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Fast fluidization of dry acid pretreated wheat straw and consequent continuous bioconversions for L-lactic acid production

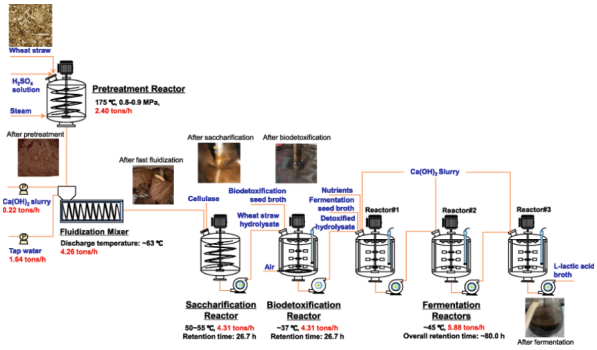
Tao Han [a], Ya Wang [b], Bin Zhang [a], Jie Bao [a,*]

[a] State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, 130 Meilong Road, Shanghai 200237, China
[b] School of Chemistry and Chemical Engineering, Shihezi University, Shihezi, Xinjiang 832000, China

HIGHLIGHTS

- A fast fluidization and continuous high-solids loading biorefinery process was developed.
- Hot pretreated wheat straw was fluidized to slurry without cellulase participation.
- The continuous biorefining process achieved high L-lactic acid production.

GRAPHICAL ABSTRACT



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ABSTRACT

Materials flow of lignocellulose biomass in fluid form suitable for transportation in pumps and pipelines is prerequisite for continuous biorefinery process at industrial scale. A continuous process for L-lactic acid production was developed starting from fast fluidization of hot pretreated wheat straw solids into homogeneous and transportable slurry at moderate temperature, followed by continuous saccharification, biodetoxification, and fermentations. The final multi-stage continuous fermentation converted all the wheat straw derived sugars into 143.1 ± 0.9 g/L of L-lactic acid at the rate of 1.79 ± 0.01 g/L/h, the yield of 0.79 ± 0.01 g/g total sugars, and the chiral purity of 99.3% ± 0.1%. The performance using industrial wheat straw feedstock at a uniform dilution rate of 0.0375 h^{-1} (residence time of ~26.7 h), zero wastewater and toxin discharges was close to that of dry-mill process using corn feedstock. This study provided a prototype of completely continuous biorefining under high solids loading of lignocellulose utilization.

1. Introduction

Continuous operation is the standard form for highly efficient industrial processes (Ruiz et al., 2020; López-Gómez et al., 2019; Brethauer et al., 2014). The prerequisite condition for operating a continuous biorefinery process is the materials flow of lignocellulose biomass in transportable fluid forms through pipelines, pumps, valves, and enclosed vessels, instead of in solid form through open transport

* Corresponding author.
E-mail address: jbao@ecust.edu.cn (J. Bao).

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belt. Biorefining process chain starts with pretreatment to disrupt the robust lignocellulose structure, followed by enzymatic saccharification to produce fermentable sugars, detoxification to remove inhibitors generated from pretreatment, fermentation (or simultaneous saccharification and co-fermentation, SSCF) to produce target products, and then purification to the refined target products (Liu et al., 2018; He et al., 2022; Duan et al., 2024). The current trend of biorefinery processing is high solids-loading from the starting pretreatment to the ending purification for the purposes of high titer, high productivity, and high yield of the target products. The high solids-loading pretreatment will delete any wastewater generation to yield the pretreated lignocellulose in solids form, and substantially reduce energy demand, toxic substance emission, and solid–liquid separation costs (Zhang et al., 2023; Shiva et al., 2022; Liu and Bao, 2017). However, the pretreated lignocellulose material flow in solids form will also create great challenges to establish a full continuous bioconversion operation.

Previous studies on continuous biorefining processes focused on individual steps of enzymatic saccharification (Brunecky et al., 2025; Brethauer et al., 2011; Lischeske and Stickel, 2019), detoxification (Singh et al., 2019; Fayet et al., 2018), or fermentation (Ahring et al., 2016; Wirawan et al., 2020; Ma et al., 2016). A major drawback in these studies is the low solid loading, which inevitably results in low product titers and productivities, along with huge wastewater generation. To achieve a continuous biorefining process under high solids-loading conditions, the pretreated lignocellulose solids should be transformed into a fluid form as soon as the discharge of the pretreated solids from pretreatment reactors. The liquid fluid or liquid slurry could be transported by mechanical pumps in enclosed pipelines or vessels to ensure an accurate quantitative flowrate control and prevent microbial contaminations during subsequent continuous operations of continuous enzymatic saccharification, detoxification, fermentation, and purifications. To achieve the fast fluidization and subsequent continuous operations of lignocellulose feedstocks under high solids-loading pretreatment, the key challenges need to be addressed include: (i) How to quickly fluidize the high-temperature pretreated solids (100–110 ℃) into a uniform fluid slurry; (ii) How to completely remove the inhibitors while minimizing the fermentable sugar loss during continuous biodetoxification; (iii) How to reduce the fermenter numbers while achieving the satisfactory product titer, yield, and the minimal residual sugars.

This study used the dry biorefining technology as the technical platform to investigate the fast fluidization and continuous bioconversion operations for L-lactic acid production using wheat straw as feedstock (Zhang et al., 2023; Han et al., 2023). The dry biorefining process maintains a high solids-loading condition from the starting dry acid pretreatment to the subsequent steps of enzymatic saccharification, biodetoxification, and fermentation with zero generations of wastewater, solids, toxins, or solvent with the similar bioconversion efficiency and techno-economic performance to those of dry mill technology using corn as the feedstock (Liu et al., 2018; Zhang et al., 2021). The results revealed that enzyme-free fast fluidization at high temperature enabling fully continuous operation. The hot dry acid pretreated wheat straw solids could be transformed into a pumpable fluidized slurry without any enzyme addition, then the formed slurry can be used for continuous L-lactic acid production through continuous saccharification, biodetoxification, and fermentation processes. This study developed a technical prototype for industrial-scale continuous biorefining processing for L-lactic acid production or other biobased products using lignocellulosic feedstocks.

2. Materials and methods

2.1. Feedstocks

Wheat Straw #1 was harvested in Jining City, Shandong Province, China in August 2022. The feedstock was stored in sealed bags after simple mechanical crushing to a particle size of approximately 10 mm. Wheat Straw #1 contained 32.33% ± 0.13% of cellulose, 21.25% ± 1.78% of xylan, 19.27% ± 4.85% of lignin, and 10.63% ± 1.89% of ash by NREL protocol (Sluiter et al., 2012).

Wheat Straw #2 was harvested in Yangqu County, Shanxi Province, China in the fall 2022 provided by Cathay Biotech Inc in Bioindustry Park, Taiyuan, Shanxi Province, China. Wheat Straw #2 was mechanically milled and de-dusted to obtain the solids with an average length of 10–30 mm. The composition was 35.42% ± 0.31% of cellulose, 24.91% ± 0.34% of xylan, 19.20% ± 1.23% of lignin, and 9.57% ± 1.74% of ash according to the same NREL protocol (Sluiter et al., 2012).

2.2. Enzymes and reagents

Cellulase Cellic CTec3 HS was purchased from Novozymes (China), Beijing, China. The protein concentration of 90.1 ± 4.4 mg/mL was determined by Bradford method (Bradford, 1976). The enzymatic activity was > 1000 Biomass Hydrolysis Units/g according to the maker's product specification instruction (Analytical Method, Biomass Hydrolysis Activity by Fluorescence Cellulose Decay (FCD), Novozymes A/S, Denmark, 2019–06-27).

Glucoamylase GA-L NEW was purchased from Genencor, Jiangsu Province, China with an enzymatic activity of 103,900 Wohlgemuth Units/mL according to the maker's instruction. The enzyme was only used in the seed culture of lactic acid bacterium strain to prevent cells flocculation (Liu et al., 2015).

Yeast extract and peptone were purchased from Oxoid, Hampshire, UK. Dextrose monohydrate, calcium hydroxide and other regular reagents were purchased from Titan Scientific Co., Shanghai, China.

2.3. Microorganisms and media

The biodetoxification strain *Paecilomyces variotii* FN89 (Chinese General Microorganism Collection Center, CGMCC, with the registry number 17665) was activated on a Potato-Dextrose-Agar (PDA) petri dish for 2–3 days, then the spores were harvested using 0.05% (w/w) Tween-80 solution. The obtained spore suspension was then inoculated into seed medium contained 2 g/L KH_2PO_4, 1 g/L $CaCO_3$, 1 g/L $(NH_4)_2SO_4$, 1 g/L yeast extract, 1 g/L $MgSO_4{\cdot}7H_2O$, and 22 g/L dextrose monohydrate. The strain was cultured at 37 ℃ and 300 rpm for 24 h as the biodetoxification seed broth.

Pediococcus acidilactici ZB220 (CGMCC M2023151) is an engineered L-lactic acid producing lactic acid bacterium with highly coordinated co-utilization capacity of pentose and hexose derived from lignocellulose feedstock. *P. acidilactici* ZB220 was inoculated into Man-Rogosa-Sharp (MRS) medium at an inoculation ratio of 10% (v/v) and activated at 42 ℃, 150 rpm for 6 h. Subsequently, the activated broth was transferred to the fresh MRS medium at the same inoculum size, and simultaneously added the glucoamylase at a dosage of 1% (v/v) and calcium carbonate at a concentration of 12 g/L, then cultured for 6 h to obtain the L-lactic acid fermentation seed broth. The MRS medium included 22 g/L dextrose monohydrate, 10 g/L peptone, 10 g/L yeast extract, 5 g/L sodium acetate, 2 g/L ammonium citrate dibasic, 2 g/L K_2HPO_4, 0.58 g/L $MgSO_4{\cdot}7H_2O$, and 0.25 g/L $MnSO_4{\cdot}H_2O$. The fermentation nutrients included:10 g/L peptone, 15 g/L yeast extract, 2 g/L ammonium citrate dibasic, and 0.25 g/L $MnSO_4{\cdot}H_2O$.

2.4. Dry acid pretreatment

Wheat Straw #1 was pretreated in a 20 L bench reactor with the solid-to-liquid ratio of 2:1 (w/w), sulfuric acid dosage of 38 mg/g dry straw, 175 ± 0.5 ℃ for 5 min under well mixing condition by helical ribbon impeller (He et al., 2014a; He et al., 2014b). The bench reactor pretreated wheat straw solids (PWS#1) contained 30.07% ± 0.39% of cellulose, 1.39% ± 0.33% of xylan, 29.20 ± 0.21 mg/g of glucose, 129.10 ± 11.89 mg/g of xylose, 17.73 ± 2.80 mg/g DM of acetic acid,

1.94 ± 1.28 mg/g DM of 5-hydroxymethylfurfural (HMF), and 5.40 ± 1.26 mg/g DM of furfural based on the dry matter weight (DM) (Sluiter et al., 2008).

