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High titer L-lactic acid production from corn stover with minimum wastewater generation and techno-economic evaluation based on Aspen plus modeling



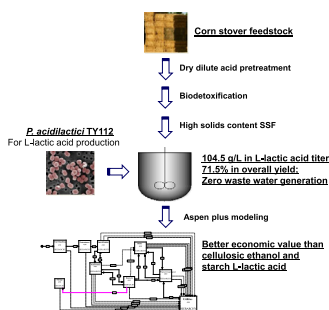
Gang Liu¹, Jiaoe Sun¹, Jian Zhang¹, Yi Tu, Jie Bao*

State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, 130 Meilong Road, Shanghai 200237, China

HIGHLIGHTS

- Obtained 104.5 g/L in L-lactic acid titer and 71.5% in overall yield by overcoming technical barriers.
- Obtained minimum water consumption by dry milling biorefinery processing (DMBP) technology.
- Developed Aspen plus model for L-lactic acid production from corn stover.
- Better economic value of cellulosic L-lactic acid than cellulosic ethanol and starch L-lactic acid.

GRAPHICAL ABSTRACT



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ABSTRACT

Technological potentials of L-lactic acid production from corn stover feedstock were investigated by experimental and techno-economic studies. An optimal performance with 104.5 g/L in L-lactic acid titer and 71.5% in overall yield from cellulose in corn stover to L-lactic acid using an engineered *Pediococcus acidilactici* strain were obtained by overcoming several technical barriers. A rigorous Aspen plus model for L-lactic acid production starting from dry dilute acid pretreated and biodetoxified corn stover was developed. The techno-economic analysis shows that the minimum L-lactic acid selling price (MLSP) was \$0.523 per kg, which was close to that of the commercial L-lactic acid produced from starch feedstock, and 24% less expensive than that of ethanol from corn stover, even though the xylose utilization was not considered. The study provided a prototype of industrial application and an evaluation model for high titer L-lactic acid production from lignocellulose feedstock.

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1. Introduction

L-lactic acid is a versatile block-building chemical used in food, pharmaceutical, leather, textile and chemical industries. Its most important application is as the monomer of biodegradable plastic polylactic acid (PLA) for substitution of petroleum-derived plastics,

such as polyethylene (PE), polypropylene (PP), and polystyrene (PS). To reach this ultimate goal, the feedstock for PLA production should be sufficient and cost-effective. Currently, approximately 90% of the commercially available L-lactic acid is produced by submerged fermentation of corn, and the corn feedstock accounts for nearly 70% of the overall production cost (Abdel-Rahman et al., 2013). Among various feedstock alternatives for corn, lignocellulose biomass is especially important for its availability and abundance.

* Corresponding author. Tel./fax: +86 21 64251799.

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

¹ These authors contributed equally to this work.

Industrial production generally requires the L-lactic acid titer over 100 g/L and optical purity over 99%, together with a high yield of L-lactic acid from glucose close to the theoretical and a high productivity for reducing overall PLA cost (Abdel-Rahman et al., 2013; Litchfield, 2009). In addition, wastewater generation should be strictly limited due to the high cost of the wastewater treatment. When lignocellulose is used as feedstock, the titer, optical purity, overall yield, and productivity of L-lactic acid, as well as wastewater generation should be close to the corn based L-lactic acid process. Hu et al. (2015) obtained 97.6 g/L of L-lactic acid from alkaline pretreated corn stover using *Bacillus coagulans* LA204. Ye et al. (2014) produced 80.6 g/L L-lactic acid from acid-catalyzed hydrolysate of empty palm fruit bunch using *B. coagulans* J112. Zhang et al. (2014) produced 38.73 g/L L-lactic acid from wheat straw using *B. coagulans* IPE22. In these studies, the major disadvantage is the massive wastewater generation from biorefinery process of lignocellulose.

According to Cargill Dow and Hycail (Cranket et al., 2005; Wee et al., 2006), the production cost of L-lactic acid could be reduced to \$1.34–1.46 per kg, but the expectation was around \$0.9 per kg for PLA use. In this study, an engineered strain *Pediococcus acidilactici* TY112 was used in the simultaneous saccharification and fermentation step (SSF) for high titer L-lactic acid fermentation from corn stover. The dry dilute acid pretreatment, biodegradation and high solids loading SSF established in our previous work were applied (Zhang et al., 2010a,b, 2011; He et al., 2014a,b). Several technical barriers were overcome and L-lactic acid titer of 104.5 g/L and yield of 71.5% were obtained. The optimal results were used as the basis of a rigorous Aspen plus modeling. The techno-economic analysis show that the minimum L-lactic acid selling price (MLSP) was \$0.523 per kg, which was close to the minimum ethanol selling price (MESP) by NREL (Humbird et al., 2011), even the xylose was not utilized. The study provides a typical case of biobased chemical production from lignocellulose and a design case for commercial scale evaluation of L-lactic acid from corn stover.

2. Methods

2.1. Raw materials and enzyme

Corn stover (CS) was obtained from Dancheng County, Henan Province, China, in fall 2013. Corn stover was water-washed to remove the impurities and air-dried, then milled using a beater pulverizer to pass through the 10-mm apertures in diameter. The milled corn stover was sealed in plastic bags and stored at room temperature until used.

