Production of Butanol from Acetyl Chloride-treated Deoiled Rice Bran by *Clostridium acetobutylicum* YM1

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Butanol was produced from pretreated deoiled rice bran (DRB) in a batch culture of *Clostridium acetobutylicum* YM1. The DRB was pretreated by acetyl chloride to produce fermentable sugars prior to butanol fermentation. Pretreatment of DRB using 1% acetyl chloride (AC-DRB) resulted in sufficient fermentable sugars (30.88 g/L), which was comparable to that produced by using 1% sulfuric acid (33.5 g/L). Pretreated AC-DRB contained 18.08 g/L glucose, 9.95 g/L xylose, and 2.86 g/L cellobiose. Detoxification of AC-DRB was performed to remove the fermentation inhibitors, such as furfural, 5-hydroxymethyl furfural (HMF), acetic acid, formic acid, and levulinic acid with the removal efficiencies of 92.98%, 98.82%, 51.53%, 38.72%, and 96.21%, respectively, using charcoal. The detoxification with charcoal was more efficient compared to that with XAD-4 resin. Acetone-butanol-ethanol (ABE) fermentation of detoxified AC-DRB (with 1% AC) by XAD-4 produced 5.64 g/L butanol, while detoxification with charcoal of AC-DRB (with 1% AC) produced 6.48 g/L butanol. In detoxified AC-DRB with charcoal, the maximum butanol and ABE yield of 6.48 g/L and 11.82 g/L, respectively, were achieved. This study is the first reported treatment of biomass using acetyl chloride, which was used as a pretreatment method for successful butanol production.

Keywords: Butanol; Deoiled rice bran; Acetyl chloride Pretreatment; Clostridium acetobutylicum YM1

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INTRODUCTION

Energy's global demand continues to increase with time. Concurrently, traditional energy sources, such as fossil fuels, are non-renewable and their availability decreases with time. The search for renewable alternative liquid fuel has sparked interest in recent decades. Butanol and ethanol are the most proposed liquid biofuels that can substitute fossil fuel. Butanol is a superior fuel compared to ethanol and has a potential to replace gasoline, as it is similar in its properties. In terms of fuel properties and compared to ethanol, butanol has many advantages: it has high energy content; it can be used in current gasoline engines without modifications, it can be shipped through current infrastructure, it exhibits low miscibility with water; and it can be blended with gasoline or used directly (Lee *et al.* 2008; Al-Shorgani *et al.* 2013).

The use of agricultural biomass as feedstock for butanol production requires pretreatment/hydrolysis to produce fermentable sugars, which are subsequently fermented to butanol by *Clostridium* strains (Ezeji *et al.* 2007). Several pretreatment methods have been reported to generate fermentable sugars from agricultural biomass, including physical

and chemical processes or a combination of both, such as acid pretreatment, alkali, radiation, wet oxidation, steam explosion, *etc*. Pretreatment steps can be done by exposing the agricultural residues to severe conditions such as high temperature and chemicals, including dilute acids and/or dilute alkali (Luo *et al.* 2002). However, the pretreatment methods resulted in the formation of fermentation inhibitors such as furfural, hydroxymethyl furfural (HMF), acetic acid, *ρ*-coumaric acid, ferulic acid, and ferulic salts (Larsson *et al.* 1999; Qureshi *et al.* 2008b).

Dilute sulfuric acid is efficient in the conversion of lignocellulosic materials into sugars and it is the most common method in the pretreatment of lignocellulosic biomass. Moreover, the aerosol and fumes of sulfuric acid are considered a human carcinogen by the International Agency for Research on Cancer (IARC) committee according to epidemiological studies (Uleckiené and Griciuté 1997). No reports on the carcinogenicity of acetyl chloride are available. The pretreatment/hydrolysis of agricultural biomasses generates hexoses and pentoses that can be utilized efficiently by solvent-producing *Clostridium spp*. for acetone-butanol-ethanol (ABE) production. Prior to fermentation, the inhibiters associated with lignocellulosic biomass hydrolysis must be detoxified for successful butanol fermentation (Palmqvist and Hahn-Hägerdal 2000).

Deoiled rice bran (DRB) is a residual of the rice processing industry after extracting the oil from the rice bran, which is abundantly available and cheap. The annual worldwide production of rice is estimated to reach 480.1 million metric tons in 2017, according to the United States Department of Agriculture statistics (Childs and Skorbiansky 2017). The DRB has limited application as an animal feed and contains large amounts of carbohydrates. The cheap price, availability, and the carbohydrate content make it a potential substrate for butanol production (Al-Shorgani *et al.* 2012b).

