Bioresource Technology 102 (2011) 1815–1821

Contents lists available at ScienceDirect

Bioresource Technology

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

Improved 1,3-propanediol production with hemicellulosic hydrolysates (corn straw) as cosubstrate: Impact of degradation products on *Klebsiella pneumoniae* growth and 1,3-propanediol fermentation

Pin Jin [a,b], Shuang Li [a], Sheng-guo Lu [a], Jian-guo Zhu [a], He Huang [a,*]

[a] State Key Laboratory of Materials-Oriented Chemical Engineering, College of Biotechnology and Pharmaceutical Engineering, Nanjing University of Technology, No. 5 Xinmofan Road, Nanjing 210009, People's Republic of China
[b] Fushun Research Institute of Petroleum and Petrochemicals, No. 31 Dandong Road, Fushun 113001, People's Republic of China

ARTICLE INFO

Article history:
Received 22 July 2010
Received in revised form 4 September 2010
Accepted 9 September 2010
Available online 17 September 2010

Keywords:
1,3-Propanediol
Corn straw
Hemicellulosic hydrolysates
Xylose
Klebsiella pneumoniae

ABSTRACT

To improve 1,3-propanediol (1,3-PD) production by an economic and efficient approach, hemicellulosic hydrolysates (HH) used as cosubstrate resulted in more biomass and higher reducing power for 1,3-PD production. The effects of primary degradation products such as individual sugars (xylose, glucose, mannose, arabinose and galactose) and major inhibitors (furfural, acetate and formate) on the *Klebsiella pneumoiae* growth and 1,3-PD production were investigated in this study. Xylose and mannose could efficiently promote the 1,3-PD production and cell growth. Furfural (0.28 g/l) and sodium acetate (1.46 g/l) in low concentration were not inhibitory to *Klebsiella pneumoniae*, rather they have stimulatory effect on the growth and 1,3-PD biosynthesis, especially the acetate. In fed-batch fermentation with HH as cosubstrate, the final 1,3-PD production, conversion from glycerol and productivity were 71.58 g/l, 0.65 mol/mol and 1.93 g/l/h, respectively, which were 17.8%, 25.0% and 17.7% higher than that from glycerol alone.

1. Introduction

Development of bio-refineries has recently attracted increasing attention as a means to provide sustainable alternative solutions to depleting petroleum resources and environmental pollution (Ji et al., 2009). Many chemicals, which could only be produced by chemical processes in the past, could potentially be generated biologically from annually renewable resources (Ragauskas et al., 2006). Biosynthesis of 1,3-propanediol (1,3-PD) with low cost renewable glycerol as a substrate has attracted worldwide interests due to that 1,3-PD is used as versatile degradable intermediate compound for the synthesis of heterocycles and a monomer for the production of polymers (Selembo et al., 2009; Zheng et al., 2008). A versatile monomer in the synthesis of PTT with excellent potential for use in textiles and carpeting that has been dubbed the "new nylon", demand of which is estimated to be 1–2 billion lb year^{-1} in 10 years (Nakamura and Whited, 2003).

Recently, the raw material costs still hinder the large scale-application of this process. Glucose, sucrose, maltose and xylose had been used as cosubstrate in 1,3-PD biosynthesis process (Abbad-Andaloussi et al., 1998; Yang et al., 2007; Ragout et al., 1996; Tong and Cameron, 1992) which all increased the 1,3-PD conversion from glycerol in different levels. Cosubstrate is also an important factor influencing the cost of 1,3-PD fermentation. Hemicellulose, the second most common polysaccharides in nature, represent about 20–35% of lignocellulosic biomass. In recent years, bioconversion of hemicellulose has received much attention because of its practical applications in various agro-industrial processes (Saha, 2003). Dilute sulfuric acid pretreatment is a popular and efficient way applied to corn straw to bring about hydrolysis. Unfortunately, during acid hydrolysis, a complex mixture of sugars and microbial inhibitors is generated. Examples of these compounds include xylose, glucose, mannose, acetic acid, furfural, etc. (Ezeji et al., 2007). A lot of studies have been conducted about efficient use of hemicellulose and various effects of inhibitors on microorganisms. However, there was no relative research work focused on the 1,3-PD production.

In this study, for effective and economic 1,3-PD production, we attempt to examine the potential use of hemicellulosic hydrolysates (HH) as cosubstrate by *Klebsiella pneumoniae*. We investigated the effects of contents in HH on cell growth and 1,3-PD biosynthesis process. By these approaches, we also got higher content of cell mass and growth rate which are advantageous to 1,3-PD synthesis.

* Corresponding author. Tel./fax: +86 25 83172094.
E-mail address: biotech@njut.edu.cn (H. Huang).

0960-8524/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2010.09.048

2. Methods

2.1. Microorganism

The strain for 1,3-PD production used in this study was *Klebsiella pneumoniae* ME-303 from *Klebsiella pneumonia*e ATCC 25955 by mutagenesis (*K. pneumoniae*), obtained from Nanjing University of Technology. The culture was maintained on Luria–Bertani (LB) agar slant at 4 °C.

2.2. Corn straw hydrolysis preparation

Corn straw was milled to a particle size no larger than 1 mm. Acid hydrolysis of corn straw was carried out in a medium containing 2% H_2SO_4 with a liquid/solid ration of 10:1 (w/w) at 100 °C for 2 h (Xu et al., 2010). Detoxification of the corn straw hydrolysate was carried out by using a modified method (overliming) with $Ca(OH)_2$ (Martinez et al., 2000). The pH of the hydrolysate was adjusted to 8.0 with $Ca(OH)_2$ at 25 °C, the supernatant was HH and it was used as a carbon source to carry out the fermentation studies. The hydrolysates used for feedstock in fed-batch fermentation were concentrated by vacuum evaporation at 40 °C till the concentration of xylose reached 50 g/l.

