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Oxidative production of xylonic acid using xylose in distillation stillage of cellulosic ethanol fermentation broth by *Gluconobacter oxydans*

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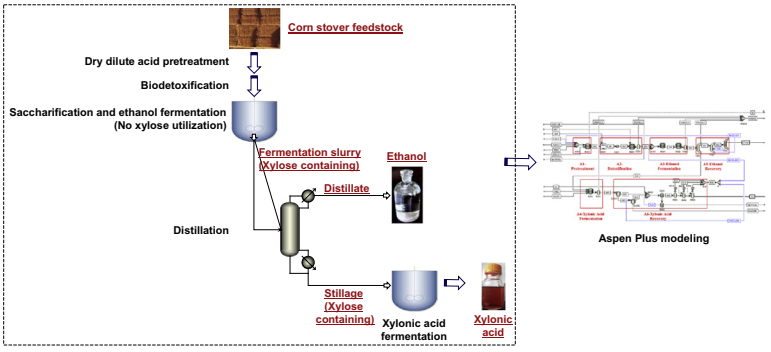
HIGHLIGHTS

- Dry dilute acid pretreatment and biodetoxification are applied on corn stover biorefining.
- 75.22 g/L of xylonic acid is produced using xylose in cellulosic ethanol distillation stillage.
- 59.80 g/L of ethanol is also obtained before xylose fermentation.
- Significant reduction of wastewater generation and energy consumption are achieved.
- Aspen Plus modeling is conducted on the flowsheet simulation of the proposed process.

GRAPHICAL ABSTRACT



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ABSTRACT

An oxidative production process of xylonic acid using xylose in distillation stillage of cellulosic ethanol fermentation broth was designed, experimentally investigated, and evaluated. Dry dilute acid pretreated and biodetoxified corn stover was simultaneously saccharified and fermented into 59.80 g/L of ethanol (no xylose utilization). 65.39 g/L of xylose was obtained in the distillation stillage without any concentrating step after ethanol was distillated. Then the xylose was completely converted into 66.42 g/L of xylonic acid by *Gluconobacter oxydans*. The rigorous Aspen Plus modeling shows that the wastewater generation and energy consumption was significantly reduced comparing to the previous xylonic acid production process using xylose in pretreatment liquid. This study provided a practical process option for xylonic acid production from lignocellulose feedstock with significant reduction of wastewater and energy consumption.

1. Introduction

Xylose is the major component of hemicellulose and its utilization represents one of the most challenging tasks in sugar platform pathway of lignocellulose biorefining (Jeffries, 1983). One way is to reduce xylose into xylulose, then enter the pentose phosphate pathway (PPP) and convert to ethanol by metabolic engineering of fermenting strains such as *Saccharomyces cerevisiae* (Ho et al., 1998) and *Zymomonas mobilis* (Zhang et al., 1995). Although engineered strains were constructed to produce ethanol from xylose, the low xylose utilization rate always results in a long fermentation process and low ethanol productivity. (Katahira et al., 2006; Ko et al., 2016). However, there exists another way of xylose conversion, oxidation of xylose into xylonic acid (Buchert et al., 1988a, b), by oxidizing xylose into xylonic acid, a value-added biobased chemical with wide applications (Werpy and Petersen, 2004) such

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[1] These authors are equally contributed to this work. HSZ performed the xylonate conversion; XSH performed the ethanol fermentation; CXW performed the Aspen Plus simulation.

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as cement additives for disperser and water reducer (Chun et al., 2006).

Oxidative production of xylonic acid from pure xylose certainly is not a practical option because fractionation of xylose and glucose of lignocellulose hydrolysate is a high cost process (Galbe et al., 2007; Larsen et al., 2008). Zhou et al. (2015) developed a high-oxygen tension bioreactor to increase xylonic acid productivity from diluted sulfuric acid pre-hydrolysates of corn stover by *G. oxydans*, showed a cost-competitive bacterial xylonic acid production. Zhu et al. (2015) tried to produce xylonic acid from xylose in the pretreatment liquid of corn stover after steam-explosion pretreatment. The xylose containing pretreatment liquid (less than 15 g/L) was concentrated to higher titer (55.00 g/L), then fermented into 54.97 g/L of xylonic acid, while the pretreated cellulose solids was fermented to ethanol. In this process design, the concentrating step of the low titer xylose from the pretreatment liquid required high energy input and the considerable wastewater was generated. Inhibitor accumulation in the xylose concentrating step also negatively affected the cell growth of the fermenting strain and xylose conversion.

To overcome the high energy consumption and wastewater generation difficulties, this study proposed a new process design for xylonic acid production from xylose in the distillation stillage of cellulosic ethanol fermentation broth after ethanol was distillated. Corn stover was dry dilute acid pretreated, biodetoxified, and then simultaneously saccharified and fermented (SSF) into ethanol using *S. cerevisiae* DQ1 (no xylose utilization function). Xylose was maintained in the distillation stillage after ethanol was distillated from the fermentation broth, and then oxidized into xylonic acid by *Gluconobacter oxydans* DSM 2003. The rigorous Aspen Plus modeling revealed that the energy consumption and wastewater generation were significantly reduced while the ethanol and xylonic acid conversion maintained at high level.

2. Materials and methods

2.1. Raw materials and enzymes

Corn stover (CS) was harvested from Bayan Nur, Inner Mongolia, China in fall 2015. The corn stover was chopped to small chippings coarsely, then washed by and sediment in 10 times (w/w) water to remove the solid dirt, sands, stones and metals, then dried and milled using a hammer crusher to pass through the 10 mm apertures in diameter, then sealed in plastic bags and stored at room temperature until use. The composition of corn stover contained 35.38% of cellulose, 24.62% of hemicellulose, 16.05% of lignin, 3.47% of ash on dry weight base (w/w) determined by Cellulose Analyzer 220 (Ankom Technology, Macedon, NY, USA).

The cellulase enzyme Youtell #7 was purchased from Hunan Youtell Biochemical Co. (Yueyang, Hunan, China). The filter paper activity of Youtell #7 was 63 FPU/g determined using the NREL protocol LAP-006 (Adney and Baker, 1996). The cellobiase activity of Youtell #7 was 344 CBU/g using the method described by Ghose (1987). The protein content of Youtell #7 was 49.5 mg per gram determined by Bradford method (Bradford, 1976).

