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Simultaneous saccharification and aerobic fermentation of high titer cellulosic citric acid by filamentous fungus *Aspergillus niger*

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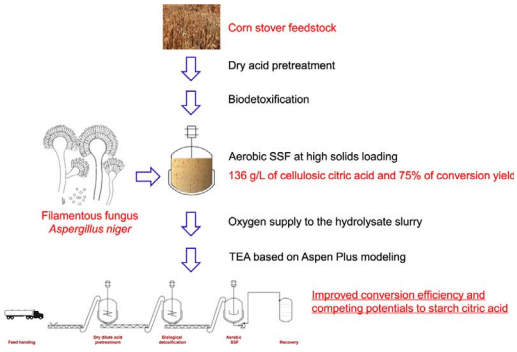
GRAPHICAL ABSTRACT



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ABSTRACT

Simultaneous saccharification and fermentation (SSF) is the most efficient operation in biorefining conversion, but aerobic SSF under high solids loading significantly faces the serious oxygen transfer limitation. This study took the first insight into an aerobic SSF by high oxygen demanding filamentous fungi in highly viscous lignocellulose hydrolysate. The results show that oxygen requirement in the aerobic SSF by *Aspergillus niger* was well satisfied for production of cellulosic citric acid. The record high citric acid titer of 136.3 g/L and the overall conversion yield of 74.9% of cellulose were obtained by the aerobic SSF. The advantage of SSF to the separate hydrolysis and fermentation (SHF) on citric acid fermentation was compared based on the rigorous Aspen Plus modeling. The techno-economic analysis indicates that the minimum citric acid selling price (MCSP) of $0.603 per kilogram by SSF was highly competitive with the commercial citric acid from starch feedstock.

1. Introduction

Simultaneous saccharification and fermentation (SSF) is the most efficient operation in lignocellulose biorefining processes for its high cellulase utilization efficiency (Balat et al., 2008). When SSF is applied to aerobic fermentations, the oxygen transfer rate from air phase to the highly viscous hydrolysate phase is one of the rate limiting steps (Chen et al., 2013; Freitas and Teixeira, 2001). The previous studies revealed that the oxygen transfer rate in the highly viscous hydrolysates significantly decreased with the increasing solids loading but still met the oxygen demand of *Gluconobacter oxydans* for gluconic acid generation (Hou et al., 2017). For the high oxygen demanding filamentous fungi such as *Aspergillus niger*, the limited oxygen transfer rate could lead to the reduced product yield even the failure of fermentation conversion in the highly viscous hydrolysates (Li et al., 2017).

Citric acid is an important organic acid product with million tons of

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annual production globally (Soccol et al., 2006). Lignocellulose biomass has been an important feedstock option for citric acid production. Two fermentation methods, the solid state fermentation and separate hydrolysis and fermentation (SHF), were conducted in the previous reports (Hang and Woodams, 1998; Liu et al., 2014; 2015; Yang et al., 2016). Solid state fermentation utilized the fermentable sugars in the feedstocks but the obtained citric acid was low (34 g/L of citric acid from pretreated sugarcane bagasse) (Khosravi-Darani and Zoghi, 2008). In the SHF operation, a maximum citric acid titer of 100 g/L was obtained from corn stover hydrolysate (Zhou et al., 2017). The major disadvantage of SHF is the considerable sugar loss and high energy consumption in the solid/liquid separation of the highly viscous hydrolysate slurry (Olofsson et al., 2008). SSF is well recognized for its advantage on relieving product (glucose) inhibition on cellulase activity (Jin et al., 2017; Kawaguchi et al., 2016; Ohgren et al., 2006). In cellulosic ethanol fermentation, SSF achieved the record high ethanol titer of 101.4 g/L (equivalent to 12.8%, v/v) from pretreated and detoxified wheat straw at moderate cellulase usage (Liu et al., 2018).

This study took the first investigation on the feasibility of SSF on cellulosic citric acid fermentation by *Aspergillus niger* under the high corn stover feedstock loading. The impact of oxygen transfer on cell growth and bioconversion of *A. niger* was evaluated. The techno-economic analysis shows that the cellulosic citric acid by SSF is competitive to the commercial citric acid from starch feedstocks.

2. Material and methods

2.1. Raw materials

Corn stover (CS) was harvested from Bayan Nur League, Inner Mongolia Autonomous Region, China in fall 2015. After collection, the materials were milled coarsely using a hammer crusher and screened through a mesh with a circle diameter of 10 mm. The milled corn stover with moisture content of 13% was washed to remove field dirt, stones and metals, then air dried at room temperature followed by drying in an oven at 105 °C until constant weight. The raw corn stover contained 35.4% of cellulose and 24.6% of hemicellulose measured according to NREL protocols (Sluiter et al., 2008; 2012).