2.3. Media and cultures

The seed medium contained yeast extract 3.0 g/l, malt 3.0 g/l, peptone 5.0 g/l, NaCl 5.0 g/l. The basic fermentation medium was composed of yeast extract 5 g/l, $K_2HPO_4{\cdot}3H_2O{\cdot}10$ g/l, $KH_2PO_4{\cdot}2$ g/l, NH_4Cl 1 g/l, NaCl 0.5 g/l, $MgSO_4{\cdot}7H_2O$ 0.1 g/l, $FeCl_3{\cdot}6H_2O$ 30 mg/l, $CoCl_2{\cdot}6H_2O$ 5 mg/l, Vitamin B_{12} 5 mg/l, glycerol 30 g/l. The cofermentation medium was based on the basic fermentation medium and supplemented HH (containing xylose 11.2 g/l). To examine the effects of four sugars and three inhibitors existed in HH on the cell growth and 1,3-PD production, the individual and mixed sugars as well as the individual inhibitors were added respectively into the basic fermentation medium aseptically at certain concentrations.

Erlenmeyer flasks (250 ml) containing 100 ml seed medium were inoculated with *K. pneumoniae* to prepare the inoculums. The flasks were incubated at 37 °C and 140 rpm for 10 h and subsequently inoculated into Erlenmeyer flasks and the fermenter at 5% (v/v). The flask fermentation was carried out in 250 ml Erlenmeyer flasks containing 100 ml fermentation medium. The batch fermentation containing the basic fermentation medium or cofermentation medium were both conducted in a 3 l stirred fermenter (BioFlo 100; New Brunswick Scientific Co., NJ, USA) with a working volume of 2 l under 0.25 vvm air-flow. The cofermentation medium was based on the basic fermentation medium supplemented with the initial HH to obtain xylose 11.2 g/l. All fermentations were carried out at initial pH 7.0, 37 °C and 300 rpm. The fed-batch fermentation was conducted in a 7 l stirred fermenter with a working volume of 4 l under 0.25 vvm air-flow. During the cofermentation of glycerol and HH, the initial HH (all contents are shown in Table 1) was added (2 l) to obtain xylose 11.2 g/l in the fed-batch fermentation medium and maintained 5–8 g/l by using the concentrated HH during the cofermentation. Among all the cofermentations, the glycerol was added initially 30 g/l, and maintained 15–20 g/l during the time courses of cultivation.

2.4. Analytical procedures

Contents in HH were detected following the procedure described by Yan et al. (2009). Formic acid and acetic acid were analyzed by a Dionex HPLC equipped with a Bio-Rad Aminex HPX-87H column and a refractive index detector, the mobile phase was 0.005 mol/l H_2SO_4 at a flow rate of 0.6 ml/min and column temperature was 60 °C. Furfural was detected by a UV detector at the wavelength of 210 nm. Sugars were analyzed by a Bio-Rad Aminex HPX-87P column and a refractive index detector, the mobile phase was water at a flow rate of 0.6 ml/min and column temperature was 85 °C.

Glycerol, 1,3-PD, acetic acid, 2,3-butanediol (2,3-BD), lactic acid, alcohol, succinic acid, and xylose were using a high-performance liquid chromatography (HPLC) system (Dionex with a Bio-Rad HPX-87H ionexclusion column) with a refractive index detector at 65 °C with 0.005 mol/l H_2SO_4 as mobile phase at 0.8 ml/min.

Biomass was determined by measuring the value of optical density at 600 nm with appropriate dilution using a UV–visible spectroscopy system. The value of the optical density was converted to cell dry weight (CDW) using a calibration equation ($CDW = 0.4183 \times OD_{600} - 0.117$).

2.5. Determination of the reducing equivalent (NAD^+ and NADH)

The concentration of intracellular NADH and NAD^+ were measured by high-performance liquid chromatography (HPLC) (column stable-C18) (Miseta et al., 2003; Sato et al., 2000; Stanley, 1986). The buffer contained 4.5 ml methanol (60%) and 0.5 ml HEPES (10%) and prepared for more than 4 h at −80 °C. A 1.0 ml sample was taken and then frozen in the prepared buffer for 5 min ensuring rapid cooling and effective inhibition of cell metabolism. The mixture was centrifuged for 5 min at 10,000*g* and 4 °C to collect the precipitate. For extracting the NADH and NAD^+, the mixture were treated with either 1.0 ml of 0.1 mol/l HCl (extracting NAD^+) or 1.0 ml of 0.1 mol/l NaOH (extracting NADH, destroying NAD^+). After incubation at 100 °C for 10 min, the mixture was centrifuged for 10 min at 10,000*g* to collect the supernatant and stored at −21 °C. The pH relative to the collected supernatant was adjusted to 6.8 with HCl or NaOH (0.1 mol/l) before HPLC column application. A mixture of 90% H_3PO_4 (pH 6.6) and 10% methanol was used as mobile phase at a flow rate of 1.0 ml/min. The wavelength of the UV detector was set at 254 nm and the column temperature was controlled at 25 °C.

3. Results and discussion

3.1. 1,3-PD production with HH as cosubstrate in batch fermentation by K. pneumoniae

HH contained xylose as main carbon source, four other sugars (glucose, arabinose, galactose, mannose) and three main inhibitors (formic acid, acetic acid, furfural) (Table 1). The fermentation efficiency for 1,3-PD was improved obviously by HH addition (Table 2). After 20 h, the 1,3-PD concentration, conversion of 1,3-PD from

Table 1
Contents in hemicellulose hydrolysates[a].

