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Process development for biological production of butanol from Eastern redcedar

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HIGHLIGHTS

- Buffer choice is critical for efficient acetone–butanol–ethanol (ABE) fermentation.
- Addition of acetate improved butanol production but citrate inhibited fermentation.
- Redcedar hydrolyzate detoxification increased butanol titer from 1 to 13 g/L.
- 19 g/L total ABE produced from detoxified hydrolyzate comparable to glucose medium.
- Sodium bisulfite in medium should be below 20 ppm not to affect butanol production.

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ABSTRACT

Eastern redcedar is an invasive softwood species in Oklahoma and across grasslands in the Central Plains of the United States and potential feedstock for butanol production. Butanol has higher energy content than ethanol and can be upgraded to jet and diesel fuels. The objective of this study was to develop a process for production of butanol from redcedar. Results showed that *Clostridium acetobutylicum* ATCC 824 and *Clostridium beijerinckii* NCIMB 8052 did not grow in fermentation medium with citrate buffer. However, both strains grew in the medium with acetate buffer, resulting in 3–4 g/L greater butanol than without acetate. Detoxification of redcedar hydrolyzate was required to increase butanol concentration from 1 to 13 g/L. Hydrolyzate was detoxified by activated carbon to remove inhibitors. Fermentations in detoxified redcedar hydrolyzate reached 13 g/L butanol and 19 g/L total ABE, comparable to glucose control. This shows the potential for redcedar use in butanol production.

1. Introduction

Eastern redcedar (*Juniperus virginiana* L., hereafter referred to as redcedar) is an invasive species of softwood in Oklahoma and the Central Plains of the United States due to its adaptability to various soils, climate conditions and topographies (Ramachandriya et al., 2013b). The encroachment of redcedar reduces ground water yields and increases the risk of wildfires, which resulted in an estimated loss of $447 million in 2012 in Oklahoma according to the National Resources Conservation Service (Starks et al., 2011). Besides essential oil that can be extracted from redcedar as a high value product (Dunford et al., 2007), biofuels such as ethanol and butanol present new opportunities for the use of this invasive tree. It was estimated that there were 11.5 million dry metric tons of above ground redcedar in northwest Oklahoma alone (Starks et al., 2011). The ethanol production potential has been reported as 43.9 gallon ethanol/metric ton of dry redcedar and it was estimated that 530 million gallons of ethanol could be produced from redcedar in northwest Oklahoma (Ramachandriya et al., 2013a). However, no studies were found on butanol production from redcedar. Butanol has higher energy content and less hygroscopicity than ethanol, which makes it more compatible with current fuel infrastructure and more easily converted to jet and diesel fuels (Harvey and Meylemans, 2011).

Butanol commercial production has a long history back to World War I and II via ABE (acetone–butanol–ethanol) fermentation using corn and molasses (Qureshi and Ezeji, 2008). However, the

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development of the petroleum industry and increasing prices of corn and molasses caused chemical synthesis to replace biological production of butanol in the 1980s (Qureshi and Ezeji, 2008). In recent years, the increase in transportation fuel prices stimulated a revival of research on butanol fermentation from low cost lignocellulosic biomass such as wood, corn fiber, corn stover, switchgrass, barley straw and wheat straw (Qureshi and Ezeji, 2008). Redcedar is a softwood with higher lignin content and biomass structure is more resistant to degradation than hardwood and agricultural waste (Ramachandriya et al., 2013b). An acid sulfite pretreatment method for redcedar has been developed and glucan to glucose theoretical yields of 87% were achieved after enzymatic hydrolysis (Ramachandriya et al., 2013b). The hydrolyzate typically contains fermentation inhibitors such as hydroxymethylfurfural (HMF) and furfural from degradation of hexose and pentose sugars during pretreatment and hydrolysis, and phenolic compounds from degradation of lignin (Mussatto and Roberto, 2006; Zhang et al., 2012). It was reported that HMF and furfural at concentrations above 3 g/L had between 5% and 10% decrease in total ABE production by *Clostridium acetobutylicum* ATCC 824 (Zhang et al., 2012). Soluble lignin content (SLC) is another indicator for the presence of inhibitors in hydrolyzate, mainly phenolic compounds. High SLC level in fermentation medium can inhibit ABE strains by increasing cell membrane fluidity and causing leakage of cellular contents (Ezeji et al., 2007a; Wang and Chen, 2011). The presence of HMF, furfural and phenolic compounds in the redcedar hydrolyzate is expected. Thus, detoxification of hydrolyzate, especially removal of phenolic compounds, is required to maximize ABE production. Detoxification methods include use of overliming, activated carbon, resin and peroxidase treatment (Wang and Chen, 2011).

The objective of this study was to develop a process to convert redcedar polysaccharides to butanol. This included selection of buffer for redcedar enzymatic hydrolysis and fermentation, identification of inhibitors and chemical species in the hydrolyzate, detoxification of hydrolyzate, and fermentation of hydrolyzate to ABE.

2. Methods

2.1. Pretreatment of redcedar

Redcedar was received in the form of chips from a local manufacturer in Oklahoma. The chips were first ground using a Thomas-Wiley mill equipped with a 2 mm screen (Arthur H. Thomas Co., Philadelphia, PA, USA). Then, the ground redcedar was impregnated at 90 °C for 3 h with pretreatment solution consisting of 3.75 g of sulfuric acid and 20 g of sodium bisulfite per 100 g of dry wood. The ratio of pretreatment solution to redcedar dry wood was 5:1 (v/w). This was followed by increasing the temperature to 200 °C with a hold time of 10 min as described previously (Ramachandriya et al., 2013b) . The solids were washed by first transferring pretreated solids into a 1 L beaker containing 500 mL DI water at 60 °C and stirring at 600 rpm for 15 min. Then, solids and water were separated by vacuum filtration using No. 4 Whatman filter paper. This procedure was repeated four times. Washed pretreated solids were stored in resealable plastic bags at 4 °C until used in enzymatic hydrolysis. The compositions of pretreated and unpretreated redcedar were analyzed using National Renewable Energy Laboratory (NREL) protocols (Sluiter et al., 2005, 2008).

2.2. Enzymatic hydrolysis of Eastern redcedar

2.2.1. Buffer selection for enzymatic hydrolysis

In order to choose a hydrolysis buffer amenable to ABE fermentation, the effects of citrate and acetate buffers (50 mM each) on ABE fermentation in glucose medium were studied. The fermentation treatments included glucose (60 g/L) medium without buffer as a control, glucose (60 g/L) medium with 50 mM citrate buffer and glucose (60 g/L) medium with 50 mM acetate buffer. *C. acetobutylicum* ATCC 824 and *Clostridium beijerinckii* NCIMB 8052 were used.

2.2.2. Enzymatic hydrolysis and selection of buffer pH

Accellerase 1500, generously provided by Genencor Inc. (Palo Alto, CA, USA), was used for enzymatic hydrolysis of redcedar. Enzymatic hydrolysis was performed in 250 mL baffled Erlenmeyer flasks with a total working weight of 100 g in each flask at 50 °C and 250 rpm in an incubator shaker (MaxQ 4450, Thermo Scientific, Dubuque, IA, USA). Acetate buffer (50 mM) was used to conduct hydrolyzate with buffer pH 5.5. In order to obtain an initial glucose concentration of ~60 g/L for ABE fermentation, a solid loading of 14% was chosen. Samples (2 mL) were withdrawn from each flask periodically and centrifuged at 16,000*g* for 10 min. The enzymatic hydrolysis was performed for 48 h. The enzyme loading was 50 FPU/g glucan as previously used for a redcedar to ethanol process (Ramachandriya et al., 2013b).

