

Article

High-Titer L-lactic Acid Production by Fed-Batch Simultaneous Saccharification and Fermentation of Steam-Exploded Corn Stover

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Abstract: Steam explosion (SE) is an effective lignocellulose pretreatment technology for second-generation L-lactic acid (L-LA) production. In this study, targeted to produce high-concentration L-LA from corn stover (CS), the fed-batch simultaneous saccharification and fermentation (SSF) of acidic, SE-pretreated CS was developed and demonstrated in a 5 L scale bioreactor under non-strict conditions (without detoxification and sterilization). The results indicated that the fed-batch SSF, with a simple pH control, realized a higher tolerance of the strains to the toxic by-products of hydrolysate, in comparison to the conventional sequential hydrolysis and fermentation (SHF), allowing for 153.8 g L⁻¹ of L-LA production, along with a productivity of 1.83 g L⁻¹ h⁻¹ in a system with a total of 40% (*w/v*) solid loading. The mass balance indicated that up to 449 kg of L-LA can be obtained from 1 t of dried CS. It exhibited obvious superiorities and laid down a solid foundation for the industrialization of second-generation L-LA production.

Keywords: steam explosion; L-lactic acid; simultaneous saccharification and fermentation; fed-batch hydrolysis; *Bacillus coagulans*



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1. Introduction

L-lactic acid (L-LA) is an important bulk chemical, which is widely used in the chemical, food, pharmaceutical, and cosmetic sectors [1]. In particular, with the implementation of sustainable development goals, along with the growing demand for biodegradable and biocompatible materials, the production of L-LA from renewable biomasses by fermentation has received increasing attention in recent decades, especially the second-generation routes from the abundant lignocelluloses [2,3].

Similar to the production of other biochemicals, a crucial step for L-LA production from lignocelluloses is developing efficient pretreatment and saccharification processes, in order to effectively depolymerize the recalcitrance structure of the lignocellulosic matrix, exposing the cellulose and improving the accessibility of the cellulase [4]. Among various candidate pretreatment techniques, steam explosion (SE) combines the physical

and chemical effects of instantaneous matter release under acidic conditions and elevated temperature and pressure, which has been well-proven in the industry [5]. Compared with other pretreatment techniques, SE exhibited the advantages of a low wastewater discharge, high efficiency, and high reliability [6]. The drawback, however, is the co-generation of a relatively large amount of phenols, organic acids, and furan derivatives as by-products of the liquidous phase, which always severely inhibit both the activity of cellulase in saccharification and the metabolism of the fermentable sugars in the subsequent fermentation unit [7].

Although remarkable achievements have been demonstrated in developing and screening the highly inhibitor-tolerant strains and employing hydrolysate detoxification strategies to improve the sugar and production yield, it is still difficult to realize a profitable lignocellulosic L-LA process in second-generation biorefinery plants [8]. A common obstacle is that the viscosity of the hydrolysate increases with increasing solid loading, which contributes to the poor mass and heat transfer of the slurry and results in low sugar yields [9]. The lower sugar concentration in hydrolysate logically causes the lower titers of lignocellulosic L-LA production in fermentation broth than the sugary and starchy processes [10] and, consequently, outputs a larger amount of wastewater that requires a more expensive downstream separation process [11]. This problem is also a prominent bottleneck in the SE-based biorefineries as most of the lignin fractions, which are undegradable but rich in hydroxyl groups, are involved in the enzymatic hydrolysis process [12]. Consequently, only a few reports realized the $>100 \text{ g L}^{-1}$ of lignocellulosic L-LA titer at the end of fermentation (Table S1) [13–15].

In this study, targeted to realize reliable and scalable bio-based L-LA production from lignocellulose, the SE of corn stover (SECS) was used as the substrate, while *B. coagulans* LA2301, a hyper strain with a high inhibitor tolerance and pentose/hexose co-assimilation ability, was served to the L-LA fermentation. To realize a high L-LA titer, along with a high yield, sequential hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) with high solid loadings were performed by detailedly comparing the kinetics in a 5 L scale bioreactor and the mass balance. The results suggested that the fed-batch SSF of SECS exhibited outstanding fermentation performances, even under non-strict conditions that were without detoxification and sterilization, which shows great promise for economically sustainable lignocellulosic L-LA production.

2. Materials and Methods

2.1. Materials and the Steam Explosion Conditions

CS was collected from local agricultural cooperatives in Hailun City, Heilongjiang, China. Both the crushing and the pretreatment of the corn stover were performed in an industrial-scale production line at the SDIC Advanced Biomass Fuel (Hailun) Co., Ltd. (Hailun, China). Briefly, the dried CS was crushed into 3–5 cm pieces, soaked with 1.5 wt% of dilute sulfuric acid, followed by being fed into a continuous tube of the SE equipment. The slurry was maintained at 170–180 °C ($\sim 0.8 \text{ MPa}$) for 15–20 min and continuous screw transmitted. Then, accompanied with a sudden decompression of the atmosphere pressure, the CS pieces were shredded into powder, namely SECS, and this was stored at $-20 \text{ }^{\circ}\text{C}$ before use. The SECS was not processed for washing or subjected to solid–liquid separation prior to enzymatic hydrolysis.

