Biochemical Engineering Journal 200 (2023) 109111

Contents lists available at ScienceDirect

Biochemical Engineering Journal

journal homepage: www.elsevier.com/locate/bej

Short communication

Improving the dissolved oxygen level in high solids loading cellulosic sugar acids fermentation by restructuring the biorefinery chain

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ARTICLE INFO

Keywords:
Gluconobacter oxydans
Sugar acids
Lignocellulose
Viscosity
Oxygen transfer rate

ABSTRACT

Industrial production of biochemicals and biofuels from lignocellulose feedstock frequently requires aerobic fermentation with high dissolved oxygen demand. Efficient oxygen transfer from the air phase to the liquid phase has been considered as a great challenge due to the high viscosity and solid particles derived from the high solids loading lignocellulosic slurry. Here we presented a solution for aerobic sugar acids (gluconic acid and xylonic acid) fermentation by *Gluconobacter oxydans* in 30% (w/w) solids loading corn stover hydrolysate without solid/liquid separation. The biorefinery chain was restructured by conducting enzymatic hydrolysis before the submerged biodetoxification instead of solid-state biodetoxification. The viscosity of corn stover slurry decreased to ~0.03 Pa·s after submerged biodetoxification and the gas-liquid oxygen transfer coefficient (k_La) reached 50.0 at the aeration rate of 0.1 vvm and agitation rate of 500 rpm, which is more efficient for sugar acids conversion than that using solid-state biodetoxified corn stover hydrolysate (only 8.8 k_La at 2.4 vvm, 500 rpm). The cellulosic sugar acids titer reached 156.0 ± 6.9 g/L at 0.1 vvm with 92.9% of the theoretical yield in the restructured biorefinery chain. The techno-economic analysis showed that the minimum selling prices of sugar acids produced by this restructured biorefinery chain was $351.7/ton which was 12.9% lower than that produced by the general biorefinery chain. This efficient process setting including one-pot hydrolysis, submerged biodetoxification, and fermentation provides a practical technical route for lignocellulose-based aerobic fermentation under high solids loading.

1. Introduction

Enzymatic hydrolysis and fermentation performing at high solids loading (generally above 15%, w/w) benefit the economics of lignocellulosic biomass conversion to biofuels and biochemicals by decreasing capital, recovery and disposal costs, energy consumption, and waste usage [1]. However, high solids loading conditions cause high viscosity resulting in a limitation in the mass transfer within the reactor [2,3]. Especially for aerobic fermentation process, the inefficient oxygen supply reduced the cell metabolism activity [4].

Oxygen transfer enhancement generally is closely related to high power consumption in aerobic fermentations. Increases in agitation rate, aeration rate, and oxygen partial pressure improve the oxygen transfer rate [5,6], but electricity consumption is increased correspondingly. Design of novel bioreactors or new oxygen vector addition also enables better oxygen supply [7–9]. In lignocellulosic biorefinery, the use of high solids loading lignocellulose hydrolysate slurry poses a significant challenge to oxygen transfer due to large amounts of solid particles and the high viscosity property. Solid particles in hydrolysate slurry and its high viscosity property facilitate bubble coalescences, as a result, reduce the gas-liquid interfacial area and gas-liquid oxygen transfer rate [10,11].

Gluconobacter oxydans is a typical obligatory aerobic Gram-negative bacterium used for the oxidation of sugars to sugar acids. This oxidation occurs under the control of oxygen transfer rate limitation [12–14]. One efficient way to improve the oxygen transfer in cellulosic sugar acids fermentation is to remove the solid particles from the lignocellulosic hydrolysate slurry. But the solid/liquid separation operation inevitably leads to high electricity consumption and unhydrolyzed cellulose fraction loss [13].

In this study, the high solids loading (30%, w/w) corn stover hydrolysate slurry was applied for sugar acids (gluconic acid and xylonic

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https://doi.org/10.1016/j.bej.2023.109111
Received 21 July 2023; Received in revised form 8 October 2023; Accepted 9 October 2023

acid) fermentation without solid/liquid separation by *G. oxydans*. To improve the oxygen transfer in the slurry, the biorefinery chain was reset by conducting the enzymatic hydrolysis at first, then the biodetoxification was conducted in the way of submerged liquid fermentation in the hydrolysate slurry instead of solid-state biodetoxification.

