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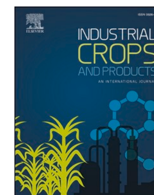

Formulating the racemic lactic acids-free nitrogen ingredients from plant proteins for cellulosic chiral lactic acid fermentation

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A B S T R A C T

Nitrogen additive is an important factor for chiral lactic acid fermentation for low-cost and high optical purity of final chiral lactic acid product. This study tried to formulate a low-cost and racemic lactic acids-free nitrogen ingredient for lactic acid bacterium strain using wheat straw as carbon source. Eight available plant proteins were evaluated as potential nitrogen additives for chiral lactic acid fermentation by proteinase catalyzed hydrolysis to release the free amino acids and short peptides. Soybean meal was selected as the most promising nitrogen additive by its high protein content and lactic acids free composition. The plant proteins derived nitrogen additives was found that the inherent deficiency of amino acids in plant proteins led to the insufficient lactic acid fermentation. A minimal yeast extract was added to the plant protein hydrolysates to compensate the amino acid deficiency, and the use of the combinational nitrogen ingredients resulted in the final cellulosic L-lactic acid titer reached 122.9 ± 0.4 g/L with the yield of 88.1% and the optical purity of 99.7% using wheat straw feedstock. Comparing with yeast extract as nitrogen additive, the combinational ingredients substituted 75% of yeast extract with 56.7% cost reduction. This study provides a cost-effective solution of nitrogen ingredients for cellulosic chiral lactic acid production in industrial biorefinery process.

1. Introduction

Lactic acid (2-hydroxypropanoic acid) has versatile applications in food, cosmetic, pharmaceutical, and other industries, presents in two enantiomeric forms of L (+) lactic acid and D (-) lactic acid (Rawoof et al., 2020; Yankov, 2022). The market demand of lactic acid presents an annual growth rate of 10%, which is mainly attributed to the utilization of lactic acid for the synthesis of polylactic acid (PLA) (Alexandri et al., 2019; Lian et al., 2023). Optically pure lactic acid is more valuable than racemic D/L-lactic acid, because the quality of the monomers lactic acid is a crucial parameter for controlling the properties of the resulting polylactic acid (PLA) product (Huang et al., 2021).

Commercial high enantiomerically pure lactic acid is produced by fermentation of starch or refined sugars (Ahmad et al., 2020). Lignocellulosic biomass, an expensive, widely available, and renewable feedstock from non-food sources, is considered to be the most attractive carbon source for future large-scale industrial production of chiral lactic acid (Cubase-Cano et al., 2018; Zhang et al., 2018). Cost-effective production of chiral lactic acid from lignocellulose feedstock should achieve a high optical purity in final product and the low cost in processing (Augustiniene et al., 2021; Tian et al., 2021).

Lactic acid bacteria require various amino acids and nutrient factors from complex nitrogen additives for their growth and lactic acid accumulation (Alves de Oliveira et al., 2018; Ren et al., 2022). Yeast extract and peptone are commonly used to provide the nutrients in the fermentation medium (de la Torre et al., 2018; Michalczyk et al., 2021), but the use of expensive yeast extract and peptone significantly increases the fermentation cost (Alves de Oliveira et al., 2018; Lian at al., 2023; Tian et al., 2019). A large number of studies have been conducted to find the cheap alternative complex nitrogen for lactic acid production, but these nitrogen additives generally contain minor racemic lactic acids and the effect of the racemic lactic acids on the chirality of final product were not fully understood in the available studies. The alternative nitrogen additives should be racemic lactic acids-free to achieve high optical purity of lactic acid product, amino acids rich to ensure the well growth of lactic acid bacteria, and price affordable for industrial applications. A systematic investigation on the relationship between chiral purity in lactic acid product and racemic lactic acid content in

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Table 1
Characterizations of common complex nitrogen sources and plant proteins.

Nitrogen additives	Grade	Proteins (%, DM)	L-Lactic acid (mg/g DM)	D-Lactic acid (mg/g DM)	Sources
(i) Complex nitrogen					
Yeast extract	Reagent	65.8 ± 0.1	1.8 ± 0.1	1.5 ± 0.2	Oxoid, Basingstoke, UK
Yeast extract	Industrial use	76.9 ± 0.9	0.9 ± 0.1	1.2 ± 0.1	Angelyeast, Yichang, Hubei, China
Peptone	Reagent	76.0 ± 1.4	1.1 ± 0.1	0.9 ± 0.2	Oxoid, Basingstoke, UK
(ii) Alternative nitrogen additive with high racemic lactic acid contents					
Corn Steep Liquor	Industrial use	45.1 ± 0.5	85.0 ± 1.6	130.2 ± 1.8	Angelyeast, Yichang, Hubei, China
(iii) Alternative plant proteins as alternative nitrogen					
Soybean meal	Animal feed	46.3 ± 0.7	1.7 ± 0.1	0.7 ± 0.1	Cofco, Lianyungang, Jiangsu, China
Cottonseed meal	Animal feed	45.0 ± 0.9	1.5 ± 0.1	0.5 ± 0.1	Jiahui BioTech, Haian, Jiangsu, China
Peanut meal	Animal feed	40.4 ± 0.8	0.5 ± 0.1	1.2 ± 0.1	Huamao Feeds, Weifang, Shandong, China
Canola meal	Animal feed	31.5 ± 0.6	0.2 ± 0.1	\	Huamao Feeds, Weifang, Shandong, China
Sesame meal	Animal feed	37.7 ± 0.4	0.2 ± 0.1	\	Huamao Feeds, Weifang, Shandong, China
Corn germ meal	Animal feed	16.1 ± 0.8	7.9 ± 0.2	10.7 ± 0.4	Huamao Feeds, Weifang, Shandong, China
Palm kernel meal	Animal feed	21.4 ± 0.3	0.2 ± 0.1	\	Huamao Feeds, Weifang, Shandong, China
Sunflower meal	Animal feed	11.8 ± 0.4	6.1 ± 0.3	1.6 ± 0.2	Huamao Feeds, Weifang, Shandong, China

alternative nitrogen additives is an important step towards the commercial production of chiral lactic acid.

Corn steep liquor is the cheap alternative nitrogen additive used in industrial fermentations, but it contains high amount of racemic lactic acids and not suitable for chiral lactic acid fermentation (Cao et al., 2020; Gao and Yuan, 2011). Plant proteins such as soybean meal (Liang et al., 2020; Tian et al., 2019), cottonseed meal (Bai et al., 2016), rapeseed meal (Brock et al., 2019), peanut meal (Wang et al., 2011) are also used as cheap alternative proteins, but the racemic lactic acid contents in these plant proteins are not been fully acknowledged, and the amino acids cocktail seems not sufficient for lactic acid fermentation due to the inherent deficiency of amino acids in plant proteins (Krull et al., 2020; Tian et al., 2020; Zhang et al., 2022, 2015). To ferment high performance chiral lactic acid product in industrial scale, formulating the combinational nitrogen ingredients with low cost and racemic lactic acid-free is very important.

In this study, eight common alternative nitrogen additives were characterized to find the lactic acids-free one with higher protein content. Soybean meal was selected and then enzymatically hydrolyzed as alternative nitrogen additives for cellulosic chiral lactic acid fermentation by the engineered lactic acid bacterium strain. The key parameters of enzymatic hydrolysis were optimized to improve the nitrogen yield and fermentation efficiency. A new combinational nitrogen ingredient was designed to compensate the nutritional deficiency of soybean meal and satisfy the minimum nutritional requirements of lactic acid bacteria. This study provides a cost-effective and racemic lactic0 acids-free nitrogen additives solution for cellulosic chiral lactic acid production in industrial biorefinery process, successfully achieving the valorization of agricultural residues.

2. Materials and methods

2.1. Feedstock, enzymes, and reagents

The wheat straw was pretreated according to our previously established dry acid pretreatment protocols (Liu et al., 2018) on pilot scale in Shanxi province, China, and provided by Cathay Industrial Biotech Inc. (Shanghai, China) in August 2023. The contents of main compositions of the pretreated wheat straw were determined by two-step acid hydrolysis method (Sluiter et al., 2008, 2012), including 310.7 ± 5.2 mg/g of cellulose, 4.4 ± 1.3 mg/g of xylan, 30.83 mg ± 0.57 mg/g of glucose, and 136.70 mg ± 1.98 mg/g of xylose based on dry matter.

Commercial cellulase Cellic CTec 3HS was purchased from Novozymes (Beijing, China) with the protein content of 90.1 mg/mL. The α-amylase HTAA was purchased from Genencor (Jiangsu, China) with the enzyme activity of 103,900 U/mL according to maker's instruction. The alkaline protease was purchased from Vland Biotech Inc. (Shandong, China) with the enzyme activity of 200,000 U/mL. Other reagents such as $(NH_4)_2C_6H_6O_7$, $(NH_4)_2SO_4$, $MnSO_4{\cdot}H_2O$ were purchased from Sinopharm Chemical Reagent Co. (Shanghai, China). The sources of yeast extract and other plant proteins were showed in Table 1.

