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Simultaneous and rate-coordinated conversion of lignocellulose derived glucose, xylose, arabinose, mannose, and galactose into D-lactic acid production facilitates D-lactide synthesis

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HIGHLIGHTS

- All lignocellulose-derived sugars were completely converted to D-lactic acid.
- Complete removal of residual sugar and inhibitor impurities facilitated the D-lactide synthesis.
- Polymer-grade D-lactide was synthesized from cellulosic D-lactic acid for the first time.
- Dry biorefinery did not generate any wastewater stream.

GRAPHICAL ABSTRACT

ARTICLE INFO

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ABSTRACT

D-lactide is the precursor of poly(D-lactide) (PDLA) or stereo-complex with poly(L-lactide) (PLLA). Lignocellulosic biomass provides the essential feedstock option to synthesize D-lactic acid and D-lactide. The residual sugars in D-lactic acid fermentation broth significantly blocks the D-lactide synthesis. This study showed a simultaneous and rate-coordinated conversion of lignocellulose derived glucose, xylose, arabinose, mannose, and galactose into D-lactic acid by adaptively evolved *Pediococcus acidilactici* ZY271 by simultaneous saccharification and co-fermentation (SSCF) of wheat straw. The produced D-lactic acid achieved minimum residual sugars (~1.7 g/L), high chirality (~99.1%) and high titer (~128 g/L). A dry acid pretreatment eliminated the wastewater stream generation and the biodetoxification by fungus *Amorphotheca resinae* ZN1 removed the inhibitors from the pretreatment. The removal of the sugar residues and inhibitor impurities in D-lactic acid production from lignocellulose strongly facilitated the D-lactide synthesis. This study filled the gap in cellulosic D-lactide production from lignocellulose-derived D-lactic acid.

1. Introduction

D-lactide is the critical precursor to produce high-performance biodegradable polylactic acid (PLA) materials, either as poly(D-lactide) (PDLA) or stereo-complexes with poly(L-lactide) (PLLA) to give desired mechanical strength, heat resistance, and hydrolysis

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resistance (Luo et al., 2020; Tsuji, 2016). The synthesis of D-lactide from lignocellulose biomass is an essential feedstock choice for commercial-scale production of high-performance polylactic acid (PLA) using D-lactide monomer from poly-condensation and de-polymerization of D-lactic acid (Abdel-Rahman et al., 2011; Wyman and Dale, 2015; Van Wouwe et al., 2016).

As the critical monomer of D-lactide, the production of D-lactic acid from lignocellulosic biomass was improved from the titer, productivity and yield (Alexandri et al., 2019; Ma et al., 2022). However, the polymerization-grade cellulosic D-lactic acid with high purity and high chirality has not been produced and the D-lactide has not been synthesized from the lignocellulose-derived D-lactic acid. The main barrier blocks the synthesis of cellulosic D-lactide is the impurity of sugars residues in the D-lactic acid broth, which is resulted from the incomplete utilization of the lignocellulose-derived sugars. The sugars from lignocellulose include glucose, xylose, arabinose, mannose, and galactose, and the non-glucose sugars approximately account for 30–40% of the whole of lignocellulose-derived sugars. However, no studies showed the wild or engineered strains co-utilized the full-spectrum of lignocellulose-derived glucose and non-glucose sugars (xylose, arabinose, mannose, and galactose) at a coordinated conversion rate to produce D-lactic acid from lignocellulose by simultaneous saccharification and fermentation (SSF). The *L. plantarum* mutant co-utilized glucose and xylose to produce 102.3 g/L D-lactic acid from hardwood pulp, but the utilization rate of xylose was much slower that of glucose and a high concentration (~15 g/L) of sugar residues remained in the fermentation broth (Hama et al., 2015). The *Sporolactobacillus inulinus* mutant realized the utilization of glucose and produced 87.3 g/L D-lactic acid from wheat bran, but no data showed the utilization of xylose, arabinose, mannose and galactose from 7.5% (w/w) of hemicellulose and resulted in the wastes of these sugars (Li et al., 2017). Engineered *Lactobacillus plantarum* co-utilized only glucose and xylose with slower xylose consumption rates than that of glucose, but no data showed the utilization of hemicellulose-derived arabinose, mannose and galactose, and finally the lower titer (22–61.4 g/L) of D-lactic acid was produced from corn stover and sorghum stalks (Zhang et al., 2016a; Zhang et al., 2016b). The *Lactobacillus bulgaricus* after evolutionary engineering only utilized glucose to produce ~108 g/L D-lactic acid, but *L. bulgaricus* unable to utilize the xylose released from 4.63% (w/w) of xylan from pretreated rice straw (Prasad et al., 2020). The incomplete utilization of lignocellulose-derived sugars not only reduced the titer, productivity and yield of cellulosic D-lactic acid, but also resulted in a plenty of residual sugars in the D-lactic acid fermentation broth. The sugar residues of D-lactic acid fermentation broth cause the major obstacle to synthesize D-lactide using D-lactic acid from lignocellulose, because the sugars are difficult and costly to be completely removed in purification step and interfere with the synthesis of D-lactide (Alves de Oliveira et al., 2019; He et al., 2022; Joglekar et al., 2006). Therefore, it is crucially important to remove residual sugars in the D-lactic acid fermentation step by coordinately and completely utilizing all glucose and non-glucose sugars.

