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Production of 2,3-butanediol from acid hydrolysates of *Jatropha* hulls with *Klebsiella oxytoca*

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ABSTRACT

Jatropha hulls were successfully for the first time used as raw materials for the production of 2,3-butanediol via dilute sulfuric acid hydrolysis and fermentation with *Klebsiella oxytoca*. Two-step hydrolysis was used to effectively hydrolyze the hulls at 150 °C after pretreatment. In the first-step, hemicellulose was hydrolyzed under mild conditions (0.5 h, 1.5% acid) to avoid secondary decomposition. The remained cellulose was further hydrolyzed in the second-step under severer conditions (1 h, 4% acid). After hydrolysis, total hydrolysis yield was 64%, which was much higher than that (37%) from the first-step. Maximum yields of 2,3-butanediol and acetoin in flask experiments were 35.6% and 41.4% from the hydrolysates of the first- and second-step hydrolysis, equivalent to 71.2% and 82.8% of the theoretical values, respectively. Similar yields were obtained in a controlled bioreactor but with higher productivities. *Jatropha* hulls are attractive raw materials for the production of 2,3-butanediol with high yield.

1. Introduction

Jatropha curcas L. grows throughout arid and semiarid tropical regions and is widely accepted to be a promising biodiesel plant (Yang et al., 2010; Deng et al., 2010; Guo et al., 2011). One tonne of *Jatropha* seeds will provide about 350-L oil and 2.4 tonnes hulls (Sharma et al., 2009). As more and more *Jatropha* seeds are used to extract crude oil for biodiesel, a large amount of hulls is generated. How to make full uses of these hulls is important for *Jatropha* biodiesel industry. Sharma et al. (2009) used *Jatropha* hulls for bioactive compost production. Duan et al. (2011) used them for the preparation of activated carbon. As *Jatropha* hulls are rich in carbohydrate, further studies are still required to utilize these polysaccharides to produce sugars for value-added products.

2,3-Butanediol can be produced by fermentation of sugars. It has potential applications in the production of printing inks, perfumes, fumigants, spandex, moistening and softening agents, explosives, plasticizers, foods and pharmaceuticals (Garg and Jain, 1995; Syu, 2001). Due to its comprehensive industry applications, 2,3-butanediol is a promising bulk chemical (Xiu and Zeng, 2008). However, the substrates account for more than half of the total production cost, and strongly influence the economy of 2,3-butanediol production (Wang et al., 2010; Celińska and Grajek, 2009; Ji et al., 2011). Consequently, it is necessary to develop an economical fermentation process based on low-cost raw materials. Some substrates such as food industry residues (e.g., starch and molasses) and glycerol (biodiesel by-product) were tested for 2,3-butanediol fermentation (Perego et al., 2003; Wang et al., 2010; Petrov and Petrova, 2009, 2010). Lignocellulosic biomass (e.g., water hyacinth, wood and corncob hydrolysates) also attracted considerable attentions as raw materials (Motwani et al., 1993; Cao et al., 1997; Cheng et al., 2010). However, most of works only used cellulose (Cao et al., 1997) or hemicellulose (Saddler et al., 1983; Cheng et al., 2010) fraction. On the other hand, relative low 2,3-butanediol concentration was obtained from these materials as compared with those using pure sugars as substrates (Li et al., 2010). No work was reported that used *Jatropha* hulls for 2,3-butanediol production.

In this work, fermentation of *Jatropha* hull hydrolysates was studied to assess their feasibility for 2,3-butanediol production. First, chemical components and composition of *Jatropha* hulls were analyzed. Two-step dilute sulfuric acid catalyzed hydrolysis was performed to effectively convert both hemicellulose and cellulose to fermentable sugars. The sugars were further fermented to 2,3-butanediol.