2.3. Enzymatic hydrolyzate detoxification

After enzymatic hydrolysis, the hydrolyzate was collected and centrifuged at 48,000*g* for 15 min at 4 °C (Avanti J-E, Beckman Coulter, Inc., Brea, CA, USA). This was repeated four times to remove suspended fine particles and obtain solids-free hydrolyzate. Then, the hydrolyzate was detoxified using 10% (w/v) activated carbon (Hydrodarco B, CABOT, Norit American, Inc., Marshall, Texas, USA) at 28 °C for 1 h (Converti et al., 1999). After detoxification, the hydrolyzate was centrifuged at 48,000*g* for 15 min at 4 °C and repeated four times to remove the carbon from the hydrolyzate. Finally, the hydrolyzate was filter-sterilized with a 0.2 μm sterile nylon filter (Nalgene Rapid-Flow™ Filter Units, ThermoFisher, Waltham, MA, USA) and stored at −20 °C before fermentation.

2.4. Microorganisms and inoculum preparation

C. acetobutylicum ATCC 824 and *C. beijerinckii* NCIMB 8052 were used in this study. Laboratory stock cultures were maintained as spore suspensions in sterile distilled water at 4 °C (Zhang et al., 2012; Han et al., 2013). Tryptone-glucose-yeast extract (TGY) medium was used to prepare the inocula for both organisms. TGY medium contained (per liter): 30 g tryptone, 20 g glucose, 10 g yeast extract, and 1 g cysteine (Ezeji et al., 2013). TGY medium was sterilized by autoclaving at 121 °C for 15 min (PRIMUS, Sterilizer Co., Inc., Omaha, NE, USA). TGY medium was then cooled to 40 °C and transferred into an anaerobic chamber (Coy Laboratory Products Inc., Ann Arbor, MI, USA). The TGY medium was allowed to attain anoxic condition by storing in the anaerobic chamber for 24 h before inoculation (Zhang et al., 2012; Ezeji et al., 2013; Han et al., 2013). The temperature in the anaerobic chamber was controlled at 35 °C. *C. acetobutylicum* ATCC 824 and *C. beijerinckii* NCIMB 8052 spores (200 μL) were heat-shocked at 75 °C for 10 min in a dry bath (Corning, Tewksbury MA, USA) and then cooled in an ice bath for 2 min (Zhang et al., 2012; Han et al., 2013). The heat shocked spores of each organism were inoculated into 10 mL of TGY medium in loosely capped Pyrex tubes in the anaerobic chamber. The precultures were incubated at 35 °C until optical density at 600 nm (OD) reached 0.9–1.1 (Zhang et al., 2012; Han et al., 2013). OD was measured using a spectrophotometer at 600 nm (UV-2100, Cole Parmer, Vernon Hills, IL, USA). Then, 6 mL of each actively growing inoculum culture were transferred into a 250 mL Pryex bottle containing 54 mL TGY medium (10% v/v

inoculation) and incubated for 3–5 h to reach an OD between 0.9 and 1.1 before being used to inoculate fermentation media.

2.5. Fermentation medium

Glucose P2 medium containing glucose (60 g/L) and yeast extract (1 g/L) was used as fermentation control (Zhang et al., 2012; Han et al., 2013). The Glucose P2 medium was autoclaved at 121 °C for 15 min and cooled down to 40 °C, before being transferred to the anaerobic chamber as described for TGY medium above. Before inoculation, glucose P2 medium was transferred aseptically into loosely capped 160 mL pre-sterilized, anoxic glass bottles (Fisher Scientific, Pittsburgh, PA, USA). Prior to inoculation, the glucose P2 medium was supplemented with 1% (v/v) pre-sterilized mineral, vitamin and buffer stock solutions as previously described (Zhang et al., 2012; Han et al., 2013). The fermentation medium was then inoculated with 6% (v/v) actively growing precultures in TGY medium.

For detoxified and non-detoxified enzymatic hydrolyzate media, 1% (v/v) filter-sterilized P2 buffer, mineral and vitamins stock solutions, and 2% (v/v) filter-sterilized yeast extract solution (50 g/L) were added to hydrolyzate. Except specified, all fermentations including glucose P2 medium, non-detoxified and detoxified hydrolyzate media were performed using 50 mL working volume with 6% (v/v) inoculum size without shaking at a temperature of 35 °C. The initial pH of all fermentation media was adjusted between 6.5 and 6.8 with 2 N KOH. Fermentations were performed in triplicate.

2.6. Analytical methods

2.6.1. Cell growth, pH and product concentrations

During fermentation, 1 mL liquid samples were taken aseptically from each bottle in the anaerobic chamber. Cell growth was measured at 600 nm using a spectrophotometer (UV-2100). The pH was monitored with a pH meter (pH 11 series, Oakton, Vernon Hills, IL, USA). A portion of the liquid sample (0.3 mL) was acidified by adding 0.3 mL 1 N HCl for solvents and organic acids measurement using Agilent GC 6890 (Agilent Technologies, Wilmington, DE, USA). The GC methods were described previously (Liu et al., 2012). The concentrations of sugars were analyzed by Agilent HPLC1200 (Agilent Technologies) as previously described (Ramachandriya et al., 2013b).

2.6.2. Inhibitors, sodium bisulfite and soluble lignin content (SLC)

The concentrations of HMF, furfural, cinnamaldehyde, vanillic acid, vanillin, hydroxybenzaldehyde, syringaldehyde, syringic acid, p-coumaric acid, and ferulic acid in the enzymatic hydrolyzates were quantified by a HPLC using a Waters 2796 Bioseparations Module (Waters, Milford, MA, USA) equipped with a photodiode array detector (Zhang et al., 2012) and a Xbridge C18 column (Waters, Milford, MA, USA) using previously described conditions (Matějíček et al., 2003). Levulinic acid and formic acid concentrations were quantified by Agilent 1200 HPLC (Agilent Technologies) with a refractive index detector using an Aminex-HPX-87H column (BioRad, Hercules, CA, USA) with 0.01 N sulfuric acid as mobile phase and a flow rate of 0.6 mL/min at 60 °C for 60 min. The sodium bisulfite concentration in redcedar hydrolyzate was measured by a sulfite kit from Chemetrics, Inc. (Midland, VA, USA) according to the supplier's protocol. The method for determination of SLC was described previously (Mussatto and Roberto, 2006).

2.7. Statistical analysis and calculations

Statistical analysis was conducted using Duncan's multiple analysis using SAS 9.3 software (Cary, NC, USA) at 95% confidence level by the GLM procedure. The statistical analysis compared the differences between treatments in terms of growth, solvents and acid production, and glucose consumption. The ABE yield was based on the amount of glucose consumed during fermentation. The calculation of glucan to glucose yield has been described previously (Ramachandriya et al., 2013b).

3. Results and discussion

3.1. Pretreatment of redcedar

The unpretreated redcedar contained 32.63 ± 0.76% glucan, 6.32 ± 0.53% xylan, 3.29 ± 0.07% galactan, 1.44 ± 0.07% arabinan, 6.90 ± 0.31% mannan, 32.65 ± 0.15% lignin, 13.24 ± 0.23% ash and 5.09 ± 0.09% extractives. Six pretreated redcedar solids were combined as Batch 1 solids. The pretreated Batch 1 solids contained 52.61 ± 0.03% glucan, 2.74 ± 0.02% xylan, 0.61 ± 0.13% galactan, 0.79 ± 0.00% mannan and 30.37 ± 0.21% lignin. Another five pretreatments of redcedar were combined as Batch 2 solids to provide additional pretreated solids. Pretreated redcedar Batch 2 solids contained 54.05 ± 0.80% glucan, 1.86 ± 0.04% xylan, 0.52 ± 0.02% galactan, 0.96 ± 0.08% mannan and 30.71 ± 0.26% lignin. Arabinan was not detected in both pretreated Batch 1 and Batch 2 solids.