The cellulase used in this study was Cellic Ctec 3 (Novozymes, with an activity of $152 \pm 15 \text{ FPU mL}^{-1}$). Chemicals of analytical grade were purchased from Macklin (Shanghai, China), while the biological reagents used throughout the experiments were obtained from Yuanye Biotech (Shanghai, China).

2.2. Enzymatic Hydrolysis and Detoxification

Firstly, the batch enzymatic hydrolysis of the SECS was performed in a 2 L conical flask with 800 mL of working volume. The solids and cellulase loadings were 10% *w/v* and 15 FPU g^{−1} (of glucan), respectively. Before the enzymatic hydrolysis was carried out, the pH of the slurry was adjusted to ~4.8 using ammonia. Then, the flask was placed in a thermostatic shaker maintained at 50 °C and 200 rpm for 72 h. The hydrolysis process was terminated by solid–liquid separation via centrifugation. The upper liquor phase was collected and stored at −20 °C for the subsequent L-LA fermentation.

To obtain a higher sugar concentration in the hydrolysate so as to increase the final L-LA concentration, fed-batch enzymatic hydrolysis was further implemented. Generally, for each 12 h of hydrolysis, an additional 10% (*w/v*) of SECS and the corresponding amount of cellulase were added into the slurry intermittently, and, finally, we realized the different overall loading rates of the SECS. All the hydrolysates were centrifuged, and the supernatant was collected and stored. Detoxified hydrolysate was obtained by feeding the supernatant liquor, and it was passed through a column that was equipped with activated carbon, as was described in previous works [16,17].

2.3. L-lactic Acid Fermentation

B. coagulans LA2301 is a lignocellulosic inhibitor-tolerant strain that is isolated through domestication and adaptive engineering screening, and it was laboratory-preserved and used throughout the experiments [18]. For the seeds' preparation, 5% (*v/v*) of the glycerol-tube-stored strains were inoculated into a 4 mL sterilized test tube containing an MRS medium (20 g L^{−1} glucose, 10 g L^{−1} peptone, 5 g L^{−1} yeast powder, 10 g L^{−1} beef extract, 2 g L^{−1} C₆H₅O₇(NH₄)₃, 5 g L^{−1} NaCl, 5 g CH₃COONa, 0.2 g L^{−1} MgSO₄, 0.05 g L^{−1} MnSO₄, 2 g L^{−1} K₂HPO₄, and 1 mL L^{−1} Tween 80). The culturing conditions were 50 °C and 200 rpm. When the OD₆₀₀ value reached ~1, the seeds were transferred into a shake flask that contained an MRS medium. All the mediums were sterilized in an autoclave at 116 °C for 25 min before inoculation. For the analysis of the inhibition effect of the toxic SE by-products on the catabolism of the *B. coagulans* strains, the plates that contained the MRS medium and the varying concentrations of each inhibitor were adopted. After 24 h of inoculation, we counted the number of colonies, and these were recorded.

SHF was conducted using the fed-batch SECSH as a substrate under different solid loadings. The fermentation was carried out in 250 mL shake flasks with 100 mL of working volume. The inoculation rate of the activated seed was 10% (*v/v*). During the cultivation process, 20 g L^{−1} of calcium carbonate was used as the neutralizer. The open SHF of L-LA was also carried out in a 5 L bioreactor with a 2 L working volume, in which process, pH-regulation was conducted in order to guarantee a relatively high yield of L-LA [18]. The fed-batch SSF was conducted in a 5 L bioreactor, in which process, the initial SECS loading in the first batch was 10% (*w/v*), while the cellulase dosage was 15 FPU g^{−1}. Similar to the conditions in the fed-batch saccharification of the SECS, for each 12 h, an additional 10% (*w/v*) of the SECS, along with the cellulase, was added into the bioreactor intermittently, until the total SECS loading reached 40% (*w/v*). Afterwards, the fermentation was terminated after an additional 60 h of cultivation. The pH of the fed-batch SSF process was first automatically adjusted to ~4.8 at the beginning of fermentation using 33 wt% of calcium hydroxide suspension. Then, a stepwise pH control strategy that increased the pH by 0.2 every 4 h was adopted, until the final pH reached 6.0, as described in previous work [18].

2.4. Analysis

The chemical composition of the CS and the dried SECS was measured by the National Renewable Energy Laboratory (NREL) standard [19]. Glucose, xylose, lactic acid, and other organic acids were detected by high-performance liquid chromatography (HPLC) that was equipped with a refractive index detector (RID) and an Aminex HP-87H column (Bio-rad, Hercules, CA, USA). The mobile phase was 5 mM H₂SO₄, and the flow rate was 0.6 mL min^{−1}. Meanwhile, the column temperature was 65 °C. Inhibitors in the SECSH, including furfural, 5-hydroxymethylfurfural (5-HMF), and vanillin, were quantitatively analyzed using HPLC (Hitachi, Tokyo, Japan) that was equipped with an ultraviolet (UV) detector set at 220 nm and a C18 column (Waters, Milford, MA, USA). The mobile phase was a 55% (*w/v*) acetonitrile solution. All the samples were assayed in triplicate.