2.2. Strains and medium

The L-lactic acid producing strain was *Pediococcus acidilactici* ZY271 (CGMCC 13611), which was derived from the *P. acidilactici* TY112 (CGMCC 8664) (Qiu et al., 2018). *P. acidilactici* ZY271 can simultaneously and rate-coordinately convert lignocellulose-derived glucose and xylose to lactic acid (He et al., 2022). *P. acidilactici* ZY271 was activated in 20 mL of simplified Man-Rogosa-Sharp (MRS) medium including 20 g/L of glucose, 10 g/L of peptone, 10 g/L of yeast extract, 5 g/L of CH_3COONa, 2 g/L of $(NH_4)_2C_6H_6O_7$, 2.6 g/L of $K_2HPO_4{\cdot}3\ H_2O$, 0.58 g/L of $MgSO_4{\cdot}7\ H_2O$, and 0.25 g/L of $MnSO_4{\cdot}H_2O$ at 42 °C, 150 rpm for 6 h. The broth was inoculated to 100 mL of fresh simplified MRS medium at the ratio of 10% (v/v) with adding 1.2 g of $CaCO_3$ for neutralization and 1% (v/v) of α-amylase to prevent flocculation (Liu et al., 2015) and cultured for 6 h as the seed. The general nutrients for cellulosic lactic acid fermentation contained 10 g/L of peptone, 15 g/L of yeast extract, 2 g/L of $(NH_4)_2C_6H_6O_7$, and 0.25 g/L of $MgSO_4{\cdot}7\ H_2O$.

The biodetoxification strain was *Paecilomyces variotii* FN89 (CGMCC 17665), which can biodegrade various lignocellulose-derived inhibitors in both pretreated solid biomass and hydrolysate (Han et al., 2023; Zhang et al., 2021). *P. variotii* FN89 was preserved on potato dextrose agar (PDA) plate. The spores of *P. variotii* FN89 were collected by washing using 0.05% (w/w) Tween 80 solution. The spores solution of *P. variotii* FN89 can be directly spread on pretreated wheat straw biomass for solid state biodetoxification (Zhang et al., 2021), or inoculated to synthetic medium at the ratio of 1% (v/v) to prepare as seed for submerged liquid biodetoxification of wheat straw hydrolysate as well (Han et al., 2023).

2.3. Enzymatic hydrolysis of plant proteins

Plant protein (5 g, dry weight) such as cottonseed meal, soybean meal, etc., was mixed with deionized water in 250 mL flask at the solid-liquid ratio of 1:4, 1:6, 1:8, 1:10, and 1:12. The pH value of the mixture was adjusted to 10.0 by adding 5 M NaOH solution. The alkaline protease powder was added at the dosage of 0.05 g/g plant protein. The

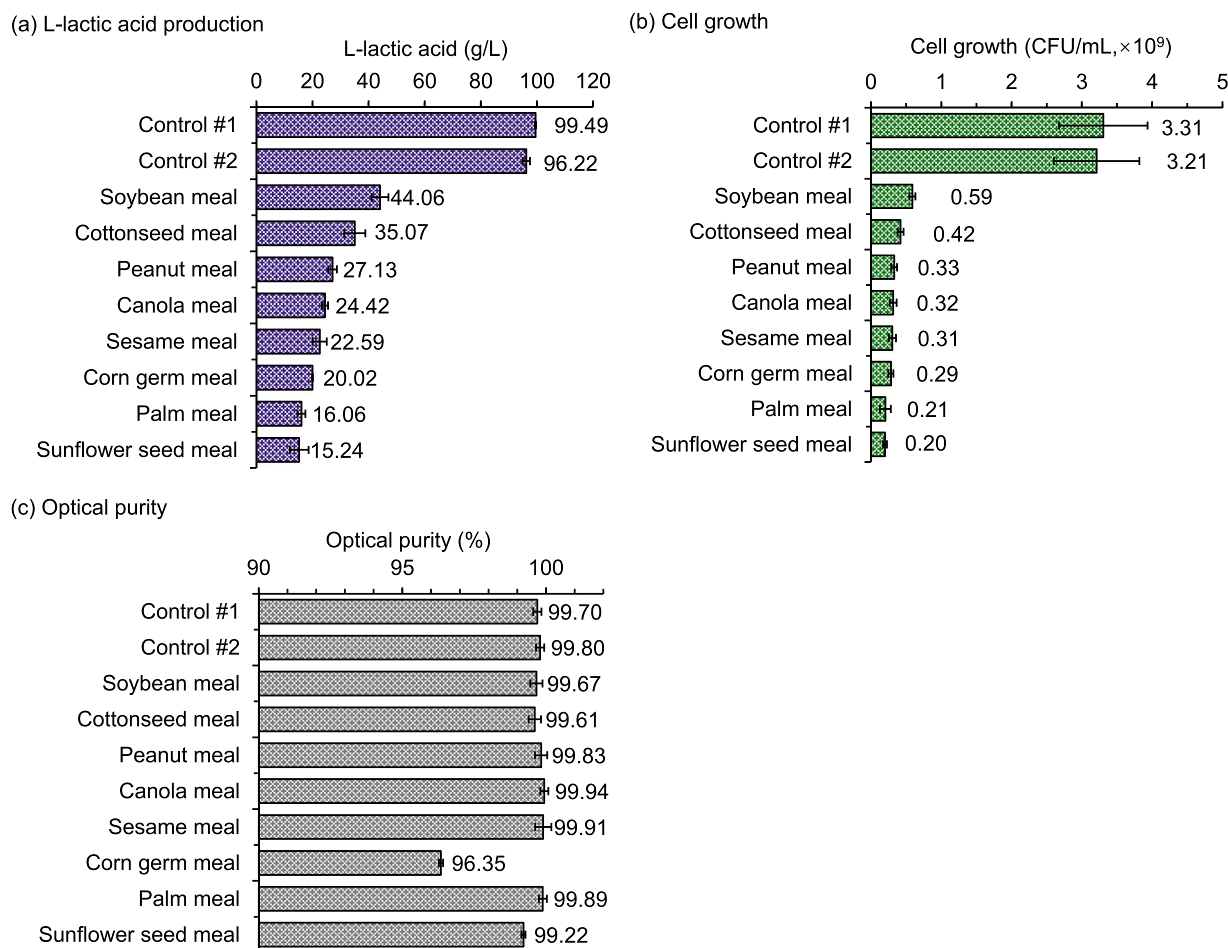

Fig. 1. Evaluation of plant proteins as alternative complex nitrogen additives for cellulosic lactic acid fermentation. (a) L-lactic acid titer; (b) cell viability for 24 h of fermentation; (c) optical purity of the lactic acid product. The enzymatic hydrolysis of plant protein was at the solid-liquid ratio of 1:4, 60°C, 150 rpm for 24 h. The dosage of alkaline protease was 5% of the mass of crude plant protein (g enzyme/g crude plant protein). The amount of plant protein added for fermentation was 20 g/L. The saccharification of wheat straw was at 25% (w/w) solids loading, 4 mg cellulase protein/g substrate, 50 °C, 150 rpm for 24 h. The fermentation was at 42℃, 150 rpm for 72 h with 10% (v/v) inoculum of *P. acidilactici* ZY271. Control #1 using 15 g/L yeast extract and 10 g/L peptone (all reagent grades) as complex nitrogen additives; Control #2 using 20 g/L yeast extract (industrial grade) as complex nitrogen additives. Each experiment was performed in triplicate. The error bar represents the standard deviation. The error bar represents the standard deviation.

enzymatic hydrolysis was conducted at 60 ℃, 150 rpm for 6–48 h. The alkaline protease was inactivated in a sterilizer at 105 ℃ for 10 min. Then the hydrolysate was stored at 4 ℃ for subsequent use.

2.4. Hydrolysis yield calculations

The hydrolysis yields of soybean meal and cottonseed meal were calculated based on the generation of free glutamic acid, because soybean meal and cottonseed meal were reported to contain abundant glutamic acid, which is a strong lactic acid bacteria growth promotor (Liang et al., 2020; Tanksley et al., 1981). The yield of glutamic acid (%) from plant protein was calculated according to the Eq. (1) as follows:

$$\text{Yield of glutamic acid } (\%) = \frac{C}{V \times M \times W \times 1000} \times 100\% \tag{1}$$

where C (g/L) is the concentration of glutamic acid in hydrolysate; V (mL) is the volume of the hydrolysate; M (g, dry weight) is the mass of plant protein; W (mg/g, dry matter) is the content of glutamic acid in plant protein.

2.5. Biodetoxification, hydrolysis, and lactic acid fermentation

The pH value of pretreated wheat straw was adjusted to 5.0 by adding $CaCO_3$. For the fermentation in flasks, the neutralized pretreated wheat straw was biodetoxified by solid state fermentation in a 15 L bioreactor at 1.0 vvm, 37 ℃ for 96 h. The biodetoxified wheat straw was hydrolyzed in 250 mL flask at 25% (w/w) solids loading, 4 mg cellulase protein/g dry matter, 50 ℃ for 12 h. Then the nutrients were added, and the seed of *P. acidilactici* ZY271 was inoculated at the ratio of 10% (v/w). The lactic acid fermentation in flask was conducted at 42 ℃, 300 rpm for 72 h. $CaCO_3$ was added at the dosage of 0.6 g/g fermentable sugars to maintained the pH value of the hydrolysate at ~5.0.