3.2. Effects of citrate and acetate buffers on ABE fermentation

Results of ABE fermentation using *C. acetobutylicum* ATCC 824 and *C. beijerinckii* NCIMB 8052 in P2 medium with glucose with and without acetate buffer solution are shown in Table 1. Both organisms grew in glucose P2 medium with and without acetate

Table 1
ABE fermentations using *C. acetobutylicum* ATCC 824 and *C. beijerinckii* NCIMB 8052 in pure glucose P2 medium containing 60 g/L glucose with and without acetate buffer after 72 h of fermentation.

Treatment	*C. acetobutylicum* ATCC 824		*C. beijerinckii* NCIMB 8052	
	Glucose P2 medium	Glucose P2 medium + 50 mM acetate buffer (pH 5.0)	Glucose P2 medium	Glucose P2 medium + 50 mM acetate buffer (pH 5.0)
Maximum OD	7.46 ± 0.48^{A}	7.48 ± 0.23^{A}	6.90 ± 0.06^{A}	7.03 ± 0.04^{A}
Final acetone (g/L)	2.50 ± 0.18^{B}	3.90 ± 0.05^{A}	2.31 ± 0.02^{B}	3.82 ± 0.22^{A}
Final ethanol (g/L)	0.28 ± 0.03^{B}	0.41 ± 0.03^{A}	0.26 ± 0.01^{B}	0.38 ± 0.01^{A}
Final butanol (g/L)	9.45 ± 0.48^{C}	13.21 ± 0.30^{A}	8.76 ± 0.10^{C}	12.30 ± 0.11^{B}
Total ABE (g/L)	12.23 ± 0.33	17.52 ± 0.38	11.33 ± 0.12	16.51 ± 0.34
Glucose consumed (g/L)	36.45 ± 0.45^{C}	50.29 ± 0.14^{A}	31.40 ± 0.75^{D}	48.44 ± 0.02^{B}
ABE yield (g/g)	0.35 ± 0.01	0.35 ± 0.02	0.36 ± 0.01	0.34 ± 0.01
Final acetic acid (g/L)	0.86 ± 0.18^{B}	1.72 ± 0.11^{A}	0.67 ± 0.06^{B}	1.90 ± 0.17^{A}
Final butyric acid (g/L)	0.92 ± 0.12^{C}	1.88 ± 0.11^{B}	0.98 ± 0.02^{C}	2.19 ± 0.03^{A}
Final total acids (g/L)	1.77 ± 0.27	3.60 ± 0.00	1.65 ± 0.07	4.09 ± 0.20

^{A,B,C,D}Duncan's multiple range group; no statistical differences between treatments with the same letter in the same row.

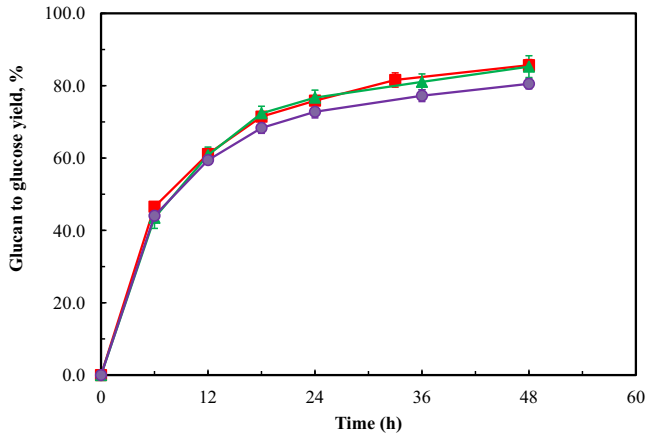

Fig. 1. Glucan to glucose yield during enzymatic hydrolysis runs with 14% solids loading in acetate buffer; (■) Batch 1 solids in acetate buffer pH 5.0, (▲) Batch 1 solids in acetate buffer pH 5.5, (●) Batch 2 solids in acetate buffer pH 5.5.

buffer. However, neither organism grew in citrate buffer-based glucose P2 medium (data not shown). Citric acid was shown to inhibit growth of *Escherichia coli* O157:H7 and *Listeria monocytogenes* by reducing the cells internal pH and proton motive force, and changing cell membrane permeability (Eswaranandam et al., 2004). Compared to *Clostridium* strains, ethanol fermentation by *Saccharomyces cerevisiae* D_5A on hydrolyzates of pretreated redcedar in citrate buffer showed the robustness of the yeast and its citrate tolerance (Ramachandriya et al., 2013a). Acetate is another buffer used for enzymatic hydrolysis (Lou et al., 2013). It was reported that *C. beijerinckii* NCIMB 8052 tolerated up to 100 mM acetate, and 80 mM acetate gave the best fermentation performance of the acetate concentrations tested, with 18 g/L of total ABE produced (Chen and Blaschek, 1999). In current study, the addition of 50 mM acetate buffer to P2 medium with glucose as carbon source resulted in the production of additional 3–4 g/L butanol, compared to control (Table 1). In addition, 38% and 54% more glucose were consumed by *C. acetobutylicum* ATCC 824 and *C. beijerinckii* NCIMB 8052, respectively, with acetate buffer compared to control ($p < 0.05$). Considering that P2 medium buffer stock solution contains 28.5 mM acetate as ammonium acetate with addition of 50 mM acetate buffer, the initial total acetate in the fermentation medium was 78.5 mM. This is similar to the 80 mM acetate reported previously (Chen and Blaschek, 1999). Therefore, acetate buffer was used for redcedar enzymatic hydrolysis. In addition, *C. acetobutylicum* ATCC 824 was used for the remaining experiments, because this strain produced 1 g/L more butanol ($p < 0.05$) and total ABE than *C. beijerinckii* NCIMB 8052 (Table 1).

3.3. Enzymatic hydrolysis and ABE fermentation

3.3.1. Enzymatic hydrolysis with acetate buffer at pH 5.0 and 5.5

Multiple enzymatic hydrolyses with Batch 1 solids containing 50 mM acetate buffer at pH 5.0 and pH 5.5 were compared. The hydrolyzates contained 70 g/L glucose after 48 h with glucan to glucose yield 85% at both pH values (Fig. 1). There were no differences in glucan to glucose yields between pH 5.0 and pH 5.5 ($p > 0.05$). The sugar concentrations in the hydrolyzates at 48 h for both pH values were similar, comprising of 1.48 ± 0.23 g/L cellobiose, 70.11 ± 1.27 g/L glucose, 2.19 ± 0.23 g/L xylose, and 0.74 ± 0.16 g/L mannose (i.e. 74.52 ± 0.95 g/L total sugars) at pH 5.0. The hydrolyzate obtained with acetate buffer at a pH of 5.5 contained 1.81 ± 0.29 g/L cellobiose, 69.96 ± 2.44 g/L glucose, 2.07 ± 0.31 g/L xylose, and 0.87 ± 0.18 g/L mannose, which adds to 74.71 ± 3.05 g/L total sugars.

