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Fermentative production of high titer gluconic and xylonic acids from corn stover feedstock by *Gluconobacter oxydans* and techno-economic analysis

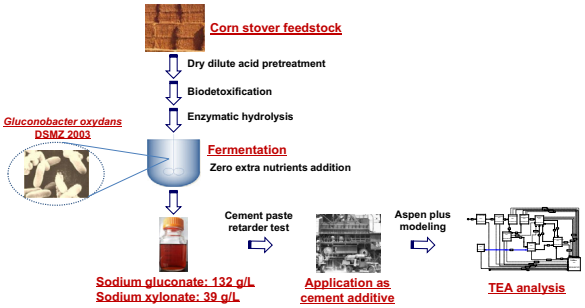
Hongsen Zhang [1], Gang Liu [1], Jian Zhang, Jie Bao *

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

HIGHLIGHTS

- Dilute acid pretreated and biodetoxified corn stover is used for this fermentation.
- Glucose and xylose are simultaneously converted by *Gluconobacter oxydans* DSM 2003.
- Highest 132.46 g/L of sodium gluconate and 38.86 g/L sodium xylonate were obtained.
- Cellulosic sodium gluconate and sodium xylonate are both used as cement additive.
- TEA based on Aspen plus is performed for the sodium gluconate/xylonate product.

GRAPHICAL ABSTRACT



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ABSTRACT

High titer gluconic acid and xylonic acid were simultaneously fermented by *Gluconobacter oxydans* DSM 2003 using corn stover feedstock after dry dilute sulfuric acid pretreatment, biodetoxification and high solids content hydrolysis. Maximum sodium gluconate and xylonate were produced at the titer of 132.46 g/L and 38.86 g/L with the overall yield of 97.12% from glucose and 90.02% from xylose, respectively. The drawbacks of filamentous fungus *Aspergillus niger* including weak inhibitor tolerance, large pellet formation and no xylose utilization were solved by using the bacterium strain *G. oxydans*. The obtained sodium gluconate/xylonate product was highly competitive as cement retarder additive to the commercial product from corn feedstock. The techno-economic analysis (TEA) based on the Aspen Plus modeling was performed and the minimum sodium gluconate/xylonate product selling price (MGSP) was calculated as $0.404/kg. This study provided a practical and economic competitive process of lignocellulose utilization for production of value-added biobased chemicals.

1. Introduction

Sodium gluconate is the major cement additive used for delaying the setting time ("retarding") of cement paste during cement casting in construction industry (Ma et al., 2015). Sodium xylonate is also a cement additive used for better fluidity by deflocculating cement granules (Chun et al., 2006). In 2014, the world cement

* Corresponding author.
E-mail address: jbao@ecust.edu.cn (J. Bao).
[1] These authors are equally contributed to this work. HSZ conducted the fermentation experiment and cement additive evaluation; GL conducted the Aspen Plus modeling and TEA calculation.

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production is estimated to be 4 billion tons and 70–80% of the cement is used in the large building casting with the requirement of retarder additive addition such as sodium gluconate (Li, 2011; Li et al., 2015). The requirement of sodium gluconate as cement retarder is approximate 1 million tons even at the minimum addition of 0.03% (w/w) (Ma et al., 2015). The future market expansion of sodium gluconate in accordance to the booming infrastructure construction in developing countries requires the alternative feedstock to substitute traditional starch and sucrose as fermentation feedstock for sodium gluconate production.

Among many feedstock options, lignocellulose biomass is the most abundant carbohydrate feedstock with positive impact on environment and climate change. In our previous study, the filamentous fungus *Aspergillus niger* SIIM M276 was used as the fermenting strain of gluconic acid from corn stover feedstock and the sodium gluconate product was successfully applied as cement retarder additive for extending the setting time of cement paste (Zhang et al., 2016). However, several disadvantages on using *A. niger* were observed including sensitive to inhibitor compounds in lignocellulosic hydrolysates, tended to the quick formation of larger fungus pellets thus oxygen transfer to the cell mycelia was reduced, and unable to utilize xylose. These drawbacks lead to the low productivity, difficulties in the multiple seed culture propagation in commercial scale application, and considerable xylose loss.

To overcome the problems on using filamentous fungus strain, a gram-negative bacterium strain *Gluconobacter oxydans* comes into the consideration for its rapid oxidation of monosaccharide into acids and ketones (Adachi et al., 1980; Matsushita et al., 1989; Silberbach et al., 2003; Elfari et al., 2005). A membrane protein of *G. oxydans*, glucose dehydrogenase, oxidizes glucose into gluconic acid and xylose into xylonic acid as well as other hexose and pentose sugars into the corresponding acids. The complete cell membrane transfer of oxygen, glucose, xylose and products across the cell membrane is partially lessened because of the location of glucose dehydrogenase on the cell membrane, instead of the intracellular location (Merfort et al., 2006; Deppenmeier et al., 2002; Toivari et al., 2012; Wei et al., 2014). In this study, we report a comprehensive and simultaneous fermentation of glucose and xylose into gluconic and xylonic acids by *G. oxydans* DSM 2003 using corn stover feedstock after it was pretreated by dry dilute sulfuric acid method (Zhang et al., 2011; He et al., 2014), biodetoxified (Zhang et al., 2010a; He et al., 2016), and enzymatically hydrolyzed into fermentable sugars. The obtained sodium gluconate and xylonate products were tested as cement retarder for extending setting time of cement paste and showed a better performance than commercial sodium gluconate from corn starch feedstock. The detailed techno-economic analysis (TEA) was performed based on the Aspen Plus modeling, minimum sodium gluconate/xylonate product selling price (MGSP) in this case was obtained, it will provide economic theoretical basis for practical industrial cellulosic sodium gluconate/xylonate production.

2. Materials and methods

2.1. Raw materials

Corn stover (CS) was harvested from Nanyang, Henan, China in fall, 2014. The collected raw corn stover materials were chopped to small chippings coarsely and washed to remove solids dirt, stones and metals, then dried in oven until constant weight. The dried corn stover materials were milled by hammer crusher with 10 mm circle diameter mesh. The composition of corn stover after pre-handling treatment contained 35.78% of cellulose, 19.36% of hemicellulose, 28.36% of lignin, 3.56% of ash on dry weight base (w/w) determined by Cellulose Analyzer (Ankom 220, Ankom Technology, Macedon, NY, USA).

