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Full Length Research Paper

Bioconversion of paper sludge with low cellulosic content to ethanol by separate hydrolysis and fermentation

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The purpose of the present work was to evaluate the possibility of converting paper sludge into ethanol using xylose-fermenting yeast SHY07-1 in separate hydrolysis and fermentation. In the enzymatic hydrolysis step, sludge on 2% (w/v, expressed in terms of total carbohydrate mass) substrate consistency was incubated with the filter-sterilized enzyme solutions (Celluclast 1.5 L on a dosage of 15 FPU/g cellulose and Novozyme™188 on a dosage of 30 CBU/g cellulose) without any pretreatment, and a total degree of saccharification of 99.65% was achieved under certain conditions. The hydrolysate produced from enzymatically-digested paper sludge (on a consistency of 2% (w/v, expressed in terms of total carbohydrate mass) was fermented by yeast SHY07-1 without prior detoxification and nutrient supplementation, and xylose was consumed well. A final ethanol concentration of 14.18 g/L was achieved, corresponding to an ethanol yield of 0.49 g/g with a fermentation efficiency of 97.68%. The results indicate that paper sludge can be enzymatically hydrolyzed with high degree of saccharification and the hydrolysate can be fermented efficiently to ethanol by using xylose-fermenting yeast SHY07-1.

Key words: Paper sludge, separate hydrolysis and fermentation, ethanol, cellulase.

INTRODUCTION

Paper sludge (PS) is a solid waste material, which composed of pulp residues and ash generated from pulping and papermaking processes. It is estimated to produce approximately 4000 dry tons PS per day in China according to paper production (Kerstetter et al., 1997). The mills dispose of their sludge by either burning in boilers, landfilling or land application, which are prohibitively expensive and environmentally harmful end solutions (Park et al., 2001; Zheng et al., 1998). Interest has therefore grown in finding novel value-added uses for this residue from the paper industry (Kadar et al., 2004; Moritz and Duff, 1996; Oral et al., 2005).Most of the sludges tested have carbohydrate content (cellulose and hemicellulose) in the range of 20 to 75% on a dry weight basis, including some lignin, coatings and fillers. The high lignocellulosic content of this sludge material offers therefore an opportunity as feedstock for bio-products (Van and Mohulatsi, 2003). Compared to traditional un-pretreated fiber such as corncobs, wheat straw, and rice straw, the polysaccharides in PS are much more susceptible to enzymatic digestion (Fan and Lynd, 2007a, b; Domke et al., 2004; Lark et al., 1997; Waymam et al., 1992; Lee et al., 2004), since sludge has already been subject to an extensive mechanical and chemical processing previously imposed on the paper raw material through pulping (during refining, bleaching and drying) (Marques et al., 2008). Lark et al. (1997) reported production of 32 and 35 g/L ethanol from 180 g/L and 190 g/L PS, respectively in 250 ml flasks operated in batch mode. Fan et al. (2003) achieved 42 g/L ethanol at a

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Abbreviations: PS, Paper sludge; **SHF,** separate hydrolysis and fermentation; **HPLC,** high-performance liquid chromatography; **DS,** degree of saccharification; **FPU,** filter paper units; **CBU,** cellobiohydrase units; **SSF,** simultaneous saccharification and fermentation.

conversion of 92% from PS by a semi continuous, solids-fed reactor system. Marques et al. (2008) obtained 19.6 g/L ethanol at 179 h by separate saccharification and fermentation (SHF) and 18.6 g/L ethanol at 48 h from 178.6 g/L of dried PS by simultaneous saccharification and fermentation (SSF). Therefore, PS is believed to be one of the most promising feedstock for near-term commercial application of technology for converting cellulosic raw materials into commodity products, such as ethanol. Cellulosic content of PS decreased with the development of papermaking industry. How to recover the PS with low cellulosic content is a challenge to paper mills. However, little information on the ethanol production from PS with low cellulosic content can be found.

SHY07-1 yeast is an intergeneric protoplast fusant between *Saccharomyces cerevisiae* and *Pichia stipitis* developed in our lab, which possesses the ability to convert not only glucose, but also xylose, into ethanol (Zhu et al., 2010, 2011). In this study, the process involving SHF was investigated for bioconversion of PS with low cellulosic content to ethanol using commercial cellulase and xylose-fermenting yeast SHY07-1.

MATERIALS AND METHODS

PS used in this study was from paper mills by chemical pulping process (Jiangmen, Guangdong), which was kept at 4°C for short-term storage and at -20°C for long-term storage. The moisture content was approximately 75.2% (based on total weight basis)., and the main compositions of PS were determined to be (on a dry weight basis): 23.6% cellulose, 4.9% hemicellulose, 38.3% ash, 4.13% acid-soluble lignin, 1.23% acid-insoluble and 1.5% protein.

Microorganism and seed culture preparation

SHY07-1 yeast (an intergeneric protoplast fusant between *Saccharomyces cerevisiae* (a gift from Sanhe ethanol factory, Zanjiang, Guangdong province) and *Pichia stipitis* (a gift from Guangzhou Sugarcane Industry Research Institute, Guangzhou), which possesses the ability to convert not only glucose, but also xylose, into ethanol, was used in this study (Zhu et al., 2010). The strain was stored in a glycerol mixture at -80°C and was grown at 30°C for two days on a YPX-agar plate containing 20 g/L yeast extract (Oxoid, Ltd., Basingstoke, Hampshire, England), 10 g/L peptone (AoboXing Universeen BioTech company Ltd., Beijing, China), and 20 g/L xylose (QiYun BioTech Company, Ltd., Guangzhou, China). The medium was sterilized by steam autoclaving at 115°C for 20 min, and the strain was periodically subculture on YPX medium to maintain its activity and purity. Subculturing was performed once a month and the subcultures were stored under sterile conditions at 4°C.