Despite having adjusted the acetate buffer to initial pH of 5.0 and 5.5 prior to the addition of the pretreated solids, the pH of the hydrolysis reaction mixture quickly decreased to 4.7 and 4.9, respectively, at the beginning of hydrolysis. This could be most plausibly accounted for carryover of acid from pretreated solids. The pH of wash water after the last wash of pretreated redcedar solids was 3.72 ± 0.06, thus, indicating that residual acids remained on the pretreated solids after washing. Although the pH of the hydrolysis reaction mixture decreased rapidly to 4.9 or 4.7, 85% glucan to glucose yield was obtained after 48 h with 14% solid loading. This is comparable to other studies with 12% or 16% redcedar solid loadings (Ramachandriya et al., 2013a,b). The pH of hydrolyzate remained between 4.7 and 4.9 from 6 to 48 h of hydrolysis. According to the manufacturer's specifications, the optimal pH for Accellerase 1500 is 5.0. In light of this, pH 5.5 was chosen for further hydrolyses, as the pH proximity of the hydrolysis reaction mixture obtained with an initial acetate buffer pH of 5.5 (i.e., 4.9) to the optimal pH for Accellerase 1500 (i.e., 5.0).

3.3.2. ABE fermentation with non-detoxified hydrolyzate from Batch 1 solids

ABE fermentations using *C. acetobutylicum* ATCC 824 were performed on non-detoxified hydrolyzate. A glucose P2 medium with 50 mM acetate buffer was used as a control to evaluate and compare butanol production using non-detoxified redcedar hydrolyzates derived from enzymatic hydrolysis using acetate buffer pH 5.0 and 5.5 obtained in Section 3.3.1. Poor fermentation was observed in non-detoxified hydrolyzates with only 1 g/L butanol and 2 g/L ABE produced, compared to 12 g/L butanol and 17 g/L ABE in glucose control medium (Table 2). A lag phase of 12 h was observed in non-detoxified hydrolyzates. The maximum OD values in the non-detoxified hydrolyzates derived from acetate buffer pH 5.0 and 5.5 were 18% and 22% of control, respectively (Table 2), thus underlining the toxicity of lignocellulose-derived

Table 2
ABE fermentation using *C. acetobutylicum* ATCC 824 in pure glucose P2 and non-detoxified redcedar hydrolyzate media containing 60 g/L glucose in acetate buffer at pH 5.0 and 5.5 after 72 h of fermentation.

Medium	Glucose P2 medium	Non-detoxified hydrolysate	Non-detoxified hydrolysate
Acetate buffer pH	pH 5	pH 5	pH 5.5
Maximum OD	7.63 ± 0.82^{A}	1.35 ± 0.44^{B}	1.66 ± 0.23^{B}
Final acetone (g/L)	3.96 ± 0.45^{A}	0.77 ± 0.25^{B}	0.78 ± 0.17^{B}
Final ethanol (g/L)	0.43 ± 0.01^{A}	0.13 ± 0.01^{B}	0.13 ± 0.01^{B}
Final butanol (g/L)	12.27 ± 0.81^{A}	1.12 ± 0.39^{B}	1.14 ± 0.32^{B}
Final total ABE (g/L)	16.66 ± 1.27	2.01 ± 0.64	2.05 ± 0.50
Glucose consumed (g/L)	50.81 ± 2.74^{A}	10.39 ± 1.79^{B}	10.30 ± 2.06^{B}
ABE yield (g/g)	0.33 ± 0.01	0.19 ± 0.03	0.20 ± 0.01
Final acetic acid (g/L)	1.49 ± 0.04^{B}	5.13 ± 0.33^{A}	5.05 ± 0.15^{A}
Final butyric acid (g/L)	0.97 ± 0.11^{B}	3.05 ± 0.16^{A}	2.91 ± 0.04^{A}
Final total acids (g/L)	2.45 ± 0.07	8.18 ± 0.16	7.96 ± 0.19

^{A,B}Duncan's multiple range group; no statistical differences among treatments with the same letter in the same row.

inhibitors in non-detoxified hydrolyzates. After 72 h, the total acids in the non-detoxified hydrolyzates were 3-fold higher than the amounts in the control cultures. Both acetic acid and butyric acid production during fermentation contributed to the increase in total acid concentrations in the non-detoxified hydrolyzates (Table 2). The SLC in the hydrolyzates in acetate buffers pH 5.0 and pH 5.5 were 9.4 ± 0.6 g/L and 10.1 ± 1.8 g/L, respectively. This indicates that phenolic compounds in non-detoxified hydrolyzates inhibited growth and favored acid production during ABE fermentation. This could be due to the decrease in the intracellular ATP as reported in *S. cerevisiae* VTT C-10883 responding to inhibition by HMF and furfural (Ask et al., 2013). It was also reported that the decrease in acid assimilation and ABE production using *C. beijerinckii* 8052 was due to redox balance disruption by HMF and furfural stress (Ujor et al., 2014).Thus, in attempt to improve the fermentation of redcedar hydrolyzate for ABE production, we sought to detoxify the hydrolyzates before fermentation.

3.4. Effect of sodium bisulfite in glucose medium on ABE fermentation

It was reported that sodium metabisulfite at concentrations as low as 200 ppm in corn steep liquor medium inhibited growth of *C. beijerinckii* BA101 and resulted in more acid instead of solvent production during ABE fermentation (Ezeji et al., 2007b). Sodium bisulfite used in pretreatment of redcedar in the present study has an antimicrobial function similar to sodium metabisulfite to inhibit bacterial growth (Babich and Stotzky, 1978). In order to evaluate the effect of sodium bisulfite on ABE fermentation, its concentration was measured in non-detoxified redcedar hydrolyzates in acetate buffer pH 5.0 and 5.5 in Section 3.3. There were 90 and 170 ppm of sodium bisulfite in non-detoxified hydrolyzates in acetate buffer pH 5.0 and 5.5, respectively. Therefore, ABE fermentation with different levels of sodium bisulfite (0, 10, 100 and 200 ppm) were performed in glucose P2 medium with acetate buffer pH 5.5 (Table 3). The amount of butanol produced in the glucose media was not significantly different among all sodium bisulfite concentrations ($p > 0.05$) (Table 3). Between 12 and 13 g/L butanol were produced in all treatments. However, total ABE contents were decreased by 14% in media with sodium bisulfite concentrations greater than or equal to 100 ppm compared to the control. Significant growth inhibition was observed at sodium bisulfite concentrations above 100 ppm. The amount of glucose consumed in the media with sodium bisulfite concentration greater than or equal to 100 ppm was 14% less than in the control. Sodium bisulfite concentrations greater than or equal to 10 ppm enhanced acid production by at least 24% compared to the control (Table 3). This observation is in agreement with the increased acid production observed in the non-detoxified hydrolyzates relative to the control. The inhibitory effect observed with sodium bisulfite was similar to the effects previously reported for sodium metabisulfite, in which fermentation cultures became more acetogenic with sodium metabisulfite at a concentrations as low as 0.2 g/L (Ezeji et al., 2007b). However, the mechanism behind increased acid production by bisulfite remains largely unknown. In order to not limit growth inhibition and decreases in ABE production, it is therefore recommended that the levels of sodium bisulfite in the hydrolyzate should be kept below 100 ppm but optimization of sodium bisulfite level may be required in the future study.

