly effective because it mitigates osmotic stress on yeast cells, enabling them to metabolize both glucose and xylose efficiently.[4,31]

2.2. Butanol fermentation with *C. tyrobutyricum*

Fig. 2 shows the time course of butanol fermentation from BSG hydrolysates using an engineered *C. tyrobutyricum* strain. Due to the presence of inhibitors (likely phenolic compounds[32] and lignin-derived aromatics[33]), CS hydrolysates were detrimental to *C. tyrobutyricum*. As a result, glucose and xylose were not consumed, and no butanol was produced. After diluting CS hydrolysates by 50%, C. *tyrobutyricum* could grow, but the butanol concentration was still lower than 2.5 g L^{-1}. In contrast, BSG hydrolysates showed no significant toxicity towards it. In the initial 48 h, the log phase during fermentation of the BSG hydrolysates could be attributed to the toxicity of the hydrolysates. Subsequently, *C. tyrobutyricum* adapted to the conditions. After 96 h, BSG-S generated a butanol concentration of 6.7 g L^{-1}, which is slightly higher than BSG-P (6.4 g L^{-1}). This could be mainly because of lower sugar concentrations in the BSG-P hydrolysate (Table 1). Furthermore, BSG-P produced a higher acetate level of 7.8 g L^{-1} compared to BSG-S (7.1 g L^{-1}), but both produced the same butyrate level of 1.6 g L^{-1} after 96 h. Overall, *C. tyrobutyricum* consumed approximately 22 g L^{-1} of glucose and only 5.5 g L^{-1} of xylose in the BSG hydrolysates. The low xylose consumption is because *C. tyrobutyricum* also exhibits carbon catabolite repression, which makes it preferentially digest glucose as its first energy source.[34]

2.3. Itaconic acid fermentation with *A. terreus*

Fig. 3 shows the time course of IA fermentation using CS and BSG hydrolysates with an *A. terreus* strain. Results indicate that *A. terreus* is subjected to carbon catabolite repression,[35] preferentially consuming glucose. CS hydrolysates led to lower IA production, with only 2.79 g L^{-1} IA for CS-P and 2.5 g L^{-1} for

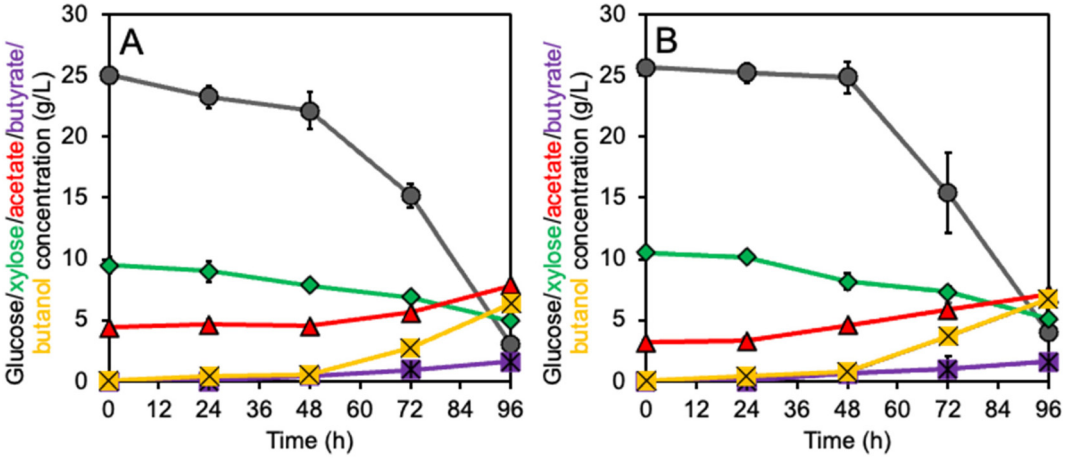

Fig. 2 Butanol fermentation of two hydrolysates (A: BSG-P and B: BSG-S) by an engineered *C. tyrobutyricum* strain.

Table 1 Chemical composition of four hydrolysis lignin residues

Sample	Glucan content (%)	Xylan content (%)	Lignin content (%)
CS-P	1.9 ± 0.3a	0.9 ± 0.2b	92.6 ± 1.8a
CS-S	1.5 ± 0.2a	1.6 ± 0.1a	90.8 ± 0.3a
BSG-P	2.6 ± 0.3b	1.5 ± 0.3a	83.5 ± 0.9b
BSG-S	2.1 ± 0.5ab	1.2 ± 0.2ab	79.1 ± 0.7b

Different letters within the same column indicate statistically significant differences ($p < 0.05$).