2.2. Enzymes and reagents

Commercial cellulase enzyme Youtell #7 was purchased from Hunan Youtell Biochemical Co., Yueyang, Hunan, China. The filter paper activity was 63.0 FPU/g cellulase according to the National Renewable Energy Laboratory (NREL) protocol LAP-006 (Adney and Baker, 1996), the cellobiase activity was 102.0 IU/g cellulase determined using the method of Ghose (1987), and the protein concentration was 49.5 mg/g cellulase detected by Bradford method using BSA as protein standard.

Chromatographic grade sodium gluconate was purchased as the standard reference chemical from Sigma-Aldrich, St. Louis, MO, USA. The sodium gluconate used as cement additive was from Shandong Xiwang Group, Zouping, Shandong, China. Calcium xylonate hydrate was from Santa Cruz Biotech., Dallas, Texas, USA. Glucose, sorbitol, 4-hydroxybenzaldehyde (HBA) and syringaldehyde were from Sangon Biotech., Shanghai, China. Furfural, 5-hydroxymethylfurfural (HMF) were purchased from J&K Scientific Co., Beijing, China. Xylose and acetic acid were from Sinopharm Chemical Reagent Co., Shanghai, China. Vanillin was purchased from Aladdin Reagents Co., Shanghai, China. The standard cement was purchased from Shandong cement Co., Shandong, China. Polycarboxylate QS8020 was from Qishuo Industry Co., Shanghai, China. The other reagents were all from Lingfeng Chemical Reagent Co., Shanghai, China.

2.3. Strains and media

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

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., 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 peeled and sliced potatoes in one liter deionized water for 30 min.

2.4. Pretreatment, biodetoxification and hydrolysate preparation

Dry dilute acid pretreatment (DDAP) method was used for pretreating the corn stover feedstock in this study (Zhang et al., 2011; He et al., 2014). Briefly, 1200 g of dried corn stove and 600 g of 5% (w/w) dilute sulfuric acid solution was co-currently fed into the 20 L pretreatment reactor under the helical agitation of the single helical impeller in the reactor. The sulfuric acid dosage was 2.5 g per 100 g of dry corn stover feedstock. The pretreatment temperature was remained at 175 ± 1 °C for 5 min under helically agitation. The pretreated corn stover contained approximately 50% (w/w) of dry solid matter (DM) and no free wastewater stream was gener-

ated. The pretreated corn stover solids (excluding water) contained 37.24% of cellulose, 8.21% of hemicellulose, 5.86% of ash determined by two-step acid hydrolysis method described in NREL protocols (Sluiter et al., 2008, 2012). The inhibitors per gram of dry pretreated corn stover (DM) include 5.64 mg of furfural, 3.54 mg of HMF, 18.43 mg of acetic acid, 0.23 mg of vanillin, 0.67 mg of syringaldehyde and 0.18 mg of HBA.

Biodetoxification of the pretreated corn stover materials was carried out in a 15 L bioreactor at 28 °C and 1 vvm of aeration for 36 h (He et al., 2016). Briefly, *A. resinae* ZN1 seeds were cultured on the pretreated corn stover materials at 28 °C for 5 days by inoculation of spores from PDA slant. Then the seed solids were inoculated at 10% (w/w) inoculation ratio onto the freshly pretreated corn stover for biodetoxification. The corn stover was briefly mixed for approximately 1 min every 6–8 h. The detoxified corn stover was disk milled before use.

Hydrolysis of the pretreated corn stover was carried out in the bioreactor equipped with helical ribbon impeller for mixing (Zhang et al., 2010b). Both the freshly pretreated corn stover and the biodetoxified and pretreated corn stover were hydrolyzed at 50 °C, pH 4.8 for 48 h at the cellulase dosage of 15 FPU/g DM. The hydrolysate slurry was centrifuged to remove the solids, then autoclaved and filtered by filter paper before use.

2.5. Gluconic and xylonic acids fermentation

G. oxydans DSM 2003 was maintained at −80 °C freezer in vials containing 30% (v/v) glycerol solution. One vial (2 mL) was inoculated into 20 mL of seed medium in 100 mL flask and cultured at 30 °C, 220 rpm for 24 h. For flask fermentation, the seed broth was inoculated at 10% volume ratio into 50 mL of fermentation medium in a 250 mL flask and fermented at 30 °C, 220 rpm for 24–72 h. For fermentor fermentation, the seed broth was inoculated at 10% (v/v) inoculation ratio into 3 L fermentor containing 900 mL corn stover hydrolysate and fermented at 30 °C, 500 rpm, 2.5 vvm of aeration for 24–72 h, where 5 M NaOH and 2 M H_2SO_4 were used for pH control. All the experiments were conducted in duplicate.

Sodium xylonate used for cement additive assay was prepared by fermenting xylose in the bottom liquid of the distillation from ethanol fermentation broth, in which only glucose was utilized and xylose was left in the broth (Zhang et al., 2010b). Xylose in the bottom liquid was converted into xylonic acid by *G. oxydans* DSM 2003 and neutralized into sodium xylonate. No sodium gluconate was detected in the sodium xylonate broth.

2.6. Analysis of sugars, acids and inhibitors

Samples were periodically taken, centrifuged and filtrated through 0.22 μm filters before analysis. Gluconic acid, xylonic acid and keto-gluconic acid (KGA) were analyzed using HPLC (LC-20AT, UV/VIS detector SPD-20A, Shimadzu, Kyoto, Japan) with an Aminex HPX-87H column (Bio-rad, Hercules, CA, USA) at 55 °C using the mobile phase of 5 mM H_2SO_4 at the flow rate of 0.4 mL/min. The detection wavelength was 210 nm.

Glucose was measured by a biosensor (SBA-40D, Shandong Academy of Agriculture, Jinan, China). Xylose was measured by HPLC (LC-20 AD, refractive index detector RID-10A, Shimadzu, Kyoto, Japan) with Aminex HPX-87H column at 65 °C using the mobile phase of 12 mM $NaHCO_3$ at the flow rate of 0.6 mL/min.

