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Butanol production by *Clostridium beijerinckii*. Part I: Use of acid and enzyme hydrolyzed corn fiber

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Abstract

Fermentation of sulfuric acid treated corn fiber hydrolysate (SACFH) inhibited cell growth and butanol production (1.7 ± 0.2 g/L acetone butanol ethanol or ABE) by *Clostridium beijerinckii* BA101. Treatment of SACFH with XAD-4 resin removed some of the inhibitors resulting in the production of 9.3 ± 0.5 g/L ABE and a yield of 0.39 ± 0.015. Fermentation of enzyme treated corn fiber hydrolysate (ETCFH) did not reveal any cell inhibition and resulted in the production of 8.6 ± 1.0 g/L ABE and used 24.6 g/L total sugars. ABE production from fermentation of 25 g/L glucose and 25 g/L xylose was 9.9 ± 0.4 and 9.6 ± 0.4 g/L, respectively, suggesting that the culture was able to utilize xylose as efficiently as glucose. Production of only 9.3 ± 0.5 g/L ABE (compared with 17.7 g/L ABE from fermentation of 55 g/L glucose-control) from the XAD-4 treated SACFH suggested that some fermentation inhibitors may still be present following treatment. It is suggested that inhibitory components be completely removed from the SACFH prior to fermentation with *C. beijerinckii* BA101. In our fermentations, an ABE yield ranging from 0.35 to 0.39 was obtained, which is higher than reported by the other investigators.

Keywords: Corn fiber hydrolysate; Sulfuric acid; Butanol; Acetone butanol ethanol (ABE); *Clostridium beijerinckii* BA101

1. Introduction

There has been an increased interest in research on the bioconversion of agricultural biomass into fuels and chemical feedstocks for two primary reasons, one being the limited supply of fossil fuels and petroleum, and the other, the increasing and fluctuating prices of oil. In order to overcome these problems, research is focused on developing bioconversion processes for fuels and chemicals. An example is production of acetone butanol ethanol (ABE or solvents) from renewable agricultural crops such as corn (Qureshi and Blaschek, 2000). Other commercial substrates that can be used for this bioconversion process include molasses (Qureshi and Maddox, 1992) and whey permeate (Ennis and Maddox, 1987). However, because of cost and availability concerns, there is also an interest in using wood and agricultural residues as feedstock for ABE production. Solventogenic ABE-producing clostridia have an added advantage over many other cultures as they can utilize both hexose and pentose sugars (Singh, 1995), which are released from wood and agricultural residues upon hydro-

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lysis, to produce ABE. Parekh et al. (1988) produced ABE from hydrolysates of pine, aspen, and corn stover using *Clostridium acetobutylicum* P262. Similarly Marchal et al. (1984) used wheat straw hydrolysate and *C. acetobutylicum*, while Soni et al. (1982) used bagasse and rice straw hydrolysates and *C. saccharoperbutylacetonicum* to convert these agricultural wastes into ABE.

Corn fiber (CF) is an agricultural residue/by-product that is generated by the corn processing industry in the mid-western region of the United States. Approximately 6.3 million tons of dry CF is produced each year in the US from wet milling. Currently CF is sold as corn gluten feed for animals at less than 5 cents/kg. Based on a carbohydrate content of 59% (dry basis), 6.3×10^6 dry tons of CF would result in the production of 4.3×10^8 gallons of ABE of which acetone and ethanol would be 1.29×10^8 gallons and 4.33×10^7 gallons, respectively. From this process 260 million gallons butanol could be produced. This is based on the use of the *C. beijerinckii* BA101 butanol producing strain which results in an ABE yield of 0.40, and an acetone, butanol, ethanol ratio of 3:6:1 (Formanek et al., 1997). The worldwide production of butanol is 2.5×10^9 kg annually of which approximately one half is produced in the United States.

In a study by Qureshi and Blaschek (2000), it was determined that butanol can be produced for \$0.55/kg from corn at \$73/ton. At this price, fermentation – derived butanol cannot compete with petrochemically produced butanol. It was also shown that substrate price has the greatest impact on the price of butanol. Use of CF for butanol production is anticipated to be more economical than corn starch. CF contains protein, fat, starch, lignin, cellulose, and hemicellulose. While lignin and fat cannot be used by the solventogenic clostridia, cellulosic and hemicellulosic hydrolysates and starch can be used to produce butanol. The objective of the present study was to produce butanol from sulfuric acid treated corn fiber hydrolysate (SACFH) and enzyme treated corn fiber hydrolysate (ETCFH) using *C. beijerinckii* BA101, a hyper-butanol producing mutant strain.