The surface morphologies of the CS and the SECS was observed using a scanning electron microscope (Hitachi S-3000N, Tokyo, Japan). The crystallinity of the cellulose was analyzed by an X-ray diffractometer (XRD, Rigaku Smart Lab, Tokyo, Japan) and calculated through the method previously reported [20]. A Fourier transform infrared spectrometer (FT-IR, Thermo-Fisher Nicolet 6700, Waltham, MA, USA) was used to analyze the functional groups and the interlinkages of the CS and the SECS.

3. Results and Discussion

3.1. The Characterization of the Steam-Exploded Corn Stover

The compositional analysis results are shown in Table 1. Generally, after acidic SE, there was still 28.4 ± 2.0 wt% of lignin remaining in the solid phase, which would negatively influence the accessibility of the cellulase within the glucan fractions [21]. The SEM images indicated that the natural CS fibers exhibited a smooth morphology (Figure S1a,b), while the transient SE and acid catalysis hydrolysis in the pretreatment stage synergistically affected the lignocellulosic structure and effectively depolymerized the dense matrix of the CS. The SECS possessed a loose and fragmented structure (shown in Figure S1c,d).

Table 1. Composition of the raw corn stover and SECS.

Samples	Chemical Composition (wt%)				Solid Recovery (%)
	Glucan	Xylan	Lignin	Others	
CS	35.5 ± 2.6	20.7 ± 2.0	27.2 ± 2.4	16.6 ± 1.2	85.0 ± 4.7
SECS	38.0 ± 1.8	21.2 ± 1.6	28.4 ± 2.0	12.4 ± 0.7	

The obvious changes in the chemical composition and morphology of the CS after the continuous SE pretreatment can be also verified by the FTIR spectrum. As is shown in Figure S1e, after SE, the peak at 1300 cm^{−1} in the CS disappeared, indicating the reduction of S-type lignin [22]. SE promotes the depolymerization of lignin because of the cleavage of β-O-4 bonds [23]. However, the lignin units were unstable under hydrothermal or acidic conditions and would undergo re-condensation [24,25]. Meanwhile, after SE, the absorption in the range of 3300–3500 cm^{−1} showed a certain degree of narrowing in the SECS compared to the CS, suggesting the cleavage of the O-H bond in glucan [26]. Besides, the peaks at 1030 cm^{−1} and 1060 cm^{−1} were significantly weakened in the SECS, indicating the substantial depolymerization of the xylan structure in acidic conditions [27]. The stretching vibration peak of the conjugated carbonyl C=O at 1640 cm^{−1} was also reduced in the SECS, revealing the delignification in the SE [28]. The effective hydrolysis of xylan and the depolymerization of lignin in the CS after acidic SE can be further verified by the XRD pattern (Figure S1f). Because of the deconstruction of the amorphous region and the

exposure of the crystal structure of the cellulose [29], the crystallinity of the SECS reached 39.39%, which was 8.1% higher than the CS.

3.2. The Enzymatic Hydrolysis of the Steam-Exploded Corn Stover

The structural characterization results indicated that the SECS could effectively depolymerize the complex lignocellulosic matrixes, thereby enhancing the reactive site in cellulose fractions. Based on fed-batch saccharification, the high concentration of monomeric sugars (mainly consisting of xylose and glucose) was hydrolyzed from the SECS and was used as the carbon source in the subsequent L-LA fermentation process. As shown in Table 2, with the increase of the total SECS loading in the fed-batch saccharification process, the mono-sugar concentration was gradually increased. A maximized 225.75 g L^{-1} of sugar (including 167.60 g L^{-1} of glucose and 58.15 g L^{-1} of xylose) was obtained in the final SECSH, which had a total of 40% solid load. Therefore, 99.1 wt% of the glucan and 68.4 wt% of the xylan in the CS were recycled in the SECSH after the acidic SE and fed-batch saccharification. However, accompanied by the hydrolysis of the carbohydrates in the SECS, the by-products possessed a high inhibition effect on the growth and L-LA production of the *B. coagulans* strains that were also released into the hydrolysate [30]. For instance, inhibitors, including 4.10 g L^{-1} of formic acid, 10.16 g L^{-1} of acetic acid, 1.57 g L^{-1} of furfural, and 3.52 g L^{-1} of 5-HMF, were detected in the hydrolysate with a 40% (*w/v*) solid loading, which were much higher than the groups with lower solid loadings. Lu et al. observed that the toxic by-products increased with the increase in the solid concentration. For example, the acetic acid concentration of 10% (*w/v*) unwashed, pretreated straw hydrolysate was less than 1.5 g L^{-1} , whilst the acetic acid concentration of the 30% (*w/v*) group reached 4.7 g L^{-1} [31].