Wheat Straw #2 was pretreated in a 10 m^3 industrial reactor in the Bioindustry Park, Taiyuan, Shanxi Province, China with the solid-to-liquid ratio of 2:1 (w/w), the sulfuric acid dosage of 40 mg/g under 175 ± 1 ℃ for 5 min using the helical ribbon impeller agitation at 10 rpm. The industrial reactor pretreated wheat straw solids (PWS#2) containing 32.42% ± 0.95% of cellulose, 4.39% ± 0.33% of xylan, 30.83 ± 0.57 mg/g of glucose, 136.70 ± 1.98 mg/g of xylose, 5.67 ± 0.68 mg/g of *gluco*-oligosaccharides, 20.38 ± 0.58 mg/g of *xylo*-oligosaccharides, 30.46 ± 0.23 mg/g of acetic acid, 3.62 ± 0.01 mg/g of HMF, 8.94 ± 0.06 mg/g of furfural based on the dry matter weight (DM) (Sluiter et al., 2008).

2.5. Fast fluidization

The fast fluidization of the freshly pretreated wheat straw solids was conducted in a screw mixer (Kunshan Kexin Machinery Co., Kunshan, Jiangsu Province, China) by simultaneously and continuously feeding the hot pretreated wheat straw solids (~100 ℃, pH ~ 2.4) with calcium hydroxide slurry (20%, w/w) and tap water (~25 ℃) into a fluidized wheat straw slurry with the solids content of ~ 30% (w/w) and the pH value of ~ 5.1.

2.6. Continuous enzymatic saccharification

The continuous enzymatic saccharification was performed in a 5 L bioreactor with helically mixing by feeding the fluidized wheat straw slurry into the reactor and discharging the saccharified wheat straw hydrolysate from the reactor every 8 h.

The saccharification was performed under the solids loading of ~ 30% (w/w) at 50 ℃ with the cellulase dosage of 4 mg total proteins/g dry solids matter and well mixing condition. Briefly, 30% of the hydrolysate in the reactor was withdrawn by a vacuum pump every 8 h (equivalent to 3.75% each hour, corresponding to a dilution rate of 0.0375 h^{-1} or a retention time of ~ 26.7 h), followed by the immediate feeding of the equal weight of the fluidized wheat straw slurry and cellulase (with same dosage above) to the reactor. The fluidized wheat straw slurry was heated to ~ 63 ℃ in an oven before the feeding to the continuous saccharification reactor to match the outlet temperature of the pretreated wheat straw solids after fast fluidization.

2.7. Continuous biodetoxification

The continuous biodetoxification was performed in 3 L bioreactor with six-blade Rushton impeller by feeding the saccharified wheat straw hydrolysate and discharging the biodetoxified wheat straw hydrolysate. The wheat straw hydrolysate from the continuous saccharification was cooled to ~ 37 ℃, then continuously fed into the biodetoxification reactor at 37 ℃, an agitation speed of 750 rpm and an aeration rate of 1 vvm. The *P. variotii* FN89 seed broth was inoculated at the ratio of 10% (v/v) and no further re-supplementation during the continuous biodetoxification. No pH adjustment was needed. The endpoint of biodetoxification was determined when the real-time pH value reached its maximum (Han et al., 2023). Briefly, the pH of the wheat straw hydrolysate was measured using a pH electrode (D09120016, Han-Star Analytical Sensor Co., Suzhou, Jiangsu Province, China), then started the new circle of the biodetoxified hydrolysate discharge and fresh hydrolysate feeding. The feeding and discharging ratio to the total hydrolysate was adjusted to let the one-circle detoxification time within 8 h.

For the wheat straw hydrolysate using PWS#1, 60% of the total hydrolysate was discharged at each biodetoxification endpoint every 8 h (equivalent to 7.5% discharge each hour, or a dilution rate of 0.075 h^{-1}), followed by the continuous feeding of the fresh wheat straw hydrolysate at equal weight. For the wheat straw hydrolysate using PWS#2 feedstock, 30% of the total hydrolysate was discharged every 8 h (equivalent to 3.75% discharge each hour, or a dilution rate of 0.0375 h^{-1}), followed by the continuous feeding of the fresh wheat straw hydrolysate at equal weight.

2.8. Continuous L-lactic acid fermentation

The multi-stage continuous L-lactic acid fermentation was performed in 5 L bioreactors by inoculating the seed culture of *P. acidilactici* ZB220 at 10% (v/v) at the start point of operation. No further re-supplementation of seed broth was conducted. The biodetoxified wheat straw hydrolysate was used for the continuous L-lactic acid fermentation in the form of simultaneous saccharification and co-fermentation (SSCF) under the participation of residual cellulase in the hydrolysate. The biodetoxified wheat straw hydrolysate at ~ 45 ℃ was continuously fed into the first fermenter of the three cascade anaerobic fermenters. For every 8 h, a portion of 30% of the total fermentation broth was discharged from the first reactor by a vacuum pump and the same weigh of the biodetoxified wheat straw hydrolysate was fed into the first bioreactor. The discharge broth from the first reactor was fed into the second reactor with the simultaneous discharge of the broth. The similar operation was conducted in the third bioreactor, and the discharged broth from the third reactor was used as the final fermentation product. The nutrients were simultaneously supplemented with the biodetoxified wheat straw hydrolysate to the first reactor including 10 g/L peptone, 15 g/L yeast extract, 2 g/L diammonium citrate, 0.25 g/L $MnSO_4{\cdot}H_2O$. For the wheat straw hydrolysates from both PWS#1 ad PWS#2 feedstocks, the dilution rate during continuous fermentation was maintained the same at 0.0375 h^{-1} (equivalent to the fermentation time 26.7 h for one single reactor, and 80.0 h for the three reactors). During the fermentation process, $Ca(OH)_2$ slurry (20%, w/w) was added for pH control/neutralization to maintain the pH at ~ 5.5.

2.9. Analytical methods

Glucose, xylose, acetic acid, furfural, 5-hydroxymethylfurfural (HMF), and lactic acid were measured using the high-performance liquid chromatography (HPLC) (Shimadzu, Kyoto, Japan) equipped with LC-20AD chromatographic pump and Bio-Rad Aminex HPX-87H column and refractive index RID-10A detector with 5 mM H_2SO_4 solution as the mobile phase at 0.6 mL/min and 65 ℃ according to the previously reported protocols (Liu et al., 2018; He et al., 2022).

The apparent viscosity of the wheat straw hydrolysate samples was measured using Brookfield DV2TLVTJ0 viscometer (Stoughton, Middleboro, USA) at the sampling volume of 5 mL and the shear rate of 43.5 s^{-1} (Wang et al., 2022). The temperature setting was 50 ℃ for the wheat straw hydrolysate samples, 37 ℃ for the biodetoxified hydrolysate samples and 45 ℃ for the fermentation broth samples. Given the liquefying nature of the hydrolysate, using the standard spindles of the viscometer were sufficient for apparent viscosity measurement.

The chiral purity of L-lactic acid therein was measured using the Megazyme D-/L- Lactic Acid Kit (Megazyme International, Wicklow, Ireland).

2.10. Aspen plus modeling

An Aspen Plus model was established for heat exchange calculation of dry acid pretreated wheat straw solids from industrial pretreatment reactors during the fast fluidization mixing with $Ca(OH)_2$ slurry and water based on our previously established Aspen Plus modeling (Wang et al., 2025a; Wang et al., 2025b). The calculated temperature data before the mixing were compared with the experimentally measured data on the industrial operation sites. The model started from the wheat straw feedstock pre-handling steps of milling and de-ashing (WS-FEED).

Sulfuric acid solution (stream H2SO4SOL, ~4% w/w) and the fresh water were sprayed onto the wheat straw feedstock (P202) in the pretreatment reactor (R201) to a solid-to-liquid ratio of 2:1 (w/w). Steam stream (STEAM-IN, ~1.6 MPa) was jetted onto the wheat straw feedstock in the pretreatment reactor. The main reactions during the pretreatment process include the excessive degradation of xylose to furfural, glucose to 5-hydroxymethylfurfural (HMF), and hydrolysis and deacetylation of hemicellulose components to acetic acid. After the pretreatment, the pretreated wheat straw solids were released by two stepwise flashing (S201 and S202) to recover the inhibitors. The screw mixer (M205) was used to fast fluidize the pretreated wheat straw solids by mixing $Ca(OH)_2$ slurry and water in which the neutralization of sulfuric acid took place (R202) and pumped to the next step of the continuous processing. The heat dissipation during the fast fluidization was ignored.

2.11. Statistical analysis

Experiments about screw mixing efficiency evaluation and physicochemical property changes were conducted with at least three independent biological replicates. As for experiments of continuous bioconversion, data represented single steady-state measurements or averages of multiple sampling points (at least 3 points). All data presented as the mean ± standard deviation (SD). Statistical significance between two groups was determined using an unpaired, two-tailed Student's *t*-test. Differences were considered statistically significant at $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$, and were not considered statistically significant when $p > 0.05$.

3. Results and discussion

3.1. Fast fluidization of dry acid-pretreated wheat straw into pumpable slurry

An integrated biorefining flowsheet of fast fluidization and continuous bioconversions was designed based on the dry biorefinery technology as shown in Fig. 1a. The dry acid-pretreated wheat straw solids were discharged from the pretreatment reactor into a receiver through sealed pipeline, then moved to the screw mixer by gravity for fast fluidization. The calcium hydroxide slurry and water were mixed with the wheat straw solids with the simultaneous neutralization and solids content adjustment. The pretreated wheat straw solids were converted to a viscous wheat straw slurry and transported to the saccharification reactor by screw pump or other pumps for transportation of highly viscous liquid slurry system to initiate the continuous saccharification, biodetoxification, and L-lactic acid fermentation.

The temperature of the pretreated wheat straw slurry after fast fluidization was calculated by Aspen Plus modeling as shown in Fig. 1b due to the inaccuracy of heat dissipation control of the on-site pretreated wheat straw solids freshly released from the pretreatment reactors. The calculated temperature (102 ℃) of the pretreated wheat straw solids released from the pretreatment reactor (R201) was approximately in agreement with the experimental data 98 ± 3 ℃, confirming the modeling was approximately correct. The calculated temperature for the pretreated wheat straw slurry after fast fluidization by mixing with $Ca(OH)_2$ slurry and water (M204 and M205), neutralization (R202), and screw transportation (P203 and P204) was ~ 63 ℃ before entering the consequent saccharification reactor.