Here we show a simultaneous and rate-coordinated conversion of the glucose and non-glucose sugars derived from lignocellulose by an adaptively evolved bacterium *Pediococcus acidilactici*, resulting in all the glucose, xylose, arabinose, mannose, and galactose from wheat straw transformed to D-lactic acid during SSCF. The residual sugars were effectively reduced to an extremely low level (~1.7 g/L) and avoided the negative effect in cellulosic D-lactic acid purification and subsequent polymerization reaction for D-lactide. A highly selective biodetoxification fungus *Amorphotheca resinae* ZN1 was applied to effectively remove the inhibitors' impurities. After the sugars and inhibitors were removed, cellulosic D-lactic acid was successfully synthesized to D-lactide for the first time by depolymerization and polycondensation reactions after a regular purification step. The properties of cellulosic D-lactide were characterized and well agreed with those of the starch-derived commercial D-lactide product. This study overcomes the barrier of residual sugar impurities for D-lactide synthesis from abundant lignocellulosic feedstock.

2. Materials and methods

2.1. Strains, media, and reagents

The adaptively evolved *P. acidilactici* ZY15 in this study was obtained after a three-stage adaptive evolution (65 days, E-Supplementary data) of *P. acidilactici* ZY15 (CGMCC #13612) engineered with the introduction of heterologous xylose-assimilating pathway in previous study (Qiu et al., 2017). The first, second and third stage lasted 38, 15, and 12 days, respectively. In the first stage, *P. acidilactici* was cultured in the simplified MRS medium containing 70 g/L glucose, 50 g/L xylose, 8 g/L arabinose, 2 g/L mannose, and 2 g/L galactose, but the xylose utilization rate of *P. acidilactici* decreased with the repeated cell transfer. In the second stage, to enhance the xylose utilization, the xylose concentration in the medium increased to 110 g/L to provide a stronger xylose driving force, while the glucose concentration reduced to 10 g/L to lessen the driving force of glucose. When the xylose utilization become stable, the sugar concentrations in the medium were regulated to the same levels in the first stage to start the third stage adaptive evolution of *P. acidilactici* until the sugars consumption recovered and stabilized. Finally, the adaptively evolved strain *P. acidilactici* ZY15 was obtained with stabilized hexose and pentose sugar utilization.

The *P. acidilactici* seeds were cultured at 42 °C, 150 rpm shaking in simplified Man-Rogosa-Sharp (MRS) medium containing 20 g/L of glucose, 10 g/L of peptone, 10 g/L of yeast exact, 5 g/L of sodium acetate, 2.0 g/L of ammonium citrate dibasic, 2 g/L of dipotassium phosphate, 0.58 g/L of magnesium sulfate heptahydrate, 0.25 g/L of manganese sulfate monohydrate. The biodetoxification fungus *Amorphotheca resinae* ZN1 (CGMCC #7452) was cultured at 28 °C on potato dextrose agar (PDA) slant containing 200 g/L of potato extract juice and 20 g/L of glucose.

