Biobutanol Production from Palm Kernel Cake (PKC) using *Clostridium saccharoperbutylacetonicum* N1-4 in Batch Culture Fermentation

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Palm kernel cake (PKC), a by-product of palm oil industry, contains glucose and mannose as hexose sugars. This study was performed to determine the feasibility of using PKC as a lignocellulosic substrate for biobutanol production by *Clostridium saccharoperbutylacetonicum* N1-4 in an acetone-butanol-ethanol (ABE) fermentation process. Moreover, the effect of tryptone-yeast extract-acetate (TYA) medium and P2 medium on biobutanol production was evaluated. Experimental results showed that butanol production of 3.05 g/L was obtained using mannose sugar, which was comparable to 3.61 g/L butanol production measured using glucose. Moreover, the maximum production of biobutanol (0.38 g/L) was obtained at a PKC concentration of 30%, indicating the possibility of PKC utilization in butanol production. ABE fermentation of PKC using distilled water, TYA medium, and P2 medium showed that the highest butanol production (0.26 g/L) with ABE production of 0.38 g/L was obtained when ABE fermentation was conducted in P2 medium.

Keywords: Palm kernel cake (PKC); Mannose; Mannan; Biobutanol production; Clostridium saccharoperbutylacetonicum N1-4

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INTRODUCTION

The continued depletion of fossil fuels has garnered the attention of many researchers studying the production of sustainable fuels. Biofuel, which is produced from various biomasses, is an effective alternative to fossil fuels. Biofuel has been described as a superior fuel to fossil fuels since it reduces greenhouse gas emissions and global warming, providing a clean energy source (Abdeshahian *et al.* 2010a). Although bioethanol is typically considered as the world's primary biofuel, biobutanol provides advantages over bioethanol such as higher efficiency of butanol for blending to gasoline, simple transportation through pipeline, higher heat emission produced from butanol vaporization than that from ethanol, and a lower risk of groundwater contamination due to low solubility in water (Ranjan and Moholkar 2012). Biobutanol is a liquid biofuel that can be produced *via* acetone-butanol-ethanol (ABE) fermentation by the solvent-producing *Clostridia* under anaerobic fermentation (Khamaiseh *et al.* 2014).

Clostridium saccharoperbutylacetonicum N1-4 is one of the solvent-producing *Clostridia* that has recently been used for the production of butanol and other solvents (Al-Shorgani *et al.* 2013). ABE fermentation is a biphasic process in which *Clostridia* starts the first phase, known as acidogenesis, followed by the second phase, known as solventogenesis. In the acidogenic phase, organic acids such as acetic acid and butyric acid are produced with the emission of hydrogen and carbon dioxide. In the solventogenic phase, solvents such as acetone, butanol, and ethanol are produced (Gheshlaghi *et al.* 2009).

Butanol production by ABE fermentation process suffers from major problems including low production of butanol due to an inhibitory effect of butanol on its production, a difficult product recovery technique, and a high production cost due to the high cost of the substrate (Kumar and Gayen 2011). However, the cost and availability of substrate always have a large impact on the production of biobutanol so that the biomass contributes to 63% of the total production cost (Jones and Woods 1986). In this sense, the utilization of low cost and renewable substances reduces butanol production costs (Ranjan and Moholkar 2012). Lignocellulosic biomass is the most promising sustainable carbon source emerging as a cheap resource for biofuel production (Razak *et al*. 2013). A variety of agricultural crops and lignocellulose-based feedstocks have successfully been used as the carbon source for ABE process such as rice bran (Al-Shorgani *et al.* 2012a), sago starch (Al-Shorgani *et al.* 2012b), palm oil mill effluent (Kalil *et al*. 2003), rice straw (Moradi *et al*. 2013), barley straw, and corn stover (Qureshi *et al*. 2013). In this regard, palm kernel cake (PKC) is a lignocelluloses-based residue, which is obtained from palm oil industry in tropical countries (Abdeshahian *et al.* 2010b).

The palm oil industry has grown in Malaysia over the recent decades. Malaysia is one of the largest producers of palm oil in the world (MPOB 2014). The huge amount of various by-products of palm oil industry are annually produced in Malaysia such as empty fruit bunch, palm oil mill effluent, palm kernel cake (PKC), fronds, mesocarp fiber, trunk and shell (Sumathi *et al*. 2008). According to the official report of Malaysian palm oil board (MPOB), 2399204 tonnes of PKC was produced in Malaysia by palm oil industry during 2012 with an increased PKC production of 2,516,664 tonnes in 2013 (MPOB 2014). This abundant lignocellulosic residue provides a great potential carbon source, which could be converted into biofuels and value-added products, giving rise to a new potential industry in Malaysia. PKC is mostly composed of cellulose (11.6%) and hemicellulose (35.2% mannan, 2.6% xylan) with 50% fermentable hexose sugars such as glucose and mannose (Swe *et al*. 2009; Cerveró *et al*. 2010; Jørgensen *et al*. 2010).