2.2. Enzyme and chemicals

Cellulase enzyme Cellic CTec 2.0 was purchased from Novozymes (China), Beijing, China. The filter paper activity was determined as 203.2 FPU/mL according to the NREL protocol LAP-006 (Adney and Baker, 1996), the cellobiase activity was 4900 CBU/mL according to Ghose (1987), and the protein concentration was 87.3 mg total proteins/mL cellulase solution according to Bradford (1976) using BSA as protein standard.

Yeast extract was purchased from Angel Yeast, Yichang, Hubei, China. Agar was purchased from Biosharp, Shanghai, China. All other chemicals KH_2PO_4, NH_4Cl, $MgSO_4{\cdot}7H_2O$, $ZnSO_4{\cdot}7H_2O$, $CuSO_4{\cdot}5H_2O$, $FeSO_4{\cdot}7H_2O$, $MnCl_2$, NaOH, H_2SO_4, $Ca(OH)_2$ were purchased from the local supplier Linfeng Chemical Reagents, Shanghai, China.

2.3. Strains and media

Aspergillus niger SIIM M288 was obtained from Shanghai Industrial Institute of Microbiology (SIIM), Shanghai, China. The spore stocks were maintained at −80 °C freezer in a synthetic medium containing 30% (v/v) glycerol solution.

Biodetoxification fungus *Amorphotheca resinae* ZN1 was isolated in our previous works and stored in China General Microorganism Collection Center (CGMCC), Beijing, China with the registration number 7452 (Zhang et al., 2010b). *A. resinae* ZN1 was stored at 4 °C on potato dextrose agar (PDA) slants.

The culture media used in this study included (1) PDA medium used for *A. resinae* ZN1 and *A. niger* SIIM M288 in petri dish with the composition of 200 g/L of potato juice, 20 g/L of glucose, 20 g/L of agar and (2) a seed medium used for seed culture of *A. niger* SIIM M288 culture with the composition of 70 g/L of glucose, 2.5 g/L of NH_4Cl, 2.5 g/L of KH_2PO_4, 0.25 g/L of $MgSO_4{\cdot}7H_2O$, 0.24 mg/L of $CuSO_4{\cdot}5H_2O$, 1.1 mg/L of $ZnSO_4{\cdot}7H_2O$, 6.4 mg/L of $FeSO_4{\cdot}7H_2O$, and 3.6 mg/L of $MnCl_2$.

2.4. Pretreatment and biodetoxification operations

Corn stover was dry sulfuric acid pretreated according to Zhang et al. (2011) and He et al. (2014). Briefly, the dry corn stover and dilute sulfuric acid solution (5%, w/w) were co-currently fed into a reactor under mild agitation using a helical impeller at a solid/liquid ratio of 2:1 (w/w) and 175 °C for 5 min. After pretreatment, the pretreated corn stover contained 37.6% of cellulose, 4.4% of hemicellulose, 2.2% of glucose and 13.8% of xylose by weight percentage.

The pretreated corn stover was milled, neutralized using 20% (w/w) of $Ca(OH)_2$ to pH 5–6, then biodetoxified by *A. resinae* ZN1 according to Zhang et al. (2010b) and He et al. (2016). The spores of *A. resinae* ZN1 were inoculated onto the pretreated corn stover and incubated for 4–7 days at 28 °C until most of inhibitors were degraded. After biodetoxification, only acetic acid and minor furfural and HMF were detected. The change in cellulose and hemicellulose was negligible, while the dissolved sugars slightly decreased, in which glucose content reduced from 2.2% to 0.6%, and xylose reduced from 13.8% to 11.8%, approximately 5.7% loss of the total sugars during biodetoxification.

2.5. Simultaneous saccharification and citric acid fermentation

A. niger SIIM M288 was activated by taking one vial of spore stock onto a PDA agar slant and cultured at 28 °C for 72 h. The spores from the PDA slant were washed by sterile water and cultured in the seed medium at 28 °C for 36 h. Spores concentration in the seed medium were counted on a haemocytometer and 2–3 × 10^5 spores per milliliter were used for inoculation.

