Production of Acetone, Butanol, and Ethanol (ABE) by *Clostridium acetobutylicum* YM1 from Pretreated Palm Kernel Cake in Batch Culture Fermentation

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The viability of most fermentation processes is very much dependent on the cheap fermentation medium used. Palm kernel cake (PKC) is an abundant biomass generated from the palm oil processing industry that can be used as the carbon source for the growth and production of acetone-butanol-ethanol fermentation (ABE) by *Clostridia*. In this study, ABE production from the fermentation of PKC using *Clostridium acetobutylicum* YM1 in a batch culture was conducted. The PKC was subjected to treatment with acids (sulphuric and hydrochloric acids), alkali (sodium hydroxide and alkaline peroxide), enzymatic hydrolysis, and hydrothermal treatment (in autoclave). The sulphuric acid-treated PKC (2% SAPKC) method produced the highest concentration of reducing sugars (30 g/L) compared with the other methods applied. The results showed that increasing the concentration of H_2SO_4 up to 3% decreased the amounts of generated reducing sugars to 20.4 g/L, which is about 32% less. The fermentation of 1%, 2%, and 3% SAPKC resulted in the production of ABE of 1.07, 5.72, and 3.48 g/L, respectively. This study showed that the pretreatment of PKC improved the content of fermentable sugars and subsequently enhanced the production of ABE by *C. acetobutylicum* YM1. This study also revealed that PKC can be regarded as a potentially low cost substrate for ABE fermentation.

Keywords: Palm kernel cake (PKC); Clostridium acetobutylicum YM1; Acetone-butanol-ethanol (ABE); Pretreatment

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INTRODUCTION

In the search for a sustainable bio-based fuel acetone-butanol-ethanol (ABE), fermentation has received increasing interest in the past decades (Lu *et al*. 2013). The ABE fermentation involves more sophisticated biotechnology to convert fermentable sugars into biobutanol by a solvent-producing bacteria, particularly the strains of *Clostridium* sp. (Chen *et al.* 2013). Substrate cost has a high impact on butanol price, which represents at least 63% of the total butanol production cost (Jones and Woods 1986). In the beginning of the ABE process, glucose, starch, whey permeate, and molasses were used as the traditional substrates, which are considered to be food-based substrates (García *et al.* 2011; Kumar and Gayen 2011; Nigam and Singh 2011). Recently, many studies have focused on finding alternative inexpensive and non-food-based substrates. In the past few years, many studies have focused on the use of lignocellulose materials, such as palm oil mill effluent

(Kalil *et al.* 2003), corn stove and barley straw (Qureshi *et al.* 2014), rice bran, and de-oiled rice bran (Al-Shorgani *et al.* 2011), as feedstocks for the ABE fermentation process.

Lignocellulosic biomass has been known to be a promising source of fermentable sugars. Lignocellulose represents an extensive renewable carbon source on the earth that is mainly composed of cellulose, hemicellulose, and lignin (Sindhu *et al.* 2016a). The diversity of these compounds depends on their original source, whether softwood, hardwood, or grass. To release the sugars stored in the form of hemicellulose and cellulose, the lignocellulosic material must first be pretreated and hydrolysed to open the polymeric structure (Jørgensen *et al.* 2007). Among different pretreatment methods, acid and alkali treatments are the most promising approaches that could enhance sugar recovery (Kumar *et al.* 2013). The pretreatment of lignocellulosic materials could constitute approximately 40% of the total biofuel production costs (Sindhu *et al.* 2016a). Therefore, it is important to develop an efficient and economically feasible pretreatment method for the cost-effective production of biofuels (Sindhu *et al.* 2016b).

Palm kernel cake (PKC) is known as a lignocellulosic residue that is obtained after the extraction of the oil from palm kernels. The PKC is one of the main by-products of the palm oil industry and is largely produced in tropical countries such as Malaysia and Indonesia. The production of PKC in Malaysia has increased in recent years so that 2,520,000 tons of PKC were produced during 2015 (MBOP 2016). The study on the PKC structure has shown that it contains a high amount of polysaccharide (50%), with a high hemicellulose content (Cerveró *et al.* 2010). Similar studies have shown that cellulose constitutes about 11.6% of PKC polysaccharide, and the hemicellulose composition of PKC includes a large amount of mannan (57.8%) with a lower quantity of xylan (3.7%) (Ong *et al.* 2004). PKC also contains protein (22% w/w), fat (4.6% w/w), and many minerals such as, potassium (6190.9 mg/kg PKC), calcium (533.5 mg/kg PKC), sodium (382.5 mg/kg PKC), iron (310.4 mg/kg PKC), manganese (161.6 mg/kg PKC), and phosphorus (0.84 mg/kg PKC) (Shukor *et al* 2016). Hence, PKC represents a potential source of fermentable sugars that could be recovered using the pretreatment methods. In this regard, in line with the utilization of lignocellulosic materials for the generation of fermentable sugars, little information is available on the pretreatment of PKC to depolymerize its lignocellulose composition and its recovery of reducing sugars.