On the other hand, fermentation medium plays a crucial role in the production of microbial metabolites since it provides a various nutrients and supplementary constituents for microbial cell growth and product formation. Hence, study of the culture medium for the enhancement of butanol production can result in lowering of the production cost, leading to a more cost-effective ABE fermentation, particularly at scale-up of a butanol synthesis process (Ranjan *et al*. 2013b; Li *et al*. 2014). In this regard, two synthetic growth media including tryptone-yeast extract-acetate (TYA) and P2 medium have been found as suitable culture media for butanol production in ABE fermentation (Al-Shorgani *et al.* 2013).

Although a large number of cellulosic feedstocks have already been utilized for butanol production as mentioned previously, new and abundant substrates need to be introduced for the sustainable production of biobutanol to meet the future global demand for biofuel. PKC is a low-cost carbon source that could be considered as a potential

substrate for butanol production. To the best of the authors' knowledge no research work has been performed so far to investigate the feasible utilization of PKC in butanol production under ABE fermentation. This research was carried out to study the use of PKC as a new and inexpensive carbon source for the production of biobutanol by *C. saccharoperbutylacetonicum* N1-4 *via* the ABE fermentation process. Moreover, the effects of two nutrient media, *i.e.* TYA and P2 medium on the biobutanol production by *C. saccharoperbutylacetonicum* N1-4 from PKC, were investigated to find better effectiveness of fermentation medium in ABE process. The findings obtained in this study could contribute to the development of ABE process for the cost-effective production of butanol in the context of sustainable fuel production.

EXPERIMENTAL

Microorganisms and Culture Condition

C. saccharoperbutylacetonicum N1-4 was obtained from the stock culture provided by Biotechnology Laboratory, Chemical and Process Engineering Department, Universiti Kebangsaan Malaysia (UKM). The stock culture was maintained in potato glucose (PG) medium, and a spore suspension obtained was kept at 4 °C (Al-Shorgani *et al*. 2012a). First, 1 mL of spore suspension was added to 9 mL of PG medium with heat shocking by boiling water at the temperature of 100 °C for 1 min, followed by cooling in ice water to activate the microorganism. The inoculum obtained was incubated at 30 °C for 1 to 2 days under anaerobic conditions. The morphology of colony was observed, and Gram-staining techniques were carried out to check the viability and purity of the inoculums. The active cultures were then transferred to sterile tryptone-yeast extract- acetate (TYA) medium that had previously been sparged with oxygen-free nitrogen gas to bring about anaerobic conditions. The inoculated cultures were then incubated for 15 to 18 h until their optical density (OD_{660}) value reached 1.1 to 1.5.

PKC and Culture Medium Preparation

PKC with no physicochemical pre-treatment was used as the sole substrate in this study. The PKC was provided by Hup Lee Oill Mill Sdn. Bhd., Klang, Malaysia. The PKC was ground and passed through a sieve with 600-µm mesh to obtain fine PKC particles. Different concentrations of PKC (10%, 20%, 30%, and 40% w/v) were prepared by the addition of defined volumes of distilled water. The prepared PKC was sterilized at 121 °C for 15 min and used in the fermentation process. In order to study the effects of nutrients on butanol production using PKC, two nutrient media, TYA and P2 medium, were utilized as they were developed by Al-Shorgani *et al*. (2012a). TYA and P2 medium were used in this study with slightly modifications. The chemical components of the P2 and TYA media are listed in Table 1.

Biobutanol Production

Biobutanol fermentation was conducted in 250-mL Schott Duran bottles (including an inlet and outlet system) with a working volume of 150 mL. The initial pH of the fermentation medium was adjusted to 6.5 before all experiments by the addition of 4 M sodium hydroxide. Fermentation medium was sterilized at 121 °C for 15 min using an autoclave. Anaerobic conditions were provided by sparging the medium with oxygen-free nitrogen gas. Fresh inoculum (18 h growth) was aseptically transferred to the medium with

an inoculum size of 10% (v/v). The culture was then incubated at 30 °C, without shaking, under anaerobic conditions at different fermentation times. All experiments were carried out in duplicate, and the average values of samples were calculated. The yield of solvents (butanol and ABE) was calculated by dividing the concentration of product (g/L) to amount of sugar consumed by clostridial cells (g/L), which was expressed as g/g.