3.5. ABE fermentation using detoxified hydrolyzate from Batch 2 solids

Poor ABE fermentation in non-detoxified redcedar hydrolyzate clearly underscored the need for detoxification. Activated carbon-mediated detoxification was explored in this study. For this experiment, Batch 2 solids were used. The enzymatic hydrolysis of Batch 2 solids achieved 80% of glucan to glucose yield after 48 h, which was similar to the redcedar hydrolyzate from Batch 1 solids (Fig. 1). The final sugar concentrations from Batch 2 were also similar to those obtained from Batch 1 solids. The combined redcedar hydrolyzate from Batch 2 solids was detoxified using 10% (w/v) activated carbon. After detoxification, the SLC level was reduced from 8.6 to 1.9 g/L. The SLC level was reduced further to 1.4 g/L after filter sterilization using 0.2 μm nylon filters. The reduction of SLC level for ABE fermentation by activated charcoal adsorption has been reported previously (Wang and Chen, 2011). It was also reported that when the SLC value in medium was above 1.77 g/L, ABE fermentation was inhibited using the enzymatic hydrolyzate derived from steam-exploded corn stover (Wang and Chen, 2011). The SLC level in detoxified redcedar hydrolyzate was 25% lower than the inhibitory level reported in corn stover hydrolyzate.

After detoxification and filter sterilization of hydrolyzate, white precipitates were formed when P2 medium stock solutions (buffer, minerals and vitamins) were sequentially added. Formation of precipitates could reduce the level of soluble amount of required nutrients for growth and ABE production. It was reported that formation of precipitate of metal ions during anaerobic digestion limited cells accessibility to these nutrients (Callander and Barford, 1983). Thus, ABE fermentation in detoxified hydrolyzates with and without precipitate was compared with a control glucose medium in 50 mM pH 5.5 acetate buffer (Table 4). The first precipitate formed after addition of P2 buffer stock solution to the hydrolyzate and was called 1st precipitate. This precipitate was removed from the hydrolyzate medium by centrifugation at 16,000*g* for 10 min. Then, a second precipitate was formed after the addition of P2 mineral stock solution to the supernatant after centrifugation and was called 2nd precipitate. The 2nd precipitate was also removed by centrifugation. The supernatant after removing 1st and 2nd precipitates was filter sterilized for fermentation. The

Table 3
Effect of sodium bisulfite on ABE fermentation using *C. acetobutylicum* ATCC 824 in glucose P2 medium containing 60 g/L glucose with acetate buffer at pH 5.5 after 72 h of fermentation.

Sodium bisulfite (ppm)	0	10	100	200
Maximum OD	8.03 ± 0.30^{A}	7.92 ± 0.37^{A}	7.05 ± 0.13^{B}	5.84 ± 0.33^{C}
Final acetone (g/L)	4.06 ± 0.64^{A}	4.42 ± 0.47^{A}	3.13 ± 0.32^{B}	2.94 ± 0.35^{B}
Final ethanol (g/L)	0.42 ± 0.04^{A}	0.39 ± 0.00^{A}	0.33 ± 0.01^{B}	0.33 ± 0.01^{B}
Final butanol (g/L)	13.24 ± 1.18^{A}	13.23 ± 0.33^{A}	11.97 ± 0.43^{A}	11.93 ± 0.09^{A}
Final total ABE (g/L)	17.73 ± 2.49	18.04 ± 0.81	15.44 ± 0.13	15.20 ± 0.45
Glucose consumed (g/L)	53.43 ± 4.26^{A}	53.97 ± 3.67^{A}	47.21 ± 0.21^{B}	46.20 ± 0.15^{B}
ABE yield (g/g)	0.33 ± 0.02	0.33 ± 0.01	0.33 ± 0.00	0.33 ± 0.01
Final acetic acid (g/L)	1.64 ± 0.18^{C}	2.06 ± 0.14^{B}	2.37 ± 0.16^{A}	2.19 ± 0.08^{AB}
Final butyric acid (g/L)	1.43 ± 0.52^{B}	1.76 ± 0.16^{B}	2.38 ± 0.33^{A}	2.43 ± 0.01^{A}
Final total acids (g/L)	3.07 ± 0.70	3.82 ± 0.30	4.75 ± 0.48	4.62 ± 0.07

^{A,B,C}Duncan's multiple range group; no statistical differences among treatments with the same letter in the same row.

Table 4
ABE fermentations using *C. acetobutylicum* ATCC 824 in pure glucose P2 and detoxified hydrolyzate media containing 60 g/L glucose with and without precipitate in acetate buffer at pH 5.5 after 72 h of fermentation.

Medium	Glucose P2 medium	Hydrolyzate with precipitate	Precipitate-free hydrolyzate
Maximum OD	8.29 ± 0.71^{A}	5.33 ± 0.17^{B}	1.82 ± 0.06^{C}
Final acetone (g/L)	4.18 ± 2.01^{B}	5.07 ± 1.97^{A}	1.03 ± 0.37^{C}
Final ethanol (g/L)	0.43 ± 0.01^{A}	0.34 ± 0.00^{B}	0.07 ± 0.01^{C}
Final butanol (g/L)	13.18 ± 0.39^{A}	13.09 ± 0.12^{A}	0.65 ± 0.02^{B}
Final total ABE (g/L)	17.78 ± 0.79	18.50 ± 0.48	1.75 ± 0.03
Glucose consumed (g/L)	51.75 ± 2.22^{A}	51.45 ± 2.81^{A}	6.75 ± 0.20^{B}
ABE yield (g/g)	0.34 ± 0.01	0.36 ± 0.01	0.26 ± 0.02
Final acetic acid (g/L)	1.75 ± 0.06^{C}	2.35 ± 0.11^{B}	2.87 ± 0.08^{A}
Final butyric acid (g/L)	$1.57 \pm 0.13^{A,B}$	1.74 ± 0.05^{A}	1.54 ± 0.09^{B}
Final total acids (g/L)	3.31 ± 0.18	4.10 ± 0.15	4.41 ± 0.08
Total sugars consumed[a] (g/L)	51.75 ± 2.22	54.43 ± 1.93	8.92 ± 0.61

[A,B,C]Duncan's multiple range group; no statistical differences among treatments with the same letter in the same row.
[a] Total sugars included cellobiose, glucose, xylose and mannose.

1st and 2nd precipitates and supernatants were analyzed by inductively coupled plasma–Atomic Emission Spectroscopy (ICP–AES).

Butanol and total ABE concentration after 72 h in hydrolyzate medium with precipitate were similar to the glucose medium (control) (Fig. 2). Butanol and total ABE in detoxified hydrolyzate with precipitate were 13.1 and 18.5 g/L, respectively (Table 4). However, only 0.7 g/L butanol and 1.8 g/L ABE were produced in precipitate-free hydrolyzate medium. Clearly, removal of precipitates from hydrolyzate medium had a negative effect on growth and ABE production, hence, demonstrating that key nutrients and buffer supplied by the buffer and mineral stock solutions, which are necessary for ABE production were retained in utilizable/functional forms in the precipitate-containing cultures.

The elemental compositions of the glucose P2 medium and hydrolyzate media (with and without precipitates) are shown in Table 5. The results show that a total of 0.86 g of solids was precipitated in 1 L of hydrolyzate medium after the addition of P2 buffer and mineral stock solutions. The elements in the greatest concentration in the 1st and 2nd precipitates were calcium and phosphorus. The 1st precipitate consisted of 14.6% calcium and 8.1% phosphorus. The 2nd precipitate consisted of 8.2% calcium and 5.0% phosphorus. The concentrations of phosphorus, iron and manganese in the precipitate-free hydrolyzate medium were 56%, 38% and 72%, respectively, lower than in the glucose P2 medium (Table 5). These elements are important for cell metabolism. Cell growth requires phosphorus for the biosynthesis of ATP, DNA, RNA, and cell membrane lipid layer (Shuler and Fikret, 2002). Iron is also an important element for hydrogenase activity and the electron carrier molecule ferredoxin (Ljungdahl, 1986), both of which affect cell redox balance, and electron transfer that enhance butanol production. Manganese is required by phosphotransacetylase for acetic acid production and ATP synthesis (Ljungdahl, 1986). Iron and manganese concentrations in precipitate-free hydrolyzate medium were 41% and 71% lower than in glucose P2 medium (Table 5), which could explain the poor butanol production when the precipitate was removed from the hydrolyzate.