CS-S (Fig. 3A and B). In contrast, glucose consumption was more rapid in BSG hydrolysates, resulting in IA titers of 5.58 g L^{-1} (BSG-P) and 8.02 g L^{-1} (BSG-S) after 72 h (Fig. 3C and D). This significant difference is most likely because BSG hydrolysates inherently contain sufficient nutrients, particularly nitrogen from proteins, to support cell growth and IA formation.[3] This is consistent with previous findings showing that *A. terreus* achieved IA titers up to 44 g L^{-1} in defined glucose–xylose mixtures, but performed poorly in lignocellulosic hydrolysates.[36] The choice of acid for pH adjustment also affected IA production. Using H_2SO_4 for pH adjustment enhanced IA production in BSG but not in CS, while using H_3PO_4 may have mitigated manganese inhibition due to phosphate limitation. Prior studies have noted that manganese tolerance is advantageous for IA-producing strains under such conditions.[36,37] Notably, acetic acid accumulation was observed in CS hydrolysates. Since acetic acid at concentrations as low as 0.6 g L^{-1} was reported to inhibit IA production and 3.2 g L^{-1} completely arrests *A. terreus* growth,[38] its presence in CS hydrolysates may partially explain the lower IA titers.

2.4. 3-Hydroxypropionic acid fermentation with *I. orientalis*

Fig. 4 presents the time course of 3-HP fermentation from CS and BSG hydrolysates using an engineered *I. orientalis* IoDY01H strain. Across all four hydrolysates, glucose was fully consumed within 12 h, and more than 90% of xylose was depleted by 48 h. These results confirm the strain's robust performance in nitrogen-deficient lignocellulosic media.[39] 3-HP production reached 7.8 g L^{-1} for CS-P, 8.1 g L^{-1} for CS-S, 9.9 g L^{-1} for BSG-P, and 10.6 g L^{-1} for BSG-S. No significant difference between the two acids used for pH adjustment suggests that the choice of acid had minimal influence on 3-HP titers in *I. orientalis* fermentations. Interestingly, although BSG hydrolysates had 31.4% lower total fermentable sugar concentrations compared to CS, they yielded 1.16-fold higher 3-HP titers. This may be attributed to the inherent nitrogen source in BSG hydrolysates, derived from proteins, which supports the *I. orientalis* IoDY01H cell growth.[3] Also, BSG hydrolysates might enhance acetate metabolism, providing amino acid precursors and redirecting carbon flux toward the 3-HP synthetic pathway.[22] Acetate was fully consumed within 24 h in all cases, and its share of total sugar equivalents was 9.5% in BSG, 1.29-fold higher than in CS. As for ethanol, the maximum titer in CS-P reached 20 g L^{-1}, 1.40-fold higher than in CS-S in 12 h. This may reflect the dissociation of H_3PO_4 into phosphate, enhancing ethanol production through improved nutrient availability.[40] In contrast, ethanol titers (9.5 g L^{-1} for BSG-P and 10.3 g L^{-1} for BSG-S) in BSG hydrolysates were similar in 12 h. To further improve 3-HP concentration and yield, future engineering strategies should consider downregulating pyruvate decarboxylase to reduce ethanol formation,[41] overexpressing the 3-HP synthetic pathway, introducing CO_2 fixation modules,[42] and blocking competitive branching pathways.[43]

2.5. Polyhydroxyalkanoates production with *H. mediterranei*

Fig. 5 shows the PHA fermentation of CS and BSG hydrolysates using a *H. mediterranei* strain. After 5 days of fermentation, CDM ranged from 7.68 to 8.62 g L^{-1} across all four hydrolysates (CS-P, CS-S, BSG-P, and BSG-S), with no substantial differences between CS- and BSG-hydrolysates (Fig. 5A). These biomass concentrations are comparable to those reported in previous studies. For instance, a study used fermented food waste permeate to obtain 7.0 g L^{-1} CDM,[44] while another study