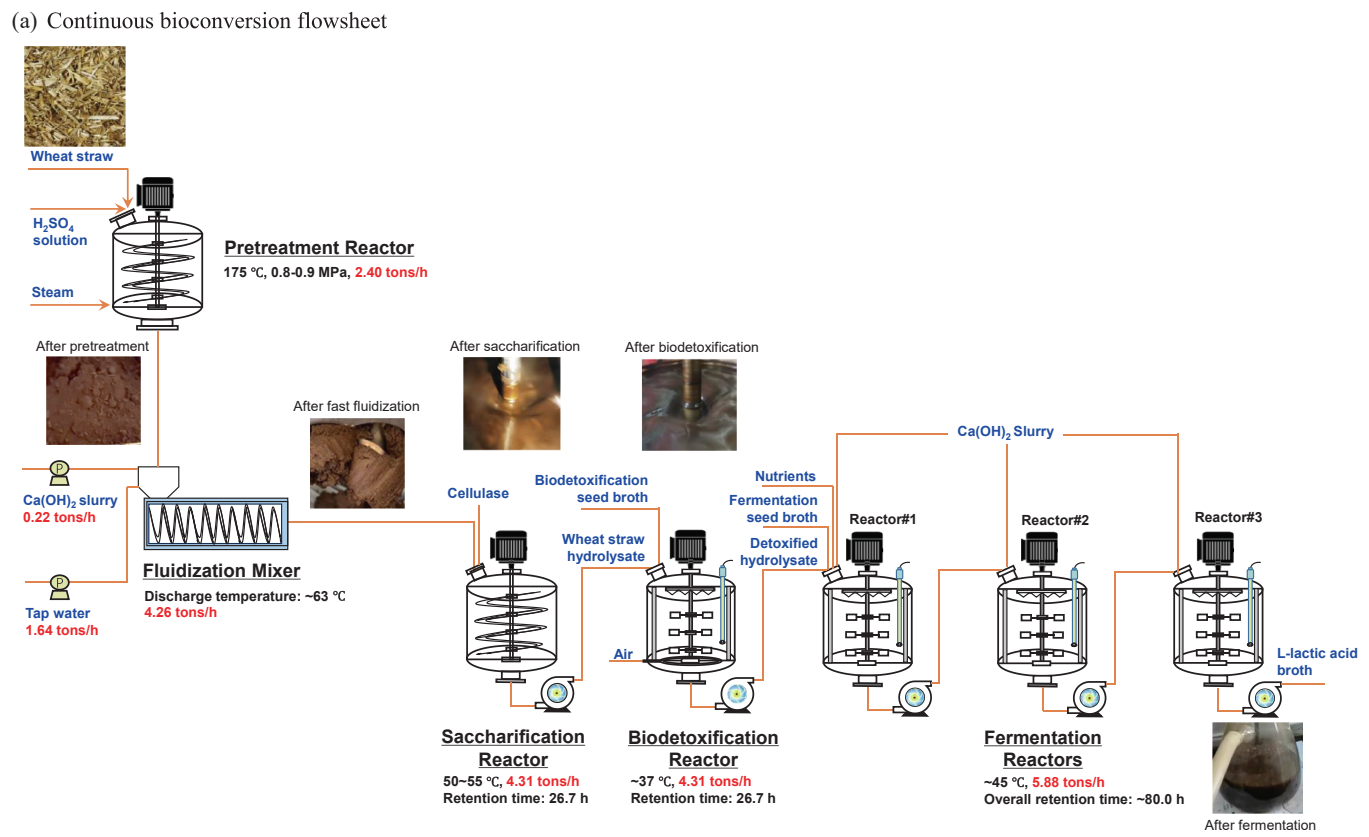
The fast fluidization was to convert the dry acid-pretreated wheat straw solids (100–130 ℃) into a transportable slurry with solids content of ~ 30% (w/w) by a specially designed screw mixer (Kexin Machinery Co., Kunshan, Jiangsu, China). Fig. 2a shows that the morphology changes of the pretreated wheat straw solids from the solid particle form into a viscous slurry form before and after fast fluidization. Fig. 2b shows that the viscous wheat straw slurry was well mixed in a short period (~3 min). The average solid contents of ~ 30% (w/w) and the average pH values of ~ 5.1 were equal to that of the thoroughly homogenized slurry control sample by manual mixing, with no statistically significant differences (*t*-test, p-values > 0.05). Fig. 2c shows that the total sugars and inhibitors contents of the pretreated wheat straw solids before and after fluidization maintained essentially unchanged, indicating no release of sugars and inhibitors into the environment, while the enzymatic hydrolysis yield maintained exactly the same with that of the thoroughly mixed slurry sample, and there were no statistically significant differences (*t*-test, p-values > 0.05). These results show that the morphology and physicochemical properties of the wheat straw slurry after fast fluidization was in a suitable condition for the subsequent continuous saccharification when the solids were converted to a transportable fluid form with the solids content from ~ 70% (w/w) to ~ 30% (w/w), the temperature from ~ 110 ℃ to ~ 63 ℃, and the pH from ~ 2.4 to ~ 5.1.

Generally, the fluidization of pretreated lignocellulose feedstock into a transportable form is difficult without cellulase enzyme participation. However, the upper limit of the thermal tolerance for commercially available cellulase enzymes is below ~ 55 ℃ while the outlet temperature of freshly released pretreated lignocellulose material is much higher than this upper limit (Fig. 1b, ~100 ℃). Therefore, the enzyme assisted fluidization is not only economically invalid, but also technically invalid. In this study, the successful fast fluidization of the dry acid-pretreated wheat straw solids may come from several favorable reasons: (i) the pretreated solids contained substantial amounts of sugars and inhibitors including monosaccharides (xylose, 13%-14%; glucose, 2%-3%), oligosaccharides (1%-3%), furfural (0.5%-1%), HMF (0.1%-0.5%), acetic acid (1%-3%) etc., which were not washed out into liquid streams due to the zero wastewater generation during unique performance of dry acid pretreatment (Liu et al., 2018; Liu and Bao, 2017; Zhang et al., 2021; He et al., 2014b). These sugars and inhibitors functioned as binding agents between insoluble solids and water for generation of transportable slurry; (ii) the freshly released pretreated solids maintained a high temperatures (above 100 ℃), enabling a further hydrolysis of hemicellulose and cellulose into oligo- or mono-saccharides under catalysis of sulfuric acid in the pretreated wheat straw solids when $Ca(OH)_2$ slurry and water was added for solids content adjustment (~70% to ~ 30%); (iii) the dilution of the system by $Ca(OH)_2$ slurry and water addition, combined with thorough screw mechanical mixing, facilitated an efficient homogenization of insoluble solids (cellulose, lignin, and others) with the soluble sugars, sulfuric acid catalyst, alkaline neutralizing agents, forming a relatively uniform viscous slurry with pumpable fluid properties.

3.2. Continuous enzymatic saccharification of fluidized wheat straw slurry

To evaluate the potential of the fluidized wheat straw slurry for the subsequent processes, the continuous saccharification were conducted in a 5 L reactor with helical mixing under the solids loading of 30% (w/w) and cellulase dosage of 4 mg enzyme proteins/g dry solids matter (DM) as illustrated in Fig. 3.

Fig. 3a shows that the thermal shock of the hot fluidized pretreated wheat straw slurry (~63 ℃) on saccharification temperature change. The fluidized slurry was heated to 60 ~ 65 ℃ and then feeding into the continuous saccharification reactor, resulted in a temperature increase of ~ 3 ℃ but quickly dropped to the control temperature of 50 ℃. During the continuous saccharification process, no gradient increase in system temperature was observed due to the continuous feeding of hot fluidized slurry, which was attributed to jacket cooling, stirring, and liquid cellulase addition. This temperature variation (50 ℃ → 53 ℃ → 50 ℃) was still well fallen into the optimal range of cellulase enzyme (50–55 ℃), indicating that the continuous feeding of the hot fluidized wheat straw slurry did not cause the negative thermal impact on cellulase activity. For industrial operations, the temperature approaching between fluidization and saccharification should be considered carefully. The fluidization temperature using Aspen Plus modeling was



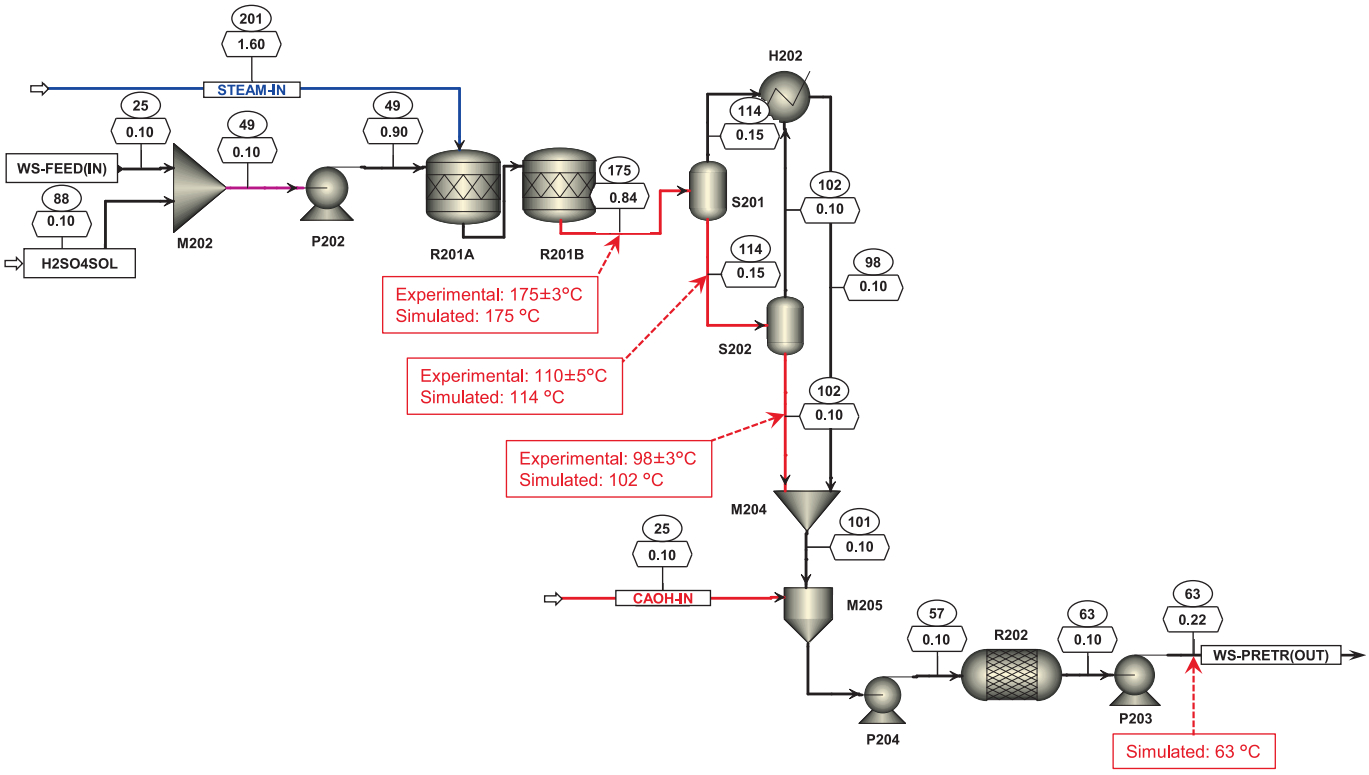


Fig. 1. Diagram of continuous fluidization and bioconversion flowsheet of dry biorefining process for production of L-lactic acid from wheat straw feedstock. (a) Continuous bioconversion flowsheet of dry biorefining process for production of L-lactic acid; (b) Aspen Plus modeling process flowsheet for temperature calculation of fluidized wheat straw slurry of dry acid pretreated wheat straw solids. The experimental conditions were shown in Materials and Method section. The pretreatment temperature was based on the measured temperature data from a 10 m^3 industrial reactor, and the continuous saccharification temperature change was based on the 5 L laboratorial small reactor.

