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Saccharification of polysaccharide content of palm kernel cake using enzymatic catalysis for production of biobutanol in acetone–butanol–ethanol fermentation

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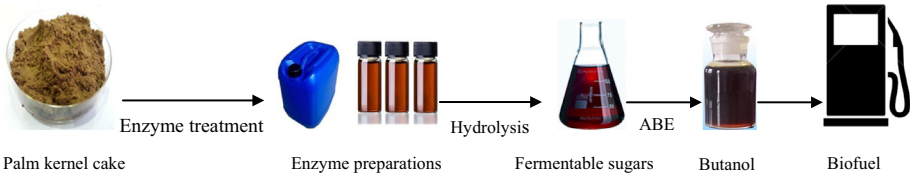
HIGHLIGHTS

- Palm kernel cake (PKC) was pretreated by liquid hot water (LHW).
- LHW–pretreated PKC was hydrolyzed by β-glucosidase, cellulase and mannanase.
- The highest quantity of the total monomeric sugars liberated was 97.81 ± 1.29 g/L.
- Maximum butanol and ABE obtained were 4.15 ± 1.18 and 7.12 ± 2.06 g/L, respectively.
- Structural change in traded PKC was studied by scanning electron microscope and FTIR.

GRAPHICAL ABSTRACT



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ABSTRACT

In this work, hydrolysis of cellulose and hemicellulose content of palm kernel cake (PKC) by different types of hydrolytic enzymes was studied to evaluate monomeric sugars released for production of biobutanol by *Clostridium saccharoperbutylacetonicum* N1-4 (ATCC 13564) in acetone–butanol–ethanol (ABE) fermentation. Experimental results revealed that when PKC was hydrolyzed by mixed β-glucosidase, cellulase and mannanase, a total simple sugars of 87.81 ± 4.78 g/L were produced, which resulted in 3.75 ± 0.18 g/L butanol and 6.44 ± 0.43 g/L ABE at 168 h fermentation. In order to increase saccharolytic efficiency of enzymatic treatment, PKC was pretreated by liquid hot water before performing enzymatic hydrolysis. Test results showed that total reducing sugars were enhanced to 97.81 ± 1.29 g/L with elevated production of butanol and ABE up to 4.15 ± 1.18 and 7.12 ± 2.06 g/L, respectively which represented an A:B:E ratio of 7:11:1.

1. Introduction

Air pollution and global warming have been major concerns about the huge consumption of the non-renewable sources, particularly fossil fuels which have resulted in the enormous emission of

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greenhouse gases such as carbon dioxide (CO_2) and nitrous oxide (N_2O) (Bansal et al., 2013; Rahimnejad et al., 2015). Biofuel is known as a renewable alternative to the fossil fuels which can mainly be produced from lignocelluosic biomass (Abdeshahian et al., 2010). Palm kernel cake (PKC) is known as agro-industrial biomass which is largely obtained from the palm oil industry in Malaysia (Hosseini and Wahid, 2012). It has been reported that Malaysia has produced 2,518,947 tons of PKC in 2014 with an average monthly PKC production of 209,912.25 tons (MPOB, 2015).

The study on the chemical composition of PKC has shown that cellulose content constitutes 11.6% of PKC fiber, while hemicellulose content forms 61.5% of PKC fiber. Similar studies have revealed that mannan forms the main component of the hemicellulose composition of PKC polysaccharide (57.8%), followed by xylan (3.7%) (Ong et al., 2004). Biobutanol is referred as a liquid biofuel, which is produced by various anaerobic bacteria in fermentative processes, namely acetone–butanol–ethanol (ABE) fermentation using carbohydrate substances. In this regard, bacterial strains of *Clostridium* genus have mainly been applied for butanol synthesis from sugar-based sources. In the context of sustainable production of butanol, various lignocellulosic substances have been utilized as carbon sources such as energy crops, agro-industrial residues and wood waste (Kiyoshi et al., 2015; Mechmech et al., 2015; Yang et al., 2015). However, the low degradability of recalcitrant lignocellulose content of biomass feedstocks has caused decisive bottlenecks for butanol production by microorganisms. In this view, different methods have already been developed including physical, chemical and biological approaches to break down lignocellulose content of plant biomass for the efficient production of fermentable sugars (Azman et al., 2015; Sartori et al., 2015). Among methods used, enzymatic treatment has received the renewed interest since it offers some advantages such as the moderate conditions required to the hydrolysis process (pH 4.5–5 and temperature 45–50 °C), the absence of corrosion of equipments and the lack of production of growth cell inhibitors like furfural and hydroxymethyl furfural (Sun and Cheng, 2002).