Furfural, acetic acid and HMF were measured by HPLC (LC-20 AD, refractive index detector RID-10A, Shimadzu, Kyoto, Japan) with Aminex HPX-87H column at 65 °C using the mobile phase of 5 mM H_2SO_4 at the flow rate of 0.6 mL/min. Vanillin, syringaldehyde and 4-hydroxybenzaldehyde (HBA) were analyzed using HPLC (LC-20AT, UV/VIS detector SPD-20A, Shimadzu, Kyoto, Japan) with YMC-Pack ODS-A column (YMC, Tokyo, Japan) at 35 °C and 270 nm at the flow rate of 1.0 mL/min. Two mobile phase solutions were 0.1% (v/v) formic acid and acetonitrile. The acetonitrile concentration in the mobile phase was adjusted in the following procedure: 10% in the first 4 min; increased from 10% to 35% from 4 to 5 min, and maintained at 35% from 6 to 15 min; reduced from 35% to 10% from 15 to 21 min, and maintained at 10% from 21 to 30 min (Khoddami et al., 2013).

2.7. Yield calculation of gluconic and xylonic acids

The gluconic acid yield is defined as the ratio of the glucose converted into the gluconic acid according to the stoichiometric equation:

$$\text{Yield } (\%) = \frac{[GA] \times V - [GA]_0 \times V_0}{[Glu]_0 \times V_0 \times 1.089} \times 100\%$$

where $[Glu]_0$, the initial glucose concentration (g/L); $[GA]_0$ and $[GA]$, the initial and final gluconic acid concentrations (g/L); 1.089 is the conversion factor for glucose to equivalent gluconic acid; V and V_0, the initial and final volumes of fermentation broth (L).

The xylonic acid yield is defined as the ratio of the xylose converted into the xylonic acid according to the stoichiometric equation:

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

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; V and V_0, the initial and final volumes of fermentation broth (L).

2.8. Assay of setting time, fluidity and strength of cement paste

The fermentation broth of gluconic and xylonic acids was purified by filtration and decoloration to remove the solids and dark colors. The obtained solution contained 132.46 g/L of sodium gluconate and 15.90 g/L sodium xylonate. The dosage of cellulosic sodium gluconate addition was based on the weight of the actual sodium gluconate and xylonate in the solution. Xylonic acid fermentation broth was prepared by fermenting xylose in the bottom liquid of the distillation from ethanol fermentation broth, was also purified by filtration and decoloration to remove the solids and dark colors. The obtained solution contained 80.50 g/L sodium xylonate. The dosage of cellulosic sodium xylonate addition was based on the weight of the actual sodium xylonate in the solution. The purity of the commercial sodium gluconate was 98% (w/w) and the dosage of commercial sodium gluconate addition was based on the weight.

The consistency of the cement paste was measured by Vicat apparatus (Luda Construction Co., Shanghai, China) and adjusted by water addition into the standard range. The setting time of the cement paste was also determined by Vicat apparatus according to Chinese Standard Protocol GB/T 1346-2011. Briefly, 500 g of the standard cement was mixed by required amount of water into cement paste mixer (NJ-160, Wuxi Construction Co., Jiangsu, China). The cement paste was mixed by the rotation at 140 rpm and the revolution at 62 rpm for 120 s, then stop for 15 s; again mixed at the rotation at 285 rpm and the revolution at 125 rpm for 120 s.

The fluidity of cement paste was determined according to the Chinese Standard Protocol GB/T 2419-2005. Briefly, 300 g of standard cement was mixed with 87 mL of water and 0.18 g polycarboxylate as water reducer into cement paste mixer (NJ-160, Wuxi construction Co., Jiangsu, China) and mixed at the rotation

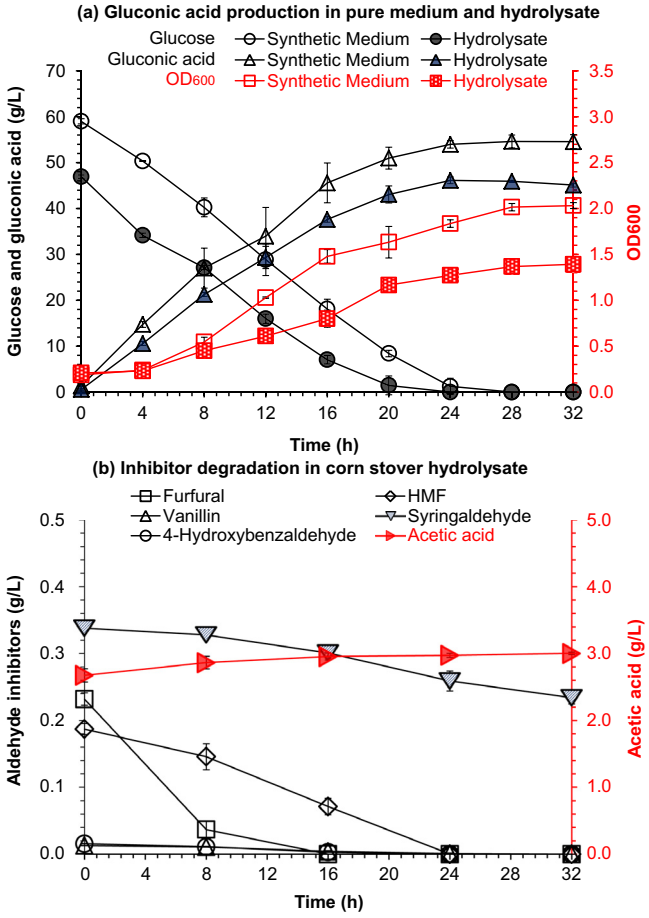


Fig. 1. Gluconic acid fermentation of *G. oxydans* DSM 2003 in flasks using synthetic medium and inhibitors containing corn stover hydrolysate. (a) Conversions; (b) Inhibitors degradation. Hydrolysate was prepared from freshly pretreated corn stover without detoxification. The fermentation was carried out at 30 °C, pH 5.5, 220 rpm and the inoculum size 10%.

at 140 rpm and the revolution at 62 rpm for 180 s. The cement paste was fully filled into a standard copper conical cylinder (60 mm in height, 50 mm in top circle diameter, and 75 mm in bottom circle diameter), then the cylinder was lifted up promptly to let the cement paste flowing on the glass plate after 30 s. The circle diameter of the cement paste slurry on the glass plate was measured as the indicator of the fluidity of cement paste (mm).

The flexural and compressive strength of the cement mortars were measured according to Chinese Standards Protocol GB/T17671-1999. Briefly, the cement paste was prepared with standard sand according to Chinese Standard GB/T17671-1999 for preparing the standard specimens (40 × 40 × 160 mm) with the weight ratio of cement, sand and water at 2:6:1. The flexural strength was carried out on the long surface of the cement mortar specimen using a cement bending tester (300KN, Jinan Kaine Testing Mechanics Co., Shandong, China) and three specimens were tested for each sample. The compressive tests were carried out on the cement pressure tester (DKZ-5000, Jinan Kaine Testing Mechanics Co., Shandong, China) and six specimens were tested for one sample to get the average value.