Xylose (g/l)	Formic acid (g/l)	Acetic acid (g/l)	Furfural (g/l)	Arabinose (g/l)	Galactose (g/l)	Mannose (g/l)	Glucose (g/l)
22.40 ± 0.85	0.83 ± 0.05	2.14 ± 0.30	0.55 ± 0.02	3.43 ± 0.67	2.05 ± 0.22	1.30 ± 0.18	2.90 ± 0.36

[a] Data with sign "±" are the average of three triplicates.

Table 2
Effects of xylose or hemicellulosic hydrolysates in batch fermentation (3 l) by *K. pneumoniae*[a].

Number	AGC (g/l)	HHC (l)	Products concentration (g/l)						Conversion (mol/mol)	Biomass (OD_{600})	Productivity (g/l/h)
			PD	BD	Suc	Ace	Eth	Lac			
1	29.05 ±1.01	–	12.1 ±0.36	2.08 ±0.10	0.97 ±0.04	1.39 ±0.08	2.36 ±0.09	1.44 ±0.05	0.45	5.48	0.61
2	30.76 ±1.35	1.00 ±0.01	14.3 ±0.53	4.28 ±0.25	1.15 ±0.05	1.21 ±0.06	2.53 ±0.13	1.54 ±0.08	0.56	7.13	0.72

[a] Data are the average of three experiments. 1: the fermentation with glycerol alone, 2: the cofermentation of glycerol and hemicellulosic hydrolysates. AGC, accumulative glycerol consumed; HHC, the initial HH consumed. Abbreviations: Lac, lactic acid; Eth, ethanol; Ace, acetic acid; BD, 2,3-butanediol; Suc, succinic acid.

glycerol and productivity with HH addition were 18.2%, 24.4% and 18.0% higher than that from glycerol alone, respectively. The byproducts production from cofermentation such as 2,3-BD, ethanol, succinic acid, lactic acid, were improved in different levels except the acetic acid. Especially, the 2,3-BD was doubly produced under HH added condition compared with that from glycerol alone. The results also showed that biomass (OD_{600}) was improved from 5.48 to 7.13, which were favorable for promoting 1,3-PD biosynthesis.

Adding sugar as cosubstrate is a feasible way to improve 1,3-PD conversion. Although the cofermentations of glycerol and other sugars were generally performed (Abbad-Andaloussi et al., 1998; Yang et al., 2007; Ragout et al., 1996; Tong and Cameron, 1992), the cosubstrates they used were still pure sugars, which inevitably resulted in high cost of 1,3-PD bio-production. As indicated in Table 2, adding HH was a feasible and economic way to improve the fermentation efficiency of 1,3-PD. Although HH could not be converted to 1,3-PD, it might be used for biomass and regeneration of reducing power whereas more glycerol and reducing power flowed to 1,3-PD pathway. And based on the effect of xylose on 1,3-PD fermentation, it might be one of the reasons to explain the positive effect of HH on 1,3-PD production. The cofermentation of glycerol and xylose in 1,3-PD production had been reported (Tong and Cameron, 1992). The 1,3-PD concentration and yield of 1,3-PD from glycerol with xylose addition in recombinant *Escherichia coli* expressed the *dha* regulon of *K. pneumoniae,* was increased from 0.67 g/l to 1.08 g/l, 0.46 mol/mol to 0.55 mol/mol, respectively. They considered it might be that the availability of reducing power was one of the factors limiting the yield of 1,3-PD from glycerol and the xylose catabolism could generate more reducing power for 1,3-PD pathway. However, there was more complexity in HH since it contained variable degradation contents including individual sugars and inhibitors. Hence, the effect of HH on reducing power generation was studied in this work as indicated in the next section.

In previous studies (Huang et al., 2002; Chen et al., 2003; Cheng et al., 2004), a positive correlation between the biomass of *K. pneumoniae* and its 1,3-PD production was demonstrated whereas more biomass, higher productivity were gained. We gained the similar results as they were described. But the 2,3-BD was more than they gained (Table 2). This might be due to three reasons: first, 2,3-BD fermentation was favorable at low pH of 6.5 (Yang et al., 2007) and especially preferred to the condition without pH control (Petrov and Petrova, 2009). These conditions were carried out most of the time during the batch fermentation in this work; second, more substrates (glycerol and HH) and NADH flowed to 2,3-BD pathway; and the last, xylose and glucose could be converted to 2,3-butanediol directly by *K. pneumoniae* (Ji et al., 2009) whereas xylose was the most abundant content in HH.

3.2. Effects of HH on internal reducing equivalent in batch fermentation

Based on the fact that manipulation of reducing equivalent by changing carbon resources can improve the 1,3-PD production, we have investigated the variation of reducing power during the cofermentation on 1,3-PD production. For cell growth from 4 to 16 h, the internal redox state in batch fermentation, as reflected by the NADH/NAD^+ ratio, was determined. The results are shown in Fig. 1A and Fig. 1B. It was found that the total reducing equivalent (total NADH pools) increased significantly by cofermentation with HH. The total NADH pool increased by 0.9–2.0 mg/g (CDW) compared with that from glycerol alone after 4 h. The reducing equivalents retained at the highest level of 9.81 mg/g (CDW) in batch fermentation with HH as cosubstrate, which was higher than that (8.97 mg/g) from pure glycerol alone. The NADH/NAD^+ ratio obtained higher levels by using HH, except that around 12 h. The result was consistent with products biosynthesis in batch fermentation, especially the 1,3-PD biosynthesis. As HH being added, 1,3-PD production and the reducing equivalent level were all