In line with the utilization of lignocellulosic biomass in the production of biofuel, PKC has been considered as a low-cost biomass feedstock with a high content of polysaccharides particularly mannan which represents a potential source of fermentable sugars for biofuel production (Fan et al., 2014). However, the development of an appropriate enzymatic treatment of PKC for the conversion of cellulose and hemicellulose content into the simple sugars that can be used easily by *Clostridium* sp. in ABE fermentation has still remained to be explored in depth. In this regard, previous studies have shown that *Clostridium saccharoperbutylacetonicum* N1-4 is capable of the efficient production of butanol in ABE using various fermentable sugars obtained from lignocellulosic substances and sugar-based sources. In an attempt, acid hydrolysis of PKC was successfully applied to produce biobutanol by *C. saccharoperbutylacetonicum* N1-4 under ABE fermentation in which the highest butanol concentration of 3.59 g/L was obtained (Shukor et al., 2014a). The cultivation of *C. saccharoperbutylacetonicum* N1-4 on palm oil mill effluent (POME) resulted in 0.9 g/L of butanol (Al-Shorgani et al., 2015). Chen et al. (2013) performed ABE fermentation for butanol production by *C. saccharoperbutylacetonicum* N1-4 using fermentable sugars obtained from enzymatic hydrolysis of rice straw to attain a butanol yield and butanol productivity of 0.22 g/g and 1.45 g/L/d, respectively. Furthermore, Thang and Kobayashi (2014) studied butanol synthesis by *C. saccharoperbutylacetonicum* N1-4 from different types of starch such as cassava starch, corn starch and wheat with using granular starch hydrolyzing enzymes.

The current work aimed to the development of the enzymatic treatment of PKC to determine the most efficient approach for the high production of fermentable sugars that could further be used in the production of butanol by *C. saccharoperbutylacetonicum* N1-4 (ATCC 13564). The effect of the pretreatment step on the enhancement of the enzymatic saccharification of the polysaccharide content of PKC was also investigated. Further studies were performed to characterize morphological changes of lignocellulose structure of PKC cell wall in relation to enzymatic hydrolysis to obtain the better understanding of the saccharification of the polysaccharide content of PKC.

2. Methods

2.1. Preparation and enzymatic hydrolysis of PKC

PKC was obtained from FELDA Pasir Gudang, Johor, Malaysia. PKC was ground and passed through a 600 μm mesh sieve to obtain fine and uniform PKC particles. The enzymatic hydrolysis of PKC was carried out in a 250 ml-Schott Duran bottle using three different enzyme preparations including 6607.143 FPU/ml Celluclast (cellulase; Navo, Malaysia), 590 IU/ml β-glucosidase Navozyme 188 (β-glucosidase; Novo Malaysia) and 1589.42 IU/ml Mannanase (Habio Bioengineering Co, Ltd). A number of experiments for the enzymatic hydrolysis of PKC were carried out using different combinations of hydrolytic enzymes studied including the binary mixtures of β-glucosidase and mannanase as well as cellulase and mannanase with a blend of all three enzymes tested. For the enzymatic treatment, 6 mL of enzyme preparations was reacted with 100 g of PKC particles using 0.2 mM sodium acetate buffer (pH 4.5). The enzymatic reaction was carried out at a temperature of 45 °C for 72 h reaction time under 170 rpm agitation. The enzyme reaction solution was then filtered by the filter paper and the filtrate obtained was kept as the PKC hydrolysate at 4 °C for sugar analysis and butanol production. Each enzymatic experiment was performed in duplicate to ensure the consistency of the results.

2.2. Microorganism preparation

Bacterial strain *C. saccharoperbutylacetonicum* N1-4 was obtained from the Biotechnology Lab in the Chemical and Process Engineering Department, Universiti Kebangsaan Malaysia (UKM). A volume of 1 ml of stock culture was transferred into the PG medium and the culture was then incubated anaerobically for 1–2 days at 30 °C. Inoculum preparation was carried out by transferring 10% (v/v) of the seed culture into the tryptone–yeast extract–acetate (TYA) medium, followed by incubating the inoculation culture at 30 °C for 18 h under anaerobic conditions. The PG medium consisted of following components (g/L): potato, 150; glucose, 10; $CaCO_3$, 3 and $(NH_4)_2SO_4$, 0.5.

Moreover, the TYA medium was composed of chemicals are as follows (g/L): tryptone, 6; yeast extract, 2; ammonium acetate, 3; KH_2PO_4, 0.5; $MgSO_4{\cdot}7H_2O$, 0.3, and $FeSO_4{\cdot}7H_2O$, 0.01 (Al-Shorgani et al., 2015).

2.3. ABE fermentation

ABE fermentation for biobutanol production was conducted in a serum bottle with a working volume of 50 mL. ABE fermentation medium was prepared by an addition of the P2 medium components into PKC hydrolysate solution. The mixture was then sterilized at 121 °C for 15 min using an autoclave. The initial pH of ABE fermentation medium was adjusted to 6.5 using a solution of 6 M NaOH. Anaerobic conditions were provided by sparging filtered oxygen-free nitrogen gas to the bottle for 10 min. ABE fermentation was carried out by transferring 10% (v/v) of the fresh inoculum of *C. saccharoperbutylacetonicum* N1-4 into the ABE fermentation medium. The inoculated medium was incubated at the temperature of 30 °C for 168 h in the anaerobic conditions. The