Commercial D-lactic acid product (with D-lactic acid of 90%) was purchased from Macklin (Shanghai, China). Stannous octoate reagent (purity of 95%) was from Adamas (Shanghai, China). A commercial cellulase Cellic CTec 2.0 was purchased from Novozymes China with the filter paper activity (203.2 FPU/ml), cellobiase activity (4900 CBU/ml) and protein concentration (87.3 mg/ml) determined by NREL protocols LAP-006 (Adney and Baker, 1996), the method of Ghose (Ghose, 1987) and Bradford method (Bradford, 1976), respectively. Total Phenol (TP) Kit was purchased from Suzhou Grace Biotech (Suzhou, China). The fine activated carbon powders with the size of 200 mesh, glucose, Bradford Protein Quantification Kit, and other general reagents were purchased from General Reagent Co. (Shanghai, China).

2.2. Wheat straw feedstock and biorefinery operations

Wheat straw feedstock was collected from Nanyang, Henan, China in 2020. The raw feedstock contained 32.9% (w/w) of cellulose, 23.3% (w/w) of hemicellulose, 21.4%(w/w) of lignin, and 11.3% (w/w) of ash determined by the method in Sluiter et al. (2008, 2012). The wheat straw was water-washed and air-dried, then coarsely milled and screened through the sieve with 10 mm circle diameter. The wheat straw was then conducted with the dry acid pretreatment and zero wastewater was generated from the high solids loading pretreatment (He et al. 2014; Zhang et al., 2011; Zhang et al., 2023). Briefly, the dry wheat straw was added into the 20L helically agitated reactor with 2.5%(w/w) dilute sulfuric acid solution. The ratio of solids to liquid was 2:1(w/w), equivalent to that 1.25 g sulfuric acid was added to per 100 g of the dry wheat straw. The pretreatment was conducted at 175 °C, 50 rpm for 5 min. The pretreated wheat straw was biodetoxified by the fungus *A. resinae* ZN1 to selectively and effectively remove the inhibitors from the pretreatment (He et al., 2016).

2.3. D-lactic acid fermentation, purification, and D-lactide synthesis

D-lactic acid was fermented using these wheat straw after pretreatment and biodetoxification by SSCF with 10% (v/v) inoculation of *P. acidilactici* ZY15 seeds (Qiu et al., 2017).

The D-lactic acid broth was removed with insoluble lignin residues by centrifugation at 11,180 g for 10 min, decolorized by activated carbon powders, crystallized to obtain the calcium D-lactate precipitates, followed by the sulfuric acid acidification to obtain the free D-lactic acid, and then cation resin adsorption to remove metal ions according to He et al. (2022). Decolorization was slightly modified into two steps, in which 7% (w/v) of activated carbon was used in the first step, then the activated carbon cake was water-washed (1/4 of the volume of fermentation broth) by vacuum filtration, followed by a second step decolorization with 3% (w/v) of activated carbon.

D-lactide synthesis was conducted using D-lactic acid after purification by polycondensation and depolymerization according to the method in He et al. (2022). Briefly, the polycondensation reaction of D-lactic acid was conducted at 150 °C for 1–2 h in a 250 mL three-port glass flask with the addition of 3%(w/w) of stannous octoate catalyst. The depolymerization was conducted by quickly increasing the temperature to 240–260 °C at vacuum condition (0.098 MPa) till no liquid was left in the glass flask.

2.4. Analyses of fermentation products

Glucose, xylose, furfural, HMF, acetic acid, and lactic acid were analyzed using HPLC (refractive index detector RID-10A, Shimadzu, Kyoto, Japan) with Aminex HPX-87H column (Bio-Rad, Hercules, CA) at 65 °C. 5 mM H_2SO_4 was used as the mobile phase with a flow rate of 0.6 mL/min.

Arabinose, mannose, and galactose were analyzed using HPLC (refractive index detector RID-10A, Shimadzu, Kyoto, Japan) with a Aminex HPX-87P column (Bio-rad, Hercules, CA) at 80 °C. The sterilized ultra-pure water was used as the mobile phase with a flow rate of 0.6 mL/min.

The phenolic aldehydes inhibitors (4-Hydroxybenzaldehyde, vanillin, and syringaldehyde) were analyzed using HPLC (UV/Vis detector SPD-20A, Shimadzu, Kyoto, Japan) at 270 nm with a YMC-Pack ODS-A column (YMC, Kyoto, Japan) at 35 °C. The determination was conducted by a gradient procedure as described in Hu et al. (2018). The mobile phase was 0.1% formic acid (eluent A) and 100% acetonitrile (eluent B) at 1.0 mL/min.