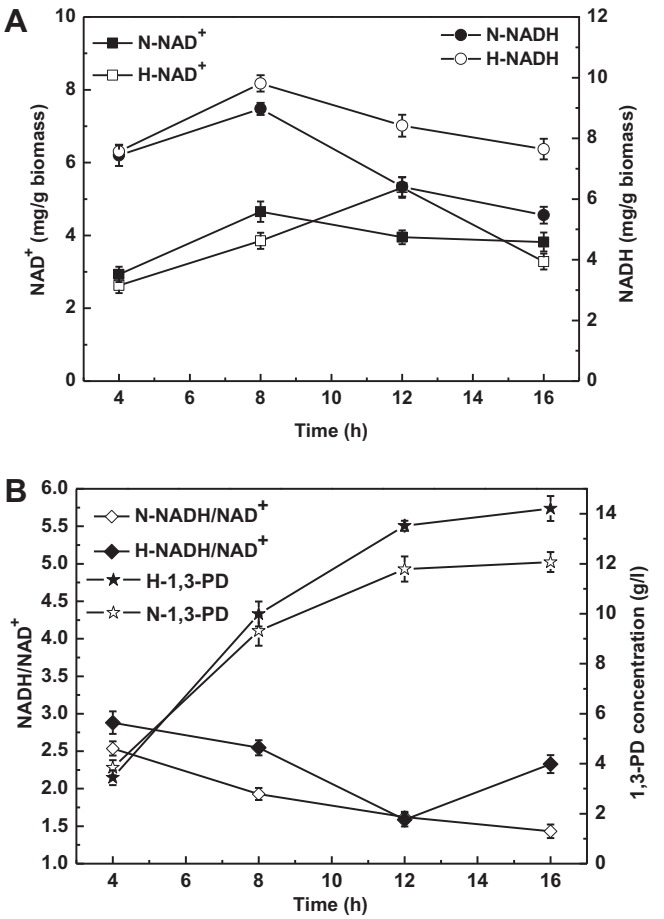

Fig. 1. Effects of hemicellulosic hydrolysates on internal redox reducing equivalent in batch fermentation. N-NAD^+, N-NADH, N-NADH/NAD^+, N-1,3-PD: all the data investigated in batch fermentation with glycerol alone; H-NAD^+, H-NADH, H-NADH/NAD^+, H-1,3-PD: all the data investigated in batch fermentation with hemicellulosic hydrolysates added.

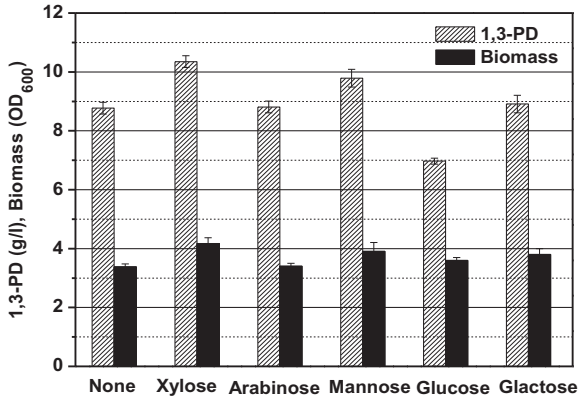

Fig. 2. Effects of individual sugars (11.2 g/l: xylose, 1.715 g/l: arabinose, 0.65 g/l: mannose, 1.45 g/l: glucose, 1.03 g/l: galactose) present in hemicellulosic hydrolysates on the 1,3-propanediol biosynthesis and cell growth.

improved. The 1,3-PD productivity decreased rapidly after 8 h that keep pace with NADH/NAD$^+$ ratio variation. The results as expected, also demonstrated that in cofactor-dependent production systems, cofactor availability and the proportion of cofactor in the active form might play an important role in dictating the overall process yield.

The 1,3-PD synthesis plays a key role in NADH consumption to regulate the intracellular reducing equivalent balance of *Klebsiella pneumoniae* (Zeng et al., 1993). Endeavor to enhance NADH generation should be taken into consideration for improving 1,3-PD production. Two distinct strategies can be used to alter the NADH pool and NADH/NAD$^+$ ratio (San et al., 2002). The first approach is based on genetic manipulations of the host cell. Recently, metabolic engineering has achieved pronounced progresses in manipulating the availability and higher intracellular concentrations of NADH (Zhang et al., 2006; Xu et al., 2009; Zhang et al., 2009). However, alteration of intracellular redox balance by genetic manipulation inevitably reduced the glycerol uptake efficiency or inhibited the