2.2. Strains and media

Xylonic acid fermenting strain *Gluconobacter oxydans* DSM 2003 was purchased from German Collection of Microorganisms and Cell Cultures (DSMZ), Braunschweig, Germany. The culture medium used for *G. oxydans* DSM 2003 included:

(1) Activation medium, containing 40.0 g of sorbitol, 10.0 g of yeast extract, 1.5 g of KH_2PO_4, 1.5 g of $(NH_4)_2SO_4$, 0.5 g of $MgSO_4{\cdot}7H_2O$, 20.0 g of agar in one liter of deionized water.
(2) Seed medium, containing 80.0 g of sorbitol, 10.0 g of yeast extract, 1.5 g of KH_2PO_4, 1.5 g of $(NH_4)_2SO_4$, 0.5 g of $MgSO_4{\cdot}7H_2O$ in one liter of deionized water.
(3) Xylose synthetic medium for fermentation, containing 80.0 g of xylose, 10.0 g of yeast extract, 1.5 g of KH_2PO_4, 1.5 g of $(NH_4)_2SO_4$, 0.5 g of $MgSO_4{\cdot}7H_2O$ in one liter of deionized water.

Ethanol fermenting strain *Saccharomyces cerevisiae* DQ1 (stored in China General Microbial Collection Center, Beijing, China, with registration number CGMCC 2528) was used for ethanol production during SSF. The culture medium used for *S. cerevisiae* DQ1 included:

Seed synthetic medium, containing 20 g glucose, 1 g yeast extract, 2 g KH_2PO_4, 1 g $(NH_4)_2SO_4$, 1 g $MgSO_4{\cdot}7H_2O$ in one liter of deionized water.

The fungus strain *Amorphotheca resinae* ZN1 (CGMCC 7452) was used for degrading inhibitors from dry dilute sulfuric acid pretreatment of corn stover (Zhang et al., 2010a). The fungus *A. resinae* ZN1 was maintained on a potato dextrose agar medium (PDA) slant. The PDA medium was prepared by boiling 200 g of cleaned, peeled and sliced potatoes in one liter deionized water for 30 min.

2.3. Dry dilute sulfuric acid pretreatment and biodetoxification operations

Corn stover was pretreated using the dry dilute sulfuric acid pretreatment (DDAP) according to Zhang et al. (2011) and He et al. (2014). Briefly, the dried prepared 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) under the helically stirring mixing, then pretreated at 175 °C for 5 min. The solids loading of the pretreated corn stover was around 50% (w/w) and no wastewater was generated. The pretreated corn stover solid contained 36.51% of glucan and 4.48% of xylan based on the dry matter weight detected by two-step acid hydrolysis method according to NREL protocols (Sluiter et al., 2008, 2012). The dissolved sugars concentration in the dry matter were discovered at 29.71 mg/g DM of glucose, 11.85 mg/g DM of gluco-oligomer, 155.07 mg/g DM of xylose and 38.91 mg/g DM of xylo-oligomer. The gluco-oligomer and xylo-oligomer were detected based on the generated glucose and xylose concentration after acid hydrolysis by 4% sulfuric acid at 121 °C for 1 h (Sluiter et al., 2008, 2012). The major inhibitors content in the dry matter was 3.56 mg/g DM of furfural, 4.02 mg/g DM of 5-hydroxymethylfurfural (HMF), 21.67 mg/g DM of acetic acid, 1.97 mg/g DM of formic acid, 2.54 mg/g DM of levulinic acid, 0.55 mg/g DM of vanillin, 0.74 mg/g DM of syringaldehyde and 0.36 mg/g DM of 4-hydroxybenzaldehyde.

The pretreated corn stover was biologically detoxified using *A. resinae* ZN1 according to Zhang et al. (2010a) and He et al. (2016). Briefly, the pretreated corn stover was neutralized with 20% (w/w) $Ca(OH)_2$ suspension slurry to pH 4.5–5.5 and disk milled, then inoculated with *A. resinae* ZN1 for biodetoxification in a helical ribbon stirring reactor at the aeration of 1.0 vvm for 48 h at 28 °C. No fresh water and nutrients were added and no wastewater was generated during biodetoxification with the approximately 50% (w/w) of solids content. The soluble sugars concentrations were 22.51 mg/g DM of glucose, 10.91 mg/g DM of gluco-oligomer glucose, 144.26 mg/g DM of xylose, and 36.53 mg/g DM of xylo-oligomer. In this detoxification process, the cellulose almost remains constant and less than one-tenth xylose and xylo-oligomer were consumed for cell growth. After the biodetoxification, the major inhibitors content in the dry

matter were reduced to 0.48 mg/g DM of HMF, 4.21 mg/g DM of acetic acid, 0.62 mg/g DM of levulinic acid, 0.25 mg/g DM of vanillin, 0.46 mg/g DM of syringaldehyde and 0.03 mg/g DM of 4-hydroxybenzaldehyde. Not furfural and formic acid were detected.