2. Methods

2.1. Materials

Jatropha hulls (Yang et al., 2010) were obtained from Xishuangbanna Tropical Botanical Garden in Yunnan, China. They

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were air-dried, milled then passed through a 20-mesh sieve. The *Jatropha* hull powders were washed with neutral detergent to remove extractives (e.g., proteins, lipids, pectins and nonfibrous carbohydrates). The neutral detergent was composed of $C_{12}H_{25}SO_4Na$ (30 g/L), Na_2EDTA (18.61 g/L), $Na_2B_4O_7{\cdot}10H_2O$ (10.81 g/L), Na_2HPO_4 (4.56 g/L) and 2-methoxyethanol (10 mL/L). *Jatropha* hull powders and neutral detergent were mixed with a ratio of 0.1 g/mL and heated at 100 °C for 1 h. The solid sample was collected by filtration, then washed with deionized water and dried as pretreated hulls for hydrolysis.

2.2. Hydrolysis of Jatropha hulls

A 500-mL high-pressure autoclave (FCFD05-30, Yantai Jianbang Chemical Mechanical Co. Ltd., Shandong, China) was used for the hydrolysis. In the first-step hydrolysis, *Jatropha* hull powders (20.0 g) and dilute sulfuric acid (200 mL; 0.5–2.5 wt.%) were loaded in the autoclave. Hydrolysis reaction was conducted at 120–160 °C for 0.5–3 h. After hydrolysis, solid residue was collected by filtration, and washed with distilled water to remove residual sulfuric acid, then air-dried for the second-step hydrolysis. In the second-step hydrolysis, the remained solid residue (25.0 g) from the first-step was hydrolyzed with higher concentrated sulfuric acid (200 mL; 2–10 wt.%) and temperatures (130–170 °C) for 0.5–2 h. The hydrolysates from the two steps were neutralized with calcium hydroxide to pH 7.0. Gypsum particles were removed by filtration. The hydrolysate solutions were concentrated under vacuum at 60–70 °C to achieve approximately sugar concentration of 100 g/L. The concentrated hydrolysate solutions (100 mL) were detoxicated in a 250-mL flask containing 2-g activated charcoal (Baoman Biochemical Co. Ltd., Shanghai). The flask was placed in a shaker at 50 °C and 200 rpm for 2 h. Finally, after removing the charcoal by filtration, the solutions were ready for fermentation.

2.3. Microorganism and media

The strain used for 2,3-butanediol production was *Klebsiella oxytoca* (CICC 22912) purchased from China Center of Industrial Culture Collection (Beijing). The culture was maintained on Luria–Bertani (LB) agar slant at 4 °C. The seed medium was composed of peptone (5 g/L), beef extract (3 g/L), NaCl (5 g/L) with pH 6.8–7.0. The fermentation medium was composed of sugar (80 g/L), urea (5.13 g/L), ethylenediamine tetraacetic acid (EDTA; 0.05 g/L), $MgSO_4{\cdot}7H_2O$ (0.5 g/L), $FeSO_4{\cdot}7H_2O$ (0.05 g/L), acetic acid (0.70 g/L), K_2HPO_4 (12.55 g/L) and KH_2PO_4 (3.9 g/L) that was obtained using response surface methodology in the previous work (Jiang et al., 2011). Urea was used as nitrogen source in fermentation because it was much cheaper than yeast extract, and only about half the cost of ammonium salts. Additionally, unlike ammonium salts, the metabolism of urea does not contribute to the acidification of medium, thus reduces the amount of base required for pH control (Ji et al., 2009a; Teixeira de Mattos and Neijssel, 1997).

2.4. Fermentation experiments in flasks and bioreactor

For seed preparation, a full loop of *K. oxytoca* from a fresh slant tube was inoculated in a 250-mL flask containing 50-mL fresh seed medium in a rotary shaker at 120 rpm for 12 h. Seed culture (5%, v/v) was then inoculated into the fermentation medium. Flask experiments were carried out in 250-mL flasks loaded with 50-mL sample solution at 150 rpm and 37 °C for 60 h. Fermentations were also conducted in a 3-L controlled bioreactor (BioTron, Korea) loaded with 1.5 L sample solution for 40 h. Similar to the previous work (Ji et al., 2009b), all cultivation conditions were at 37 °C and 200 rpm stirring with 1.5 L/min airflow. The value of pH was maintained at 6.5 by automatic addition of 3 M H_3PO_4 or 6 M KOH using a program-controlled peristaltic pump. Samples were collected periodically for product concentration analyses. The reported values were averaged from the triplicate and twice experiments in flask and the bioreactor, respectively.