The current study was conducted to evaluate the different pretreatment methods of PKC for the efficient sugar recovery from the polysaccharides of PKC as an economically viable feedstock. Furthermore, the hydrolysate obtained from the pretreatment methods tested was utilized for the ABE fermentation by *C. acetobutylicum* YM1. The *C. acetobutylicum* YM1 was recently isolated from a Malaysian agricultural soil and found as a hyper-butanol producing strain (Al-Shorgani *et al.* 2015, 2016)

EXPERIMENTAL

Microorganism and Medium Preparation

Clostridium acetobutylicum YM1 was provided by the biotechnology lab, Department of Chemical and Process Engineering, Universiti Kebangsaan Malaysia (Bangi, Malaysia). A tryptone-yeast extract-acetate (TYA) medium was used for the culture of *C. acetobutylicum* YM1. The medium was sterilized at 121 °C for 15 min, and its initial pH was adjusted to 6.2. This medium was used to prepare the inoculum of *C. acetobutylicum* YM1. Microbial inoculum was prepared by transferring 1 mL of the spore

suspension of *C. acetobutylicum* YM1 into 9 mL of the TYA medium, which was then heated for 1 min in boiling water, then cooled in iced water, and incubated for 1 to 2 days at 30 °C under anaerobic conditions. This culture was then subcultured in the TYA medium and incubated for 18 h to 20 h to be used as an inoculum source.

The TYA composition consisted of the following components: tryptone 6 g/L; yeast extract 2 g/L; ammonium acetate 3 g/L; KH_2PO_4 0.5 g/L; $MgSO_4.7H_2O$ 0.3 g/L; and $FeSO_4.7H_2O$ 0.01 g/L (Komonkiat and Cheirsilp 2013). To investigate the ability of *C. acetobutylicum* YM1 to consume PKC-derived sugars, the TYA medium was supplemented with 30 g/L of different sugars including glucose, mannose, and a mixture of glucose and mannose.

PKC Pretreatment

The PKC was provided by the Malaysia palm oil board (MPOB). The PKC was first ground and passed through a sieve with a 500-µm mesh to obtain fine particles. It was then subjected to the different pretreatment methods, including acid pretreatment (sulphuric and hydrochloric acids), alkali pretreatment (sodium hydroxide and alkaline peroxide), enzymatic hydrolysis, and hydrothermal pretreatment to generate various samples labeled as SAPKC, HAPKC, SHPKC, HPPKC, EHPKC, and HPKC, respectively.

For the sulphuric acid pretreatment, 100 g of PKC was added to 1 L of 1%, 2%, and 3% (v/v) sulphuric acid solutions in 2-L Scott Duran bottles individually and was heated for approximately 45 min at 121 °C to generate sulphuric acid-pretreated PKC (SAPKC 1%, 2%, and 3%). For comparison, the PKC was also pretreated with hydrochloric acid (HCl) by the addition of 100 g of PKC to 1 L of 1% and 2% HCl (v/v) solutions and was heated (121 °C and 45 min) to generate hydrochloric acid-pretreated PKC (HAPKC) sample.

The alkali pretreatment of PKC was performed using sodium hydroxide 1% (w/v) and alkaline peroxide separately to generate sodium hydroxide-pretreated PKC (SHPKC 1%) and hydrogen peroxide-pretreated PKC (HPPKC) samples, respectively. For the sodium hydroxide pretreatment, PKC was added to a 1% NaOH solution to obtain a PCK loading of 10% (w/v). It was mixed well and heated at 80 °C for 1 h using a water bath. Subsequently, the mixture was centrifuged at 5000 rpm under room temperature for 15 min to remove the residual solids and the supernatant was kept at 4 °C for the reducing sugar analysis and ABE fermentation. The PKC was also pretreated with hydrogen peroxide as described by (Qureshi *et al.* 2008). Prior to fermentation, the pH was adjusted to 6.2 using concentrated HCl. For the hydrothermal pretreatment, the PKC was subjected to hot steam using an autoclave set at 121 °C for 60 min.

Enzymatic Saccharification

Enzymatic hydrolysis was performed in 250-mL Duran bottles with 10% dry matter of PKC in a 0.2 mM sodium acetate buffer (pH 4.5). Thereafter, 1589.4 IU/mL mannanase (Habio Bioengineering Co., Ltd. Mianyang, China) enzyme was added to provide 5% and 10% ($w/w_{substrate}$) enzyme loadings. The enzymatic treatment was performed at a temperature of 60 °C for 72 h reaction time under 200 rpm agitation to generate enzyme-treated PKC hydrolysate (EHPKC). Samples were withdrawn at regular intervals, filtered using a filter paper, and kept at 4 °C for sugar analysis. After enzymatic hydrolysis, the pH was adjusted to 6.2 and was used directly for ABE fermentation by *C. acetobutylicum* YM1.

ABE Fermentation

The ABE fermentations were performed using PKC-derived sugars, namely glucose, mannose, and the mixture of glucose and mannose as the carbon source. About 100 mL of the TYA medium containing 30 g/L of these sugars was transferred into 250-mL Scott Duran bottles. The initial pH of the medium was adjusted to 6.2 by 10 M of NaOH solution. The medium was sterilized by autoclaving at 121 °C for 15 min. Before inoculation, the medium was flushed with oxygen-free nitrogen to make anaerobic conditions. The TYA medium was inoculated with 10% (v/v) of a fresh suspension of *C. acetobutylicum* YM1 (grown bacterial cells in TYA medium for 18 h to 20 h at 30 °C under anaerobic conditions), and the culture was incubated at 30 °C for 72 h. The culture samples were withdrawn for the measurement of sugars and generated solvents during ABE fermentation. Similarly, the ABE fermentation was performed using 100 mL of PKC-derived hydrolysate in 250-mL Scott Duran bottles.

Analytical Methods

The culture samples were centrifuged at 10,000 rpm for 5 min to separate the sediments, and the clear supernatant was stored at -18 °C. The ABE solvents and organic acids (acetic acid and butyric acid) were measured using a gas chromatography system (7890A GC-System; Agilent Technologies, 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). The injector and detector temperatures were set at 250 °C and 280 °C, respectively. Helium was used as the carrier gas, and it was set at a flow rate of 1.5 mL/min.