For the lactic acid fermentation in 3 L bioreactor, the neutralized pretreated wheat straw was first enzymatically hydrolyzed at 30% (w/w) solids loading. The seed of *P. variotii* FN89 was then inoculated to the hydrolysate at the ratio of 10% (v/w). The biodetoxification was conducted by submerged liquid fermentation at 1.0 vvm, 37 ℃, 750 rpm for 36 h. The biodetoxified hydrolysate was used for lactic acid production by simultaneous saccharification and co-fermentation (SSCF). SSCF was conducted at 42 ℃, 300 rpm for 72 h. The fermentation pH was controlled at 5.5 by automatically adding 25% (w/w) $Ca(OH)_2$ slurry.

2.6. Analytical methods

The protein content of plant proteins and free nitrogen content of supernatant were determined by semi-automatic Kjeldahl apparatus

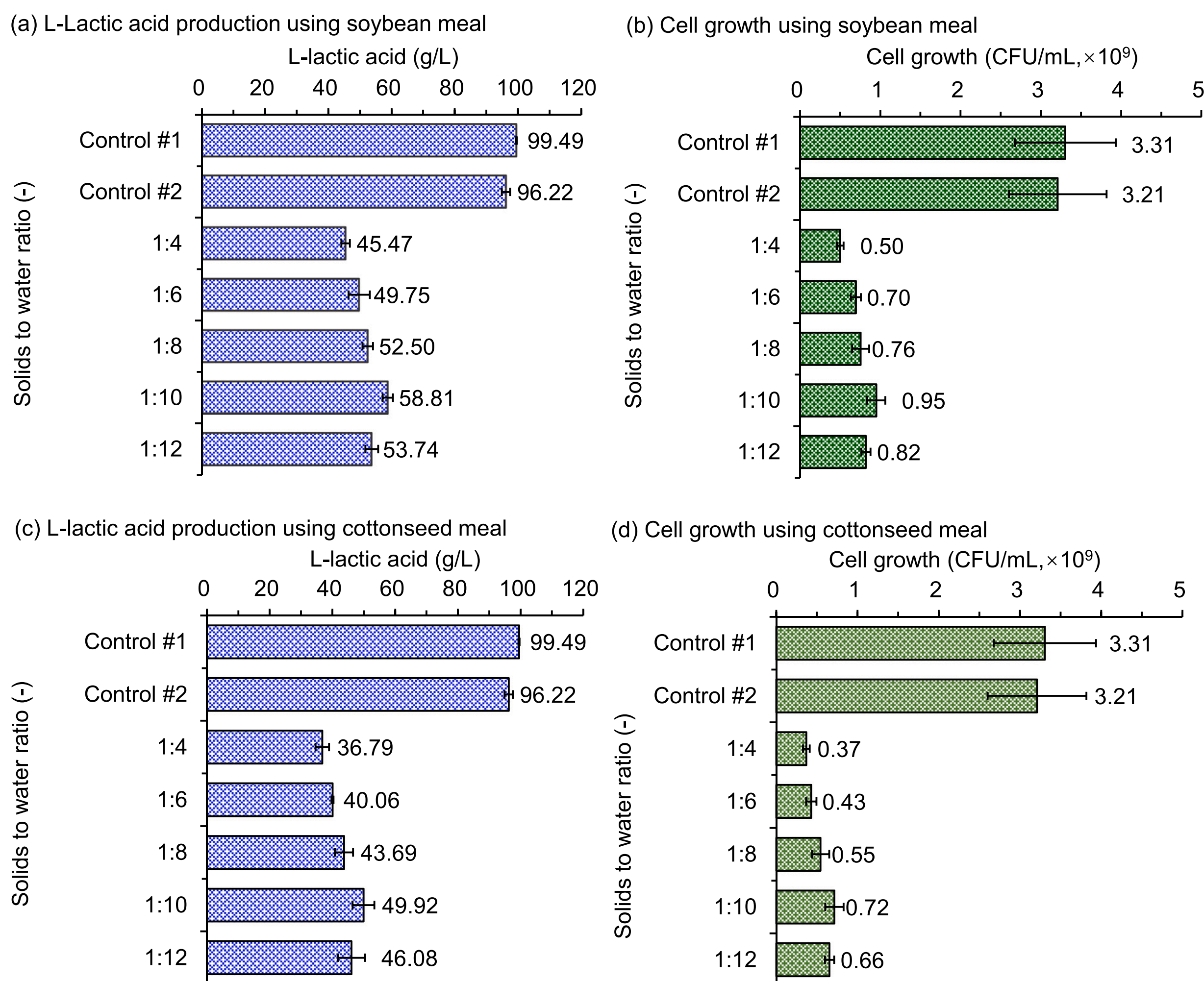

Fig. 2. Plant protein hydrolysate with different solids to water ratio (-) as complex nitrogen additives for cellulosic lactic acid fermentation in flasks. (a), (b) indicate the L-lactic acid titer and cell viability using soybean meal hydrolyzed at different solids to water ratios (-) as complex nitrogen additives; (c), (d) indicate the L-lactic acid titer and cell viability using cottonseed meal hydrolyzed at different solids to water ratios (-) as complex nitrogen additives. The plant protein hydrolysis was at 60°C, 150 rpm for 24 h. The dosage of alkaline protease was 5% of the mass of crude plant protein (g enzyme/g crude plant protein). The amount of plant protein added for fermentation was 20 g/L.

(PeiOu Analysis Instrument, Shanghai, China). Glutamate was detected by SBA-90D biosensor analyzer (Shandong Academy of Sciences, Shandong, China). The amino acid content was determined using Hitachi (Japan) L-8900 Amino Acid Analyzer equipped with 2622#PH ion exchange column. The cell growth in fermentation was determined by counting the colony-forming units (CFU). Glucose, xylose, acetic acid, 5-hydroxymethyfurfual (HMF), furfural, lactic acid was measured by HPLC method. The HPLC system was equipped with LC-20AD pump (Shimazu, Kyoto, Japan), RID-10A detector (Shimazu, Kyoto, Japan), and Aminex HPX-87 H column (300 mm × 7.8 mm, Bio-Rad, Hercules, USA). The column temperature was 65 ℃, the mobile phase was 0.5 mM sulfuric acid with the flow rate of 0.6 mL/min. D-lactic acid was also measured by HPLC equipped with SPD-20A detector (Shimazu, Kyoto, Japan) and MCI GEL CRS10W column (4.6 mm × 50 mm, Mitsubishi, Japan). The wavelength of the UV detector was set at 254 nm. The column temperature was 25 ℃, the mobile phase was 2 mM copper sulfate with the flow rate of 0.5 mL/min.

3. Results and discussion

3.1. Screening the potential plant proteins as alternative nitrogen additive for chiral lactic acid fermentation

Eight common plant proteins were screened as the potential alternative nitrogen additives for providing the amino acids by alkaline proteinase enzyme for cellulosic chiral lactic acid production. Table 1 shows that the protein content of the selected plant proteins varied widely from 11.8% (w/w) to 46.3% (w/w) and the content of mixed lactic acids (both L-lactic acid and D-lactic acid) among the selected plant proteins were very low and similar to these in yeast extract and peptone, except for corn germ meal. The results indicate that the use of these plant proteins as alternative nitrogen additives would not give a significant impact to the chiral purity of the final chiral lactic acid product. For corn steep liquor, the content of L-lactic acid and D-lactic acid reached 85.0 ± 1.6 mg/g DM and 130.2 ± 1.8 mg/g DM, respectively, indicating that the use of corn steep liquor as nitrogen additive certainly led to a decrease the optical purity of lactic acid product.

The plant proteins were enzymatically hydrolyzed by alkaline proteinase to release free amino acids as nitrogen additive for cellulosic lactic acid fermentation in flasks. Yeast extract and peptone were selected as the control. *P. acidilactici* ZY271 produced 129.4 g/L L-lactic

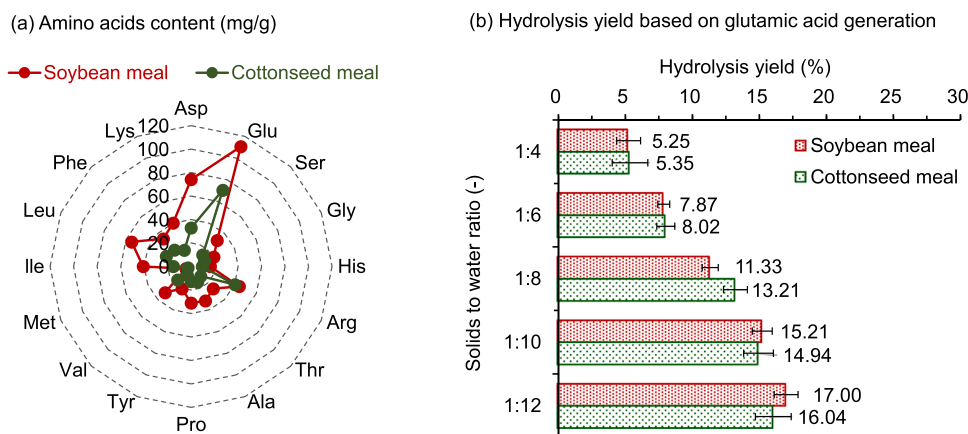

Fig. 3. Hydrolysis yield of soybean meal and cottonseed meal at different solids to water ratio (-). (a) Amino acids content in soybean meal and cottonseed meal; (b) hydrolysis yield based on glutamic acid generation. The plant protein hydrolysis was at 60°C, 150 rpm for 24 h. The dosage of alkaline protease was 5% of the mass of crude plant protein (g enzyme/g crude plant protein).

