Frontiers in Bioscience Elite

Front. Biosci. (Elite Ed) **2026**; 18(3): 49846
https://doi.org/10.31083/FBE49846

Original Research

Enhanced Lactic Acid Production From Soybean Waste Using a Xylose-Fermenting *Lactiplantibacillus pentosus* Strain

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Academic Editor: Baohong Zhang
Submitted: 9 January 2026 Revised: 20 February 2026 Accepted: 12 March 2026 Published: 8 September 2026

Abstract

Background: The high carbohydrate content of the cellulose and hemicellulose fractions in agricultural crops, along with their by-products and derivative residues (e.g., soybean straw, hulls, sugarcane bagasse, and corn stover), offers a promising resource for biorefinery industries. These lignocellulosic materials, generated during harvesting and mechanical processing, support large-scale biological production of renewable energy, sustainable chemicals, and alternative food sources. **Methods:** This study investigated lactic acid fermentation of liquid hydrolysates from soybean straw hydrothermally pretreated with either sodium hydroxide (NaOH) or hydrogen peroxide (H_2O_2), and assessed the impact on cellulolytic enzyme activity and subsequent sugar release. Soybean straw (10% w/v, dry weight basis) was subjected to moderate-severity hydrothermal pretreatment at 121 °C for 60 min in the presence of either 1% (v/v) NaOH or H_2O_2 as representative dilute chemical benchmarks. The resulting pretreated solids were enzymatically hydrolyzed using a cellulolytic enzyme mixture at a loading rate of 50 mg enzyme protein/g cellulose (equivalent to 90 IU/g glucan) at 50 °C for 72 h under shaking incubation at 150 rpm. **Results:** Scanning electron microscopy (SEM) revealed that both NaOH and H_2O_2 pretreatments effectively disrupted the surface morphology and biomass structure of the soybean straw, increasing surface roughness and exposure of reactive sites, thereby enhancing enzymatic accessibility and improving the conversion of glucan and xylan to glucose and xylose, respectively. NaOH pretreatment achieved hydrolysis yields of 66.8% for cellulose and 45.7% for hemicellulose, whereas H_2O_2-treated samples yielded only 52.3% (cellulose) and 38.1% (hemicellulose). In comparison, the untreated control yielded only 21.6% cellulose and 16.4% hemicellulose conversion. A positive control using Solka-Floc (a lignin-free cellulose powder) yielded 83.5% glucose and 62.6% xylose conversion. Subsequent lactic acid fermentation of the NaOH-pretreated hydrolysates using a xylose-co-fermenting *Lactiplantibacillus pentosus* strain produced L-lactic acid at 80.5% of the theoretical yield (12.72 g/L), with productivity of 0.35 g/L/h and a yield of 0.2544 g LA/g of NaOH-pretreated straw solids. **Conclusions:** These results demonstrate that NaOH pretreatment is more effective than H_2O_2 in enhancing the enzymatic saccharification of soybean straw. Moreover, the resulting hydrolysates can be efficiently converted into L-lactic acid using a *Lactiplantibacillus pentosus* strain capable of co-fermenting xylose, supporting the feasibility of this integrated process for value-added bioconversion of lignocellulosic biomass.

Keywords: lignocellulosic biomass; soybeans; enzymatic hydrolysis; fermentation; *Lactiplantibacillus pentosus*

1. Introduction

Soybean is recognized as one of the most important agricultural crops, and its global production has steadily increased to meet the rising demand for both traditional food products and a wide range of industrial applications. In recent years, growing interest in functional foods, natural ingredients, and dietary supplements has further stimulated worldwide soybean consumption, while also driving continued improvements in seed quality and productivity [1,2]. Nutritionally, soybeans are valued for their high protein content, which represents about 35–40% of the dry weight, presenting one of the richest plant-based protein sources. They provide a well-balanced amino acid composition, including all nine essential amino acids such as lysine and methionine, which contribute to their role as a high-quality protein source. Specifically, soy protein is capable of lowering low-density lipoprotein (LDL) cholesterol and raising high-density lipoprotein (HDL) cholesterol, supporting heart health as well as reducing risk of coronary heart disease. In addition to protein, soybean oil is rich in unsaturated fatty acids, particularly omega-3 and omega-6 fatty acids, making it an important dietary source of essential fatty acids [3,4].

Of particular interest are green soybeans, which contain a relatively high protein concentration (approximately 35–38 g per 100 g on a dry weight basis). This high protein content makes them suitable for a broad range of functional and nutritional applications in both food processing and animal feed. These include their use in plant-based meat analogs, protein-fortified foods, and specialized diets as a gluten-free and lactose-free protein source. In addition, processed products such as fat-free soybean meal and textured vegetable protein (TVP) serve as affordable, amino acid–balanced protein supplements for animal feed, while

Table 1A. Chemical compositions of representative plant-derived lignocellulosic biomass types.

(A)	Composition (% by dry weight basis)							
	Cellulose	Hemicellulose	Lignin	Solid recovery (%)	Cellulose loss (%)	Hemicellulose loss (%)	Delignification (%)	Reference
Soybean straw (as received)	27.2	13.0	26.9	-	-	-	-	Current work (adapted from [11])
NaOH pretreated	39.7	14.8	25.2	57.8	15.6	34.2	45.9	-
H_2O_2 pretreated	33.8	14.7	29.8	78.8	2.1	10.9	12.7	-
Soybean hull	34.2	16.5	6.7	-	-	-	-	-
Sugarcane bagasse	36.4–37.9	21.1–21.5	26.1–27.2	-	-	-	-	[12]
Corn stover	37.0	22.7 (xylan)	18.6	-	-	-	-	[10]
Corn pericarp	22.5	23.7	4.7	-	-	-	-	[13]
Switchgrass	39.4	17.3	21.2	-	-	-	-	[14]

Table 1B. Various pretreatment strategies applied to soybean straw.

(B) Pretreatment	Conditions	Hydrolysis conditions	Glucose yield (%)	Reference
Hydrothermal with dilute chemicals	1% (v/v) NaOH or H_2O_2	90 IU/g cellulose (50 mg protein/g cellulose), 50 °C, 72 h, 150 rpm	66.8 (NaOH-pretreated solids) 52.3 (H_2O_2-pretreated solids)	Current work
Liquid hot water	170–210 °C, 3–10 min	20 FPU/g solids, 50 °C, 48–72 h, 130 rpm	~70.76	[16]
Dilute acid (H_2SO_4)	4% (w/v) H_2SO_4, 121 °C, 1 h	19.8 FPU/g biomass, 50 °C, 72 h, 150 rpm	~93.9	[15]
Dilute acid (H_2SO_4) or alkaline (NaOH)	1–4% (w/v) H_2SO_4 or 0.3–1.5% (w/v) NaOH, 121 °C, 30 min	15 FPU/g substrate, 50 °C, 72 h, 150 rpm	~78.7	[17]
Ammonia liquor	10% (w/v) ammonia liquid, soaking for 24 h, room temp	50 FPU/g substrate, 50 °C, 36 h	~51.22	[18]

FPU, filter paper unit.

also offering viable plant-based alternatives to conventional meat and dairy products [5]. The economic and agricultural significance of soybeans is further underscored by production statistics. According to the United States Department of Agriculture (USDA), global soybean production for the 2024/2025 season is projected at approximately 15.57 billion bushels (423.97 million metric tons). In the United States, soybean production is estimated at 4.37 billion bushels (118.84 million metric tons), representing a 5% increase compared to the 2023/2024 season and accounting for 28% of the global harvest (https://www.fas.usda.gov/data/production/commodity/2222000).