Table 1. Chemical Compositions of P2 and TYA Media

P2 medium*		TYA medium	
Component	Concentration (g/L)	Component	Concentration (g/L)
Yeast Extract	1	Yeast Extract	2
$MnSO_4.4H_2O$	0.01	Tryptone	6
K_2HPO_4	0.75	CH_3COONH_4	3
$MgSO_4.7H_2O$	0.4	$MgSO_4.7H_2O$	0.3
KH_2PO_4	0.75	KH_2PO_4	0.5
$FeSO_4.7H_2O$	0.01	$FeSO_4.7H_2O$	0.01
NH_4NO_3	2		
Cysteine	0.5		
Resazurin	0.001		

* Finally biotin (80µg/L) and 1 mL of solution containing 4-Aminobenzoic Acid (1mg/L H_2O) were added per 1 L P2 medium

Analytical Methods

Samples collected at appropriate time intervals were kept in a centrifuge tube and centrifuged at 10,000 rpm for 5 min. The supernatant was analyzed to determine the ABE and organic acid concentrations. The concentrations of ABE and acids (acetic and butyric) were measured using a gas chromatograph (7890A GC-System; Agilent Technologies, Palo Alto, CA, USA) equipped with a flame ionization detector and a 30-m capillary column (Equity 1; 30 m × 0.32 mm × 1.0 µm film thickness; Supelco, Bellefonate, PA, USA). The oven temperature was programmed to increase from 40 to 130 °C at a rate of 8 °C/min. The injector and detector temperatures were set at 250 and 280 °C, respectively. Helium, the carrier gas, was set at a flow rate of 1.5 mL/min.

RESULTS AND DISCUSSION

Growth of *C. saccharoperbutylacetonicum* N1-4 using Mannose and Glucose as Carbon Sources for ABE Fermentation

As mentioned previously, PKC content is composed of different amounts of mannose and glucose. On the other hand, it has been found that glucose is a superior carbohydrate source over other fermentable sugars such as mannose for microbial cells so that it is utilized predominantly by solvent producing *Clostridia* in ABE process in comparison to other hexose and pentose sugars (Ezeji *et al*. 2007a). In order to investigate the ability of *C. saccharoperbutylacetonicum* N1-4 for consumption of mannose and glucose content of PKC in ABE fermentation as well as to study acidogenesis phase and solventogenesis phase of ABE process in relation to growth of *C. saccharoperbutylacetonicum* N1-4 for butanol production, two sets of ABE fermentation were performed. In the first experiment this clostridial strain was grown in a culture

medium with 30 g/L mannose. Another set of ABE fermentation was conducted in such a way that *C. saccharoperbutylacetonicum* N1-4 was cultivated in culture medium containing 30 g/L glucose as the control experiment. TYA medium was used as culture medium in these experiments.

Figure 1 illustrates the growth profile of *C. saccharoperbutylacetonicum* N1-4 using mannose and glucose as the carbon sources for batch ABE fermentation. As shown, similar patterns of clostridial cell growth were obtained when glucose and mannose were used as substrate. The short lag phase (less than 4 h) and early onset of exponential phase for both sugars (as shown in Fig. 1) could be attributed to sufficient carbon source and nutrients in culture medium for growth of *C. saccharoperbutylacetonicum* N1-4 (Ni *et al*. 2012; Rajagopalan *et al*. 2014). Moreover, the exponential growth phase (log phase) occurred between 8 and 32 h for both sugars with a maximum optical density (OD_{660nm}) of 1.746 for mannose and 1.815 for glucose. As can be observed, the log phase was followed by a stationary growth phase, which lasted until 72 h for glucose and 96 h for mannose. It is evident that the production of butanol from mannose and glucose began at the exponential growth phase and decisively increased with the highest butanol production at the stationary growth phase. This finding could be attributed to the fact that the metabolic pathway for solventogenesis phase in clostridial cells mainly was associated with the stationary phase of cell growth, resulting in the highest butanol concentration in culture medium during the stationary phase (Amiri *et al.* 2014). As can be found, maximum butanol concentration produced from glucose (3.61 g/L) was comparable to the highest butanol obtained from mannose (3.05 g/L), suggesting that mannose is a suitable alternative carbohydrate source for butanol production in ABE process.