The cellulase enzyme Youtell #6 was purchased from Hunan Youtell Biochemical Co. (Yueyang, Hunan, China). The filter paper activity of Youtell #6 was 135 FPU/g determined using the NREL protocol LAP-006 (Adney and Baker, 1996), the cellobiase activity was 344 CBU/g (Sharma et al., 1991), and the protein content was 90 mg/g.

2.2. Strains and culture

L-lactic acid producing strain *P. acidilactici* TY112 (CGMCC 8664) was engineered from the wild strain *P. acidilactici* DQ2 (CGMCC 7471) by the knockout of D-lactic acid dehydrogenase encoding gene *ldhD*. *Amorphotheca resiniae* ZN1 (CGMCC 7452) was used as the biodegradation strain for degrading inhibitors existing in the dry dilute sulfuric acid pretreated corn stover via solid state fermentation (Zhang et al., 2010b).

The simplified MRS medium contained 20 g/L of glucose, 5 g/L of yeast extract, 2 g/L of triammonium citrate, 5 g/L of sodium acetate ($\text{CH}_3\text{COONa}\cdot 3\text{H}_2\text{O}$), 0.6 g/L of $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$, 2 g/L of K_2HPO_4 , 0.25 g/L of $\text{MnSO}_4\cdot 7\text{H}_2\text{O}$. The activated *P. acidilactici* TY112 in sim-

plified MRS medium was aliquoted into 1.5 mL vials containing 30% (w/w) glycerol and stored at -80°C freezer. At each seed culture operation, one vial of *P. acidilactici* TY112 was completely inoculated into the seeding culture in order to keep the same inoculation size and quality. The optical density at 600 nm (OD_{600}) of the inoculum culture of *P. acidilactici* TY112 was 6.61 ± 0.35 when glucoamylase was added. The OD_{600} was only 3.38 ± 0.13 without glucoamylase addition because of flocculation of cells reduced the dispersed suspension of cells.

A two-step seeds cultivation method in flasks was used: one vial of *P. acidilactici* TY112 was inoculated into a 100 mL Erlenmeyer flask containing 20 mL of sterilized simplified MRS medium, cultured in a shaking incubator at 42°C , 150 rpm for 12 h; then the cells were transferred into a 500 mL Erlenmeyer flask containing 200 mL medium with 10% inoculation ratio, cultured at the same condition.

For seeds cultivation in bioreactors, the procedure was same to that in flasks in the first two steps, and the third step was conducted in 3-L bioreactors at 42°C and 150 rpm for 8 h before the culture broth was used as the fermentation seeds. The pH value during cultivation in bioreactors was controlled at 5.5 with 5 M sodium hydroxide.

2.3. Dry milling biorefinery process operation

Corn stover was pretreated using dry dilute sulfuric acid pretreatment (DDAP) in a 20-L reactor according to Zhang et al. (2011) and He et al. (He et al., 2014a,b). Briefly, dry corn stover and dilute sulfuric acid solution at 5.0% (w/w) were co-currently fed into the reactor at a solid/liquid ratio of 2:1 (w/w) with helically stirring at 50 rpm, then pretreated at 175°C for 5 min. The solids content of the pretreated slurry was around 50% (w/w) and no wastewater stream was generated.

The pretreated corn stover was detoxified using *A. resiniae* ZN1 according to Zhang et al. (2010b). Briefly, the pretreated corn stover was neutralized with 20% (w/w) $\text{Ca}(\text{OH})_2$ suspension slurry added to the biodegradation reactor, then mixed by helical ribbon stirrer agitation with the pretreated corn stover for neutralizing the sulfuric acid added to pH value of 5–6. The filamentous *A. resiniae* ZN1 cells was inoculated on the pretreated corn stover at 10% (w/w) ratio and cultured at 28°C for six days. No fresh water and nutrients were added during biodegradation, and the solids content of the biodegraded corn stover material was kept at about 50% (w/w). The accelerated biodegradation process was developed by feeding sterilized air at 1.0 vvm and a periodical agitation every 12 h in the helical-stirring bioreactor during shortened 48 h biodegradation. The inhibitor contents of the freshly pretreated corn stover included 4.81 mg of furfural, 3.34 mg of 5-hydroxymethylfurfural (HMF), and 16.79 mg acetic acid per gram of dry pretreated corn stover. After 48 h biodegradation, furfural, HMF, and acetic acid were reduced to 0, 0.43, 3.81 mg per gram of dry pretreated corn stover, respectively.

A 5-L bioreactor equipped with a helical ribbon stirrer was used for handling high solids loading saccharification or SSF of lignocellulose materials (Zhang et al., 2010a). The dry dilute acid pretreated and biodegraded corn stover was fed into the reactor and the prehydrolysis was performed for 6 h at 50°C . Then the SSF was started by inoculating the *P. acidilactici* TY112 at 45°C for 72 h. Unless otherwise stated, the cellulase dosage was 15 FPU/g DM and the pH was maintained at 5.5 by automatic regulation with 5 M NaOH. The samples were withdrawn at regular intervals, centrifuged at 11,167g for 5 min and the supernatant was analyzed.