The seed culture for the fermentation inoculum was prepared from culture by transferring a loopful of active SHY07-1 cells to 10 mL of YPX medium in sterile test tubes and incubating them at 30°C, 200 rpm for 12 to 18 h in an incubator shaker (C24KC refrigerated incubator shaker, Edison, New Jersey, United States). Then 10 ml of the active cells were aseptically transferred to 100 ml of sterile YPX medium in a 250 ml Erlenmeyer flask. The flask was incubated at 30°C, 200 rpm for 12 h in the same incubator shaker. After cultivation, the cells were harvested by centrifugation in a 50 ml sterilized centrifuge tube for 10 min at 5000 rpm using a TDL-5000B centrifuge (Shanghai Anke company, Ltd., China). The pellets were resuspended in a sterilized 0.9% NaCl solution to obtain a cell suspension with a cell mass concentration of 200 g wet weight per liter. The time between cell harvesting and initiation of the SHF was no longer than 2 h.

Composition analysis of PS

PS was analyzed gravimetrically for water (by oven drying at 105°C to constant weight) and ash content (by igniting at 575°C and burning for 5 h). Protein content was estimated by the Kjeldahl method using a nitrogen-to-protein conversion factor of 6.25. Hemicellulose and cellulose contents analysis was conducted according to the method described by Fan et al (Fan et al., 2003), and reducing sugars were measured by high-performance liquid chromatography (HPLC) using an Aminex HPX-87P column (300 mm × 7.8 mm) and a Carbo-P Micro-Guard column (30 mm × 4.6 mm, Bio-Rad, Hercules CA, United States), operating at 60°C with ultrapure water as the mobile phase at a flow rate of 0.6 ml/min.

Assay of enzyme activity

Total cellulase activity was described by filter paper units (FPU) with the Whatman No. 1 filter paper strip, 1.0 × 6.0 cm (approximately 50 mg) as a substrate. Xylanase activity in the cellulase was assayed using 1% (w/w) oat spelts xylan (Sigma, St. Louis, USA) as substrate. The total reducing sugars were estimated by the 3, 5-dinitrosalicylic acid (DNS) method (Miller, 1959). Enzyme activity was expressed in international units (U) as the amount of enzyme required to release 1 μmol of either glucose (FPU/ml) or xylose (U/ml) per minute under the assay conditions (pH 4.8, 50°C).

Determination of β-glucosidase (EC 3.2.1.21) enzyme activity was measured by HPLC. Because common sugar-determination methods such as DNS method couldn't distinguish cellobiose from glucose, both of which were reducing sugars. An international unit of β-glucosidase enzyme activities (cellobiohydrase units (CBU), IU / ml) was represented for the amount of enzyme required to produce 2 μmol glucose per minute in standard conditions (pH 4.8, 50°C).

Analysis of soluble carbohydrate and ethanol

For the determination of ethanol and monomeric sugars, reactor samples were acidified with 10 wt% sulfuric acid, and centrifuged at 12000 rpm for 15 min, and then filtered through a membrane filter of 0.22 μM pore size. The supernatant (pH 1~3) was analyzed for soluble sugar and ethanol using a Waters 2695 (Millford LA, USA) equipped with a refractive index detector. An Aminex HPX-87H column and a Cation H Cartridge Micro-Guard column (Bio-Rad, Hercules CA, United States) were used, operating at 60°C with 2.5 mM H_2SO_4 as the mobile phase at a flow rate of 0.6 ml/min (Lynd et al., 1989).

Enzymatic hydrolysis trials

The PS was enzymatically-hydrolyzed by commercial enzymes mixtures. The cellulase mixture consisted of Celluclast 1.5 L cellulolytic complex (Novozymes, Denmark, exhibiting a cellulase activity of 12.54 FPU/ml, a β-glucosidase activity of 26.42 CBU/ml and a xylanase activity of 0.97 U/ml) as well as Novozyme™188 (Sigma-Aldrich Co., 192 CBU/ml), a fungal β-glucosidase, was used to supplement the β-glucosidase activity of the cellulase. The PS

Table 1. Summary of parameters for four experiments examining the effects of pH; temperature; enzyme loadings and substrate loadings on enzymatic hydrolysis.

Experiment parameter	Effect of pH	Effect of temperature	Effect of enzyme loading	Effect of adding Novozyme™188	Effect of substrate loading
Initial pH	3.0, 4.0, 5.0, 6.0, 7.0	5.0	5.0	5.0	5.0
Temperature (°C)	40	30, 35, 40, 45, 50	40	40	40
Residence time (h)	96	96	96	72	96, 120, 144
Enzyme loadings (FPU/g cellulose)	25	25	15, 25, 35, 45	15	15
Novozyme™188* (CBU/g cellulose)	-	-	-	+	+
Substrate loadings %(w/v)	2	2	2	2	2,4,6

* "-"without Novozyme™188: "+" add Novozyme™188, and add Novozyme™188 in approximately a 1:2 ratio of cellulase units (15 FPU/g cellulose of cellulase, plus 30 CBU/g cellulose of Novozyme™188).

contained calcium carbonate that rendered the resulting suspensions alkaline. Therefore, PS was neutralized to different pH values with hydrochloric acid prior to use. The neutralized PS at 2% consistency (w/v, expressed in terms of total carbohydrate mass) was steam sterilized by autoclaving at 121°C for 30 min. Sludge was incubated with the filter-sterilized enzyme solution at different temperature (30, 35, 40, 45 and 50°C), different pH values (the natural pH value, 6.0, 5.0, 4.0 and 3.0), different enzymes loadings (15, 25, 35 and 45 FPU/g cellulose), in an orbital shaker (250 rpm) (Table 1). Aseptic conditions were maintained throughout the experiments. Each hydrolysis was conducted at a working volume of 50 ml in a 100 ml serum bottle. The hydrolysis mixture was centrifuged twice at 8000 rpm for 30 min to separate the liquid solution from unhydrolyzed solids. The supernatant (not sterilized again) was used for fermentation. The hydrolysates obtained, after residual solid removal by filtration (through a membrane filter of 0.22 µM pore size), were analyzed for sugar profiles by HPLC.