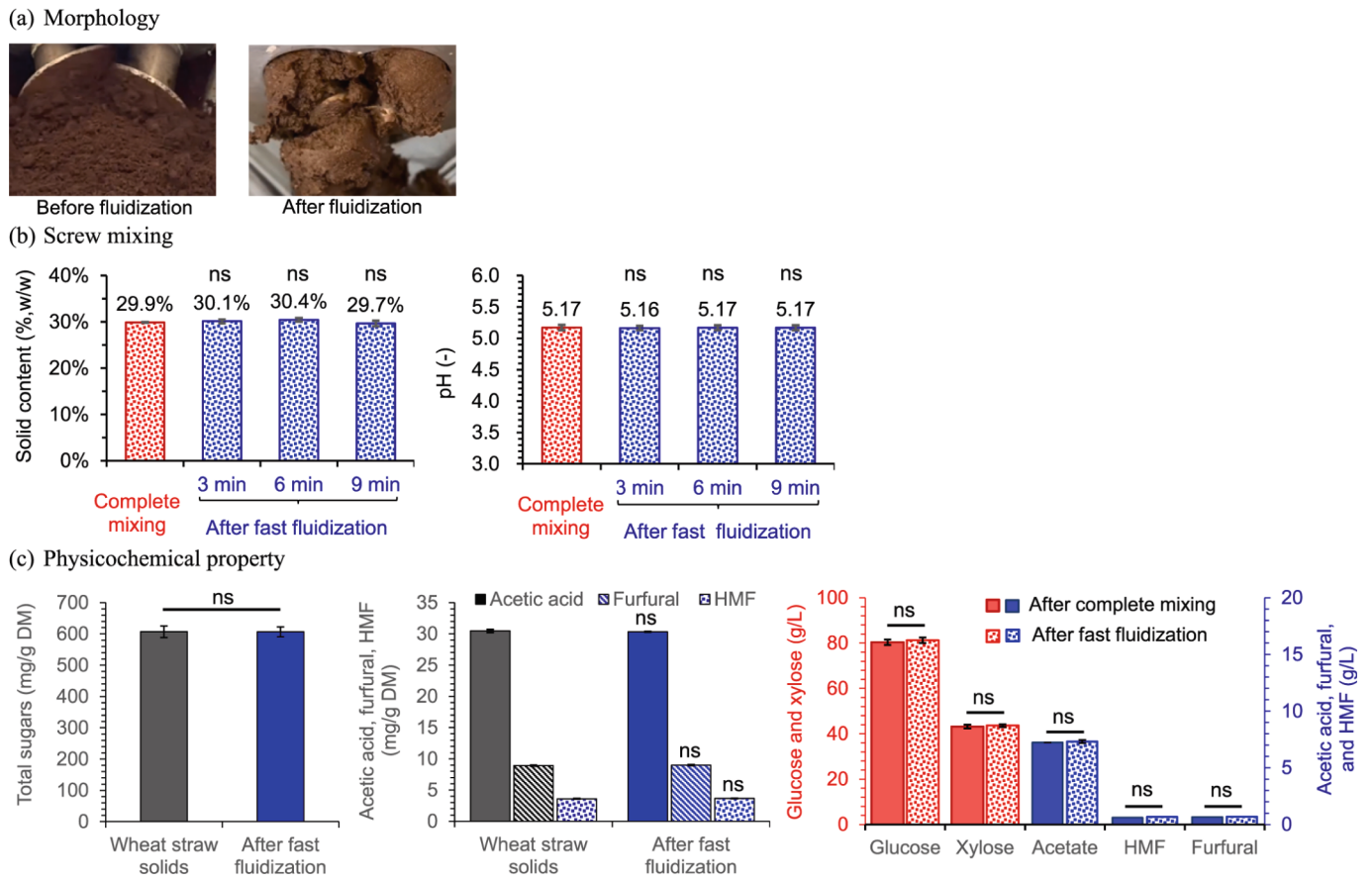


Fig. 2. Morphology and property changes of dry acid pretreated wheat straw solids before and after fast fluidization. (a) Morphological changes; (b) Screw mixing efficiency evaluation; (c) Physicochemical property changes. The dry acid pretreated wheat straw #2 (PWS #2) was used as the raw material for the fluidization experiment. The fluidization experiment was carried out in a screw mixer by simultaneously and continuously feeding the hot PWS#2 solids (~100 ℃, pH ~ 2.4) and calcium hydroxide slurry (20%, w/w) and tap water into a fluidized wheat straw slurry within 9 min. Screw-mixed samples were collected every 3 min, 1 kg each time, and then multi-point (at least 10 points) sampling analysis including solid content and pH measurement was conducted. The measurement of solid content was carried out by the differential weight method (overnight drying at 105 ℃). The complete mixing involves manually thoroughly blending PWS#2, water and calcium hydroxide slurry (with a total weight of 500 g). For wheat straw solids (PWS #2) and wheat straw slurry (after fast fluidization), the total sugar, acetic acid, furfural, and HMF contents were expressed as milligrams per gram of dry solids matter (mg/g DM). The total sugars included monosaccharides (glucose and xylose) and their oligomers. The enzymatic hydrolysis evaluations of the two mixed slurries (after complete mixing and after fast fluidization) were performed at the solids content of 20% (w/w), 50 ℃, 200 rpm, cellulase dosage of 4 mg enzyme proteins/g dry weight. Data represent averages of multiple sampling points. Statistical significance was set at *p < 0.05 and **p < 0.01; "ns" denotes no significant difference (p > 0.05). Data were presented as mean ± standard deviation.

calculated without considering heat dissipation, and the temperature difference was 8–13 °C (63 °C → 50–55 °C). If the heat loss during the fluidized slurry conveying and the jacket cooling of the screw mixer and saccharification reactor were considered, the temperature would easily approach the optimal saccharification range (~50 °C).

Fig. 3b shows that the continuous saccharification using the industrial reactor pretreated wheat straw solids (PWS#2) under a dilution rate of 0.0375 h^{-1} (equivalent to saccharification time ~ 26.7 h). The operation reached a steady state after ~ 24 h with 95.55 ± 1.13 g/L of glucose, 61.26 ± 0.54 g/L of xylose, 12.00 ± 0.24 g/L of acetic acid, 0.89 ± 0.07 g/L of furfural, and 0.93 ± 0.07 g/L of HMF, respectively, at which the apparent viscosity was 0.201 ± 0.005 Pa·s. Fig. 3c shows that the continuous saccharification using the bench reactor pretreated wheat straw solids (PWS#1) under the same dilution rate of 0.0375 h^{-1} reached a steady state after ~ 32 h with 84.94 ± 0.73 g/L of glucose, 46.43 ± 0.53 g/L of xylose, 7.94 ± 0.16 g/L of acetic acid, 0.68 ± 0.08 g/L of furfural, and 0.80 ± 0.04 g/L of HMF, and the apparent viscosity at 0.310 ± 0.013 Pa·s, respectively. For the continuous saccharification process using PWS#2 (instead of PWS#1), the resulting saccharified hydrolysate had a significantly higher total monosaccharide release concentration (156.81 ± 1.59 g/L vs. 131.64 ± 1.31 g/L) and a relatively lower apparent viscosity (0.201 ± 0.005 Pa·s vs. 0.310 ± 0.013 Pa·s), as determined by *t*-test (p-values < 0.001). This indicated that the pretreatment severity (or the damage degree to lignocellulose feedstocks) in the industrial reactor was higher than that in the bench reactor even under the same operation parameters, which was conducive to achieving higher cellulase accessibility. The major reason for the higher pretreatment severity may come from the reduced heat dissipation in large industrial reactors (10 m^3) than that in small bench reactors (20 L), resulting in higher average temperature distributions inside the reactors. A viscosity of 0.2 ~ 0.3 Pa·s of the wheat straw hydrolysate enabled an enclosed pipeline transportation using standard centrifugal pumps (up to ~ 0.5 Pa·s) and ensured a continuous hydrolysate transfer to the subsequent biodetoxification stage.

The total glucose and xylose titer during the steady-state period reached 156.81 ± 1.59 g/L (Fig. 3b) or 131.64 ± 1.31 g/L (Fig. 3c), which was comparable to that by the batch saccharification with the completely inhibitor removal (137.17 ± 9.54 g/L, glucose + xylose) (Hou et al., 2018; Hou et al., 2019; Jin and Bao, 2021), indicating that high inhibitor levels was negligible on cellulase activity and saccharification efficiency during high solids-loading continuous saccharification.