Simultaneous saccharification and fermentation (SSF) of the pretreated and detoxified corn stover feedstock was carried out in two 5 L bioreactors. The corn stover was fed into the first bioreactor equipped with a helical impeller and pre-hydrolyzed at solids loading of 20–30% with a cellulase dosage of 4 or 6 mg total protein per gram of dry solid matter (DM) at 50 °C and pH 4.8 for 12–48 h (Zhang et al., 2010a). Then the prehydrolysate slurry was transferred to the second bioreactor equipped with two Rushton impellers for SSF at an aeration rate of 1 vvm and 10% (v/v) inoculation of *A. niger* seed culture. The samples were withdrawn at regular intervals, centrifuged at 11,167 × *g* for 5 min and the supernatant was analyzed for sugars and citric acid. The viability of *A. niger* strain was measured by counting the colony forming units (CFU) on the petri dish of the diluted fermenting slurry, in which hypha formatted dispersive colony after 36 h culture (Javed et al., 2010).

2.6. Citric acid yield calculation

The conversion yield of cellulose to citric acid is defined as the ratio of citric acid formed to stoichiometric citric acid from cellulose in corn stover feedstock:

$$Citric\ acid\ yield = \frac{[CA]\times M\times [Water]-[CA]_0\times M_0\times [Water]_0}{M_0\times(1-[Water]_0)\times[Cellulose]\times 1.111}\times\frac{M_{Glu}}{M_{CA}}\times 100\% \quad (1)$$

where $[CA]$ and $[CA]_0$ are citric acid concentration at the end and the starting of fermentation (g/L), M and M_0 are the total weight of fermentation system at the end and starting point (g), $[Water]$ and $[Water]_0$ are the water fraction content of fermentation system at the end and starting point (g/g), $[Cellulose]$ is the cellulose fraction of dry

feedstock (g/g), 1.111 is the conversion factor for cellulose to equivalent glucose, M_{Glu}/M_{CA} (0.9375) is the conversion factor of glucose to citric acid on the basis of stoichiometric biochemistry (Zhou et al., 2017).

2.7. Strain morphology

The fermentation slurry was diluted tenfold by deionized water and then the diluted slurry was directly observed on a light microscope (Olympus BX51, Tokyo, Japan) under 20 × magnification. *A. niger* was distinguished from corn stover fibers based on morphological difference, in which the hyphae of *A. niger* presented as the curved filamentous lines and compacted pellets, while corn stover particles were rodlike fibers.

2.8. Particle size distribution

Particle size distribution in the fermentation slurry was measured on Malvern Mastersizer 2000 particle size analyzer (Malvern Instruments, Worcestershire, UK). The detection range was 0.02 μm–1000 μm.

2.9. Rheological property measurement

The rheology of fermentation slurry was measured by a rotational viscometer (DV2T, spindle SC4-16, Brookfield, Middleboro, MA). The apparent viscosity (η_a) was obtained at shear rate (γ) range from 11.6 to 46.7 s^{-1} at 25 °C. The rheological property was fitted into power law model $\eta_a = K_p \cdot \gamma^{n-1}$, where n was the dimensionless power-law index (-), K_p was the consistency coefficient (Pa·s^n) (Hou et al., 2016). The linearized power law model was shown in Eq. (2) and the K_p and the n values were calculated by measuring the intercept and the slop of Eq. (2):

$$\log_{10}\eta_a = \log_{10}K_P + (n-1)\log_{10}\gamma \quad (2)$$

2.10. Assay of sugars, citric acid and inhibitors

Glucose, xylose, citric acid, acetic acid, furfural and HMF were analyzed using HPLC (LC-20AD, refractive index detector RID-10A, Shimadzu, Kyoto, Japan) with a Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA) at 65 °C using a mobile phase of 5 mM H_2SO_4 at a rate of 0.6 mL/min.

2.11. Process model on Aspen Plus platform and economic analysis method

The process model was developed using Aspen Plus software (AspenTech, Cambridge, MA) based on the cellulosic ethanol model by NREL (Humbird et al., 2011). The major modifications included: (1) pretreatment from conventional dilute acid pretreatment to dry acid pretreatment (Zhang et al., 2011; He et al., 2014); (2) detoxification from ammonia overliming into biodetoxification (He et al., 2016); (3) saccharification and fermentation from SHF for ethanol production at 20% (w/w) solids loading into SHF or SSF for citric acid production at 25% (w/w) solids loading; and (4) product recovery from evaporation for ethanol into heating (80 °C), solid/liquid separation, alkali neutralization, acidolysis, decoloration and evaporative crystallization for citric acid. The plant size was 37.5 metric tons processing capacity of corn stover each hour (300,000 metric tons annually) with the annual operation time of 8000 h.