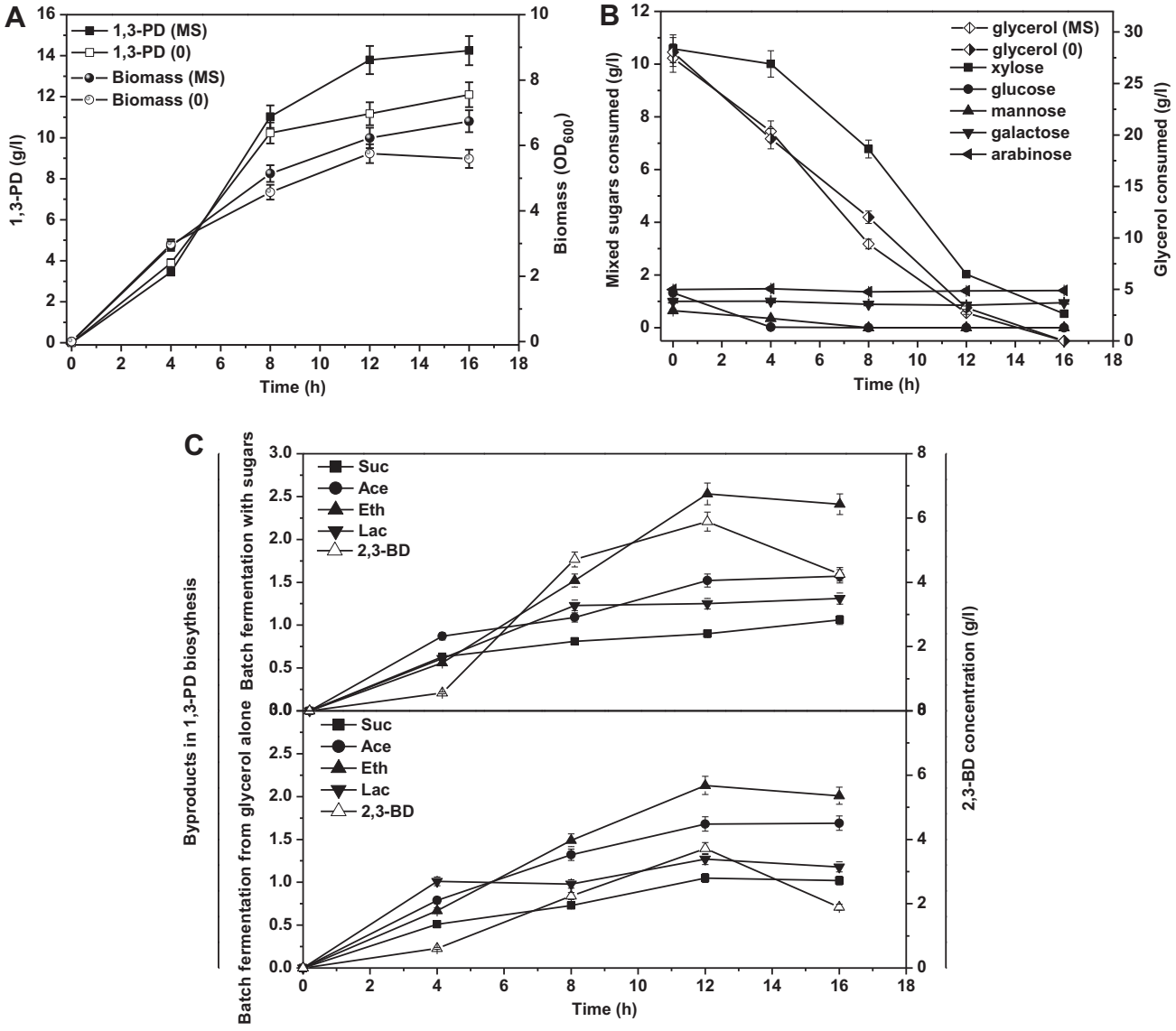

Fig. 3. Effects of mixed sugars simulating hemicellulosic hydrolysates on the 1,3-PD biosynthesis and cell growth. All the data are the average of three experiments. Abbreviations: Lac, lactic acid; Eth, ethanol; Ace, acetic acid; 2,3-BD, 2,3-butanediol; Suc, succinic acid. 1,3-PD (MS), Biomass (MS) and glycerol (MS): all the data come from batch fermentation with mixed sugars as cosubstrate; 1,3-PD (0), Biomass (0) and glycerol (0): all the data come from batch fermentation with glycerol alone.

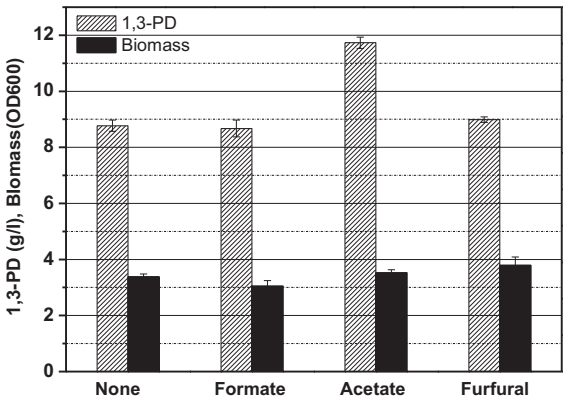

Fig. 4. Effects of main inhibitors (0.61 g/l: formate, 1.46 g/l: acetate, 0.28 g/l: furfural) present in hemicellulosic hydrolysates on the 1,3-propanediol production and cell growth. Abbreviations: 1,3-PD, 1,3-propanediol.

growth of producer or obtained a limited effect on conversion and production. It was also demonstrated that the metabolite distribution can be influenced by a change in the NADH/NAD^+ ratio as mediated by the oxidation state of the carbon source used (San et al., 2002). In this project, we focused on the latter approach to increase the reducing equivalent concentration.