Nevertheless, no reports are available in the literature pertaining to the pretreatment of lignocellulosic biomass using acetyl chloride. In this study, DRB was pretreated by acetyl chloride prior to fermentation and the pretreated DRB was then fermented to butanol using a local aerotolerant strain of *Clostridium acetobutylicum* YM1.

EXPERIMENTAL

Materials

Microorganism

Clostridium acetobutylicum YM1, a local aerotolerant strain provided by Pilot Plant Biotechnology Lab, Department of Chemical and Process Engineering, UKM, was cultivated at 30 ºC in a tryptone–yeast extract–acetate medium (TYA) as previously reported (Al-Shorgani *et al.* 2015b). The TYA medium used to prepare the inoculum consisted of: 20 g/L glucose, 6 g/L tryptone, 3 g/L ammonium acetate, 2 g/L yeast extract, 0.5 g/L potassium dihydrogen phosphate, 0.3 g/L magnesium sulfate heptahydrate, and 0.01 g/L ferrous sulfate heptahydrate. All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Fermentation

Batch fermentation experiments were conducted in 100-mL serum bottles equipped with rubber caps and crimped with aluminum seals with a working volume of 80 mL under anaerobic condition by sparging the medium with nitrogen gas. The pretreated DRB was supplemented with TYA components (without glucose) and the pH of the medium was

adjusted to 6.2 before sterilization. After autoclave sterilization, the medium was left to cool to room temperature, then inoculated with a fresh inoculum of *C. acetobutylicum* YM1 (10% v/v) and then incubated at 30 °C for 72 h.

Pretreatment of DRB

Rice bran was obtained from the Abidin Rice Mill Sdn. Bhd., Perlis, Malaysia, and kept at 4 °C until use. Deoiled rice bran was obtained by extracting the oil from rice bran using hexane (J.T. Baker Chemical Co. Phillipsburg, NJ, USA) as reported by Al-Shorgani *et al.* (2012b). The pretreatment by acetyl chloride was performed by soaking 10% (w/v) of DRB in acetyl chloride (AC) (J.T. Baker Chemical Co. Phillipsburg, NJ, USA) solution and then autoclaved (at 121 °C /15 psi) for 1 h. The solid materials after pretreatment were separated by filtration and the pH of AC-DRB hydrolysate was adjusted to 6.2 by using sodium hydroxide (NaOH) (10 M).

Detoxification of DRB hydrolysate

The DRB hydrolysate was detoxified to reduce the inhibitory effect of the fermentation inhibitory compounds such as furfural, HMF, acetic acid, formic acid, and levulinic acid. The hydrolysate (pH 6) was passed through charcoal or XAD-4 [Amberlite XAD-4 (Sigma-Aldrich, St. Louis, MO, USA)] that were packed in a glass column (60 cm × 2 cm). Approximately 500 mL of hydrolysate passed through 10 g of charcoal or XAD-4 resin. The pH of the detoxified hydrolysates was adjusted to a pH of 6.2 before sterilization.

Methods

Analysis of solvents (acetone, butanol, and ethanol) and acids (acetic and butyric) were conducted using gas chromatography (7890A GC-System, Agilent Technologies, Palo Alto, CA, USA) equipped with a flame ionization detector and a 30-m capillary column (Equity1; 30 m × 0.32 mm × 1.0 µm film thickness; Supelco Co., Bellefonte, PA, USA) as previously described (Al-Shorgani *et al.* 2015a).

Fermentation inhibitor compounds (furfural, HMF, acetic acid, formic acid, and levulinic acid) were detected using high-performance liquid chromatography (HPLC; 12000 Series, Agilent Technologies, Palo Alto, CA, USA) equipped with a Phenomenex C18 column (250 x 4.6 mm ID; Phenomenex Inc., Torrance, CA, USA). The concentrations were measured using a UV detector at 220 nm (UV-D; 1200, Agilent Technologies, Palo Alto, CA, USA) at 40 °C with a flow rate 1 mL/min of mobile phase which contains a mixture of 20 mM sulfuric acid and acetonitrile with a ratio of 95:5, respectively.