The input data in pretreatment process contained the sulfuric acid dosage per dry corn stover at 2%, the solids/liquid ratio at 2:1 (w/w), pretreatment temperature at 175 °C, pretreatment time at 5 min and pretreatment conversion yield at 4% for glucose from glucan, 40% for xylose from xylan, 3.3% for furfural from xylan and 60% for acetic acid hydrolysis ratio. The input data in detoxification process contained detoxification temperature at 28 °C, detoxification time at 48 h and detoxification conversion yield at 100% for furfural, 70% for acetic acid and 5% for glucose (Zhang et al., 2016). The input data in saccharification and fermentation as well as product recovery process was based on the following premises: (1) the glucan conversion yield to glucose in SHF was calculated based on our previous study (Zhou et al., 2017) while the yield in SSF was calculated using the same citric acid yield from glucose with SHF; (2) the citric acid yield from glucose in SHF was cited from our previous study (Zhou et al., 2017) while the yield in SSF was set as same; (3) the solid/liquid separation yield of hydrolysate slurry and fermentation slurry, as well as alkali neutralization yield and acidolysis yield in both cases were experimentally measured.

The material and energy balance data from Aspen Plus modeling were used to design equipment and determine chemical usage. The year of 2013 was used as the reference year. The exchange rate from US dollar ($) to Chinese Yuan (CNY) used was 1: 6.6 according to the current average rate (http://data.stats.gov.cn/). The general equipment of pumps, conveyors and evaporators were quoted from the NREL model (Humbird et al., 2011). The specific equipment of reactors, fermentors and agitators were modified according to actual situation in China. A discounted cash flow rate of return to determine the minimum citric acid selling price (MCSP, $/kg) required a net present value of zero for 8% internal rate of return after taxes.

3. Results and discussion

3.1. Feasibility and validation of SSF for aerobic citric acid fermentation by A. niger

The feasibility of aerobic SSF for cellulosic citric acid fermentation by *A. niger* SIIM M288 under high feedstock solids loading was evaluated. In the first stage, the dry acid pretreated and biodetoxified corn stover feedstock was pre-hydrolyzed into the corn stover hydrolysate slurry in a specially designed helical impeller (Zhang et al., 2010a). In the second stage, the liquid suspension slurry was transferred into the second bioreactor equipped with two Rushton impellers, then the *A. niger* seed culture was inoculated and aeration was initiated to supply oxygen for the fungus growth, marking the start of the SSF operation for citric acid production (Fig. 1). Glucose was rapidly generated from cellulose hydrolysis and consumed gradually to a very low level, while citric acid started to accumulate till the end of fermentation, indicating a successful SSF was operated. The dissolved oxygen (DO) level sharply decreased to approximately 10% of saturation and then gradually increased at the late stage of the SSF, indicating that the oxygen transfer from air phase to the highly viscous hydrolysate slurry phase for the growth and metabolism of the high oxygen demanding filamentous fungus strain was satisfied. The high cell viability indicates that the

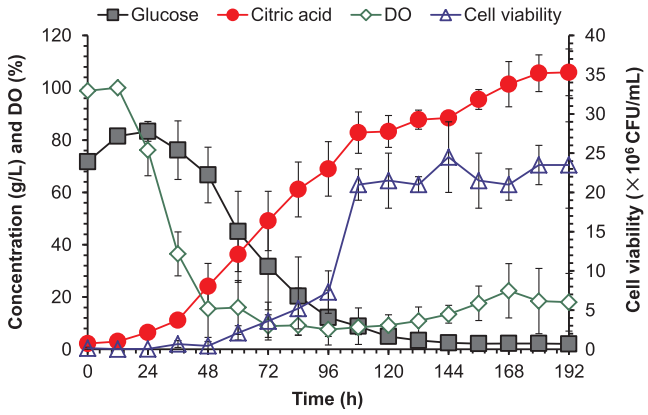

Fig. 1. SSF for cellulosic citric acid fermentation by *A. niger* from corn stover feedstock. Prehydrolysis conditions: 25% solids loading, 6 mg cellulase protein/g DM, 50 °C, pH 4.8 for 12 h. SSF conditions: inoculum size 10%, 1vvm, 33 °C for 192 h.