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

The process model was developed using Aspen Plus software (AspenTech Co., Cambridge, MA, USA). The basic model was

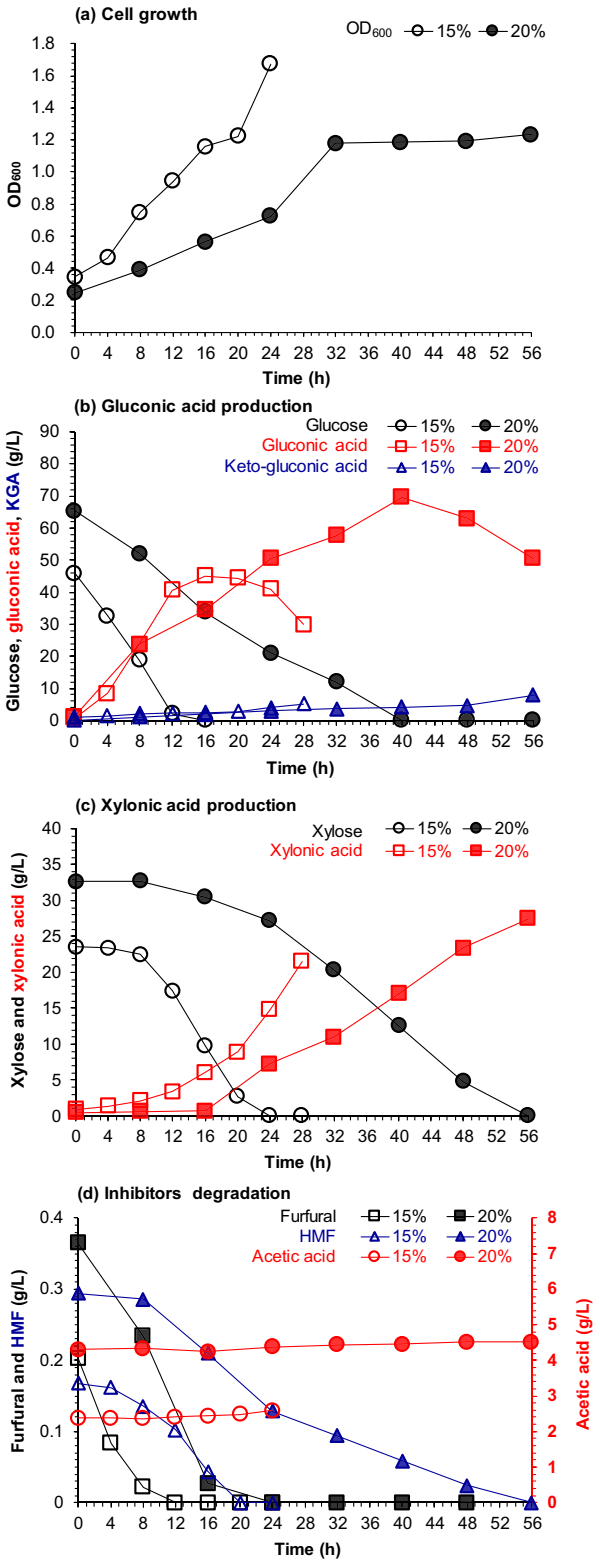


Fig. 2. Gluconic and xylonic acids fermentation of *G. oxydans* DSM 2003 in fermentors using inhibitor containing corn stover hydrolysates. (a) Cell growth; (b) Gluconic acid generation; (c) Xylonic acid generation; (d) Inhibitors degradation. The fermentation was carried out at 30 °C, pH 5.5, 500 rpm, 2.5 vvm, inoculum size 10%. Corn stover hydrolysate contained: (1) 15% solids loading, 54.49 g/L of glucose, 23.53 g/L of xylose, 3.24 g/L of acetic acid, 0.20 g/L of HMF, 0.31 g/L of furfural; (2) 20% solids loading, 72.20 g/L of glucose, 37.60 g/L of xylose, 4.28 g/L of acetic acid, 0.48 g/L of HMF, 0.51 g/L of furfural.