Table 2. The main components and inhibitors of the fed-batch SECSHs with different solid loadings.

Concentrations	Solid Loading (% <i>w/v</i>)			
	10	20	30	40
Glucose (g L^{-1})	36.48 ± 0.83	66.69 ± 1.16	109.38 ± 2.29	167.60 ± 3.47
Xylose (g L^{-1})	15.98 ± 0.06	37.88 ± 0.17	48.27 ± 2.78	58.15 ± 3.53
HMF (g L^{-1})	0.82 ± 0.01	1.72 ± 0.11	2.82 ± 0.03	3.52 ± 0.01
Furfural (g L^{-1})	0.32 ± 0.01	0.62 ± 0.05	1.08 ± 0.09	1.57 ± 0.09
Formic acid (g L^{-1})	1.21 ± 0.03	2.18 ± 0.30	3.72 ± 0.91	4.10 ± 0.65
Acetic acid (g L^{-1})	2.36 ± 0.43	4.65 ± 0.36	8.09 ± 0.64	10.16 ± 0.58
Vanillin (mg L^{-1})	14.04 ± 1.58	25.38 ± 2.89	46.73 ± 1.17	61.53 ± 4.40

3.3. L-lactic Acid Fermentation Using the Steam-Exploded Corn Stover Hydrolysate

The deficiency of a high inhibitor content in SE hydrolysate has long been criticized in previous reports, which had a negative influence on the biochemical production in fermentation and was recognized as one of the bottlenecks for large-scale SE applications in the industry [32]. Fortunately, in contrast to other industrial microbes that were sensitive to the SE inhibitors, *B. coagulans* was suggested to have relatively high tolerances to the toxic compounds, such as the mono-phenols, organic acids, and furan derivatives. This was due to the fact that *B. coagulans* cells have multiple genes that are upregulated under the pressure of high concentrations of inhibitors, including oxidoreductases that can oxidize/reduce aldehyde groups in inhibitors [33].

Hence, to investigate whether the undetoxified SECSH can be directly used as a substrate for L-lactic acid fermentation or not, before we carried out the SHF, pre-experiments were conducted to analyze the colony growth of *B. coagulans* LA2301 strains in plates with inhibitor stress. Generally, the results illustrated that acetic acid and vanillin exhibited the stronger inhibition of the growth of *B. coagulans* LA2301, compared to other toxic by-products of the

SECSH (Figure S2). However, the tolerance of these two compounds by *B. coagulans* was higher than their concentrations in the fed-batch enzymatic hydrolysate. It was illustrated that the presence of a low concentration of furfural in the substrate could promote morphological changes and improve the cell fitness and, consequently, promote the L-LA production [34]. This phenomenon was also observed in the current work. The strains still grew well, even when the furfural concentration was increased to 4 g L^{-1} in the substrate.

The pre-experiment results indicated that the *B. coagulans* LA2301 strains exhibited high tolerances to the toxic compounds in the SECSH. Although the synergistic inhibition effect of the inhibitors in the SECSH were not investigated, the promising results prompted us to evaluate the L-LA fermentation performances using the undetoxified SECSH directly. As shown in Figure 1, both glucose and xylose can be used as carbon sources by *B. coagulans*. However, because of the carbon catabolite repression effect, no matter the initial sugar concentration in the fed-batch SECSH, the xylose conversion in the batch SHF was always less than the metabolism of glucose [35]. Moreover, with the increase of the sugar concentration in the SECSHs with increased solid loadings, the residual xylose concentration in the final fermentation broth was also increased. This could be explained by the higher inhibitor concentration with the increase of the SECS loading in the fed-batch saccharification process, which indeed possessed a severer toxicity to the strains. Consequently, the price for the higher L-LA concentration ($147.65 \pm 5.54 \text{ g L}^{-1}$) in the higher solid loading (40% , w/v) groups was a lower productivity ($2.27 \text{ g L}^{-1} \text{ h}^{-1}$) and yield (0.85 g g^{-1}). There was still 16.05 g L^{-1} of xylose remaining in the final broth of the 40% solid loading group, while there were no residual sugars in the broth in the 10% (w/v) solid loading group. Table 3 gives the parameters of each SHF group.