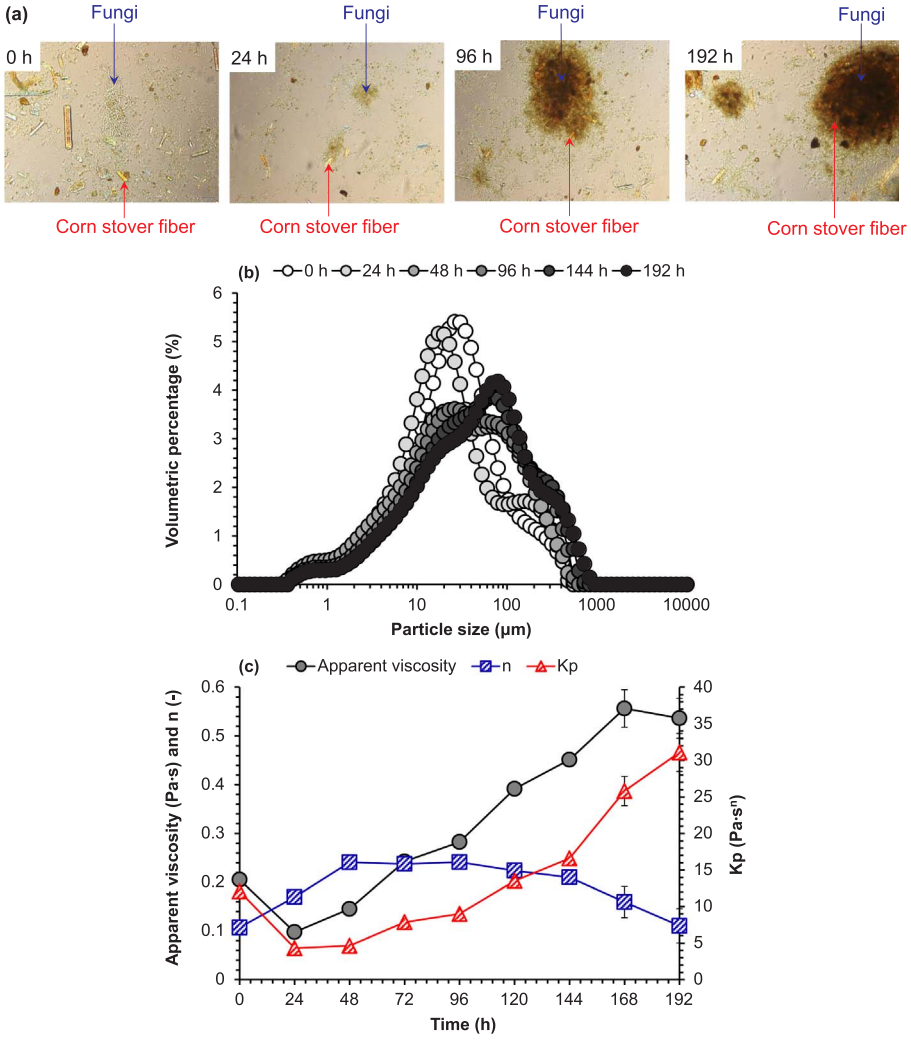

Fig. 2. Evolution of strain morphology (a), particle size distribution (b) and rheology (c) in the SSF of corn stover by *A. niger*. The enlargement of the photos in (a) was 20 folds. Prehydrolysis conditions: 25% solids loading, 6 mg cellulase protein/g DM, 50 °C, pH 4.8 for 12 h. SSF conditions: inoculum size 10%, 1vvm, 33 °C for 192 h.

fungus cell growth was not negatively affected by the shear stress of solid particles and air bubbles. Citric acid generation continued when apparent glucose was almost exhausted after 132 h, perhaps due to glucose release from the residual cellulose.

We also noticed that the aerobic SSF by *A. niger* showed slightly different from the typical anaerobic SSF such as ethanol (Liu et al., 2018) or lactic acid (Qiu et al., 2017; 2018) fermentations. In anaerobic SSF, generally the rate limiting step was the hydrolysis of pretreated lignocellulose feedstock in which the initial glucose was consumed quickly from the beginning to a near zero level till the end of SSF due to the faster ethanol or lactic acid formation rate. However, the glucose generation rate in the aerobic SSF by *A. niger* was greater than the consumption rate, as indicated by a peak glucose accumulation at 24 h (Fig. 1).

In the high solids content SSF, the mycelia of the filamentous *A. niger* fungus were strongly entangled with the rodlike corn stover fibers (Fig. 2a). The average size of the entangled mycelium-fiber bodies (balls) slightly decreased from 59.2 μm to 53.7 μm but quickly increased to 104.5 μm during the SSF (Fig. 2b), while the viscosity also decreased from 0.2 Pa·s to 0.1 Pa·s then sharply increased to 0.5 Pa·s (Fig. 2c). The entangled mycelium-fiber bodies negatively affected the oxygen transfer as well as the catalytic efficiency of the fungus cells. The phenomenon partially explained the slow citric acid fermentation rate by *A. niger* strains in the high solids loading and highly viscous corn stover hydrolysate. On the other hand, the final average size of entangled mycelia-fiber bodies was less than 0.5 mm in diameter, which was still suitable for citric acid fermentation (Papagianni, 2007; Snell and Schweiger, 1951).