The sugar acids fermentation performances by *G. oxydans* were evaluated in general or restructured biorefinery chain at different agitations and aerations. And the oxygen transfer coefficient (k_La) was further measured. The technol-economic analysis was carried out, showing the potential of applying the new restructured biorefinery chain for high-titer cellulosic sugar acids production.

2. Material and methods

2.1. Feedstock

Raw corn stover was harvested in Nanyang City, Henan Province, China, which contained 34.36% of cellulose, 26.12% of xylan, 15.91% of lignin, and 8.05% of ash based on dry weight (w/w). Cellulase Cellic CTec 2.0 was purchased from Novozymes Biotechnology Co., Ltd (Beijing, China) with the protein content of 81.5 mg/mL.

2.2. Strains

The sugar acids producing strain *G. oxydans* DSM 2003 was cultured in sorbitol medium containing 80 g/L of sorbitol, 20 g/L of YE, 0.5 g/L of $MgSO_4·7\ H_2O$, 1.5 g/L of KH_2PO_4, and 1.5 g/L of $(NH_4)_2SO_4$. No nutrients were added during cellulosic sugar acids fermentation.

The biodetoxification strain *Paecilomyces variotii* FN89 (CGMCC 17665) was preserved on PDA plant. The seed medium for *P. variotii* FN89 contained 20 g/L of glucose, 1.0 g/L of YE, 1.0 g/L of $MgSO_4·7\ H_2O$, 2.0 g/L of KH_2PO_4, and 1.0 g/L of $(NH_4)_2SO_4$.

2.3. Pretreatment and biodetoxification

The pretreatment and solid-state biodetoxification were carried out according to the previous protocols [15]. The toxins such as furfural, HMF, and acetic acid generated in pretreated were removed by solid-state/submerged biodetoxification. For submerged biodetoxification, the pretreated corn stover was hydrolyzed at 30% (w/w) solids loading, 4 mg protein/g substrate, 50 rpm for 12 h. The spores suspension was inoculated into seed medium at the ratio of 1% (v/v), and cultured at 37 ℃, 300 rpm for 18 h as the seed. The seed was inoculated into the corn stover hydrolysate at the ratio of 10% (v/w). The submerged biodetoxification was conducted at 37 ℃, 750 rpm, 1.0 vvm for 18 h. Then the biodetoxified hydrolysate was anaerobically maintained at 50 ℃ for 12 h to inactivate *P. variotii* FN89.

2.4. Cellulosic sugar acids fermentation

G. oxydans DSM 2003 was inoculated into the seed medium, and cultured at 30 ℃, 220 rpm for 24 h. The seed culture was inoculated into the hydrolysate slurry at the inoculum ration of 10% (v/w). The fermentation was performed at 30 ℃, 250–500 rpm, 0.1–2.4 vvm for 24 h. The fermentation pH was controlled at 5.0 by automatically adding 5 M NaOH solution.

2.5. Volumetric oxygen mass transfer coefficient (k_La) measurement

The same volume of water as the seed was added in corn stover slurry instead of inoculation, and then k_La was measured. The volumetric oxygen mass transfer rate (h^{-1}) in corn stover hydrolysate was measured by the dynamic oxygen desorption method [13]. The k_La values (h^{-1}) were calculated according to Eq. (1):

$$\frac{dC}{dt} = k_La \quad (C^* - C) \tag{1}$$

where C^* is the saturation oxygen concentration (mol/L); C is the measured oxygen concentration (mol/L).

2.6. Energy consumption calculation

Energy consumption of different aerations was calculated by the simulation on Aspen Plus software (Aspen Tech Co., Cambridge, MA, USA). The working pressure of air compressor was set at 2.0 bar. The density of air was 1.29 kg/m^3.