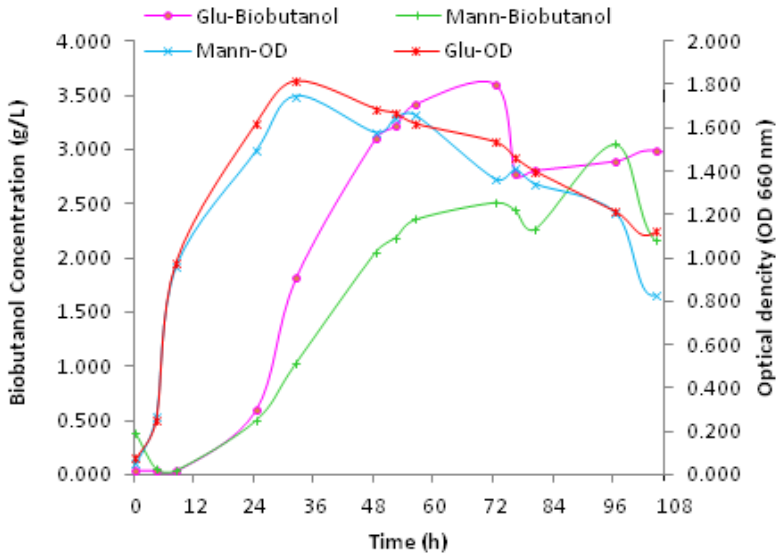

Fig. 1. Growth profiles of *C. saccharoperbutylacetonicum* N1-4 using mannose and glucose (control culture) in ABE fermentation at 30 °C (OD: optical density, Mann: mannose, Glu: glucose)

In order to study acidogenesis phase in ABE process using glucose and mannose, the profile of pH changes in culture during ABE fermentation was plotted. Figure 2 shows variations in pH of culture during the time course of ABE fermentation. As mentioned previously, the pH of two cultures was initially adjusted to 6.5. However, after sterilization, the pH of media decreased to 5.71 and 5.72 for glucose and mannose, respectively. As shown, the pH of the cultures continuously decreased during the log phase of clostridial

cell growth. The pH value reached 5.08 and 5.0 in the culture supplemented by mannose and glucose, respectively, at early exponential phase. The drop in pH drastically continued to reach the lowest value of 4.5 for the both culture at 24 h incubation, which was consistent with the early exponential growth phase (Fig. 1). The decrease in pH was owing to the fact that in acidogenesis of biphasic ABE fermentation, organic acids, such as acetic acid and butyric acid were produced, which resulted in a decrease in pH of the culture (Khamaiseh *et al.* 2014). When the acid content reached a critical level (acid accumulation) in exponential growth phase (Fig. 1), the reassimilation of organic acids occurred, resulting in the production of solvents (acetone, butanol, and ethanol), which was concurrent with the stationary growth phase with an increase in pH (Figs. 1 and 2) (Gheshlaghi *et al.* 2009).

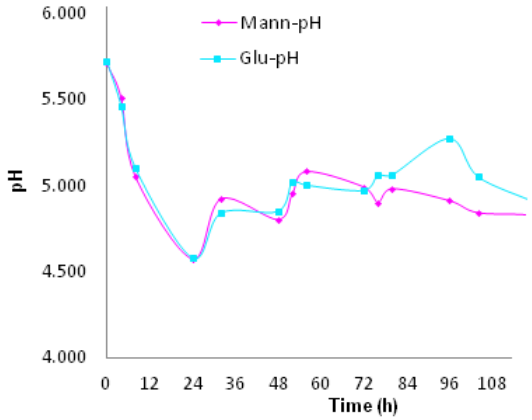

Fig. 2. pH profile of *C. saccharoperbutylacetonicum* N1-4 using mannose and glucose for ABE fermentation, with glucose as the control (Mann: Mannose, Glu: Glucose)

Table 2 shows the comparision of solvents and organic acids produced in ABE fermentation of mannose over the incubation time with those obtained from ABE fermentation of glucose. As can be found from Table 2, biobutanol and ABE production from mannose were as high as 3.05 g/L and 4.36 g/L, respectively, whereas the utilzation of glucose was triggered 3.61 g/L biobutanol and 5.29 g/L ABE. This finding indicated that *C. saccharoperbutylacetonicum* efficently cosumed mannose in ABE process similar to that when glucose was used as carbohydrate source. Furthermore, butanol yield and productivity of 0.10 g/g and 0.03 g/L/h was produced using mannose, which was comparable with butanol yield and productivity obtaned from glucose (control sugar) with the values of 0.12 g/g and 0.05 g/L/h, respectively. These observations suggested that mannose-based feedstocks such as PKC can be used for biobutanol production *by C. saccharoperbutylacetonicum* N1-4 to decrease butanol production costs.