A significant difference between the present SSF using fungus strain and the previous SSF using yeast or bacterial strain was observed on the rheology behaviors. In general SSF operations, the apparent viscosity of fermentation slurry decreased with the progress of SSF because of the reduced molar mass of cellulose fragments (Hou et al., 2016; Zhang et al., 2010a). However, in this SSF, the fungal mycelia entangled with the corn stover particles and generated larger particle bodies, resulting in the increased viscosity of fermentation slurry and decreased oxygen transfer rate.

3.2. Maximizing citric acid yield and productivity by regulating operation parameters

The fermentation parameters of SSF on cellulosic citric acid production were carefully examined for maximizing the conversion yield and productivity (Fig. 3). The prolonged prehydrolysis time elevated the initial glucose concentration but the final citric acid yield was approximately the same (Fig. 3a). The suitable temperature for enzymatic hydrolysis was 50 °C, but the proper growth temperature for *A. niger* is in the range of 25–30 °C (Angumeenal and Venkappayya, 2013). The higher temperature varying from 33 °C to 37 °C is preferred in SSF to match the high hydrolysis temperature (Fig. 3b). During the SSF, xylose was slowly released from the residual hemicellulose and gradually utilized for cell growth and metabolism, and the maximum citric acid titer (122.7 g/L) appeared at the moderate 35 °C. Cellulase enzyme usage is one of the key factors on the bioconversion cost and a low

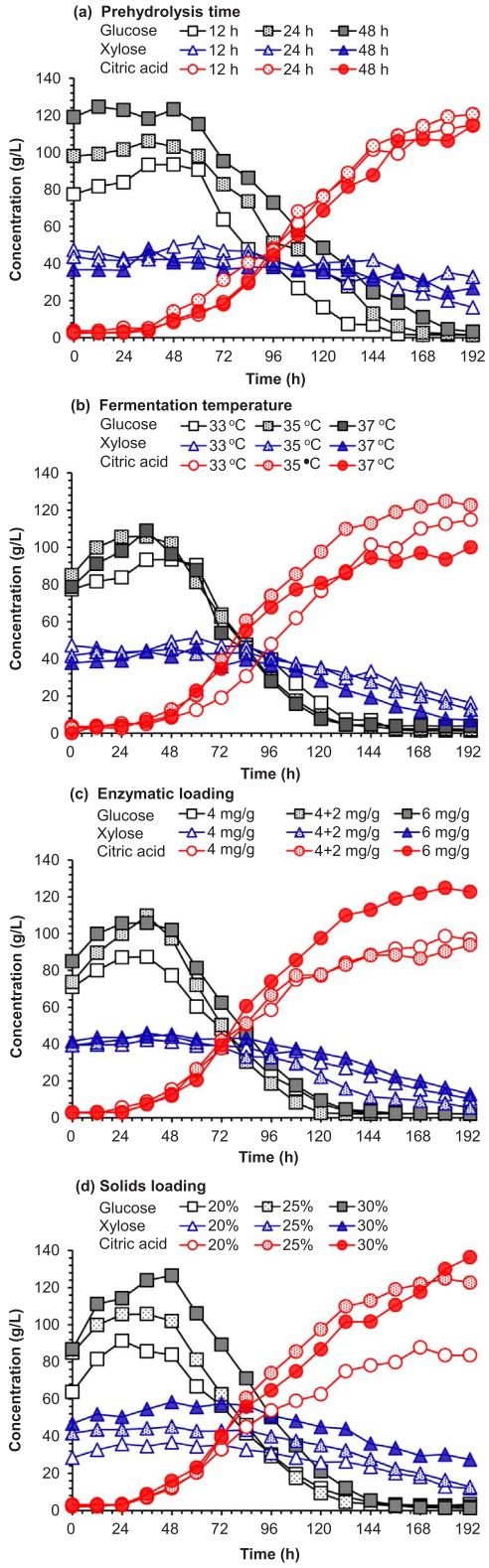

Fig. 3. Parameter optimization of SSF by *A. niger* on cellulosic citric acid production. Prehydrolysis conditions: 50 °C and pH 4.8; SSF conditions: inoculum size 10%, 1 vvm for 192 h. (a) Prehydrolysis time. 25% (w/w) solids loading, 6 mg cellulase protein/g DM, fermentation at 35 °C (b) SSF temperature. 25% (w/w) solids loading, 6 mg cellulase protein/g DM, prehydrolysis for 12 h. (c) Enzymatic loading. 4 + 2 mg/g indicated 4 mg cellulase protein/g DM added in prehydrolysis stage and 2 mg cellulase protein/g DM added in fermentation stage, 25% (w/w) solids loading, prehydrolysis for 12 h, fermentation at 35 °C. (d) Solid loading. 6 mg cellulase protein/g DM, prehydrolysis for 12 h, fermentation at 35 °C. Agitation rates were 400, 500 and 600 rpm at the solids loading of 20%, 25 and 30%, respectively.