Proteins were measured by Bradford method (Bradford, 1976) and total phenolics were determined by Folin & Ciocalteu method (Ainsworth and Gillespie, 2007). The ions (Ca^{2+}, K^{+}, Na^{+}, Mg^{2+}, Cl^{-}) contents were determined by the Inductively Coupled Plasma-Optical Emission Spectrometer (725 ICP-OES, Agilent) (Han and Bao, 2018).

2.5. D-lactide characterization

The molecular structure (^{1}H NMR spectrum), functional groups (FT-IR spectrum), melting point (DSC curve), molecular weight (MS-spectra), and element contents of D-lactide were characterized by the methods in He et al. (2022). The chiral purity was determined by HPLC (SPD-20A UV detector, Shimadzu, Kyoto, Japan) at 220 nm with a chiral column Superchiral S-IG (Chiralway Biotech, Shanghai, China) at 40 °C. The mobile phase was a 90/10/0.05 (volume) ratio of n-hexane/ethanol/trifluoroacetic acid solution at 0.9 mL /min flow rate.

3. Results and discussion

3.1. Bioconversion of wheat straw into D-lactic acid by dry biorefinery processing

Dry acid pretreatment was performed on wheat straw with zero wastewater stream generation by the protocols in previous study (Table 1) (He et al., 2014; Zhang et al., 2011). Most of hemicellulose was removed in the pretreatment and there was a considerable accumulation of inhibitors in the pretreated wheat straw (23.0 ± 1.0 mg acetic acid; 5.8 ± 0.0 mg furfural; 3.5 ± 0.0 mg 5-hydroxymethylfurfural (HMF); 0.4 ± 1.0 mg 4-hydroxybenzaldehyde (HBA); 3.3 ± 0.0 mg syringaldehyde; 3.3 ± 0.0 mg vanillin /g dry wheat straw). Fungus *A. resinae* ZN1 was applied to degrade inhibitors to carbon dioxide and water with the soluble sugars well preserved (He et al., 2016; Yi et al., 2019), in which furfural, HMF and acetic acid were totally eliminated and most of phenolic inhibitors were degraded (54%-82%). The residual phenolics (syringaldehyde, 1.5 ± 0.1 mg/g; vanillin, 0.6 ± 0.1 mg/g; HBA, 0.1 ± 0.0 mg/g) were totally eliminated after the following decolorization step. The inhibitor impurities, which negatively affect the purity and yield of cellulosic D-lactic acid, were thus solved. There are at least five types of monosaccharide sugars released from the pretreatment and enzyme hydrolysis of lignocellulose, including glucose and the four non-glucose monosaccharides (xylose, arabinose, mannose, and galactose). Incomplete utilization of fermentable sugars results in sugar residues and these sugar residues are hard to remove completely by regular downstream purification, which then causes obstacles to synthesize the chiral lactide (Joglekar et al., 2006). To achieve complete sugar consumption, the adaptively evolved strain *P. acidilactici* ZY15 with the pentose metabolic pathway was applied to convert the fermentable sugars from wheat straw into D-lactic acid by SSCF (Fig. 1). The full spectrum of lignocellulose-derived hexose and pentose sugars were simultaneously and coordinately transformed to high titer D-lactic acid (128.1 g/L) at the observable equal consumption rate (Fig. 1). The D-lactic acid showed the high chirality (99.07%) with only minor residual sugars left (glucose, ~0.7 g/L; xylose, ~0.6 g /L; arabinose + mannose, ~0.2 g /L; galactose, ~0.3 g /L). The efficient conversion of all the sugars is important as the residual sugars cause significant downstream processing problems in purifying cellulosic D-lactic acid and synthesizing D-lactide.

3.2. Purification of cellulosic D-lactic acid broth

Cellulosic D-lactic acid broth underwent five conventional purification processes (solids and liquid separation, decolorization, crystallization, acidification, and adsorption) to remove the sugar, protein, phenolics, and ion impurities in the same way as L-lactic acid purification (He et al., 2022), but a modified two-step decolorization was introduced to remove the higher concentratiion of phenolics. The total sugar residues (43.0 ± 3.4 mg/g lactic acid), the proteins (3.1 ± 0.2 mg/

Table 1
Wheat straw compositions before and after dry acid pretreatment and biodetoxification.