2.4. Analysis

The cellulose and hemicellulose contents of the corn stover were analyzed using a two-step H_2SO_4 hydrolysis method. The

dry dilute sulfuric acid pretreated corn stover contained 37.57% of glucan and 2.68% of xylan.

Glucose, lactic acid, acetic acid, furfural and HMF contained in the taken samples were analyzed on HPLC (LC-20AD pump, RI detector RID-10A, Shimadzu, Kyoto, Japan) with Bio-Rad Aminex HPX-87H column operated at 65 °C and 0.6 mL/min of 5 mM H₂SO₄ as the mobile phase.

2.5. L-lactic acid yield calculation

L-lactic acid yield was defined as the ratio of the actually obtained L-lactic acid to the theoretical L-lactic acid calculated based on the chemical stoichiometry from cellulose in two steps of saccharification and fermentation:

$$\text{L-lactic acid yield} = \frac{[LA] \times V - [LA]_0 \times V_0}{f \times [\text{Biomass}] \times 1.111} \times 100\%$$

where [Biomass] is the dry feedstock content at the beginning (g/L), f is the cellulose fraction of dry feedstock (g/g), 1.111 is the conversion factor for cellulose to equivalent glucose, [LA] is the L-lactic acid concentration at the end (g/L) and [LA]₀ is the starting concentration (g/L) (comes from the seed inoculation) in the liquid fraction (not the whole slurry), V is the volume of the SSF system including NaOH solution added, V_0 is the volume of the SSF system at the starting point. The liquid volume was calculated based on the water mass balance of SSF operation.

2.6. Process model establishment

Process model was developed using Aspen plus software (AspenTech, Cambridge, MA). The NREL design for ethanol production from corn stover was used as the basis after re-designing the unit operations to meet the dry milling biorefinery technology requirement for L-lactic acid production (Humbird et al., 2011). Similar to NREL model, the overall flowsheet of the present model in Fig. 1 consists of 10 areas, including feedstock handling, pretreatment, biodetoxification, enzymatic hydrolysis and fermentation, cellulase production, product recovery, wastewater treatment, residue combustion, storage, and utilities system. The physical

data of L-lactic acid and its derivative salts were cited from Aspen plus built-in database. As shown in Table 1, the major changes compared with the NREL model include:

- (1) In Area 100, 900 metric tons of dry corn stover is handled each day, instead of 2000 tons in NREL model, to meet the agricultural feature in China (the composition of corn stover feedstock is set to be same in appendix Table A-1).
- (2) In Area A200, the dry dilute acid pretreatment method is applied with 67% of corn stover solids and 33% of dilute sulfuric acid solution (5% sulfuric acid in weight percentage) at the beginning, and 50% solids content to 50% liquid at the end (due to the steam condensation), instead of 30% solids content in the pretreated solids in NREL design. No wastewater is generated thus no solid-liquid separation is needed because of the high solid content in this design, while a solid-liquid separation by flash-cooled is required in NREL design. The equipment is also re-designed for changing the capital investment.
- (3) In Area A300, the solid state biodetoxification by culturing filamentous *A. resiniae* ZN1 on the pretreated corn stover is performed to remove the inhibitors, instead of ammonia conditioning on liquid pretreated hydrolysate in NREL design. The corresponding equipment is also changed.
- (4) In Area A400, enzymatic hydrolysis is carried out at 30% (w/w) solids content and L-lactic acid fermentation by *P. acidilactici* TY112 is performed, while in NREL design the hydrolysis is carried out at 20% solids content and ethanol fermentation is followed.
- (5) In Area A500, L-lactic acid purification is performed to obtain 88% (w/w) solution by solid/liquid separation, ultrafiltration, electro dialysis and multi-effect evaporation successively (Madzingaidzo et al., 2002), instead of the two column ethanol distillation and one column molecular sieve absorption in NREL design. At the same time, NaOH solution produced in electro dialysis is recycled to A400 for pH control.

The rest of the process areas including the on-site cellulase enzyme production in Area B400, the residue combustion in Area