3.3. Effects of degradation sugars in HH on the growth of K. pneumoniae and 1,3-PD biosynthesis

Hemicellulosic hydrolysates, containing a lot of sugars and inhibitors, could distinctly improve the yield of 1,3-PD from glycerol. The effects of sugars in HH on the flask fermentation were tested at certain concentrations (Figs. 2 and 3). The flask fermentations were run for 29 h. Both 1,3-PD concentration (10.35 g/l) and biomass (4.17, OD_{600}) could reach the highest level with xylose (11.2 g/l) as cosubstrate. It was found for the first time that mannose (0.65 g/l) added can improve the 1,3-PD production (9.79 g/l) and the biomass (3.90, OD_{600}) significantly. Although low concentration of mannose in HH, the effect of mannose on 1,3-PD biosynthesis and the microbial growth was obvious. It may be due to mannose catabolism in *K. pneumoniae* was used more efficiently for generation of reducing power. The galactose (1.025 g/l) and arabinose (1.715 g/l) have no influence on the process. Glucose (1.45 g/l) has the negative effects on 1,3-PD production compared with that of glycerol alone: biomass was enhanced by 6.5%; but the production of 1,3-PD was declined by 20.5%. The inhibition of glucose on 1,3-PD production could be explained by the "glucose effect" that glycerol could not be used in time unless glucose was to be exhausted by bacteria (Yang et al., 2007).

Mixed sugars simulating HH were tested for their combined effects on batch fermentation with glucose, mannose, arabinose, galactose and xylose at the control concentrations as 1.45 g/l, 0.65 g/l, 1.715 g/l, 1.025 g/l, and 11.20 g/l, respectively. The 1,3-PD production profiles by *K. pneumoniae* over the course of 16 h showed that the culture produced 14.25 g/l 1,3-PD and 6.74 of biomass (OD_{600}), which was 17.8% and 20.6% higher than that from glycerol alone, respectively (Fig. 3A). The utilization of sugars by *K. pneumoniae* was shown in Fig. 3B. Most sugars such as xylose, glucose and mannose were concurrently consumed with specific rate during the fermentation. Galactose and arabinose could not be utilized by *K. pneumoniae*. The simultaneous uptake and metabolism of these sugars for 1,3-PD production is very desirable feature since the sugar composition of HH is diverse. The rapid glucose utilization is not surprising since glucose represents the preferred substrates in the metabolism of many microorganisms (Ezeji et al., 2007). It should be noted the glycerol utilization was not inhibited by mixed sugars which was similar to that happened in glycerol alone. The rate of glycerol utilization with mixed sugars, was a little low before 4 h. And then the rate was much higher than that from glycerol alone during 4–8 h while 1,3-PD production was rapidly accumulated during this period. The byproducts in 1,3-PD biosynthesis with mixed sugars addition were similar to that of HH as cosubstrate. The most abundant byproduct, the final 2,3-butanediol concentration was increased by 124.9% with mixed sugars added, while the acetic acid was decreased by 7.1%. All the rest byproducts were improved in low level.

3.4. Effects of inhibitors in HH on the growth of K. pneumoniae and 1,3-PD biosynthesis

Furfural, formate and acetate, the main inhibitors in HH, usually give negative effects on cell growth and products biosynthesis. Their effects on the cell growth and 1,3-PD biosynthesis were shown in Fig. 4. Cell growth of *K. pneumoniae* in the presence of 0.28 g/l furfural was improved by 12.1% compared with no inhibitors added. However, 1,3-PD production was slightly improved. The unexpected stimulatory effect may be due to convert furfural to less inhibitor (Horváth et al., 2003) and disrupt energy metabolism which stimulate energy production through glycerol metabolism. In the presence of 0.61 g/l formate, both the cell growth and 1,3-PD production were slightly inhibited while the biomass and 1,3-PD concentration were decreased by 9.8% and 1.1%, respectively. Interestingly, in the presence of 1.46 g/l acetate, the cell growth was slightly improved. The final concentration of 1,3-PD was enhanced from 8.77 g/l to 11.73 g/l. From the metabolites tested in the cofermentation with acetate added, we found that acetate acid formation, one of the byproducts in 1,3-PD biosynthesis, was much lower than that from glycerol alone (data not shown).

Previous study of effects of acetate on 1,3-PD fermentation focused on experiments under acetate stress with high concentration. In the study by Xue et al. (2010), 1,3-PD production was enhanced by the supply of organic acids (citrate, fumarate and succinate) whereas the byproducts, especially the lactic acid and ethanol, decreased significantly. However, the 1,3-PD production was inhibited as 10 g/l acetic added in their study. Cheng et al. (2005) also reported the acetate was the main inhibitor metabolite during the 1,3-PD fermentation under anaerobic condition while the critical concentration of acetate was 15 g/l. To our knowledge, there have been some relative research works on impact of acetate and finally gained similar results or phenomena. Lü et al. (2008) had studied the impact of acetate on product formation of fermenting polysaccharide-rich organic waste. They found ethanol was favorably produced with dosed acetate at pH 6, as well as the production methanol and formate. The impact of elevated concentrations of acetate on ethanolic fermentation was also reported by He et al. (2009). They showed an stimulatory effect on ethanolic fermentation by *Thermoanaerobacter ethanolicus* with enhancing ethanol production by up to 394%. As well, production of butanol from acetate by Clostridium acetobutylicum and production of butanediol from acetate by *Klebsiella aerogenes* had also been reported (Booth, 1985).

To sum up, the effect of acetate with concentration stimulating HH on this study was demonstrated that low contention of acetate could improve the 1,3-PD production efficiently for first time. The related research works of the metabolic pathway of acetic ions by *K. pneumoniae* are in progress in our laboratory.