Fermentation studies

Hydrolysate (containing (g/L) 0.35 cellobiose, 21.99 glucose, and 6.77 xylose; some increasing sugars resulted from the addition of enzyme solution) obtained from sludge on 2% consistency (w/v, expressed in terms of total carbohydrate mass) was used as fermentation medium, with no prior detoxification and nutrient supplementation. The mock sugars mixture (total sugars, 110 g/L) contained 10 g/L cellobiose, 60 g/L glucose and 40 g/L xylose as well as 20 g/L yeast extract, 10 g/L peptone and 0.3% corn steep liquor.

Fermentations were conducted at a working volume of 50 ml in a 100 ml serum bottle, at different temperatures (25, 30, 35 and 37°C), different pH values (3.5, 4.5, 5.5 and 6.5) and different initial cell density (2, 4,6 and 8 g/L) in an orbital shaker (200 rpm) for 72 h (Table 2). Samples were taken during the course of fermentation. Glucose, xylose, cellobiose, and ethanol profiles were measured using HPLC as previously described. Experiments were performed in duplicate to ensure consistency and accuracy of results.

Calculations and statistical method

$$DS^{*}_{glucan} = \frac{\text{Glucose concentration}^{**}, g/L}{\text{Cellulose concentration}, g/L} \times \frac{162}{180} \times 100\%$$

$$DS_{xylan} = \frac{\text{Xylose concentration}^{**}, g/L}{\text{Hemicellubse concentration}, g/L} \times \frac{132}{150} \times 100\%$$

$$DS_{total} = \frac{(\text{Glucose concentration}^{**}, g/L + \text{xylose concentration}^{**}, g/L) \times 0.9}{\text{Substrate loadings}^{***}, g/L} \times 100\%$$

$$\text{Fermentation efficiency} = \frac{\text{Ethanol formed concentration, g/L}}{\text{Theoretical ethanol concentration}^{****}, g/L} \times 100\%$$

$$\text{Theoretical ethanol concentraton} = (\text{Glucose concentraton} + \text{Cellobiose concentraton} \times \frac{360}{342}) \times 0.51 + \text{Xylose concentraton} \times 0.46, g/L$$

*The degree of saccharification, ** sugars from enzyme solution was deducted from the concentration of sugar in hydrolysate, ***expressed in terms of total carbohydrate mass, ****sugars from enzyme solution were calculated in theoretical ethanol concentration.

All experiments were conducted in duplicate and data are presented as mean values ± standard deviation. Statistical analysis was carried out by the Microsoft Excel using Students t-test and results were considered statistically significant at 95% confidence ($p < 0.05$).

RESULTS

Enzymatic hydrolysis of PS

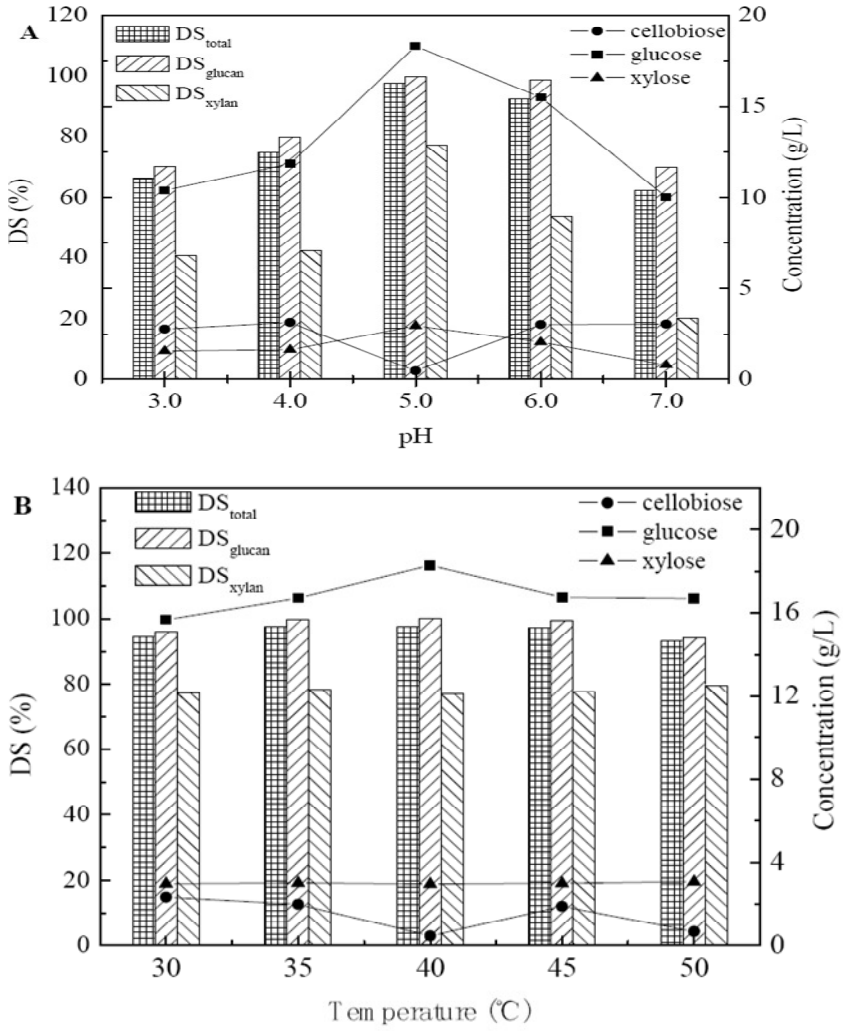
Trials were carried out to investigate the effect of (A) pH, (B) temperature, (C) enzyme loadings, and (D) substrate loadings on enzymatic hydrolysis through the calculation of DS. DS was based on the total sugar concentration in the final hydrolysate (corrected for the concentration in the control assay) relative to the content of polysaccharides (potential glucose and xylose) in the substrate.