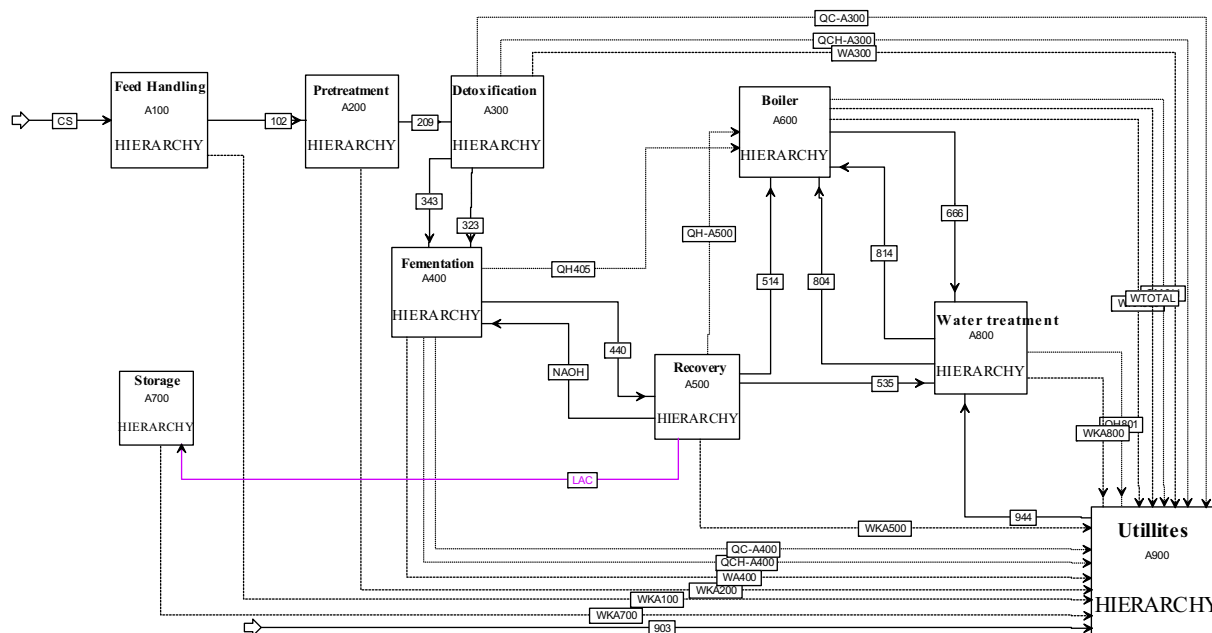


Fig. 1. Flowchart of cellulosic L-lactic acid production from corn stover feedstock on Aspen plus platform. The detailed mass balance, heat balance and work balance data were listed in the Supplemental Materials.

Table 1
Main process parameters in Aspen plus models.

	NREL model	DMBP model (this study)
<i>Pretreatment</i>		
Sulfuric acid loading	22 mg/g dry biomass	25 mg/g dry biomass
Temperature	158 °C	175 °C
Pressure (MPa)	0.56	1.25
Residence time	5 min	5 min
Total solids loading	30% (w/w)	50% (w/w)
<i>Detoxification</i>		
	Ammonia conditioning	Biodetoxification
<i>Saccharification and fermentation</i>		
Strain	<i>Z. mobilis</i> 8b (for ethanol)	<i>P. acidilactici</i> TY112 (for L-lactic acid)
Temperature and residence time	48 °C, 84 h (hydrolysis)	50 °C, 6 h (prehydrolysis)
	32 °C, 36 h (fermentation)	45 °C, 72 h (SSF)
Total solids loading	20% (w/w)	30% (w/w)
Cellulase loading	20 mg protein/g cellulose	28 mg protein/g cellulose
Glucan conversion to glucose (%)	90	85
Ethanol and Lactate yield from glucose (%)	95	87
Ethanol and Lactate yield from xylose (%)	85	0
<i>Product recovery</i>		
Final product concentration (%)	99.5% (v/v) ethanol	88% (w/w) L-lactic acid

600, the storage in Area 700, the wastewater treatment in Area 800 and the utilities in Area 900 are maintained the same with the NREL model.

2.7. Techno-economic analysis methodology

The mass and energy balance data are calculated based on Aspen plus simulation. The number and size of equipment, the chemical usage, and the utility usage are determined by the simulation data. The price quotations of general equipment such as pumps, conveyors, and evaporators were cited from NREL database and other sources (Humbird et al., 2011; Tao et al., 2012; Akerberg and Zacchi, 2000). The pretreatment reactors, fermentation tanks and helical agitators used in the dry milling biorefinery process technology were quoted from manufacturers in China in 2014, as shown in appendix Table A-2. Chemical prices and labor cost were cited from National Bureau of Statistics of the People's Republic of China (<http://www.stats.gov.cn/>) in appendix Table A-3. The year of 2013 is used as the reference year. The exchange rate of US dollar to Chinese Yuan (RMB) is set to approximately 6.2 according to the average exchange rate in 2013 (<http://data.stats.gov.cn/easyquery.htm?cn=C01&zb=A060J&sj=2013>).

The total capital investment is calculated based on the total equipment cost, and then variable and fixed operating costs are determined according to plant capacity. With these costs, a discounted cash flow rate of return analysis to determine the minimum L-lactic acid selling price (MLSP, \$/kg) required to obtain a net present value of zero with 8% internal rate of return after taxes. The parameters used in the discounted cash flow analysis are shown in appendix Table A-4.

3. Results and discussion

3.1. L-lactic acid fermentation at high solids loading of corn stover feedstock

For development a high titer L-lactic acid production process using corn stover feedstock, several technical difficulties are required to give practical solutions, including reducing seed inoculation ratio, obtaining high cell density seed culture, and accelerating biodetoxification in the simultaneous saccharification and fermentation (SSF) of corn stover at 30% solids loading using the engineered strain *P. acidilactici* TY112.