acid with 99.6% optical pure from 30% (w/w) solids loading wheat straw using 15 g/L reagent grade yeast extract and 10 g/L reagent grade peptone as complex nitrogen in 3 L fermenter (He et al., 2022). Industrial grade yeast extract was further used to replace the reagent grade yeast extract and peptone. The results showed that the fermentation indicators using 20 g/L industrial grade yeast extract were similar to those using 15 g/L reagent grade yeast extract and 10 g/L reagent grade peptone (Fig. S1). When yeast extract and peptone were used as nitrogen additives, either in reagent grade (Control #1) or industrial grade (Control #2), the L-lactic acid titer, cell viability and product optical purity were similar, reaching over 95.0 g/L (Fig. 1a), 3.0×10^9 CFU/mL (Fig. 1b) and 99.7% (Fig. 1c), respectively, from 25% (w/w) solids loading wheat straw in flasks. Among the plant proteins tested, the highest L-lactic acid titer and cell viability were obtained with soybean meal and cottonseed meal as nitrogen additives due to their higher protein contents (Table 1). The chiral purity of the lactic acid product using plant proteins as nitrogen additives reached over 99.5%, except for corn germ meal (96.4%, Fig. 1c). However, the lactic acid titer and cell viability using plant proteins as alternative nitrogen additives were lower than the levels of the control groups. The key parameters of enzymatic hydrolysis of soybean meal and cottonseed meal were further optimized to improve the hydrolysis yield and fermentation performance.Where "/" indicated that the content of D-lactic acid below 0.05 mg/g DM.

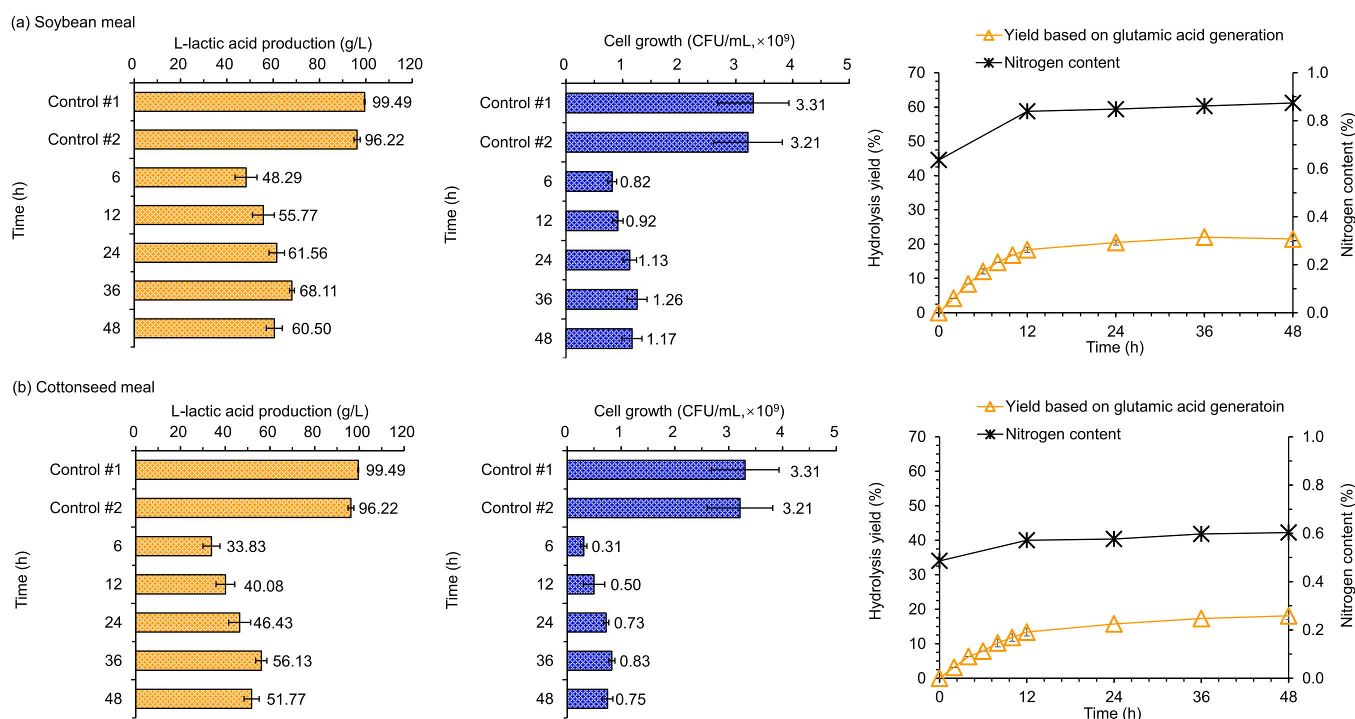

Fig. 4. Plant proteins with different enzymatic hydrolysis times as complex nitrogen additives for cellulosic lactic acid fermentation in flasks. (a) L-lactic acid titer, cell viability, and hydrolysis yield using soybean meal hydrolyzed with different times as complex nitrogen additives. (b) L-lactic acid titer, cell viability, and hydrolysis yield using cottonseed meal hydrolyzed with different times as complex nitrogen additives. The degree of hydrolysis was defined based on the glutamate content of the plant protein hydrolysate. The plant protein hydrolysis was at the solid-liquid ratio of 1:10, 60°C, 150 rpm for 8–48 h. The dosage of alkaline protease was 5% of the mass of crude plant protein (g enzyme/g crude plant protein). The amount of plant protein added for fermentation was 20 g/L.

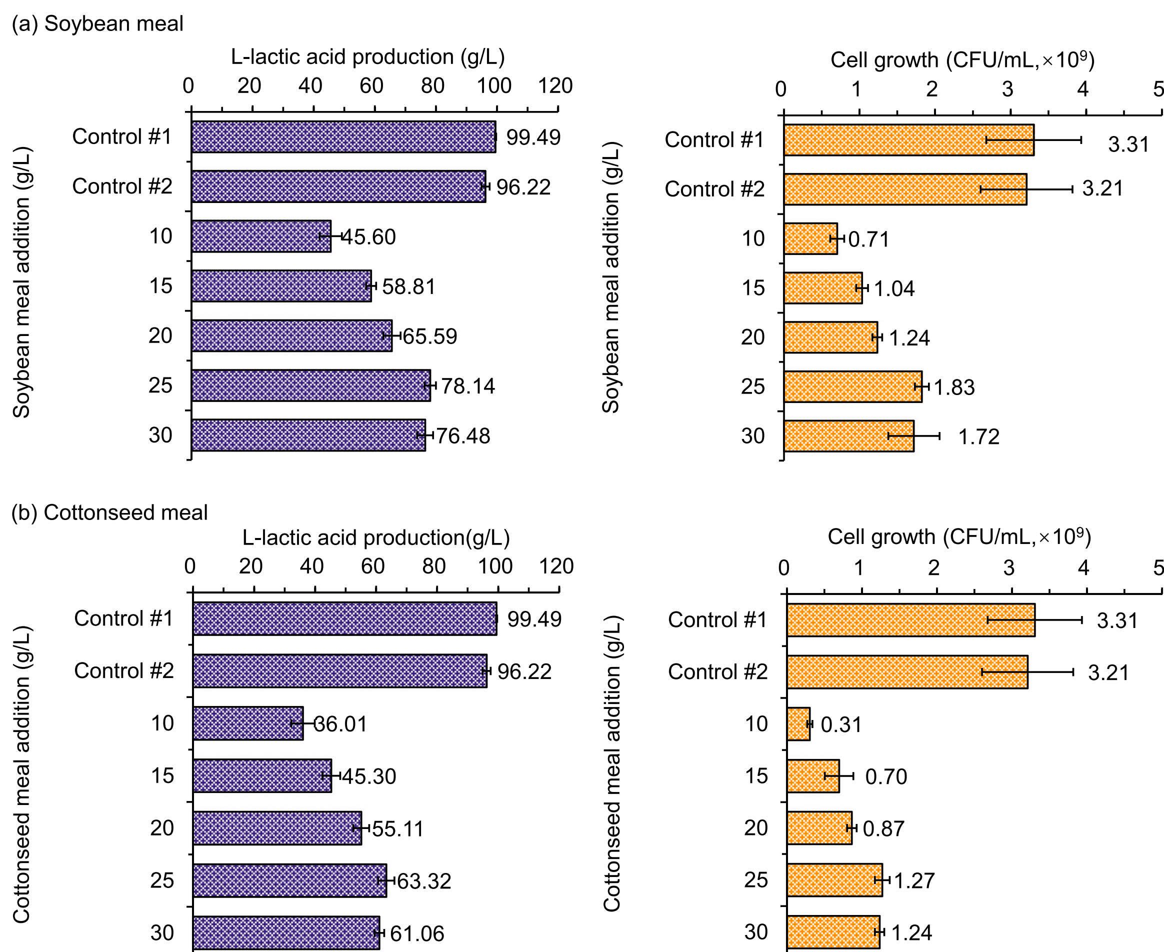

Fig. 5. Cellulosic L-lactic acid fermentation using different dosage of plant protein as complex nitrogen additives in flasks. (a) L-lactic acid titer and cell viability, using soybean meal hydrolyzed with different dosages (10–30 g/L based on the dry weight of soybean meal). (b) L-lactic acid titer and cell viability using cottonseed meal hydrolyzed with different dosages (10–30 g/L based on the dry weight of soybean meal). The plant protein hydrolysis was at the solid-liquid ratio of 1:10, 60°C, 150 rpm for 36 h. The dosage of alkaline protease was 5% of the mass of crude plant protein (g enzyme/g crude plant protein).