Soybean straw is the major lignocellulosic residue generated during soybean grain harvesting and processing. Because of its relatively high carbohydrate content, particularly cellulose (35–45%) and hemicellulose (10–25%), soybean straw has attracted interest as a feedstock for renewable energy and sugar-platform industries [6,7,8]. In addition to energy applications, its composition also makes it a promising raw material for biochemicals, nanocellulose, and biodegradable materials [9]. A comprehensive comparison of soybean straw with other commonly utilized lignocellulosic biomass feedstocks is outlined in Table 1A (Ref. [10,11,12,13,14]) with various pretreatment applications and their glucose yields (Table 1B, Ref. [15,16,17,18]). Despite this potential, the inherent biomass recalcitrance, stemming from the tight association of crystalline cellulose with hemicellulose and lignin within the cell wall matrix, limits direct enzymatic hydrolysis of soybean straw. Effective pretreatment is therefore essential to reduce this structural resistance. Such treatments aim to solubilize hemicellulose, disrupt covered lignin–carbohydrate complexes, and partially decrystallize cellulose, thereby improving enzyme accessibility and saccharification efficiency [10,15]. A range of pretreatment technologies have been evaluated on soybean straw and other legume residues. For instance, subcritical water hydrolysis pretreatment has been reported to significantly disrupt the lignocellulosic matrix and enhance fermentable sugar release [19]. Mechanical methods such as ball milling reduce particle size and crystallinity, improving hydrolysis efficiency [20]. Chemical routes, including alkaline and dilute-acid pretreatments, have also demonstrated effectiveness in delignification and hemicellulose removal, leading to improved enzymatic digestibility [21]. More recently, our work presented that hydrothermal pretreatment of soybean straw with either 1% (v/v) sodium hydroxide (NaOH) or hydrogen peroxide (H_2O_2) effectively improved glucose yields [11].

Lactic acid (LA) is a multifunctional C3 platform chemical widely applied in the food, pharmaceutical, and cosmetic industries, and in recent years, it has gained exceptional importance as the principal monomer for polylactic acid (PLA), a leading bio-based and biodegradable polymer [22,23,24]. The global lactic acid market was valued at approximately USD 3.5–4.0 billion in 2023 and is projected to exceed USD 8–10 billion by 2030, with a compound annual growth rate (CAGR) of 7–9%, driven largely by expanding applications in biodegradable plastics, food preservation, pharmaceuticals, and personal care products (Grand View Research, 2024, https://www.grandviewresearch.com/industry-analysis/lactic-acid-and-poly-lactic-acid-market). The PLA market alone is expected to surpass USD 5–6 billion by 2030 due to increasing regulatory pressure to reduce petroleum-based plastics and rising consumer demand for sustainable packaging materials. As feedstock costs account for a substantial fraction (30–50%) of total fermentation production costs, the use of low-cost, non-food lignocellulosic residues such as soybean straw represents an economically attractive alternative to food-grade glucose or starch hydrolysates. The rapid global expansion of the PLA market, driven by regulatory restrictions on petroleum-based plastics and rising consumer demand for sustainable alternatives, has intensified research efforts to improve lactic acid production. Traditionally, lactic acid has been produced via microbial fermentation using food-grade sugars such as glucose, sucrose, and starch hydrolysates; however, this approach raises concerns over feedstock cost,

food-versus-fuel competition, and sustainability [25,26]. Consequently, lignocellulosic biomass including agricultural residues (e.g., corn stover, soybean straw, sugarcane bagasse), dedicated energy crops (e.g., switchgrass, miscanthus), and industrial by-products (e.g., sawdust, paper mill waste) have emerged as attractive alternative feedstock for lactic acid production due to their low cost, abundance, and non-competitive nature with food systems [27]. In addition to economic benefits, the valorization of lignocellulosic residues aligns with circular bioeconomy strategies, contributing to carbon reduction, waste minimization, and renewable polymer production, thereby making lignocellulose to lactic acid conversion a high-priority target for both research and industrial commercialization [28].

Although numerous studies have examined alkaline or oxidative pretreatment strategies and lactic acid fermentation from various lignocellulosic feedstocks, comparatively fewer studies have systematically benchmarked these approaches specifically for soybean straw under consistent hydrolysis and fermentation conditions. In particular, direct comparisons of NaOH and H_2O_2 pretreatment effects on saccharification efficiency, inhibitor profiles, and downstream co-fermentation performance remain limited for this feedstock [29]. Therefore, rather than introducing new pretreatment chemistry or fermentation pathways, the present study aims to provide a controlled comparative evaluation of these established strategies applied to soybean straw. By integrating compositional analysis, scanning electron microscopy (SEM) characterization, enzymatic hydrolysis, and C5/C6 sugar co-fermentation using *Lactiplantibacillus pentosus*, this work provides feedstock-specific performance data and benchmarking against other agricultural residues. Such information is essential for assessing the realistic bioconversion potential of soybean straw within an integrated biorefinery context.

2. Materials and Methods

2.1 Materials

Soybean plants were cultivated and harvested in 2019, and the resulting soybean straw byproducts were generously provided by Peace and Plenty Farm (Rocky Ridge, Frederick, MD, USA). The raw soybean straw was mechanically milled into smaller, uniform particles using an all-steel Wiley cutting mill (Thomas Scientific, Swedesboro, NJ, USA) and subsequently sieved through a 30-mesh screen (0.595 mm). The ground straw samples were collected and oven-dried at 50 °C overnight. Moisture content (<5%) was determined using a Halogen moisture analyzer (Mettler Toledo HB 43, Columbia, OH, USA). A commercial cellulolytic enzyme preparation, Cellic CTec2 (SAE 0020, Lot# SLCG4389), was obtained from Sigma-Aldrich (St. Louis, MO, USA). The different enzyme activities and protein concentrations were determined following previously described methods and summarized in Table 2 [12]. All other chemicals and reagents used in this study were purchased from Fisher Scientific (Hampton, NH, USA), Sigma-Aldrich (St. Louis, MO, USA), and Thermo Fisher Scientific (Waltham, MA, USA).

2.2 NaOH and H_2O_2 Pretreatment of Soybean Straw

Following our previous work [11], soybean straw (10%, w/v) was subjected to hydrothermal pretreatment in the presence of either 1% (v/v) NaOH or H_2O_2 at 121 °C for 60 min. Briefly, the pretreated soybean straw slurry was filtered under vacuum using a Buchner funnel onto a Whatman No. 1 filter paper (MilliporeSigma, Bedford, MA, USA, Catalog number: WHA1001045) and separated into pretreated solid and liquid fractions. To remove potential soluble inhibitors (e.g., furans, phenolic compounds), the residual solids were washed three times with 100 mL of distilled water (room temperature) and vacuum filtered through the same filter paper. The washed solids were then oven-dried at 50 °C overnight (moisture content: <5%) and stored for subsequent hydrolysis experiments and further scanning electron microscopy analysis.