2. Methods

2.1. Corn fiber hydrolysis

Corn fiber [moisture content 16.0% (w/w), starch content $0.36 \pm 0.1\%$ (w/w)] was obtained from A.E. Staley Manufacturing Company (Decatur, IL, USA). The CF was hydrolyzed by dilute sulfuric acid treatment (called SACFH), enzyme hydrolysis (called ETCFH), or both as described by Grohmann and Bothast (1997). Sixty three to 84 g CF (with 16.0% moisture) was soaked in 1 L of 0.5% (v/v) sulfuric acid (unless specified) for 15 min in a 2 L Pyrex screw capped bottle followed by heating at 121 °C for 60 min in an autoclave. Upon cooling, liquid samples were collected for total sugar analysis. Dilute sulfuric acid treatment at 121 °C hydrolyzes the xylan (composed primarily of pentose sugars) fraction of the fiber and leaves behind most of the cellulosic fraction unhydrolyzed (Grohmann and Bothast, 1997).

Hydrolysis of the cellulose fraction from both acid and hot water treated CF was accomplished via enzyme treatment. For hot water pretreatment which serves as pretreatment control, 63–84 g CF (with 16.0% moisture) was soaked in 1 L of distilled water for 15 min followed by autoclaving at 121 °C for 60 min. Hot water treatment releases some pentose from xylan while leaving behind cellulose. Prior to enzymatic treatment, the pH of both hot water pretreated CF and SACFH was adjusted to 4.5 using 10 M NaOH solution. This was followed by transferring the CF suspension to a 2 L bioreactor (New Brunswick Scientific Company, New Brunswick, NJ, USA) equipped with an agitator and temperature control. The temperature was controlled at 45 °C while agitating the mixture vigorously (200 rpm). At this stage enzyme suspensions [cellulase (celluclast – 0.7 FPU/g, density 1.2 g/mL: added 1 mL/100 g corn fiber) and cellobiase (Novozym 188; 250 U/g: added 1 mL/100 g corn fiber): both from Sigma Chemicals, St. Louis, MO, USA] were added to breakdown the cellulosic fraction. Enzymatic hydrolysis of hot water treated CF (ETCFH) and sulfuric acid treated CF (SACFH) was carried out for 72 h at 45 °C to generate enzyme treated corn fiber hydrolyzate (ETCFH) and sulfuric acid hydrolyzate (SACFH), respectively. Glucose and reducing sugars were measured during the course of hydrolysis (see Section 2.6).

2.2. Inhibitor removal

Solids from the cooled SACFH were removed using cheese cloth. The liquid was then centrifuged (Sorvall, Newtown, CT, USA) at 4000 rpm (1700*g*) for 15 min. The centrifuged supernatant (SACFH) was adjusted to pH 10 with $Ca(OH)_2$ followed by adding 1 g/L Na_2SO_3 (overliming). The mixture was heated to 90 °C for 30 min with intermittent mixing. This was followed by cooling to room temperature and adjusting pH to 7.0 with concentrated H_2SO_4. The hydrolysate was then centrifuged again at 4000 rpm (1700*g*) for 15 min. Fermentation inhibitors were removed by pumping the clear supernatant through a nonionic polymeric adsorbent resin [Amberlite XAD-4 (Sigma Chemicals St. Louis, MO)] column (510×15 mm, BioRad, Hercules, CA, USA) at a flow rate of 8 mL/min. The amount of XAD-4 resin packed in the column was 60 g. XAD-4 was prepared prior to use as recommended by the supplier. Approximately 400 mL SACFH was passed through the column. Previous studies suggested that 60–80% of furfural, hydroxymethyl furfural (HMF) and ferulic compounds (inhibitors) are removed under these conditions (Weil et al., 2002).