2.4. Simultaneous saccharification and ethanol fermentation (SSF) and distillation

SSF for ethanol production was performed in the helical ribbon stirring bioreactor as described in Zhang et al. (2010b) and Liu et al. (2015). Briefly, A three-step adaptation procedure for SSF using pretreated and biodetoxified corn stover was followed: first, a vial (2 mL) of *S. cerevisiae* DQ1 was inoculated into 20 mL of sterilized synthetic medium in a 100 mL Erlenmeyer flask, cultured in a shaker at 30 °C, 150 rpm, for 18 h; then culture (2 mL) was inoculated into 20 mL of sterilized medium containing 50% of corn stover hydrolysate and 50% of synthetic medium (pH 5.0) in a 100 mL Erlenmeyer flask, cultured at 30 °C, 150 rpm for 15 h; finally, the culture (20 mL) was inoculated into 200 mL of sterilized corn stover hydrolysate medium in a 500 mL flask, cultured at 30 °C, pH 5.0, 150 rpm for 15 h. The yeast cells were harvested by centrifuging at 12000×*g* for 10 min, and the precipitates were resuspended in 20 mL sterilized water. Then the yeast suspension was used for SSF step as seed. The pretreated and biodetoxified corn stover material was loaded into the bioreactor at 20–30% (w/w) solids content (dry basis) with helically stirring, followed by the pre-hydrolysis for 12 h at 50 °C, pH 4.8 after the cellulase was added. Then the temperature was reduced to 37 °C and the *S. cerevisiae* DQ1 seed was inoculated into the bioreactor at 10% inoculum size (v/v) to start the SSF at pH 4.8 for 60 h. Samples were taken periodically for analysis of ethanol, glucose and xylose. The cell concentration of *S. cerevisiae* DQ1 in this SSF process was not detected because of the water insoluble solid (WIS) in ethanol fermentation slurry would influence the OD_{600} value.

The whole ethanol fermentation broth slurry was directly fed into the bottom kettle of glass distillation column (50 mm in the inner diameter packed with theta ring packing carrier). The distillation operation was conducted at atmospheric pressure at the reflex ratio of 3:1. Ethanol was recovered as the distillate and xylose was maintained in the stillage together with solids residue. The stillage was centrifuged to remove the solids to get the xylose containing liquid as the xylonic acid fermentation feedstock, or the stillage was directly used as the feedstock without solids removal.

2.5. Xylonic acid fermentation

One vial (2 mL) of *G. oxydans* DSM 2003 in 30% (v/v) glycerol was inoculated into 20 mL of the seed medium in 100 mL flask and cultured at 30 °C for 24 h. The seed culture was inoculated at 10% (v/v) inoculum size into 3 L fermentor containing 1 L of ethanol fermentation broth distillation stillage and fermented at 30 °C, 2.5 vvm of aeration in duplicate. When the whole cells were used for catalytic conversion, the seed culture was centrifuged at 14400×*g* for 5 min and collected, then inoculated into 3 L fermentor containing 1 L ethanol fermentation broth distillation stillage at 30 °C, 2.5 vvm of aeration in duplicate. The initial cell concentration was maintained at about 2.5 g/L. The pH in fermentors was controlled by 5 M NaOH and 2 M H_2SO_4.

2.6. Analysis and calculation

Glucose, ethanol, acetic acid, furfural and HMF were analyzed on HPLC (LC-20AD pump, refractive index detector RID-10A, Shimadzu, Kyoto, Japan) with Bio-Rad Aminex HPX-87H column at 65 °C and 0.6 mL/min of 5 mM H_2SO_4 as the mobile phase. Xylose was measured by HPLC (LC-20 AD, detector RID-10A, Shimadzu, Kyoto, Japan) with Aminex HPX-87H column at 65 °C using the mobile phase at the flow rate of 0.6 mL/min of 12 mM $NaHCO_3$ to replace 5 mM H_2SO_4 to eliminate interference from the peak of xylonic acid at the similar relative retention time. Xylonic acid was analyzed using HPLC (LC-20AD, detector RID-10A, Shimadzu, Kyoto, Japan) with a Shodex Rspak JJ50-4D column (Showa Denko, Tokyo, Japan) at 40 °C using the mobile phase of 12 mM $NaHCO_3$ at the rate of 0.5 mL/min.

Ethanol yield based on cellulose of pretreated corn stover was calculated using the method deduced by Zhang and Bao (2012) specifically for the high solids loading and high ethanol titer SSF process:

$$\text{Ethanol yield}(\%) = \frac{[C_1] \times W}{967.9 - 0.804 \times [C_1]} \times \frac{1}{0.511 \times f \times [Biomass] \times \text{m} \times 1.111} \times 100\%$$

where $[C_1]$ is the ethanol concentration (g/L) of fermentation broth at the end of SSF; W is the total water input into the SSF system (g); f, the cellulose content of dry pretreated corn stover (g/g); [*Biomass*], dry pretreated corn stover weight content in the starting of SSF (g/g); m, total weight of pretreated materials at the beginning of the operation (g); 976.9 and 0.804 are the correction factors; 1.111 is the conversion factor for cellulose to equivalent glucose; 0.511 is the conversion factor for glucose to ethanol based on the stoichiometric biochemistry of yeast.

The xylose recovery yield based on pretreated corn stover in SSF process was calculated according to:

$$\text{Xylose recovery yield}(\%) = \frac{[C_1] \times W}{967.9 - 0.804 \times [C_1]} \times \frac{1}{(Xylose + Xylooligomer \times 1.136 + Xylan \times 1.136) \times \text{m}} \times 100\%$$

where $[C_1]$ is the xylose concentration (g/L) of fermentation broth in SSF; W is the total water input into the SSF system (g); *Xylose*, the xylose content of dry pretreated corn stover (g/g); *Xylooligomer*, the xylo-oligomer content of dry pretreated corn stover (g/g); *Xylan*, the xylan content of dry pretreated corn stover (g/g); m, total weight of pretreated materials at the beginning of the operation (g); 976.9 and 0.804 are the correction factors; 1.136 is the conversion factor for xylan to equivalent xylose based on the stoichiometric balance.

The xylonic acid yield is defined as the ratio of the xylose converted into xylonic acid to the total initial xylose:

$$\text{Xylonic acid yield}(\%) = \frac{[XA] \times V - [XA]_0 \times V_0}{[Xyl]_0 \times V_0 \times 1.107} \times 100\%$$

where $[Xyl]_0$, the initial xylose concentration (g/L); $[XA]_0$ and $[XA]$, the initial and final xylonic acid concentrations (g/L); 1.107 is the conversion factor for xylose to equivalent xylonic acid based on the stoichiometric balance; V_0 and V, the initial and final volumes of fermentation broth (L). The sodium xylonate yield equals to the xylonic acid yield.