The mixing power was calculated according to the method described by Zhang et al. with modifications [16]. The dimension power number N_p was expressed as the function of Reynolds number Re_m under the laminar flow according to Eq. (2):

$$N_p = C \times \mathrm{Re}_m^x \tag{2}$$

where C is geometric dimension parameter (147.12); x is dimensionless factor (−1). Re_m was calculated according to Eq. (3).

$$\mathrm{Re}_m = \frac{Nd^2\rho}{\mu_a} \tag{3}$$

where N is agitation rate (rev/s); d is impeller diameter (m); ρ is fluid density (kg/m^3); μ_a is apparent viscosity (Pa·s). The mixing energy consumption (P, W) without aeration was calculated according to Eq. (4):

$$P = N_pN^3d^5\rho f \tag{4}$$

where f is correction factor (0.55). The mixing energy consumption (P_g, W) with aeration was calculated by introducing a ventilation criterion (N_a) according to Eq. (5):

$$N_a = \frac{Q_g}{Nd^3} \tag{5}$$

where Q_g is aeration rate (m^3/s); when $N_a < 0.035$, $P_g/P = 1 - 12.6\ N_a$; when $N_a \geq 0.035$, $P_g/P = 0.62 - 1.85\ N_a$.

2.7. Analysis methods

The rheological property of corn stover slurry was measured by a rotational viscometer (DV2T, model LV, spindle SC4 −16, Brookfield Engineering Laboratories Inc., Middleboro, MA, USA) at room temperature and shear rate of 58 s^{-1}, corresponding to the reactor agitation of 200 rpm. The apparent viscosity of the hydrolysate was measured and analyzed at the same shear rate, and the average apparent viscosity of the process (hydrolysis-biodetoxification-fermentation) was taken to calculate the Reynolds number. Glucose, xylose, furfural, HMF, and acetic acid was determined by refractive index RID-10A detector and Bio-rad Aminex HPX-87 H column [15]. Gluconic acid and xylonic acid were determined by UV/VIS detector SPD-20A [17].

3. Results and discussion

3.1. Changing biorefinery chain for a reduced viscosity of high solids loading hydrolysate

The general dry biorefinery chain was restructured by conducting enzymatic hydrolysis at first, and then biodetoxification in submerged liquid hydrolysate slurry (Fig. 1a). The one-pot hydrolysis, submerged biodetoxification and fermentation saved the capital costs [18]. The biodetoxification strain *P. variotii* FN89 can degrade the toxic compounds including acetic acid, furfural, and HMF before fermentable sugars consumption [15,18].