2.3 SEM Analysis

The surface morphology and structural characteristics of raw soybean straw, pretreated with either NaOH or H_2O_2, as well as the resulting hydrolyzed solids, were analyzed using a JCM-6000 benchtop scanning electron microscope (Model JCM 6000-OG-2, JEOL, Peabody, MA, USA). Oven-dried samples were mounted on aluminum stubs with double-sided carbon tape and sputter-coated with a gold–palladium (Au/Pd) alloy using a Cressington sputter coater (Ted Pella Inc., Redding, CA, USA) to improve surface conductivity. SEM observations were performed at an accelerating voltage of 15 kV, with magnifications ranging from 100× to 500×, to evaluate structural changes induced by enzymatic hydrolysis [11,29].

2.4 Enzyme-Mediated Hydrolysis of Pretreated Soybean Straw Solids

Pretreated, washed, and oven-dried solids were suspended at 5% (w/v, dry basis) in 100 mL of 50 mM sodium citrate buffer (pH 4.8) and subjected to enzymatic hydrolysis with a loading of 50 mg enzyme protein/g of cellulose (equivalent to 90 IU/g cellulose) at 50 °C for 72 h in a shaking incubator. As a control, Solka-Floc (purified lignin-free cellulose fiber: 80% cellulose and 20% hemicellulose) was hydrolyzed under identical conditions. Enzymatic hydrolysis was conducted at a solid loading of 5% (w/v, dry basis), which is commonly used in laboratory-scale saccharification studies to minimize mass-transfer limitations, excessive viscosity, and mixing heterogeneity. At higher solids loadings, substrate aggregation and reduced enzyme mobility can confound interpretation of pretreatment effectiveness by introducing diffusion and rheological constraints. The 5% (w/v) loading therefore allowed intrinsic differences in substrate accessibility and lignin removal be-

Table 2. (A) Enzyme activity and corresponding protein composition, and (B) generalized enzyme activities calculated at an enzyme loading of 50 mg protein per gram of soybean straw cellulose.

(A)	Protein (mg/mL)	Filter paper unit (FPU)	Endo-glucanase	β-glucosidase	Xylanase	β-xylosidase
Activity (units/mL)	186.5	50.4	2179.9	351.6	288.3	35
(B)	–	Filter paper unit (FPU)	Endo-glucanase	β-glucosidase	Xylanase	β-xylosidase
Generalized (unit/g cellulose)	50	13.5	584.4	94.3	77.3	9.4

Generalized enzyme activity units (units per gram of substrate) were calculated as follows: enzyme activity (units per mg protein) multiplied by the applied enzyme dosage (mg of enzyme protein per gram of soybean straw cellulose).

tween pretreatment strategies to be evaluated under well-controlled and reproducible conditions. This loading was selected for comparative benchmarking rather than volumetric productivity optimization.

2.5 Preparation of Microbial Culture and Inoculum

The recombinant *Lactobacillus pentosus* strain (*Lactiplantibacillus pentosus* 124-2, ATCC 8041) was obtained from the American Type Culture Collection (Manassas, VA, USA). This type strain has a fully sequenced genome, is non-motile, rod-shaped, and capable of fermenting both hexoses and pentoses while producing organic acids. The dehydrated strain was rehydrated and cultivated in 100 mL of ATCC 416 liquid medium at 37 °C overnight in a 5% CO_2 incubator under static conditions with agitation at 150 rpm. The Lactobacilli MRS broth medium was prepared with 10 g proteose peptone #3, 10 g beef extract, 5 g yeast extract, 20 g dextrose, 1 g sorbitan monooleate, 2 g ammonium citrate, 5 g sodium acetate, 0.05 g manganese (II) sulfate monohydrate ($MnSO_4 \cdot H_2O$), and 2 g sodium hydrogen phosphate (Na_2HPO_4) per liter of distilled water, with the final pH adjusted to 6.5. After cultivation, the culture was centrifuged (10 min, 25 °C, 5000 rpm) to remove the supernatant. The cell pellet was collected, washed, and resuspended in distilled water to achieve a fresh-cell suspension with an optical density (OD_{600}) of approximately 0.6. A 10% (v/v) inoculum of this suspension was then added to a 250 mL Erlenmeyer flask containing 100 mL of vacuum-filtered hydrolysate and incubated for lactic acid fermentation at 35 °C for 36 h under micro-anaerobic conditions in a 5% CO_2 incubator with shaking at 150 rpm.

2.6 Analytical Procedures

During the enzymatic hydrolysis and lactic acid fermentation, liquid samples were collected at defined time intervals and the generated glucose, xylose, and lactic acid concentrations were quantified using a GOPOD colorimetric assay (K-GLUG, 700004297), a D-xylose analysis (K-XYLOSE, 700004355), and a D-/L-lactic acid assay kit (K-DLATE, 700004276), respectively (Megazyme, Wicklow, Ireland).

D-Glucose concentrations were measured using a glucose oxidase/peroxidase (GOPOD) assay kit (K-GLUC; Megazyme). In this colorimetric assay, D-glucose is oxidized by glucose oxidase to D-gluconate and hydrogen peroxide (H_2O_2). The produced H_2O_2 subsequently reacts with *p*-hydroxybenzoic acid and 4-aminoantipyrine in the presence of peroxidase to form a quinoneimine dye complex. The absorbance of the resulting pink chromophore was measured spectrophotometrically at 510 nm, with the intensity directly proportional to the D-glucose concentration in the sample. D-Xylose concentrations were quantified using a D-xylose assay kit (K-XYLOSE; Megazyme). D-Xylose is enzymatically oxidized by D-xylose dehydrogenase in the presence of nicotinamide adenine dinucleotide (NAD^+) to D-xylonate. The amount of NADH formed in this redox reaction is stoichiometric with the amount of D-xylose present and was determined by measuring the increase in absorbance at 340 nm. D- and L-Lactic acid concentrations were determined using a D-/L-lactic acid assay kit (K-DLATE; Megazyme). In this enzymatic method, D-lactate and L-lactate are oxidized to pyruvate by D-lactate dehydrogenase and L-lactate dehydrogenase, respectively, in the presence of NAD^+. The concomitant formation of NADH, which corresponds stoichiometrically to the total lactic acid concentration, was measured spectrophotometrically by the increase in absorbance at 340 nm [11].

3. Results and Discussion

3.1 Hydrothermal Pretreatment of Soybean Straw in the Presence of Dilute NaOH or H_2O_2

To build upon the previous work, the chemical compositions of raw soybean straw and its NaOH- and H_2O_2-pretreated solids were analyzed according to the biomass compositional analysis procedures established by the National Renewable Energy Laboratory (NREL) and adapted from our earlier study [11,30]. As received soybean straw exhibited a typical herbaceous lignocellulosic composition, containing 27.2% cellulose, 13% hemicellulose, and 26.9% lignin (Table 1A). This relatively high content, comparable to that of other agricultural residues such as sugarcane bagasse and switchgrass (Table 1A), contributes to biomass recalcitrance and limits enzymatic saccharification efficiency without pretreatment. Alkaline (NaOH) pretreatment substantially increased the cellulose fraction to 39.7%, reflecting partial delignification and enhanced exposure of structural carbohydrates. In contrast, H_2O_2 pretreatment resulted in a more modest increase in cellulose

content (33.8%) and a relative increase in lignin proportion (29.8%), suggesting lignin modification rather than effective solubilization. Hemicellulose content remained relatively stable following both pretreatments (14.7–14.8%), indicating that carbohydrate preservation was largely maintained. When compared to other agricultural residues commonly evaluated for bioenergy and biochemical production, soybean straw demonstrates competitive carbohydrate content and a favorable balance between cellulose and hemicellulose (Table 1A).