3.2. Optimizing plant protein hydrolysis as nitrogen additives for chiral lactic acid fermentation

The enzymatic hydrolysis of soybean meal and cottonseed meal were investigated to achieve the maximum amino acids yield and lactic acid fermentation performance. Soybean meal and cottonseed meal were hydrolyzed by 5% (w/w, based on dry weight of plant proteins) alkaline proteinase at varying solid-liquid ratios (from 1:4–1:12) (Fig. 2) and hydrolysis times (from 6 h to 48 h) (Fig. 4), then used as nitrogen additives for cellulosic lactic acid fermentation.

The results showed that the L-lactic acid titer and cell viability were improved when the hydrolysis solid-liquid ratio was decreased from 1:4–1:10. Further reduction of the solid-liquid ratio to 1:12 showed no enhancement in lactic acid production and cell growth (Fig. 2). The hydrolysis yield of the plant proteins at varying solid-liquid ratios were calculated based on the free glutamic acid generation (Fig. 3). The amino acids profile of soybean meal and cottonseed meal indicates that glutamic acid content was the highest among the fifteen amino acids measured, reaching 110.6 ± 0.6 mg/g and 70.4 ± 0.4 mg/g, respectively (Fig. 3a). The hydrolysis yield of soybean meal and cottonseed meal was around 15% based on the free glutamic acid generation when the solid-liquid ratio of the hydrolysis was 1:10, but further reduction of solid-liquid ratio showed no improvement on the hydrolysis yield (Fig. 3b). The trend of hydrolysis yields based on free glutamic acid generation at varying solid-liquid ratios was consistent with that of lactic acid production and cell growth.

The enzymatic hydrolysis of soybean meal and cottonseed meal were further conducted at changing hydrolysis times (6–48 h) (Fig. 4). Prolonged hydrolysis time of soybean meal and cottonseed meal from 6 h to 36 h improved the L-lactic acid titer and cell viability, but further increase to 48 h showed no positive effect on lactic acid fermentation. The hydrolysis yields at different hydrolysis times were calculated based on the free glutamic acid generation. The generally used titration method (pH-stat method) was not applied to determine the hydrolysis yield (Adler-Nissen, 1986; Wang et al., 2018), owing to the presence of gossypol in cottonseed meal (Zhang et al., 2024). The released gossypol during the enzymatic hydrolysis of cottonseed meal would neutralize the sodium hydroxide for titration, resulting in inaccurate hydrolysis yield calculation. For soybean meal, the hydrolysis yield increased from 0 h to 36 h and remained essentially unchanged after 36 h. The free nitrogen contents were almost unchanged after 12 h, indicating that most of soluble peptides were generated during the first 12 h of the hydrolysis (Fig. 4a). For cottonseed meal, the free nitrogen content and hydrolysis yield were also basically not changed after 12 h and 36 h, respectively. But the overall free nitrogen contents and hydrolysis yields of cottonseed meal were lower than those of soybean meal (Fig. 4b).

The optimized hydrolysates of soybean meal and cottonseed meal improved the L-lactic acid production by 54.6% and 60.1%, respectively (Fig. 4), but was still lower than that using yeast extract and peptone as complex nitrogen additives. Although the optical purity of lactic acid product using soybean meal and cottonseed meal as nitrogen additives was still maintained over 99.7%, more efforts are needed to further

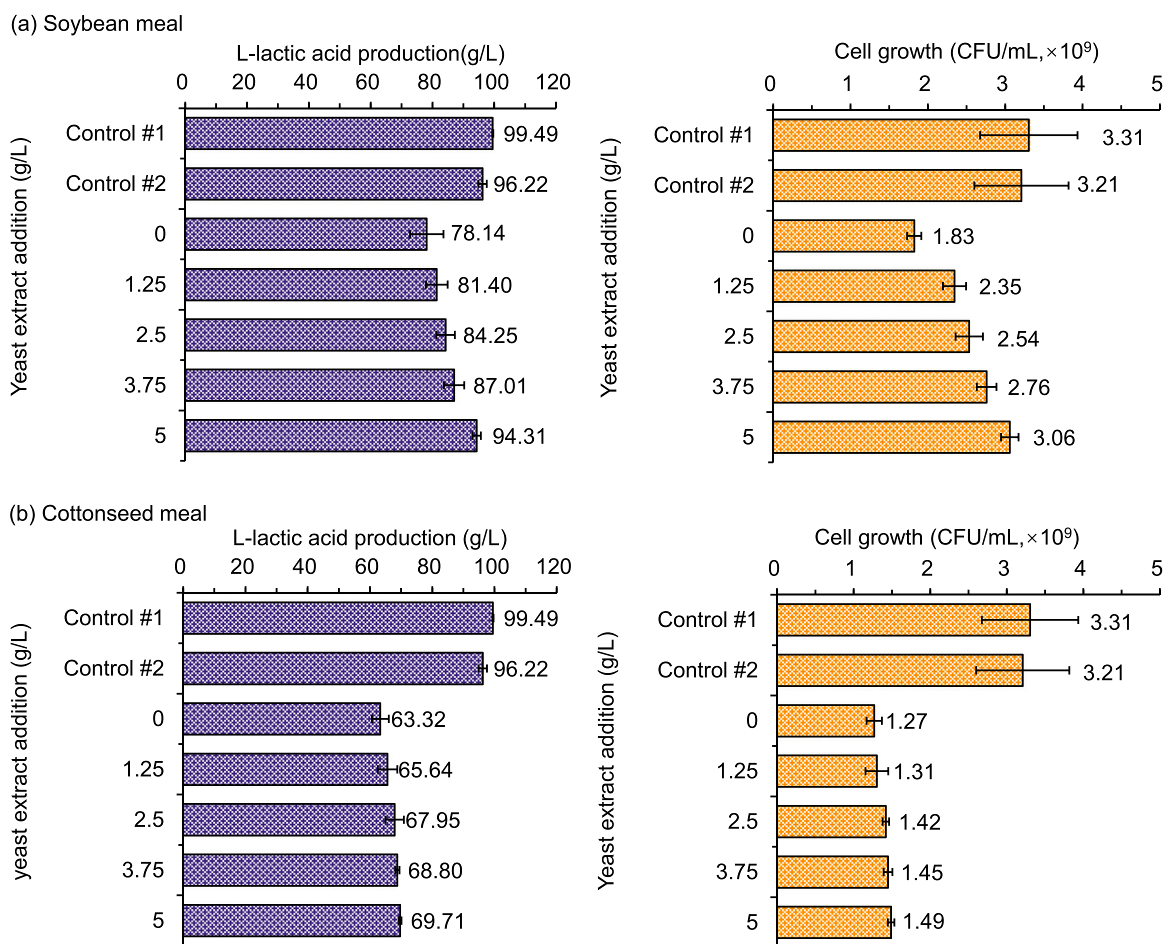

Fig. 6. Cellulosic L-lactic acid fermentation using plant protein and different dosages of industrial grade yeast extract as complex nitrogen additives in flasks. (a) L-lactic acid titer and cell viability, using soybean meal hydrolyzed and different dosages of YE (0–5 g/L). (b) L-lactic acid titer and cell viability using cottonseed meal hydrolyzed and different dosages of yeast extract (0–5 g/L). The plant protein hydrolysis was at the solid-liquid ratio of 1:10, 60°C, 150 rpm for 36 h. The dosage of alkaline protease was 5% of the mass of crude plant protein (g enzyme/g crude plant protein). The amount of plant protein added for fermentation was 25 g/L.

improve the fermentation indicators using plant protein as the nitrogen additives to reach the standard of those using yeast extract and peptone as complex nitrogen additives.

3.3. Formulation of combinational nitrogen ingredients for chiral lactic acid fermentation

Increasing dosages of soybean meal and cottonseed meal increased the lactic acid production with the peak values (63.3–78.1 g/L) at the dosage of 25 g/L (Fig. 5), but were still significantly lower than those using yeast extract and peptone (96.2–99.5 g/L), indicating that the plant proteins might not be sufficient in nutritional requirements of lactic acid bacteria.