Components	Virgin wheat straw (mg/g DM)	After pretreatment (mg/g DM)	After biodetoxification (mg/g DM)
Cellulose	323.3 ± 0.7	335.0 ± 5.2	/
hemicellulose	231.9 ± 2.4	31.7 ± 3.2	/
Acetic acid	ND	23.0 ± 1.0	ND
Furfural	ND	5.8 ± 0.0	ND
5-Hydroxymethylfurfural (HMF)	ND	3.5 ± 0.0	ND
4-Hydroxybenzaldehyde (HBA)	ND	0.4 ± 1.0	0.1 ± 0.0
Vanillin	ND	3.3 ± 0.0	0.6 ± 0.1
Syringaldehyde	ND	3.3 ± 0.0	1.5 ± 0.1

DM, dry feedstock matter; ND, not detected by HPLC; /, the cellulose and hemicellulose contents in the biodetoxified wheat straw were not determined.

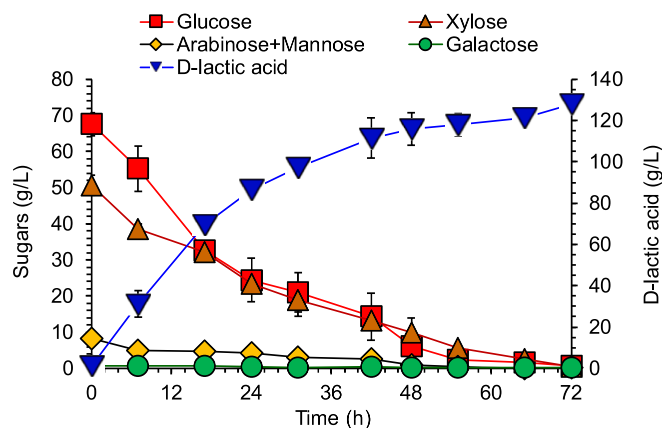

Fig. 1. Simultaneous saccharification and co-fermentation (SSCF) of cellulosic D-lactic acid by adaptively evolved *P. acidilactici* ZY15. The pretreated and biodetoxified wheat straw at 30%(w/w) of solids loading was pre-hydrolyzed at 50 °C, 150 rpm stirring for 6 h in a 5 L bioreactor. The cellulase dosage was 4 mg cellulase proteins per gram of dry wheat straw. The SSCF was conducted at 42 °C, pH 5.5 with 10% (v/v) inoculum size. The pH was controlled by adding 25% (w/w) $Ca(OH)_2$ slurry. Due to the close retention time of arabinose and mannose peaks on Aminex HPX-87P column, the minor mannose peak was partly overlapped by the arabinose peak, thus the mannose and arabinose were approximately calculated as one component.

g), the residual phenolics (29.6 ± 1.4 mg/g), and the acetate (as fermentation ingredient, 9.4 ± 2.2 mg/g) in the cellulosic D-lactic acid broth were completely eliminated (Fig. 2a). The removal of residual sugars, proteins, and acetate is probably due to the modified twice-activated carbon decolorization step with the first 7% (w/v) and the second 3% (w/v) of the activated carbon adsorption. The repeated decolorization exhibited a better effect of removing impurities than that of conducting only one decolorization using 10% (w/v) of the activated carbon adsorption in the previous study (He et al., 2022). The improved decolorization effect brought about a chain reaction, in which the calcium D-lactate crystals were easier to precipitate from the mother liquor with fewer impurities attached to the surface of the crystals, and the impurities were removed more thoroughly. The relative high contents of Ca^{2+} (305.8 ± 12.0 mg/g) and K^{+} (69.4 ± 2.8 mg/g) were strongly reduced to below 3.6 mg/g; the contents of Na^{+} (20.7 ± 1.5 mg/g) and Cl^{-} (32.6 ± 2.3 mg/g) were reduced to below 2.8 mg/g, respectively; the low Mg^{2+} content (5.7 ± 0.3 mg/g) was completely removed (Fig. 2b). No significant D-lactic acid loss was observed in any of the purification steps, except in the decolorization step using the activated carbon powders adsorption. The activated carbon filter cake after decolorization was water-washed only once, leading to a rather high loss of D-lactic acid (~21%) after this step (Supplementary Materials). This loss can likely be avoided in industrial processes by multiple washing and a higher recovery yield should be obtained. After the regular purification steps, the impurities remained in cellulosic D-lactic acid broth (sugars, proteins, total phenolics, and ions) had been effectively removed and purified cellulosic D-lactic acid was prepared.