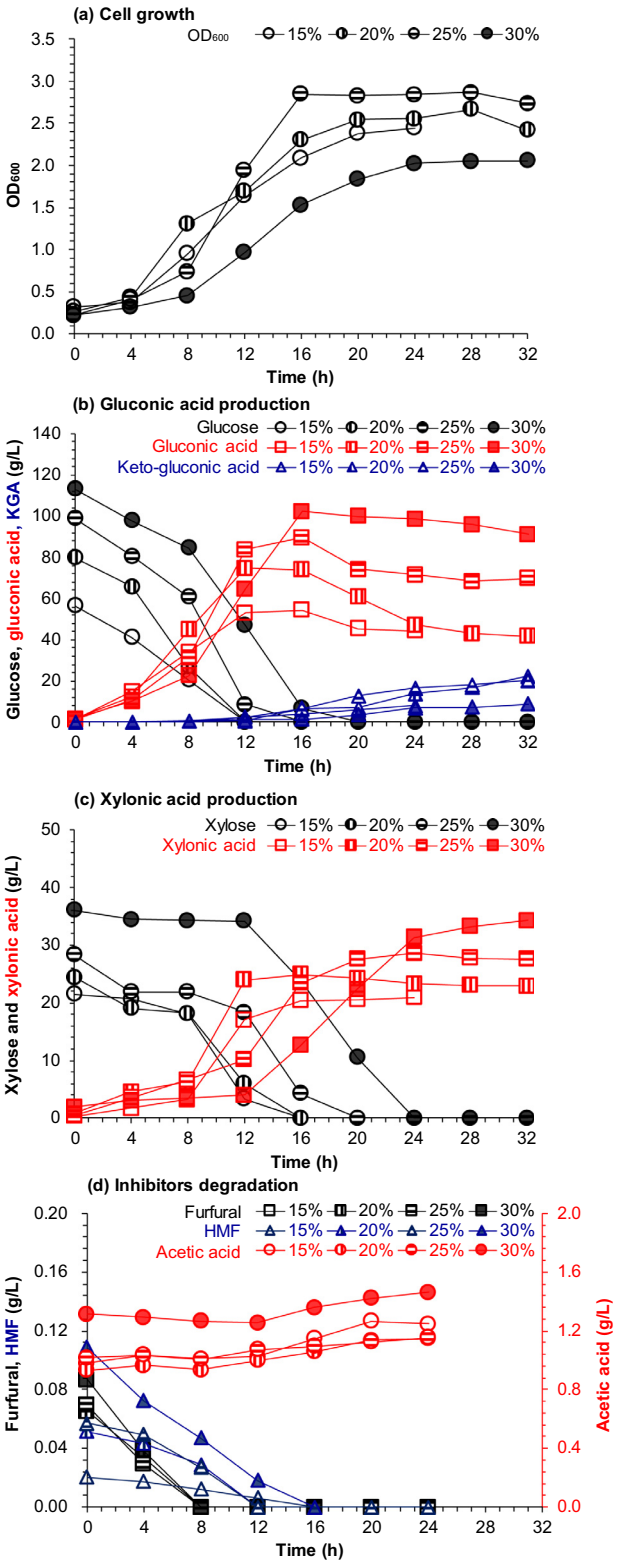


Fig. 3. Gluconic and xylonic acids fermentation of *G. oxydans* DSM 2003 in fermentors using biodetoxified corn stover hydrolysates. (a) Cell growth; (b) Gluconic acid generation; (c) Xylonic acid generation; (d) Inhibitors degradation. Conditions: 30 °C, pH 5.5, 500 rpm, 2.5 vvm, the inoculum size 10%, 1 L liquid in 3 L fermentor. Corn stover hydrolysates: (1) 15% solids, 62.72 g/L of glucose, 23.88 g/L of xylose, 1.09 g/L of acetic acid, 0.02 g/L of HMF, 0.07 g/L of furfural; (2) 20% solids, 88.47 g/L of glucose, 27.13 g/L of xylose, 1.03 g/L of acetic acid, 0.06 g/L of HMF, 0.07 g/L of furfural; (3) 25% solids, 109.78 g/L of glucose, 31.46 g/L of xylose, 1.13 g/L of acetic acid, 0.06 g/L of HMF, 0.08 g/L of furfural; (4) 30% solids, 124.80 g/L of glucose, 40.03 g/L of xylose, 1.46 g/L of acetic acid, 0.12 g/L of HMF, 0.10 g/L of furfural.

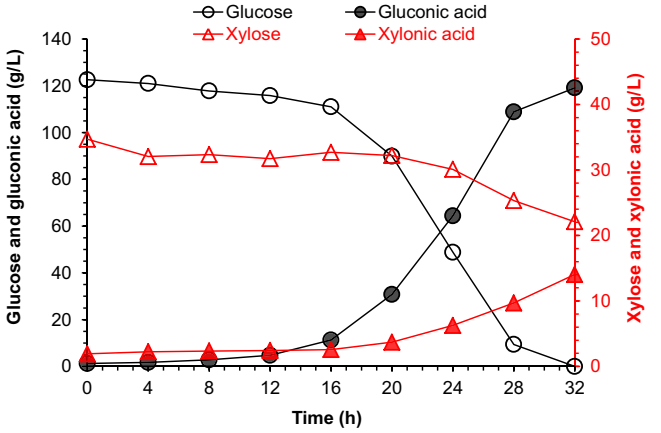


Fig. 4. Large scale sodium gluconate fermentation in 50 L fermentor in corn stover hydrolysate from 30% (w/w) pretreated and biodetoxified corn stover. The fermentation was carried out at 30 °C, pH 5.5, 300 rpm, 1.5 vvm, inoculum size 1%, liquid volume 35 L in 50 L fermentor. Corn stover hydrolysate contained 124.80 g/L of glucose, 40.03 g/L of xylose, 1.46 g/L of acetic acid, 0.12 g/L of HMF, 0.10 g/L of furfural. 119.10 g/L gluconic acid and 14.04 g/L xylonic acid were obtained at 32 h, equivalent to 132.46 g/L of sodium gluconate and 15.90 g/L of sodium xylonate, respectively.

applied the NREL design report (Humbird et al., 2011) with the changes in four areas: (1) The pretreatment area was changed from the conventional dilute acid pretreatment in the NREL report into the dry dilute acid pretreatment (DDAP) (Zhang et al., 2011); (2) the detoxification area was changed from ammonia overliming into the biodetoxification (He et al., 2016); (3) the saccharification and fermentation area was changed from 20% (w/w) solids loading into 30% (w/w) solids loading; (4) the product recovery area was changed from ethanol into sodium gluconate and xylonate. The plant size is 900 tons processing capacity of corn stover each day (300,000 tons annually) with an annual operation of 8000 h.

The detailed Aspen plus flowsheet contains ten process areas (Supplemental materials Fig. S1): feedstock handing, pretreatment, biodetoxification, enzymatic hydrolysis and fermentation, cellulase production, product recovery, wastewater treatment, residue combustion, storage, and utilities system. Area 200 is the dry dilute acid pretreatment (DDAP) unit, in which the feedstock is treated by dilute acid at the corn stover solids of 66.7% (w/w). Area 300 is the biodetoxification using *A. resinae* ZN1 to remove most of the inhibitors in 36 h. Area 400 is the enzymatic hydrolysis and fermentation, in which the pretreated and biodetoxified corn stover is hydrolyzed and fermented to sodium gluconate and xylonate by *Gluconobacter oxydans* DSM 2003 at 30% (w/w) solids loading. Area 500 is the product recovery of sodium gluconate and xylonate to meet the technical grade of 98% (w/w) by solid/liquid separation, decoloration and multiple evaporation steps. Others areas in the Aspen plus flowsheet are maintained the same with the NREL model.

The material and energy balance data from Aspen plus modeling are used to design the equipment and determine the chemical usage. The year of 2013 is used as the reference year. The exchange rate from US dollar ($) to Chinese Yuan (CNY) is 1: 6.2. The general purpose equipment of pumps, conveyors and evaporators are quoted from the NREL price (Humbird et al., 2011). The specific equipment of the pretreatment reactors, fermentors and helical agitators, chemicals, and staff wages are modified according to actual situation in China. The raw material composition, main equipment and chemical used in the process and their prices are shown in Supplemental materials Tables S1, S2 and S3. Note that the price of net electricity to the grid is 0.75 CNY/kWh based on the government regulation for renewable electricity pricing ("Reg-

Table 1
Assay of sodium gluconate and xylonate as cement retarder additives.