Glucose, xylose, and cellobiose were also measured by HPLC (12000 Series, Agilent Technologies, Palo Alto, CA, USA) using a Shodex Asahipak NH_2P-50 4E column (4.6 mm ID × 250 mm; Shodex, Kanagawa, Japan). The concentrations were detected with a refractive index detector (RID; 1200, Agilent Technologies, Palo Alto, CA, USA) at 30 °C with a flow rate 1 mL/min of a mixture of acetonitrile (J.T. Baker Chemical Co. Phillipsburg, NJ, USA) and water (H_2O/CH_3CN = 40/60) as a mobile phase.

The total reducing sugar concentrations were estimated using the 3,5-dinitrosalicylic acid (DNS) assay according to the Miller method (Miller 1959).

RESULTS AND DISCUSSION

Pretreatment of DRB

The DRB (10% w/v) was pretreated with AC (1% v/v and 2% v/v), H_2SO_4 (1% v/v), and HCl (1% v/v), and the total sugar released from the pretreatment was compared. Table 1 shows that the non-treated DRB contained only 4.23 g/L of total sugars, while the pretreatment of DRB by AC, H_2SO_4, and HCl noticeably increased the sugar productions. The pretreatment of DRB by 1% AC released 30.9 g/L of total sugars and that pretreated by HCl produced less sugar (25.7 g/L), while the highest sugar concentration (33.5 g/L) was generated when 1% H_2SO_4 was used.

Deoiled rice bran (DRB) is a residual of the rice bran after extracting the oil. Rice bran is rich in cellulose and hemicellulose in addition to small fractions of starch and lignin. The hemicelluloses in rice bran are complex and contain mainly pentoses (59%), which are mainly xylose and arabinose (Luh *et al.* 1991).

In this study, pretreatment of DRB by dilute acetyl chloride (1%) could efficiently hydrolyze cellulose and hemicellulose in DRB and generated 30.9 g/L of total sugars including monosaccharides such as glucose (58.5%) and disaccharides such as cellobiose (9.3%) were released from cellulose fraction whereas hemicellulose fraction was converted to pentoses such as xylose (32.2%). The data in this study showed that the chemical pretreatment process of DRB increased the sugar content approximately 86%.

Table 1 shows that besides sugars production, many other compounds were also produced during the chemical treatment by AC, HCl, and H_2SO_4. Aliphatic carboxylic acids, such as acetic acid, formic acid, levulinic acid, and furan aldehydes including HMF and furfural, were produced during the pretreatment process due to the further degradation of sugars. These compounds are known as inhibitory compounds for microbial growth and ABE fermentation. Furfural, HMF, levulinic acid, formic acid, and acetic acid were reported to be strong inhibitor compounds for *Clostridium* growth and subsequently lead to the failure fermentation of butanol (Larsson *et al.* 1999; Kudahettige-Nilsson *et al.* 2015).

Table 1. Comparison of Sugar and Inhibitors Production from DRB Pretreated with 1% AC and 2% AC, 1% Sulfuric Acid, and 1% Hydrochloric Acid

Pre-treatment Method	Sugar (g/L)	Fermentation Inhibitors (g/L)				
		Furfural	HMF	Levulinic Acid	Formic Acid	Acetic Acid
No treatment	4.23	-	-	-	-	-
AC 1%	30.88	0.05	0.036	0.07	0.41	3.22
AC 2%	39.00	0.35	0.357	0.85	0.31	3.55
H_2SO_4 1%	33.50	0.34	0.460	1.18	0.10	3.17
HCl 1%	25.72	0.36	0.405	0.74	0.30	2.82

Moreover, phenolic inhibitors, such as ferulic acid, ρ-coumaric acid, and syringaldehyde, were reported as strong fermentation inhibitors compared to furfural and HMF (Yao *et al.* 2017). In contrast, Ezeji *et al.* (2007) found that the presence of low concentrations of HMF and furfural supported the production of butanol; however, the

production of butanol was decreased and the growth of *C. beijerinckii* BA101 was inhibited when the concentrations of HMF and furfural exceeded the optimal level. However, the presence of 0.3 g/L rho-coumaric and ferulic acids resulted in the significant decrease in *C. beijerinckii* BA101 growth and ABE production (Ezeji *et al.* 2007).