2.3. Organism, culture maintenance, and inocula preparation

A hyper-butanol producing mutant strain of *C. beijerinckii* BA101 was used in these studies (Annous and Bla-

schek, 1991; Formanek et al., 1997). A laboratory stock of *C. beijerinckii* BA101 was routinely maintained as spore suspensions in sterile double distilled water at 4 °C. *C. beijerinckii* BA101 spores (200 μL) were heat shocked for 10 min at 80 °C followed by cooling in ice cold water. Cells were inoculated into 20 mL tryptone–glucose–yeast extract (TGY) medium (in 50 mL screw capped Pyrex bottle) and incubated anaerobically for 15–16 h at 36 ± 1 °C (Formanek et al., 1997).

2.4. Fermentation

Fermentation studies were conducted in 125 mL screw capped bottles containing 100 mL of P2 medium with glucose, xylose or SACFH / ETCFH as carbon source/s. Prior to autoclaving the medium, the pH was adjusted to 6.8 using 1 M NaOH. The medium containing carbon source and yeast extract (1 g/L; Sigma Chemicals St. Louis, MO, USA) was sterilized at 121 °C for 15 min. On cooling to 35 °C under oxygen-free nitrogen atmosphere (in an anaerobic chamber), filter-sterilized P2 stock solutions [(buffer: KH_2PO_4, 50 g/L; K_2HPO_4, 50 g/L; ammonium acetate, 220 g/L), (vitamin: para-amino-benzoic acid, 0.1 g/L; thiamin, 0.1 g/L; biotin, 0.001 g/L), and (mineral: $MgSO_4 \cdot 7H_2O$, 20 g/L; $MnSO_4 \cdot H_2O$, 1 g/L; $FeSO_4 \cdot 7H_2O$, 1 g/L; NaCl, 1 g/L)] (Qureshi and Blaschek, 1999) were added (1 mL each) followed by inoculation with highly motile cells of *C. beijerinckii* BA101 (5 mL cell suspension in 100 mL medium). One mL samples were withdrawn at intervals for acetone butanol ethanol (ABE) and sugar analysis. Prior to analysis, the samples were centrifuged at 14,000*g* in a microcentrifuge for 1 min. Unless otherwise stated fermentation time is 72 h.

2.5. Experimental design

The experimental design is schematically illustrated in Fig. 1. Corn fiber was treated with dilute sulfuric acid, cellulosic enzymes, and sulfuric acid + enzymes (Section 2.1) to generate SACFH, ETCFH, and SACFH + enzyme treatments, respectively. The SACFH was pumped through an XAD-4 column to remove some of the inhibitory compounds (Section 2.2) to generate SACFH + XAD-4 treatment. These corn fiber hydrolysates were used as carbon sources for ABE fermentation by *C. beijerinckii* BA101. In addition, pure sugars (glucose, 25 and 55 g/L, and xylose, 25 g/L) were used for ABE fermentation by *C. beijerinckii* BA101. The ABE values obtained from the fermentation of pure sugars and corn fiber hydrolysates were compared.

2.6. Analyses

Cell concentration was estimated by optical density and cell dry weight method using a predetermined correlation between optical density (540 nm) and cell dry weight. ABE and acids (acetic and butyric) were measured using

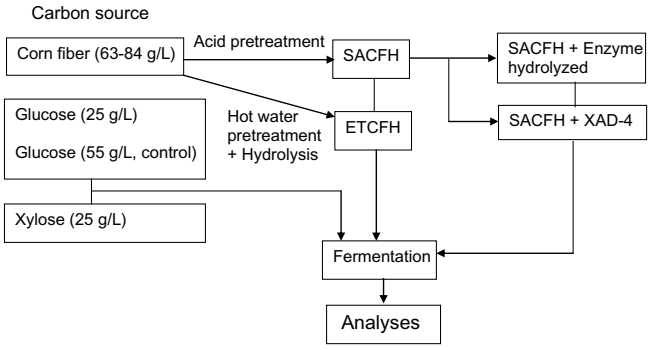

Fig. 1. The experimental design. SACFH, Sulfuric acid corn fiber hydrolysate; ETCFH, Enzyme treated corn fiber hydrolysate; SACFH + XAD-4, SACFH treated with Amberlite XAD-4 resin to remove inhibitory compounds.

a 6890 Hewlett–Packard gas chromatograph (Hewlett–Packard, Avondale, PA, USA) equipped with a flame ionization detector (FID) and 6 ft × 2 mm glass column (10% CW-20M, 0.01% H_3PO_4, support 80/100 Chromosorb WAW). Productivity was calculated as the maximum ABE concentration achieved (g/L) divided by the fermentation time (when fermentation ceased). Yield was defined as grams of ABE produced per gram of glucose or sugar utilized.