cellulase dosage was used (4–6 mg cellulase protein/g dry corn stover matter, DM) (Fig. 3c). The higher cellulase dosage facilitated citric acid formation, but the fed-batch addition of cellulase at the initial SSF stage did not make any improvement (4 mg/g DM in prehydrolysis plus 2 mg/g DM in SSF). The increase of feedstock solids loading certainly increased the initial glucose concentration and the final citric acid yield (Fig. 3d), but the further increase from 25% to 30% (w/w) gave a very limited increase, and the overall conversion yield from cellulose to citric acid also decreased from 86.8% to 74.9%. The maximum citric acid titer of 136.3 g/L was obtained under the solids loading of 30% (w/w) at 6 mg cellulase protein/g DM and 35 °C. However, SSF for citric acid production at the relatively lower solids loading of 25% could be more economic due to the higher conversion yield.

3.3. Techno-economic analysis (TEA) on cellulosic citric acid production from corn stover

The Aspen Plus model for production of cellulosic citric acid was established by processing 37.5 metric tons dry corn stover each hour. The two typical operation cases of SHF and SSF for cellulosic citric acid production were evaluated using the same corn stover feedstock and biorefining processing technology (dry acid pretreatment and biodetoxification, DryPB) (Liu et al., 2018). The SHF case was cited from our previous study (Zhou et al., 2017), in which the dry acid pretreated and biodetoxified corn stover feedstock was enzymatically hydrolyzed at the solids loading of 25% for 48 h, and then the corn stover hydrolysate was obtained by solid/liquid separation and sent for citric acid fermentation (Table 1). The SSF case was cited from Fig. 3b, in which the corn stover feedstock was pre-hydrolyzed at the same solids loading and then directly sent for simultaneous saccharification and fermentation (SSF) without solid/liquid separation. The SSF case obtained the higher citric acid titer (120 g/L) and conversion yield (84.8%) in a short overall time (168 h) than the SHF case (100 g/L, 62.3%, 240 h, respectively).

The process diagram of the two cases was shown in Fig. 4. The materials balance shows that the solid/liquid separation of hydrolysate slurry in SHF caused a considerable sugar loss (1.697 tons of glucose, 1.071 tons of xylose and 3.426 tons of glucan and xylan per hour), while no sugar loss in SSF by eliminating the solid/liquid separation (Fig. 4a). SSF produced 327 kg of citric acid (98%, w/w) from one ton of dry corn stover, but SHF produced 260 kg. Besides, 14.559 tons of wastewater was generated in SHF for producing one ton of citric acid product, while only 12.284 tons of wastewater was generated in SSF (Fig. 4a). The more wastewater generation in citric acid product was since that product purification by alkali neutralization and acidolysis generated abundant of non-recyclable water containing dark color impurities. The solid/liquid separation and the longer overall operating time in SHF also led to increase of electricity consumption by 18.4% than that in SSF (Fig. 4b).

The TEA calculation based on the established Aspen Plus model shows that the minimum citric acid selling prices (MCSP) by SHF was

Table 1
Main process input data for establishing the Aspen Plus model and conversion costs.

	SHF	SSF
Solids loading (%)	25	25
Cellulase dosage (mg protein/g dry feedstock)	6	6
Temperature for hydrolysis (°C)	50	50
Residence time for hydrolysis (h)	48	12
Temperature for fermentation (°C)	33	35
Residence time for fermentation (h)	192	156
Citric acid concentration (g/L)	100	120
MCSP ($/kg citric acid product)	0.851	0.603
Feedstock	0.280	0.222
Enzyme	0.098	0.077
Non-enzyme conversion	0.473	0.304