Table 2. ABE Fermentation using Mannose and Glucose (Control) in Batch Cultures of *C. saccharoperbutylacetonicum* N1-4

Carbon source (30 g/L)	Fermentation time (h)	Production (g/L)				Butyric acid (g/L)	Acetic acid (g/L)	Yield (g/g)		Productivity (g/L/h)	
		A	B	E	ABE			B	ABE	B	ABE
Mannose	96	1.26	3.05	0.06	4.36	0.76	0.06	0.10	0.15	0.03	0.05
Glucose (Control)	72	1.55	3.61	0.14	5.29	0.45	0.04	0.12	0.18	0.05	0.07

Abbreviations: A: Acetone, B: Butanol, E: Ethanol; ABE: Acetone- Butanol- Ethanol

Glucose is a fermentable sugar that is widely present in carbohydrate-based feedstocks such as sugar-based crops (sugarcane and beet), starch-based crops (grain and root plants), and by-products of sugar-based industry (molasses). However, these glucose sources are utilized as food in the human diet (Abdeshahian *et al*. 2010a; Naik *et al*. 2010). Hence, finding cost–competitive carbohydrate sources such as mannose reduce the cost of butanol production. It has been demonstrated that substrate is a critical factor that affects the price of butanol, therefore, economically feasible substrate reduces the required cost of biomass-to-butanol process (Ezeji *et al.* 2007b).

Biobutanol Production from PKC

As already shown in this work, *C. saccharoperbutylacetonicum* N1-4 was able to consume pure mannose sugar in production of butanol in ABE process as efficiently as that from glucose. On other hand, it was also mentioned that PKC content is composed of glucose and mannose. In order to study the capability of *C. saccharoperbutylacetonicum* N1-4 in consumption of mannose and glucose content of PKC for biobutanol production process, PKC was fermented by *C. saccharoperbutylacetonicum* N1-4 in an ABE process. In this regard, *C. saccharoperbutylacetonicum* N1-4 was cultivated on dissolved PKC in distilled water with a concentration of 10% (w/v) at 30 °C for 192 h. Figure 3 depicts the profile of solvent and organic acids produced during the course of ABE process.

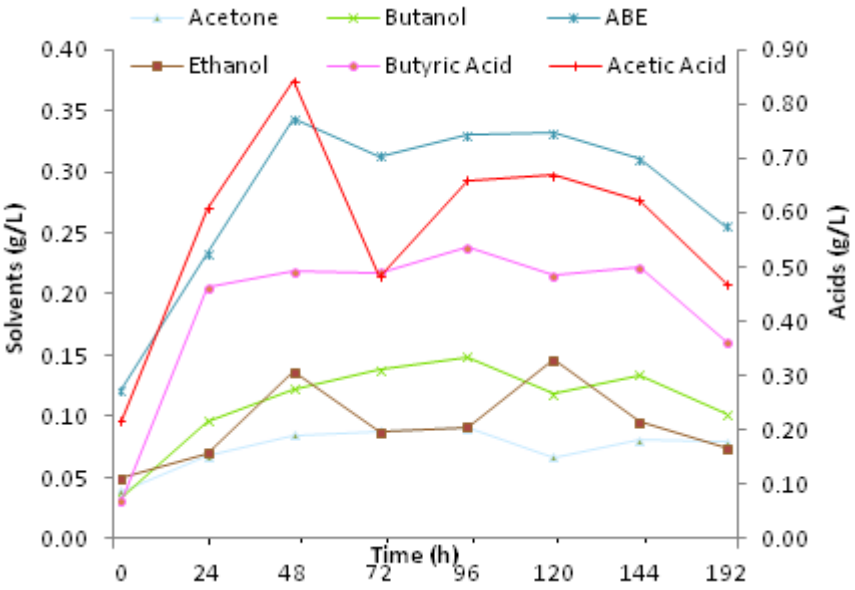

Fig. 3. Biobutanol production profiles of 10% (w/v) PKC by *C. saccharoperbutylacetonicum* N1-4

As shown in Fig. 3, the high levels of acetic acid and butyric acid were produced at 48 h ABE process coroborating the fact that acidogenesis phase occurred at early ABE process using PKC (Khamaiseh *et al.* 2014). The highest concentration of biobutanol (0.15 g/L) was attained at 96 h of fermentation time with a total ABE production of 0.34 g/L. These results indicated that as the culture proceeded, a solventogenesis phase occurred at late ABE fermentation (Amiri *et al.* 2014). The low production of biobutanol using PKC can be attributed to the high concentration of hemicellulose component (mannan) which hampered the consumption of glucose and mannose by *C. saccharoperbutylacetonicum* N1-4. It has been found that *Clostridium saccharoperbutylacetonicum* N1-4 is unable to

directly degrade mannan content to release fermentable sugars for butanol production because mannan has a crystaline structure similar to cellulose, and mannan-degrading enzymes have not been identified in *Clostridium saccharoperbutylacetonicum* N1-4 (Jørgensen *et al*. 2010).