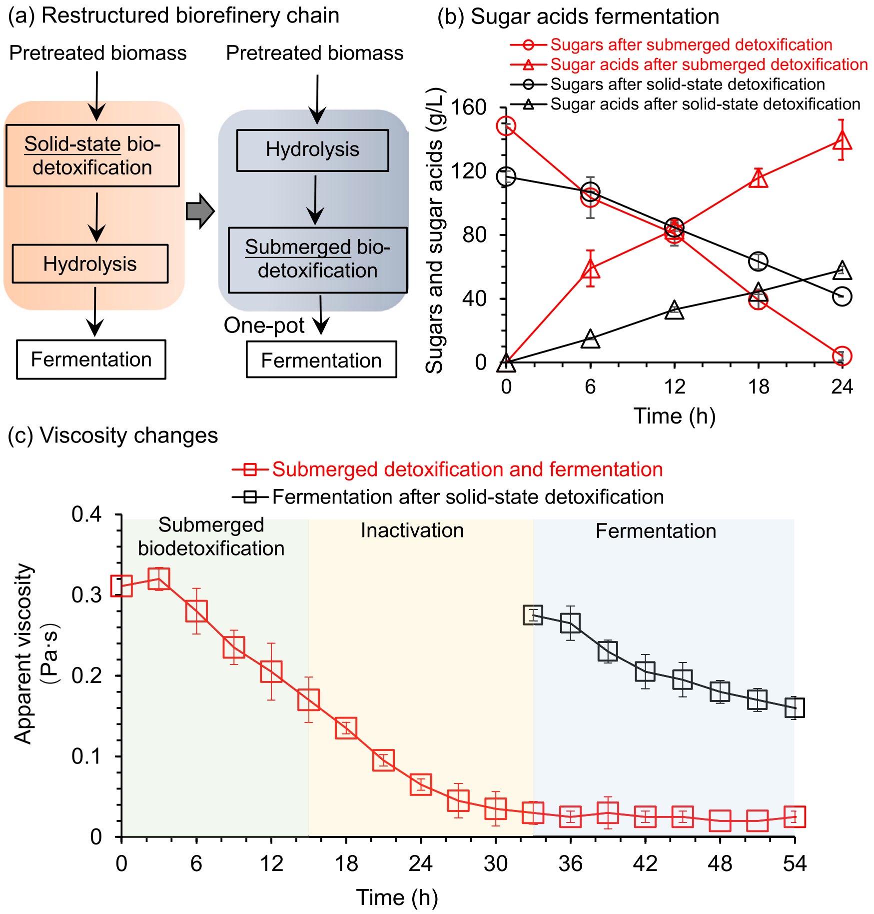


Fig. 1. Viscosity changes during sugar acids fermentation at 30% (w/w) solids loading of corn stover. (a) Flowsheet; (b) sugar acids fermentation; (c) viscosity changes. Fermentation conditions: 30 ℃, 2.4 vvm, 500 rpm.

The cellulosic sugar acids fermentation was performed at 2.4 vvm, 500 rpm using biodetoxified corn stover slurry (without solid/liquid separation) (Fig. 1b). Although these fermentation conditions have been well demonstrated for high-titer sugar acids production from clarified corn stover hydrolysate prepared by solid-state biodetoxification, hydrolysis, and centrifugation [19], only 66.1% of fermentable sugars were consumed and 58.1 ± 2.2 g/L of sugar acids were generated from the slurry after solid-state biodetoxification and hydrolysis without solid/liquid separation. In the restructured biorefinery chain, enzymatic hydrolysis was placed before biodetoxification. All sugars were consumed and finally, 139.7 ± 12.6 g/L of sugar acids were produced at 24 h.

The changes in viscosity of slurry during submerged biodetoxification and sugar acids fermentation were determined (Fig. 1c). The initial apparent viscosity of slurry at 30% (w/w) solids loading after enzymatic hydrolysis was around 0.3 Pa·s. During the fermentation after solid-state biodetoxification and hydrolysis, the apparent viscosity gradually decreased to 0.15 Pa·s within 24 h due to the continuous hydrolysis of cellulase. In the restructured biorefinery chain, the submerged biodetoxification prolonged the hydrolysis period, facilitating the decrease in viscosity of the slurry. The process aiming to heat inactivate the biodetoxification strain further reduced the apparent viscosity of the slurry to around 0.03 Pa·s.

3.2. *New biorefinery chain improved the oxygen transfer rate in high solids loading hydrolysate*

The oxygen transfer coefficient k_La in 30% (w/w) solids loading corn stover hydrolysate was experimentally measured at different aeration rates (0.1–2.4 vvm) and agitations rates (0–500 rpm) after solid-state or submerged biodetoxification (Fig. 2). Hou et al. [13] reported that the oxygen transfer rate is the determinative factor for cellulosic sugar acids fermentation, and a minimum threshold value of k_La was around 10 h^{-1} for *G. oxydans*. Fig. 2a shows that the k_La values were consistently below 10 h^{-1} at 100–500 rpm and 1.0–2.4 vvm after solid-state biodetoxification and enzymatic hydrolysis, resulting in low dissolved oxygen level and sugar acids titer (Fig. 1b). The oxygen transfer rate was significantly improved after submerged biodetoxification in restructured biorefinery chain. The k_La value reached 12.4 ± 1.8 h^{-1} even at 0.1 vvm and 300 rpm (Fig. 2b). The reduced apparent viscosity and enhanced oxygen transfer rate of hydrolysate in restructured biorefinery chain allow the aerobic sugar acids fermentation to be performed at a lower aeration and agitation rate, thus would reduce the electricity consumption.