2.5. Analytical methods

Chemical components and composition of biomass materials were analyzed by a Fiber Analyzer (ANKOM 200, USA) using Van Soest Fiber Analysis System (Goering and Soest, 1970) and an Organic Elemental Analyzer (Elementar Analysensysteme GmbH D-63452 Hanau, Germany). Gas chromatography–mass spectrometry (GC–MS) analysis was carried out with a Trace GC Ultra-DSQ system equipped with an Agilent DB-1MS column (30 m × 0.25 mm, 0.25 μm) and a flame ionization detector. Helium was used as carrier gas at a constant flow rate of 0.8 mL/min. Samples (1 μL) were injected at injector temperature of 250 °C. Oven temperature was 220 °C. The temperature of transfer line was held at 280 °C and that of the source at 250 °C. Electron impact mass spectra were acquired at 70 eV. Hydrolysate solutions of *Jatropha* hulls were filtrated and neutralized with NaOH before analysis. Dry cell weight (DCW, g/L) was determined by measuring the absorbance of broth at 600 nm using an ultraviolet (UV)–visible spectrophotometer (Shimadzu, UV1800, Japan). One unit of optical density was estimated to be equal to 0.45 g/L DCW. Reducing-sugar concentration of hydrolysate solutions was measured by 3,5-dinitrosalicylic acid (DNS) method using an UV–visible spectrophotometer (Miller, 1959). Glucose concentration was determined by a glucose analyzer (Biosensor SBA-50, Shandong). Concentrations of 2,3-butanediol and acetoin were measured by high-performance liquid chromatography (HPLC; LC-20A, Shimadzu, Japan) fitted with a refractive index detector and Aminex HPX-87H column (Bio-Rad, USA) at 60 °C with 0.005 M H_2SO_4 as mobile phase at a flow rate of 0.6 mL/min. Furfural and 5-hydroxymethylfurfural (5-HMF) were analyzed by an HPLC UV detector at 280 nm. The carbon amount in the aqueous phase was measured by a total organic carbon (TOC) analyzer (TOC-5000A, Shimadzu, Japan). Hydrolysis yield (wt.%) was calculated as follows:

$$\text{Hydrolysis yield } (\%) = (\text{carbon mass of water soluble organic compounds})/(\text{carbon mass of pretreated } \textit{Jatropha} \text{ hulls}) \times 100\%$$

Both 2,3-butanediol and its precursor acetoin were considered as the target products in 2,3-butanediol production, and expressed as diol yield (wt.%):

$$\text{Diol yield } (\%) = (\text{mass of } 2,3\text{-butanediol} + \text{mass of acetoin})/(\text{mass of consumed reducing sugars}) \times 100\%$$

3. Results and discussion

3.1. Chemical components and composition of Jatropha hulls

The main components and elemental composition of *Jatropha* hulls were analyzed and listed in Table 1. In original *Jatropha* hulls, organic elements (C, O, H, N, S) occupied 92.0 wt.% but three main biopolymer components (cellulose, hemicellulose and lignin) only accounted for 67.1 wt.%. Therefore, *Jatropha* hulls contained high content of light-molecular extractives that may produce many inhibitors after acid hydrolysis. In order to confirm its feasibility as feedstock, original *Jatropha* hulls were directly hydrolyzed at 150 °C for 0.5 h with 1.5 wt.% sulfuric acid. The hydrolysate solution was condensed and detoxicated, and used for fermentation experiment. After fermentated for 60 h, only 3.71 g/L DCW, 4.11 g/L diol were produced, with diol productivity of 0.069 g/L h and diol yield of 5.5%. It indicated that some chemicals in the hulls

Table 1
Main components and elemental composition of *Jatropha* hulls.