Higher seeds inoculation ratio was found to be important for reaching a high lactic acid titer from corn stover when the wild strain *P. acidilactici* DQ2 was used (Zhao et al., 2013). In this study, the effect of seeds inoculation ratio on the engineered strain *P. acidilactici* TY112 for L-lactic acid production was tested as shown in Fig. 2(a). Similar to its wild parent, the high inoculation ratio led to the increased L-lactic acid yield using the engineered strain *P. acidilactici* TY112 (Table 2). L-lactic acid titer was only slightly increased due to the dilution of the high seeds inoculation volume on the product titer in the fermentation broth.

For practical operations in industrial fermentation, high seed inoculation is not preferred thus an alternative is to increase the cell density of the seed culture to maintain the required cell density in fermentation medium. However, seed cells in bioreactors aggregated into small pieces and attached to impeller parts and fermentor wall, then led to the low cell viabilities and practically poor fermentation performance (Fig. 2(b)). The cell bridging by polysaccharide, protein, and lipid is considered as the major reasons of flocculation (Zhang et al., 2010c; Li et al., 2014). Therefore, glucoamylase was supplemented during cell cultivation in bioreactors to break the polysaccharide links among the cell aggregations and the cell aggregation disappeared with the uniform cell distribution after glucoamylase addition. L-lactic acid fermentation performance was also improved using the seed culture with glucoamylase addition. Using such a seed cells culture method, the commonly used 10% inoculation ratio of seeds is sufficiently enough for obtaining a satisfactory L-lactic acid fermentation (Fig. 2(c) and Table 2).

For shortening the detoxification time of pretreated corn stover feedstock from conventional 6 days into 2 days, an accelerated biodetoxification was performed using low aeration onto the biodetoxification fungus *A. resiniae* ZN1 and regular agitation rate. At the accelerated detoxification conditions, all the observable inhibitors were degraded within 48 h from the original 144 h without aeration and mixing. Fig. 2(d) shows that the L-lactic acid titer of 96.3 g/L and yield of 74.3% were obtained at 30% solids loading with 10% inoculation, similar to the results of conventional biodetoxified corn stover.

By solving these technical barriers for high titer L-lactic acid fermentation, the maximum titer of 96.3 g/L and yield of 74.3% were obtained at 30% (w/w) solids loading of corn stover. More importantly, no any wastewater was generated through the complete process starting from pretreatment to biodetoxification and fermentation. This result was compared to the previous L-lactic acid