Glucose concentration was determined using a hexokinase and glucose-6-phosphate dehydrogenase (Sigma Chemicals, St. Louis, MO, USA) coupled enzymatic assay (Ezeji et al., 2005). Total reducing sugar concentration was measured according to the 3,5-dinitrosalicyclic acid method (Bergmeyer and Grassel, 1983; Jesse et al., 2002). One milliliter of SACFH/ETCFH was diluted 20-fold before following the measurement procedure. Starch content of the CF was determined as described by Holm et al., 1986; and Ezeji et al., 2005. CF (250 mg) was suspended in distilled water (15 mL) in a 50-mL beaker prior to adding 100 μL heat-stable α-amylase (Sigma Chemicals, St. Louis, MO, USA).

2.7. Statistical analyses

Multiple one-way analyses of variance (ANOVA) were conducted to investigate the effect of different corn fiber treatments on ABE production. The type of treatment was used as the independent variable while the dependent variables were ABE concentration, ABE productivity, ABE yield, cell concentration and cell yield. Unless otherwise stated all results were expressed as mean ± SD ($n \geqslant 3$). Pair-wise comparisons test the null hypotheses that two experiments have the same effect. Because multiple tests were conducted, Tukey's adjustment was applied to the pair-wise comparisons to determine significant difference ($P < 0.05$) among treatments. All the analyses were conducted using the General Linear (GLM) Model procedure of SAS Version 9.1.3 (SAS Institute Inc., Cary, NC, USA).

3. Results and discussion

3.1. ABE production in glucose-based medium

Prior to carrying out SACFH or ETCFH fermentations, control batch fermentation was conducted using 55 g/L glucose as the carbon source. Although the fermentation was run for 72 h, the culture stopped producing butanol within 60 h leaving behind 7.8 g/L residual glucose (Fig. 2). The fermentation likely stopped due to butanol inhibition. During 60 h of fermentation 18.1 ± 1.5 g/L total ABE was produced of which 13.2 g/L was butanol. The maximum cell concentration that was achieved during the fermentation was 3.37 ± 0.29 g/L. The fermentation resulted in a productivity and ABE yield of 0.30 ± 0.03 g/L h (based on 60 h fermentation) and 0.38 ± 0.006, respectively. Based on total glucose utilization, cell yield was 0.07 ± 0.006 g/g. The results obtained during the batch fermentation of glucose (55 g/L) showed that the ABE concentration, productivity, and yield are similar to previous batch fermentations conducted in our laboratory using this concentration of glucose (Ezeji et al., 2004).

3.2. ABE fermentation in non-detoxified SACFH-based medium

The result obtained following fermentation of the SACFH (total sugar concentration 29.8 g/L; glucose, 4.3 g/L) is shown in Table 1. A maximum cell concentration of 0.75 g/L was obtained as compared to 3.37 ± 0.29 g/L (Table 2) obtained in the control glucose fermentation. Cell growth data suggested that the culture was strongly inhibited by the hydrolysate. The culture did not produce more than 1.7 ± 0.2 g/L total ABE. The culture became acidogenic and produced more acetic acid compared to the control. Fermentation of sulfuric acid plus enzyme hydrolyzed CF (SACFH + enzyme, initial total sugar 54.3 g/L; glucose 22.4 g/L) did not produce more than 1.6 ± 0.4 g/L ABE (Table 2). In this experiment, a maximum cell concentration of 0.65 g/L was obtained. A comparison of the two fermentations suggested that presence of low sugar concentration (29.8 g/L) in the first experiment was not the reason for unsuccessful fermentation. Soni et al. (1982) reported that bagasse and rice straw hydrolysates were inhibitory to *C. saccharoperbutylacetonicum*.

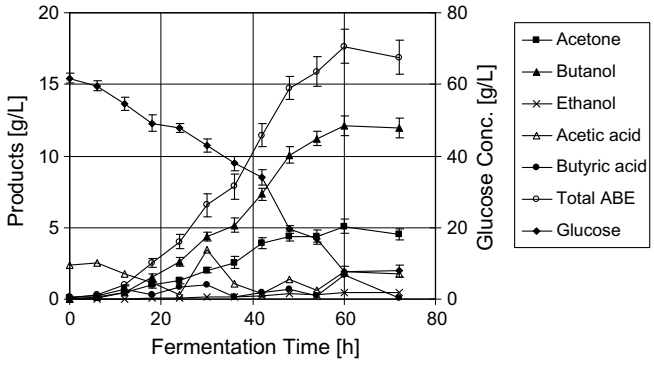

Fig. 2. Production of ABE from glucose when using *C. beijerinckii* BA101.