Figure 1 shows the effect of different conditions on enzymatic hydrolysis. The concentration of glucose in hydrolysate and the DS_{total} rose first and decreased afterwards with the increasing pH from 3.0 to 7.0 (Figure

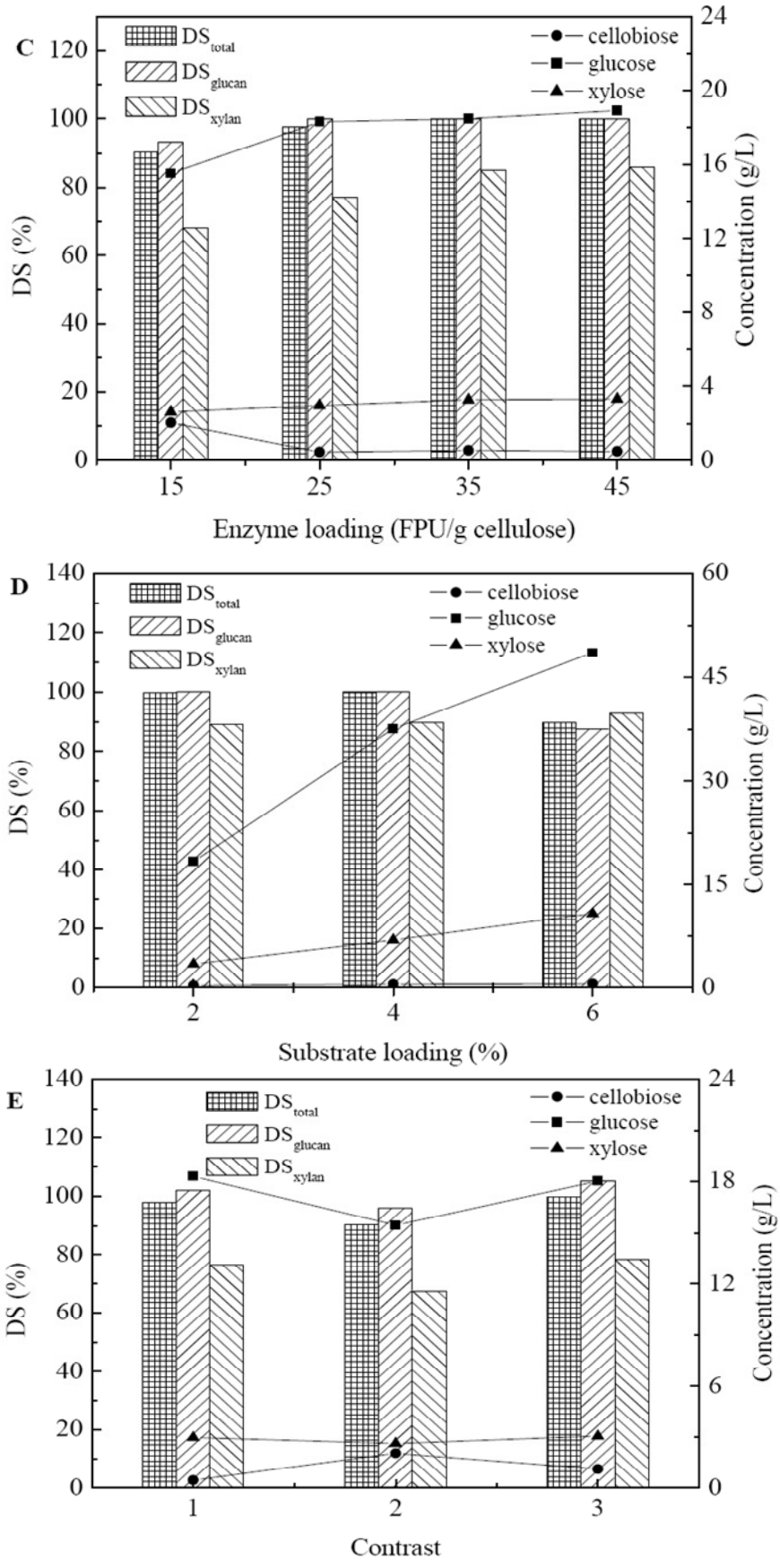
Table 2. Summary of parameters for four experiments examining the effects of pH, temperature, initial cell density, and nutrient supplementation on fermentation of hydrolysate from PS.

Experiment parameter	Effect of pH	Effect of temperature	Effect of initial cell density	Effect of nutrient supplementation
Initial pH	3.5, 4.5, 5.5, 6.5	5.5	5.5	5.5
Temperature, (℃)	35	25, 30, 35, 37	35	35
Residence time, (h)	72	72	72	72
Initial cell density, (g/L)	2	2	2, 4, 6, 8	2
Nutrient supplementation	-	-	-	+

* "-" Without nutrient supplementation; "+" nutrient supplementation with 3 g/L corn steep liquor.