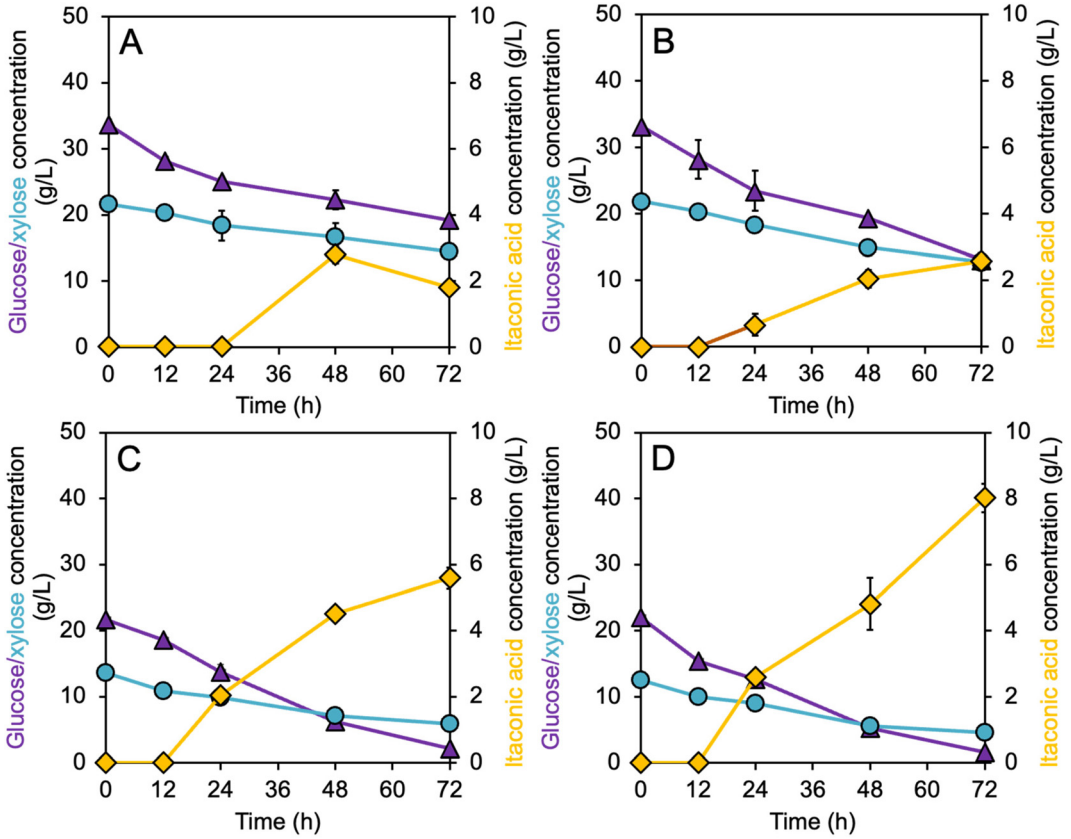

Fig. 3 IA fermentation of four hydrolysates (A: CS-P; B: CS-S; C: BSG-P; and D: BSG-S) by an *A. terreus* strain.