Hydrothermal treatment of non-food lignocellulosic resources, such as agricultural residues and woody biomass, is known to promote delignification, solubilize hemicellulose, and enhance the enzymatic conversion of cellulose into fermentable monomeric sugars. For instance, Wan et al. [16] reported that the highest glucose yield (70.7%) from pretreated soybean straw was achieved under hydrothermal conditions of 210 °C for 10 min (severity factor: 4.24), whereas a lower yield (38.6%) was obtained at a lower severity factor of 2.54 (170 °C for 3 min), demonstrating that cellulose solubilization and hemicellulose digestibility progressively increased with pretreatment severity. Additional pretreatment approaches applied to soybean straw, along with their hydrolysis conditions and resulting glucose yields, are outlined in Table 1B. Lignins, particularly kraft and softwood types, typically exhibit thermoplastic behavior between 120 °C and 170 °C, while substantial chemical modification or thermal decomposition occurs above 200 °C. Consequently, pretreatments conducted at elevated temperatures (>200 °C) and longer reaction times are more effective at disrupting the complex lignocellulosic structure [31]. However, severe reaction conditions (e.g., high temperature and pressure, concentrated acids or alkalis, and extended residence times) can also promote carbohydrate degradation and generate inhibitory byproducts, including furan aldehydes, carboxylic acids, phenolic compounds, and lignin-derived intermediates. Previous studies have demonstrated that soluble phenolic compounds generated during alkaline pretreatment can significantly suppress enzymatic hydrolysis efficiency. Earlier, our soybean straw literature reported that phenolics released from NaOH-pretreated soybean straw (716 mg/L total phenols) reduced glucose yield from 57% to 20% when hydrolysis was performed in the presence of the NaOH liquid hydrolysate instead of a buffer control [11]. To further assess inhibitor generation under the selected moderate-severity conditions, total phenolic concentrations in the pretreatment liquid fractions were quantified. NaOH- and H_2O_2-pretreated soybean straw generated approximately 720 mg/L and 630 mg/L total phenolics, respectively. Although both pretreatments produced substantial levels of soluble phenolic compounds, the slightly higher phenolic concentration in the NaOH liquor is consistent with its stronger delignification capability. Importantly, because the pretreated solids were thoroughly washed prior to enzymatic hydrolysis, these measured phenolics primarily reflect compounds solubilized into the pretreatment liquor rather than those retained in the solid fraction. Therefore, differences in downstream hydrolysis and fermentation performance are more likely associated with residual lignin structure and inhibitor retention within the solid matrix rather than total phenolic concentration in the removed liquid fraction. Such inhibitory compounds not only decrease enzyme activity but also increase the required enzyme loading and necessitate additional detoxification or conditioning steps to alleviate their adverse effects on subsequent hydrolysis and fermentation. In consideration of these challenges, the present study employed moderate pretreatment conditions either at 1% (v/v) NaOH or 1% (v/v) H_2O_2, 121 °C for 60 min (severity factor = 2.40) to achieve effective structural disruption of soybean straw while minimizing inhibitor formation, thereby improving downstream enzymatic saccharification and lactic acid fermentation performance.

The selected pretreatment parameters (1% NaOH or 1% H_2O_2 at 121 °C for 60 min; severity factor: 2.40) were chosen as moderate-severity benchmark conditions rather than optimized settings [11,29]. Dilute alkaline concentrations in the range of 0.5–2% and temperatures near 121 °C are widely reported for laboratory-scale evaluation of agricultural residues, providing sufficient delignification while limiting excessive carbohydrate degradation and inhibitor generation [12]. Similarly, a 60 min residence time represents a balance between structural disruption and preservation of fermentable sugars. These conditions were selected to enable direct comparison with previously reported soybean straw and related lignocellulosic pretreatment studies under comparable autoclave-based hydrothermal systems. Therefore, the objective of this work was to evaluate relative pretreatment effectiveness under standardized, representative conditions, rather than to define optimal industrial severity parameters.

3.2 SEM Characterization of Hydrothermal Pretreatment of Soybean Straw

To evaluate the morphological and structural alterations of soybean straw before and after pretreatment, SEM was employed (Fig. 1A–F). The untreated soybean straw (Fig. 1A,B) displayed a compact, intact surface with tightly bound fiber bundles and minimal porosity, indicative of the dense lignin–carbohydrate complex typical of raw lignocellulosic biomass. This rigid and continuous morphology acts as a physical barrier, limiting enzyme accessibility and penetration, as similarly observed in untreated rice straw and sugarcane bagasse [11,17]. After hydrothermal pretreatment with either NaOH or H_2O_2, the soybean straw exhibited marked structural disintegration (Fig. 1C–F). The fibers became increasingly fragmented and peeled, with longitudinal cracks and exposed fibrillar networks visible across the cell wall surfaces. Such disruptions sug-

gest partial delignification and the removal of hemicellulosic layers, allowing cellulase enzymes to access the internal cellulose microfibrils. These observations are consistent with previous findings for NaOH- and H_2O_2-pretreated hemp residues and dual-impeller liquefied soybean straw, where enzymatic or oxidative treatments caused delamination, fibrillation, and increased surface roughness, all of which enhance saccharification efficiency [11,12,17]. At higher magnification (Fig. 1D,F), the pretreated soybean straw revealed a distinctly porous and irregular structure, with visible voids, disrupted fiber orientations, and a sponge-like texture. The removal of amorphous lignin and hemicellulose fractions during enzymatic action likely contributed to this increased porosity and surface area, facilitating greater enzyme adsorption and hydrolytic efficiency. Similar pore development and fibril exposure were also reported in liquid hot water-pretreated sugarcane bagasse [12] and alkaline-pretreated switchgrass [32], reinforcing that the observed morphological breakdown is a key indicator of improved enzyme accessibility. The progressive transformation from the dense, rigid matrix of raw soybean straw (Fig. 1A,B) to the fragmented, porous structure after pretreatment (Fig. 1C–F) confirms that enzymatic hydrolysis alone, even in the absence of harsh chemical pretreatment, can induce substantial physical disruption. This enhanced microstructural accessibility supports the higher cellulose conversion and sugar yields observed in subsequent hydrolysis and fermentation steps.