The concentrations of reducing sugars were measured by high-performance liquid chromatography (HPLC 12,000 Series; Agilent technologies, Palo Alto, CA, USA) using a SUPELCOGEL C-611 HPLC column (300 mm × 7.8 mm ID). The sugar concentrations were detected using a refractive index detector (RID, Agilent Technologies, Alto, CA, USA) at 60 °C and a flow rate of 0.5 mL/min using 10^{-4} M sodium hydroxide solution as the mobile phase. The cell growth of *C. acetobutylicum* YM1 was detected at 600 nm using a UV-vis spectrophotometer (Genesys 10, Thermo Spectronic, USA).

RESULTS AND DISCUSSION

Butanol Production using PKC-derived Sugars

The ABE experiments were first performed to investigate the ability of *C. acetobutylicum* YM1 to grow and produce butanol from the main sugars that could be recovered from the hydrolysis of PKC. The experiments were conducted in 250-mL Scott Duran bottles containing 100 mL of a TYA medium supplemented with 30 g/L of individual sugars (glucose, mannose, and/or a mixture of both). The cultures were then incubated at 30 °C for 72 h. The experimental results showed that the culture supplemented with glucose produced the highest concentration of butanol (6.22 g/L) and ABE (10.27 g/L) solvents after 54 h of incubation time, while the culture supplemented with mannose stopped the production of butanol within 72 h, and approximately 4.70 g/L butanol and 7.83 g/L ABE were produced (Table 1). A similar consumption of glucose (29.7 g/L) and mannose (28.6 g/L) was observed by the YM1 strain. It was reported that glucose is the preferred sugar for the clostridial cells in relation to the ABE process (Ezeji *et al.* 2007). Evidently, the ABE fermentation using the glucose-based medium exhibited a higher yield

and productivity of ABE with values as high as 0.35 g/g and 0.19 g/L.h, respectively. However, in the culture with mannose, the yield and productivity of ABE were 0.27 g/g and 0.11 g/L.h, respectively. This indicated that mannose could be used as an important sugar derived from hemicellulose for an efficient ABE fermentation.

Table 1. ABE Fermentation of Glucose and Mannose by *C. acetobutylicum* YM1

Fermentation Characteristics	Glucose	Mannose	Glucose + Mannose[a]
Sugar Consumed (g/L)	29.2	28.6	29.1
Butanol Concentration (g/L)	6.22	4.70	5.24
ABE Concentration (g/L)	10.27	7.83	8.24
Total Acids (g/L)	1.24	1.40	2.48
ABE Yield (g/g)	0.35	0.27	0.28
ABE Productivity (g/L·h)	0.19	0.11	0.11

Note: [a] The ratio of glucose and mannose is 1: 7

The results obtained from the fermentations of glucose and mannose showed that *C. acetobutylicum* YM1 could utilize the main sugars in PKC (mannose and glucose) efficiently for the production of butanol. To investigate the ability of *C. acetobutylicum* YM1 to ferment the mixture of glucose and mannose to butanol, an experiment was performed using the mixed sugars of glucose and mannose at a ratio of 1:7 (as in the H_2SO_4 pretreated hydrolysate) dissolved in the TYA medium. After 72 h of ABE fermentation, the cultures stopped the production of butanol due to the utilization of the sugars. The experimental results showed that 5.24 g/L butanol and 8.24 g/L ABE were produced during this time. It was noteworthy that, although the amount of glucose in the mixture was low, the ABE yield (0.28 g/g) was slightly increased, while the ABE productivity (0.11 g/L.h) was equal to that of the ABE productivity obtained from the medium with solely mannose.

The cultivation of *C. beijerinckii* BA101 in the P2 medium containing 55 g/L glucose resulted in 13.5 g/L butanol and 17.8 g/L ABE, while similar cultivation using pure mannose (55 g/L) triggered 12 g/L butanol and 14.20 g/L ABE (Ezeji *et al.* 2007), respectively.

Another batch of ABE culture was performed using raw PKC as a carbon source without pretreatment and nutrient supplementation. After PKC sterilization, a small amount of sugar was released (0.79 g/L), and then the ABE fermentation was run by *C. acetobutylicum* YM1 for 72 h. The experimental results revealed the production of 0.21 g/L and 0.33 g/L butanol and ABE, respectively. The low ABE and butanol concentrations produced could be attributed to the low sugar concentration in untreated PKC. It was reported that less than 10 g/L of glucose was unable to shift the metabolites from acid to solvent production in ABE fermentation by solveno-genic clostridial strains (Long *et al.* 1984; Fond *et al.* 1985).

In another attempt, the PKC was pretreated using hot steam by autoclaving of PKC at 121 °C for 1 h. The pretreatment of PKC by hot steam showed that 1.10 g/L of reducing sugars were produced. As shown in Table 2, the amount of ABE and butanol produced was similar to that using untreated PKC with values of 0.34 g/L and 0.21 g/L, respectively. The hot steam pretreatment did not considerably improve the sugar generation from PKC, and the same concentrations of butanol and ABE were obtained from the hot steam pretreated- and untreated- PKC samples (Table 2).