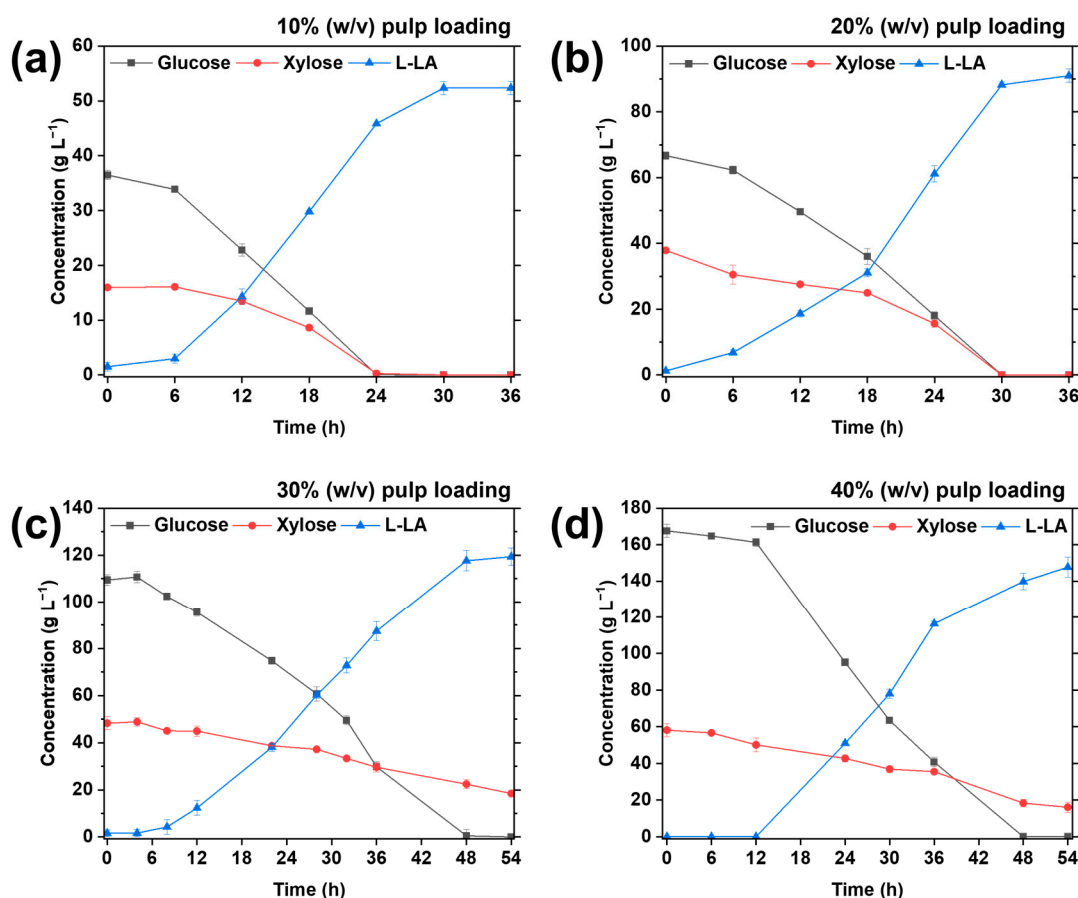


Figure 1. The time course of the SHF for L-LA using the undetoxified fed-batch SECSH with an overall (a) 10% (w/v), (b) 20% (w/v), (c) 30% (w/v), and (d) 40% (w/v) solid loading.

Table 3. The SHF of L-LA using the fed-batch SECSH with different solid loadings.

Parameters	Solid Loading (<i>w/v</i>)			
	10	20	30	40
Initial glucose (g L ⁻¹)	36.48 ± 0.83	66.69 ± 1.16	109.38 ± 2.29	167.60 ± 3.67
Initial xylose (g L ⁻¹)	15.98 ± 0.06	37.88 ± 0.17	48.27 ± 2.78	58.15 ± 3.53
Fermentation period (h)	30	36	54	54
Residual sugars (g L ⁻¹)	0.00	0.00	18.46 ± 1.15	16.05 ± 2.76
L-LA conc. (g L ⁻¹)	52.36 ± 1.19	90.98 ± 2.04	119.39 ± 3.66	147.65 ± 5.54
Productivity (g L ⁻¹ h ⁻¹)	1.88	3.04	2.45	2.27
L-LA yield (g g ⁻¹)	0.97	0.95	0.93	0.85

Therefore, it was necessary to further improve the L-LA fermentation performances when using the SECSH containing high concentrations of sugars and inhibitors. As was illustrated in previous studies, enhancing the nutrient supplementation in the substrate could increase the robustness of strains, so as to improve the tolerance to the inhibitors in the lignocellulose hydrolysate. To this end, batch L-LA fermentation using the SECSH, with additional peptone as a nutrient and nitrogen source, was practiced. As expected, the catabolism of mono-sugars in the undetoxified SECSH was rapidly prompted (Figure S3). Nonetheless, even though the xylose consumption in the 10% (*w/v*) solid loading group was increased by 25.40% after adding 20 g L⁻¹ of peptone, the L-LA yield and concentration were not significantly increased compared to the control group without any peptone addition. Therefore, the nutrient supplementation in the SECSH was excessive, as the substrate redirected more carbon sources towards biomass synthesis. Comparatively, adding 10 g L⁻¹ of peptone into the 40% (*w/v*) SECSH exhibited higher L-LA productivity and a higher yield.