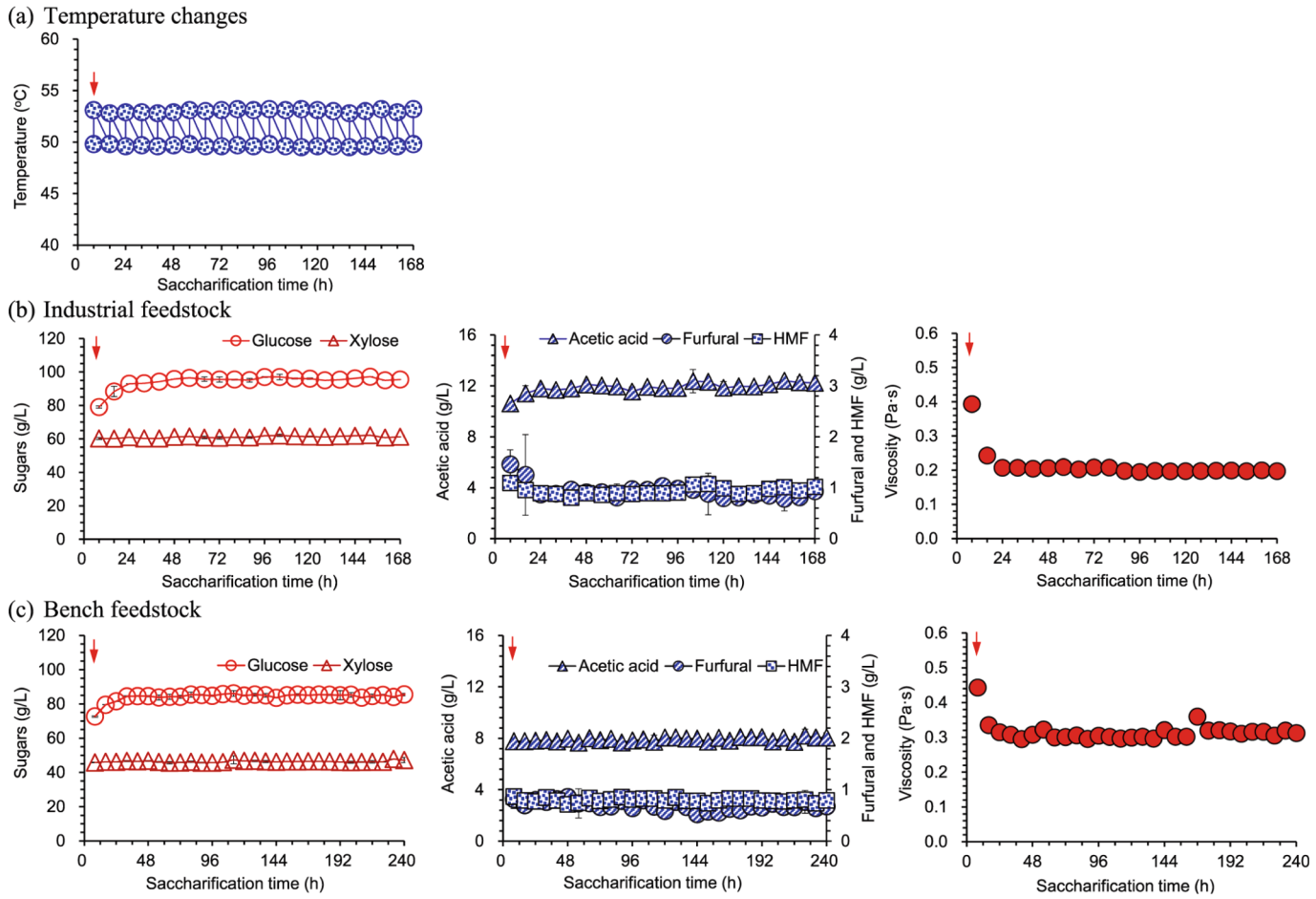


Fig. 3. Continuous saccharification of the fluidized wheat straw slurry. (a) Temperature changes of saccharification system with continuously supplement of hot fluidized wheat straw slurry; (b) Time courses of sugars, inhibitors, and apparent viscosity of continuous saccharification using the fluidized wheat straw slurry from industrial reactor pretreated feedstock; (c) Time courses of sugars, inhibitors, and apparent viscosity of continuous saccharification using fluidized wheat straw slurry from the bench reactor pretreated feedstock. The fluidized wheat straw slurry was obtained from the fast fluidization as in Fig. 2. The continuous saccharification operation was conducted at 30% (w/w) solids loading, 50 ℃, stirring rate of 200 rpm and cellulase dosage of 4 mg total proteins per gram of dry matter (DM). The 5 L reactors were equipped with pH and temperature control, and a helical ribbon agitator. Red arrow (↓) indicates the starting point of continuous saccharification operation by feeding the fluidized wheat straw slurry and discharging the saccharified wheat straw hydrolysate. The feeding and discharging of the hydrolysate accounted for 30% (w/w) of the total every 8 h. The continuous saccharification using the industrial-reactor or bench-reactor pretreated wheat straw solids under the same dilution rate of 0.0375 h^{-1} (equivalent to saccharification time ~ 26.7 h). Data represent averages of multiple sampling points.

3.3. Continuous biodetoxification of wheat straw hydrolysates

For dry-acid pretreatment operations, all the inhibitors generated were eventually accumulated into the pretreated wheat straw solids because of zero free aqueous stream generations, and entered the wheat straw hydrolysate after saccharification. In this work, the inhibitors in the wheat straw hydrolysates from the continuous saccharification were removed by the continuous submerged liquid biodetoxification using the biodetoxification strain *P. variotii* FN89 (Zhang et al., 2021). Two aspects were examined, one was how to maintain a steady-state with decreasing inhibitor content in the hydrolysate, and the other was how to determine the endpoint accurately to avoid unnecessary sugar loss by excessive biodetoxification.

Fig. 4a shows that the continuous biodetoxification with the dissolved oxygen transfer of $k_L a$ value above 10 h^{-1} and the dilution rate of 0.0375 h^{-1} (equal to a detoxification time ~ 26.7 h) by discharging 30% of the total biodetoxified wheat straw hydrolysate and re-supplementing the equal weight of wheat straw hydrolysate every 8 h. The endpoint of biodetoxification was determined when the pH value of the biodetoxified hydrolysate reached the peak value of 7.01 ± 0.09 (~24 h) (Han et al., 2023; Li et al., 2024), where furfural and HMF were completely removed, and acetic acid reduced from 10.29 ± 0.27 g/L to 3.82 ± 0.15 g/L. Then the hydrolysate discharge and re-supplementation were repeatedly done to enter a steady-state continuous operation. Fig. 4b shows that the continuous biodetoxification of wheat straw hydrolysate using bench reactor pretreated wheat straw solids with a higher dilution rate of 0.0750 h^{-1} (equals to a detoxification time ~ 13.3 h) by discharging and re-supplementing 60% of the total biodetoxified wheat straw hydrolysate every 8 h. The endpoint pH was 6.51 ± 0.06, furfural and HMF were completely removed and acetic acid was reduced from 6.65 ± 0.01 g/L to 0.68 ± 0.07 g/L. For both wheat straw hydrolysates, the sugar titers increased with the biodetoxification time due to the ongoing enzymatic hydrolysis in the continuous biodetoxification stage. On the other hand, the pretreated wheat straw hydrolysate using industrial reactor pretreated feedstock showed the lower apparent viscosity, which should be easier transportable to the subsequent fermentation process.

Relatively, the wheat straw hydrolysate using industrial reactor pretreated feedstock contained significantly higher inhibitors concentration (especially acetic acid, 11–13 g/L vs. 7–8 g/L) than that using bench reactor pretreated feedstock, as determined by *t*-test (p-values < 0.001). Therefore, if acetic acid of the former is removed to an extremely low level (<1 g/L) like the detoxification of hydrolysate using bench reactor pretreated feedstock, more detoxification time will be required.

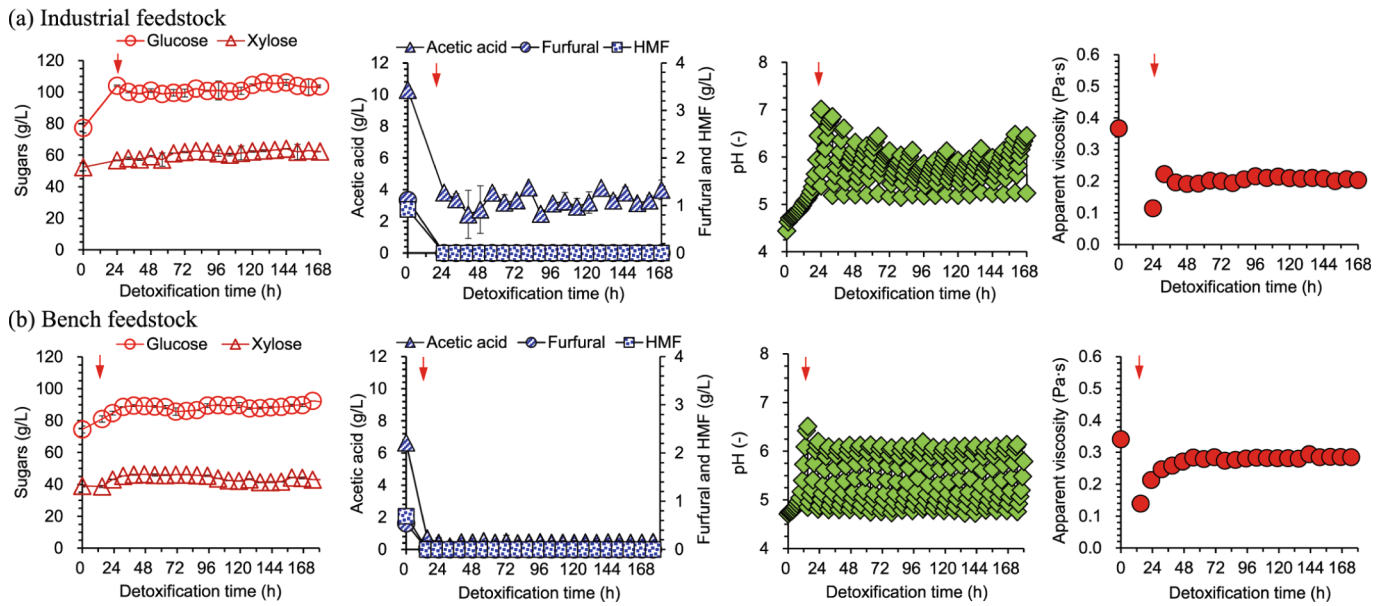


Fig. 4. Continuous biodetoxification of the saccharified wheat straw hydrolysate. (a) Time courses of sugars, inhibitors, pH and apparent viscosity of the industrial reactor pretreated wheat straw hydrolysate with 30% discharge and re-supplementation of the total biodetoxified wheat straw hydrolysate every 8 h (equivalent to a dilution rate of 0.0375 h^{-1} or a retention time of ~ 26.7 h); (b) Time courses of sugars, inhibitors, pH and apparent viscosity of the bench reactor pretreated wheat straw hydrolysate with 60% discharge and re-supplementation of the total biodetoxified wheat straw hydrolysate every 8 h (equivalent to a dilution rate of 0.075 h^{-1} or a retention time of ~ 13.3h). Red arrow (↓) indicates the start of the continuous biodetoxification by saccharified hydrolysate feeding and biodetoxified hydrolysate discharging. The biodetoxification operation was conducted at ~ 30% (w/w) solids loading, 37 ℃, aeration of 1.0 vvm, stirring rate of 750 rpm and inoculation size of 10% (v/v) in a 3 L bioreactor equipped with two six-blade impellers and one anti-foam paddle. Data represent averages of multiple sampling points.