2.7. Flowsheet model establishment on Aspen Plus platform

Two models were developed on Aspen Plus platform (Aspen-Tech Co., Cambridge, MA, USA) based on the NREL model for cellulosic ethanol production (Humbird et al., 2011). The models have been modified in several areas including pretreatment from the conventional dilute acid pretreatment into the dry dilute acid pretreatment (DDAP) (Zhang et al., 2011), the detoxification from ammonia overliming into biodetoxification (He et al., 2016), and the saccharification and fermentation from 20% (w/w) of solids

loading into 30% (w/w). The major difference between these two models is on the biofractionation of cellulose and hemicellulose. Process One simulates the process design in this study, in which xylose is recovered from the distillation stillage of ethanol fermentation broth, followed by xylonic acid fermentation. Process Two simulates the process design of Zhu et al. (2015), in which xylose is recovered from the pretreatment liquid, then concentrated and fermented into xylonic acid, while solid part is sent for ethanol fermentation. In the two processes, the same conversion or recovery yields are given to the sections of dilute acid pretreatment, hydrolysis, ethanol fermentation, xylonic acid fermentation, as well as product recovery for evaluating the two process design of the two processes on the same basis.

3. Results and discussion

3.1. Ethanol fermentation from dry dilute acid pretreated and biodetoxified corn stover and xylose containing stillage recovery

Corn stover feedstock was dry dilute acid pretreated, biodetoxified, enzymatic hydrolyzed, and fermented into ethanol using *S. cerevisiae* DQ1 by simultaneous saccharification and fermentation (SSF) at the solids loading of 20%, 25% and 30% (w/w) (Fig. 1a) to obtain the ethanol at 40.65 g/L, 50.24 g/L and 56.23 g/L with the yield of 87.68%, 83.06% and 71.74%, respectively. The ethanol titer obtained at 30% solids loading (56.23 g/L, equivalent to 7.1% by volumetric concentration) satisfied the requirement of minimum ethanol titer in the consequent distillation (Galbe et al., 2007; Larsen et al., 2008). *S. cerevisiae* DQ1 was not able to utilize xylose, therefore xylose was maintained in the fermentation broth, then left in the stillage stream after ethanol was distillated. Xylose concentration in the fermentation broth was 39.28 g/L, 50.43 g/L, and 56.30 g/L with the recovery yield 68.56%, 66.18%, and 64.32% based on total xylose of the pretreated corn stover at the solids content of 20%, 25%, 30%, respectively (Fig. 1a). The decrease of xylose concentration during ethanol fermentation was caused by total liquid volume increase with the increasing ethanol generation. The overall xylose was approximately in mass balance during the ethanol fermentation period and no major xylose loss was detected (Fig. 1a). The major focus of this study is for xylonic acid production thus the high xylose concentration is required. Higher solids loading results in the higher xylose titer in the ethanol distillation stillage, and the higher xylose titer consequently leads to the higher xylonic acid titer without additional concentration step. In addition, the ethanol productivity of SSF at 30% is higher than that

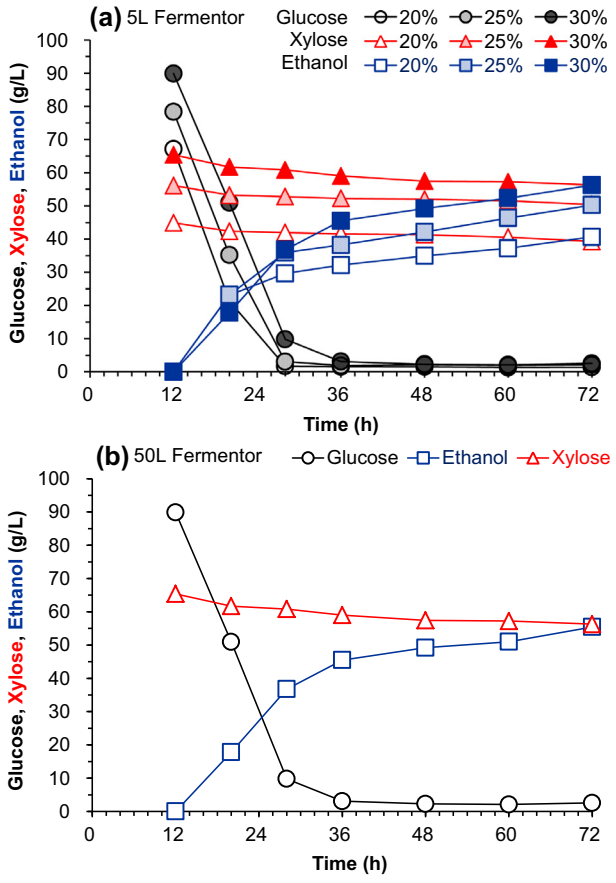


Fig. 1. Simultaneous saccharification and fermentation (SSF) of corn stover by *S. cerevisiae* DQ1. (a) in 5 L fermentor; (b) in 50 L fermentor. SSF was operated at the pre-hydrolysis with 20%, 25%, 30% solids loading (w/w) of pretreated and biodetoxified corn stover in 5 L fermentors, cellulase dosage of 15 FPU/g DM, 50 °C, pH 4.8, 150 rpm for 12 h; then SSF with 10% (v/v) inoculum size of *S. cerevisiae* DQ1 at 37 °C, pH 4.8. In 50 L fermentor, SSF was operated at the pre-hydrolysis with 30% (w/w) solids loading under same conditions except the inoculum size of *S. cerevisiae* DQ1was reduced to 3% (v/v).

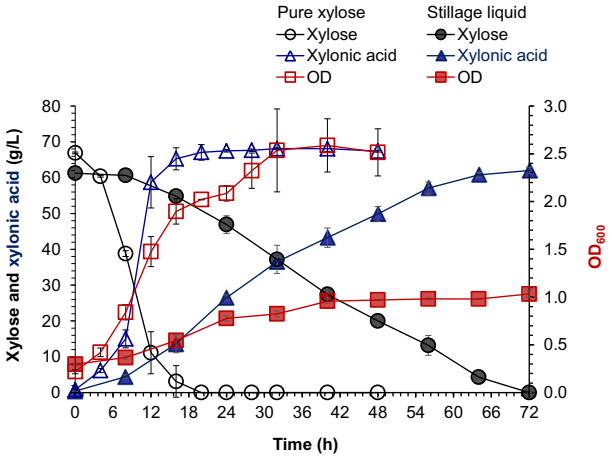


Fig. 2. Xylonic acid fermentation by *G. oxydans* DSM 2003 in fermentors using different substrate. Pure xylose: xylose containing synthetic medium. Stillage liquid: liquid supernatant of cellulosic ethanol distillation stillage containing 66.87 g/L of xylose, 2.08 g/L of glucose, 2.12 g/L of acetic acid, 0.07 g/L of HMF, no ethanol and furfural were detected. Conditions: 30 °C, pH 5.5, 500 rpm, 2.5 vvm, the inoculum size 10% (v/v), 1 L liquid in 3 L fermentor.