Effect of PKC Concentration on Biobutanol Production

To investigate the effect of PKC concentration on biobutanol production, different PKC concentrations ranging from 10% to 40% (w/v distilled water) were utilized. The initial pH of each culture was adjusted to 6.5. The culture was then incubated at 30 °C for 144 h under anaerobic conditions. The effect of varied PKC concentrations on biobutanol production is illustrated in Fig. 4.

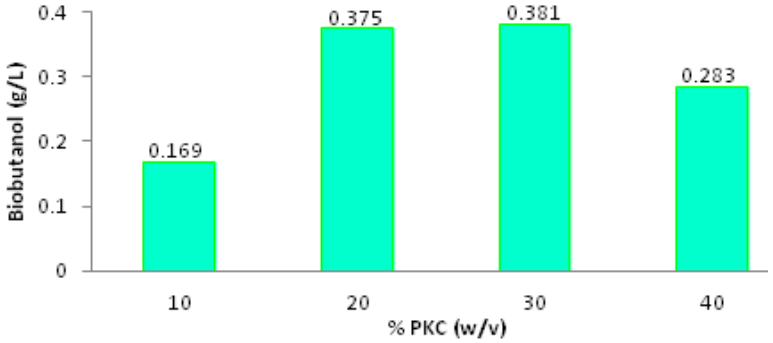

Fig. 4. Effect of different concentrations of PKC on biobutanol production by *C. saccharoperbutylacetonicum* N1-4

As shown in Fig. 4, when PKC concentration increased from 10% to 30% (w/v) the production of biobutanol also increased. Fermentation of PKC with a concentration of 30% (w/v) revealed the highest biobutanol production and total ABE production of 0.38 g/L and 0.57 g/L, respectively. Kalil *et al*. (2003) observed that *C. saccharoperbutylacetonicum* N1-4 produced 1.82 g/L total ABE when it was cultivated on palm oil mill effluent as the substrate.

The fermentation of PKC using different initial concentrations revealed a direct effect of PKC concentration on the production and productivity of both biobutanol and total ABE. In this study, solvent production increased concurrently with increases in PKC concentration (Table 3). However, when PKC concentration increased from 30% to 40%, biobutanol production decreased to 0.28 g/L. A similar result was obtained for the yield and productivity of biobutanol at this PKC concentration. The yield and productivity were reduced to 0.005 g/g and 0.002 g/L/h, respectively, at 40% PKC concentration. The total ABE production was 0.416 g/L with a yield and productivity of 0.007 g/g and 0.003 g/L/h, respectively, when 40% PKC concentration was used. An analytical experiment on PKC concentration of 30% was performed to determine glucose and mannose content of PKC. It was revealed that PKC concentration of 30% contained 1.25 g/L glucose and 0.02 g/L mannose. This finding indicated that even the low content of glucose and mannose could support microbial cell growth and butanol production by *C. saccharoperbutylacetonicum* N1-4. The reduction in butanol production at a PKC concentration of 40% (w/v) was possibly due to the point that in this concentration, substrate saturation in culture broth occurred and therefore too high PKC viscosity was developed in the culture medium. This could cause an inhibitory effect on the achievement of adequate mixing and transferring

nutrients in the culture, which in turn resulted in the decrease of microbial cell growth and product formation (Venkateswarlu *et al.* 2000). On the other hand, no acetic acid was detected for PKC concentrations between 10 and 30%, whereas 0.4 g/L butyric acid was produced using 40% PKC concentration.