Properties		Addition (%, w/w)	Commercial sodium gluconate	Cellulosic sodium gluconate	Cellulosic sodium xylonate
Setting Time (min)	Initial	0	163 ± 4	163 ± 4	163 ± 4
		0.01	185 ± 0	186 ± 6	165 ± 3
		0.02	223 ± 4	239 ± 5	170 ± 0
		0.03	308 ± 4	325 ± 7	230 ± 4
		0.06	>390	>390	225 ± 4
		0.1	>390	>390	ND
	Final	0	220 ± 0	220 ± 0	220 ± 0
		0.01	248 ± 4	253 ± 4	215 ± 4
		0.02	288 ± 4	303 ± 4	225 ± 4
		0.03	355 ± 7	380 ± 7	280 ± 7
		0.06	>390	>390	275 ± 4
		0.1	>390	>390	ND
Fluidity (mm)		0	243 ± 1	243 ± 1	243 ± 1
		0.01	271 ± 2	276 ± 4	246 ± 3
		0.02	281 ± 1	282 ± 4	244 ± 4
		0.03	289 ± 1	286 ± 1	246 ± 1
		0.06	253 ± 4	240 ± 6	240 ± 7
		0.1	198 ± 4	153 ± 4	ND
Flexural strength (MPa)	3 days	0.01	5.4 ± 0.3	5.3 ± 0.3	ND
		0.02	6.1 ± 0.4	5.6 ± 0.4	ND
		0.03	5.5 ± 0.5	5.7 ± 0.3	ND
	28 days	0.01	9.1 ± 0.4	8.8 ± 0.2	ND
		0.02	8.6 ± 0.3	8.6 ± 0.3	ND
		0.03	8.8 ± 0.2	8.7 ± 0.2	ND
Compressive strength (MPa)	3 days	0.01	28.5 ± 0.8	26.4 ± 0.6	ND
		0.02	28.3 ± 0.8	26.1 ± 0.6	ND
		0.03	26.6 ± 0.6	26.9 ± 0.9	ND
	28 days	0.01	53.8 ± 1.1	50.3 ± 0.8	ND
		0.02	52.4 ± 0.8	51.0 ± 0.6	ND
		0.03	50.2 ± 1.0	50.9 ± 0.5	ND

Fluidity and setting time assays were conducted according to Chinese Standard Protocol GB/T 1346-2011 and GB/T 2419-2005, respectively. Strength assay was conducted according to Chinese Standard Protocol GB/T 17671-1999. Cellulosic sodium gluconate fermentation broth contained 132.46 g/L of sodium gluconate and 15.90 g/L of sodium xylonate. Cellulosic sodium xylonate fermentation broth contained 80.50 g/L sodium xylonate. Polycarboxylate was supplemented into the cement paste at the dosage of 0.06% (w/w) when the fluidity was tested as water reducer. The final setting time over 390 min was not counted according to Chinese Standard Protocol GB/T 2419-2005. ND demonstrated not detected.

ulations on Electricity Prices on Renewable Energy Use", the National Development and Reform Commission of China, http://www.gov.cn/ztzl/2006-01/20/content_165910.htm).

A discounted cash flow rate of return to determine the minimum sodium gluconate/xylonate product selling price (MGSP, $/kg) requires a net present value of zero for 8% internal rate of return after taxes. Table S4 shows the assumptive parameters in the discounted cash flow analysis.

3. Results and discussion

3.1. Gluconic and xylonic acids fermentation by G. oxydans cells using corn stover feedstock

Gluconic acid fermentability of *G. oxydans* DSM 2003 was evaluated in both synthetic medium and corn stover hydrolysate (without inhibitor removal) in flasks (Fig. 1). Gluconic acid productivity and the cell growth of *G. oxydans* DSM 2003 in the inhibitor containing corn stover hydrolysate were almost same to that in synthetic medium, even without any nutrients or inorganic salts addition to the hydrolysate (Fig. 1a). *G. oxydans* cells showed stronger inhibitor degradation capacity than *A. niger* SIIM M276 (Zhang et al., 2016), and well adapted to the hydrolysate environment by maintaining stable cell morphology (Fig. 1b).

Then the fermentation was conducted in fermentors with accurate control of pH, temperature, dissolved oxygen level (Fig. 2). In the hydrolysate prepared from 15% (w/w) of the freshly pretreated corn stover, gluconic acid productivity in fermentors increased significantly to 2.82 g/(L·h) from 1.90 g/(L·h) in flasks (Fig. 2a) and 0.28 g/(L·h) in flasks by *A. niger* (Zhang et al., 2016). When the sugars increased by increasing solids content of freshly pretreated corns stover hydrolysate preparation to 20% (w/w), the cell growth rate decreased with the inhibitors increased but gluconic acid productivity was not inhibited obviously comparing with the one in 15% (w/w) freshly pretreated corn stover hydrolysate (Fig. 2b). Xylose started to convert to xylonic acid (Fig. 2c) after the complete consumption of glucose, but also gluconic acid started to convert to keto-gluconic acid (KGA). The existence of xylonic acid in the fermentation products is a positive factor because sodium xylonate is an excellent water reducer of cement (Chun et al., 2006), but the function of keto-gluconic acid is not clear as cement additive. Furfural and HMF inhibitors were quickly degraded, and acetic acid was almost constant or slightly increased but no obvious inhibition on gluconic acid productivity was observed (Fig. 2d). However, when the sugar concentration increased by increasing solids content in hydrolysis step to 25% (w/w), *G. oxydans* DSM 2003 was not able to grow.

To obtain the maximum yield and titer of gluconic and xylonic acids, the inhibitors in the pretreated corn stover were removed as completely as possible by applying the biodetoxification on the solid pretreated corn stover (Fig. 3). The residue furfural and HMF were completely degraded in the first 16 h of the fermentation (Fig. 3d). *G. oxydans* cells growth in the detoxified corn stover hydrolysates increased 2–3 folds than that in the hydrolysate without inhibitor removal, even similar to that in synthetic medium (Fig. 3a). Gluconic acid productivity maintained considerably high with the maximum titer of 102.10 g/L and yield of 91.13% (Fig. 3b), and xylose was converted to xylonic acid with the maximum titer of 34.31 g/L and yield of 90.02% (Fig. 3c). The maximum gluconic acid and xylonic acid could be obtained by avoiding unnecessary conversion of gluconic acid to keto-gluconic acid at specific time point.