To further decrease the inhibition of the higher concentration of toxic compounds in the 40% solid loading group and improve the lignocellulosic L-LA production, SHF using the detoxified SECSH was also practiced. Here, the active carbon was adopted, in which process, the crude SECSH was passed through a package-bed column. Most of the furan derivants and phenolic compounds (86.62% of furfural, 85.51% of HMF, and 96.25% of vanillin) in the SECSH were adsorbed onto the surface of the active carbon, while the organic acids were also partially separated (13.17% of formic acid and 21.65% of acetic acid). Consequently, as illustrated in Figure 2, when using the detoxified SECSH with the 40% solid loading as the substrate, the fermentation period was shortened by 6 h, while there were 159.68 ± 3.57 g L⁻¹ of L-LA in the final broth (increased by 7.53%). At the same time, L-LA productivity (3.33 g L⁻¹ h⁻¹) was also obviously increased compared to that of the SHF using the undetoxified SECSH (2.27 g L⁻¹ h⁻¹).

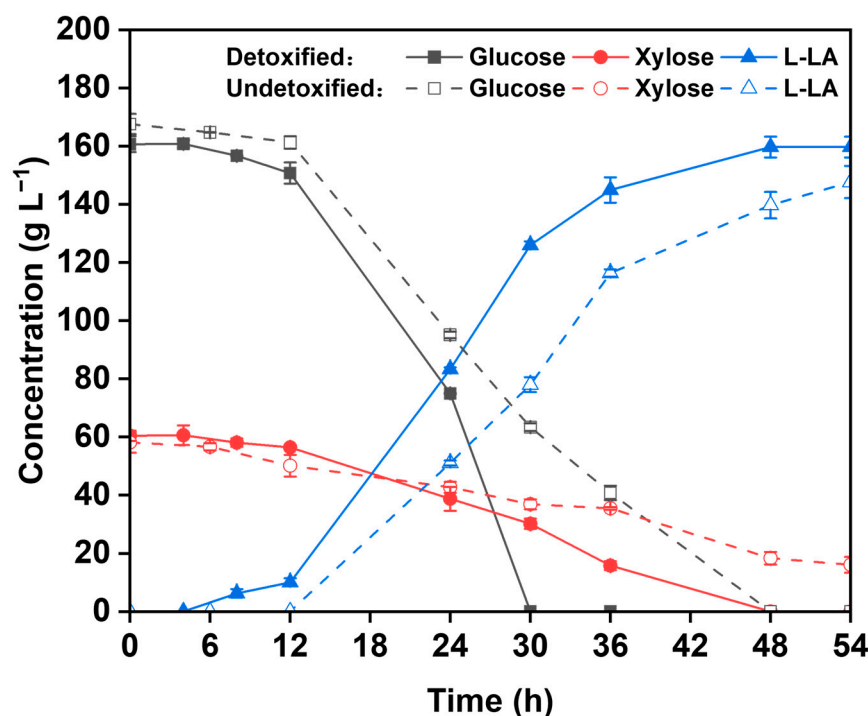


Figure 2. The time course of the SHF for L-LA using the detoxified fed-batch SECSH with an overall 40% (*w/v*) solid loading. Also, 10 g L⁻¹ peptone was added as an additional nutrient and a nitrogen source.

3.4. Fed-Batch Simultaneous Saccharification and Fermentation for L-Lactic Acid Production

SSF allowed the simultaneous hydrolysis of carbohydrates and bioconversion in a one-pot process. The negative influence of hyper-osmosis, caused by high-sugar conditions, on the growth of the strains can be avoided by SSF, whilst the viscosity of the slurry can be low, which results in good mass and heat transfer in the bioreactor [36]. Meanwhile, before the feeding operation, the inhibitor concentration was maintained at a relatively low level in the initial adaptive and the pre-logarithmic stages of cell growth, in which the strains were highly sensitive to the toxic compounds [37]. This avoided inhibition due to the large accumulation of inhibitors accompanying the pulp addition. On this basis, we speculated that L-LA production can be further improved by adopting fed-batch SSF, even though this was absent from the detoxification process of the liquidous fraction.

The time course of the fed-batch SSF is shown in Figure 3, in which process, a pH-adjusting strategy was conducted to realize a high carbohydrate saccharification rate and a high L-LA yield. Similarly to the phenomenon in fed-batch saccharification, after the initial 12 h in batch mode, additional SECSs were intermittently loaded into the bioreactor in the following period of SSF, until the overall SECS loading reached 40%. As can be seen from Figure 3, during the first 48 h of SSF, a low concentration of sugars was detected in the fermentation broth, indicating the carbohydrates' hydrolysis rate was higher than the consumption rate. Over the following 36 h, the catabolism of the sugars was faster than their generation. Consequently, no mono-sugars were detected in the broth until the termination of the SSF. Finally, 153.94 ± 5.51 g L⁻¹ of L-LA was obtained at the end of the SSF, with a productivity of 1.83 g L⁻¹ h⁻¹. Furthermore, only 1.70 ± 0.54 g L⁻¹ of residual xylose was detected in the fermented broth. These results were attractive when compared to the previous results using the SE hydrolysate of various lignocelluloses, even when considering the systems based on other pretreatment strategies (Table S1).