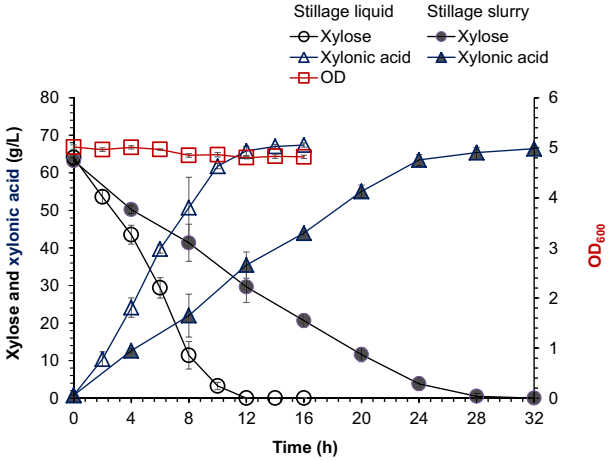
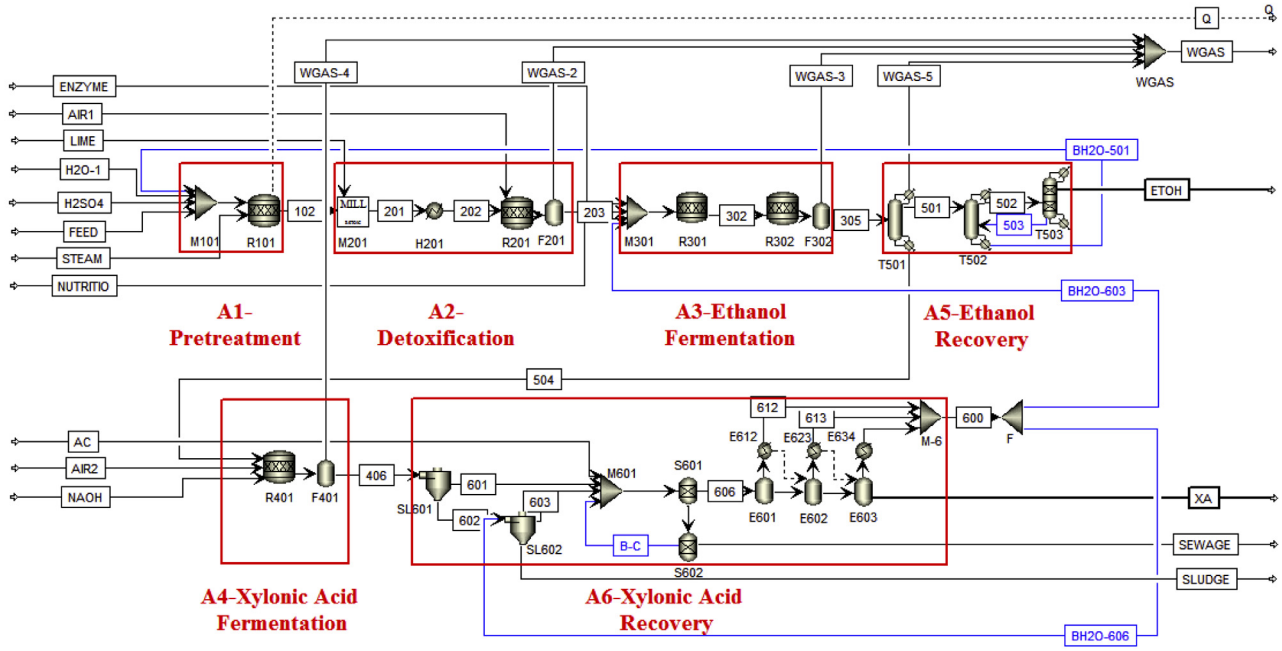


Fig. 3. Xylonic acid conversion by whole cell catalysis of *G. oxydans* DSM 2003 in fermentors. Stillage liquid: liquid supernatant of cellulosic ethanol distillation stillage. Stillage slurry: ethanol distillation stillage without solid/liquid separation. Conditions: 30 °C, pH 5.5, 500 rpm, 2.5 vvm, the initial cell concentration maintained at about 2.5 g/L (OD_{600} about 5.0), 1 L liquid in 3 L fermentor.