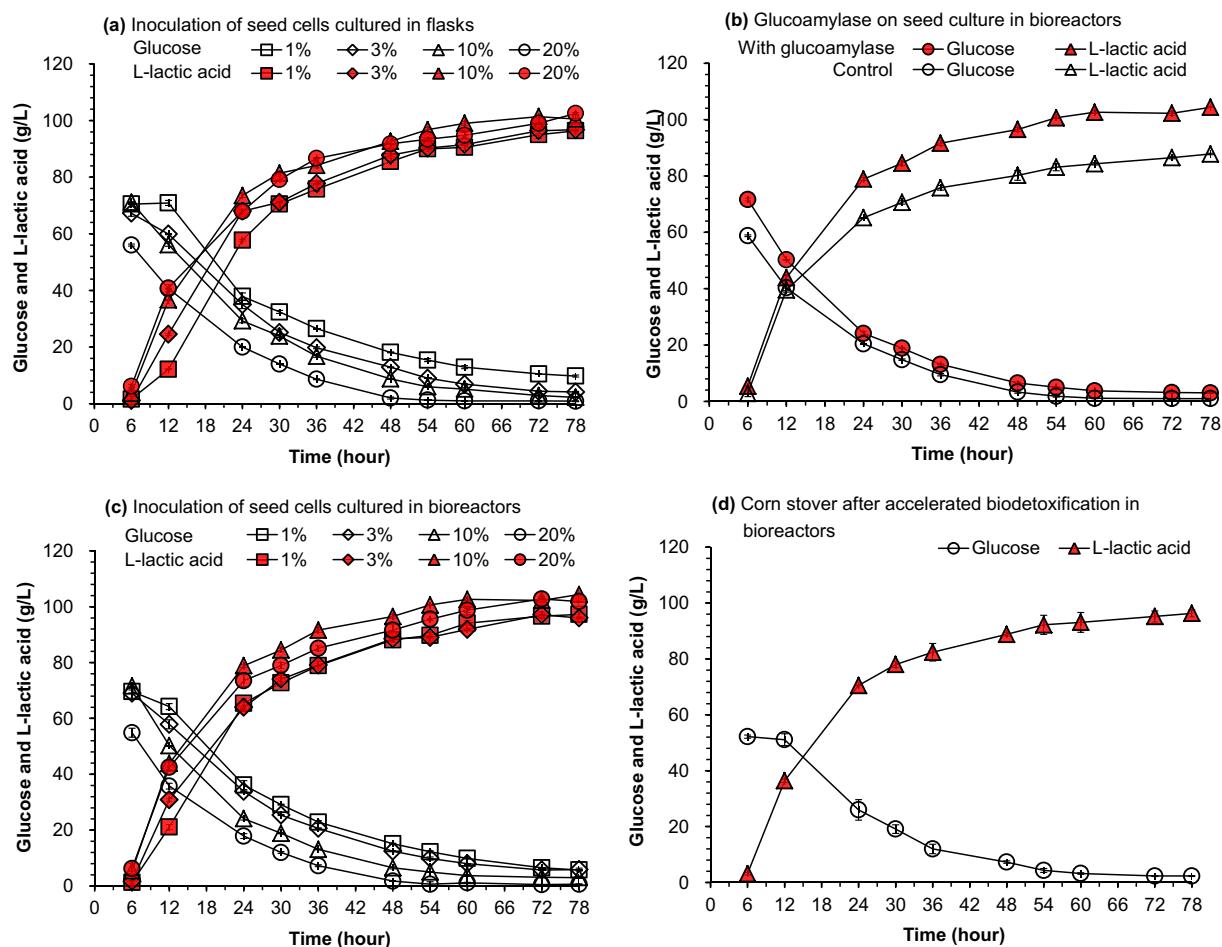


Fig. 2. SSF of *P. acidilactici* TY112 at 30% solids loading of corn stover feedstock. (a) Inoculation of seed cells cultured in flasks; (b) Effect of glucoamylase on seed culture in bioreactors; (c) Inoculation of seed cells cultured in bioreactors; and (d) Corn stover after accelerated biodegradation in bioreactors. The detailed seeds cultivation method and the accelerated biodegradation were described in Section 2. SSF was operated at the prehydrolysis with 30% solids loading (w/w) of pretreated and biodegraded corn stover, cellulase dosage of 15 FPU/g DM, 50 °C, pH 4.8, 150 rpm for 6 h; then SSF with inoculation of *P. acidilactici* TY112 cultivated with different methods at 45 °C, pH 5.5, 150 rpm for 72 h.

Table 2

Evaluation of seed culture on L-lactic acid fermentation in SSF of corn stover.

Seeds culture mode	Glucoamylase (% v/v)	Inoculum ratio (% v/v)	Titer (g/L)	Yield (%)	Productivity (g/L/h)
In flasks	0	1	96.4 ± 0.2	61.8 ± 0.1	1.34 ± 0
	0	3	96.8 ± 0.0	64.5 ± 0.0	1.34 ± 0
	0	10	101.4 ± 0.5	71.5 ± 0.2	1.41 ± 0.01
	0	20	102.5 ± 0.4	76.6 ± 0.2	1.42 ± 0.01
In bioreactor	0	10	87.8 ± 0.3	62 ± 0.1	1.22 ± 0
	1	1	97.3 ± 0.7	63.8 ± 0.3	1.35 ± 0.01
	1	3	97.1 ± 1.2	64.7 ± 0.4	1.35 ± 0.02
	1	10	104.4 ± 0.2	71.5 ± 0.1	1.45 ± 0
	1	20	102.7 ± 1.1	77.2 ± 0.4	1.43 ± 0.02

1% (v/v) glucoamylase solution was added into the seeds culture broth in the bioreactor. The SSF conditions: prehydrolysis with 30% solids loading of pretreated and detoxified corn stover, cellulase dosage of 15 FPU/g DM, 50 °C, pH 4.8, 150 rpm for 6 h. SSF was at 45 °C, pH 5.5, 150 rpm for 72 h.

fermentations as summarized in Table 3. In this comparison, only L-lactic acid titer greater than 30 g/L was considered because the lower titer of L-lactic acid is not meaningful for practical use. These fermentation cases were divided into two categories: (1) all the solids and pretreatment liquid were utilized for L-lactic acid production (the whole pretreated slurry), thus no wastewater was generated from pretreatment to fermentation; (2) only the solids of the pretreated feedstock was utilized for fermentation, then wastewater was released as pretreatment liquid and washing

liquid to obtain “clean” solids. It should be noted that the fermentation cases in Table 3 were not operated at the exactly same conditions such as nutrient additions, seed inoculations, and cellulase dosage etc.

In the first category with the whole pretreated slurry either for SSF or SHF (separate enzymatic hydrolysis and fermentation), Ye et al. (2014) obtained 80.6 g/L of L-lactic acid from SSF of acid-catalyzed pretreated empty fruit bunch at 16.7% solids loading. The fresh water usage during pretreatment and detoxification

Table 3
Comparison of high titer L-lactic acid fermentation from various lignocellulose feedstocks.

Feedstocks	Solids content (% w/w)	Pretreatment methods	Titer (g/L)	Yield (%)	Productivity (g/L/h)	Water consumption ^a (kg/kg)	Sources
<i>Whole package of the pretreated materials (solids and liquid) were used in the L-lactic acid fermentation</i>							
Corn stover	30	Dilute acid with biotodetoxification	104.4	71.5	1.45	1.0 + 0	This study
Empty palm fruit bunch	16.7	Acid-catalyzed hydrolysis	80.6	~77.9	3.4	3.0 + 2.0	Ye et al. (2014)
Oil palm trunk	8	Hydrothermal	~40	89.5	~0.36	125.0 + 0	Eom et al. (2015)
Wheat straw ^b	~10	Acid pretreatment	38.7	~76.7	0.65	10.0 + 0	Zhang et al. (2014)
<i>Solids only (after solids/liquid separation) were used in the L-lactic acid fermentation</i>							
Corn stover ^c	10	Steam explosion with water washed	75.0	75	1.04	1 + 10	Ouyang et al. (2013)
Corn stover	14.4	Sodium hydroxide with water washed	97.6	72.6	1.63	5 + 8	Hu et al. (2015)
Wheat straw	15.9	Alkaline	40.7	43	0.74	10 + 0	Maas et al. (2008)
Corn stover	22.1	Aqueous Ammonia with water washed	74.8	65	0.26	10 + 8	Zhu et al. (2007)
Sugarcane bagasse cellulose ^d	8	Steam and alkali pretreatment	67	83	0.93	~10	Adsul et al. (2007)

^a Water consumption included the water used in pretreatment (acid, alkali or solvent solutions and the steam condensation water), as well as the water used for washing the pretreated lignocellulosic materials for inhibitors removal.