The fermentation was further scaled up in a 50 L bioreactor (35 L of working volume) from the 3 L fermentors (1 L of working volume) using the corn stover hydrolysate prepared at 30% (w/w) solids loading of the pretreated and biodetoxified corn stover feedstock (Fig. 4). 119.10 g/L of gluconic acid (equivalent to 132.46 g/L of sodium gluconate) with 4.14 g/(L·h) of productivity and 97.12% of yield within 32 h were received. The productivity was slightly low because of the reduced inoculum size (10% in 3 L, but 1% in 50 L) and oxygen transfer (2.5 vvm of aeration and 500 rpm of agitation rate in 3 L, but 1.5 vvm and 300 rpm in 50 L) for considerations of practical commercial scale operation. The fermentation was stopped at 32 h to prevent the possible conversion of gluconic acid to keto-gluconic acid, thus xylose conversion to xylonic acid was not complete (14.04 g/L of xylonic acid, equivalent to 15.90 g/L of sodium xylonate).

The robust inhibitor tolerance, easy cell growth, and co-production of high titer sodium gluconate and xylonate by *G. oxydans* DSM 2003 successfully overcome the drawbacks of low productivity, difficult seed propagation, and xylose loss by *A. niger* SIIM M276.

3.2. Sodium gluconate and xylonate product from corn stover as cement retarder additives

Sodium gluconate broth produced using corn stover feedstock in the 50 L fermentor was directly used as the cement retarder additive without any concentrating operation. The cement additive assay was conducted by testing the addition of sodium gluconate broth on the setting time, fluidity and strength of cement paste and compared with the commercial sodium gluconate from corn

Table 2
Main process input data for the established Aspen plus simulation model.

Features	Values
Pretreatment	
Sulfuric acid dosage (%)	2.5
Residence time (min)	5
Temperature (°C)	175
Pressure (MPa)	0.89
Solids after pretreatment (%)	50
Glucose yield from glucan (%)	4
Hemicellulose sugar yields (%)	40
Furfural yield from xylan (%)	3.3
Acetic acid hydrolysis ratio (%)	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 fermentation	
Temperature for hydrolysis (°C)	50
Temperature for fermentation (°C)	33
Residence time for hydrolysis (h)	48
Residence time for fermentation (h)	24
Solids loading (%)	30
Cellulase dosage (mg protein/g cellulose)	28
Glucan conversion to glucose (%)	87
Xylan conversion to xylose (%)	82
Sodium gluconate yield from glucose (%)	97
Sodium xylonate yield from xylose (%)	90
Glycerol yield from glucose (%)	1
Glucose consumed for cell growth (%)	2
Sodium gluconate concentration (g/L)	132
Sodium xylonate concentration (g/L)	39
Product recovery	
Purity (sodium gluconate and sodium xylonate)	98% (w/w)
Water content	2% (w/w)

starch feedstock (Table 1). The initial setting time indicates the start of cement paste setting, hardening, and plasticity loss; and the final setting time indicates the complete loss of plasticity and behaving structural strength. The fluidity indicates the uniformity and stability of cement paste. The corn stover derived sodium gluconate/xylonate showed the obvious cement retarding properties of longer setting time and greater fluidity than that of the commercial sodium gluconate in the range of sodium gluconate dosage of 0.01%–0.03% (w/w) addition. When the sodium gluconate dosage exceeded 0.03% (w/w), the plasticity of the cement paste was too high and the setting time was too long (more than 390 min). The addition of sodium xylonate only also showed considerable retarding property for extending setting time in the range of 0.01%–0.06% (w/w) addition.

Strength test indicates the ability to withstand external force to cement mortar. The sodium gluconate/xylonate showed similar flexural strength and the compressive strength reached almost the same with the commercial sodium gluconate when the sodium gluconate dosage was 0.03% (w/w).

The results indicate that the crude sodium gluconate/xylonate broth from corn stover feedstock by *G. oxydans* DSM 2003 was a satisfactory cement retarder additive, similar to the commercial product from corn starch.

3.3. Techno-economic analysis (TEA) of cellulosic sodium gluconate product

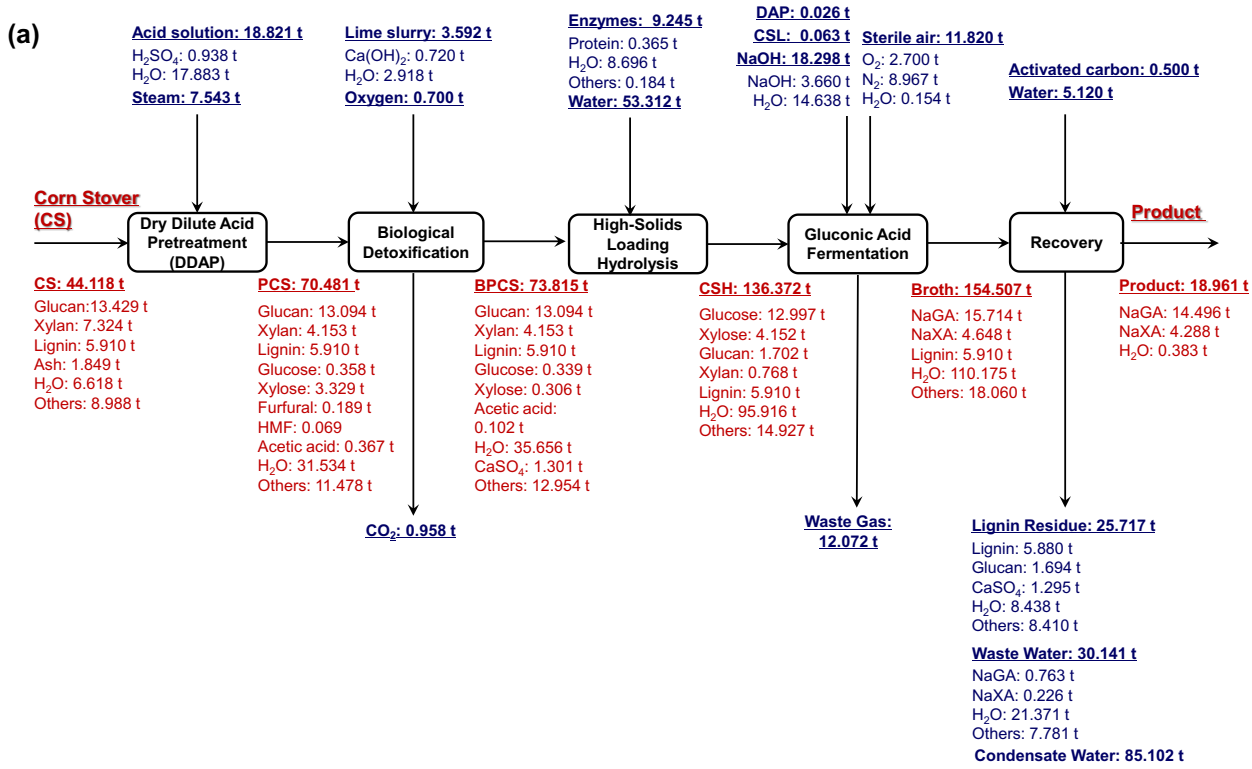
The Aspen Plus model was established for sodium gluconate and xylonate production as described in the Method section based on the experiments results and the relevant TEA study on L-lactic acid product from lignocellulose (Liu et al., 2015). The main process input data are listed in Table 2. The sodium gluconate and sodium xylonate in fermentation broth are 132 g/L and 39 g/L in the process simulation, respectively, according to the maximum experimental results. The recovery yield of sodium gluconate and xylonate in the product recovery area is assumed to be the same with the sodium lactate recovery from corn stover (92%) (Liu et al., 2015). The overall processing capacity is 300,000 tons of corn stover annually and the total product output is 151,688 tons annually (75.65% of sodium gluconate, 22.35% of sodium xylonate, and 2.00% of water, w/w).