The concentrations of the inhibitory compounds were varied based on the pretreatment method used (type of chemical) and the concentration of the chemical used for pretreatment (Table 1). Pretreatment of DRB with 1% AC produced the lowest concentrations of the inhibitory compounds, whereas the sulfuric acid pretreatment (1% v/v) produced the highest concentrations of sugars and microbial inhibitors. These inhibitors were produced due to the extreme degradation of biomass by chemicals, and should be removed or detoxified to conduct successful butanol fermentation.

In a trial to improve the sugar generation from DRB, the concentration of AC was increased up to 5 % and the sugars were measured after the pretreatment. The results showed that increasing the concentration of AC up to 4% led to an improvement in the production of sugars and the highest sugar generation was 40 g/L with an AC concentration of 3% and 4%, while beyond that the sugar concentration was decreased (Fig. 1).

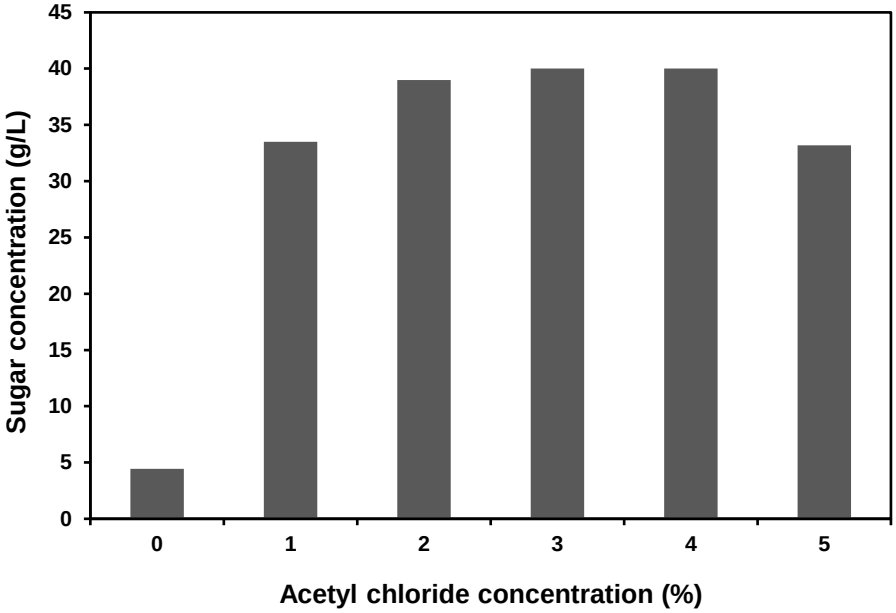

Fig. 1. Effect of acetyl chloride (AC) concentration on sugar production from DRB (10%)

Pretreatment with 2% AC produced 39 g/L of total reducing sugars and the sugar concentration did not increase much with 3% and 4% of AC, while only 40 g/L of sugar was obtained as shown in Fig. 1. However, a significant decrease of reducing sugars was observed when the acetyl chloride concentration increased beyond 4%. This could be the result of extreme hydrolysis, which leads to the degradation of sugars into carboxylic acids and furan compounds and results in a decrease of reducing sugars.

Butanol Fermentation of Pretreated DRB

The ABE fermentation of non-treated DRB, DRB treated by 1% sulfuric acid (SA-DRB), HCl-DRB, and AC-DRB was conducted in a batch culture of *C. acetobutylicum* YM1. Non-treated DRB was used as a control and contained 4.43 g/L of total reducing sugar. In addition, the TYA medium with 30 g/L glucose was also used as a control. All

fermentation culture were supplemented with TYA components and inoculated with 10 % (v/v) fresh inoculum, and then incubated at 30 ºC. Fermentation of non-treated DRB produced 5.15 g/L of total solvent and 3.30 g/L butanol, which were similar to that reported previously by Al-Shorgani *et al.* (2012b) when non-treated DRB was fermented by *C. saccharoperbutylacetonicum* N1-4. The SA-DRB hydrolysate contained the highest sugars content and produced the highest concentrations of ABE and butanol as 13.08 g/L and 7.53 g/L, respectively. The cultivation of *C. acetobutylicum* YM1 with 1% AC-DRB produced 10.56 g/L total ABE containing 4.55 g/L acetone, 5.60 g/L butanol, and 0.41 g/L ethanol. Fermentation of TYA containing 30 g/L glucose, as a comparison to the pretreated DRB that contained a similar amount of sugar, resulted in the production of 6.22 g/L butanol with 9.10 g/L total ABE, which was higher than that produced from AC-DRB (with 1% AC) and lower than that obtained from SA-DRB. In terms of butanol productivity and yield, SA-DRB was the best material for the fermentation (Table 2).