In fact, to ensure that the continuous operation intervals during the biodetoxification period were all 8 h, there was indeed a residue of 2–4 g/L of acetic acid in the hydrolysate, which was no longer considered as a major inhibitor for cell growth and metabolism of most fermenting strains. Notably, the sugar loss generated at the endpoints of the continuous biodetoxification according to the pH detection method reported before was essentially negligible (<2% of the total sugars) (Han et al., 2023), which were similar to the batch submerged liquid

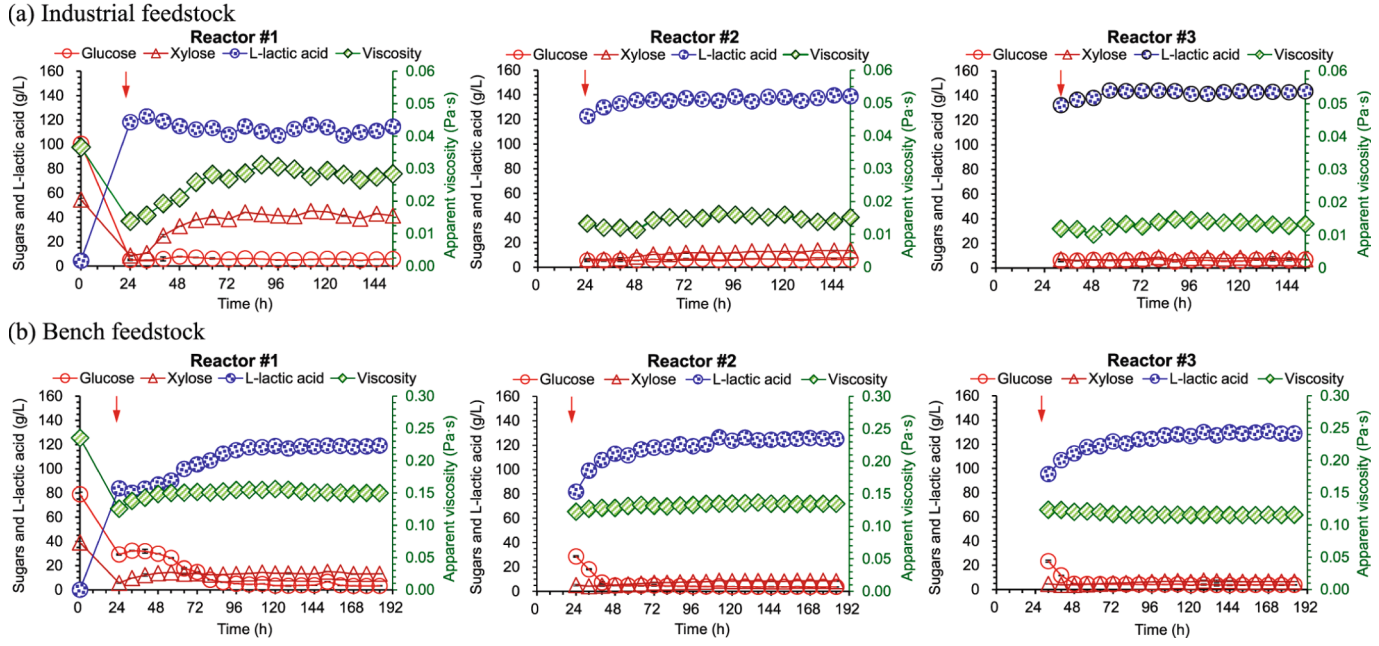


Fig. 5. Multi-stage continuous simultaneous saccharification and co-fermentation (SSCF) of the biodetoxified wheat straw hydrolysate for L-lactic acid production. (a) Time courses of sugars, L-lactic acid and apparent viscosity using industrial reactor pretreated wheat straw hydrolysate; (b) Time courses of sugars, L-lactic acid and apparent viscosity using bench reactor pretreated wheat straw hydrolysate. Red arrow (↓) indicates the start of the continuous operation by biodetoxified wheat straw hydrolysate feeding and fermentation broth discharging. For each 5 L reactor, the feeding and discharging of the broth accounted for 30% (w/w) of the total every 8 h (equivalent to a dilution rate of 0.0375 h^{-1} or a retention time of ~ 26.7h). The operation was conducted at ~ 30% (w/w) solids loading, 45 °C, 180 rpm, inoculation ratio of 10% (v/v), pH 5.5 by adding 25%(w/w) $Ca(OH)_2$ slurry automatically in 5 L bioreactor with helical ribbon impeller. Data represent averages of multiple sampling points.

biodetoxification using *P. variotii* FN89 (Han et al., 2023; Zhang et al., 2021).

3.4. Continuous L-lactic acid fermentation using biodetoxified wheat straw hydrolysate

The biodetoxified wheat straw hydrolysate was used for the continuous fermentation in the form of simultaneous saccharification and co-fermentation (SSCF) for L-lactic acid production. Two critical aspects were concerned including how to achieve a fast steady-state continuous SSCF with high L-lactic acid titer, productivity, yield, and how to reduce the residual sugars to a minimal level to avoid the complicated purification steps. This study used three 5 L serial fermenters operated at 45 ℃ with the seed inoculation of *P. acidilactici* ZB220 only to the first fermenter. The dilution rate for all fermenters was adjusted at 0.0375 h^{-1} by discharging 30% of the total fermentation broth from the first fermenter to the next fermenter till the last one every 8 h with the re-supplementation of the pretreated wheat straw hydrolysate. The equivalent average fermentation time was ~ 26.7 h for each of the three fermenters, with the total fermentation time of ~ 80.0 h for the three.

Fig. 5 shows that continuous fermentations of the industrial reactor and bench reactor pretreated wheat straw hydrolysate in the three serial fermenters. Fig. 5a shows that when the industrial reactor pretreated wheat straw hydrolysate was used, the first fermenter reached the steady state after 64 h with 111.51 ± 3.08 g/L of L-lactic acid, 5.54 ± 0.51 g/L of residual glucose, 41.87 ± 2.08 g/L of residual xylose and the apparent viscosity of 0.029 ± 0.001 Pa·s; the second fermenter reached the steady state after 56 h with 136.89 ± 1.61 g/L of L-lactic acid, 6.23 ± 0.35 g/L of residual glucose, 12.28 ± 1.11 g/L of residual xylose and the apparent viscosity of 0.015 ± 0.001 Pa·s; the third fermenter reached the steady state after 56 h with 143.05 ± 0.93 g/L of L-lactic acid, 6.30 ± 0.55 g/L of residual glucose, 7.70 ± 0.69 g/L of residual xylose and the apparent viscosity of 0.014 ± 0.001 Pa·s. The overall L-lactic acid productivity of the fermentation step was 1.79 ± 0.01 g/L/h and the yield was 0.79 ± 0.01 g L-lactic acid per gram sugars converted from wheat straw cellulose and hemicellulose. The key bioconversion performance indicators of the final continuous fermentation (titer of 143.05 ± 0.93 g/L, rate of 1.79 ± 0.01 g/L/h, and yield of 78.8% ± 0.5%) were comparable to those of L-lactic acid batch fermentation (titer of 130.80 ± 1.60 g/L, rate of 1.82 ± 0.00 g/L/h and yield of 67.7% ± 0.3%) (Qiu et al., 2018). The L-lactic acid chiral purity in the final fermenter was determined at 99.3% ± 0.1% (He et al., 2022). Fig. 5b shows that when the bench reactor pretreated wheat straw hydrolysate was used, the first fermenter reached the steady state after 104 h with 118.34 ± 0.70 g/L of L-lactic acid, 4.00 ± 1.05 g/L of residual glucose, 13.69 ± 0.67 g/L of residual xylose and the apparent viscosity of 0.152 ± 0.003 Pa·s; the second fermenter reached the steady state after 112 h with 125.17 ± 0.91 g/L of L-lactic acid, 3.80 ± 0.16 g/L of residual glucose, 8.50 ± 0.22 g/L of residual xylose and the apparent viscosity of 0.135 ± 0.001 Pa·s; the third fermenter reached the steady state after 112 h with 128.83 ± 1.26 g/L of L-lactic acid, 3.83 ± 0.24 g/L of residual glucose, 6.66 ± 0.31 g/L of residual xylose and the apparent viscosity of 0.115 ± 0.000 Pa·s. The overall L-lactic acid productivity of the fermentation step was 1.61 ± 0.02 g/L/h and yield was 0.80 ± 0.00 g L-lactic acid per gram sugars converted from wheat straw cellulose and hemicellulose. The L-lactic acid chiral purity in the final fermenter was determined at 99.5% ± 0.1% (He et al., 2022). Relatively, the pretreated wheat straw under industrial operation scenario (instead of bench operation scenario), was helpful to achieve the faster steady-state fermentation performance (~56 h vs ~ 112 h), higher L-lactic acid titer (143.05 ± 0.93 g/L vs. 128.83 ± 1.26 g/L) and lower apparent viscosity (0.01–0.03 Pa·s vs 0.135 ± 0.001 Pa·s) in the third fermenter, as determined by *t*-test (p-values < 0.001).

Fig. 5a shows that the overall L-lactic acid yield was ~ 60.7% in the first fermenter, ~76.2% in the second fermenter, and ~ 78.8% in the third fermenter. The L-lactic acid titer increased and the residual sugar (glucose and xylose) decreased with increased serial fermenter numbers. In conclusion, the results indicated that three serial fermenters were completely enough to achieve the steady-state continuous L-lactic acid fermentation with favorable L-lactic acid titer (>140 g/L) and minimal residual sugar level (<14 g/L), while reducing the difficulty of subsequent lactic acid purification steps and lowering the investment in fermenters. Moreover, these results also confirmed that the multi-stage configuration (e.g., using three bioreactors arranged in a cascade rather than a single bioreactor) is highly beneficial for enhancing productivity, process stability and overall economic feasibility.

3.5. Mass balance of continuous bioconversions from fast fluidization to L-lactic acid fermentation

Fig. 6 shows a complete mass balance of the continuous biorefinery conversion of wheat straw feedstock starting from the fast fluidization till the fermentative production of L-lactic acid. The mass balances of each component in the material streams were calculated based on the experimentally measured data during the steady-state period.

The dry acid pretreatment of 1,000 kg wheat straw feedstock produced 1,752 kg of dry acid-pretreated wheat straw solids (53.71% of the dry solids) with a dry matter loss of 47 kg (equivalent to ~ 5% of dry wheat straw feedstock), corresponding to a glucose/glucan recovery of 99.64%, xylose/xylan recovery of 73.14%, and a total sugar recovery of 88.56%.