Table 2. ABE Fermentation of Untreated PKC and Hot Steam Pretreated PKC using *C. acetobutylicum* YM1

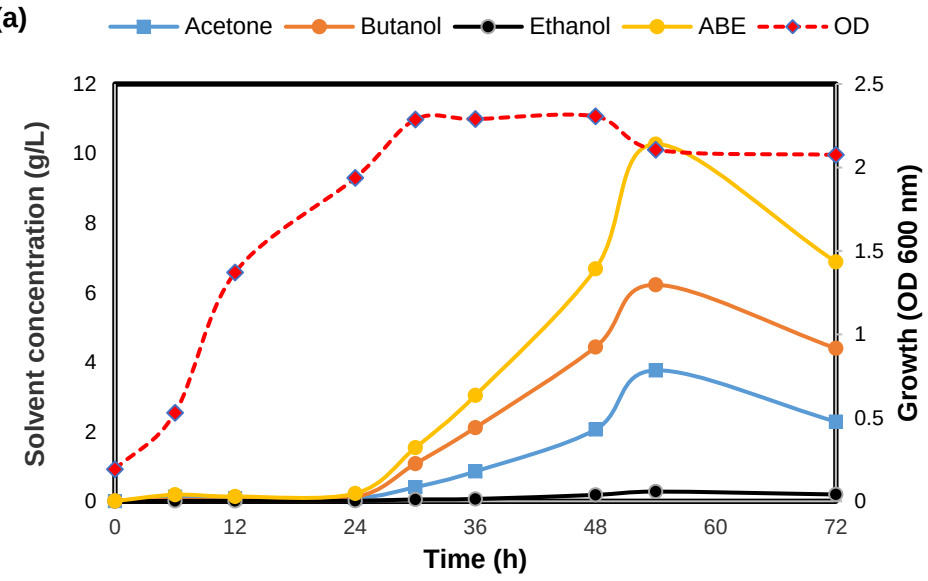
PKC Type	Total Sugars Liberated (g/L)	Butanol	ABE (g/L)	ABE Yield (g/g)	ABE Productivity (g/L·h)
Untreated	0.79	0.21	0.33	[a] NA	0.005
[b] Autoclave	1.10	0.22	0.34	0.30	0.005

Note: [a] NA: Not available; [b] Autoclave of PKC at 121 °C for 60 min

Growth Profile of *C. acetobutylicum* YM1 using Different Sugars in the ABE Process

To study the growth of *C. acetobutylicum* YM1 in relation to the acidogenesis and solvenogenesis phases, three sets of ABE fermentations were performed in which *C. acetobutylicum* YM1 was grown in a TYA medium with glucose, mannose, and mixed sugars (glucose and mannose) at a ratio of 1:7.

Figures 1 to 3 show the growth profiles of *C. acetobutylicum* YM1 with variations in the solvents, organic acids, and culture pH measured using various sugars tested during 72 h of the batch ABE fermentations. The cell growth profile was monitored by measuring the optical density (OD 600) of clostridial cells. As shown in Figs. 1 to 3, after a short lag phase, the growth entered the logarithmic (exponential) growth phase that lasted until 30 h. The logarithmic phase was followed by the stationary phase, during which no considerable increase in OD was recorded. This phase lasted until 48 h in all cultures. It was clear that the concentrations of the total organic acids drastically increased during 25 h of ABE fermentation, in which the clostridial cells were in the exponential growth phase. In contrast, the concentrations of butanol and ABE were decisively enhanced during the stationary growth phase. This could have been attributed to the biphasic ABE process that occurred in clostridial cells, which includes two phases-the initial acidogenic phase followed by the solvenogenic phase (Gheshlaghi *et al.* 2009).



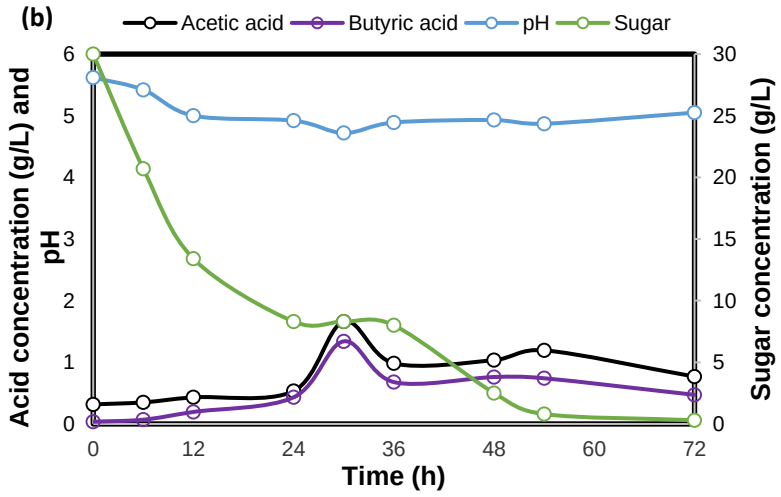


Fig. 1. The profile of ABE fermentations of glucose as the carbon source by *C. acetobutylicum* YM1: (a) solvents and OD; (b) sugar, pH, and acids concentration