1A). Similar trends were observed as temperature increased from 30 to 50℃ (Figure 1B). The glucose content and the DS_{total} increased by 18.45 and 8.09%, respectively as the enzyme loading increased from 15 FPU/g cellulose to 25 FPU/g cellulose, however, a slight increase could be seen when the enzyme loading increased from 25 FPU/g cellulose to 45 FPU/g cellulose (Figure 1C). Despite of the high degree of sludge hydrolysis, a lower concentration of reducing sugar was obtained due to a low substrate loading. In order to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DS (%)
Concentration (g/L)
DS_total
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Substrate loading (%)
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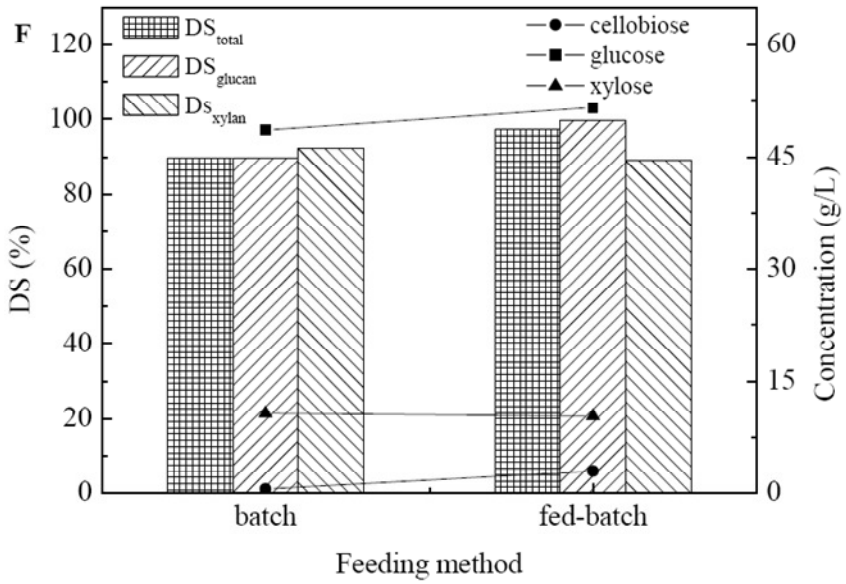


Figure 1. Effect of different conditions on enzymatic hydrolysis. The sugar concentration (cellobiose, glucose, xylose) in the final hydrolysate have been corrected for concentration in the control assay. (A) Effect of pH on enzymatic hydrolysis: Experiments were carried out with 2% substrate loading (w/v, expressed in terms of total carbohydrate mass) at 40°C for 96 h, and the enzyme loading was 25 FPU celluclast and 1.5 L/g cellulose. (B) Effect of temperature on enzymatic hydrolysis. Experiments were carried out at 2% substrate loading (w/v, expressed in terms of total carbohydrate mass) for 96 h with an initial pH 5.0, and the enzyme loading was 25 FPU celluclast 1.5 L/g cellulose. (C) Effect of enzyme loadings on enzymatic hydrolysis. Experiments were carried out with 2% substrate loading (w/v, expressed in terms of total carbohydrate mass) at 40°C for 96 h with an initial pH 5.0. (D) Effect of substrate loadings on enzymatic hydrolysis. 2%: experiments were carried out at 40°C for 72 h with an initial pH 5.0, and the enzyme loadings were: 15 FPU celluclast 1.5 L + 30 CBU Novozyme™188/g cellulose; 4%: experiments were carried out at 40°C for 120 h with an initial pH 5.0, and the enzyme loadings were: 15 FPU celluclast 1.5 L + 30 CBU Novozyme™188/g cellulose; 6%: experiments were carried out at 40°C for 120 h with an initial pH 5.0, and the enzyme loadings were: 25 FPU celluclast 1.5 L + 50 CBU Novozyme™188/ cellulose. (E) Effect of Novozyme™188 on enzymatic hydrolysis. Experiments were carried out at 2% substrate loading (w/v, expressed in terms of total carbohydrate mass) for 96 h with an initial pH 5.0. Contrast 1: enzyme loading was: 25 FPU celluclast 1.5 L/g cellulose; Contrast 2: enzyme loading was: 15 FPU celluclast 1.5 L/g cellulose; Contrast 3: enzyme loadings were: 15 FPU celluclast 1.5 L + 30 CBU Novozyme™188/g cellulose. (F) Effect of feeding method on enzymatic hydrolysis. Experiments were carried out with 6% substrate loading (w/v, expressed in terms of total carbohydrate mass) at 40°C for 96 h, and the enzyme loading was: 25 FPU celluclast 1.5 L/g cellulose. Fed-batch: 4% (w/v, expressed in terms of total carbohydrate mass) as for the substrate concentration, and began to flow 10 g/L substrates every 12 h until the substrate concentration reached 6%.

maximize the concentration of reducing sugar, the substrate concentration was increased to 6% (w/v, expressed in terms of total carbohydrate mass) and the hydrolysis time was extended to 144 h. However, it was not possible to have a suspension due to the high amount of solids. Sludge cannot be completely hydrolyzed after 144 h; DS_{total} reached only 89.92%, when the substrate loadings reached to 6% (Figure 1D). Although, the cellulase contains β-glucosidase activity, the activities of this enzyme are generally insufficient to prevent the accumulation of cellobiose, resulting in product inhibition of endoglucanases and cellobiohydrolases. Product inhibition is particularly problematic when high substrate consistencies are used in order to produce more concentrated glucose syrup for fermentation (Marques et al., 2008). Consequently, cellulase preparations were typically supplemented with Novozyme™188, a cellobiase (β-glucosidase), in order to

maximize the sludge conversion to monosaccharide (glucose) and to prevent cellobiose accumulation minimizing product inhibition (Tu et al., 2006). With this enzyme formulation, cellobiose decreased from the hydrolysate maintaining DS_{tatol} at 99.65%, indicating that almost all the cellulose present in the substrate was completely hydrolyzed to glucose (Figure 1E). Meanwhile, the addition of Novozyme™188 could reduce the amount of cellulase (from 25 FPU/g cellulose to 15 FPU/g cellulose) and shorten the hydrolysis time (from 96 to 72 h).

An increase of DS_{glucan} and DS_{total} by 11.18 and 8.35% respectively could be seen as compared batch process with fed-batch process, indicating that fed-batch process can contribute to enhance the degree of saccharification. Moreover, it was reducing the amount of enzyme loading (from 25 to 15 FPU/g cellulose) and shortening the hydrolysis time (from 144 to 120 h) (Figure 1F).