3.3 Enzymatic Hydrolysis of Pretreated Soybean Straw

A commercial cellulolytic/hemicelluloytic formulation Cellic CTec2 was employed for hydrolysis of the pretreated soybean straw samples. The enzyme was added at a loading of 50 mg protein/g cellulose to both NaOH- and H_2O_2-pretreated soybean straw, following thorough washing to eliminate residual pretreatment reagents that could interfere with enzyme activity. This blended enzyme contains endoglucanase, xylanase, β-glucosidase, and β-xylosidase, which act synergistically to depolymerize insoluble plant polymers, cellulose and hemicellulose, into monomeric soluble sugars, primarily glucose and xylose. The identified enzyme activities and their corresponding generalized enzyme activity units are summarized in Table 2. In our previous studies with soybean hulls, the combination of a cellulase blend and Multifect Pectinase significantly improved saccharification efficiency and reduced enzyme loadings from 45 to 10 mg enzyme protein/g solids. Even with a moderate enzyme dosage of 10 mg enzyme protein/g solids, efficient sugar release was achieved, resulting in 90.6% cellulose-to-glucose conversion. This yield was comparable to that obtained under the higher enzyme loading of 45 mg enzyme protein/g solids, reaching 94.4% yield [29]. This effect was attributed to the disruption of the gel-like pectic matrix in the plant cell walls and the reduction of biomass viscosity enabled by the addition of pectinase, together with improved mixing performance provided by the dual-impeller system rather than shaking incubation [11,13,29]. The dual elephant-ear impeller blades improved hydrodynamic mixing by breaking up biomass clumps, maintaining a homogeneous suspension, and facilitating consistent enzyme access to cellulose and hemicellulose surfaces, thereby enhancing substrate-enzyme interactions and overall hydrolysis efficiency. This strategy was particularly effective for low-lignin substrates such as soybean hulls, which contain only about 5% lignin, and enabled efficient hydrolysis without the need for pretreatment. In contrast, soybean straw retains more than 25% lignin even after pretreatment (Table 1A), and therefore the combined enzyme approach was not applied in the current study. This decision was based on the increased formation of lignin-derived degradation products and other inhibitory compounds generated during pretreatment, which could negatively affect enzymatic hydrolysis [12,13].

The lignin-free cellulose powder, Solka-Floc, was used as the positive control, while raw, untreated soybean straw served as the negative control. As expected, the highest sugar yields were obtained with Solka-Floc, achieving 83.5% conversion of cellulose to glucose (36.74 g/L) and 62.6% conversion of hemicellulose to xylose (6.89 g/L). In contrast, the raw soybean straw samples exhibited markedly lower conversion efficiencies, yielding only 21.6% glucose release (3.23 g/L) and 16.4% xylose release (1.17 g/L), respectively (Fig. 2). Compared to control tests, the NaOH-treated straw achieved 66.8% cellulose conversion (14.59 g/L glucose) and 45.7% hemicellulose conversion (3.72 g/L xylose). These values represent 14.5% and 7.6% higher yields, respectively, than those obtained from the H_2O_2-treated samples, demonstrating the superior effectiveness of alkaline pretreatment in disrupting biomass structure and enhancing enzymatic saccharification efficiency. It is worth noting that alkaline pretreatment with NaOH is often more effective than H_2O_2 oxidative pretreatment for improving saccharification because it targets lignin and ester linkages in plant cell walls more aggressively, leading to deeper structural deconstruction. NaOH selectively saponifies ester bonds between lignin and hemicellulose, disrupts lignin-carbohydrate complexes (LCCs), and causes lignin swelling and partial solubilization, which together increase biomass porosity and expose a greater fraction of accessible cellulose microfibrils [33,34]. This enhanced cell-wall deconstruction substantially reduces crystallinity and increases the surface area available for enzyme adsorption; two key factors strongly correlated with glucose release efficiency [35]. However, H_2O_2 pretreatment relies primarily on oxidative delignification through the generation of hydroxyl radicals. While effective at removing lignin, it is less efficient at cleaving ester linkages in lignin–carbohydrate complexes and often results in limited disruption of cellulose crystalline regions, thereby restricting enzyme accessibility [12]. Similar trends were observed in our previous

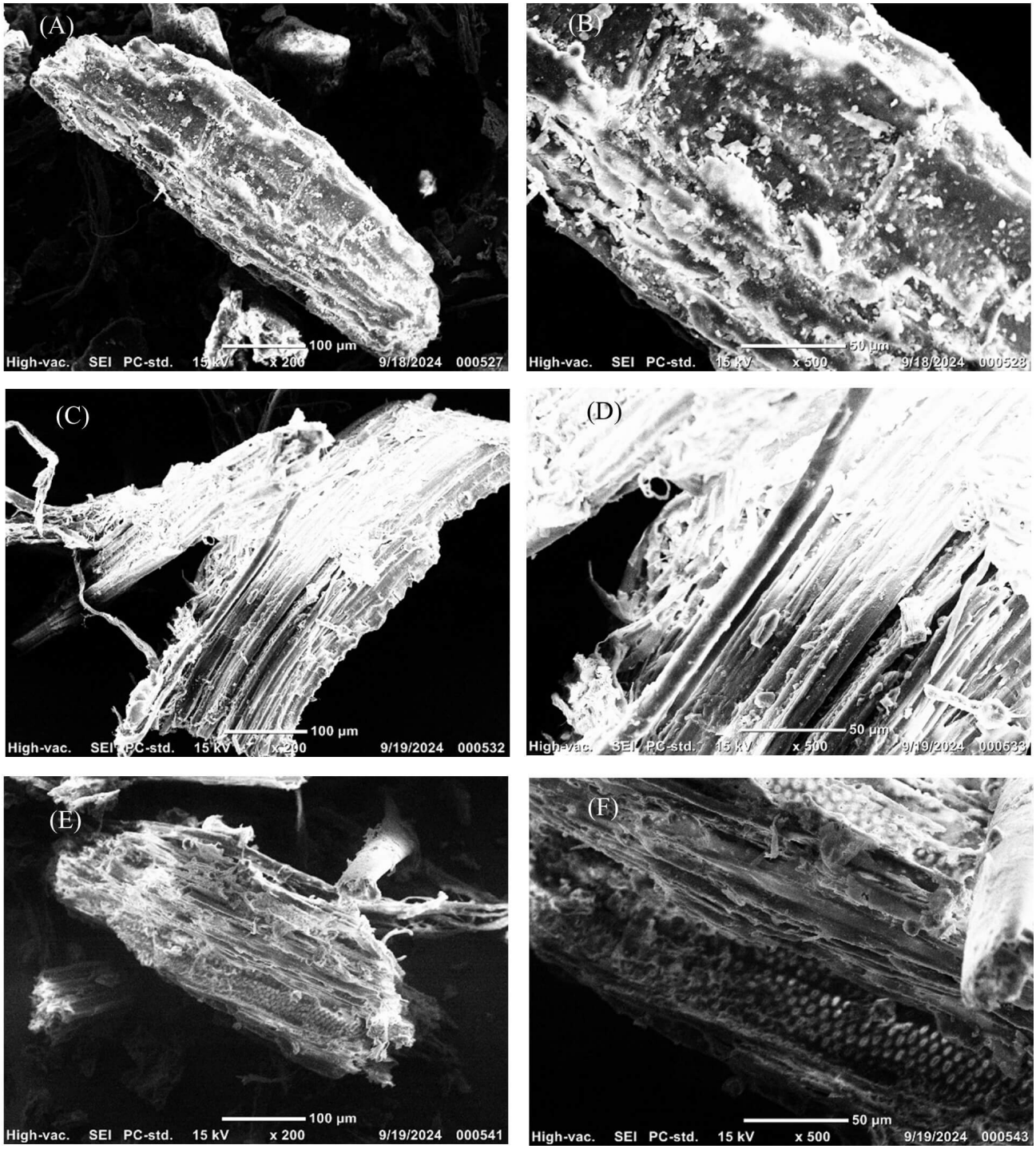

Fig. 1. Morphological structure and surface characteristics of untreated and pretreated soybean straw. Scanning electron microscopy (SEM) images of soybean straw: (A,B) untreated raw sample, (C,D) NaOH-pretreated, and (E,F) H_2O_2-pretreated samples following hydrothermal treatment at 121 °C for 60 min. Images were captured at 200× and 500× magnifications, with scale bars and magnification levels indicated below each image. (A,C,E) Scale bar: 100 µm; (B,D,F) Scale bar: 50 µm.