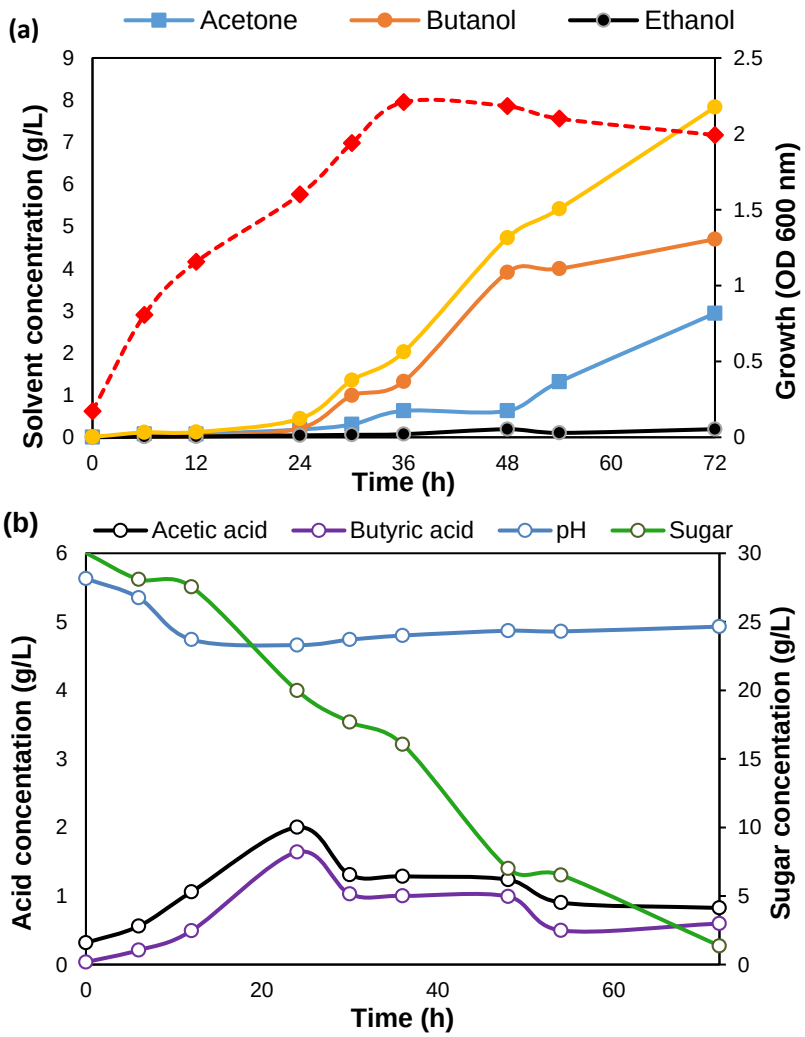


Fig. 2. The profile of ABE fermentation of mannose as the carbon source by *C. acetobutylicum* YM1: (a) solvents and OD; (b) sugar, pH, and acids concentration

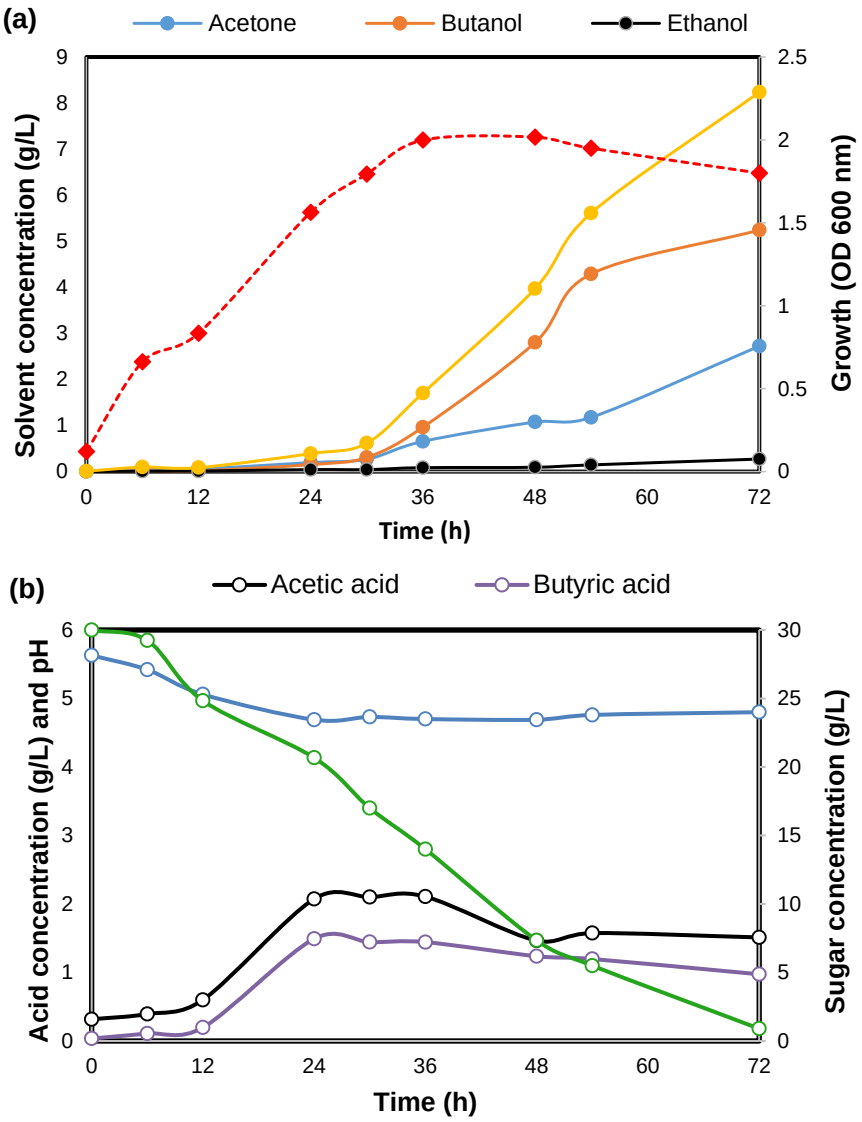


Fig. 3. The profile of ABE fermentation of mixed sugar (glucose and mannose) as the carbon source by *C. acetobutylicum* YM1: (a) solvents and OD; (b) sugar, pH, and acids concentration

As can be seen in Fig. 1, the acidogenic phase of the glucose-based culture was accompanied by a decrease in pH value (4.72) and an increase in the total acid concentration (3 g/L). The second phase was the solvenogenic phase in which the acids were reassimilated, resulting in the production of solvents with an increase in pH value. A similar trend was observed for the cultures that also contained mannose and mixed sugars. The acidogenic phase led to the production of 3.64 g/L and 3.57 g/L of total acids in the mannose-based culture and the culture that contained the mixture of glucose and mannose, respectively (Figs. 2 and 3).