Fermentation of hydrolysates from PS

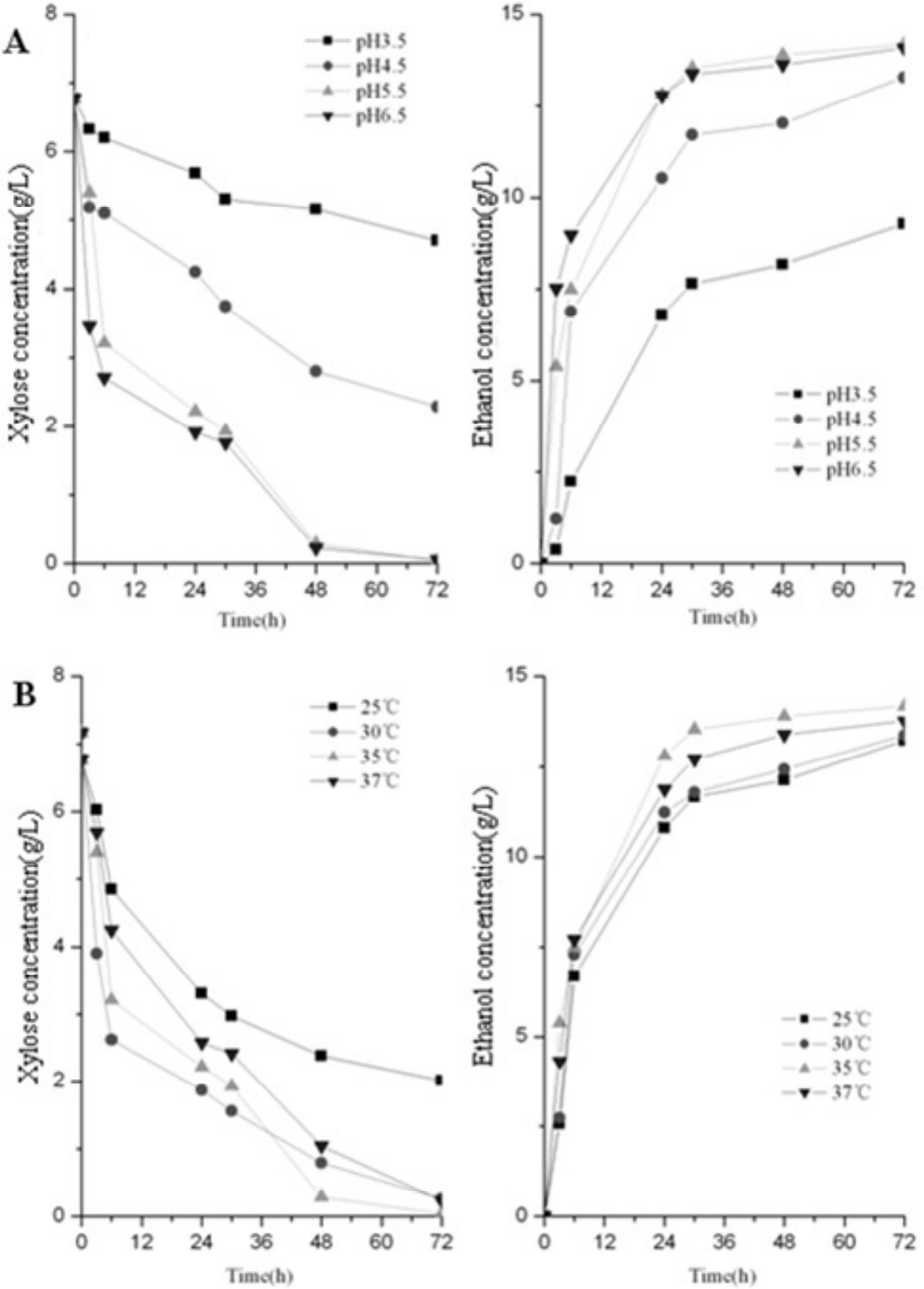
Several fermentations were carried out to investigate the effect of (A) pH, (B) temperature, (C) initial cell density, and (D) nutrient supplementation on fermentation of hydrolysates. Figure 2 shows the effect of different conditions on fermentation. Fermentation at pH 6.5 exhibited the highest xylose consumption rate among the tested pH values with the ethanol productivity of 1.2 g/ (L h) during the first 3 h. However, the ethanol concentration of 14.09 g/L, corresponding to an overall ethanol yield of 0.45 g/g, was somewhat less than those at pH 5.5, 14.18 g/L ethanol and overall ethanol yield of 0.46 g/g, respectively (Figure 2A). Therefore, pH 5.5 was determined to be the optimal pH. Regarding temperature optimization, fermentation at 30°C exhibited the highest xylose consumption rate with the ethanol productivity of 1.1 g / (L h) during the first 3 h. However, the overall ethanol yield of 0.43 g/g at 72 h was lower than 0.46 g/g at 35°C (Figure 2B). The initial xylose consumption rate was correlated to the initial cell density. However, the final ethanol yield was almost irrelevant to initial cell density among the tested cell densities. Relatively low cell density was used for further investigation (Figure 2C). Nutrient supplementation showed no significant effect on sugars consumption and ethanol production for the fermentation of the hydrolysate from PS (Figure 2D), indicating that the sludge hydrolysate contained the essential nutrients to support yeast growth and ethanol production, requiring no further supplementation (mainly N source).

The profiles over time obtained for the fermentation of pure sugars with certain nutrients are presented in Figure 3. Glucose can be consumed completely in 12 h and xylose consumption was slightly slower than glucose. However, xylose was also consumed completely in 24 h, which showed that SHY07-1 had good xylose-fermenting ability. The concentration of ethanol increased quickly with the consumption of glucose and xylose before 24 h. An ethanol yield of 0.49 g/g was the same as the fermentation of the hydrolysate without extra nutrient supplementation. However, the fermentation efficiency of 98.37% was slightly higher than 97.68% in the hydrolysate. It was indicated that the hydrolysate from PS with low cellulosic content was still suitable for the ethanol fermentation.