Table 3. ABE Fermentation Results using Different Concentrations of PKC

PKC (%)	Production (g/L)				Butyric Acid (g/L)	Acetic Acid (g/L)	Yield (g/g)		Productivity (g/L/h)	
	Acetone	Butanol	Ethanol	ABE			Butanol	ABE	Butanol	ABE
10	0.040	0.169	0.094	0.303	0.080	0.000	0.011	0.020	0.001	0.002
20	0.051	0.375	0.131	0.557	0.114	0.000	0.013	0.019	0.003	0.004
30	0.051	0.381	0.147	0.579	0.261	0.000	0.008	0.013	0.003	0.004
40	0.051	0.283	0.082	0.416	0.403	0.042	0.005	0.007	0.002	0.003

The Effect of Medium Composition

TYA medium is a typical medium for *Clostridium saccharoperbutylacetonicum* N1-4; it is rich in nitrogen (Ishizaki *et al*. 1999). P2 medium is a semi-synthetic medium that contains minerals, vitamins, and yeast extract with a buffer pH. P2 medium was specifically formulated for saccharolytic *Clostridia* (Annous and Blaschek 1990). Supplementing substrates with P2 medium will enhance the production and productivity of biobutanol and ABE. It was found that a sufficient amount of nitrogen from yeast extract as well as high vitamins (*p*- aminobenzoic acid) and minerals in P2 medium resulted in the better growth of *Clostridium saccharoperbutylacetonicum* N1-4 so that it induced various enzymes from clostridial cells involved in solvent production during the course of ABE fermentation (Leclerc *et al*. 1998).

In order to study the effects of different nutrient medium on butanol production from PKC, P2 and TYA medium were used with the addition of 10% (w/v) PKC. Figure 5 shows biobutanol and ABE production profiles for PKC dissolved in H_2O as the control culture as well as for TYA and P2 medium using 10% (w/v) PKC concentration. Culture was set at 30 ^oC and initial pH of 6.5. As can be seen, P2 medium produced greater butanol and total ABE concentration after 144 h of fermentation time than TYA medium and control culture. As shown, butanol and total ABE produced from PKC supplemented with TYA had no increase compared to those obtained from the control culture. Similarly, it was found that the addition of cassava starch and maltodextrin to P2 medium increased the production of ABE (Formanek *et al.* 1997; Thang *et al*. 2010).

Table 4 shows the production of biobutanol and total ABE with their respective yields and productivities following 144 h of incubation. As shown, the maximum yield of ABE observed was 0.0038 g/g when P2 medium was used. As can be seen, a culture with TYA medium produced the lowest butanol and ABE concentration with values of 0.116 g/L and 0.275 g/L, respectively. However, a culture including P2 medium produced the butanol concentration of 0.261g/L and ABE concentration of 0.381g/L (Table 4). On the other hand, the control culture produced much more biobutanol (0.134 g/L) than the culture including TYA medium (0.116 g/L). This finding suggests that supplementation of PKC with nutrients is a key factor in the enhancement of butanol production by *C. saccharoperbutylacetonicum* N1-4 in ABE fermentation. In this regard, it has been found that PKC contains a large amount of trace mineral and nitrogen (Alimon 2004; Sundu *et*

al. 2006; Ezieshi and Olomu 2007; Iluyemi *et al*. 2010) so that it could support the production of biobutanol and ABE process.

The variations in butanol production using culture medium studied could be related to the fact that growth media tested were comprised of various microbial cell growth components such as trace elements (Mn and Fe), vitamins (biotin and *p*- aminobenzoic acid) and amino acids (tryptophan and cysteine), which affected growth of clostridial cells and microbial metabolism for synthesis of products. Therefore, P2 medium increased butanol production by providing more growth factors and nutrient components for clostridial cells compared to TYA and PKC with no medium supplementation (Ranjan *et al*. 2013a). The results of the current study were in good agreement with the previous study fulfilled by Al-Shorgani *et al*. (2012), who observed that P2 medium improved ABE production more than TYA medium.

Ranjan *et al*. (2013a) showed that an addition of 3 g/L yeast extract and 4 mg/L *p*-aminobenzoic acid in P2 medium supported rice straw hydrolysate fermentation with an optimum production of 6 g/L butanol and 8.7 g/L total solvents. Li *et al*. (2014) revealed that a butanol production of 11.3 g/L was obtained by mutant *Clostridium acetobutylicum* PW12 using P2 medium in batch culture of ABE fermentation process. The same study showed that a butanol concentration of 15.8 g/L was obtained in which P2 medium was supplemented with biotin (0.05 mg/L) using cassava flour as carbon source, so that the butanol produced was 21% more than that of the P2 medium with no biotin supplementation. Qureshi *et al*. (2010) studied the production of butanol from barley straw hydrolysate by *Clostridium beijerinckii* P260 using P2 medium. They observed that a butanol concentration of about 4.0 g/L was produced with a total ABE concentration of 7.09 g/L in a batch fermentation process. Ranjan *et al*. (2013b) added yeast extract (3 g/L) and para-aminobenzoic acid (2 mg/L) to rice straw hdrolysate to support growth of *Clostridium acetobutylicum* MTCC 481 for the production of butanol under ABE fermentation. They found that a butanol concentration of 9.28 g/L was produced with a total solvent (ABE) concentration of 12.05 g/L.