3.5. 1,3-PD production with HH as cosubstrate in fed-batch fermentation

For large-scale application of HH and efficient production of 1,3-PD, fed-batch cultures of *K. pneumoniae* were performed with HH

Table 3
Effects of xylose or hemicellulosic hydrolysates on fed-batch fermentation by *K. pneumoniae* (the culture time was 37 h).[a]

Number	AGC (g/l)	AXC (g/l)	Products concentration (g/l)						Conversion (mol /mol)	Biomass (OD_{600})	Productivity (g/l/h)
			PD	BD	Suc	Ace	Eth	Lac			
1	143.16	–	60.78	4.1	5.81	9.76	9.24	31.17	0.52	1.64	10.45
	±5.24	–	±2.65	±0.21	±0.24	±0.39	±0.41	±1.56			
2	133.12	15.26	71.58	8.4	7.23	10.35	11.24	41.02	0.65	1.93	15.09
	±5.64	±0.69	±3.28	±0.40	±0.33	±0.45	±0.52	±1.87			

[a] Data with sign "±" are the average of three experiments. 1: the fermentation with glycerol alone, 2: the cofermentation of glycerol and hemicellulosic hydrolysates. AGC accumulative glycerol consumed, AXC accumulative xylose consumed (xylose come from the initial and concentrated hemicellulosic hydrolysates). Abbreviations: Lac, lactic acid; Eth, ethanol; Ace, acetic acid; BD, 2,3-butanediol; Suc, succinic acid; PD, 1,3-propanediol.

as cosubstrate. During fed-batch culture, the pH was controlled at 7.0 by automatic addition of 10 mol/l NaOH. In this study, we regulate the redox state variation online by the feeding rate (control the xylose from HH 5–8 g/l) during the fed-batch cofermentation. The results were shown in Table 3. HH added led to the rapid cell growth after 5 h (data not shown). The final biomass (37 h) with HH as cosubstrate reached 15.09 (OD_{600}) that was much higher than that (10.45) from glycerol alone.

HH added increased the 1,3-PD concentration, the conversion and productivity respectively from 60.78 to 71.58 g/l, 0.52 to 0.65 mol/mol, 1.64 to 1.93 g/l/h. The result was similar with that performed in batch fermentation, except for 2,3-BD and lactate. The lower 2,3-BD production was performed in fed-batch fermentation since the pH controlled at 7.0 was unfavorable for 2,3-butanediol formation. However, the lactate formation was preferable to the pH control condition (pH 7.0) and more reducing power and substrates (glycerol and HH) flowed to lactate pathway.

4. Conclusion

HH used as cosubstrate was efficient and economic for 1,3-PD biosynthesis process. HH added resulted in higher reducing power and more biomass for 1,3-PD fermentation. With the addition of HH, maintaining the xylose concentration at 5–8 g/l, 1,3-PD concentration, conversion and productivity increased to 71.58 g/l, 0.65 mol/mol, 1.93 g/l/h respectively in fed-batch fermentation, which were 17.8%, 25.0% and 17.7% higher than that from glycerol alone. Among the degradation products in HH including individual sugars and main inhibitors, xylose, mannose and acetate increased the 1,3-PD production efficiently by 18.0%, 11.6% and 33.8%, respectively.

Acknowledgements

This work was financially supported by the key program of National Natural science Foundation of China (No. 200936002), the national basic Research program of China (No. 2009CB724701), National High Technology Research and Development Program of China (No. 2006AA020103).

References

Abbad-Andaloussi, S., Amne, J., Ferard, P., Petitdemange, H., 1998. Effect of glucose on glycerol metabolism by *Clostridium butyricum*. J. Appl. Microbiol. 84, 515–522.

Booth, I.R., 1985. Regulation of cytoplasmic pH in bacteria. Microbiol. Rev. 49, 359–378.

Chen, X., Zhang, D.J., Qi, W.T., Gao, S.J., Xiu, Z.L., Xu, P., 2003. Microbial fed-batch production of 1,3-propanediol by *Klebsiella pneumoniae* under micro-aerobic conditions. Appl. Microbiol. Biotechnol. 63, 143–146.

Cheng, K.K., Liu, D.H., Sun, Y., Liu, W.B., 2004. 1,3-propanediol production by *Klebsiella pneumoniae* under different aeration strategies. Biotechnol. Lett. 26, 911–915.

Cheng, K.K., Liu, H.J., Liu, D.H., 2005. Multiple growth inhibition of *Klebsiella pneumoniae* in 1,3-propanediol fermentation. Biotechnol. Lett. 27, 19–22.

Ezeji, T., Qureshi, N., Blaschek, H.P., 2007. Butanol production from agricultural residues: Impact of degradation products on *Clostridium beijerinckii* growth and butanol fermentation. Biotechnol. Bioeng. 97, 1460–1469.

He, Q., Lokken, P.M., Chen, S., Zhou, J.Z., 2009. Characterization of the impact of acetate and lactate on ethanolic fermentation by *Thermoanaerobacter ethanolicus*. Bioresour. Technol. 100 (23), 5955–5965.

Horváth, I.S., Franzén, C.J., Mohammad, J., Taherzadeh, M.J., Niklasson, C., Lidén, G., 2003. Effects of furfural on the respiratory metabolism of *Saccharomyces cerevisiae* in glucose-limited chemostats. Appl. Environ. Microbiol. 69, 4076–4086.

Huang, H., Gong, C.S., Tsao, G.T., 2002. Production of 1,3-propanediol by *Klebsiella pneumoniae*. Appl. Biochem. Biotechnol. 98–100, 687–698.

Ji, X.J., Huang, H., Du, J., Zhu, J.G., Ren, L.J., Hu, N., Li, S., 2009. Enhanced 2, 3-butanediol production by *Klebsiella oxytoca* using a two-stage agitation speed control strategy. Biores. Technol. 100, 3410–3414.

Lü, F., He, J.P., Shao, L.M., Lee, H.D., 2008. Stress of pH and acetate on product formation of fermenting polysaccharide-rich organic waste. Biochem. Eng. J. 39, 97–104.