Consequently, the data showed that the pretreatment of DRB was an essential step for the generation of fermentable sugars that subsequently improved the fermentation efficiency of butanol.

Table 2. Butanol Fermentation of TYA, SA-DRB (1% SA), and AC-DRB (1% AC) in Batch Culture of *C. acetobutylicum* YM1

Parameters	Medium			
	TYA	DRB	SA-DRB	AC-DRB
Initial Sugar (g/L)	30	4.43	33.50	30.88
Acetone (g/L)	2.53	1.66	4.83	4.55
Butanol (g/L)	6.22	3.30	7.53	5.60
Ethanol (g/L)	0.35	0.20	0.72	0.41
ABE (g/L)	9.10	5.16	13.08	10.56
B:A Ratio	2.46	1.99	1.56	1.23
Acetic Acid (g/L)	0.35	0.15	1.74	0.69
Butyric Acid (g/L)	0.50	1.26	0.20	0.92
Total Acids (g/L)	0.83	1.41	1.94	1.61
Butanol Yield (g/g)	0.21	0.74	0.22	0.22
Butanol Productivity (g/L·h)	0.086	0.046	0.105	0.078

Based on the data presented in Tables 2 and 4, the concentrations of acetone produced from the DRB hydrolysates were higher than that gained in the control cultures. In addition, the ratios of butanol to acetone (B:A) were less than 2, while the normal ratio of B:A in ABE fermentation is 2:1 (Jones and Woods 1986). In the control experiment using the TYA medium, the ratio of B:A was 2.46, while the lower B:A ratios were obtained from the fermentation of the DRB hydrolysates. Thermal degradation of lignocellulosic biomass released some organic acids, such as acetic acid, and it was noticeable that the concentrations of acetic acid were higher than 3 g/L (Table 1) in all

DRB hydrolysates. Acetic acid is the precursor of acetone in ABE fermentation by solvent-producing *Clostridium*. In the ABE fermentation pathway, acetic acid and acetoacetyl-CoA are converted into acetoacetate by acetoacetyl-CoA:acetate transferase, which then converts to acetone *via* acetoacetate decarboxylase (Wiesenborn *et al.* 1989; Petersen and Bennett 1990). Hence, the high production of acetone in DRB hydrolysates and subsequently low B:A ratios can be attributed to the high initial acetic acid concentration in the DRB hydrolysates.

Detoxification of Acetyl Chloride-pretreated DRB

As mentioned above, fermentation inhibitory compounds, such as furfural, HMF, acetic acid, formic acid, and levulinic acid, were detected in DRB hydrolysates due to the chemical pretreatment by AC. In this study, two different detoxification methods, using activated charcoal and a nonionic polymeric adsorbent XAD-4 resin, respectively, were applied to reduce the inhibitors. The detoxified AC-DRB were then fermented to produce butanol with a batch culture of *C. acetobutylicum* YM1.

Based on the results presented in Table 3, it is clear that activated charcoal showed a high ability to reduce the inhibitory compounds, which is remarkably better than XAD-4 resin. The concentrations of furfural, HMF, acetic acid, formic acid, and levulinic acid were reduced by 93.0%, 98.8%, 51.5%, 38.7%, and 96.2%, respectively, with charcoal used as the detoxification agent. The XAD-4 did not show similar efficiency as a detoxification method to reduce HMF, acetic acid, formic acid, and levulinic acid, while it only showed similar reduction efficiency of furfural compared to that of charcoal (Table 3). It was reported that the detoxification of corn fiber hydrolysate by XAD-4 did not remove the fermentation inhibitors completely, and it was suggested that these compounds should be completely removed for successful butanol fermentation prior to fermentation with *C. beijerinckii* BA101 (Qureshi *et al.* 2008a). This is in agreement with the results obtained in this study.