3.3. ABE fermentation using detoxified SACFH-based medium

In an attempt to circumvent the inhibition problem, SACFH was treated with the resin XAD-4 prior to fermentation. This resin has been previously shown to adsorb furfural and hydroxymethyl furfural (HMF) (Weil et al., 2002). In the beginning of the fermentation, 46.3 g/L total reducing sugar was present in the medium. It took 88 h to produce 2.7 g/L acetone, 6.4 g/L butanol and 0.2 g/L ethanol (Fig. 3). At zero time 8.8 g/L acetic acid was present which reduced to 5.7 g/L by the end of fermentation. During this run 9.3 ± 0.5 g/L total ABE was produced resulting in a productivity of 0.10 ± 0.006 g/L.h and a yield of 0.39 ± 0.015. This yield is based on utilization of 23.6 g/L reducing sugar. At the end of fermentation 22.7 g/L residual sugar was measured. Also SACFH contained pentose sugars which were expected to be fermented by *C. beijerinckii* BA101. It should be noted that *C. beijerinckii* has the ability to utilize both pentose and hexose sugars (Ezeji et al., unpublished). Analysis ($p < 0.0001$) of ABE results obtained after fermentation of XAD-4 treated SACFH revealed that ABE concentration was significantly higher than ABE concentration obtained with non-treated SACFH (Table 2). Production of ABE from the XAD-4 treated SACFH demonstrated that components that inhibited fermentation of untreated SACFH were, at least par-

Table 1
Butanol production by *C. beijerinckii* BA101 following fermentation of SACFH[a]

Time (h)	Cell conc. (g/L)	Acetone (g/L)	Butanol (g/L)	Ethanol (g/L)	Acetic acid (g/L)	Butyric acid (g/L)	Total acids (g/L)	Total ABE (g/L)
0	0.02	ND	ND	ND	6.5	0.2	6.7	0.0
24	0.58	0.0	0.1	0.1	7.8	0.4	8.2	0.2
54	0.75	0.2	1.4	0.1	13.3	0.4	13.7	1.7
72	0.60	0.1	1.0	0.2	9.5	0.2	9.7	1.3
96	0.31	0.1	0.9	0.2	9.2	0.2	9.4	1.2

ND – not detected.
[a] The data represent the average of duplicate determinations.

Table 2
Fermentation characteristics of CFH and wood sugars using *C. beijerinckii* BA101

Experiment	Substrate/conc. (g/L)	ABE conc. (g/L)	Productivity (g/L h)	ABE yield (g/g)	Cell conc. (g/L)	Cell yield (g/g)
Control	Glucose (55.0)	18.1 ± 1.5^{a}	0.3 ± 0.03^{a}	0.38 ± 0.006^{a}	3.37 ± 0.29^{a}	0.07 ± 0.006^{a}
SACFH	CF sugars (29.8)	1.7 ± 0.2^{c}	x	x	0.75	x
SACFH + enzyme hydrolyzed	CFH CF sugars (54.3)	1.6 ± 0.4^{c}	x	x	0.65	x
SACFH + XAD-4 treated	CFH CF sugars (46.3)	9.3 ± 0.5^{b}	0.1 ± 0.006^{c}	0.39 ± 0.015^{a}	x	x
ETCFH	CF sugars (25)	8.6 ± 1.0^{b}	0.1 ± 0.011^{c}	0.35 ± 0.01^{b}	1.44 ± 0.16^{b}	0.06 ± 0.006^{a}
Glucose (comparison)	Glucose (25)	9.9 ± 0.4^{b}	0.17 ± 0.006^{b}	0.39 ± 0.025^{a}	1.44 ± 0.16^{b}	0.06 ± 0.006^{a}
Xylose (comparison)	Xylose (25)	9.6 ± 0.4^{b}	0.16 ± 0.01^{b}	0.39 ± 0.011^{a}	1.44 ± 0.17^{b}	0.06 ± 0.006^{a}

CFH – corn fiber hydrolysate. x – not measured or parameters not calculated due to poor growth or fermentation. abc – means ± SD with same superscript are not significantly different.