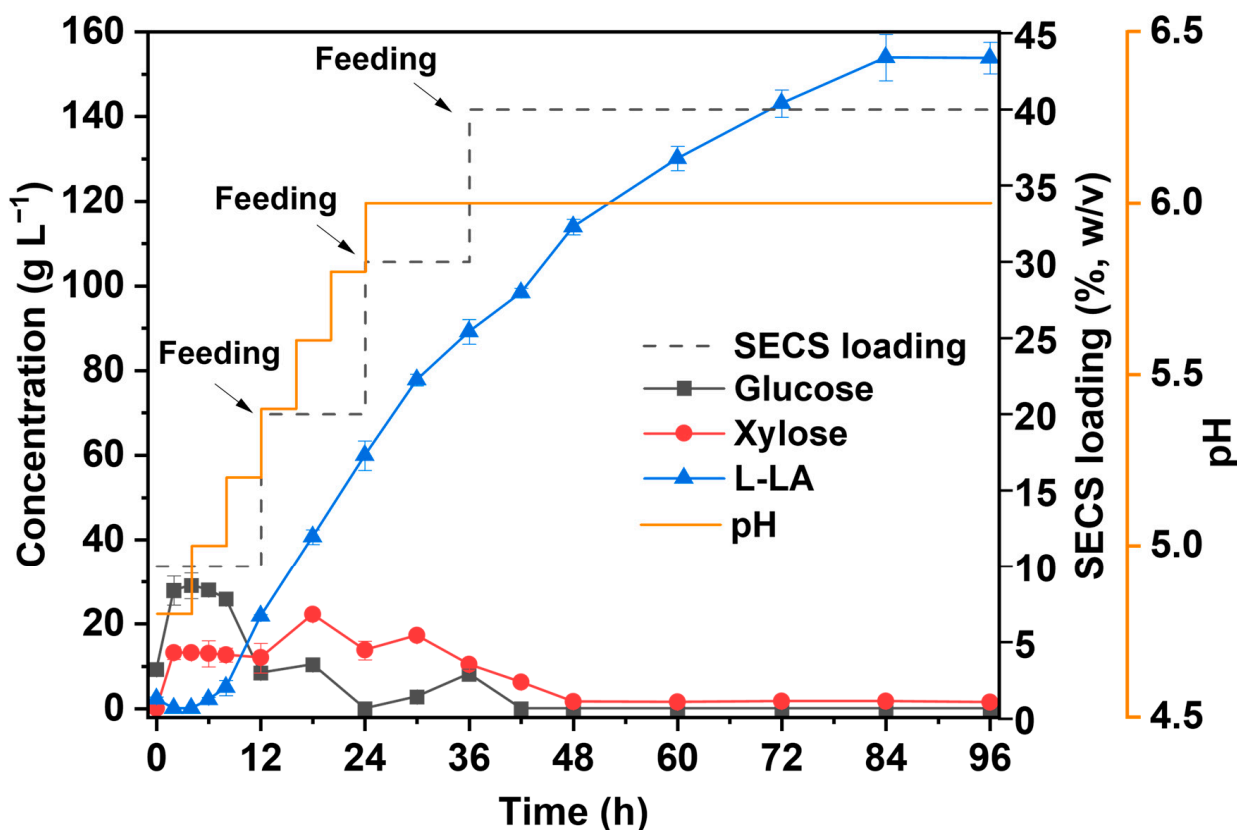


Figure 3. L-LA production by *B. coagulans* LA2301, based on the fed-batch SSF of SECS without autoclaving and detoxification.

Therefore, the high concentration and yield of L-LA confirmed the superiority of the fed-batch SSF technique, which also revealed the speculation of the lower inhibition of the *B. coagulans* metabolism. The mass balance indicated that the fed-batch SSF was much more effective than that based on the SHF of the SECSH under a similar solid loading, whether practicing detoxification or not. As Figure 4 shows, although the SHF of the detoxified SECSH resulted in a higher L-LA concentration, the high sugar-losing rate (11.90%) in the detoxification process lead to this process only outputting 370 kg of L-LA from 1 t of dried CS, which was still 10.84% lower than the SHF of the undetoxified SECSH. Remarkably, up to 449 kg t⁻¹ of L-LA can be yielded from the fed-batch SSF of the SECS. Compared with the previous results shown, the current work exhibited superiorities of a high L-LA concentration, yield, and productivity, and the entire pretreatment and fermentation process was not subjected to washing, detoxification, or sterilization, which indicates the strong potential of the low process cost and many environmental benefits (Table S1). Moreover, the excellent fermentation results and the simple operation conditions make the fed-batch SSF very feasible to be scaled to large-scale operations.