Table 3. Reduction of Fermentation Inhibitors of Detoxified DRB-treated with AC

Inhibitor Reduction (%)	Detoxification Method	
	Charcoal	XAD-4
HMF	98.82	16.49
Furfural	92.96	92.53
Acetic Acid	51.54	4.96
Formic Acid	38.72	13.39
Levulinic Acid	96.21	18.62

It is noticeable that charcoal and XAD-4 could reduce the inhibitory compounds, while negligible sugars were reduced, which is a good property to avoid the loss of sugars during the detoxification process. This is in agreement with a study conducted by Kudahettige-Nilsson *et al.* (2015), who found that detoxification resulted in 99% to 100% recovery of xylose using activated charcoal of hardwood kraft black liquor hydrolysate. Activated charcoal is a cost effective detoxification method and it has high ability to adsorb inhibitor compounds with less effect on reducing sugar concentration (Mussatto and Roberto 2004; Kamal *et al.* 2011). The results in this study indicated that activated charcoal

had a high ability to minimize HMF and furfural concentrations compared to XAD-4 resin, where it was able to remove 98.8% of HMF and 93.0% of furfural. Guo *et al*. (2013) found that detoxification of spruce hydrolysate with charcoal removed 94% of furfural and HMF, which is similar to the results obtained in this study.

Butanol Fermentation from Detoxified AC-DRB Hydrolysates

Detoxified AC-DRB hydrolysates were fermented by *C. acetobutylicum* YM1 for butanol production in a batch culture. As shown in Table 4, butanol production from non-detoxified AC-DRB and detoxified AC-DRB by XAD-4 was similar. In addition, the butanol yield and productivity were also similar as 0.22 g/g and 0.08 g/L·h, respectively.

In regards to the AC-DRB hydrolysate detoxified by activated charcoal, the butanol concentration obtained was 6.48 g/L with a total ABE of 11.82 g/L, which was higher than that found from non-detoxified hydrolysate and hydrolysate detoxified by XAD-4 with a butanol yield and productivity of 0.24 g/g and 0.09 g/L·h, respectively (Table 4). Moreover, titers of butanol and ABE found from detoxified AC-DRB with charcoal were higher than those obtained from control culture of TYA containing 30 g/L glucose as shown in Table 2.

Table 4. Effect of Detoxification of DRB Pretreated by 1% AC on Butanol Production

Parameters	Detoxification Method		
	Control	Charcoal	XAD-4
Initial Sugar (g/L)	30.88	30.29	30.15
Growth (OD_{600nm})	1.95	2.23	2.20
Acetone (g/L)	4.55	4.84	3.79
Butanol (g/L)	5.60	6.48	5.64
Ethanol (g/L)	0.41	0.50	0.34
ABE (g/L)	10.56	11.82	9.77
B:A ratio	1.23	1.34	1.49
Acetic Acid (g/L)	0.69	1.83	0.56
Butyric Acid (g/L)	0.92	1.21	0.63
Total Acids (g/L)	1.61	3.04	1.18
Residual Sugar (g/L)	5.74	2.96	4.35
Butanol Yield (g/g)	0.22	0.24	0.22
Butanol Productivity (g/L·h)	0.08	0.09	0.08

Pretreatment of DRB by 1% AC resulted in the production of a mix of sugars including glucose (18.08 g/L), xylose (9.95 g/L), and cellobiose (2.86 g/L). Solvent-producing *Clostridium* strains are able to consume hexoses and pentoses sugars simultaneously for the production of butanol (Liu *et al.* 2010).

The increase of the AC concentration to 2% resulted in the decrease of butanol fermentation efficiency, although the reducing sugar content was high. The removal of

fermentation inhibitors led to the improvement of bacterial growth and ABE yield, however, the production of butanol and ABE was still less than that obtained from AC-DRB with 1% AC. This may have been attributable to the increase of chloride ion in the hydrolysate due to the high concentration of AC (2%). The reaction of AC with water results in the production of acetic acid and HCl according to Eq. 1,

$$CH_3COCl + H_2O \rightarrow CH_3COOH + HCl \quad (1)$$

A high concentration of chloride was reported to have an inhibitory effect on bacterial growth, butanol fermentation, and biohydrogen production (Wang *et al.* 1995; Al-Alawi 2007; Al-Shorgani *et al.* 2012a).