3.6. ABE fermentations in detoxified hydrolyzate medium with addition of $CaCO_3$

Addition of $CaCO_3$ (4 g/L) was previously reported to improve sugar consumption, butanol and total ABE production in glucose P2 medium, which was related to $CaCO_3$ buffering effect, increase in solventogenesis enzymes activities by Ca^{2+} and improvement in sugar transport (Han et al., 2013). Therefore, the effect of addition of 4 g/L $CaCO_3$ to detoxified hydrolyzate medium derived from pretreated redcedar Batch 1 and Batch 2 solids on ABE fermentation was studied. Results of the $CaCO_3$-supplementation of the hydrolyzates are shown in Fig. 3. The precipitates that formed after addition of P2 stock buffer and mineral solutions to the hydrolyzate medium were not removed. ABE fermentations in glucose media containing acetate buffer pH 5.5 and hydrolyzate media from both Batch 1 and Batch 2 solids with and without addition of $CaCO_3$ were compared (Fig. 3). Enzymatic hydrolysis was performed using acetate buffer pH 5.5. Glucan to glucose yield after 48 h and total sugar concentration in the hydrolyzates from Batch 1 and Batch 2 solids were over 80% and 70 g/L, respectively (Fig. 1).

There were no differences ($p > 0.05$) in butanol concentration and glucose consumed in glucose P2 media with and without addition of $CaCO_3$ (Table 6). However, the OD, acetone and total ABE concentrations increased by 33%, 53% and 14%, respectively, in the glucose P2 medium with $CaCO_3$ compared to glucose P2 medium without $CaCO_3$. ABE fermentation in hydrolyzate medium with $CaCO_3$ from Batch 1 and Batch 2 solids resulted in similar butanol and ABE concentrations of about 10 and 16 g/L, respectively. The hydrolyzate media from Batch 1 and Batch 2 solids without $CaCO_3$ had butanol and total ABE concentrations of about 13 and 19 g/L, which were 30% and 19%, respectively, higher than hydrolyzate medium with $CaCO_3$. Hydrolyzate media without $CaCO_3$ resulted in comparable butanol and total ABE concentration with glucose P2 medium without $CaCO_3$. However, the hydrolyzate media from Batch 1 and Batch 2 solids with $CaCO_3$ resulted in 25% and 21% lower butanol formation, respectively, than the glucose P2 medium with $CaCO_3$. The addition of $CaCO_3$ into either glucose P2 medium or hydrolyzate media improved growth as shown by the higher maximum OD when compared to the media without $CaCO_3$ (Fig. 3 and Table 6). Similar to previous experiments (Table 4), there were at least 35% more total acids produced in hydrolyzate media compared to glucose media (Table 6). The addition of $CaCO_3$ into the hydrolyzate media increased total acids production by around twofold compared to hydrolyzate media without $CaCO_3$. This could be due the fast depletion of sugars in the hydrolyzate media with $CaCO_3$ at 36 h (Fig. 3B) and no additional NADH from sugars were available to reduce acids to solvents. In addition, the higher maximum OD values measured in the hydrolyzate media with $CaCO_3$ could indicate more ATP production to support growth, which is associated with acid production.

Through detoxification and filter sterilization of hydrolyzate media from Batch 1 and Batch 2 solids, SLC concentrations reduced by 85% and 87% to 1.2 and 1.0 g/L, respectively. The SLC reduction in this experiment was consistent with that described in Section 3.5. The sodium bisulfite levels in the hydrolyzate media from Batch 1 and Batch 2 solids after detoxification decreased from 160.0 to 20.0 ppm and from 160.0 to 17.5 ppm, respectively. This showed that in order to obtain comparable butanol and total ABE concentrations in glucose P2 medium, the SLC and sodium bisulfite

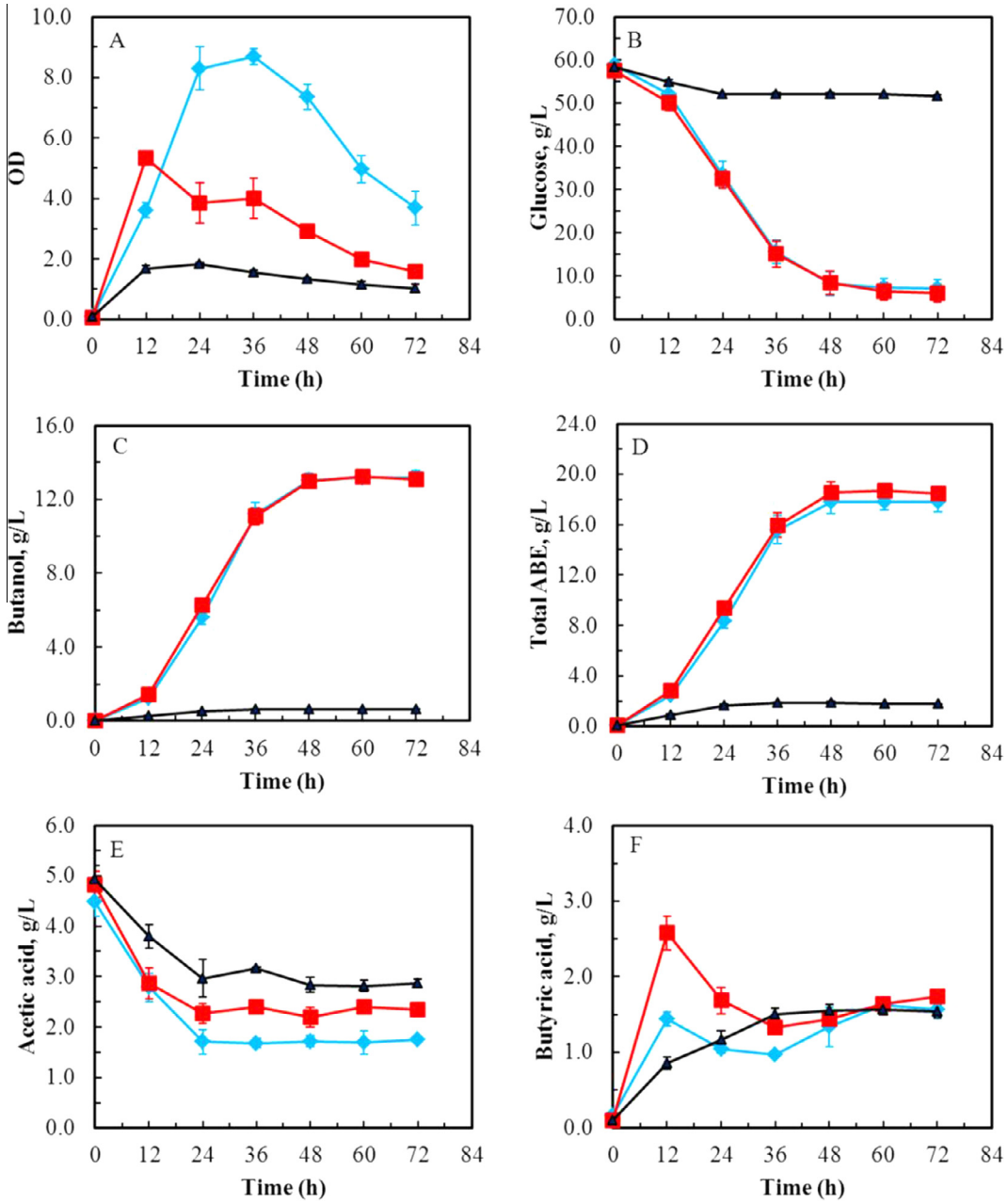

Fig. 2. (A) Growth (B) glucose (C) butanol (D) total ABE (E) acetic acid (F) butyric acid profiles during ABE fermentation using *C. acetobutylicum* ATCC 824 in (♦) glucose P2 medium, (■) hydrolysate medium containing precipitate, (▲) precipitate-free hydrolysate medium.