3.3. Synthesis and characterization of D-lactide

The cellulosic D-lactic acid after purification was used to synthesize D-lactide catalyzed by the regular stannous octoate and the synthesized cellulosic D-lactide were characterized by detail with the starch-derived D-lactide samples as control (E-Supplementary data). The purified D-lactide appeared as white powders, similar to that of a commercial D-lactide synthesized from starch-derived D-lactic acid. The cellulosic D-lactide was characterized with the control of starch-derived D-lactide sample. The molecular weight of cellulosic D-lactide was determined as 144.0581 and same as the control (144.0581). The H-NMR spectrogram displayed peaks that were consistent with the control, including the double peaks at $\delta = 1.63–1.64$ and the quadruple peaks at $\delta = 5.03–5.06$ with 1:1 and 1:3:3:1 as the intensity ratio, respectively. The element contents of cellulosic D-lactide were 49.72 ± 0.00% (carbon) and 5.49 ± 0.01% (hydrogen), respectively, which were consistent with that of the control (49.76 ± 0.02% and 5.39 ± 0.12%). The infrared spectrum charts showed that the characteristic peaks were well agreed with those of the control, including the stretching vibration peaks (1099 cm^{-1} and 1272 cm^{-1}) of C–O–C ester, the bending vibration peaks of –CH (1384 cm^{-1}) and $–CH_3$ (1457 cm^{-1}), the stretching vibration peaks of ester C=O (1766 cm^{-1}), –CH (2933 cm^{-1}) and $–CH_3$ (2998 cm^{-1}), and the ring skeleton vibration peaks (651 cm^{-1} and 935 cm^{-1}). The melting point of the cellulosic D-lactide was 96.1 °C, which was comparable to the values reported in other studies (95–98 °C) and the control (95.2 °C) (Cunha et al., 2022). The determination of chiral purity was conducted to cellulosic D-lactide and the value was calculated by measuring the peak area for D-lactide at 9.86 min using HPLC with a chiral column Superchiral S-IG. A single peak of D-lactide appeared on the chromatogram and the chiral purity of D-lactide was calculated based on the peak area as 99.99%. In summary, the characterizations of the cellulosic D-lactide well agreed with that of the starch-derived D-lactide, confirming that D-lactide was successfully produced using D-lactic acid from lignocellulose.

In this study, the main purpose is to evaluate the feasibility of the D-lactide synthesis from lignocellulose-derived D-lactic acid, and D-lactide synthesis was conducted in a 250 mL three-port glass flask. However, this experimental setup is not suitable for the quantification of D-lactide. The insufficient insulation of the experimental setup resulted in the accumulation of most of the synthesized D-lactide in the entry port section and the cooling tube when D-lactide was vaporized at 240–260 °C. The same phenomenon occurred in the D-lactide synthesis using both cellulosic and commercial D-lactic acid. The accurate quantitation of D-lactide yield will be conducted when the setup and catalyst are improved.

Here the cellulosic D-lactic acid production (Table 2) was compared with previous cellulosic L-lactic acid production (He et al., 2022). High titers (~130 g/L), high chiral purities (99.07–99.60%) and minor sugar residues (1.7–2.2 g/L) were achieved by the engineered *P. acidilactici* ZY15 and *P. acidilactici* ZY271 both in cellulosic D- and L-lactic acid production. The yield and productivity of D-lactic acid were equally high as those of L-lactic acid (0.70 g/g and 1.80 g/L/h, respectively). These results indicated that equally high titer and high chirality of lactic acid were achieved with minimal sugar residues by applying the biorefining strategy (dry-acid pretreatment and biodetoxification) to lignocellulose with the followed SSCF by *P. acidilactici*. Also, zero wastewater was produced during the biorefining processes. The cellulosic D-lactic acid production was also compared with that in the recently reported studies (E-Supplementary data). Complete consumption of the sugars from lignocellulose is a precondition for obtaining D-lactic acid free of sugar residues used for D-lactide synthesis. The utilization of the five lignocellulose-derived pentose and hexose sugars was not well satisfied in previous studies (Campos et al.,2021; Prasad et al., 2020; Utrilla et al., 2016; Zhang et al., 2016a; Zhang et al., 2016b) and this study showed the only exception for highly simultaneous and coordinate utilization of all sugars. Engineered *Lactobacillus plantarum* produced D-lactic acid (22–61.4 g/L) from corn stover and sorghum stalks, but only glucose and xylose were co-utilized and the xylose consumption rate was much slower than that of glucose (Zhang et al., 2016a; Zhang et al., 2016b). Engineered *Escherichia coli* showed the utilization of xylose and arabinose with glucose, but no data for mannose and galactose. More importantly, the consumption rates of xylose and arabinose were not coordinated with glucose consumption, resulting in relatively high concentration of sugar residues (~7 g/L xylose and arabinose) and lower D-lactic acid titer (~60 g/L) from corn stover (Utrilla et al., 2016). Engineered *P. acidilactici* strains and the consequent adaptive evolved strains had been used for utilization of