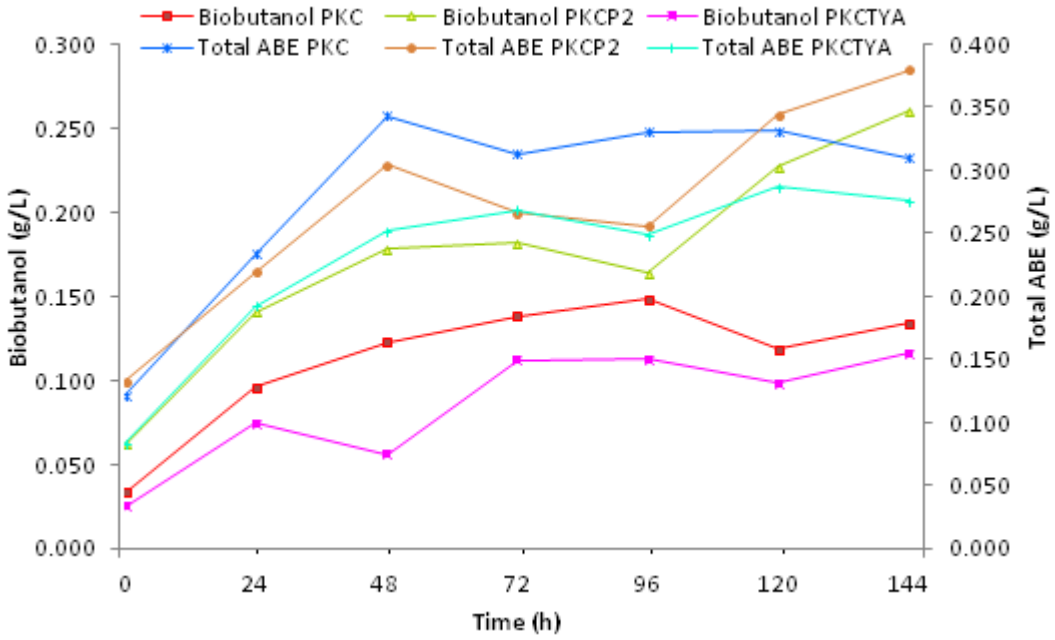

Fig. 5. Biobutanol production profiles of *Clostridium saccharoperbutylacetonicum* N1-4 using PKC (10% v/w) mixed in H_2O, TYA, and P2 media in ABE fermentation

Table 4. Results of 144 Hours of ABE Fermentation of Palm Kernel Cake using *C. saccharoperbutylacetonicum* N1-4 with Different Nutrient Systems

Culture medium	Production (g/L)				Butyric Acid (g/L)	Acetic Acid (g/L)	Yield (g/g)		Productivity (g/L/h)	
	A	B	E	ABE			B	ABE	B	ABE
PKC+ d. H_2O (Control)	0.080	0.134	0.096	0.310	0.499	0.623	0.0013	0.0031	0.0009	0.0022
PKC+ P2	0.057	0.261	0.063	0.381	0.496	0.000	0.0026	0.0038	0.0018	0.0026
PKC+ TYA	0.087	0.116	0.072	0.275	0.455	0.640	0.0012	0.0028	0.0008	0.0019

Abbreviations: d. H_2O: Distilled water, TYA: tryptone-yeast extract-acetate, A: Acetone, B: Butanol, E: Ethanol; ABE: Acetone, butanol, and ethanol combined

CONCLUSIONS

The present work was a basic trial to evaluate the feasibility of palm kernel cake (PKC) utilization in butanol production process under acetone-butanol-ethanol (ABE) fermentation. The production of butanol from PKC as a new and low-cost carbon source was successfully conducted utilizing the solvent-producing clostridia *Clostridium saccharoperbutylacetonicum* N1-4. The findings of this study revealed that the utilization of PKC with a concentration of 30% (w/v) resulted in the highest butanol production of 0.38 g/L with the ABE concentration of 0.579 g/L. A comparison study on the effect of nutrient media on butanol production from PKC showed that P2 medium had a better effect on the enhancement of butanol production compared to that using TYA medium and PKC with no nutrient supplementation. This finding revealed the importance of the type of medium in microbial cell growth and product formation. The current study indicated that PKC could be used for butanol production as a promising biomass feedstock contributing to the cost-effective production of biofuel. Further study could be performed to elevate butanol production from PKC by the optimization of operating variables (such as incubation temperature and initial pH value of culture) which decisively affect butanol production in an ABE process.

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