Table 5
Elements found in pure glucose P2 medium, detoxified hydrolyzate medium before addition of nutrients (buffer, minerals and vitamins) and detoxified hydrolyzate medium after addition of nutrients and removal of precipitates.

Treatments	Elements (ppm)								
	Na	Ca	Mg	K	S	P	Fe	Zn	Mn
Glucose P2 medium	1048.9	1.1	20.4	456.6	112.8	221.7	2.2	0.1	3.1
Hydrolysate before addition of nutrients	1173.8	531.2	56.7	88.5	718.4	3.1	0.6	0.3	0.2
Precipitate-free hydrolyzate medium after addition of nutrients	1087.6	299.2	69.9	544.0	682.5	98.3	1.3	0.5	0.9

levels in the detoxified redcedar hydrolyzate should be reduced to below 1.5 g/L and 20 ppm, respectively (Table 6).

In this experiment, the results showing concentrations of individual inhibitors reported after pretreatment and during enzymatic hydrolysis (Table 7). The major inhibitors in the prehydrolyzate generated during the acid bisulfite pretreatment were HMF, furfural and cinnamaldehyde. During enzymatic hydrolysis from 6 to 48 h, there were increases in the concentrations of these inhibitors ranging from 50% to 120% (Table 7), which could be due to the breakdown of biomass lignocellulose structure and release

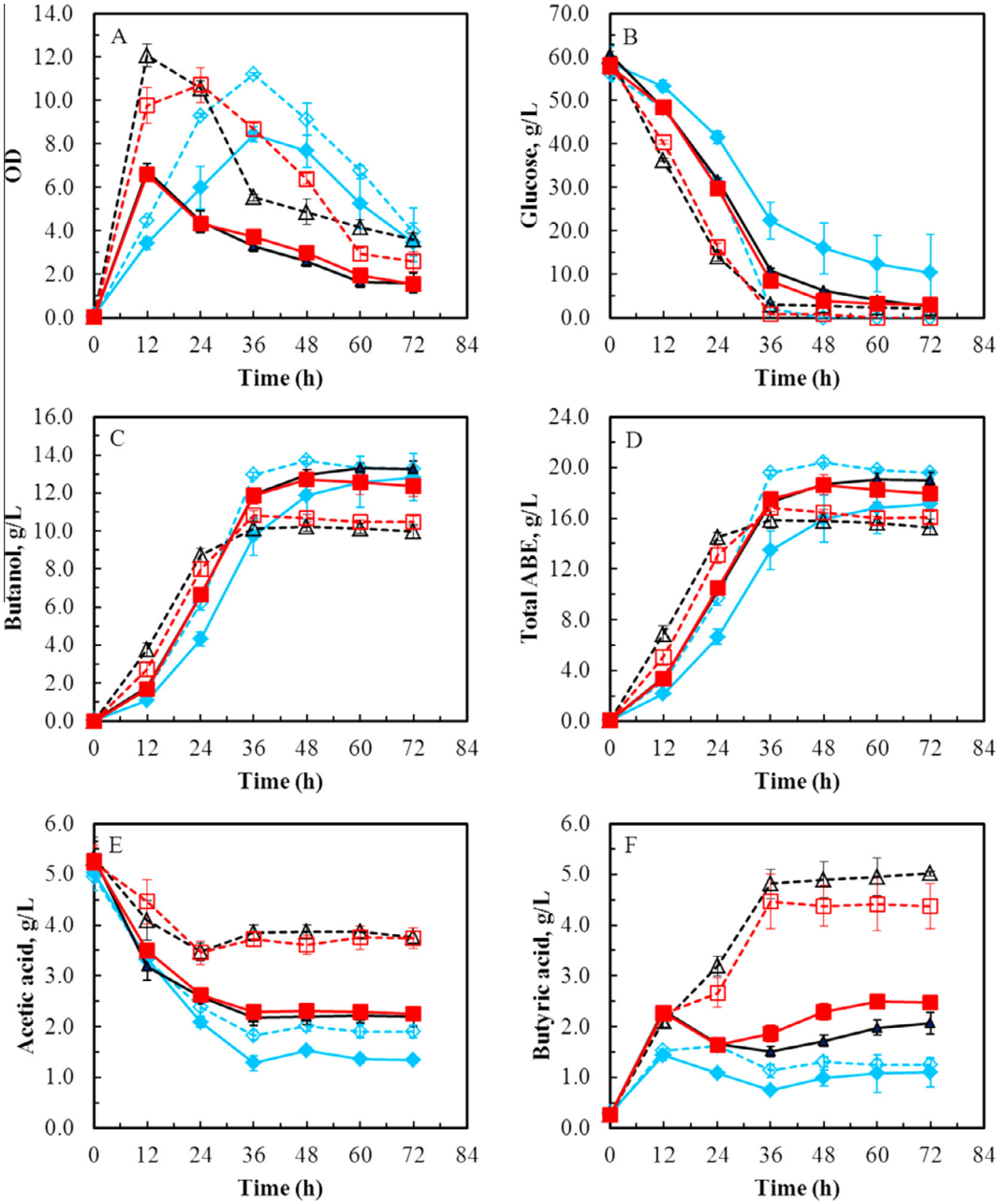

Fig. 3. (A) Growth (B) glucose (C) butanol (D) total ABE (E) acetic acid (F) butyric acid profiles during ABE fermentation using *C. acetobutylicum* ATCC 824 in (♦) glucose P2 medium (▲) Batch 1 derived hydrolysate medium containing precipitate (■) Batch 2 derived hydrolysate medium containing precipitate without $CaCO_3$ (solid line) and with $CaCO_3$ (dash line).

of more lignin derived inhibitors into the hydrolyzate. The release of inhibitors attached to pretreated redcedar solids after pretreatment and washing steps could have also contributed to the increase of inhibitors concentrations during enzymatic hydrolysis. Generally, concentration of inhibitors in hydrolyzate after 48 h of enzymatic hydrolysis was below 100 ppm. Also, hydroxybenzaldehyde detected in the prehydrolyzate was not detected during enzymatic hydrolysis (Table 7). Levulinic acid and formic acid also were not detected in enzymatic hydrolyzates. The detoxification of hydrolyzate with activated carbon resulted in 100% removal of the inhibitors listed in Table 7.

Previous studies showed that syringaldehyde concentration less than 1 g/L and HMF and furfural less than 3 g/L had no effect on either growth and ABE production using *C. acetobutylicum* ATCC 824 (Ezeji and Blaschek, 2008). Less than 0.07 g/L of HMF and furfural were observed in hydrolyzates, indicating they were not the cause of inhibition in non-detoxified hydrolyzate. Vanillic acid concentration of more than 900 ppm caused 50% inhibition of *C. acetobutylicum* growth and ABE production in another study (Ropars et al., 1992). In the present study, vanillic acid concentration was below 4 ppm in the non-detoxified hydrolyzate (Table 7). Cinnamaldehyde at a level above 0.25 g/L inhibited the growth of *E. coli* O157:H7 in a previous study (Kim et al., 2004). Non-detoxified hydrolyzate media contained between 0.03 and 0.20 g/L of cinnamaldehyde, which could have contributed to poor ABE fermentation (Tables 2 and 7). However, the combined effect of multiple inhibitors in the non-detoxified hydrolyzate media can have additive inhibitory effects on growth and ABE production.