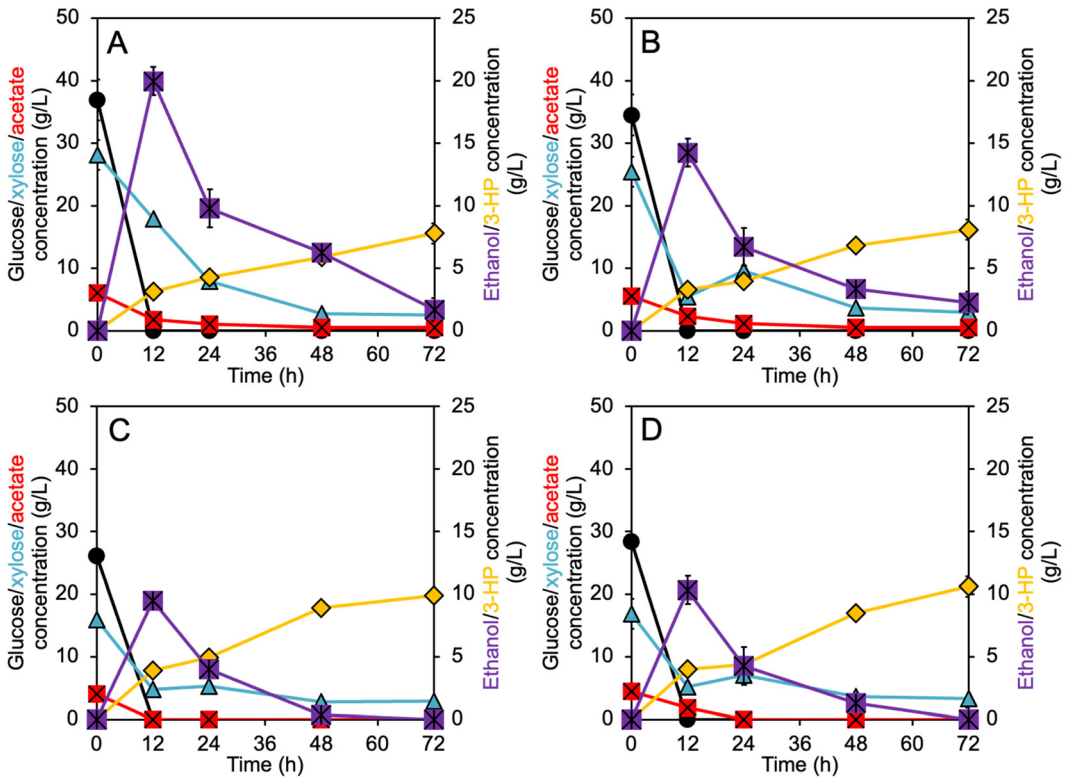

Fig. 4 3-HP fermentation of four hydrolysates (A: CS-P; B: CS-S; C: BSG-P; and D: BSG-S) by an *I. orientalis* strain.

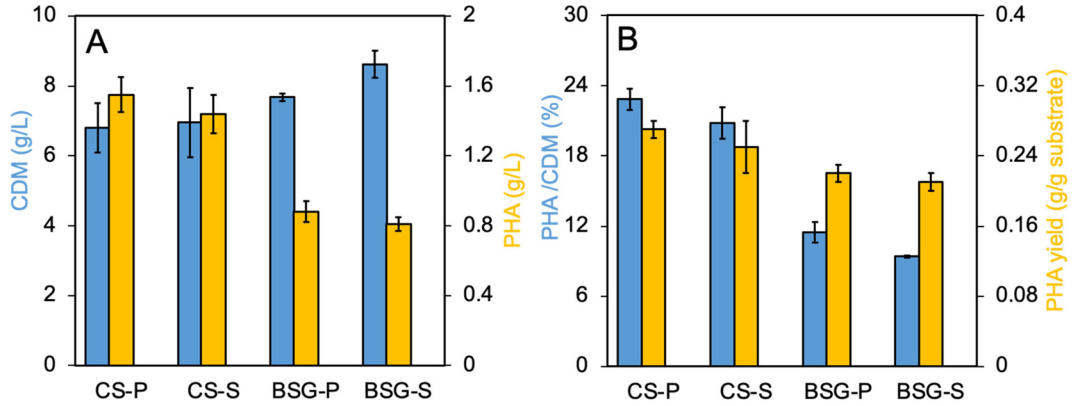

Fig. 5 PHA production from four hydrolysates by an engineered *H. mediterranei* strain (A: CDM and PHA concentrations; B: percentage of PHA content in CDM and PHA yield).

used nanofiltration retentate of ricotta whey to obtain 6.0 g L^{-1} CDM.[45] In terms of PHA production, CS-P produced the highest titer (1.55 g L^{-1}), which is slightly higher than CS-S (1.44 g L^{-1}) but significantly higher than BSG-P (0.88 g L^{-1}) and BSG-S (0.81 g L^{-1}) (Fig. 5A). It is important to note that the PHA tiers obtained from this study were significantly lower than the industrial benchmarks (over 100 g L^{-1}) when using different carbon sources.[46] CS hydrolysates outperformed BSG hydrolysates, likely due to their higher sugar concentrations (Table 1), and using H_3PO_4 for pH adjustment proved more effective to promote PHA production than using H_2SO_4, given that phosphate is a known essential nutrient for cell growth and PHA synthesis. Moreover, a trade-off between biomass accumulation and PHA synthesis was evident, in line with previous observations that PHA accumulation is often inversely related to rapid cell growth in *H. mediterranei* cultures.[47,48] In addition, PHA content as a proportion of CDM for CS hydrolysates (20.81%–22.84%) was higher than that (9.39%–11.47%) for BSG hydrolysates (Fig. 5B). Overall, CS-P (0.27 g g^{-1} substrate) and CS-S (0.25 g g^{-1} substrate) showed higher PHA yields than BSG-P (0.22 g g^{-1} substrate) and BSG-S (0.21 g g^{-1} substrate).