The detailed curves of sugars consumption and sugar acids production at 0.1 vvm, 250–500 rpm after submerged biodetoxification were showed in Fig. 3. The higher agitation rate leads to the higher sugar acids titer. The agitation rate lower 300 rpm severely hindered the metabolism of *G. oxydans*. The conversion rate of 92.6% of fermentable sugars and sugar acids titer of 115.0 ± 11.2 g/L were achieved at 0.1 vvm and 400 rpm (Fig. 3b), which was only 17.7% lower than the fermentation titer at 2.4 vvm and 500 rpm (Fig. 1b). Further improvement of agitation rate to 500 rpm led to the higher sugar acids titer of 156.0 ± 6.9 g/L at 24 h, which was 92.9% of the theoretical yield. Under the same agitation conditions (500 rpm), the sugar acids titer was higher at lower aeration (0.1 vvm) than that at higher aeration (2.4 vvm), perhaps owing to the decrease in the generation of the byproduct 2-keto-gluconic acid.

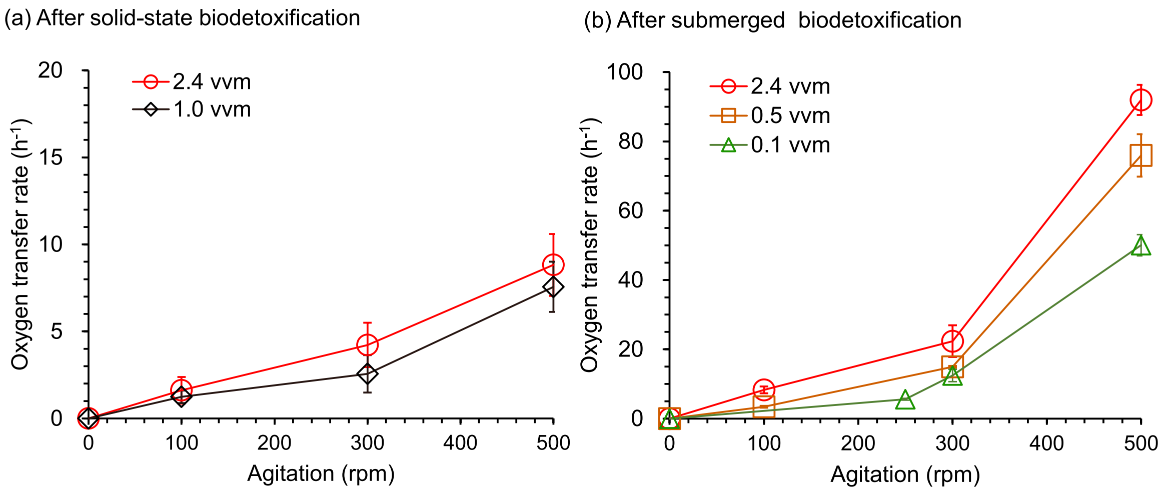


Fig. 2. k_La measurements at different agitations and aerations after solid-state or submerged biodetoxification. (a) Slurry obtained after solid-state biodetoxification and hydrolysis; (b) slurry obtained after hydrolysis and submerged biodetoxification.

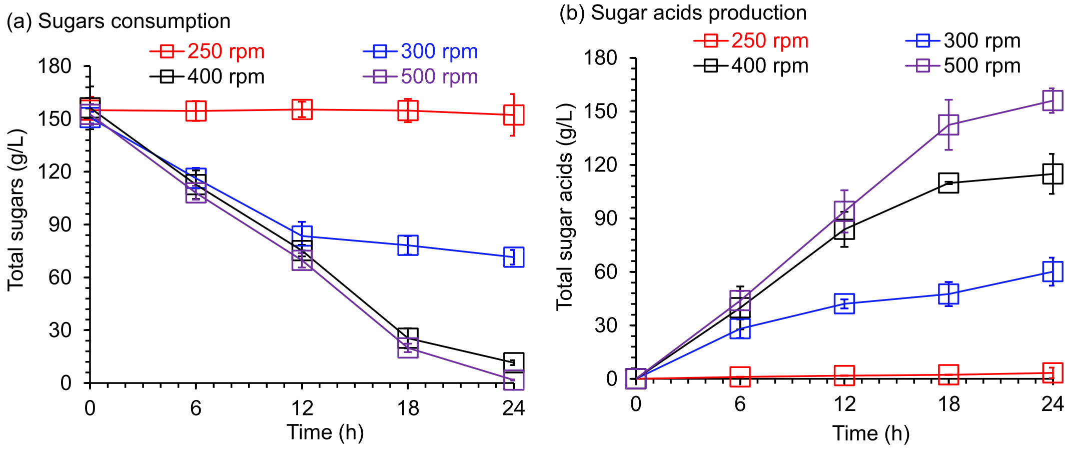


Fig. 3. Sugar acids fermentation at 0.1 vvm, 250–500 rpm after submerged biodetoxification. (a) Sugars consumption; (b) sugar acids production.