ABE Fermentation using Acid-pretreated PKC

In this study, the PKC was pretreated with sulphuric acid at different concentrations ranging from 1% to 3% (v/v) for 45 min at 121 °C. The amount of dry PKC loaded was

10% (w/v). Table 3 shows the results of the acid pretreatment of PKC. The acid hydrolysis experiments revealed that 2% SAPKC produced the highest reducing sugar concentrations with a value as high as 30 g/L that contained 3.78 g/L glucose and 26.22 g/L mannose, while the PKC pretreatment with 1% H_2SO_4 generated 9.97 g/L total sugar that contained 1.06 g/L glucose and 8.91 g/L mannose. When the concentration of H_2SO_4 was increased up to 3%, the amount of the total reducing sugar recovery decreased to 21.9 g/L, including 3.12 g/L glucose and 18.78 g/L mannose. A study by Noparat *et al.* (2015) reported that a more severe pretreatment resulted in low sugar recovery due to the degradation of the sugars to hydroxymethyl furfural and furfural.

Table 3. Hydrolysate Obtained from the Acid Pretreated PKC and the Subsequent ABE Fermentation of the Hydrolysate by *C. acetobutylicum* YM1

Treatment Method	Sugars Liberated (g/L)			Butanol (g/L)	ABE (g/L)	ABE Yield (g/g)	ABE Productivity (g/L·h)
	Glucose	Mannose	Total				
SAPKC 1%	1.06	8.91	9.97	0.73	1.07	0.14	0.01
SAPKC 2%	3.78	26.22	30	3.55	5.72	0.24	0.08
SAPKC 3%	3.12	18.78	21.9	2.63	3.48	0.24	0.05
HAPKC 1%	-	5.69	5.69	0.23	0.40	0.09	0.005
HAPKC 2%	1.74	13.28	15.02	1.68	2.63	0.18	0.04

The results obtained from hydrochloric acid-pretreated PKC (HAPKC) showed that acid hydrolysis of PKC by 1% HCl recovered 5.69 g/L of total sugar (Table 3). When the HCl concentration was increased to 2% it enhanced the total sugars up to 15.02 g/L, which contained 1.74 g/L glucose and 13.28 g/L mannose. This result also indicated that the sulphuric acid pretreatment of PKC was more efficient to recover sugars than using a hydrochloric acid pretreatment. The pretreatment of de-oiled jatropha waste by acids also revealed that there was a positive relationship between the quantity of sugars released and the concentration of acids used for pretreatment (HCl (0.5% to 10%) and H_2SO_4 (0.5% to 5%)), so that the total sugar generated ranged from 1.4 g/L to 1.7 g/L and 1.4 g/L to 7.8 g/L using HCl and H_2SO_4, respectively (Kumar *et al.* 2013).

The SAPKC and HAPKC samples were filtered using cheese cloth prior to fermentation to remove the sediments. The supernatant liquid was collected, and the pH was adjusted to 6.2 using 10 M NaOH solution and used as such for ABE fermentation. The results obtained from the fermentation of SAPKC showed that the highest concentrations of ABE (5.72 g/L) and butanol (3.55 g/L) were obtained when 2% SAPKC was used, followed by 3% SAPKC with the production of 3.48 g/L ABE and 2.63 g/L butanol (Table 3). This could be attributed to the high total of sugars recovered from 2% SAPKC compared with that using other acid pretreatments tested. The fermentation of hydrolysate from 2% HAPKC produced 2.63 g/L ABE, and 1.68 g/L butanol, concentrations that were considered low compared with the results obtained from the fermentation of 2% SAPKC. The ABE fermentation of 2% SAPKC was performed using H_2SO_4-pretreatment hydrolysate as medium produced ABE (5.72 g/L) and butanol (3.55 g/L), while the ABE fermentation of mixed sugars was performed in TYA medium

supplemented with mixed sugar produced ABE (8.24 g/L) and butanol (5.24 g/L), which suggested that the culture may have been inhibited due to the inhibitors that were generated resulting from the hydrolysis process by sulphuric acid. Ezeji *et al.* (2007) reported that corn fibre treated with sulphuric acid inhibited butanol production by *Clostridium beijerinckii* BA101. Previous studies have reported that, during acid treatment, there are some byproducts produced as fermentation-inhibitor compounds. These byproducts are also known as microbial inhibitors, including furfural, 5-hydroxymethyl-2-furaldehyde (HMF), acetic acid, formic acid, and levulinic acid (Larsson *et al.* 1999; Noparat *et al.* 2015).

Table 3 shows that the highest ABE yield obtained was from 2% and 3% SAPKC with the same value of 0.24 g/g. The lowest ABE yield (0.09 g/g) was obtained when 1% HAPKC was utilized. It was clear that the 2% SAPKC gave the highest productivity value (0.08 g/L.h) compared with that when 3% SAPKC was used (0.05 g/L.h).