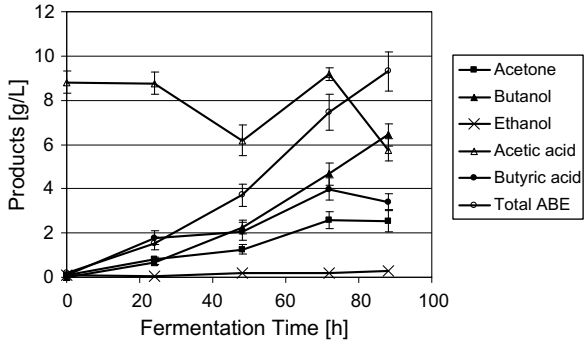

Fig. 3. Production of ABE from XAD-4 treated SACFH when using *C. beijerinckii* BA01.

tially, removed by the XAD-4 column resin. In a similar study Soni et al. (1982) improved performance of bagasse and rice straw hydrolysate significantly when these hydrolysates were treated with ammonium sulfate and activated carbon. In their study, an ABE concentration of 18.1 ± 1.5 g/L was obtained. A comparison of our results (treated and untreated) suggests that SACFH was more toxic than their hydrolysates.

Fermentation of wood (pine and aspen), bagasse, rice straw, wheat straw, and corn stover to ABE has been investigated by various groups (Table 3). Bagasse (Soni et al., 1982), rice straw (Soni et al., 1982), and wheat straw (Marchal et al., 1984) hydrolysate fermentations resulted in the production of 18.1 ± 1.5, 13.0, and 17.7 g/L ABE, respectively, using different solventogenic strains. These values are comparable to 17.7 g/L ABE achieved in our control fermentation using *C. beijerinckii* BA101. The maximum value of 9.3 ± 0.5 g/L ABE obtained in the XAD-4 treated SACFH fermentation is lower than that reported by the above authors. It is likely that the different nature of CF, presence of the inhibitors still present in the hydrolysate or both may have contributed to the inefficient utilization of the sugars present in the XAD-4 treated SACFH for ABE production. A comparison of the fermentation kinetic parameters in the above runs is shown in Table 2.

An investigation on the inhibitory components that are present in SACFH and their affects on butanol fermentation will be published elsewhere. However, it should be noted that ABE yield, which is an important factor in a biological process, was higher in our fermentations than that reported by other authors (Tables 2 and 3). Utilization of CFH and other wood and agricultural residues is a positive step toward conversion of agricultural biomass into fuels and chemicals. However, fermentation of such substrates should be further examined.

3.4. ABE fermentation in ETCFH-based medium

The CF was hydrolyzed using enzymes already described in the materials and methods and the hydrolysate was fermented. The ETCFH initially contained 25 g/L total sugars. The hydrolysate was centrifuged prior to fermentation to remove suspended solids. Following this, the clear supernatant was fermented to produce ABE (Fig. 4A). During the 72 h fermentation, 24.6 g/L sugar was consumed leaving behind 0.4 g/L sugar. The fermentation stopped due to lack of sugars in the broth. During the fermentation 8.6 ± 1.0 g/L total ABE and 6.5 g/L butanol were produced. In the present experiment, a productivity and yield of 0.10 ± 0.011 g/L h and 0.35 g/g were obtained, respectively. Cell concentration in the fermentation broth was 1.65 ± 0.40 g/L. Based on total sugar utilization, a cell yield of 0.07 ± 0.03 g/g was obtained. A comparison of cell concentration and cell yield values for this and that of glucose (25 g/L) and xylose (25 g/L) fermentation showed that there was no significant difference among the three fermentations suggesting that ETCFH did not inhibit *C. beijerinckii* BA101 growth.