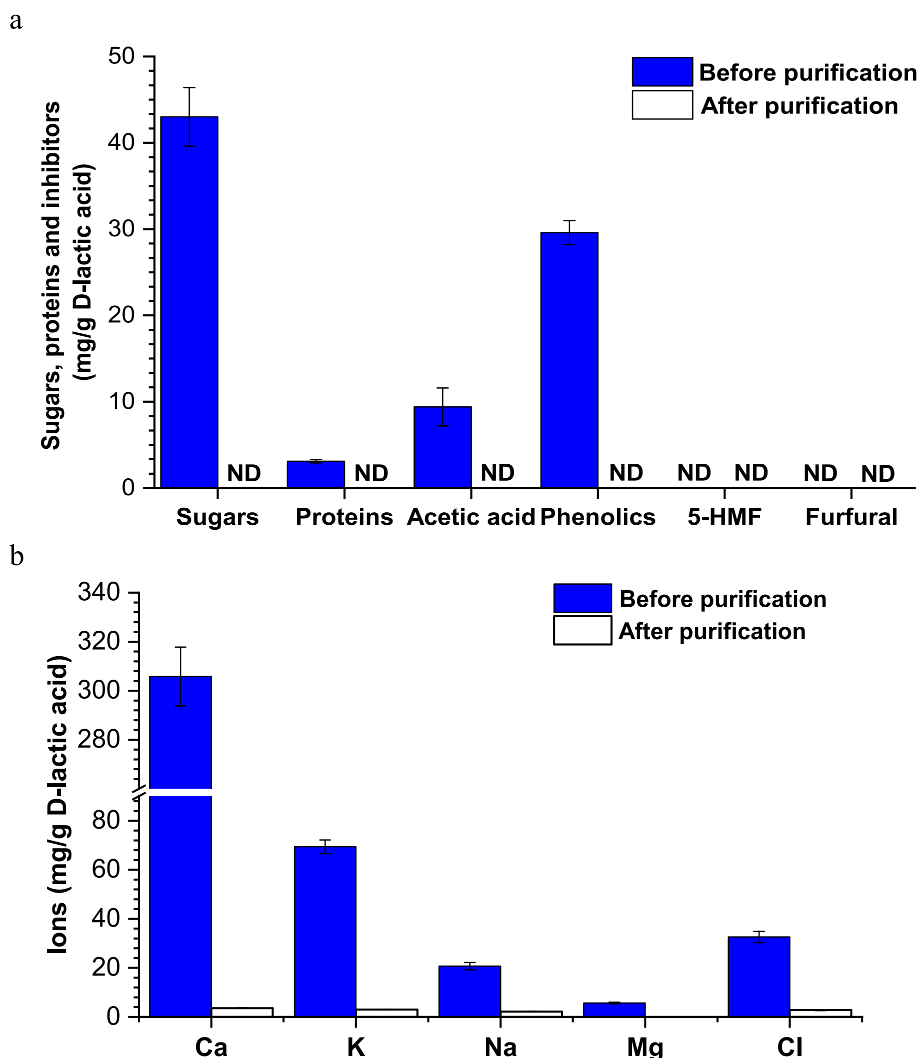

Fig. 2. Purification of cellulosic D-lactic acid broth and the impurities changes. (a) Changes in the residual sugars, proteins, acetic acid, phenolics, HMF, and furfural contents in the cellulosic D-lactic acid broth before and after the purification; (b) Changes of ions contents in the cellulosic D-lactic acid broth before and after the purification. The purification and the analysis methods were described in the Materials and Methods. All the assays were performed in duplicate.