3.3. *Preliminary technol-economic evaluation*

Much work focused on using engineered whole-cell catalysis for sugar acid production [20–23]. However, it is practically not possible to recovery the cells from solid lignin residue in high-solids loading lignocellulose hydrolysate. Table 1 summarized the fermentation parameters and results of sugar acid produced by *G. oxydans*. The vigorous agitation (over 500 rpm) and adequate aeration (over 1.0 vvm) are generally prerequisites for efficient production of sugar acids by *G. oxydans*. The solid/liquid separation operation was required to obtain the clarified hydrolysate for sugar acids fermentation. This study restructured general biorefinery chain, the titer of sugar acids increased from 58.1 g/L to 156.0 g/L using corn stover slurry without solid/liquid separation, whereas the aeration was reduced from 2.4 vvm to 0.1 vvm.

The energy consumptions (kW/m^3) in biodetoxification and sugar acids fermentation were preliminarily calculated in general or restructured biorefinery chains (Table 2). The energy consumption of mixing and aeration for solid-state biodetoxification was not calculated because of the heterogeneity of the substrate. The aeration accounts for a higher share of energy consumption in detoxification and fermentation using high solids loading corn stover slurry compared with agitation. The decrease of the aeration rate from 2.4 vvm to 0.1 vvm during sugar acids fermentation in restructured biorefinery chain led to a 95.6% reduction in energy consumption compared to that in general biorefinery chain. Besides, different from the difficult discharge and conveying of solid biomass after solid-state biodetoxification, the hydrolysate before or after submerged biodetoxification with the apparent viscosity of 0.03–0.32 Pa·s can be efficiently transported by the regular pump in

Table 1
Summary of sugar acids production by *G. oxydans*.

Substrate	S/L[b]	Conditions	Titer (g/L)[c]	Reference
Corncob	Y	1.5 vvm, 500 rpm	GA, 98.8	[26]
Pure xylose	/	~1.3 vvm. 500 rpm	XA, 108.2	[27]
Corn stover	Y	Pure oxygen, 0.5 vvm, 500 rpm	XA, 102.3; DHA, 40.6	[28]
Wheat straw	Y	Pure oxygen, 0.5 vvm, 500 rpm	XA, 104.4	[29]
Pure xylose	/	~1.3 vvm, 728 rpm	XA, ~88.4	[30]
Corn stover	Y	2.5 vvm, 500 rpm	GA and XA, 171.3	[19]
Corn stover	N	0.1 vvm, 500 rpm	GA and XA, 156.0	This study

[a] The corn stover was hydrolyzed for ethanol fermentation. The ethanol-fermented waste liquid containing residual xylose and glycerol was mixed with fresh corn stover hydrolysate and used for xylonic acid and 1,3-dihydroxyacetone (DHA) fermentation.

[b] Where Y indicates that after the solid/liquid separation of the hydrolysate, the clarified hydrolysate was used for sugar acid fermentation; N indicates that there was no solid/liquid separation of the hydrolysate.

[c] Where GA indicates gluconic acid; XA indicates xylonic acid; DHA indicates 1,3-dihydroxyacetone.

Table 2
Energy consumption of biodetoxification and sugar acids fermentation in general or restructured biorefinery chain.