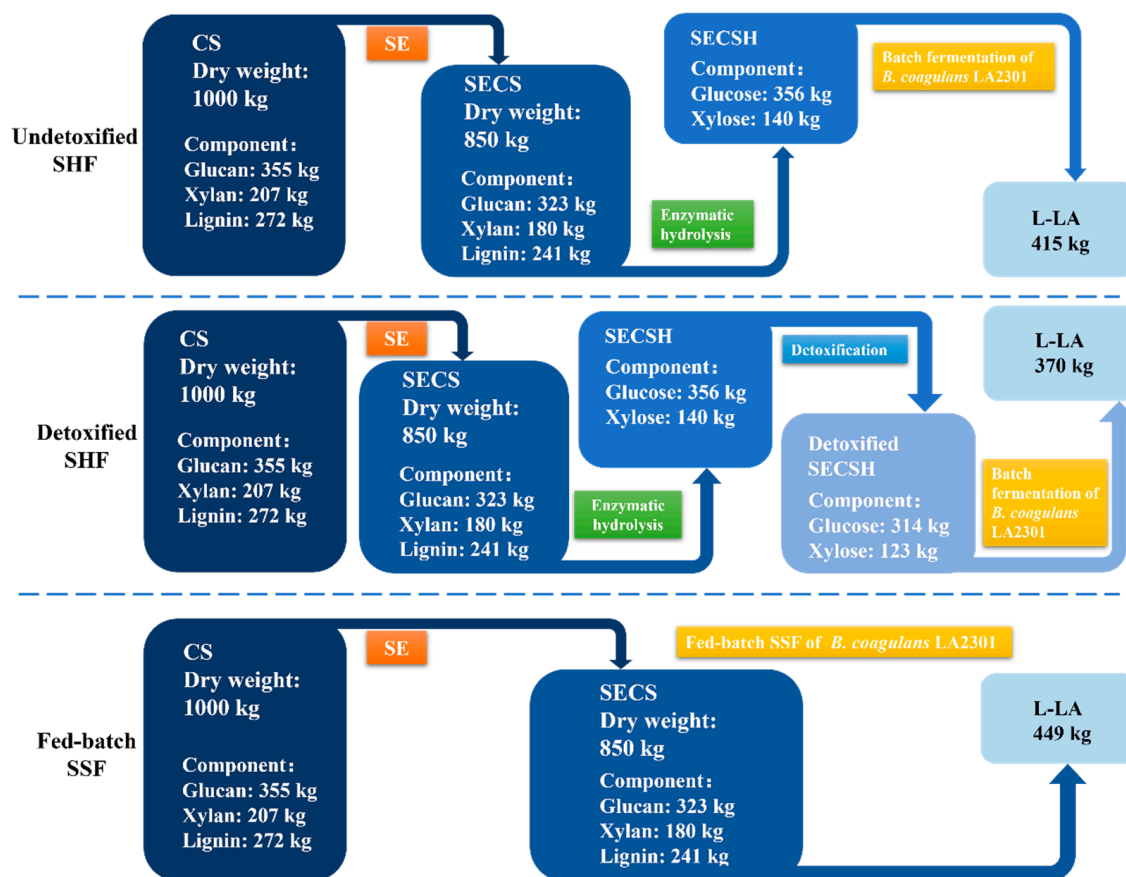


Figure 4. Mass balance of L-LA based on different fermentation strategies. The overall SECS loadings were 40% (w/v).

4. Conclusions

High-concentration L-LA was produced by employing fed-batch SSF using the SECS and *B. coagulans* LA2301, without detoxification and sterilization. Because of the high tolerance of the strains and the mitigation of the inhibitions, the carbohydrate fractions in the SECS were efficiently hydrolyzed and catabolized into L-LA in a one-pot process. Remarkably, up to 153.8 g L^{-1} of L-LA was generated within 84 h of inoculation, with a productivity of $1.83 \text{ g L}^{-1} \text{ h}^{-1}$. The mass balance indicated that the fed-batch SSF allowed for 449 kg of L-LA production from dried CS, which was obviously higher than that of SHF (415 kg). Therefore, the outstanding L-LA fermentation performance and the simple operation offer practical advantages.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/fermentation11010025/s1>, Figure S1: Structural characterization of the SECS. SEM images of (a,b) CS and (c,d) SECS. Comparison of (e) FTIR spectrum and (f) XRD patterns of the CS and SECS. Figure S2: Colony tolerance of *B. coagulans* LA2301 to typical inhibitors containing in steam-exploded corn stover hydrolysate (SECSH). Figure S3: Time course of sequential hydrolysis and fermentation (SHF) of L-LA by *B. coagulans* LA2301 using undetoxified fed-batch SECSH with the additional peptone as nutrient and nitrogen source. (a) Glucose and (b) xylose concentrations in fermentation broth, and (c) L-LA concentration in broth. Table S1: Current advances of lignocellulosic L-LA production based on various pretreatment strategies. Table S2: Comparison of key parameters for fed-batch SHF of L-LA by *B. coagulans* LA2301 using SECSH. Refs. [38–42] are cited in the supplementary materials.

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C.S. and D.C.; Supervision, D.C.; Funding acquisition, B.W. All authors have read and agreed to the published version of the manuscript.

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