alkaline pretreatment study of soybean straw, where NaOH treatment significantly enhanced fiber swelling and reduced biomass recalcitrance, resulting in higher glucose yields than H_2O_2-treated straw solids at 1% (w/v glucan) hydrolysis in the presence of 25 mg enzyme protein/g glucan (57% vs. 39%) [11]. In addition, oxidative pretreatment of lignin-rich biomasses can generate inhibitory aromatic byproducts, such as hydroxycinnamic acid derivatives, which negatively impact downstream enzymatic saccharification and microbial fermentation, whereas alkaline pretreatment pro-

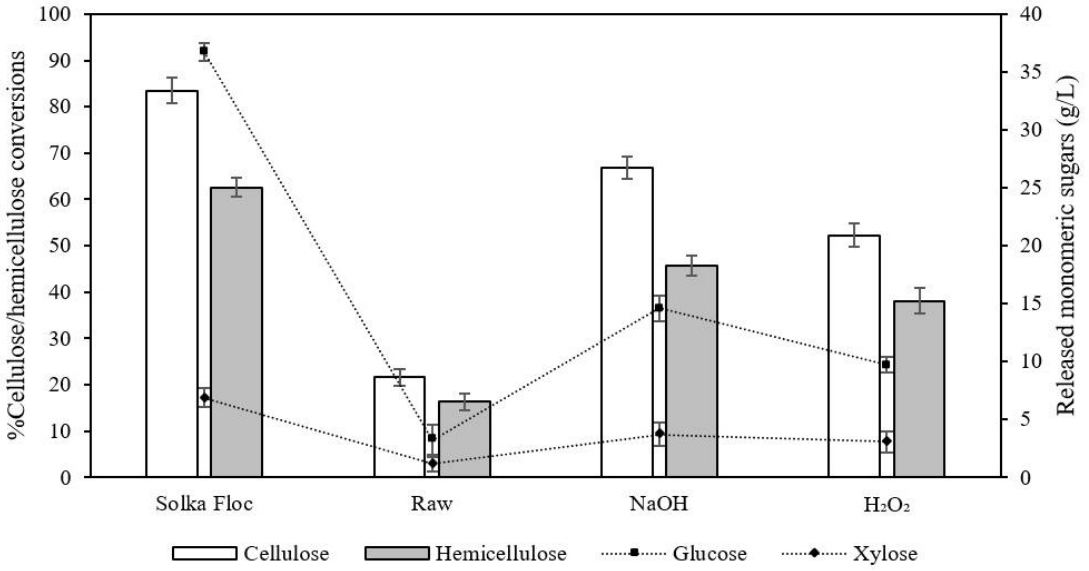

Fig. 2. Enzymatic saccharification of raw and pretreated soybean straw was conducted at a solid loading of 5% (w/v, dry-weight basis). Hydrolysis was performed using Cellic CTec2 at a dosage of 50 mg enzyme protein/g cellulose. Reactions were carried out at 50 °C for 72 h under micro-anaerobic conditions with agitation at 150 rpm in a 5% CO_2 shaking incubator. Following hydrolysis, aliquots were collected, filtered, and analyzed for released monomeric sugars. All experiments were conducted in triplicate, and results are reported as mean values with corresponding 95% confidence intervals.

duced fewer fermentation inhibitors [33]. Our results in the current work align with the broader literature showing that NaOH-treated agricultural residues, including corn stover, wheat straw, and soybean straw, exhibit markedly higher enzymatic hydrolysis yields than those subjected to oxidative pretreatment [36].

A quantitative comparison of compositional changes and hydrolysis performance further supports the mechanistic interpretation. NaOH pretreatment reduced lignin content from 26.9% (raw) to 25.2%, whereas H_2O_2 pretreatment resulted in a relative lignin content of 29.8% (Table 1A). Although the absolute lignin removal was modest under moderate-severity conditions, even small differences in lignin content and structure can significantly influence enzyme accessibility due to non-productive enzyme adsorption onto lignin surfaces. The higher cellulose enrichment (39.7%) and lower residual lignin proportion in NaOH-pretreated solids correlated with a 14.5% higher cellulose-to-glucose conversion compared to H_2O_2-treated samples. This compositional–conversion relationship supports the hypothesis that lignin accessibility and structural modification, rather than total phenolic concentration alone, are key determinants of saccharification efficiency.

3.4 Lactic Acid Fermentation of Soybean Straw Hydrolysates

One of the central bottlenecks in lignocellulosic bioconversion is the poor utilization of pentose sugars, particularly xylose, during microbial fermentation. Most conventional lactic acid-producing microorganisms, such as *Lactobacillus delbrueckii*, *Lactobacillus acidophilus*, and *Lactococcus lactis*, preferentially metabolize hexose sugars and exhibit little to no capacity for fermenting pentoses to lactic acid. This metabolic limitation has hindered efficient valorization of agricultural residues enriched in hemicellulose-derived sugars. To overcome this challenge, extensive metabolic engineering efforts have produced recombinant strains of *Escherichia coli*, *Saccharomyces cerevisiae*, and *Bacillus coagulans* capable of co-utilizing hexoses and pentoses for improved lactic acid production. To enable the simultaneous utilization of both pentose and hexose sugars present in soybean straw hydrolysates, this study used a recombinant *Lactiplantibacillus pentosus* strain procured from the American Type Culture Collection. *L. pentosus* is well suited for mixed-sugar bioconversion because it naturally harbors the enzymatic machinery necessary to metabolize C5 sugars, a capability absent in many traditional homofermentative lactic acid bacteria. Briefly, *L. pentosus* efficiently metabolizes glucose via the classical Embden–Meyerhof–Parnas (EMP) pathway. Glucose is transported into the cell primarily through phosphoenolpyruvate-dependent phosphotransferase systems (PTS), where it is phosphorylated to glucose-6-phosphate and subsequently metabolized through glycolysis to pyruvate. Under micro-anaerobic conditions, pyruvate is reduced to L-lactic acid by NADH-dependent lactate dehydrogenase (LDH), thereby regenerating NAD^+ required for continued glycolytic flux [37,38,39]. When