Minimum industrial grade yeast extract dosage (0–5 g/L) was added into the soybean meal hydrolysate to compensate the deficiency of the specific amino acids in soybean meal and cottonseed meal (Fig. 6). The results showed that the addition of yeast extract to soybean meal hydrolysate was effective in improving the chiral lactic acid fermentation efficiency. At the addition of 5 g/L yeast extract to soybean meal hydrolysate, the L-lactic acid titer and cell viability improved to 94.3 ± 1.4 g/L and 3.1 ± 0.1 CFU/mL, close to the chiral lactic acid production indicators using 25 g/L of industrial grade yeast extract (Control #2) as the complex nitrogen additives. In contrast, the addition of 5 g/L yeast extract to the cottonseed meal hydrolysate only slightly enhanced the L-lactic acid titer and cell viability to 69.7 ± 0.4 g/L and 1.5 ± 0.0 CFU/mL (Fig. 6b). Therefore, soybean meal was a better choice as the alternative complex nitrogen additives for chiral lactic acid production by *P. acidilactici* ZY271. The lactic acids-free combinational nitrogen ingredients including 25 g/L of soybean meal hydrolysate and 5 g/L of industrial grade yeast extract satisfied the nutritional requirements of lactic acid bacteria *P. acidilactici* ZY271 with similar results to those using yeast extract and peptone as nitrogen additives.

The soybean meal hydrolysate was used as nitrogen ingredients for L-lactic acid fermentation from 30% (w/w) solids loading wheat straw feedstock. The soybean meal hydrolysate was added to wheat straw hydrolysate as water supplement for saccharification and biodetoxification. All acetic acid, HMF and furfural were consumed until 36 h. The addition of soybean meal did not accelerate the biodetoxification rate, while 35.1% of free nitrogen was consumed by the biodetoxification strain *P. variotii* FN89 (Fig. 7a). The sugars concentration during the biodetoxification was slightly increased (Fig. S2), owing to (i) the biodetoxification strain can degrade inhibitors prior to sugars (Zhang et al., 2021), and (ii) the continues hydrolysis of wheat straw solids by cellulase. The total sugars concentration reached ~170 g/L (~120 g/L of glucose and ~50 g/L of xylose) after biodetoxification. But the initial total sugars concentration in fermentation was decreased to ~150 g/L due to the dilution of seed inoculation. After the supplementation of 5 g/L yeast extract in the fermentation stage, the L-lactic acid titer still reached 122.9 ± 0.4 g/L, which was similar to that of Control #1 (121.9 ± 0.6 g/L) and Control #2 (123.0 ± 0.5 g/L) (Fig. 7b). And the optical purity of lactic acid product using combinational nitrogen ingredients and wheat straw reached 99.7%. This result

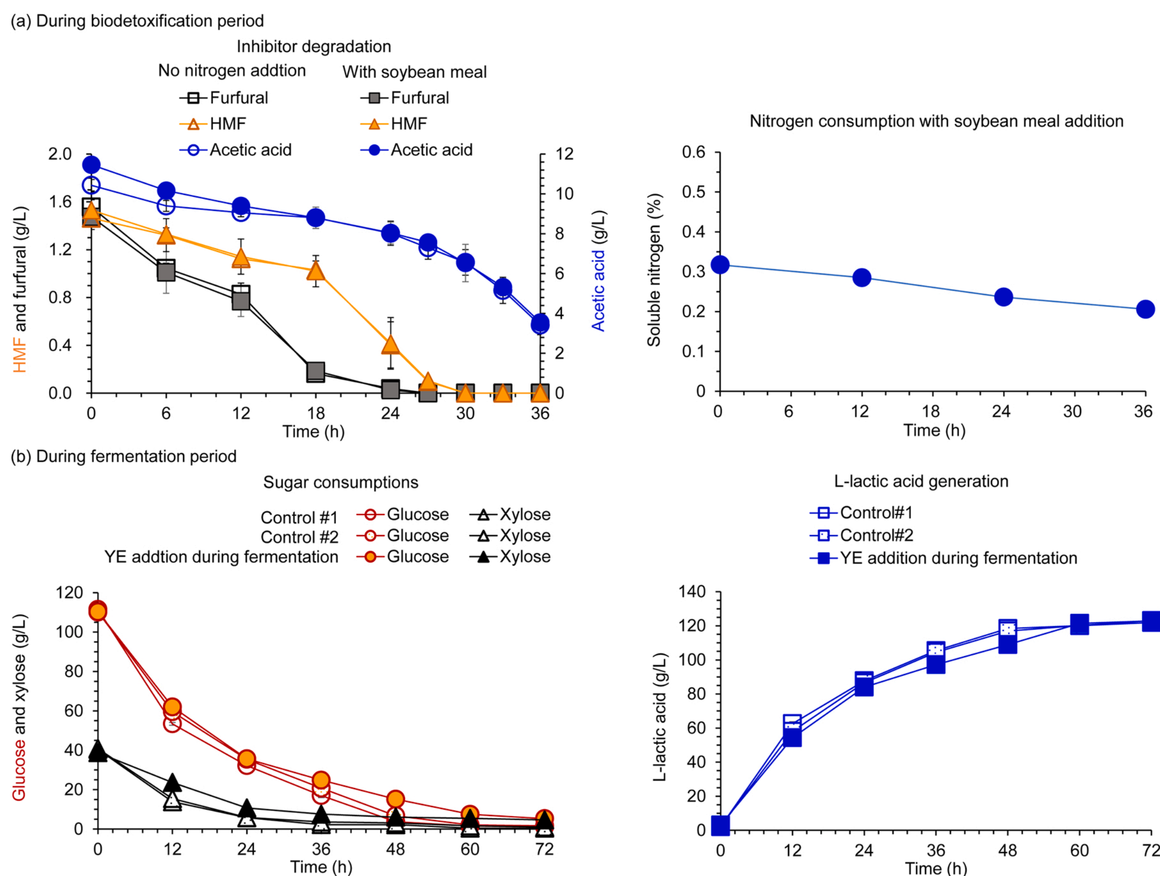

Fig. 7. SSCF for cellulosic L-lactic acid production at 30% solids loading of wheat straw feedstock in bioreactors. (a) Effect of the addition of nitrogen additives on the inhibitors degradation and nitrogen consumption during the biodetoxification process; (b) effect of the addition of 5 g/L yeast extract on sugars consumption and L-lactic acid production during fermentation. Soybean hydrolysate was added to replace partial water during saccharification.

Table 2
Summary of chiral lactic acid production using cheap carbon source and complex nitrogen source.

Strain	Carbon source	Complex nitrogen	Titer (g/L)	Productivity (g/L/h)	Yield (g/g sugars)	Chirality (%)	Ref
Sporolactobacillus inulinus YBS1–5	Corncob residues	Cottonseed meal	107.2[a]	1.19	0.85	99.2	Bai et al., (2016)
Lactobacillus delbrueckii ssp.	Orange peel waste	Corn steep liquor	56.4	2.35	0.92	> 95	de la Torre et al., (2018)
Lactobacillus thermophilus A69	Sweet sorghum juice	Soybean hydrolysate[b]	118.8	3.71	0.97	\	Tian et al., (2020)
Microbial consortium CEE-DL15[c]	Sugarcane molasses	Corn steep liquor powder	122.3	4.49	0.81	95.6	Sun et al., (2019)
Lactobacillus delbrueckii S–NL31	Sugarcane molasses	Soybean meal, yeast extract	112.3[a]	2.40	0.98	99.6	Liang et al., (2020)
P. acidilactici ZY271	Wheat straw	Cottonseed meal[d]	96.5	1.32	0.31[e]	99.7	Zhang et al., (2022)
P. acidilactici ZY271	Wheat straw	Soyben meal, yeast extract	122.9	1.71	0.88	99.7	This study

[a] The fermentation was carried out by fed-batch mode.
[b] An addition of 72 mM vitamin C was supplemented.
[c] The consortium mainly consisted of *Clostridium sensustricto* (57.29%), *Escherichia* (34.22%), and *Enterococcus* (5.32%)
[d] The cottonseed meal was hydrolyzed by sulfuric acid.
[e] The yield was calculated based on g/g raw feedstock.

indicated that although the biodetoxification strain consumed a portion of free nitrogen in soybean meal hydrolysate, this fraction of nutrients derived from soybean meal may be in excess for the fermentation strain, and therefore did not influence the fermentation efficiency. In addition, the L-lactic acid titer was only 65.3 ± 0.9 g/L without the supplementation of yeast extract (only soybean meal hydrolysate) (Fig. S3). This result suggested that this portion of nutrients from yeast extract is necessary for efficient lactic acid biosynthesis by *P. acidilactici* ZY271, demonstrating the rationality and feasibility of the designed combinational nitrogen ingredients for cellulosic lactic acid production.

The typical cases of chiral lactic acid production using cheap carbon source and complex nitrogen source were summarized and showed in Table 2. Most of these studies used molasses or plant juice as carbon source, which resulted in higher productivity (Liang et al., 2020; Sun et al., 2019; Tian et al., 2020). The previous studies also demonstrated that corn steep liquor was not suitable for chiral lactic acid production because it caused the reduction in the chirality of the product (de la Torre et al., 2018; Sun et al., 2019). Bai et al. (2016) reported that *Sporolactobacillus inulinus* YBS1–5 produced 107.2 g/L of D-lactic acid using corncob residues as carbon source and cottonseed meal as complex nitrogen. But the corncob residues hydrolysate was firstly concentrated and then used for fed-batch fermentation, which is not an energy-effective process. Compared to acid hydrolysis, the enzymatic hydrolysis provided more moderate conditions and fewer undesirable side reactions or product (Marinova et al., 2014). The lactic acid titer using soybean meal hydrolysate obtained by enzymatic hydrolysis were

Table 3
Preliminary technoeconomic evaluation of cellulose L-lactic acid production using plant proteins nitrogen ingredients.