(a) Process One: Xylose in ethanol distillation stillage



(b) Process Two: Xylose in pretreatment liquid

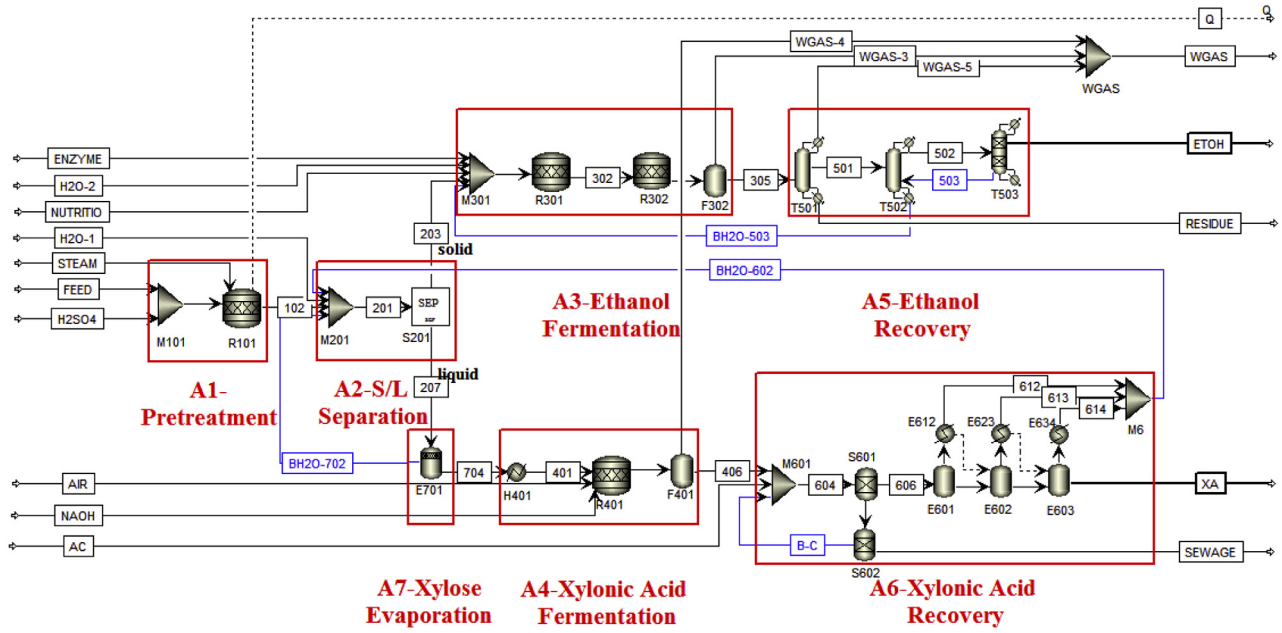


Fig. 4. Flowsheet illustrations on Aspen Plus platform for xylonic acid production. (a) Process One using xylose in cellulosic ethanol distillation stillage (this study); (b) Process Two using xylose from pretreatment liquid (Zhu et al., 2015). Abbreviations: ETOH, ethanol; XA, xylonic acid; SEP, solid/liquid separation; AC, active carbon; WGAS, waste gas.

at 20%, which was also helpful for the reduction of steam energy cost in the downstream distillation. Therefore, the higher solids loading at 30% (w/w) was selected at the first ethanol fermentation step to yield the higher xylose concentration in the distillation stillage liquid and to produce higher xylonic acid titer and yield with better economic competitiveness.

The above process was scaled up to 50 L fermentor at 30% solids loading (Fig. 1b), in which the inoculum size of *S. cerevisiae* was decreased from 10% (v/v) to 3% (v/v) for considerations of practical operation factor. Only a slight decrease in ethanol productivity was observed the overall performance in 50 L fermentor was approximately the same with that in the 5 L fermentor with the titer and yield of ethanol were 59.80 g/L and 73.88% (based on cellulose of pretreated corn stover), while xylose was 55.35 g/L in titer and 64.31% in recovery yield (based on total xylose of pretreated corn stover). The purpose of ethanol fermentation in 50 L fermentor is to provide xylose for xylonic acid production. The similar SSF for ethanol production had been investigated for multiple times and the result in this study is in agreement with the previous experiments (Gu et al., 2014). After ethanol was distillated, the xylose titer was increased to 65.39 g/L in the distillation stillage and not changed after solid/liquid (S/L) separation to remove the solids.

3.2. Conversion of xylose in the distillation stillage into xylonic acid

Xylonic acid fermentation using the xylose containing distillation stillage without extra nutrient addition was carried out in 3 L fermentor by *G. oxydans* DSM 2003 which showed excellent xylonic acid fermentability in corn stover hydrolysate (Zhang et al., 2016) (Fig. 2). Comparing to pure xylose, the xylonic acid productivity in the stillage liquid decreased to 0.89 g/(L h) from 3.24 g/(L h) and the maximum cell growth decreased by 60%. Nevertheless, the xylonic acid yield and titer were almost the same using the stillage liquid. The nutrient addition to the stillage liquid did not show obvious change on cell growth rate and xylonic acid productivity (data not shown). The result demonstrated the nutrition was not the key factor of low xylonic acid productivity in ethanol stillage liquid. Instead, the residual inhibitors and metabolite from ethanol fermentation by *S. cerevisiae* may negatively inhibit the growth of *G. oxydans*, besides the reduced oxygen transfer rate by the existence of solids particles in xylonic acid production process.

To improve the productivity of xylonic acid production from the xylose in the fermentation broth, the whole cell catalysis was carried out utilizing the xylose dehydrogenase of *G. oxydans* cells as biocatalyst (Fig. 3). When the stillage liquid was used, 2.5 g/L of dry cell weight was inoculated (equivalent to 5.0 of the OD value at 600 nm, approximately five folds greater than that of the final OD value of the fermentation case). Xylonic acid productivity was remarkably increased from 0.89 g/(L h) of the fermentation case to 5.42 g/(L h) of the whole cell catalysis and the overall conversion time was reduced from 72 h to 12 h when xylose was completely converted. Then the stillage slurry of ethanol fermentation broth (together with water insoluble solids) was directly used for saving xylose in the solids portion in S/L separation (Fig. 3). When the stillage slurry was used, xylonic acid productivity by the whole cell catalysis maintained a high level at 2.31 g/(L h) while the final xylonic acid titer was almost the same. The stillage slurry contained high content water insoluble solid (WIS), which could reduce the oxygen transfer rate in this cell biocatalysis reaction for xylonic acid production. The stillage liquid was the liquid part of the stillage slurry after the WIS was removed out by solid/liquid separation, which was more beneficial to oxygen transfer in biocatalysis reaction. The result indicates that the oxygen transfer required by xylose oxidation was not significantly affected by the existence of high water insoluble solids (WIS) in the stillage slurry. 63.25 g/L of xylose was consumed completely within 32 h and 66.42 g/L of xylonic acid was obtained with 97.55% of the overall yield. The initial whole cell concentration was same with that in the stillage liquid catalysis, but the time course of cell mass was not recorded because of the high solids in the stillage slurry.