^b Because the composition of wheat straw was not included in Zhang et al. (2014), 60 g of fermentable sugars were assumed from 100 g of wheat straw.

^c The solid/liquid ratio of the steam explosion pretreatment used was not revealed in Ouyang et al. (2013). Glucose was supplemented during L-lactic acid fermentation, and the L-lactic acid yield was based on glucose (from corn stover and from glucose supplementation).

^d The sugarcane bagasse cellulose contained 99.82% of cellulose and 0.18% of lignin. The details of the pretreatment were unknown and a commonly liquid–solid ratio for fractionation was assumed to be 10:1 by Sattar et al. (2015).

steps (5.0 kg/kg dry feedstock) was more than 5-folds of the present case (1.0 kg/kg). The other two cases were relatively low in L-lactic acid titer (around 40 g/L) and high in water consumption (125 and 10 kg/kg dry feedstock matter) (Eom et al., 2015; Zhang et al., 2014).

In the second category with the solids portion used only, a harsh pretreatment was carried out followed by a solid/liquid separation to remove pretreatment liquor. In the detoxification step, fresh water was used to thoroughly wash the pretreated materials to remove the inhibitor compounds and again a solid–liquid separation was followed. Finally the almost “pure cellulose” was obtained for the next step hydrolysis and fermentation. Several cases reached the L-lactic acid titer of 75.0, 97.6, 40.7, 74.8, and 67.0 g/L with the yield of 75.0%, 72.6%, 43.0%, 65.0% and 83.0%, respectively (Ouyang et al., 2013; Hu et al., 2015; Maas et al., 2008; Zhu et al., 2007; Adsul et al., 2007). However, 11, 13, 10, 18 and 10 kg water was also consumed handling per kg dry feedstock at each case above. Also, the loss of fine solid particles in the two solid/liquid separations was not calculated due to insufficient data.

3.2. Techno-economic analysis of L-lactic acid production from corn stover

The techno-economic evaluation was carried out based on the Aspen plus modeling (shown in Table 4) as described in Section 2. The L-lactic acid product refers to the L-lactic acid solution at 88% (w/w). The minimum L-lactic acid selling price (MLSP) is \$0.559 per kg, in which the contributions of feedstock, enzyme, and non-enzyme conversion were \$0.237, \$0.130 and \$0.192 per kg, respectively. Comparing to the minimum ethanol selling price (MESP) of \$0.72/kg (\$2.15 per gal) from the similar process flowsheet (Humbird et al., 2011), the cost of L-lactic acid product was 24% less expensive than that of ethanol. The reason for the reduced cost of L-lactic acid production from corn stover mainly lies in the great improved theoretical product yield, namely, the theoretical lactic acid yield is 1.0 g/g glucose and almost doubled than that of ethanol (0.51 g/g glucose). Xylose conversion to L-lactic acid is not considered in the present calculation because the microorganism of *P. acidilactici* TY112 is not a xylose utilizing strain. It is expected that the cost of L-lactic acid should

Table 4
Summary of L-lactic acid yields and conversion costs.

Feedstock handling rate	900 metric tons/day
Annual operation time	8000 h/yr
L-lactic acid yield	269 kg/dry ton
Total capital investment	\$186 million
Plant water usage	6.1 kg/kg L-lactic acid product
Plant electricity use	1.502 kW h/kg L-lactic acid product
Minimum lactic acid selling price	\$0.559/kg L-lactic acid product
Feedstock contribution	\$0.237/kg L-lactic acid product
Enzyme contribution	\$0.130/kg L-lactic acid product
Non-enzyme conversion contribution	\$0.192/kg L-lactic acid product

be further reduced after the fermenting strain is metabolically engineered to convert xylose into L-lactic acid.

Akerberg and Zacchi (2000) reported that the production cost of L-lactic acid from wheat flour was around \$0.833/kg of 70% (w/w) lactic acid product. Gonzalez et al. (2007) reported that the production cost of food grade lactic acid from ultrafiltered whey was \$1.25/kg of 50% (w/w) L-lactic acid product. Sikder et al. (2012) reported that production cost of L-lactic acid from sugarcane juice was \$3.15/kg of 80% (w/w) L-lactic acid product. Apparently, the production cost of L-lactic acid from corn stover at \$0.56/kg of 88% (w/w) L-lactic acid product was comparable and competitive to that from food crops.