Table 2
The production of cellulosic D-lactic acid and D-lactide using lignocellulose feedstock.

Lactic acid properties	D-lactic acid
Feedstock	Wheat straw
Pretreatment	Dry acid pretreatment
Detoxification	Biodetoxification by *A. resinae* ZN1
Fermentation	SSCF by *P. acidilactici* ZY15 at 30% (w/w) solids loading
Chiral purity (%)	99.07
Yield (g/g sugars)	0.69
Product titer (g/L)	128.1
Productivity (g/L/h)	1.78
Residual sugars (g/L)	~1.7
Wastewater (g/g lactic acid)	0
Lactide properties	**D-lactide**
Element composition (%)	C: 49.72 ± 0.00% H: 5.49 ± 0.01%
Molecular weight	144.0581
Melting point (°C)	96.10
Chiral purity (%)	99.99[a]

[a] Calculated as the ratio of the peak area values of D-lactide and that of the L-lactide and D-lactide.

various lignocellulose-derived glucose and xylose (Qiu et al., 2017; Qiu et al., 2022; Qiu et al., 2023). *P. acidilactici* ZP26 was discovered to be capable of utilization of both glucose and mannose from spruce (Campos et al., 2021). In this study, the adaptive evolved strain *P. acidilactici* simultaneously converted the full spectrum of lignocellulose-derived glucose, xylose, arabinose, mannose and galactose to high titer of D-lactic acid (128.1 g/L) with extremely low level of sugar residues (~1.7 g/L). The consumption rates of xylose, arabinose, mannose, and galactose were coordinated with glucose consumption: all sugars were consumed at almost one point in time (72 h) (Fig. 1). This study provides an excellent *P. acidilactici* candidate to produce cellulosic D-lactic acid and negligible sugar residues was remained, which reduces the difficulties of polymerization-grade D-lactic acid purification and promotes the sequent D-lactide synthesis. For D-lactide synthesis, this study is the only successful D-lactide synthesis using D-lactic acid from lignocellulose feedstock.

Here the synthesized D-lactide was compared with previous cellulosic L-lactide synthesis (He et al., 2022). The similar element compositions (C: ~49.70%, H: 5.49–5.67%), melting point values (~96 °C) and the same molecular weight values (144.0581) were found between cellulosic D- and L-lactide. High chirality was achieved at 99.99% and 98.99% in the production of cellulosic D- and L-lactide, respectively. All the characterizations of cellulosic D- and L-lactide were consistent with

the theoretical values and reported values.

Lignocellulosic biomass is likely the most suitable substitute for starch to produce chiral lactic acid. However, the residual sugar impurities, caused by the incomplete utilization of sugars during D-lactic acid fermentation, made it difficult to obtain polymerization-grade cellulosic D-lactic acid to synthesize D-lactide. This study makes up for the vacancy of cellulosic D-lactide production using cellulosic D-lactic acid and laid the groundwork for producing high-performance PDLA from abundant lignocellulosic biomass. Till now, chiral lactide were successfully produced from cellulosic chiral lactic acid, which promotes the development of industrial-scale high-performance PLA production from lignocellulose as carbon neutral carbohydrates feedstock.

4. Conclusions

The adaptively evolved bacterium *P. acidilactici* ZY15 was applied to coordinately and effectively utilize the hexose and pentose sugars from lignocellulose and produce D-lactic acid with high titer. Importantly, the residual sugars were reduced to an extremely low level (~1.7 g/L), which overcome the crucial technical barrier of residual sugars for D-lactide synthesis and promoted successful synthesis of polymerization grade D-lactide.

CRediT authorship contribution statement

Niling He: Conceptualization, Methodology, Validation, Data curation, Visualization, Writing – original draft, Writing – review & editing. **Mingxing Chen:** Methodology, Data curation, Formal analysis, Visualization, Writing – original draft. **Zhongyang Qiu:** Conceptualization, Data curation, Supervision. **Chun Fang:** Conceptualization, Data curation, Supervision. **Gunnar Lidén:** Conceptualization, Writing – review & editing. **Xiucai Liu:** Conceptualization, Writing – review & editing. **Bin Zhang:** Writing – review & editing. **Jie Bao:** Conceptualization, Methodology, Supervision, Writing – review & editing, Funding acquisition, Project administration.

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. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2023.128950.

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