One of the problems observed during activated carbon detoxification was the loss of sugars. In the present study, the loss

Table 6
Effect of addition of $CaCO_3$ on ABE fermentations using *C. acetobutylicum* ATCC 824 in pure glucose P2 and detoxified hydrolyzate media from pretreated redcedar Batch 1 and Batch 2 solids containing 60 g/L glucose and acetate buffer pH 5.5 after 72 h fermentation.

Medium	Glucose P2 medium		Hydrolyzate from Batch 1 solids		Hydrolyzate from Batch 2 solids	
	No $CaCO_3$	4 g/L $CaCO_3$	No $CaCO_3$	4 g/L $CaCO_3$	No $CaCO_3$	4 g/L $CaCO_3$
Maximum OD	8.44 ± 0.34^{C}	11.23 ± 0.07^{B}	6.79 ± 0.29^{D}	12.06 ± 0.52^{A}	6.60 ± 0.25^{D}	10.71 ± 0.79^{B}
Final acetone (g/L)	3.95 ± 0.67^{C}	6.04 ± 0.09^{A}	5.46 ± 0.19^{A,B}	5.13 ± 0.31^{B}	5.34 ± 0.21^{B}	5.36 ± 0.20^{B}
Final ethanol (g/L)	0.37 ± 0.03^{A}	0.33 ± 0.00^{B}	0.28 ± 0.01^{C}	0.20 ± 0.01^{E}	0.25 ± 0.02^{D}	0.21 ± 0.01^{E}
Final butanol (g/L)	12.82 ± 1.25^{A}	13.25 ± 0.10^{A}	13.24 ± 0.43^{A}	9.96 ± 0.35^{B}	12.37 ± 0.56^{A}	10.50 ± 0.27^{B}
Final total ABE (g/L)	17.14 ± 1.95	19.61 ± 0.13	18.98 ± 0.63	15.30 ± 0.66	17.96 ± 0.79	16.06 ± 0.45
Glucose consumed (g/L)	48.23 ± 11.97^{A}	56.28 ± 0.89^{A}	57.94 ± 2.32^{A}	58.11 ± 1.40^{A}	54.86 ± 0.47^{A}	58.13 ± 1.86^{A}
ABE yield (g/g)	0.36 ± 0.05	0.35 ± 0.01	0.33 ± 0.00	0.26 ± 0.01	0.33 ± 0.01	0.28 ± 0.01
Final acetic acid (g/L)	1.34 ± 0.07^{D}	1.89 ± 0.12^{C}	2.19 ± 0.20^{B}	3.76 ± 0.11^{A}	2.26 ± 0.03^{B}	3.75 ± 0.20^{A}
Final butyric acid (g/L)	1.09 ± 0.29^{D}	1.24 ± 0.13^{D}	2.06 ± 0.21^{C}	5.02 ± 0.05^{A}	2.48 ± 0.13^{C}	4.38 ± 0.45^{B}
Final total acids (g/L)	2.43 ± 0.34	3.14 ± 0.25	4.25 ± 0.41	8.78 ± 0.16	4.74 ± 0.12	8.13 ± 0.63
Total sugars consumed[a] (g/L)	48.23 ± 11.97	56.28 ± 0.89	61.11 ± 2.54	60.75 ± 1.34	57.86 ± 0.41	60.67 ± 1.98

^{A,B,C,D,E}Duncan's multiple range group; no statistical differences among treatments with the same letter in the same row.
[a] Total sugars included cellobiose, glucose, xylose and mannose.

Table 7
Inhibitor in prehydrolyzate and hydrolyzate generated from 14% solid loading during enzymatic hydrolysis of pretreated redcedar Batch 1 and Batch 2 solids before detoxification.

	Inhibitor concentrations[a]					
	HMF	Furfural	Cinnamaldehyde	Syringaldehyde	Vanillic acid	Hydroxybenzaldehyde
			Batch 1 solids			
Prehydrolyzate (g/L)	5.05 ± 0.52	4.45 ± 0.48	5.92 ± 4.06	0.34 ± 0.05	0.54 ± 0.30	0.22 ± 0.12
Hydrolyzate at 6 h (mg/L)	33.05 ± 1.09	4.76 ± 0.62	115.03 ± 3.53	12.86 ± 1.11	1.54 ± 0.18	ND[b]
Hydrolyzate at 24 h (mg/L)	37.11 ± 2.34	8.49 ± 0.55	127.58 ± 12.95	15.79 ± 1.39	2.08 ± 0.14	ND[b]
Hydrolyzate at 48 h (mg/L)	50.08 ± 6.55	10.27 ± 1.05	202.65 ± 15.28	16.84 ± 1.92	3.25 ± 0.40	ND[b]
			Batch 2 solids			
Prehydrolyzate (g/L)	4.97 ± 0.71	3.79 ± 1.41	14.89 ± 2.00	0.36 ± 0.04	0.27 ± 0.08	0.39 ± 0.02
Hydrolyzate at 6 h (mg/L)	67.78 ± 3.65	24.93 ± 1.55	29.78 ± 3.15	1.93 ± 0.28	3.00 ± 0.34	ND[b]
Hydrolyzate at 24 h (mg/L)	72.60 ± 4.71	26.02 ± 2.25	27.71 ± 1.93	2.73 ± 0.41	3.28 ± 0.03	ND[b]
Hydrolyzate at 48 h (mg/L)	70.54 ± 2.76	26.29 ± 1.50	33.87 ± 7.33	6.28 ± 1.25	3.59 ± 0.44	ND[b]

[a] None of the listed inhibitors were detectable in the detoxified hydrolyzates used in ABE fermentation.
[b] Not detectable.

of sugars in the hydrolyzate media due to detoxification with activated carbon was between 2% and 8%. This can be minimized in future studies by reducing activated carbon loading below 10% (w/v). Also, carbon recycling and reactivation are plausible strategies that can improve the feasibility of butanol production from redcedar. The recycle and reactivation of activated charcoal for three cycles was previously reported during production of xylitol from oak wood (Converti et al., 1999). Based on the maximum theoretical glucose that can be obtained from unpretreated redcedar and the experimental butanol yield of 0.23 g butanol/g glucose, 27.4 gallon butanol/Mg dry redcedar can be produced. Therefore, 315.1 million gallons of butanol can potentially be produced from 11.5 million dry metric tons of above ground redcedar biomass in northwest Oklahoma.

4. Conclusions

Acetate buffer was selected for enzymatic hydrolysis because citrate buffer inhibited ABE fermentation. Detoxification of redcedar hydrolyzate was required to remove inhibitors, enhance growth and butanol production. Butanol (13 g/L) and total ABE (19 g/L) were produced from detoxified redcedar hydrolyzate media, which were comparable to glucose medium. Sodium bisulfite concentration in the hydrolyzate medium should be below 20 ppm not to affect butanol production. The addition of $CaCO_3$ in the hydrolyzate medium did not improve butanol and ABE production. An estimated butanol yield of 27.4 gallons/Mg dry redcedar was calculated, showing the potential for butanol production from softwood.

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