2.6. Characterization of lignin-rich hydrolysis residues

The solid residues from the enzymatic hydrolysis of the pretreated CS and BSG could be readily available, low-cost byproducts for the production of new value-added products. Since the structure of lignin plays a decisive role in its feasibility of further conversion and the ultimate properties of the lignin-derived products, the lignin-enriched hydrolysis residues obtained in this study were examined to gain knowledge that can maximize the valorization of biomass. Table 1 summarizes the composition of lignin-enriched hydrolysis residues derived from CS and BSG. CS residues exhibited substantially higher lignin content (90.8–92.6%) than BSG residues (79.0–83.0%), consistent with the elevated protein and extractive fractions typically present in BSG. Conversely, CS residues had lower glucan content (1.5–1.9%) compared with BSG (2.1–2.6%), while xylan levels were similarly low in both feedstocks (0.9–1.6%). These values confirm efficient enzymatic removal of carbohydrate fractions across all samples. CS-derived lignins had slightly lower M_n (619–659 g mol^{-1}) and M_w (4999–5142 g mol^{-1}) compared with kraft lignin standard (M_n: 875 g mol^{-1}; M_w: 6039 g mol^{-1}), but exhibited higher dispersity ($Đ_M$: 8.0–8.3 *vs.* 7.2). These elevated $Đ_M$ values suggest that CS lignins are highly polydisperse, encompassing a broad range of oligomeric and polymeric structures. The M_w values reported here are notably higher than literature values for ethanol-extracted (2609 g mol^{-1})[49] and enzymatic hydrolysis lignins from CS (3580 g mol^{-1}),[50] likely due to the acetylation of lignin in this study before GPC analysis. In contrast, BSG-derived lignins displayed markedly lower M_n (365–412 g mol^{-1}) and M_w (551–841 g mol^{-1}), with narrow $Đ_M$ (1.5–2.0), indicating high structural homogeneity. These M_w values are significantly lower than those previously reported for BSG lignins extracted using deep eutectic solvents (3280–3890 g mol^{-1}).[50] The lower M_n and M_w values of BSG lignins may result from extensive depolymerization during pretreatment or the presence of structurally distinct lignin–carbohydrate complexes. Additionally, the residual xylan content linked with lignin may have limited the solubilization of higher-molecular-weight fractions, further contributing to the observed trends.

Fig. 6 shows the NMR spectra of lignins extracted from CS and BSG hydrolysis residues. No significant differences were observed between the neutralization methods (H_3PO_4 *vs.* H_2SO_4) for either CS- or BSG-derived lignins. The CS lignin was primarily composed of 42–46% syringyl (S), 25–29% guaiacyl (G), and 16–19% *p*-hydroxyphenyl (H) units, along with small amounts of hydroxycinnamates (*p*CA and FA) relative to the total SGH aromatic units. In the aliphatic region, CS lignin was dominated by β-O-4′ linkages, which accounted for ~80% of total side-chain linkages. These results are consistent with the reported structural characteristics of CS lignin, although slight variations can arise due to differences in tissue origin and isolation methods.[51] In contrast, BSG lignin also exhibited a typical SGH-type structure but with a markedly different com-