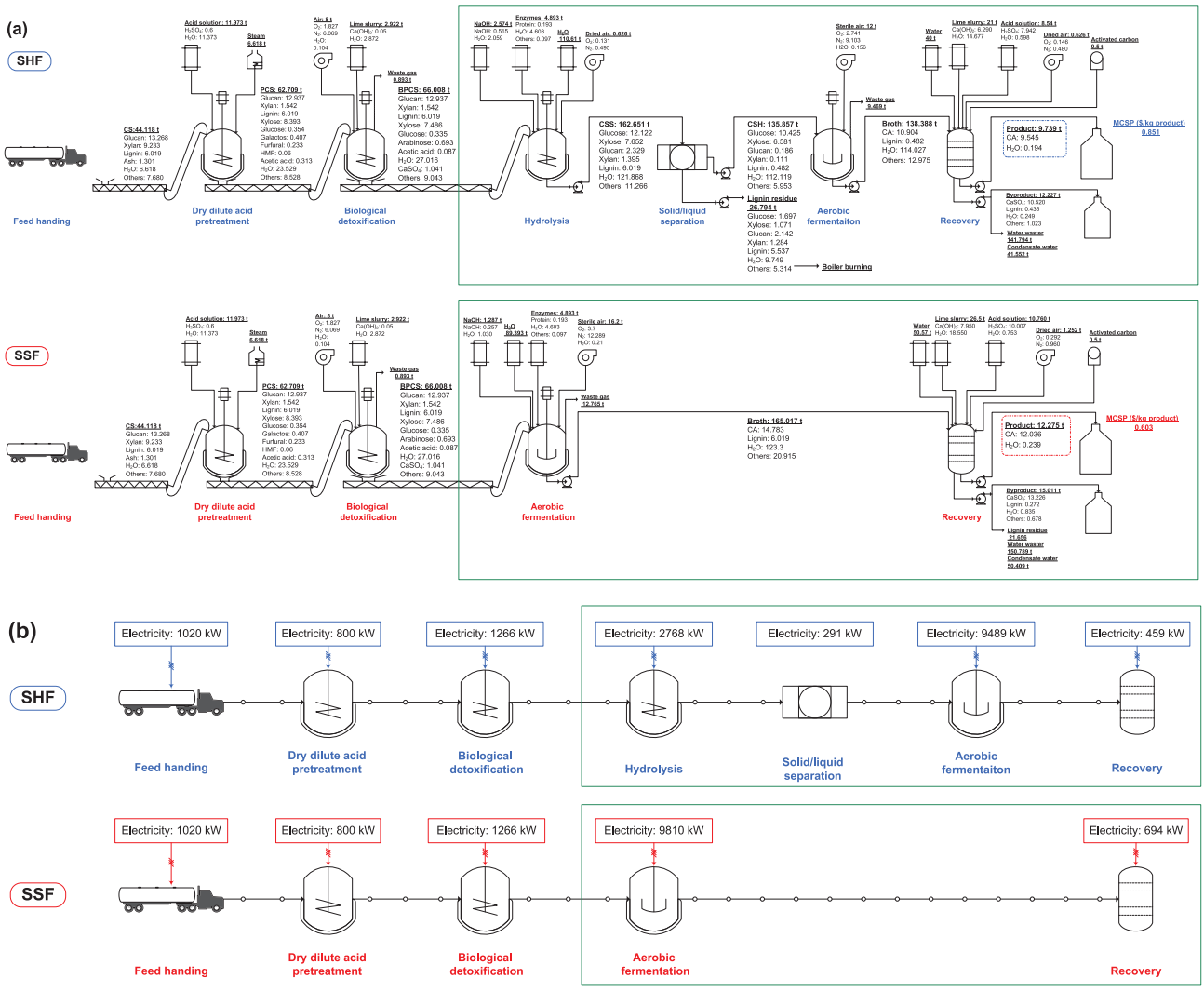

Fig. 4. Materials and energy balance of cellulosic citric acid production from corn stover on the hour basis (metric tons per hour). (a) Materials balance; (b) Energy balance. Abbreviations: CS, Corn stover; PCS, Pretreated corn stover; BPCS, Biodetoxified and pretreated corn stover; CSS, Corn stover hydrolysate slurry; CSH, Corn stover hydrolysate; CA, Citric acid; MCSP, Minimum citric acid selling price. *All patterning used in this Figure were abstract representation of processing flowsheet and not the real equipment drawing.

$0.851 per kg, in which the contribution of feedstock, enzyme and non-enzyme conversion were $0.280, $0.098 and $0.473 per kg (Table 1), respectively. The greater non-enzyme conversion in citric acid production was mainly caused by the higher power consumption, longer fermentation period, and more complicated product recovery operations. For the SSF case, the MCSP of $0.603 per kg just turned the profit of cellulosic citric acid below the current market price ($0.68 per kg, Alibaba Enterpriser website https://www.1688.com, cited from Weifang Ensign Industry Co).

4. Conclusion

The feasibility of aerobic SSF by the high oxygen demanding *A. niger* fungus for cellulosic citric acid production was evaluated. The high citric acid titer and the conversion yield of 84.8% by SSF resulted in the minimum citric acid selling price (MCSP) from corn stover feedstock, which is competitive to the commercial citric acid produced from starch or sugar feedstocks.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2018.01.011.

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