DISCUSSION

The purpose of the present work was to evaluate the possibility of converting PS with low cellulosic content, an industrial residue stream with strong environmental impact, into valuable ethanol fuels by SHF in our country. The results demonstrate that the biological conversion of sludge to ethanol was efficient even with no pretreatment, detoxification and nutrient supplementation. Although, high degree of saccharification was achieved in this study, further improvements in overall yield and titer required making this technology more commercially-attractive. This study confirmed that enzymatic hydrolysis of PS was the rate-limiting step in the SHF, and results presented herein suggested that efforts to increase the availability of fermentable sugars at high concentrations deserve the highest priority. Enzymatic hydrolysis at high solids loading was identified as the primary bottleneck affecting overall ethanol yield and titer (Lau and Dale, 2009). Since a progressive decrease on slurry viscosity was observed during the course of batch conversion, the bioconversion of PS might be conducted under fed-batch conditions, with periodic addition of fresh substrate, so as to overcome the limit to high substrate concentrations imposed by mixing constraints (Marques et al., 2008). This operational procedure will allow the achievement of higher yield and titer at lower enzyme loading, and therefore reducing the product recovery cost.

The SHF process was completed for 144 h (hydrolysis for 72 h and fermentation for 72 h) of incubation allowing the production of 14.18 g/L of ethanol from hydrolysate with the PS loadings of 2% (w/v, expressed in terms of total carbohydrate mass), which was considerably lower than the ethanol concentration (19.6 g/L) attained in the cited work (Marques et al., 2008). However, it has to be noted that these authors used a substantially higher substrate concentration in terms of carbohydrate mass (7.5% instead of 2% (w/v) in the present study). The ethanol production of 49.6 g/kg PS obtained in this study was lower than 109.7 g/kg PS as noted by Marques et al. (2008), which was mainly caused by low cellulosic content in our PS. The PS in our research consisted of 28% (w/w) of cellulose and hemicellulose, on a dry matter basis. This value is distinct from the average carbohydrate content found in a detailed analysis of 15 kinds of PS on a previously reported study (Lynd et al.,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Xylose concentration(g/L)
Time(h)
pH3.5
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Ethanol concentration(g/L)
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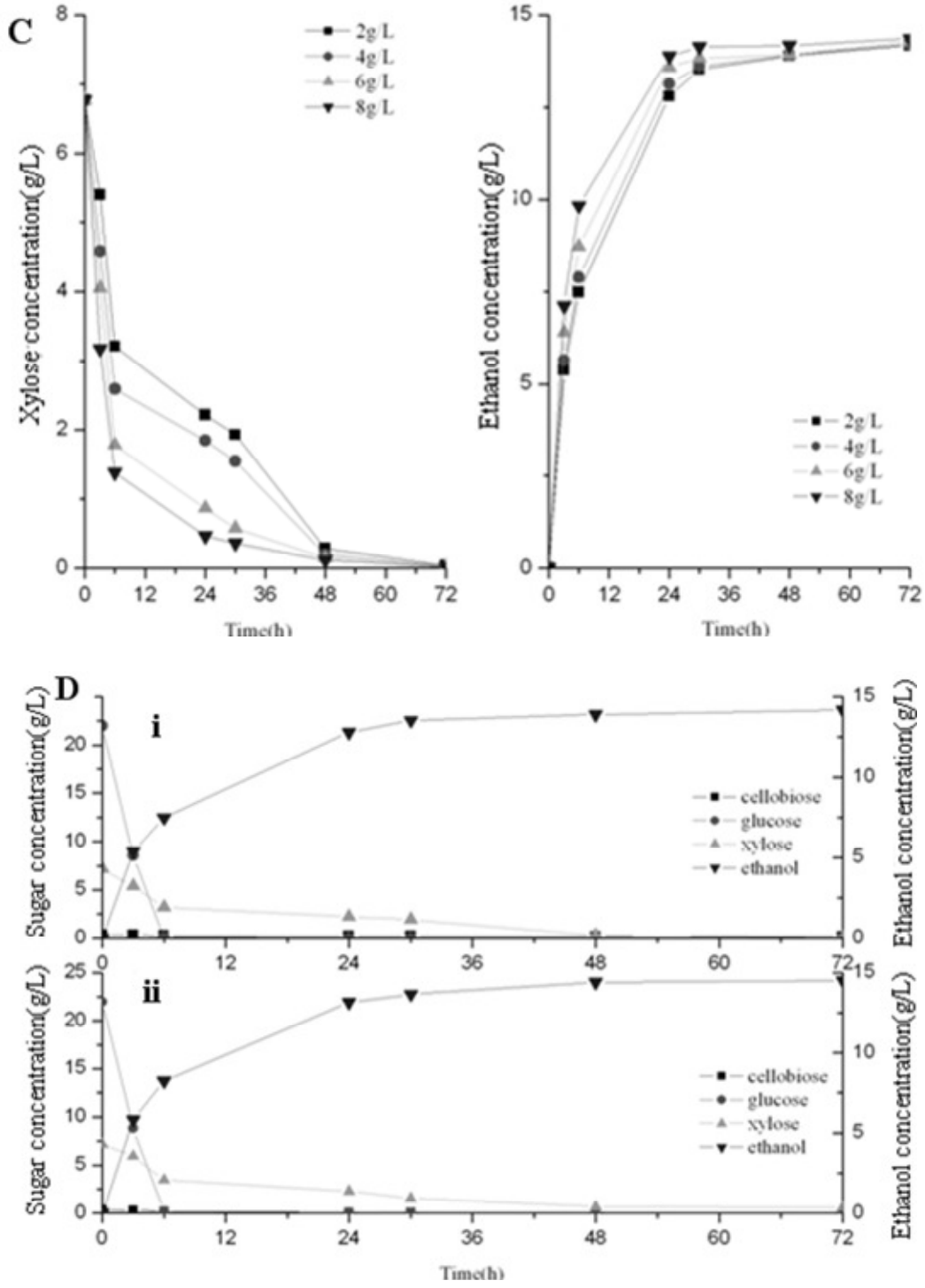