In this regard, Al-Shorgani *et al.* (2011) pretreated rice bran with 1% (v/v) H_2SO_4 and 1% (v/v) HCl for further utilization in the ABE process. The pretreatment results revealed that when H_2SO_4 and HCl were used, the concentrations of total sugar recovered from pretreated rice bran were 31.43 g/L and 9.7 g/L, respectively. The ABE fermentation of hydrolysates obtained from acid-pretreated rice bran showed that 6.32 g/L butanol and 8.61 g/L ABE were produced using the hydrolysate of sulphuric acid pretreatment. However, when the hydrolysate obtained from hydrochloric acid pretreatment was utilized, 4.93 g/L and 5.96 g/L of butanol and ABE were obtained, respectively.

The TYA medium was the best for butanol production by *C. acetobutylicum* YM1 (Al-Shorgani *et al.* 2013). To study the efficiency of the nutrients present in SAPKC for ABE fermentation compared with the TYA medium, the SAPKC was fermented to ABE with and without the TYA medium using *C. acetobutylicum* YM1. Then the ABE production was compared between these two cultures with the culture of TYA (control). The results obtained showed that the supplementation of SAPKC with TYA improved the production of ABE by 11.4%. The supplementation of SAPKC with the TYA (SAPKC as the only carbon source) medium enhanced the ABE production to 6.37 g/L (3.99 g/L butanol) compared with 5.72 g/L when SAPKC was used without the TYA supplement (Table 4). These results suggested that the low ABE production from SAPKC (5.72 g/L) compared with 8.23 g/L from culture with the TYA medium (glucose and mannose) may have been due to not only the presence of microbial inhibitors in SAPKC but also to the insufficient nutrient contents in the SAPKC sample.

Table 4. The Effect of TYA Medium Supplementation on ABE Production using SAPKC

Medium	Acetone (g/L)	Butanol (g/L)	Ethanol (g/L)	ABE (g/L)	Butyric Acid (g/L)	Acetic Acid (g/L)	ABE Yield (g/g)	ABE Productivity (g/L·h)
SAPKC	1.97	3.55	0.20	5.72	0.79	0.75	0.24	0.08
SPKC + TYA	2.14	3.99	0.24	6.37	0.95	0.97	0.26	0.09
TYA	2.72	5.24	0.27	8.24	0.97	1.05	0.32	0.11

ABE Fermentation using Alkali-pretreated PKC

Pretreatment of lignocellulosic biomass with sodium hydroxide is one of the chemical-pretreatment methods that has been used to hydrolyse the structure of these materials by removing the lignin and dissolving hemicelluloses (Zhao *et al.* 2008). In this study, PKC was pretreated with a 1% NaOH (1% SHPKC) solution. The experimental results showed that a low amount of total sugar was released (2.15 g/L) using 1% SHPKC. However, only 0.82 g/L total sugars were released when PKC was pretreated with alkaline hydrogen peroxide. These results showed that the alkali pretreatment was not efficient at hydrolysing the lignocellulosic content of PKC and for recovering high quantities of the reducing sugars.

The hydrolysate obtained from SHPKC and HPPKC was used for ABE fermentation by *C. acetobutylicum* YM1 after adjustment of the pH to 6.2 with concentrated HCl. No growth was observed; subsequently, no ABE production occurred during the 72 h of ABE fermentation. The failure of *C. acetobutylicum* YM1 to grow and produce butanol could have been attributed to the inhibition of clostridial cells by the phenolic compounds, which may have been produced from lignin degradation during the alkali pretreatment. In this view, Jonsson *et al.* (1998) observed that inhibition of fermentation was decreased by removing phenolic compounds from willow hydrolysate.

The study conducted by Qureshi *et al.* (2008) also revealed that alkaline peroxide pretreatment of wheat straw produced a high concentration of sugars, while the growth of *C. beijerinckii* P260 was inhibited during ABE fermentation, likely because the alkaline pretreatment resulted in the production of a toxic substances that inhibited the growth of the strain.

An attempt was made to use alkali-pretreated rice straw for butanol production. Results for the co-culture of the *C. thermocellum* and *C. saccharoperbutylacetonicum* strain N1-4 on alkali-pretreated rice straw hydrolysate showed that 5.5 g/L butanol was produced during 7 days of ABE fermentation (Kiyoshi *et al.* 2015).

ABE Fermentation in EHPKC-based Medium

Most of the PKC content was mannan, with some cellulose and a small amount of arabinoxylan and 4-O-methyl-glucoronoxylan (Cerveró *et al.* 2010; Jørgensen *et al.* 2010). Preliminary enzymatic hydrolysis of PKC was evaluated using mannanase enzyme with various enzyme loadings, namely 5% and 10% ($w/w_{substrate}$), to determine the effect of enzyme loading on the amount of sugars released from pretreated-PKC. The result obtained from this study showed that the use of an enzyme concentration of 5% $w/w_{substrate}$ gave 11 g/L of reducing sugar (9.9 g/L mannose, 1.1 g/L glucose). The reducing sugar concentration was 11.9 g/L (only mannose) when the saccharification was performed with an enzyme loading of 10% w/w (Fig. 4). These findings showed that there was no substantial increase in the reducing sugar formation with an increase in the enzyme concentration. The reduction of reducing sugar production at a higher enzyme loading could have been due to the saturation of the substrate surface with the enzyme (Xu *et al.* 2007).