Table 5. Effect of Detoxification of DRB Pretreated by 2% AC on Butanol Fermentation by *C. acetobutylicum* YM1

Parameters	Detoxification		
	Non	Charcoal	XAD-4
Initial Sugar (g/L)	39.80	39.20	39.33
Inhibitors (g/L)			
HMF	0.357	0.04	0.298
Furfural	0.35	0.02	0.03
Acetic Acid	3.22	1.22	3.05
Levulinic Acid	0.85	0.03	0.69
Formic Acid	0.13	0.08	0.69
ABE Fermentation			
Growth (OD_{600nm})	0.31	2.62	2.54
Acetone (g/L)	0	3.85	3.11
Butanol (g/L)	0	3.48	3.30
Ethanol (g/L)	0	0.50	0.45
ABE (g/L)	0	7.83	6.86

Heavy metal ions, such as iron, copper, nickel, and chromium can be formed during the acidic pretreatment of cellulosic biomass due to the corrosion of pretreatment equipment under high temperature and acidic conditions. These ions can be inhibitory to microbial fermentation. In addition, cations such as calcium, magnesium, and sodium, can appear from chemical pretreatment or from the adjustment of pH (Watson *et al.* 1984; Jönsson and Martín 2016). Another possibility of inhibition is the high concentration of NaCl that may be formed due to the availability of chloride ion and sodium ion. It was reported that cell growth and sugar uptake were halted at high sodium concentrations (Zhao *et al.* 2016). The effect of sodium chloride on cell growth and solvent production was attributed to the osmotic pressure, which dehydrates the cell periphery and therefore causes damage to cell membrane permeability (Shi *et al.* 2011).

Production of furan compounds, as well as carboxylic acids, are associated with biomass pretreatment methods, which decreased the sugars yields. Hence, it is desirable to minimize the generation of these inhibitory compounds by manipulating the pretreatment

process conditions, such as the concentration of chemical, temperature, and time of pretreatment. However, in regards to butanol production and productivity, it was better to use AC-DRB (with 1% AC) in butanol fermentation.

A summary of solvent production from various agricultural substrates by solventogenic strains of clostridia including corn fiber, distillers dried grains and soluble, wheat bran, rice straw and palm kernel cake is provided in Table 6.

Table 6. Production of Butanol from Various Agrowastes by Solventogenic Clostridium

Substrate	Strain	Treatment	Sugars (g/L)	ABE (g/L)	Butanol (g/L)	Reference
Corn Fiber	*C. beijerinckii* BA101	Dilute sulfuric acid + enzyme hydrolyzed + detoxification by XAD-4	46.3	9.3	6.4	(Qureshi *et al.* 2008a)
Wheat Bran	*C. beijerinckii* ATCC 55025	Dilute sulfuric acid	53.1	11.8	8.8	(Liu *et al*. 2010)
Distillers Dried Grains and Soluble	*C. beijerinckii* BA101	Ammonium fiber expansion + enzyme hydrolyzed	41.4	10.4	7.9	(Ezeji and Blaschek 2008)
Palm Kernel Cake	*C. saccharoperb-utylacetonicum*	Dilute sulfuric acid	35.97	5.89	3.59	(Shukor *et al.* 2014)
Rice Straw	*C. acetobutylicum* NCIM 2337	Dilute sulfuric acid with shear stress	39.88	20.56	13.5	(Ranjan *et al.* 2013)
Deoiled Rice Bran	*C. acetobutylicum* YM1	Dilute acetyl chloride	30.88	11.82	6.48	This study

In our study, utilization of detoxified-SADRB produced a maximum ABE concentration of 11.82 g/L which is higher than produced from using corn fiber hydrolysate (Qureshi *et al.* 2008a), palm kernel cake hydrolysate (Shukor *et al.* 2014), and distillers dried grains and soluble hydrolysate (Ezeji and Blaschek 2008). A similar ABE concentration was obtained when wheat bran hydrolysate was consumed as a substrate by *C. beijerinckii* ATCC 55025 (Liu *et al.* 2010). However, Ranjan *et al.* (2013) reported higher ABE concentration from fermentation of rice straw hydrolysate by C. *acetobutylicum* NCIM 2337 (Table 6). The differences in ABE production can be attributed to the differing nature of the feedstock, the content of sugar concentration, the *Clostridium* strains used and the presence of the fermentation inhibitory compounds.

CONCLUSIONS

1. This study demonstrated a successful pretreatment method of DRB by dilute acetyl chloride that led to an improvement of the sugar content for butanol fermentation.
2. Pretreatment of DRB with 2% acetyl chloride increased the sugar production approximately 10 times compared to non-pretreated DRB.
3. Detoxification of pretreated DRB was essential to decrease the fermentation inhibitors and enhance the butanol productivity.
4. Pretreatment of DRB with 1% acetyl chloride was the best for butanol production, in which 6.48 g/L of butanol and 11. 82 g/L of ABE were obtained after detoxification by charcoal.

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