	Original material (wt.%)	Pretreated material (wt.%)
Components		
Cellulose	42.8 ± 0.6	58.4 ± 0.3
Hemicellulose	14.7 ± 0.5	20.9 ± 0.8
Lignin	9.6 ± 0.6	12.5 ± 0.3
Total	67.1	91.8
Elements		
Carbon	39.2 ± 2.6	41.8 ± 1.6
Oxygen	46.0 ± 0.7	46.7 ± 2.0
Hydrogen	5.4 ± 0.01	5.5 ± 0.2
Nitrogen	1.0 ± 0.1	0.3 ± 0.1
Sulfur	0.4 ± 0.2	0.2 ± 0.4
Total	92.0	94.5

after hydrolysis had significant unfavorable influence on the 2,3-butanediol metabolic pathway and biological activities. Many kinds of long-chain fatty acids and phenolics were detected in the extractives (by ethyl acetate) of original *Jatropha* hulls and their hydrolysates by GC–MS (Fig. S1). The main chemicals found were summarized in Table 2. These chemicals after hydrolysis were harmful for the fermentation. So, they need to be removed. Neutral detergent can easily dissolve extractives (e.g., proteins, lipids, pectins and nonfibrous carbohydrates), leaving fibrous residues that were primarily cell wall components (i.e., biopolymers: cellulose, hemicellulose and lignin) and indigestible nitrogenous matter (Mertens, 2002). So the neutral detergent was used in the pretreatment of original *Jatropha* hulls. After washed by neutral detergent, biopolymers increased to 91.8 wt.% (Table 1), while fatty acids in the pretreated hulls and phenolics in the hydrolysates were significantly reduced. So, the pretreated *Jatropha* hulls were used for all the following experiments.

3.2. First-step hydrolysis

Effects of reaction variables on hydrolysis including reaction time, temperature and sulfuric acid concentration were studied in the first-step hydrolysis (Fig. 1). In Fig. 1A, when reaction time increased from 0.5 to 2 h, concentration of reducing-sugars decreased slightly. Concentration of reducing-sugars decreased significantly at temperature higher than 160 °C or acid concentration higher than 1.5 wt.% (Fig. 1B and C) due to the decomposition of sugars at severe conditions. The degradation products such as 5-HMF and furfural were fermentation inhibitors. Therefore, the optimal conditions for the first-step hydrolysis were selected as: reaction time 0.5 h, temperature 150 °C and acid concentration 1.5 wt.%. At the conditions, the maximum reducing-sugar concentration was 34.01 g/L, containing 10.27 g/L glucose. The hydrolysate solution also contained 0.26 g/L furfural and 0.14 g/L 5-HMF. Concentration of soluble carbon was 15.35 g/L and hydrolysate yield was 37.0%. After the first-step hydrolysis, the remained solid residue contained only 0.52% hemicellulose but 69.08% cellulose by the fiber analyzer. This indicated that hemicellulose was nearly completely hydrolyzed with little hydrolysis of cellulose. In the first-step, less severe conditions were used to hydrolyze amorphous hemicellulose to avoid secondary decomposition. The second-step at severe conditions was needed for the hydrolysis of the remained cellulose in the solid residue.

Table 2
Main chemicals in *Jatropha* hulls and their hydrolysates extracted by ethyl acetate and analyzed by GC–MS in Fig. S1.

	Retention time (min)	Molecular formula	Relative content (area %)
Fig. S1A	4.07	$C_{16}H_{32}O_2$	21.0
	6.18	$C_{18}H_{34}O_2$	45.6
	7.67	$C_{21}H_{42}O_2Si$	2.3
Fig. S1B	2.76	$C_{11}H_{14}O_4$	4.4
	3.88	$C_{12}H_{18}O_4$	2.1
	5.08	$C_{10}H_6N_4S$	1.1
	8.30	$C_{14}H_{12}O_4$	0.8
Fig. S1C	1.97	$C_{12}H_{26}O$	1.1
	2.50	$C_{16}H_{34}O$	1.2
	6.02	$C_{18}H_{34}O_2$	0.4
Fig. S1D	2.39	$C_{10}H_{14}O_3$	1.6
	3.88	$C_{12}H_{18}O_4$	0.4

3.3. Second-step hydrolysis

Cellulose with crystal structure was more difficult to be hydrolyzed. Effects of reaction variables on hydrolysis of the solid residue from the first step were illustrated in Fig. 2. Concentration of reducing-sugars was significantly affected when temperature increased from 130 to 170 °C and acid concentration from 2 to 10 wt.%. The optimal conditions were selected as: reaction temperature 150 °C, acid concentration 4 wt.% and reaction time 1 h. The maximum reducing-sugar concentration was 31.77 g/L, containing 31.29 g/L glucose. The hydrolysate solution also contained 0.33 g/L 5-HMF. Soluble carbon concentration in the second-step hydrolysate solution was 20.50 g/L. After the two-step hydrolysis, total hydrolysis yield was 64.0%, which was much higher than that (37.0%) from the first-step hydrolysis only.