The fast fluidization (including neutralization and screw mixing) generated a pretreated wheat straw slurry and then pumped to the continuous saccharification step to generate 3,072 kg of the pretreated wheat straw hydrolysate (36.49% of the dry solids), in which 69.72% of glucan and 65.45% of xylan were hydrolyzed into glucose and xylose.

The continuous submerged liquid biodetoxification produced 3,358 kg of the biodetoxified wheat straw hydrolysate by removing all furfural and HMF, and ~ 70% of acetic acid with a minor sugar loss less than ~ 1% due to the inhibitor priority of the biodetoxification strain to the fermentable sugars. The glucose/glucan recovery, xylose/xylan recovery, and total sugar recovery in this step were 99.53%, 72.24%, and 87.41%, respectively.

The final continuous fermentation produced 4,497 kg of L-lactic acid fermentation broth containing 564 kg calcium L-lactate (equivalent to ~ 466 kg of pure L-lactic acid), along with 21 kg of the residual glucose and 25 kg of the residual xylose. The overall L-lactic acid yield was 0.788 g per gram of sugars converted from the total cellulose and hemicellulose in the wheat straw feedstock. The polymer-grade L-lactic acid was purified by solid–liquid separation for lignin solid removal, decolorization, multi-effect evaporation, crystallization, acidification, solid–liquid separation for $CaSO_4$ removal, and ion adsorption before the lactide synthesis (He et al., 2022).

3.6. Expectations of the fast fluidization and continuous dry biorefinery processing technology

This study investigated a continuous bioconversion of wheat straw feedstock to L-lactic acid by a fast fluidization of the hot dry-acid-pretreated wheat straw solids to a pumpable fluidized slurry using a screw mixer, then the fluidized slurry was pumped through an enclosed pipeline to the consequent reactors for continuous saccharification, biodetoxification, and fermentation to produce L-lactic acid with high titer (143.1 ± 0.9 g/L), high productivity (1.79 ± 0.01 g/L/h), and high yield (78.8% ± 0.5%). To achieve a full flowsheet continuous biorefinery processing, several problems should be taken into considerations.

The missing step for a complete continuous biorefinery process is continuous pretreatment under high solids loading. The reported continuous pretreatment examples were operated at low solid contents (~5%-10%, w/w) by mixing lignocellulose feedstocks with large amount of water or solvent to form a relatively continuous slurry stream

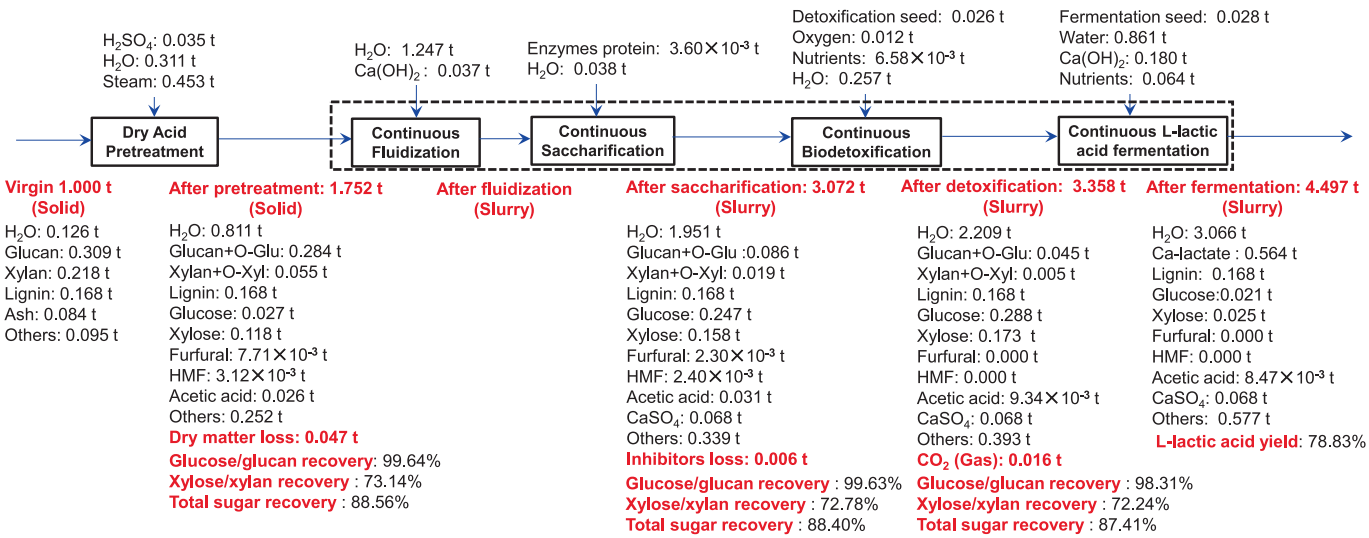


Fig. 6. Mass balance of the overall continuous bioconversion for L-lactic acid production. The data were based on the steady state process stages using the industrial reactor pretreated wheat straw feedstock. Glucose/glucan and xylose/xylan recoveries were calculated based on the total glucose or xylose contents divided by the total glucose or xylose contents in the virgin wheat straw feedstock (polysaccharides and oligosaccharides were all converted to the monomers), respectively. The total sugar recoveries were calculated by the total sugars obtained divided by the total sugars in the virgin wheat straw (all carbohydrates were converted to monosaccharides). L-lactic acid yield was calculated by the L-lactic acid in the broth of Reactor #3 divided by the theoretical L-lactic acid from the conversion from the total sugars in the raw wheat straw feedstocks (all carbohydrates were converted to monosaccharides prior to calculation).

(Chen et al., 2013; Cheng et al., 2019; Cha et al., 2015). However, low solids-loading pretreatments are no longer the choice of practical industrial processes for its massive wastewater generation, release of toxic inhibitors, and losses of soluble sugars (Zhang et al., 2023). For the high solids-loading pretreatment operation in this study, its advantages have been well demonstrated including no wastewater or waste residues, high sugar yield and high pretreatment severity, and minimal corrosion to pretreatment reactors due to the absence of aqueous solutions (Shao et al., 2017). However, a significant technical difficulty arises from the nature of very high solids-loading at the very beginning: ~70% solids loading lignocellulose feedstock certainly exists in a scattered dry particle form. The continuous transport of these loosely bounded solid particles from an ambient conveyor into a pressurized pretreatment reactor proposed a great engineering challenge. On the other hand, the discharge of the pretreated solids from the reactor to the ambient containers is not difficult by the driving force of the minor positive internal pressure of the reactors after pretreatment. A novel design of the continuous pretreatment reactor for the very high solids-loading feedstock should be proposed to solve this engineering difficulty of scattered particle transport to a pressurized reactor.

The second problem for a complete biorefinery processing is superhigh thermal tolerant cellulase enzyme used for fast fluidization of the hot pretreated lignocellulose solids as soon as the solids released from the pretreatment reactors, similar to the high-temperature liquefaction (fluidization) of starch using thermal tolerant α-amylase (95–105 ℃) (Xu et al., 2016). The regular pretreatment is operated under harsh conditions with the high temperature range from 160 to 200 ℃, and the temperature of discharged pretreated solids still maintains over 100 ℃. The cellulase enzyme used for the immediate fluidization of these hot solids should be tolerant to ~ 100 ℃ or higher. However, the maximum thermal tolerance of the conventional industrial cellulases is only in the range of 50–55 ℃. The development of thermotolerant cellulases capable of ~ 100 ℃ or higher would significantly enhance the efficiency of continuous bioconversion by rapidly transform the solids into a highly fluidized liquid. In this study, we tried various industrial thermostable cellulases for fast hydrolysis of the hot pretreated wheat straw solids at ~ 100 ℃ or higher temperature, but all the trials failed. Therefore, the development of thermostable cellulases tolerant to ~ 100 ℃ or higher should be an important topic for future cellulase enzyme studies.

The third problem is the frequent temperature switching in biorefinery processing chain. This study tried to narrow the temperature range but still involves several transitions including ~ 100 ℃ of the pretreated wheat straw solids to ~ 63 ℃ of the pretreated wheat straw slurry in the fast fluidization, then to 50 ℃ of the continuous saccharification to 37 ℃ of the continuous biodetoxification, and to 45 ℃ of the continuous fermentation. In industrial reactors with hundred or thousand cubic meters scale, a prolonged operation time and reduced productivity are inevitable with considerable energy input associated with reactor heating and cooling. The ideal scenario to this temperature transition problem is to elevate the overall process temperature to a relatively high and uniform level, close to the saccharification and fermentation steps around 45–50 ℃, then energy consumptions and process time could be significantly reduced.

The final concern is the viscosity of lignocellulose slurry after fast fluidization. The slurry viscosity should fall into the working range of conventional mechanical pumps. The most commonly used pump is centrifugal pump with the suitable fluid viscosity less than 500 cP. Other pumps include piston pumps with the suitable viscosity, 10,000 cP; diaphragm pumps and peristaltic pumps, 50,000 cP; gear pumps, progressive cavity pumps, and screw pumps, up to 1,000,000 cP. For the pretreated slurry after fast fluidization, the fluid is in such a high solids-loading slurry form, which is difficult to measure the accurate apparent viscosity due to the high solids content. Therefore, a screw pump or driving mechanics might be a suitable choice. For the continuous bioconversion steps, the apparent viscosity of the fluids varied from 200 to 450 cP in the continuous saccharification, 100–300 cP in the continuous biodetoxification, and 10–150 cP in continuous fermentation, which perfectly fell in the range of the conventional centrifugal pumps and adequately satisfy the pumping requirements for fluidized slurry across all bioconversion stages.

4. Conclusion

This study achieved a fast fluidization of the dry-acid-pretreated wheat straw solids and the consequent continuous bioconversions for L-lactic acid production. The fast fluidization rapidly converted the hot pretreated wheat straw solids (100–110 ℃) into a homogeneously pumpable fluid slurry with moderate temperature (~63 ℃) and pH

(~5.1), followed by the continuous enzymatic saccharification and continuous biodetoxification to a wheat straw hydrolysate with a low apparent viscosity (0.2 ~ 0.3 Pa·s) and high monosaccharides concentrations (glucose + xylose, 130 ~ 170 g/L). The multi-stage continuous fermentation achieved L-lactic acid production by three fermenters with the final titer of 143.1 ± 0.9 g/L, productivity of 1.79 ± 0.01 g/L/h, yield of 78.8% ± 0.5%, and chiral purity of 99.3% ± 0.1%. This work provided the technical foundation for future commercial biorefining of lignocellulosic biomass for the low-cost L-lactic acid and polylactic acid (PLA) production.