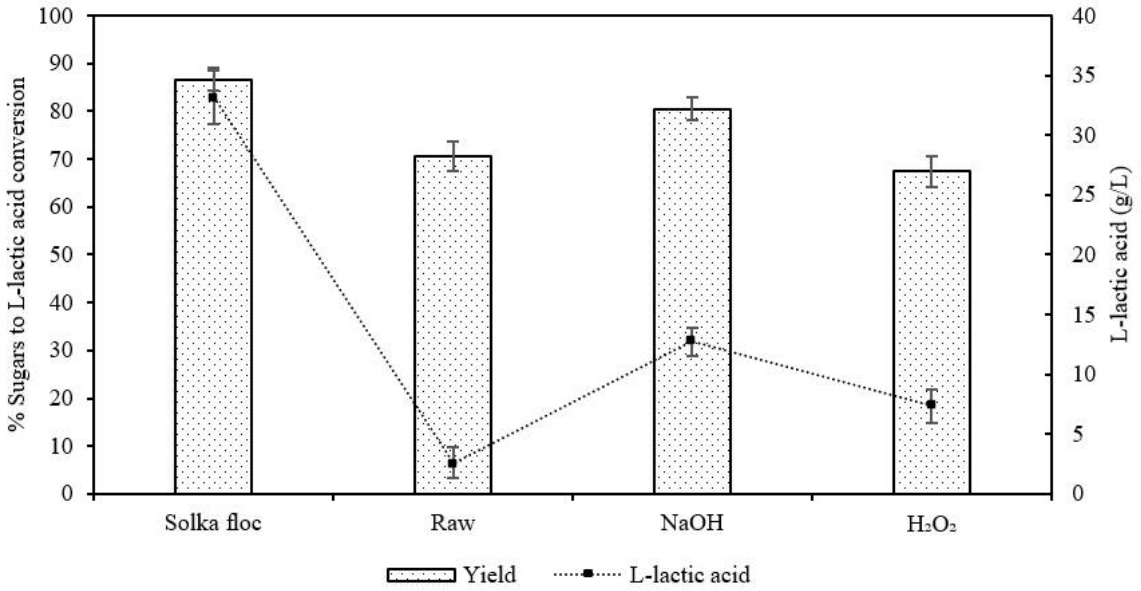

Fig. 3. Conversion of total fermentable sugars to L-lactic acid by *Lactiplantibacillus pentosus* using hydrolysates derived from raw and pretreated soybean straw. Fermentations were performed in duplicate at 35 °C for 36 h under micro-anaerobic conditions with agitation at 150 rpm in a 5% CO_2 shaking incubator. Data represent the mean of duplicate independent experiments, and results are presented as mean values with corresponding 95% confidence intervals.

metabolizing xylose, *L. pentosus* utilizes the phosphoketolase pathway (PKP), where xylose is converted to xylulose-5-phosphate and cleaved by phosphoketolase into glyceraldehyde-3-phosphate and acetyl-phosphate. Xylose uptake occurs via high-affinity permeases, after which intracellular xylose is converted to xylulose by xylose isomerase (XI) and subsequently phosphorylated by xylulokinase (XK) to form xylulose-5-phosphate. This key intermediate is then cleaved by xylulose-5-phosphate phosphoketolase, generating acetyl-phosphate and glyceraldehyde-3-phosphate [40,41]. These products are further metabolized to lactic acid and acetic acid or ethanol under more reducing fermentation conditions, facilitating efficient pentose catabolism from lignocellulosic substrates. This metabolic versatility underpins the strain's suitability for fermenting pentose-rich hydrolysates derived from soybean straw.

Subsequent lactic acid fermentation of soybean straw hydrolysates was conducted under micro-anaerobic conditions in a 5% CO_2 shaking incubator for 36 h. Total sugar consumption (glucose + xylose) and conversion to lactic acid are plotted in Fig. 3. In all cases, fermentable sugars were completely consumed within 36 h. The highest lactic acid concentration was obtained from the lignin-free Solka-Floc hydrolysate, reaching 33.18 g/L, corresponding to 86.63% of the maximum theoretical yield. Among the pretreated biomass samples, NaOH-treated soybean straw hydrolysates achieved a lactic acid concentration of 12.7 g/L, equivalent to 80.5% of the theoretical yield. In contrast, lower lactic acid yields were observed for H_2O_2-treated (7.3 g/L, 67.4%) and raw soybean straw hydrolysates (2.6 g/L, 70.6%). Notably, NaOH-treated samples exhibited not only higher final lactic acid concentrations, but also superior sugar-to-lactic acid conversion efficiencies compared to H_2O_2-treated samples, suggesting differences in hydrolysate quality and residual inhibitor profiles between the pretreatment and hydrolysis strategies. Despite post-pretreatment washing, H_2O_2-pretreated soybean straw solids may retain or subsequently release inhibitory compounds during enzymatic hydrolysis due to incomplete disruption of LCCs and the nature of oxidative delignification. Oxidative pretreatment primarily modifies lignin through partial oxidation rather than extensive cleavage of ester and ether linkages that anchor lignin to polysaccharides. As a result, phenolic fragments and oxidized lignin derivatives can remain covalently or physically associated with the solid matrix. During enzymatic hydrolysis, progressive enzymatic disruption of the cell wall structure can mobilize these retained compounds, leading to the delayed release of phenolic inhibitors into the hydrolysate. In addition, residual oxidized lignin fragments generated during H_2O_2 pretreatment may persist within biomass pores and are not readily removed by water washing alone. Their subsequent liberation during hydrolysis can inhibit both cellulolytic enzymes and lactic acid bacteria, thereby reducing sugar-to-lactic acid conversion efficiency. In contrast, NaOH pretreatment more effectively cleaves LCC ester linkages and solubilizes lignin, enabling more thorough removal of inhibitory compounds prior to fermentation and resulting in hydrolysates with improved fermentability [14,16].

Table 3. Comparative performance of *Lactiplantibacillus pentosus* in lactic acid fermentation using hydrolysates derived from soybean straw and other agricultural residues.

Feedstock	Pretreatment	Lactic acid (g/L)	Productivity (g/L/h)	Yield (g LA/g sugars)	Notes	Ref.
Solka-Floc	No pretreatment; 80% cellulose + 20% hemicellulose	33.18	0.92	0.87	Theoretical benchmark	Current work
Soybean straw	No chemical pretreatment	2.62	0.073	0.71	-	Current work
Soybean straw	1% (v/v) NaOH, 121 °C, 60 min; washed prior to hydrolysis	12.72	0.35	0.81	Highest fermentability	Current work
Soybean straw	1% (v/v) H_2O_2, 121 °C, 60 min; washed prior to hydrolysis	7.34	0.20	0.67	Possible residual phenolics	Current work
Sugarcane bagasse (SCB)	1% H_2SO_4, 121 °C, 27 min + addition of 4% (w/v) NaOH 121 °C, 30 min	42.4	1.02	0.71	Hemicellulose hydrolysate from sugarcane bagasse, batch system	[42]
Sugarcane bagasse	1% H_2SO_4, 121 °C, 27 min + addition of 4% (w/v) NaOH 121 °C, 30 min	64.8	1.01	0.93	Hemicellulose hydrolysate + partially delignified cellulignin, SSCF*	[42]
Sugarcane bagasse	Two step steam-exploded SCB total hydrolysate	28.9	0.83	0.78	2 L bioreactor	[43]
Vine-trimming wastes	1–3% (w/v) or 1–2% (w/v) H_2SO_4 at 130 °C, 15 min; hydrolyzed vine-trimming hemicellulose liquor	60–65	1.0–1.2	0.90–0.93	Continuous fermentation in high xylose content	[44]
Wheat straw	Wet-oxidation with Na_2CO_3 at 195 °C, 10–15 min; hemicellulose-rich hydrolysate	9–10	-	0.88	Co-cultivation of *L. pentosus* and *L. brevis* enabled up to 95% pentose sugar utilization	[45]

*SSCF: Saccharification and co-fermentation with an initial enzymatic hydrolysis.