Martinez, A., Rodriguez, M.E., York, S.W., Preston, J.F., Ingram, L.O., 2000. Effects of $Ca(OH)_2$ treatments ("overliming") on the composition and toxicity of bagasse hemicellulose hydrolysates. Biotechnol. Bioeng. 69, 526–536.

Miseta, A., Tokes-Fuzesi, M., Aiello, D., Bedwell, D., 2003. A Saccharomyces cerevisiae mutant unable to convert glucose to glucose-6-phosphate accumulates excessive glucose in the endoplasmic reticulum due to core oligosaccharide trimming. Eukaryot Cell. 2, 534–541.

Nakamura, C.E., Whited, G.M., 2003. Metabolic engineering for the microbial production of 1,3-propanediol. Curr. Opin. Biotechnol. 4, 454–459.

Petrov, K., Petrova, P., 2009. High production of 2, 3-butanediol from glycerol by *Klebsiella pneumoniae* G31. Appl. Microbiol. Biotechnol. 84, 659–665.

Ragauskas, A.J., Williams, C.K., Davison, B.H., Britovsek, G., Cairney, J., Eckert, C.A., Frederick, W.J., Hallett, J.P., Leak, D.J., Liotta, C.L., Mielenz, J.R., Murphy, R., Templer, R., Tschaplinski, T., 2006. The path forward for biofuels and biomaterials. Science 311, 484–498.

Ragout, A., Sineriz, F., Diekmann, H., Valdez, G.F., 1996. Shift in the fermentation balance of *Lactobacillus reuteri* in the presence of glycerol Fermentation. Biotechnol. Lett. 18, 1105–1108.

Saha, B.C., 2003. Hemicellulose bioconversion. J. Ind. Microbiol. Biotechnol. 30, 279–291.

San, K.Y., Bennett, G.N., Berríos-Rivera, S.J., Vadali, R.V., Yang, Y.T., Horton, E., Rudolph, F.B., Sariyar, B., Blackwood, K., 2002. Metabolic engineering through cofactor manipulation and its effects on metabolic flux redistribution in *Escherichia coli*. Metab. Eng. 4, 182–192.

Sato, K., Yoshida, Y., Hirahata, T., 2000. On-line measurement of intracellular ATP of *Saccharomyces cerevisiae* and pyruvate during sake mashing. J. Biosci. Bioeng. 90, 294–301.

Selembo, P.A., Perez, J.M., Lloyd, W.A., Logan, B.E., 2009. Enhanced hydrogen and 1,3-propanediol production from glycerol by fermentation using mixed cultures. Biotechnol. Bioeng. 104, 1098–1106.

Stanley, P.E., 1986. Extraction of adenosine triphosphate from microbial and somatic acid. Methods Enzymol. 133, 14–22.

Tong, I., Cameron, D.C., 1992. Enhancement of 1,3-propanediol production by cofermentation in *Escherichia coli* expressing *Klebsiella pneumoniae* dha regulon gene. Appl. Biochem. Biotech. 34–35, 149–159.

Xu, Y.Z., Guo, N.N., Zheng, Z.M., Ou, X.J., Liu, H.J., Liu, D.H., 2009. Metabolism in 1,3-propanediol fed-batch fermentation by a D-Lactate deficient mutant of *Klebsiella pneumoniae*. Biotechnol. Bioeng. 104, 965–972.

Xu, Q., Li, S., Fu, Y.Q., Tai, C., Huang, H., 2010. Two-stage utilization of corn straw by *Rhizopus oryzae* for fumaric acid production. Bioresour. Technol. 101, 6262–6264.

Xue, X.D., Li, W., Li, Z.M., Xia, Y.L., Ye, Q., 2010. Enhanced 1,3-propanediol production by supply of organic acids and repeated fed-batch culture. J. Ind. Microbiol. Biotechnol. 37, 681–687.

Yan, L.S., Zhang, H.M., Chen, J.W., Lin, Z.X., Jin, Q., Jia, H.H., Huang, H., 2009. Dilute sulfuric acid cycle spray flow-through pretreatment of corn stover for enhancement of sugar recovery. Bioresour. Technol. 100, 1803–1808.

Yang, G., Tian, J., Li, J., 2007. Fermentation of 1,3-propanediol by a lactate deficient mutant of *Kelbsiella oxytoca* under microaerobic conditions. Appl. Microbiol. Biotechnol. 73, 1017–1024.

Zeng, A.P., Biebl, H., Schlieker, H., Deckwer, W.D., 1993. Pathway analysis of glycerol fermentation by *Klebsiella pneumoniae*: Regulation of reducing equivalent balance and product formation. Enzyme. Microbiol. Technol. 15, 770–779.

Zhang, Y.P., Li, Y., Du, C.Y., Liu, M., Cao, Z.A., 2006. Inactivation of aldehyde dehydrogenase: A key factor for engineering 1,3-propanediol production by *Klebsiella pneumoniae*. Metab. Eng. 8, 578–586.

Zhang, Y.P., Huang, Z.H., Du, C.Y., Li, Y., Cao, Z.A., 2009. Introduction of an NADH regeneration system into *Klebsiella oxytoca* leads to an enhanced oxidative and reductive metabolism of glycerol. Metab. Eng. 11, 101–106.

Zheng, Z.M., Hu, Q.L., Hao, J., Xu, F., Guo, N.N., Sun, Y., Liu, D.H., 2008. Statistical optimization of culture conditions for 1,3-propanediol by *Klebsiella pneumoniae* AC 15 via central composite design. Biores.Technol. 99, 1052–1056.