Figure 2. Effect of different conditions on fermentation of hydrolysates from enzymatically-digested PS. Hydrolysis experiments were conducted with 2% substrate loading (w/v, expressed in terms of total carbohydrate mass) at 40°C for 96 h, and the enzyme loading was: 25 FPU celluclast 1.5 L/g cellulose. (A) Effect of pH on fermentation of hydrolysates. Fermentation was conducted at 35°C for 72 h with an initial cell density of 2 g/L. (B) Effect of temperature on fermentation of hydrolysates. Fermentation was conducted at initial pH of 5.5 for 72 h with an initial cell density of 2 g/L. (C) Effect of initial cell density on fermentation of hydrolysates. Fermentation was conducted at 35°C for 72 h with an initial pH of 5.5. (D) Effect of nutrient supplementation on fermentation of hydrolysates. Fermentation was conducted at 35°C for 72 h with an initial pH of 5.5. (i) hydrolysate without corn steep liquor; (ii) hydrolysate with 3 g/L corn steep liquor.

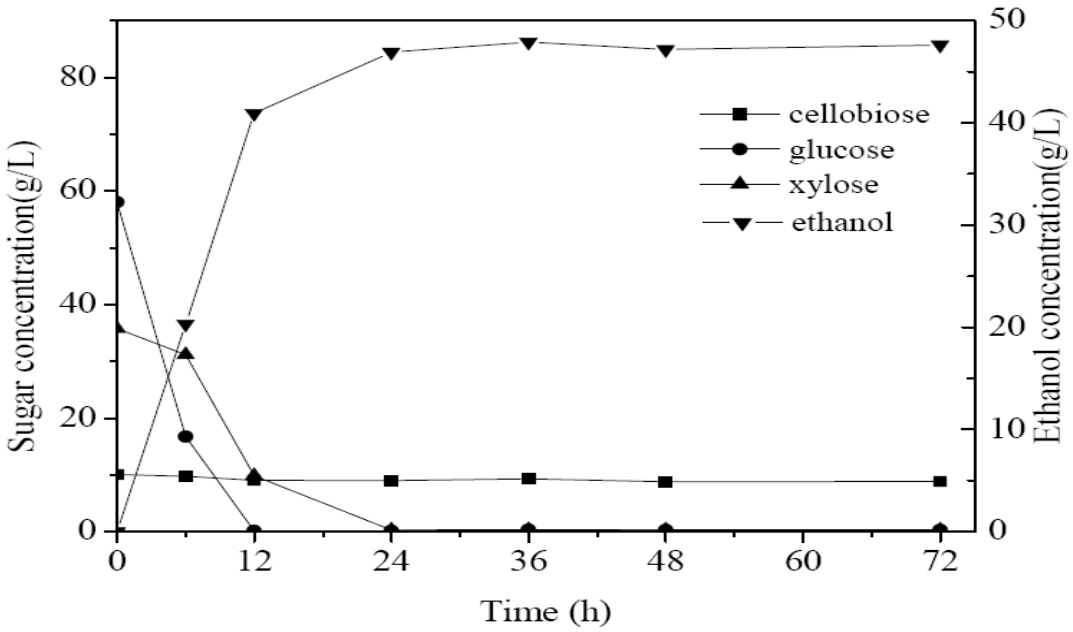

Figure 3. Trends over time of substrate and product concentrations in the fermentation of pure sugars medium. Results are means of duplicate experiments. Fermentations were conducted at 30°C, initial pH 5.5 and 200 rpm agitation for 72 h. 20 g/L yeast extract, 10 g/L peptone and 0.3% corn steep liquor were added in the mock sugars mixture (contained 10 g/L cellobiose, 60 g/L glucose and 40 g/L xylose).

2001), which resulted from the high ash content. It is generally considered that the following three steps should be completed prior to enzymatic hydrolysis (Lau and Dale, 2009): (1) enzyme diffuse in the liquid, (2) enzyme transfer to the substrate surface from the liquid, (3) enzymes were absorbed by cellulose and enzyme-cellulose complex were formed. A higher substrate loading might result in higher mass and heat transfer resistance in the liquid or between liquid and the solid, being not conducive to full contact between enzyme and substrate and sugar release, which thus affects the cellulose hydrolysis and lowers cellulose conversion rate (Marques et al., 2008; Lau and Dale, 2009).

Biotechnological recovery of this potentially attractive substrate requires the conversion of its major components (cellulose and hemicellulose) to fermentable sugars, which could be further converted to ethanol. This process may thereby represented an opportunity for the reduction of an important residue stream generated from the wastewater treatment unit of paper mills, having direct benefits in the reduction of land-filling costs while producing a commercial product. However, the chemical characteristics of the sludge are determined by the pulping process and source of furnish (Kerstetter et al., 1997). Outstanding issues for further evaluation of this possibility include economic analysis, which is underway, analysis of the amenability of hemicellulose sugars to conversion, assessment of the feasibility and profitability of mineral recovery from the residues remaining after enzymatic hydrolysis and on-site versus off-site cellulase production. In conclusion, PS represents a potential resource to be used by other industries to obtain useful value-added products. In order to realize industrialization as soon as possible, it is important to pursue more effective techniques to increase the conversion of cellulosic ethanol, cut back production costs and achieve the maximization of resource utilization.

Conclusion

This study thus far, confirmed the feasibility of PS with low cellulosic content from wastewater treatment as feedstock for conversion to ethanol fuels by separate hydrolysis and fermentation using xylose-fermenting yeast SHY07-1 as an ethanologenic strain. Biological conversion of the sludge to ethanol is efficient even without pretreatment, detoxification and nutrient supplementation. The optimum conditions of enzymatic hydrolysis were: pH 5.0, 40°C, 15 FPU cellulase/g cellulose and 30 CBU β-glucosidase/g cellulose while the optimum conditions of fermentation were: pH 5.5, 35°C and 2 g/L inoculum.

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