	General biorefinery	Restructured biorefinery
Biodetoxification	Solid-state	Submerged
Conditions	37 ℃, 1.0 vvm	37 ℃, 1.0 vvm, 750 rpm
Period	48 h	18 h
Energy consumption (kW/m^3)	/	0.69 for mixing; 19.43 for aeration
Sugar acids fermentation		
Conditions	30 ℃, 2.4 vvm, 500 rpm	30 ℃, 0.1 vvm, 500 rpm
Period	24 h	24 h
Energy consumption (kW/m^3)[a]	0.24 for mixing; 60.75 for aeration	0.03 for mixing; 2.65 for aeration

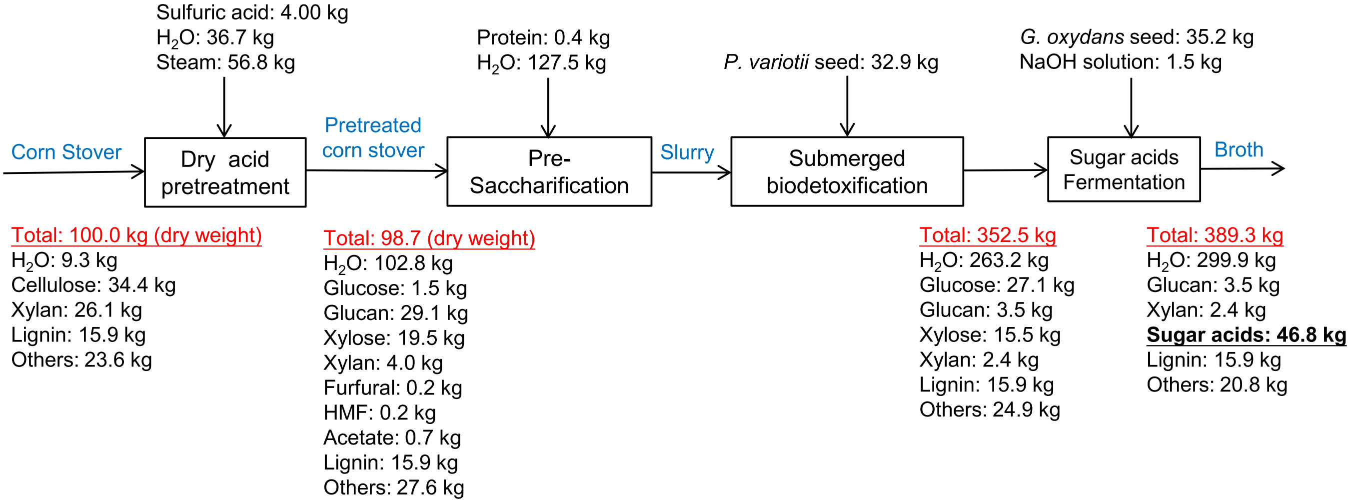
[a] It is worth noting that the mixing energy consumption was calculated based on the results of fermentation in 5 L bioreactor. Linearly projecting production costs at a commercially-relevant scale from laboratory-scale experiments is not sufficiently accurate, although the Eqs. (2–5) adopted for mixing energy consumption calculation take into account the impeller diameter of the reactor [16, 31].

closed vessels and pipelines [24], meanwhile, the problem of contamination can thus be avoided.

The overall mass balance of sugar acids production by this restructured biorefinery chain was conducted from 100 kg of corn stover (dry weight) (Fig. 4a). The raw corn stover was pretreated at ~70% (w/w) solids loading, and the pretreated corn stover are still in solid particle form. Most of xylan was hydrolyzed, while most of cellulose fraction was well preserved during the pretreatment. Meanwhile, the inhibitors including ~0.2 kg of furfural, ~0.2 kg of HMF, and ~0.7 kg of acetic acid were generated. Then the pre-saccharification was conducted at 30% (w/w) solids with the cellulase dosage of 4 mg protein/g dry matter. The slurry without solid/liquid separation was used for submerged biodetoxification. All of these inhibitors were removed but the fermentable sugars loss was below 10% (w/w). Then the fermenting strain *G. oxydans* DSM 2003 converted all free fermentable sugars into corresponding sugar acids. Total 46.8 kg of sugar acids were produced with the yield of 0.47 g/g raw corn stover. The fermentation broth was simply solid/liquid separated to obtain the liquid fraction, which can be concentrated and directly used as cement retarder [25].