From an industrial point of view, the removal of suspended solids by centrifugation would add additional cost to the production of butanol from the CF. Therefore fermentation of enzyme hydrolyzed CF without removal of solids was investigated using *C. beijerinckii* BA101 (Fig. 4B). At zero time 25 g/L total sugars were present, which were used (residual sugar 0.6 g/L) by the culture in 48 h, thereby producing 6.1 g/L butanol and 8.3 g/L total ABE. The cell concentration could not be measured due to the presence of suspended solids. However, a compari-

Table 3
Production of butanol from wood or agricultural residue hydrolysates

Substrate	Hydrolysis method	Treatment to remove inhibitors	Culture	ABE produced (g/L)	Yield (g ABE/g sugar)	Productivity (g/L h)	Reference
Wood[a]							
Pine[c]	SO_2-catalysed prehydrolysis + enzyme hydrolysed	None	*C. acetobutylicum* P262	17.6	0.36	0.73	Parekh et al. (1988)
Aspen[d]	Above	None	Above	20.1–24.6	0.31–0.34	0.84–1.03	Parekh et al. (1988)
Bagasse	Alkali pretreated + enzyme hydrolysed	Ammonium sulfate + activated carbon	*C. saccharoperbutylacetonicum* ATCC 27022	18.1	0.33	0.30	Soni et al. (1982)
Rice straw	Above	Above	Above	13.0	0.28	0.15	Soni et al. (1982)
Wheat straw	Alkali pretreated + enzyme hydrolysed	None	*C. acetobutylicum* IFP 921	17.7	0.18	0.47	Marchal et al. (1984)
Corn stover[b]	SO_2-catalysed prehydrolysis + enzyme hydrolysed	None	*C. acetobutylicum* P262	25.8	0.34	1.08	Parekh et al. (1988)
Corn fiber	Dilute sulfuric acid	XAD-4 resin	*C. beijerinckii* BA101	9.3	0.39	0.10	This work

[a,b] The batch fermentation was integrated with cell recycle and product recovery, which as expected resulted in high ABE concentration and productivity.
[c,d] Yield reported as 298 L acetone butanol per ton of pine, and 255 L AB per ton of aspen, respectively.

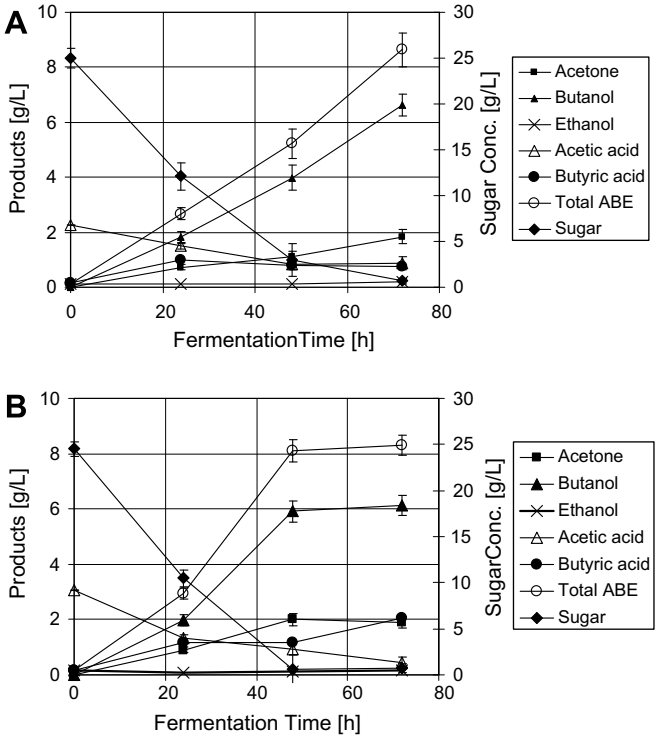

Fig. 4. Production of ABE from ETCFH using *C. beijerinckii* BA101. (A) Centrifuged hydrolysate; (B) hydrolysate containing solids.

son between this and the previous experiment suggests that a substantial amount of cell growth occurred. An ABE productivity and yield of 0.17 g/L h and 0.36 were obtained, respectively. In the ETCFH, the level of initial sugars was lower than in the control fermentation (glucose 55 g/L). Hence, it is difficult to compare the performance of the two systems.