3.3. Modeling of integrated ethanol and xylonic acid production from corn stover on Aspen Plus platform

In this study, a new process for xylonic acid production from xylose in the distillation stillage of cellulosic ethanol fermentation broth was designed starting from dry dilute acid pretreatment and biodetoxification (Process One). In an earlier study, Zhu et al. (2015) proposed a xylonic acid process starting from xylose in the pretreatment liquid starting from steam explosion pretreatment (Process Two). The two processes were compared by simulating the process flowsheet on Aspen Plus platform (Fig. 4). The same processing parameters and conversion yields were applied for the accurate comparison of process design concept (Table 1).

(1) Process One simulation

Corn stover was dry dilute acid pretreated in the pretreatment reactor R101 (Area 1); the pretreated corn stover was milled and neutralized by 20% (w/w) $Ca(OH)_2$ solution (M201), and then was fed into the detoxification reactor R201 to remove inhibitors (Area 2); the detoxified corn stover was fed into the pre-hydrolysis reactor R301 together with cellulase, then to the SSF reactor R302 for simultaneous saccharification and fermentation (SSF) into ethanol (Area 3). The fermentation broth was distillated into 40% (v/v) ethanol stream and the stillage slurry retained (Area 5). The stillage slurry stream was feed into the fermentation reactor R401 for production of xylonic acid from xylose by *Gluconobacter oxydans* DSM 2003 (Area 4). Xylonic acid product was recovered in Area 6 in the form of sodium xylonate.

(2) Process Two simulation

Corn stover was steam explosion pretreated in the pretreatment reactor R101 (Area 1) and the pretreatment liquid was obtained by solid/liquid separation SEP at the solid-to-liquid ratio of 1:15 (Area 2); the solid corn stover was fed into the prehydrolysis reactor R301 for hydrolysis and the SSF reactor R302 for ethanol fermentation (Area 3). Same ethanol recovery flowsheet was followed as in Process One (Area 5). The pretreatment liquid was concentrated by evaporation to 55 g/L of xylose (Area 7) and then sent to the fermentation reactor R401 for production of xylonic acid from xylose by *Gluconobacter oxydans* DSM 2003 (Area 4). Same xylonic acid recovery process was followed as in Process One (Area 6).

Table 1
Main process input data for the established Aspen Plus simulation model.

Features	Values
Pretreatment	
Sulfuric acid usage (g/kg dry feedstock)	25
Residence time (min)	5
Temperature (°C)	175
Pressure (MPa)	0.89
Solids content of pretreated corn stover (%)	50
Glucan conversion to glucose (%)	4
Hemicellulose conversion to xylose (%)	40
Xylan conversion to furfural (%)	3.3
Acetyl group conversion to acetic acid (%)	60
Biodetoxification	
Temperature (°C)	28
Residence time (h)	36
Furfural conversion (%)	100
Acetic acid conversion (%)	70
Glucose consumed for cell growth (%)	5
Xylose consumed for cell growth (%)	90
H_2SO_4 neutralized (%)	100
Saccharification and ethanol fermentation	
Temperature for pre-hydrolysis (°C)	50
Temperature for fermentation (°C)	37
Residence time for pre-hydrolysis (h)	12
Residence time for fermentation (h)	84
Solids loading (%)	30
Cellulase dosage (mg protein/g cellulose)	28
Glucan conversion to glucose (%)	87
Xylan conversion to xylose (%)	82
Ethanol yield from glucose (%)	93
Glycerol yield from glucose (%)	1
Glucose consumed for cell growth (%)	2
Ethanol concentration (g/L)	60
Xylonic acid fermentation	
Temperature for fermentation (°C)	30
Residence time for fermentation (h)	32
Sodium xylonate concentration (g/L)	75
Sodium xylonate yield from xylose (%)	98
Product recovery	
Purity (ethanol)	99% (w/w)
Purity (sodium xylonate)	98% (w/w)

(3) Mass and energy balances

The detailed mass balance calculations of the two processes are shown in Fig. 5 at the plant size of 300,000 tons processing capacity of dry corn stover annually with an annual operation time of 8000 h. Similar ethanol and xylonic acid products are obtained in the two processes: 142 kg of ethanol and 151 kg of sodium xylonate from one ton of dry corn stover are produced in Process One, while 131 kg of ethanol and 142 kg of sodium xylonate are produced in Process Two.

The major differences between the two processes are on the wastewater generation and energy consumption (Table 2). In Process One, 9.13 tons of wastewater is generated for producing one ton of sodium xylonate. In Process Two, 16.41 tons of wastewater is generated for producing one ton of sodium xylonate, approximately 80% more wastewater than that of Process One. For heating steam usage, 34.758 GJ of steam is consumed for producing one ton of sodium xylonate in Process One, mainly in the areas of pretreatment, ethanol distillation, and the sodium xylonate concentrating step. In Process Two, 239.989 GJ is consumed for producing one ton of sodium xylonate, approximately eight folds greater than that of Process One. Among the huge steam energy consumption in Process Two, nearly 90% is on the concentrating step of low xylose containing pretreatment liquid (less than 10 g/L) to the meaningful value for xylonic acid fermentation (55 g/L).