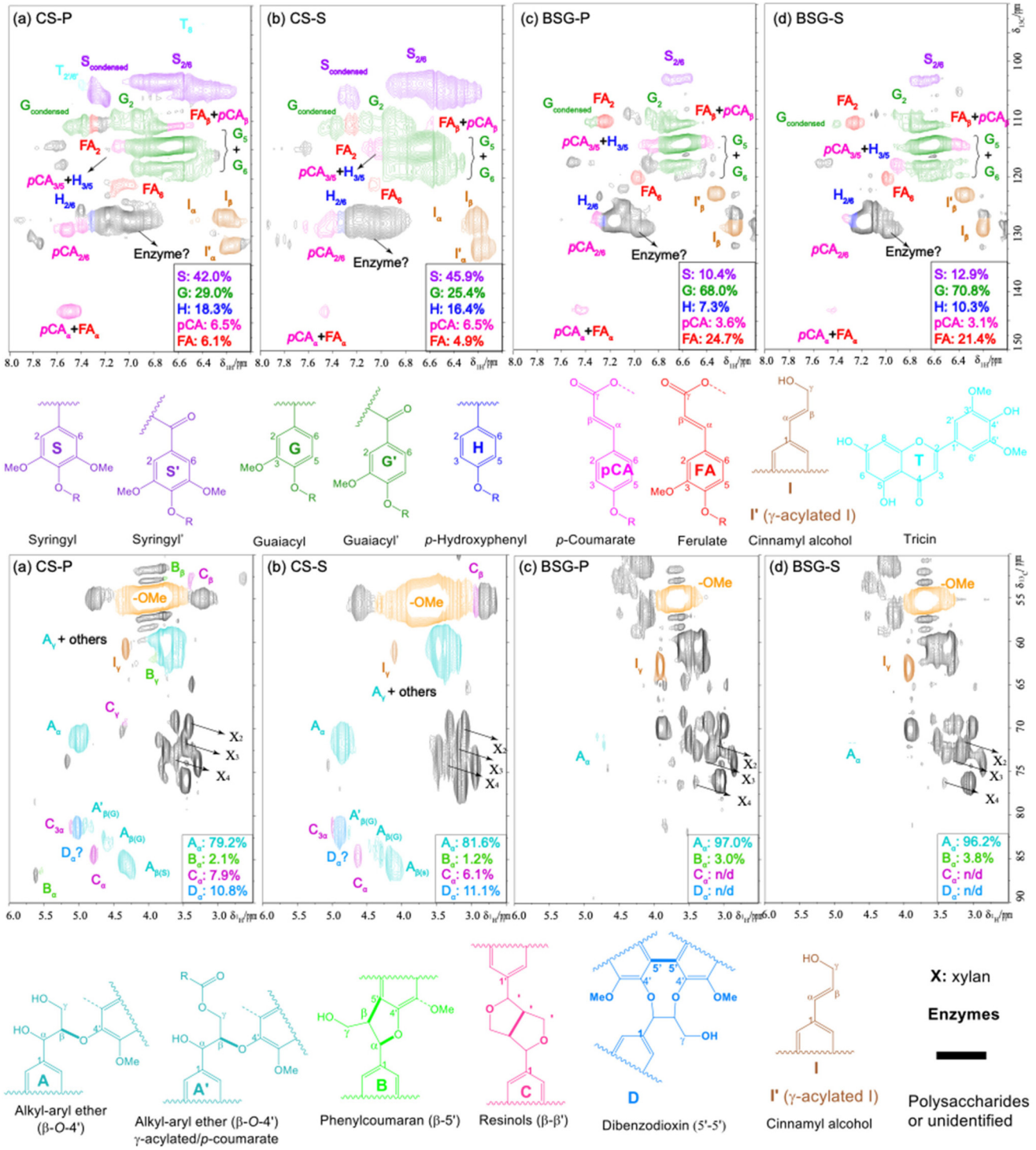

Fig. 6 Short-range aromatic (top) and aliphatic (bottom) region $^{13}C–^{1}H$ correlation HSQC spectra and semi-quantification of compositional units and side chains (insets) of the DMSO-extracted lignins from the hydrolysis residues from CS-P (a), CS-S (b), BSG-P (c), and BSG-S (d) in DMSO-d_6. Contours corresponding to color-coded structures in this region are used to measure the relative abundance of side chains. δ, chemical shift; ppm, parts per million.

position: 10–13% S, 68–71% G, and 7–10% H units, and minor hydroxycinnamates. Unlike CS lignin, BSG lignin contained very few side-chain linkages, suggesting that its aromatic units were largely present as monomers, dimers, or low-molecular-weight oligomers. This interpretation aligns with the GPC results showing substantially lower molecular weights for BSG

Table 2 Molecular weights and dispersity of indulin AT reference and lignin-enriched residues. (M_n: number-average molecular weight; M_w: weight-average molecular weight; $Đ_M$: molar-mass dispersity)

Sample	M_n (g mol^{-1})	M_w (g mol^{-1})	$Đ_M$
Lignin reference	875 ± 87a	6039 ± 924a	7.2
CS-P	659 ± 31b	4999 ± 218a	8.0
CS-S	619 ± 54b	5142 ± 313a	8.3
BSG-P	412 ± 31c	841 ± 91b	2.0
BSG-S	365 ± 23c	551 ± 75b	1.5

Different letters within the same column indicate statistically significant differences ($p < 0.05$).