3.5. ABE fermentation under low concentrations of pure glucose and xylose conditions

To compare the performance of ETCFH (initial sugar concentration 25 g/L) and fermentation of pentose (xylose) by the mutant *C. beijerinckii* BA101, two experiments, one with glucose and the other with xylose, were conducted. In the glucose fermentation, an initial substrate level of 25 g/L was used. During the 72 h of fermentation all the glucose was utilized thus producing 9.9 ± 0.4 g/L total ABE of which butanol was 6.1 g/L (Fig. 5A). Acetone and ethanol were 3.9 and 0.4 g/L, respectively. Productivity and a yield of 0.17 ± 0.006 g/L h (fermentation stopped at 60 h) and 0.39 ± 0.025 were obtained, respectively. In the xylose fermentation (25.0 g/L initial xylose) 9.6 ± 0.4 g/L total ABE was produced with 0 g/L residual sugar (productivity 0.16 ± 0.01 g/L h for 60 h fermentation time, and yield 0.39 ± 0.011). Acetone and ethanol concentrations were 2.6 and 0.5 g/L, respectively (Fig. 5B). The 8.6 ± 1.0 g/L ABE produced from the fermentation of ETCFH was approximately 15% lower than the pure glucose (25 g/L) fermentation ABE values and 11.6% lower than the pure xylose (25 g/L) fermentation ABE values. However, the levels of ABE that were produced during the pure sugar fermentations were not significantly different ($P > 0.38$) from the ABE concentrations obtained from ETCFH fermentations (21 g/L glucose + 2.7 g/L xylose + 1.3 g/L arabinose). We observed that the mean ABE yield for the treatment ETCFH is significantly less than those of other

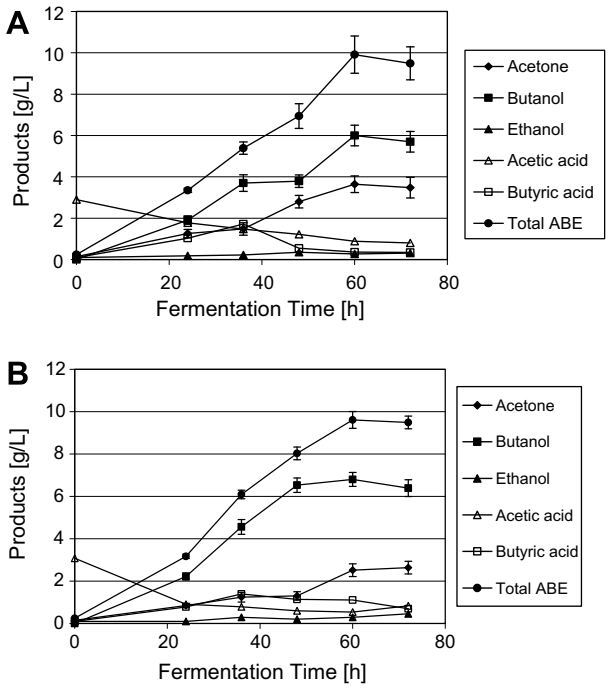

Fig. 5. Production of ABE from sugars equivalent in concentration to that reported in Fig. 4. (A) Glucose; (B) xylose.

treatments and is the only treatment whose mean is significantly different from the others. These results demonstrated that both glucose and xylose were efficiently utilized by the culture.

4. Conclusions

Fermentation of SACFH inhibited cell growth and butanol fermentation (produced 1.7 ± 0.2 g/L ABE) using *C. beijerinckii* BA101. Treatment of SACFH with XAD-4 resin removed some of the inhibitors which resulted in the production of 9.3 ± 0.5 g/L ABE and a yield of 0.39 ± 0.015. Fermentation of ETCFH did not show any signs of cell inhibition and resulted in the production of 8.6 ± 1.0 g/L ABE from 24.6 g/L total sugars used. Fermentation of 25 g/L glucose and 25 g/L xylose produced 9.9 ± 0.4 and 9.6 ± 0.4 g/L ABE, respectively suggesting that the culture was able to utilize xylose equally to that of glucose. Production of 9.3 ± 0.5 g/L ABE (compared to 17.7 g/L in control) from XAD-4 treated SACFH suggested that some fermentation inhibitors may still be present after the treatment. Studies examining the inhibitory components present in SACFH will be published elsewhere. It is suggested that inhibitory components be completely removed from the SACFH prior to fermentation. Another possibility could be that a new culture capable of fermenting SACFH be developed. In our fermentations ABE yields ranging from 0.35 to 0.39 were obtained which is higher than reported by the other investigators (Tables 2 and 3). It is suggested that fermentation of SACFH be further developed to achieve the highest possible level of ABE. In addition, fermentation integration with novel product removal techniques (Ezeji et al., 2003, 2004; Maddox et al., 1995) may be beneficial to the process of butanol production from agricultural residues. These studies suggested that fermentation of corn fiber hydrolysate (CFH) to ABE was successful.

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