	Complex nitrogen additives	Hydrolysis catalyst	Inorganic nitrogen additives	Titer (g/L)	Yield (%)	Productivity (g/L/h)	Optical purity (%)	Feasibility as industrial technology
(a) Fermentation evaluation								
Case 1	15 g/L yeast extract and 10 g/L peptone (both in reagent grade)	\	2 g/L $(NH_4)_2C_6H_6O_7$	121.5	88.7	1.69	99.7	No (nitrogen ingredients for lab use only)
Case 2	20 g/L yeast extract (industrial grade)	\	10 g/L $(NH_4)_2SO_4$	123.3	90.1	1.71	99.8	Yes
Case 3	25 g/L corn steep liquor	\	10 g/L $(NH_4)_2SO_4$	98.5	68.3	1.37	96.0	No (low optical purity)
Case 4	25 g/L soybean meal	6.25 g/L Sulfuric acid	10 g/L $(NH_4)_2SO_4$	62.0	39.8	0.86	99.7	No (low production)
Case 5	25 g/L soybean meal	1.25 g/L Alkaline protease	10 g/L $(NH_4)_2SO_4$	65.9	41.6	0.92	99.8	No (low production)
Case 6	25 g/L soybean meal and 5 g/L yeast extract (industrial grade)	1.25 g/L Alkaline protease	10 g/L $(NH_4)_2SO_4$	122.9	88.1	1.70	99.7	Yes

(b) Cost evaluation of nitrogen additives for cases with industrial application potential	Complex nitrogen additives (¢/kg lactic acid)	Hydrolysis catalyst (¢/kg lactic acid)	Inorganic nitrogen additives (¢/kg lactic acid)	Total nitrogen cost (¢/kg lactic acid)
Case 2	44.7	\	1.1	45.7
Case 6	16.9	1.8	1.1	19.8

Notes: The fermentation was conducted in fermenter. The yields in Table 3a were calculated based on the theoretical yield. The acid hydrolysis of soybean meal was referred to the report by Zhang et al. (2022). The exchange rate of dollar to Chinese Yuan was set to 7.11. The prices of these raw materials were provided by local suppliers including yeast extract (reagent grade) $ 42194.1/ton, peptone (reagent grade) $ 59915.6/ton, yeast extract (industrial grade) $ 3938.1/ton, corn steep liquor $ 98.5/ton, soybean meal $ 395.2/ton, sulfuric acid $ 66.1/ton, alkaline protease $ 2531.7/ton, $(NH_4)_2C_6H_6O_7$ $ 2320.7/ton, $(NH_4)_2SO_4$ $ 189.9/ton.

much higher that obtained by acid hydrolysis (Zhang et al., 2022). This study achieved high level cellulosic L-lactic acid production from wheat straw and soybean meal through a mature biorefinery process without solid-liquid separation and concentration steps. The chiral cellulosic lactic acid titer, productivity and yield in this study are obviously improved compared to those in previous studies (Bai et al., 2016; Zhang et al., 2022).

3.4. Preliminary technoeconomic evaluation of plant proteins nitrogen ingredients

In summary, after the optimization of enzymatic hydrolysis conditions and the design of combinational nitrogen ingredients, the soybean meal hydrolysate achieved the substitution of 75% of yeast extract, and the lactic acid titer reached 122.9 ± 0.4 g/L with the optical purity of 99.7%. Three key criteria were used to evaluate the feasibility of nitrogen ingredients for L-lactic acid fermentation: L-lactic acid titer greater than 100 g/L, optical purity greater than 99.5%, and the minimal costs. The key indicators using different nitrogen ingredients were shown in Table 3a. Case 1 was excluded for practical because of the use of high amounts of expensive reagent grade yeast extract (15 g/L) and peptone (10 g/L), which was only suitable for lab bench use, although the product titer and optical purity met the criteria. Case 2 was acceptable because of the use of relatively cheap industrial grade yeast extract, and the product titer and optical purity were well satisfied. Case 3 was excluded because of the low optical purity (~96%, far below 99.5% in the criteria), although the ingredients were cheap and product titer was almost satisfied. Case 4 and Case 5 were excluded because of the low product titer (far below the minimal criteria of 100 g/L), although 25 g/L of soybean meal hydrolysates were cheap and the optical purity met the criteria. Case 6 was acceptable because of the moderate cost with high product titer and optical purity by using the combination of industrial grade yeast extract and soybean meal.

The two acceptable cases (Case 2 and Case 6) were used for preliminary technoeconomic evaluation by accounting the costs of organic nitrogen additives, hydrolysis catalyst and inorganic nitrogen additives (Table 3b). Case 4 is a general nitrogen ingredient used in industrial lactic acid fermentation, while Case 6 is the modified combined nitrogen ingredients formulated in this study. Based on the prices provided by local suppliers, the cost of the soybean meal was $395.2/ton with the usage of 25 g/L; the alkaline protease was $2,531.7/ton with the usage of 0.05 g/g soybean meal; the industrial grade yeast extract was $3,838.1/ton with the usage of 5 g/L; and the ammonium sulfate was $189.9/ton with the usage of 10 g/L. In Case 2, the total nitrogen ingredients cost to produce one kilogram of L-lactic acid was $0.457, while in Case 6 the total nitrogen ingredients cost was only $0.198/kg, equivalent to 43.3% of the Case 2.

4. Conclusion

After the screening of lactic acids-free plant proteins, the optimizations of enzymatic hydrolysis conditions, and the design of combinational nitrogen ingredients, 25 g/L of soybean hydrolysate was used to replace 75% of yeast extract for chiral L-lactic acid production by *P. acidilactici* from wheat straw. The L-lactic acid titer reached 122.9 ± 0.4 g/L with the yield of 88.1% from 30% (w/w) solids loading of pretreated wheat straw and optical purity of 99.8%, which are similar to the fermentation indicators using yeast extract as nitrogen additive. The total cost of the alternative nitrogen additives to produce one kilogram of chiral lactic acid was only ¢ 19.8, reducing the cost by 56.7% compared to those using yeast extract. This study provides a cost-effective alternative nitrogen source solution for cellulosic chiral lactic acid production in industrial biorefinery process.

CRediT authorship contribution statement

Jie Bao: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization. **Ying Han:** Writing – review & editing, Writing – original draft, Visualization,

Validation, Resources, Methodology, Investigation, Formal analysis, Data curation. **Bin Zhang:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

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.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.indcrop.2024.118821.

References

Adler-Nissen, J., 1986. Enzymic Hydrolysis Offood Proteins. Elsevier Applied Science, London, UK.

Ahmad, A., Banat, F., Taher, H., 2020. A review on the lactic acid fermentation from low-cost renewable materials: Recent developments and challenges. Environ. Technol. Innov. 20, 101138 https://doi.org/10.1016/j.eti.2020.101138.

Alexandri, M., Schneider, R., Mehlmann, K., Venus, J., 2019. Recent Advances in d-lactic acid production from renewable resources: Case studies on agro-industrial waste streams. Food Technol. Biotechnol. 57 (3), 293–304. https://doi.org/10.17113/ftb.57.03.19.6023.

Alves de Oliveira, R., Komesu, A., Vaz Rossell, C.E., Maciel Filho, R., 2018. Challenges and opportunities in lactic acid bioprocess design—From economic to production aspects. Biochem. Eng. J. 133, 219–239. https://doi.org/10.1016/j.bej.2018.03.003.

Augustiniene, E., Valanciene, E., Matulis, P., Syrpas, M., Jonuskiene, I., Malys, N., 2021. Bioproduction of l- and d-lactic acids: advances and trends in microbial strain application and engineering. Crit. Rev. Biotechnol. 42 (3), 342–360. https://doi.org/10.1080/07388551.2021.1940088.

Bai, Z., Gao, Z., Sun, J., Wu, B., He, B., 2016. d-Lactic acid production by *Sporolactobacillus inulinus* YBS1-5 with simultaneous utilization of cottonseed meal and corncob residue. Bioresour. Technol. 207, 346–352. https://doi.org/10.1016/j.biortech.2016.02.007.

Brock, S., Kuenz, A., Prüße, U., 2019. Impact of hydrolysis methods on the utilization of agricultural residues as nutrient source for d-lactic acid production by *Sporolactobacillus inulinus*. Fermentation 5 (1), 12. https://doi.org/10.3390/fermentation5010012.

Cao, X., Chen, Z., Liang, L., Guo, L., Jiang, Z., Tang, F., Yun, Y., Wang, Y., 2020. Co-valorization of paper mill sludge and corn steep liquor for enhanced n-butanol production with *Clostridium tyrobutyricum* Δcat1::adhE2. Bioresour. Technol. 296, 122347 https://doi.org/10.1016/j.biortech.2019.122347.

Cubase-Cano, E., González-Fernández, C., Ballesteros, M., Tomás-Pejó, E., 2018. Biotechnological advances in lactic acid production by lactic acid bacteria: lignocellulose as novel substrate. Biofuels Bioprod. Bioref. 12 (2), 290–303. https://doi.org/10.1002/bbb.1852.

de la Torre, I., Ladero, M., Santos, V.E., 2018. Production of d-lactic acid by *Lactobacillus delbrueckii* ssp. *delbrueckii* from orange peel waste: Techno-economical assessment of nitrogen sources. Appl. Microbiol. Biotechnol. 102 (24), 10511–10521. https://doi.org/10.1007/s00253-018-9432-4.