In the present model, cellulase enzyme was supplied through on-site production, and the cost contributed to the second largest portion of the overall cost. A scenario of varied cellulase prices on minimum L-lactic acid selling price (MLSP) was evaluated when cellulase was supplied by different production modes and makers. Five cases of cellulase purchase prices were considered based on the publically available sources, \$1.25/kg enzyme protein (Klein-Marcuschamer et al., 2012), \$5.07/kg enzyme protein (Kazi et al., 2010), \$6.27/kg enzyme protein (<http://novozymes.com/en/news/news-archive/Pages/45713.aspx>), \$10.14/kg enzyme protein (Klein-Marcuschamer et al., 2012), and \$23.30/kg enzyme protein (Fang et al., 2013), respectively. Table 5 shows that the MLSP increased from the base case of \$0.559/kg, to \$0.477, \$0.616, \$0.634, \$0.795 and \$1.166 per kg correspondingly. At the lowest cellulase price (Case 1) same to the price of soybean protein, the enzyme cost is \$0.039/kg L-lactic acid and the MLSP is only \$0.477/kg, but this case was apparently less practical

Table 5
Estimation of minimum L-lactic acid selling price (MLSP) and the impact of cellulase price on MLSP.

Enzyme price case	Base case	Case 1	Case 2	Case 3	Case 4	Case 5
Enzyme unit price (\$/kg protein)	4.24	1.25	5.07	6.27	10.14	23.30
Enzyme cost (\$/kg product)	0.130	0.039	0.177	0.195	0.355	0.724
Feedstock cost (\$/kg product)	0.237	0.237	0.237	0.237	0.237	0.237
Conversion cost (\$/kg product)	0.192	0.201	0.202	0.202	0.203	0.205
MLSP (\$/kg product)	0.559	0.477	0.616	0.634	0.795	1.166

Base case: Cellulase enzyme is produced by “on-site” production mode with the same design in NREL model.

Case 1: Assume that the cellulase enzyme protein with the same value to soy protein, the cheapest protein on the market (Klein-Marcuschamer et al., 2012): \$1.25/kg protein (2011\$).

Case 2: Assume that the cellulase enzyme protein is purchased under the price estimated by Kazi et al. (2010): \$507 per metric ton of the enzyme broth containing 10% proteins, equivalent to \$5.07/kg (2007\$).

Case 3: Assume that the cellulase enzyme is purchased from Novozymes with the claimed enzyme cost of \$0.50/gal ethanol (<http://novozymes.com/en/news/news-archive/Pages/45713.aspx>). In the proposed process (Humbird et al., 2011), the ethanol production is 21,672.41 kg/h and the enzyme usage is 579 kg protein/h, which are equivalent to 7256.35 gal ethanol/h (21,672.41 kg/h is divided by the ethanol density 0.789 then transformed to gal). The enzyme protein price is equivalent to $7256.35 \times 0.50/579 = \$6.27/\text{kg}$ (2010\$).

Case 4: Assume that the cellulase enzyme protein is purchased under the price estimated by Klein-Marcuschamer et al. (2012) on the fungal cellulase production: \$10.14/kg (2007\$).

Case 5: Assume that the cellulase enzyme is Youtell #6 purchased from Chinese enzyme market at 13 Chinese Yuan (RMB)/kg enzyme with the protein content of 9% (Fang et al., 2013), equivalent to $13/6.2$ (the present exchange rate of dollar to RMB)/0.09 = \$23.30/kg (2013\$).

because the proteins from microbial fermentation are certainly more expensive than soybean protein. On the other extreme, the highest cellulase price (Case 5) used a commercial cellulase price (Youtell Biochemical Co.) on Chinese industrial enzyme market with the open price tag (Fang et al., 2013). The enzyme cost is \$0.724/kg L-lactic acid product and approximately the same with that of textile and animal feed cellulase, then the corresponding MLSP is up to \$1.166/kg. The base case represents the on-site enzyme production cost and is cited from the NREL design using filamentous fungus as fermenting strain and glucose as the primary carbon source (Humbird et al., 2011). The enzyme cost is only \$4.24/kg protein and the MLSP is \$0.559/kg, although the total installed equipment cost increases at the certain extent. The results indicate that similar to the cellulosic ethanol production, the enzyme cost is still the main concern for the L-lactic acid production from corn stover, and the on-site cellulase production seems most reasonable because the multiple steps of fungus cell removal (cells die in the anaerobic ethanol fermentation), enzyme concentrating and purifying steps, as well as logistic transportation and storage could be deleted (Kovacs et al., 2009). However, these advantages still requires the validation of industrial scale operations.

4. Conclusion

The high L-lactic acid titer and yield of 104.5 g/L and 71.5% were obtained using the engineered strain *P. acidilactici* TY112 from the dry milling biorefinery processed corn stover. The techno-economic analysis based on the Aspen plus model showed that the minimum L-lactic acid selling price (MLSP) was \$0.523 per kg, which was close to the minimum ethanol selling price estimated by NREL, even without xylose utilization. In addition, the cellulase enzyme contributed nearly a quarter to the MLSP, and the on-site cellulase enzyme supplying way was the most economical and reasonable.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.biortech.2015.09.098>.

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