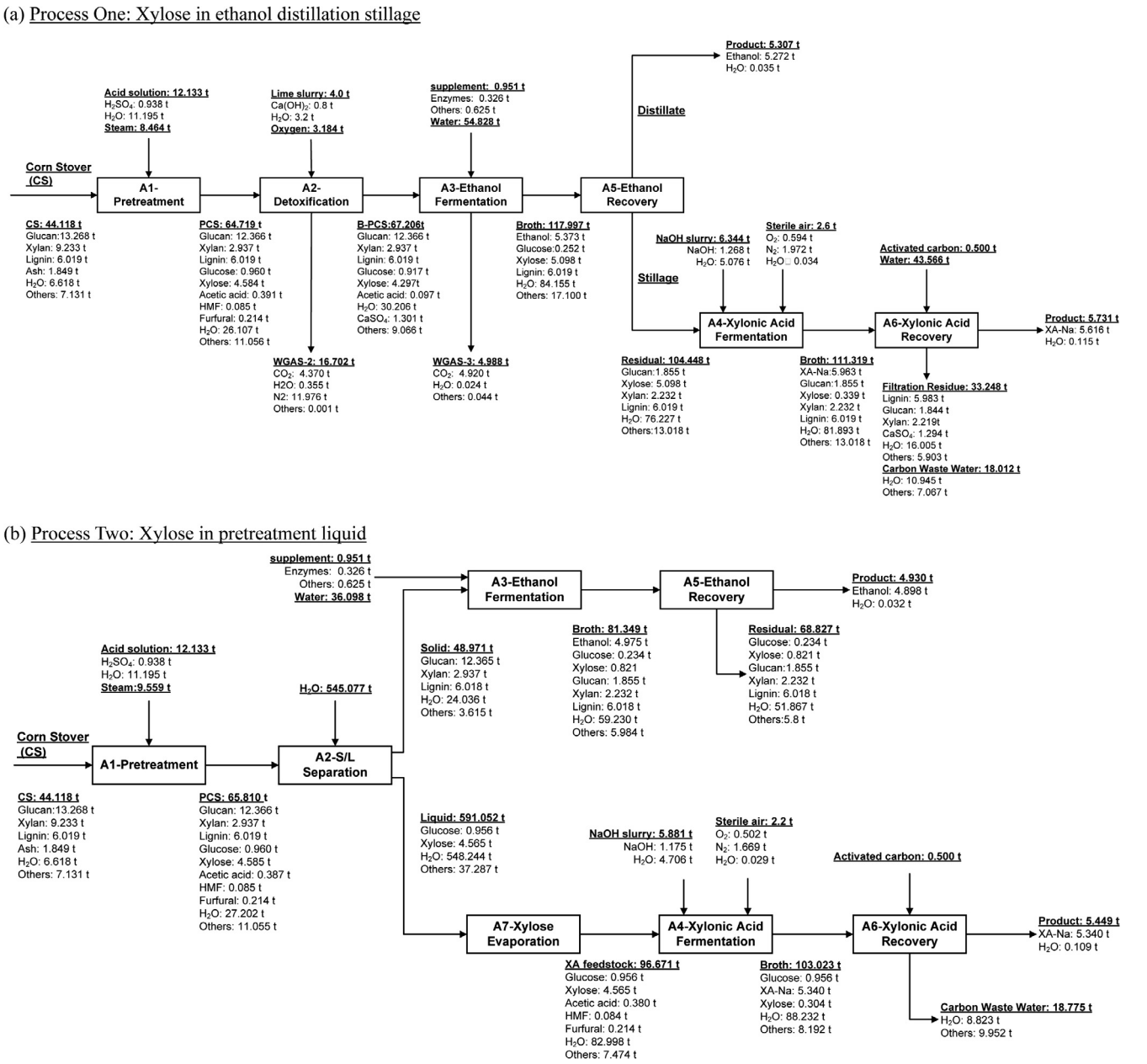


Fig. 5. Mass balance of xylonic acid production processes on Aspen Plus platform. (a) Process One using xylose in cellulosic ethanol distillation stillage (this study); (b) Process Two using xylose from pretreatment liquid (Zhu et al., 2015). Abbreviations: ETOH, ethanol; XA, xylonic acid; SEP, solid/liquid separation; AC, active carbon; WGAS, waste gas. Process One, the major process input data are listed in Table 1, the recovery yield of ethanol is 98% (Zhang et al., 2013) and the recovery yield of sodium xylonate in the product recovery area is assumed to be the same with the sodium gluconate recovery from corn stover, 92% (Zhang et al., 2016). The recovery yield of active carbon is assumed as 90%, the other 10% is discharged as wastewater. Process Two, the titers of ethanol and sodium xylonate are based on Zhu et al. (2015), 73 g/L and 60 g/L, respectively. The recovery yield was assumed as same as Process One. Abbreviations: PCS, pretreated corn stover; B-PCS, biodetoxified and pretreated corn stover; XA-NA, sodium xylonate.

Table 2
Wastewater generation and steam energy consumption per ton of sodium xylonate production calculated by Aspen Plus modeling.

Contents	Areas	process one	Process two
Wastewater (ton/ton sodium xylonate)	Xylonic acid recovery	5.921	0
	Ethanol distillation	0	12.890
	Decoloration	3.207	3.517
	Total	9.117	16.406
Steam energy consumption (MJ/ton sodium xylonate)	Pretreatment	5671	6736
	Ethanol distillation	10,202	8432
	Ethanol Rectification	1171	1259
	Triple effect evaporation	17,714	15,218
	Prehydrolysate evaporation	0	208,343
	Total	34,758	239,989

(4) Process evaluation

Although the similar product yields are obtained in the two processes, Process One generates only 55.64% of wastewater generation and consumes 14.48% of heating energy of Process Two. In Process One, xylose from hemicellulose hydrolysis in pretreatment is moved to the consequent biodetoxification, saccharification and ethanol fermentation together with cellulose solids. Finally the xylose is recovered in the distillation stillage of the cellulosic ethanol fermentation broth, then used for xylonic acid fermentation. In Process Two, xylose from hemicellulose hydrolysis in pretreatment is separated as a liquid stream from cellulose solids immediately after pretreatment. Xylose containing pretreatment liquid is then sent for concentrating step, which is energy intensive. Then the concentrated xylose containing liquid is fermented into xylonic acid, while the cellulose solids are sent for hydrolysis and ethanol fermentation. The rigorous evaluation of the two processes based on Aspen Plus modeling suggest that Process One using xylose from distillation stillage of cellulosic ethanol fermentation broth is similar in ethanol and xylonic acid with Process Two using xylose from pretreatment liquid, but the energy consumption and wastewater generation are significantly reduced and leads to a practical process design and application.

4. Conclusion

A new xylonic acid production process using xylose in cellulosic ethanol distillation stillage was designed and investigated. 59.80 g/L of ethanol was obtained in the simultaneous saccharification and ethanol fermentation step, then 66.42 g/L of xylonic acid was obtained from xylose in distillation stillage of ethanol fermentation broth. Aspen Plus modeling shows that the current process is similar in ethanol and xylonic acid with the process using xylose from pretreatment liquid, but the energy consumption and wastewater generation are significantly reduced and leads to a practical process design and application.

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