Gao, Y., Yuan, Y.J., 2011. Comprehensive quality evaluation of corn steep liquor in 2-keto-L-gulonic acid fermentation. J. Agric. Food Chem. 59 (18), 9845–9853. https://doi.org/10.1021/jf201792u.

Han, T., Zhang, B., Yang, H., Liu, X., Bao, J., 2023. Changes in pH values allow for a visible detection of the end point in submerged liquid biodetoxification during biorefinery processing. ACS Sustain. Chem. Eng. 11 (46), 16608–16617. https://doi.org/10.1021/acssuschemeng.3c04986.

He, N., Jia, J., Qiu, Z., Fang, C., Liden, G., Liu, X., Bao, J., 2022. Cyclic L-lactide synthesis from lignocellulose biomass by biorefining with complete inhibitor removal and highly simultaneous sugars assimilation. Biotechnol. Bioeng. 119, 1903–1915. https://doi.org/10.1002/bit.28082.

Huang, S., Xue, Y., Yu, B., Wang, L., Zhou, C., Ma, Y., 2021. A review of the recent developments in the bioproduction of polylactic acid and its precursors optically pure lactic acids. Molecules 26 (21). https://doi.org/10.3390/molecules26216446.

Krull, S., Brock, S., Prüße, U., Kuenz, A., 2020. Hydrolyzed agricultural residues—Low-cost nutrient sources for L-lactic acid production. Fermentation 6 (4), 97. https://doi.org/10.3390/fermentation6040097.

Lian, T., Zhang, W., Cao, Q., Wang, S., Yin, F., Zhou, T., Zhang, F., Dong, H., 2023. Efficient production of lactic acid from anaerobic co-fermentation of starch and nitrogen-rich agro-industrial waste using a batch system. Chem. Eng. J. 471, 144689 https://doi.org/10.1016/j.cej.2023.144689.

Liang, S., Jiang, W., Song, Y., Zhou, S.F., 2020. Improvement and metabolomics-based analysis of d-lactic acid production from agro-industrial wastes by *Lactobacillus delbrueckii* submitted to adaptive laboratory evolution. J. Agric. Food Chem. 68 (29), 7660–7669. https://doi.org/10.1021/acs.jafc.0c00259.

Liu, G., Sun, J., Zhang, J., Tu, Y., Bao, J., 2015. High titer L-lactic acid production from corn stover with minimum wastewater generation and techno-economic evaluation based on Aspen plus modeling. Bioresour. Technol. 198, 803–810. https://doi.org/10.1016/j.biortech.2015.09.098.

Liu, G., Zhang, Q., Li, H., Qureshi, A.S., Zhang, J., Bao, X., Bao, J., 2018. Dry biorefining maximizes the potentials of simultaneous saccharification and co-fermentation for cellulosic ethanol production. Biotechnol. Bioeng. 115 (1), 60–69. https://doi.org/10.1002/bit.26444.

Marinova, M., Cuc, N.T.K., Tchnorbanov, B., 2014. Enzymatic hydrolysis of soy protein isolate by food grade proteinases and aminopeptidases of plant origin. Biotechnol. Biotechnol. Equip. 22 (3), 835–838. https://doi.org/10.1080/13102818.2008.10817563.

Michalczyk, A.K., Garbaczewska, S., Morytz, B., Białek, A., Zakrzewski, J., 2021. Influence of nitrogen sources on d-lactic acid biosynthesis by *Sporolactobacillus laevolacticus* DSM 442 Strain. Fermentation 7 (2), 78. https://doi.org/10.3390/fermentation7020078.

Qiu, Z., Gao, Q., Bao, J., 2018. Engineering *Pediococcus acidilactici* with xylose assimilation pathway for high titer cellulosic L-lactic acid fermentation. Bioresour. Technol. 249, 9–15. https://doi.org/10.1016/j.biortech.2017.09.117.

Rawoof, S.A.A., Kumar, P.S., Vo, D.N., Devaraj, K., Mani, Y., Devaraj, T., Subramanian, S., 2020. Production of optically pure lactic acid by microbial fermentation: a review. Environ. Chem. Lett. 19 (1), 539–556. https://doi.org/10.1007/s10311-020-01083-w.

Ren, Y., Wang, X., Li, Y., Li, Y., Wang, Q., 2022. Lactic acid production by fermentation of biomass: Recent achievements and perspectives. Suatainability 14 (21), 14434. https://doi.org/10.3390/su142114434.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., 2008. Determination of sugars, byproducts, and degradation products in liquid fraction process samples. NREL, Golden, CO, NREL/TP-510–42623. Retrieved from ⟨http://www.nrel.gov/docs/gen/fy08/42623.pdf⟩.

Sluiter, A., Hames, B., Scarlata, C., Sluiter, J., Templeton, D., 2012. Determination of structural carbohydrates and lignin in biomass national renewable. NREL, Golden, CO, NREL/TP-510–42618. Retrieved from ⟨http://www.nrel.gov/docs/gen/fy08/42618.pdf⟩.

Sun, Y., Xu, Z., Zheng, Y., Zhou, J., Xiu, Z., 2019. Efficient production of lactic acid from sugarcane molasses by a newly microbial consortium CEE-DL15. Process Biochem 81, 132–138. https://doi.org/10.1016/j.procbio.2019.03.022.

Tanksley Jr., T.D., Knabe, D.A., Purser, K., Zebrowska, T., Corley, J.R., 1981. Apparent digestibility of amino acids and nitrogen in three cottonseed meals and one soybean meal. J. Anim. Sci. 52 (4), 769–777. https://doi.org/10.2527/jas1981.524769x.

Tian, X., Chen, H., Liu, H., Chen, J., 2021. Recent advances in lactic acid production by lactic acid bacteria. Appl. Biochem. Biotechnol. 193 (12), 4151–4171. https://doi.org/10.1007/s12010-021-03672-z.

Tian, X., Hu, W., Chen, J., Zhang, W., Li, W., 2020. The supplement of vitamin C facilitates L-lactic acid biosynthesis in *Lactobacillus thermophilus* A69 from sweet sorghum juice coupled with soybean hydrolysate as feedstocks. Ind. Crop. Prod. 146, 112159 https://doi.org/10.1016/j.indcrop.2020.112159.

Tian, X., Jiang, A., Mao, Y., Wu, B., He, M., Hu, W., Chen, J., Li, W., 2019. Efficient L-lactic acid production from purified sweet sorghum juice coupled with soybean hydrolysate as nitrogen source by *Lactobacillus thermophilus* A69 strain. J. Chem. Technol. Biotechnol. 94 (6), 1752–1759. https://doi.org/10.1002/jctb.5939.

Wang, L., Zhao, B., Li, F., Xu, K., Ma, C., Tao, F., Li, Q., Xu, P., 2011. Highly efficient production of D-lactate by *Sporolactobacillus* sp. CASD with simultaneous enzymatic hydrolysis of peanut meal. Appl. Microbiol. Biotechnol. 89 (4), 1009–1017. https://doi.org/10.1007/s00253-010-2904-9.

Wang, S., Meng, D., Wang, S., Zhang, Z., Yang, R., Zhao, W., 2018. Modification of wheat gluten for improvement of binding capacity with keratin in hair. R. Spc. Open Sci. 5 (2), 171216 https://doi.org/10.1098/rsos.171216.

Yankov, D., 2022. Fermentative lactic acid production from lignocellulosic feedstocks: from source to purified product. Front. Chem. 10, 823005 https://doi.org/10.3389/fchem.2022.823005.

Zhang, B., Khushik, F.A., Zhan, B., Bao, J., 2021. Transformation of lignocellulose to starch-like carbohydrates by organic acid-catalyzed pretreatment and biological detoxification. Biotechnol. Bioeng. 118 (10), 4105–4118. https://doi.org/10.1002/bit.27887.

Zhang, B., Wu, L., Liu, X., Bao, J., 2022. Plant proteins as an alternative nitrogen source for chiral purity l-lactic acid fermentation from lignocellulose feedstock. Fermentation 8 (10), 546. https://doi.org/10.3390/fermentation8100546.

Zhang, L., Zheng, H., Zhang, X., Chen, X., Liu, Y., Tang, Y., Zhang, W., Wang, Z., Zhao, L., Guo, Y., 2024. Effective degradation of free gossypol in defatted cottonseed meal by

bacterial laccases: performance and toxicity analysis. Foods 13 (4), 566. https://doi.org/10.3390/foods13040566.
Zhang, Y., Vadlani, P.V., Kumar, A., Hardwidge, P.R., Govind, R., Tanaka, T., Kondo, A., 2015. Enhanced D-lactic acid production from renewable resources using engineered *Lactobacillus plantarum*. Appl. Microbiol. Biotechnol. 100 (1), 279–288. https://doi.org/10.1007/s00253-015-7016-0.
Zhang, Y., Yoshida, M., Vadlani, P.V., 2018. Biosynthesis of D-lactic acid from lignocellulosic biomass. Biotechnol. Lett. 40 (8), 1167–1179. https://doi.org/10.1007/s10529-018-2588-2.