3.4. Fermentation

After condensation and detoxification by charcoal adsorption, furfural and 5-HMF were not detected. This result was consistent with the report of Cheng et al. (2010).

To evaluate the 2,3-butanediol fermentability, the hydrolysate was used for 2,3-butanediol production. As shown in Fig. 3A, fermentation in flasks was undertaken using the collected first-step hydrolysate with the initial reducing-sugar concentration of 74.4 g/L. After 60 h fermentation, 4.8 g/L DCW and 25.03 g/L diol were achieved, giving a diol productivity of 0.42 g/L h and a yield of 35.6% (equivalent to 71.2% of the theoretical value). All the fermentation data were summarized in Table 3. After washed with neutral detergent, diol yield increased by 5.5 times (35.6% *vs.* 5.5%). The concentration and yield of target products were much higher than those for the untreated hulls, which indicated that the treatment with neutral detergent had significant influence on the fermentation reactions. Co-fermentation experiments of glucose and xylose mixtures were performed previously for 2, 3-butanediol production (Ji et al., 2009a). Under the optimum conditions, 25.45 g/L diol were obtained, giving a maximal diol yield of 42.8% (or 85.6% of theoretical value) that was higher than the present study with *Jatropha* hulls (Table 3). But, the pure glucose and xylose were much expensive than the hulls. In the work of Grover et al. (1990), wood acid hydrolysate which was neutralized with $Ca(OH)_2$ had been used for 2,3-butanediol production, and relatively 2,3-butanediol concentration was achieved, obtaining 13.3 g/L 2,3-butanediol with a yield of 29% and a productivity of 0.28 g/L h. Their diol yield was much lower than this work. Cheng et al. (2010) used corncob acid hydrolysate as substrate, which was detoxificated by boiling, overliming and activated charcoal adsorption. A maximal 35.7 g/L 2,3-butanediol was obtained after 60 h of fed-batch fermentation, giving a diol yield of 50% and a productivity of 0.59 g/L h. However, this process mainly used the hemicellulose hydrolysate and most cellulose fraction was wasted.

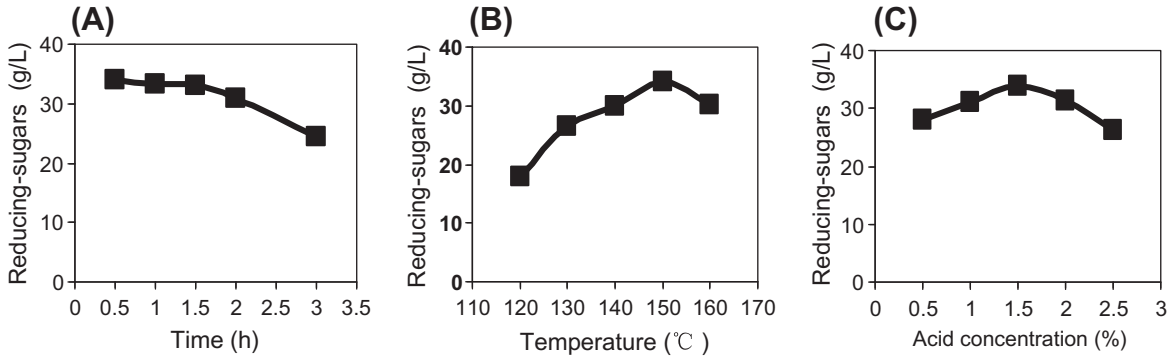

Fig. 1. Effects of reaction variables on hydrolysis in the first-step: (A) reaction time (reaction temperature 150 °C, acid concentration 1.5%); (B) reaction temperature (reaction time 0.5 h, acid concentration 1.5%); (C) acid concentration (reaction time 0.5 h, reaction temperature 150 °C).