The minimum selling price of sugar acids produced by restructured biorefinery chain was calculated according to our previously established method [19]. The cost breakdowns of equipment and installation capital expenditures, and total operating expense were showed in Fig. 4b and c.

(a) Overall mass balance



(b) Cost breakdown of equipment and installation capital expenditures

(c) Cost breakdown of total operating expense

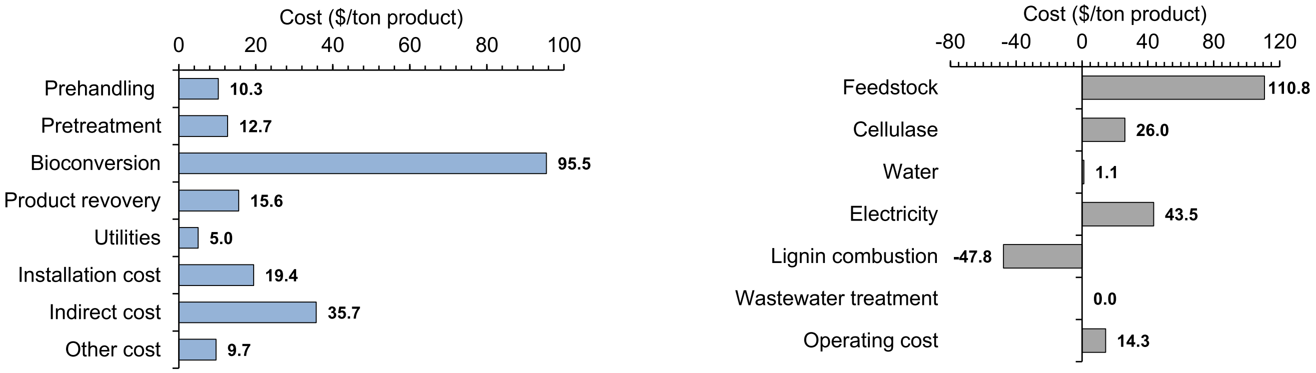


Fig. 4. Technol-economic analysis of sugar acids production by restructured biorefinery chain. (a) Overall mass balance. (b) Cost breakdown of equipment and installation capital expenditures. The indirect cost includes equipment maintenance, site overheads, freight, insurance, etc. The other cost includes land use charges and working capital. The main reactor types for cost calculation include two 10 m^3 pretreatment reactors, eight 300 m^3 bioreactors for saccharification, biodetoxification and fermentation, and one 500 m^3 broth storage tank. (c) Cost breakdown of total operating expense. The residual lignin was combustion for electricity generation. The generated electricity was sold to the grid for benefit. The fermentation broth was solid/liquid separated to obtain the liquid fraction rich in sugar acid, which was multiple evaporated to the product containing ~50% (w/w) of sugar acids and directly sold as cement retarder [25].

The minimum selling price of sugar acids produced by restructured biorefinery chain was $351.7/ton, which was 12.9% low than that produced by general biorefinery chain ($404.0/ton) [19]. The one-pot of saccharification and biodetoxification, and the lower energy consumption for fermentation contributed to the production cost savings in this restructured biorefinery chain compared to the general biorefinery chain.

4. Conclusions

The general biorefinery was restructured by adopting submerged biodetoxification instead of solid-state biodetoxification. The apparent viscosity of the slurry was significantly decreased, which improved the oxygen transfer coefficient, allowing the sugars acids fermentation to be performed at 0.1 vvm. The cellulosic sugar titer reached 156.0 ± 6.9 g/L at 0.1 vvm with 92.9% of the theoretical yield. This restructured biorefinery chain saves at least 62.6% of total electricity consumption in biodetoxification and sugar acids fermentation, providing a practical processing for lignocellulose-based aerobic fermentation under high solids loading.

CRediT authorship contribution statement

Bin Zhang and Hucheng Yang conducted the experiments. Ya Wang did the calculation; Jie Bao designed and conceived the study. Bin Zhang and Jie Bao wrote the manuscript. All authors contributed to the revisions of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

Acknowledgements

This research was supported by the National Natural Science Foundation of China (21978083, 32301269), and the Yangfan Project of Science and Technology Committee of Shanghai Municipality (23YF1409900).

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