3.5 Limitations

The findings presented here do not introduce new pretreatment chemistries or fermentation pathways; rather, they provide a feedstock-specific validation of established lignocellulosic bioconversion strategies applied to soybean straw. By maintaining consistent hydrolysis and fermentation parameters, this study enables direct comparison of pretreatment effectiveness and inhibitor-related impacts on downstream fermentability. Such benchmarking is critical for identifying realistic performance expectations and guiding process optimization for underutilized agricultural residues. In addition, it should be noted that while total phenolic concentrations were quantified, individual inhibitory species (e.g., vanillin, syringaldehyde, furfural, or hydroxymethylfurfural) were not specifically profiled in this study. In addition, a complete carbon mass balance tracking carbohydrate solubilization, degradation, and product formation was not performed. Therefore, although correlations between lignin content, sugar release, and fermentation performance are supported by quantitative trends, further mechanistic resolution would benefit from detailed inhibitor speciation analysis and full mass balance closure in future investigations.

A wide range of feedstocks, pretreatment strategies, and their subsequent fermentation performances have been investigated in previous works (Table 1A and Table 1B; Table 3, Ref. [42,43,44,45]) to improve lactic acid production. Collectively, these comparisons position soybean straw as a competitive yet underutilized feedstock for lactic acid bioproduction. Further performance gains are expected through (i) optimization of alkaline pretreatment severity to maximize delignification while minimizing carbohydrate loss, (ii) incorporation of mild detoxification strategies (e.g., overliming, activated carbon, or biological detoxification) to reduce residual phenolics, (iii) increased solids loading to enhance volumetric productivity, and (iv) refinement of enzymatic hydrolysis using tailored enzyme cocktails to improve pentose release. In parallel, adaptive

evolution or metabolic engineering of *L. pentosus* to enhance inhibitor tolerance and acid productivity could further improve process robustness. Together, these strategies provide a clear pathway for advancing soybean straw as a sustainable feedstock for lactic acid and downstream PLA production within an integrated biorefinery framework.

4. Conclusions

This study provides a comparative evaluation of alkaline and oxidative pretreatment strategies for soybean straw and benchmarks their downstream saccharification and lactic acid fermentation performance under controlled conditions. As summarized in Table 3, NaOH pretreatment followed by enzymatic hydrolysis produced the most favorable fermentation outcomes among soybean straw-derived substrates, yielding 12.72 g/L lactic acid with a productivity of 0.35 g/L/h and a sugar-to-lactic acid yield of 0.81 g/g. These values approach the theoretical benchmark achieved with Solka-Floc cellulose (0.87 g/g sugars) and fall within the yield range reported for well-established agricultural residues such as corn stover and wheat straw, which typically achieve 0.8–0.88 g/g sugars following effective alkaline pretreatment or wet-oxidized hemicellulose-rich hydrolysate [22,23,25]. When normalized to raw biomass input, NaOH-pretreated soybean straw achieved approximately 0.35 g/L/h, comparable to sugarcane bagasse (0.4 g/L/h and 0.8 g/g) and within the lower range reported for corn stover (0.7–0.9 g/L/h, and 0.8 g/g). This performance is comparable to reported values for several established agricultural residues, indicating that soybean straw can achieve competitive yields when appropriate pretreatment strategies are applied [27,36].

In contrast, H_2O_2-pretreated soybean straw hydrolysates resulted in lower lactic acid titers (7.34 g/L), productivities (0.2 g/L/h), and yields (0.67 g/g sugars), despite extensive post-pretreatment washing. This reduced performance is consistent with prior reports indicating that oxidative pretreatments can generate or retain phenolic compounds and oxidized lignin fragments that are not readily removed by water washing and may be released during enzymatic hydrolysis, thereby inhibiting both cellulolytic enzymes and lactic acid bacteria [46]. It is worth noting that lignin and associated acetylated and phenolic compounds form complex, amorphous polyphenolic networks that impart structural rigidity to plant cell walls and serve as an intrinsic defense mechanism against microbial and enzymatic attack [28]. During pretreatment and enzymatic hydrolysis, partial depolymerization or modification of lignin can generate soluble phenolic acids, aldehydes, and oxidized lignin fragments that persist in hydrolysates and act as potent inhibitors. These compounds are known to adsorb cellulolytic enzymes non-productively, reduce catalytic efficiency, and interfere with microbial metabolism by disrupting membrane integrity, redox balance, and key enzymatic pathways. Consequently, incomplete delignification or ineffective removal of lignin-derived inhibitors can significantly compromise both saccharification efficiency and downstream lactic acid fermentation. As previously demonstrated by Ladeira Ázar et al. [12], even modest differences in lignin content can result in disproportionately large impacts on cellulose conversion efficiency. In their study, a reduction in lignin content from 22.3% to 20.6% in sugarcane bagasse increased enzymatic hydrolysis yields from 68.3% to 89.5%, highlighting the high sensitivity of cellulose accessibility to lignin abundance and structure. These findings are directly relevant to the present work, where differences in pretreatment effectiveness influenced both sugar release and lactic acid fermentation performance. Compared with high-performing systems based on dilute acid and alkaline-pretreated sugarcane bagasse hydrolysates, which report lactic acid titers of 42–65 g/L, productivities exceeding 1.0 g/L/h, and sugar-based yields above 0.7 g/g [42], the soybean straw systems evaluated here are primarily limited by lower fermentable sugar concentrations rather than intrinsic limitations of *L. pentosus*. Importantly, once inhibitory effects are minimized, *L. pentosus* efficiently converts both hexose and pentose sugars from soybean straw hydrolysates at yields comparable to those obtained with other agricultural residues, including steam-exploded sugarcane bagasse and alkaline-treated cereal straws [11,22,43]. Similar trends have been reported for other lignin-rich feedstocks such as barley straw, where partial delignification improves fermentation performance only when combined with effective inhibitor control [47,48].

Availability of Data and Materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

DK, investigation experimental, data curation, formal analysis, writing original draft, conceptualization, funding acquisition, project administration, supervision, and validation; EC, investigation experimental, data curation, formal analysis; EK, investigation experimental, data curation, formal analysis; SK, methodology, supervision, formal analysis. All authors contributed to editorial changes in the manuscript. Additionally, all authors have reviewed and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Under US federal and Maryland state agricultural regulations, wild-collection/harvesting permits apply only to wild or protected flora on public lands; wild-collection permits are not required for privately cultivated agricultural

materials such as soybeans, and no specific ethical approval was needed. This study involved soybean (*Glycine max*) straw obtained from Peace and Plenty Farm (Rocky Ridge, MD, USA). The plants were grown and harvested in 2019. Agricultural crop residue was provided directly with permission from the property owner at the farm. The plant material was confirmed as cultivated soybean (*Glycine max*) based on standard agricultural characteristics and farm origin records.

Acknowledgment

The authors gratefully acknowledge Peace and Plenty Farm for generously providing the soybean straw samples used in this study. We also thank the Center for Coastal & Watershed Studies at Hood College for their valuable support in coordinating and conducting the scanning electron microscopy (SEM) analyses. The Graphical Abstract was created using FigureLabs (Web version, Cactus Communications Inc., Princeton, NJ, USA; available at https://figurelabs.com).

Funding

This research was supported by the Maryland E-Nnovation Initiative Fund (MEIF) administered by the Maryland Department of Commerce, the 2024 Maryland Soybean Board Research Program (Project No. 2024-15), and the 2025 Board of Associates McCardell Professional Development Grant from Hood College, which provided additional equipment and research support.

Conflicts of Interest

The authors declare no conflicts of interest. Although SK is affiliated with Mthera Pharma Co., Ltd., the judgments in data interpretation and writing were not influenced by this relationship.

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