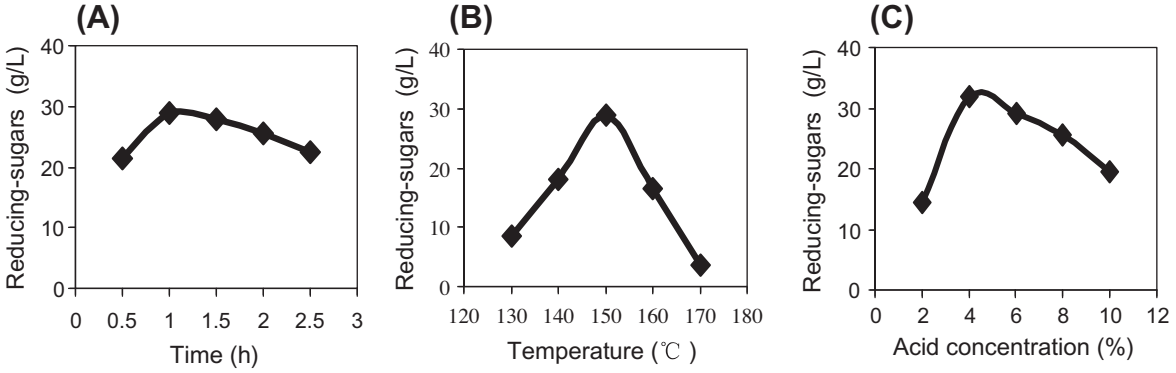

Fig. 2. Effects of reaction variables on hydrolysis in the second-step: (A) reaction time (reaction temperature 150 °C, acid concentration 6%); (B) reaction temperature (reaction time 1 h, acid concentration 1.5%); (C) acid concentration (reaction time 1 h, reaction temperature 150 °C).

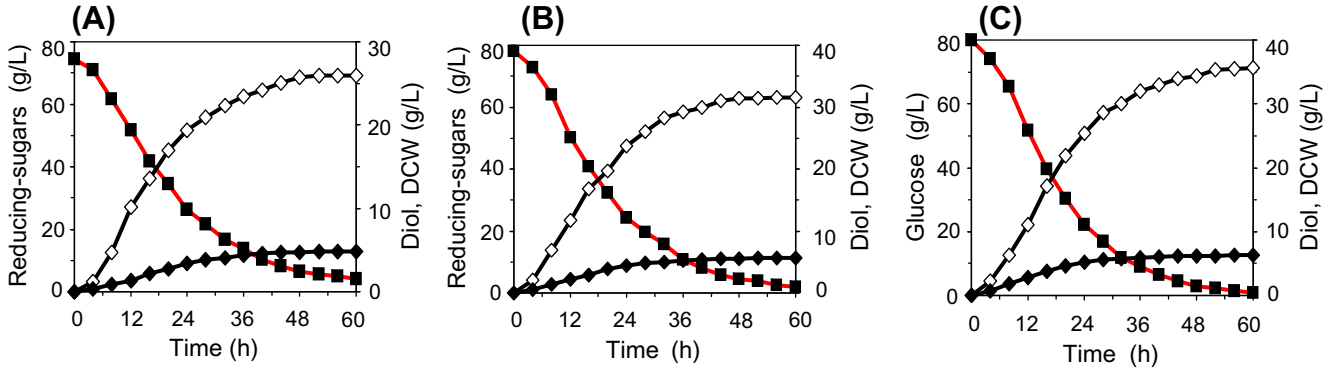

Fig. 3. Production of 2,3-butanediol by *K. oxytoca* fermentation for 60 h using different substrates in flasks: (A) hydrolysate solution from the first-step hydrolysis; (B) hydrolysate solution from the second-step hydrolysis; (C) glucose solution. Symbols: reducing-sugars (■); DCW (♦); diol (◇).