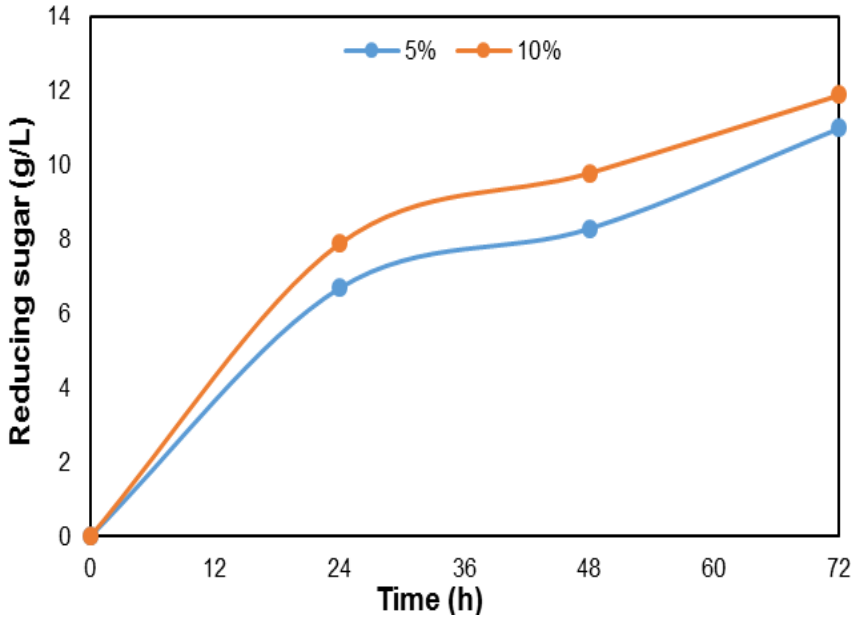


Fig. 4. The effect of enzyme loadings on total reducing sugar content

The potential to produce butanol from enzymatic scarification of PKC was performed in this study, and the results are presented in (Fig. 5). The fermentation of EHPKC with an initial reducing sugar concentration of 11 g/L for 72 h was able to produce 1.06 g/L ABE and 0.74 g/L butanol within 48 h, leaving behind 0.54 g/L sugar, with an ABE productivity and yield of 0.02 g/L.h and 0.07 g/g, respectively. The fermentation stopped because of the lack of sugar in the broth, and the culture could not shift completely to the solvenogenic phase, as indicated by the high acid accumulation (2.03 g/L) in the broth.

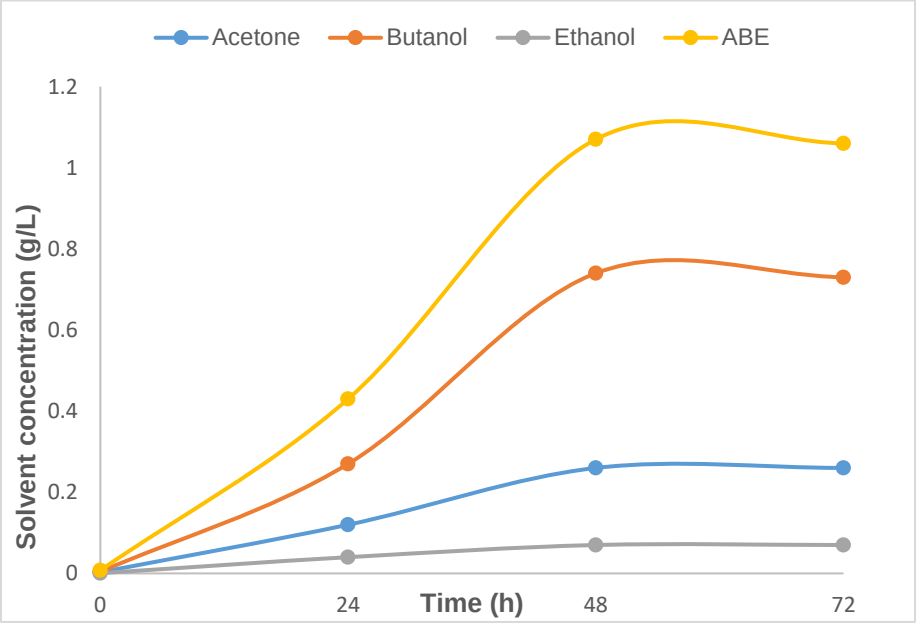


Fig. 5. ABE fermentation of hydrolysate from enzymatic saccharification of PKC

To evaluate the performance of hydrolysis with a low sugar concentration, control experiments were performed with a low concentration of pure sugars 11 g/L (glucose and mannose). During 72 h of fermentation, both cultures produced the highest butanol within 48 h, the glucose culture produced 1 g/L ABE, while the mannose culture produced 0.87 g/L ABE. The ABE production from glucose was similar to that produced from EHPKC and 1% SAPKC. The productivity and yield resulting from the fermentation of glucose were 0.02 g/L.h and 0.08 g/L, respectively. The fermentation of mannose resulted in a productivity and yield of 0.02 g/L.h and 0.07 g/L.h, respectively. The similar performance of EHPKC and 1% SAPKC to that of the control culture may have been due to the low concentrations of microbial inhibitors produced during the low severity pretreatment.

CONCLUSIONS

1. *C. acetobutylicum* YM1 utilized glucose, mannose, and a mixture of glucose and mannose (1:7), and it produced different amounts of ABE solvents. Fermentation of 30 g/L glucose and 30 g/L mannose resulted in the production of 10.27 g/L and 7.88 g/L of ABE, respectively.
2. This study approved that PKC is a suitable substrate for ABE fermentation.
3. Reducing sugar released in 2% H_2SO_4-pretreatment hydrolysate (of PKC) gave the highest ABE and butanol products.
4. Nutrients supplementation of the 2% H_2SO_4-pretreatment hydrolysate increased the ABE and butanol yield.

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