CRediT authorship contribution statement

Tao Han: Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. **Ya Wang:** Writing – review & editing, Methodology, Investigation. **Bin Zhang:** Writing – review & editing, Writing – original draft, Investigation, Funding acquisition. **Jie Bao:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, 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.

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Data availability

The source data supporting the findings of this study are available within the paper.

References

Ahring, B.K., Traverso, J.J., Murali, N., Srinivas, K., 2016. Continuous fermentation of clarified corn stover hydrolysate for the production of lactic acid at high yield and productivity. Biochem. Eng. J. 109, 162–169. https://doi.org/10.1016/j.bej.2016.01.012.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254. https://doi.org/10.1016/0003-2697(76)90527-3.

Brethauer, S., Studer, M.H., Wyman, C.E., 2014. Application of a slurry feeder to 1 and 3 stage continuous simultaneous saccharification and fermentation of dilute acid pretreated corn stover. Bioresour. Technol. 170, 470–476. https://doi.org/10.1016/j.biortech.2014.07.049.

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

Brunecky, R., Li, Y., Decker, S.R., Li, Y.D., Decker, S.R., Himmel, M.E., 2025. Advancing continuous enzymatic hydrolysis for improved biomass saccharification. Biotechnol. Biofuels Bioprod. 18, 82. https://doi.org/10.1186/s13068-025-02680-z.

Cha, Y.K., Yang, J.W., Park, Y., An, G.H., Ahn, J.W., Moon, Y.H., Yoon, Y.M., Yu, G.D., Choi, I.H., 2015. Continuous alkaline pretreatment of Miscanthus sacchariflorus using a bench-scale single screw reactor. Bioresour. Technol. 181, 338–344. https://doi.org/10.1016/j.biortech.2015.01.079.

Chen, W.H., Tsai, C.C., Lin, C.F., Tsai, P.Y., Hwang, W.S., 2013. Pilot-scale study on the acid-catalyzed steam explosion of rice straw using a continuous pretreatment system. Bioresour. Technol. 128, 297–304. https://doi.org/10.1016/j.biortech.2012.10.111.

Cheng, M.S., Dien, B.S., Lee, D.K., Singh, V., 2019. Sugar production from bioenergy sorghum by using pilot scale continuous hydrothermal pretreatment combined with disk refining. Bioresour. Technol. 289, 121663. https://doi.org/10.1016/j.biortech.2019.121663.

Duan, Y., Chen, L.M., Ma, L.X., Amin, F.R., Zhai, Y.D., Chen, G.F., Li, D.M., 2024. From lignocellulosic biomass to single cell oil for sustainable biomanufacturing: current advances and prospects. Biotechnol. Adv. 77, 108460. https://doi.org/10.1016/j.biotechadv.2024.108460.

Fayet, A., Teixeira, A.R.S., Allais, F., Bouix, M., Lameloise, M.L., 2018. Detoxification of highly acidic hemicellulosic hydrolysate from wheat straw by dianafiltration with a focus on phenolic compounds. J. Membr. Sci. 566, 112–121. https://doi.org/10.1016/j.memsci.2018.08.045.

Han, T., Zhang, B., Yang, H.C., Liu, X.C., 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, 16608–16617. https://doi.org/10.1021/acssuschemeng.3c04986.

He, N.L., Jia, J., Qiu, Z.Y., Fang, C., Liden, G., Liu, X.C., 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.

He, Y.Q., Zhang, J., Bao, J., 2014a. Dry dilute acid pretreatment by co-currently feeding of corn stover feedstock and dilute acid solution without impregnation. Bioresour. Technol. 158, 360–364. https://doi.org/10.1016/j.biortech.2014.02.074.

He, Y.Q., Zhang, L.P., Zhang, J., Bao, J., 2014b. Helically agitated mixing in dry dilute acid pretreatment enhances the bioconversion of corn stover into ethanol. Biotechnol. Biofuels Bioprod. 7, 1. https://doi.org/10.1186/1754-6834-7-1.

Hou, W.L., Kan, J.L., Bao, J., 2019. Rheology evolution of high solids content and highly viscous lignocellulose system in biorefinery fermentations for production of biofuels and biochemicals. Fuel 253, 1565–1569. https://doi.org/10.1016/j.fuel.2019.05.136.

Hou, W.L., Zhang, M.F., Bao, J., 2018. Cascade hydrolysis and fermentation of corn stover for production of high titer gluconic and xylonic acids. Bioresour. Technol. 264, 395–399. https://doi.org/10.1016/j.biortech.2018.06.025.

Jin, C., Bao, J., 2021. Lysine production by dry biorefining of wheat straw and cofermentation of *Corynebacterium glutamicum*. J. Agric. Food Chem. 69, 1900–1906. https://doi.org/10.1021/acs.jafc.0c07902.

Li, Z.B., Zhang, L.X., He, N.L., Zhang, B., Bao, J., 2024. Competition between biodetoxification fungus and lactic acid bacterium in the biorefinery processing chain for production of cellulosic L-lactic acid. Bioresour. Bioprocess. 11, 54. https://doi.org/10.1186/s40643-024-00772-6.

Lischeske, J.J., Stickel, J.J., 2019. A two-phase substrate model for enzymatic hydrolysis of lignocellulose: application to batch and continuous reactors. Biotechnol. Biofuels Bioprod. 12, 299. https://doi.org/10.1186/s13068-019-1633-2.

Liu, G., Bao, J., 2017. Maximizing cellulosic ethanol potentials by minimizing wastewater generation and energy consumption: Competing with corn ethanol. Bioresour. Technol. 245 (Part A), 18-26. doi: 10.1016/j.biortech.2017.08.070.

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

Liu, G., Sun, J.E., 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.

López-Gómez, J.P., Alexandri, M., Schneider, R., Venus, J., 2019. A review on the current developments in continuous lactic acid fermentations and case studies utilising inexpensive raw materials. Process Biochem. 79, 1–10. https://doi.org/10.1016/j.procbio.2018.12.012.

Ma, K., Hu, G.Q., Pan, L.W., Wang, Z.C., Zhou, Y., Wang, Y.W., Ruan, Z.Y., He, M.X., 2016. Highly efficient production of optically pure l-lactic acid from corn stover hydrolysate by thermophilic *Bacillus coagulans*. Bioresour. Technol. 219, 114–122. https://doi.org/10.1016/j.biortech.2016.07.100.

Qiu, Z.Y., Gao, Q.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.

Qiu, Z.Y., Gao, Q.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.

Ruiz, H.A., Conrad, M., Sun, S.N., Sanchez, A., Rocha, G.J.M., Romani, A., Castro, E., Torres, A., Rodriguez-Jasso, R.M., Andrade, L.P., Smirnova, I., Sun, R.C., Meyer, A. S., 2020. Engineering aspects of hydrothermal pretreatment: from batch to continuous operation, scale-up and pilot reactor under biorefinery concept. Bioresour. Technol. 299, 122685. https://doi.org/10.1016/j.biortech.2019.122685.

Shao, S., Zhang, J., Bao, J., 2017. Reduction of reactor corrosion by eliminating liquid phase existence in dry dilute acid pretreatment of corn stover. Energy Fuels 31, 6140–6144. https://doi.org/10.1021/acs.energyfuels.7b00446.

Shiva, Barba, F.C., Rodríguez-Jasso, R.M., Sukumaran, R.K., Ruiz, H.A., 2022. High-solids loading processing for an integrated lignocellulosic biorefinery: Effects of transport phenomena and rheology–a review. Bioresour. Technol. 351, 127044. https://doi.org/10.1016/j.biortech.2022.127044.

Singh, A., Bedore, S.R., Sharma, N.K., Lee, S.A., Eiteman, M.A., Neidle, E.L., 2019. Removal of aromatic inhibitors produced from lignocellulosic hydrolysates by Acinetobacter baylyi ADP1 with formation of ethanol by Kluyveromyces marxianus. Biotechnol. Biofuels Bioprod. 12, 91. https://doi.org/10.1186/s13068-019-1434-7.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., 2008. Determination of Sugars, Byproducts, and Degradation Products in Liquid Fraction Process Samples; National Renewable Energy Laboratory (NREL) Technical Report NREL/TP-510-42623; NREL: Golden, CO.

Sluiter, A., Hames, B., Scarlata, C., Sluiter, J., Templeton, D., 2012. Determination of structural carbohydrates and lignin in biomass national renewable. NREL/TP-510-42618. National Renewable Energy Laboratory, Gloden, CO.

Wang, Y., Yang, H.C., Zhang, B., Liu, X.C., Bao, J., 2022. Continuous enzymatic saccharification and its rheology profiling under high solids loading of lignocellulose biomass. Biochem. Eng. J. 186, 108543. https://doi.org/10.1016/j.bej.2022.108543.

Wang, Y., Zhang, B., Bao, J., 2025a. Rigorous calculation of greenhouse gases (GHG) in sustainable L-lactic acid production from lignocellulosic biomass based on advanced biorefinery processing technology. ACS Sustain. Chem. Eng. 13, 6186–6196. https://doi.org/10.1021/acssuschemeng.4c10527.

Wang, Y., Zhang, B., Yang, H.C., Liu, X.C., Bao, J., 2025b. Balanced water and heat energy recycling by full evaporation of wastewater (FEW) in dry biorefining processes of lignocellulose biomass. Waste Manag. (Oxf.) 193, 307–316. https://doi.org/10.1016/j.wasman.2024.12.018.

Wirawan, F., Cheng, C.L., Lo, Y.C., Chen, C.Y., Chang, J.S., Leu, S.Y., Lee, D.J., 2020. Continuous cellulosic bioethanol co-fermentation by immobilized *Zymomonas mobilis* and suspended pichia stipitis in a two-stage process. Appl. Energy 266, 114871. https://doi.org/10.1016/j.apenergy.2020.114871.

Xu, Q.S., Yan, Y.S., Feng, J.X., 2016. Efficient hydrolysis of raw starch and ethanol fermentation: a novel raw starch-digesting glucoamylase from Penicillium oxalicum. Biotechnol. Biofuels Bioprod. 9, 216. https://doi.org/10.1186/s13068-016-0636-5.

Zhang, B., Ahemed, F., Zhan, B.R., Bao, J., 2021a. 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., Liu, X.C., Bao, J., 2023. High solids loading pretreatment: the core of lignocellulose biorefinery as an industrial technology – an overview. Bioresour. Technol. 369, 128334. https://doi.org/10.1016/j.biortech.2022.128334.

Zhang, B., Zhan, B.R., Bao, J., 2021b. Reframing biorefinery processing chain of corn fiber for cellulosic ethanol production. Ind. Crop. Prod. 170, 113791. https://doi.org/10.1016/j.indcrop.2021.113791.