As shown in Fig. 3B, flask fermentation was undertaken using the collected second-step hydrolysate with the initial reducing-sugar concentration of 78.2 g/L. After 60 h fermentation, 5.66 g/L DCW and 31.57 g/L diol were produced, giving a diol productivity of 0.53 g/L h and a yield of 41.4% (equivalent to 82.8% of the theoretical value). As comparison, fermentation was undertaken using glucose as substrate with initial concentration of 80 g/L (Fig. 3C). After fermentation for 60 h, 6.26 g/L DCW and 35.62 g/L diol were produced, giving a diol productivity of 0.59 g/L h. The diol yield reached 45.1%, corresponding to 90.2% of the theoretical value. The concentrations of products were similar to those from the second-step hydrolysis. This indicated that after treated by neutral detergent, acid hydrolysis and detoxification by charcoal adsorption, the inhibitors in the hydrolysate were removed cleanly to some extent. Cao et al. (1997) used corncob as substrate. After pre-treatment with dilute ammonia and hydrochloric acid, 90% of cellulose was hydrolyzed to glucose, which was then used as the substrate for 2,3-butanediol fermentation. A 2,3-butanediol concentration of 25 g/L was obtained, giving a diol yield of 31% and productivity of 0.36 g/L h. However, the conversion of corncob into 2,3-butanediol made use of the cellulose fraction only, and the hemicellulose fraction was wasted.

Table 3
Production of 2,3-butanediol from various substrates by different microorganisms.

Substrates	Organisms	2,3-Butanediol + acetoin			References
		Concentration (g/L)	Productivity (g/L h)	Yield (%)	
Glucose and xylose	*K. oxytoca*	25.45	0.58	85.6 (42.8)[a]	Ji et al. (2009a)
Wood acid hydrolysate	*K. pneumoniae*	13.3	0.28	58 (29)[a]	Grover et al. (1990)
Corncob acid hydrolysate	*K. oxytoca*	35.7	0.59	100 (50)[a]	Cheng et al. (2010)
Corncob cellulose hydrolysate	*K. oxytoca*	25.0	0.36	62 (31)[a]	Cao et al. (1997)
Fermentation in flasks					
Original *Jatropha* hulls hydrolysate	*K. oxytoca*	4.11	0.069	11 (5.5)[a]	This study
First-step hydrolysate from pretreated *Jatropha* hulls	*K. oxytoca*	25.03	0.42	71.2 (35.6)[a]	This study
Second-step hydrolysate from the solid residue of the first step	*K. oxytoca*	31.57	0.53	82.8 (41.4)[a]	This study
Glucose	*K. oxytoca*	35.62	0.59	90.2 (45.1)[a]	This study
Fermentation in bioreactor					
First-step hydrolysate from pretreated *Jatropha* hulls	*K. oxytoca*	26.26	0.66	73.4 (36.7)[a]	This study
Second-step hydrolysate from the solid residue of the first step	*K. oxytoca*	31.41	0.79	80.4 (40.2)[a]	This study
Glucose	*K. oxytoca*	36.21	1.13	91.6 (45.8)[a]	This study

[a] *Note:* The data in brackets are actual diol yields, which are equivalent to (actual diol yields)/0.5 of their corresponding theoretical values.

Furthermore, controlled experiments in the bioreactor were conducted and similar results as those in flasks but with higher productivities were obtained (Table 3 and Fig. 4). Total fermentation time decreased from 60 in flasks to 40 h in bioreactor (Fig. 3 *vs.* Fig. 4) due to the continuous feeding of air for promoting bioreactions.

In the present study, two-step dilute sulfuric acid hydrolysis was performed. This strategy could not only effectively convert *Jatropha* hulls to fermentable sugars but also provide an opportunity to study the influence of the two acid hydrolysis steps on sugar yield and the production of 2,3-butanediol. Concentrations of DCW and diol from the second-step hydrolysis were higher than those from the first-step. It can be explained that glucose was more suitable material for fermentation than other sugars (Saha and Bothast, 1999; Wang et al., 2010). Therefore, the diol productivity from the second-step hydrolysate solution was higher than that from the first-step. On the other hand, the solid residue after pretreated with neutral detergent and first-step hydrolysis may produce fewer inhibitors for fermentation.