The detailed material balance of the overall process is shown in Fig. 5a at the time scale of hour. The rigorous calculation shows that 506 kg of sodium gluconate product is obtained from one ton of dry corn stover. For fresh water usage, 4.92 tons of fresh water is consumed for producing one ton of product, but only 1.20 tons of fresh water is required if the water cycling is taken into account in the overall process. The pretreatment area and the enzyme hydrolysis area consume 19.16% and 57.12% of the total fresh water, respectively. For waste water generation, 1.88 tons of waste water is generated for producing one ton of product. No free waste water stream is released from dry dilute acid pretreatment, and equivalently 1.58 tons of waste water per ton of product is released in product recovery area to the waste water area.

The detailed energy balance of the overall process is shown in Fig. 5b at the time scale of hour. For heating steam usage, 6349 MJ of heating value is required for producing one ton of product, which is equivalent to 2.18 tons of hot steam (273 °C, 1.3 MPa). The pretreatment steam and the triple-effect evaporation in the recovery area account for 18.05% and 73.65% of heating steam usage, respectively, while the hydrolysis step takes 8.3% of heating steam. For electricity consumption, the electricity consumption is approximately 504 kWh for producing one ton of product. The disk milling of pretreated corn stover, drying and grinding of fresh corn stover and the hydrolysis mixing of highly viscous material account for 26.51%, 21.36%, and 11.73%, respectively. On the other hand, the combustion of lignin residue in boiler area generates sufficient heat and electricity to meet all the needs of the overall processing with 251 kWh of excessive electricity to the grid as by-product credit for producing one ton of product.

Fig. 5c illustrates the detailed contribution of capital, operations and fixed costs to the overall cost. The dry dilute acid pretreatment and biodetoxification cost is significantly reduced to $0.030 per kg product compared to other pretreatment technologies (Humbird et al., 2011). The cost of corn stover and sodium hydroxide is $0.122 and $0.090 per kg product, respectively, accounting for 78% of the cost of saccharification and fermentation area, as well as 52% of the total cost. The cost of combustion step is only $0.024 per kg product due to electricity credit. The total capital investment is about $166.9 MM for this biorefinery plant with 300,000 tons of corn stover feedstock annually. The minimum sodium gluconate/xylonate product selling price (MGSP) is calculated to be $0.404 per kg product, in which the feedstock, enzyme production and non-enzyme conversion cost were $0.122, $0.095 and $0.187 per kg, respectively. Compared with a typical commercial sodium gluconate product using corn feedstock on Chinese market ($0.476/kg, Xiwang Group Co. on Alibaba Enterpriser website https://www.1688.com), the cellulosic sodium gluconate/xylonate product show a certain competitive.

Dry milling biorefining process (DMBP) played the key role on realizing a competitive production of sodium gluconate/xylonate to the corn based product by its low capital investment, low steam and water usage, low waste water generation, and high fermentation performance (Zhang et al., 2010a, 2011; He et al., 2014, 2016). Note that no extra nutrients were required for fermentation and



Summary	Minimum Sodium Gluconate Selling Price (MGSP)	$0.404 per kg product
	Feedstock contribution	$0.122 per kg product
	Enzyme contribution	$0.095 per kg product
	Non-enzyme conversion contribution	$0.187 per kg product

Fig. 5. Materials, energy and cost balance of cellulosic sodium gluconate production from corn stover feedstock on the hour basis (tons per hour). (a) Materials balance; (b) Energy balance; (c) Cost contribution details from each process area (per kg sodium gluconate/xylonate product). Abbreviations: CS, Corn stover; DDAP, Dry dilute acid pretreatment; PCS, Pretreated corn stover; BPCS: Pretreated biodetoxification corn stover; CSL, Corn steep liquor; CSH, Corn stover hydrolysate; DAP: Ammonium phosphate; NaGA, Sodium gluconate; NaXA, Sodium xylonate.

the crude fermentation broth only was an excellent cement retarder additive product, which provides an important alternative for traditional sodium gluconate from corn. The minimum sodium gluconate/xylonate selling price (MGSP) based on the Aspen plus modeling is only $0.404 per kg, which was obviously competitive than ethanol from corn stover (MESP at $2.15 per gallon, equivalent to $0.720 per kg, Humbird et al., 2011) using the same calculation principle and similar processing flowsheet, and competitive to the commercial sodium gluconate ($0.476 per kg) from starch in China market. The biorefining technology practice on value added biochemical production provides the practical and competitive economic benefits on the future utilization of lignocellulose biomass.

4. Conclusion

Gluconobacter oxydans DSM 2003 showed an excellent gluconic and xylonic acids fermentability using the dry dilute acid pretreated and biodetoxified corn stover feedstock. Maximum titer of sodium gluconate at 132.46 g/L and sodium xylonate at 38.86 g/L were obtained and the fermentation was scaled up. Sodium gluconate/xylonate product from corn stover showed competitive performance as cement retarder than the commercial sodium gluconate. The techno-economic analysis based on Aspen plus modeling demonstrated high economic and technical competitiveness to the corn starch based commercial technology. This study provided a potential of lignocellulose biomass commercially applicable for sodium gluconate and xylonate industry production.

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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.2016.07.068.

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