lignin compared with CS lignin (Table 2). The spectra further indicate that the structural features of BSG lignin vary considerably depending on the pretreatment method. Finally, residual signals confirmed the presence of small amounts of xylan in the BSG hydrolysis residues. Currently, less than 2% of lignin streams from paper and biorefinery operations are utilized for commercial products, primarily in dispersants, adhesives, and additives. High-molecular-weightM_w lignin is typically directed toward carbonaceous applications, such as spun carbon fibers, through thermochemical conversion due to its greater thermal and mechanical stability.[52] In contrast, the BSG- and CS-derived lignins obtained in this study exhibit relatively low molecular weights M_w, which have several processing advantages. These include enhanced solubility in solvents, improved processability, and higher chemical reactivity, likely due to a greater abundance of hydroxyl groups. Such properties also increase compatibility with other polymers, facilitating chemical modifications (*e.g.*, esterification) and promoting uniform film formation. Therefore, these features suggest strong potential for developing lignin-derived coating materials with desirable adhesion and flexibility.[53,54]

3. Conclusions and outlook

This study demonstrates the novelty of a one-pot CaO pretreatment with direct pH adjustment that eliminates washing and solid–liquid separation steps, thereby enabling the direct microbial fermentation of hydrolysates into a diverse portfolio of bioproducts. The approach highlights the potential of BSG and CS as feedstocks for integrated biorefineries, while revealing that different biomass sources confer distinct advantages —CS being more favorable for sugar-based products such as ethanol and PHA, and BSG supporting higher titers of nitrogen-sensitive products such as 3-HP and IA. The toxicity effects in CS hydrolysates limited butanol fermentation, highlighting the need for further optimization of strains and processes. BSG lignins exhibited significantly lower M_w and $Đ_M$ compared to CS lignins. These lignins, which exhibit typical SGH lignin structural features, have the potential to be compatible with other polymers. This compatibility allows for chemical modifications, such as esterification, and promotes uniform film formation, making them suitable for developing coating materials with desirable adhesion and flexibility.

The scalability and economic viability of the one-pot CaO pretreatment process for producing various products are critical considerations for translating this laboratory demonstration into industrial practice. CaO is inexpensive, abundant, and already produced at scale for construction and agricultural applications, making it an attractive catalyst for biomass pretreatment. From an economic standpoint, using H_2SO_4 for pH adjustment would be significantly more cost-effective compared to using H_3PO_4. The elimination of washing and solid–liquid separation steps reduces water and chemical inputs, minimizes wastewater treatment requirements, and lowers operational complexity—factors that could directly translate into cost savings in large-scale biorefineries. Furthermore, generating fermentation-ready hydrolysates without extensive detoxification has the potential to streamline integration with diverse microbial platforms, thereby reducing both capital and operating expenditures. Despite these advantages, several challenges probably remain for scaling up and achieving commercialization. The low market values of commodity products such as ethanol and butanol might offset the environmental sustainability benefits if production costs during pretreatment, separation, and purification are high. In contrast, products such as IA, 3-HP, and PHAs have greater market prices but are currently produced at low titers, which will significantly increase downstream processing costs. Addressing these barriers might necessitate strategies such as utilizing pelleted biomass for pretreatment under high solid loadings to increase fermentable sugar concentrations and engineering robust microbial strains capable of efficiently co-utilizing glucose and xylose to enhance the concentration and yield of bioproducts. Additionally, integrating a multi-product biorefinery framework presents a promising avenue for enhancing economic viability. By harnessing the complementary strengths of multiple product streams, we can overcome the limitations inherent in single-product systems, thereby improving the overall economic viability and environmental sustainability of lignocellulosic biorefineries. Future research should also extend beyond laboratory-scale validation to include technoeconomic analysis (TEA) and life-cycle assessment (LCA) of the one-pot CaO pretreatment process. TEA will be critical for identifying cost drivers, evaluating process integration with downstream fermentation, and quantifying the economic competitiveness of producing both commodity (e.g., ethanol and butanol) and higher-value (3-HP and IA) bioproducts. Complementary LCA studies will provide insight into the environmental footprint of the process, including impacts on energy use, water consumption, and greenhouse gas emissions, thereby elucidating its sustainability against conventional pretreatment methods.

Conflicts of interest

There are no conflicts to declare.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

Supplementary information, including materials and methods, is available. See DOI: https://doi.org/10.1039/d5gc04325e.

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