Biological production of 2,3-butanediol on an industrial-scale is still in its early stage but with strong prospects of growth (Celińska and Grajek, 2009). The utilization of waste materials (e.g., *Jatropha* hulls) from renewable sources made the process economically feasible. Still, biological synthesis technology needs to be developed to compete with less expensive chemical routes. Based on the current study on *Jatropha* hulls, the main challenges are the high cost in pretreatment and hydrolysis processes. First of all, it was difficult to recover and reuse neutral detergent, which increases cost and causes pollution. Therefore, development of new pretreatment methods that can use low-cost and recyclable solvents (e.g., hydrothermal, supercritical CO_2, ionic liquid and other green solvent treatments) or detoxification techniques that can detoxicate the hydrolysate from untreated *Jatropha* hulls for fermentation, will lower the overall process cost. On the other hand, the two-step hydrolysis at 150 °C can be simplified to one-step process that is performed at higher temperatures (180–190 °C) in a percolating semi-flow reactor to effectively hydrolyze both hemicellulose and cellulose and to avoid secondary decomposition by removing sugars continuously. This one-step dilute acid hydrolysis was commercialized even before 1950s in the former Soviet Union (Nanjing Forestry Institute, 1961) and could reduce hydrolysis cost. Although vacuum evaporation was successful in increasing sugar concentrations in this work, the process consumed high energy. Membrane techniques may be used to concentrate and separate sugars from hydrolysate solutions (Weng et al., 2010; Huang et al., 2008). All these novel techniques would offer significant opportunities to further decrease production cost.

4. Conclusions

In this study, *Jatropha* hulls were for the first time used as raw materials for 2,3-butanediol production via two-step acid hydrolysis and fermentation with *K. oxytoca*. Two-step hydrolysis resulted in a full utilization of hemicellulose and cellulose in *Jatropha* hulls. The hydrolysates from both steps were further fermented successfully to 2,3-butanediol in both flasks and bioreactor. It could be

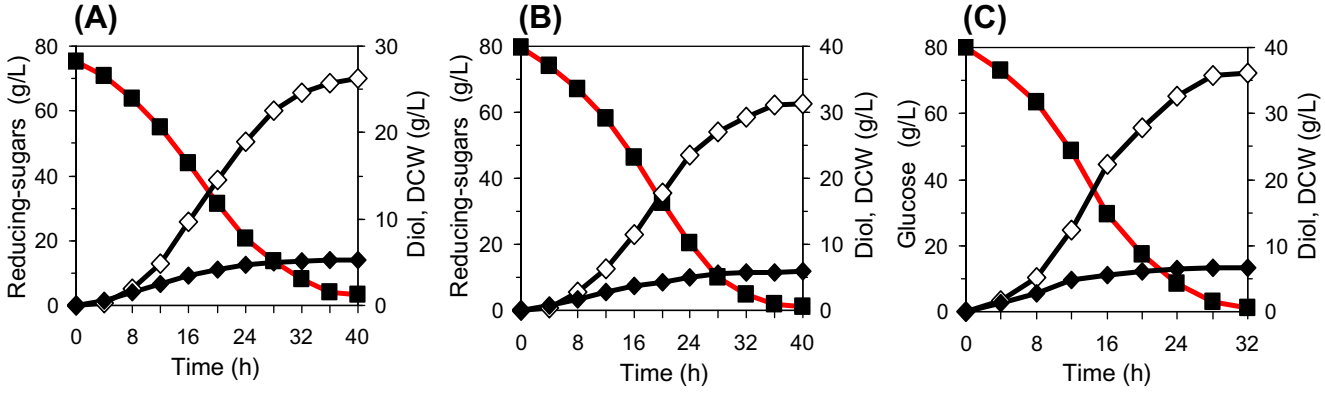

Fig. 4. Production of 2,3-butanediol by *K. oxytoca* fermentation for 40 h using different substrates in bioreactor: (A) hydrolysate solution from the first-step hydrolysis; (B) hydrolysate solution from the second-step hydrolysis; (C) glucose solution. Symbols: reducing-sugars (■); DCW (♦); diol (◇).

concluded that after pretreatment by neutral detergent, *Jatropha* hulls were attractive raw materials for the production of 2,3-butanediol with relative high yield.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biortech.2011.12.083.

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