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Efficient utilization of oil palm frond for bio-based products and biorefinery

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ABSTRACT

The prospect of oil palm frond (OPF) juice as fermentation feedstock was investigated by taking two bioproducts, i.e. poly(3-hydroxybutyrate), P(3HB) and bioethanol as example. P(3HB) was successfully produced by *Cupriavidus necator* NCIMB 11599 from OPF juice through fed-batch fermentation with cell dry mass and PHB content of 40 g/l and 75 wt.%, respectively. On the other hand, bioethanol fermentation from OPF juice was conducted by using Baker's yeast, with and without nitrogen source supplementation. Ethanol yield of 0.49 g/g sugars was recorded when OPF juice was supplemented with nitrogen source. Furthermore, OPF pressed fiber obtainable after pressing the OPF juice was saccharified in order to obtain more fermentable sugars from OPF petiole. Hydrolysis of OPF fiber holocellulose into sugars was very high at 95%, contributed by the low lignin content in OPF and pre-treatment by wet disc-mill. Apart from fermentation, OPF pressed fiber is also useful for bio-based plastics, ruminant feed, reinforce material for biocomposites and bio-briquettes. Efficient utilization of OPF petiole proposed herewith can be an alternative pathway to the contribution of green and sustainable biorefinery.

1. Introduction

Biomass utilization for value-added products has been widely discussed. Lignocellulose materials are the most favorable substrate for bioconversion as they are renewable and abundantly available. In Malaysia, oil palm biomass is generated daily either at the palm oil mill or at the plantation (MIA, 2011). Oil palm frond (OPF) is the most generated biomass from the palm oil industry (MPOC, 2010; Zahari et al., 2012a). We recently demonstrated that sugary juice extracted from OPF petiole can be used as renewable fermentation feedstock for valued added-products (Zahari et al., 2012a). It was exhibited that microbial poly(3-hydroxybutyrate), P(3HB) bioplastic was successfully produced by wild-type *Cupriavidus necator* strain CCUG52238^T from OPF juice (Zahari et al., 2012a). Subsequent experiment on optimization of P(3HB) production in shake flask using the aforementioned strain resulted a 40% increment on P(3HB) content compared to the P(3HB) produced under non-optimized condition, followed by cultivation in a 2-L bioreactor (1-L working volume) yielded CDW of 11.37 g/L and P(3HB) content of 44 wt.% (Zahari et al., 2012b). However, these results are considered too low for commercialization. This is due to the fact that P(3HB) content gives significant effect on the recovery cost of P(3HB) and eventually, total operating cost. For instance, Lee and Choi (1998) reported that a relatively low P(3HB) content of 50% resulted in a recovery cost of USD 4.2/kg P(3HB), which contributed to more than 60% of the total operating cost. On the other hand, higher P(3HB) content at 88.3% reduced the recovery cost of P(3HB) to only USD 0.65/kg P(3HB), which was approximately 36% of the total operating cost. This comparison shows that lower P(3HB) content contributed to higher recovery cost. This is mainly due to the use of larger amount of surfactant and hypochlorite and the increase in waste treatment cost (Lee and Choi, 1998).

In addition, P(3HB) obtained in our previous study was lower compared to the other literature which reported that higher

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accumulation of polyhydroxyalkanoates (PHA) at more than 50 wt.% can be obtained from *C. necator* through batch fermentation (Doi et al., 1988; Rusendi and Sheppard, 1995; Park et al., 1995). Thus, one of the objectives of this study was to further enhance P(3HB) production using mutant strain of *C. necator* NCIMB 11599 from OPF juice. Batch experiment was conducted in shake flasks in order to examine the effect of various OPF juice concentration on P(3HB) production, followed by batch and fed-batch fermentations in 2 L bioreactor to observe the growth and P(3HB) production profile by the mutant strain of *C. necator* NCIMB 11599.

In order to further evaluate the potential of OPF juice as fermentation feedstock, bioethanol production by *Saccharomyces cerevisiae* was tested. In large-scale bioethanol production, the cost of substrate and its pre-treatment are very crucial. Hence, the use of simple sugar resource such as sugar cane juice is preferable. However, since sugar cane is classified as food crop, the food versus fuel issue has urged the researcher to find alternatives to the food crops for bioethanol production.

Pressed OPF fiber is the by-product from OPF pressing process. Pressed OPF fiber contains substantial amount of carbohydrate, which is also useful as fermentation feedstock. This paper demonstrates efficient utilization of OPF petiole as fermentation feedstock to produce value-added products, whereby the potential of both the OPF juice and OPF fiber as fermentation feedstock is discussed. P(3HB) and bioethanol were taken as examples of bioproducts. OPF fiber which consists high cellulose content is also useful for other products such as bio-based plastic, ruminant feeds, bio-briquettes and biocomposites. A scheme for efficient utilization of OPF is proposed in this paper, considering potential uses of both the OPF juice and OPF fiber.

2. Materials and methods

2.1. Raw materials

OPF petioles were collected from an oil palm plantation located in Universiti Putra Malaysia, Serdang, Selangor. OPF juice was obtained by pressing fresh OPF petioles following the method described earlier (Zahari et al., 2012a).

Fiber residue obtained after pressing (OPF pressed fiber) was sun-dried and ground using a hammer mill (Hsiangtai) with 2 mm screen size. Hammer-milled OPF fiber was then further treated using a disc mill (Ishiusu) in wet condition. Water was added to OPF fiber in a ratio of 1:20 in order to assist the disc-milling process. Wet disc milling was repeated for 20 cycles until the mixture become homogenized in a paste form. Both the hammer-milled OPF and OPF fiber paste were kept at 4 °C until further use.

2.2. Bacterial strains

For P(3HB) production, *C. necator* NCIMB 11599 was obtained from the National Collection of Industrial, Food and Marine Bacteria (NCIMB), Aberdeen, Scotland, and used for the production of P(3HB). *C. necator* NCIMB 11599 is a mutant of *C. necator* wild strain H16 obtained from UV and spontaneous mutagenesis. The mutagenesis allows *C. necator* NCIMB 11599 to utilize glucose, compared to its wild strain which is deficient in consuming glucose (Orita et al., 2012). The culture was kept in −80 °C as frozen stock in 20% glycerol and used throughout of this study. For the preparation of inoculum, 24 h old slant cultures incubated at 31 °C were transferred into a 20 ml sterile nutrient rich medium in a 100 ml flask containing (per liter of distilled water): nutrient broth, 8 g; peptone, 5 g; and yeast extract, 3 g. The pH value was set at 7.0 with 2 M NaOH or H_2SO_4. The flasks were incubated at 31 °C under aerobic condition at 200 rpm for 24 h and the broth was used as 10% inoculum for the P(3HB) production medium.

As for the ethanol production, *S. cerevisiae* used in this study was obtained from Mauri-Pan, Instant yeast, AB Mauri Malaysia Sdn. Bhd. The yeast was inoculated on YPD agar, consisted of glucose (20 g/l), peptone (20 g/l), yeast extract (10 g/l) and technical agar (10 g/l). This culture was incubated at 30 °C for 24 h and stored at 4 °C prior to use. The inoculum was developed in two stages. In the first stage, a loopful of yeast was pre-cultured in 100 ml of YPD medium containing 20 g peptone, 10 g yeast extract and 20 g glucose per liter. The inoculum was cultivated on a rotary shaker (150 rpm) at 30 °C for 6–8 h. In the second stage of inoculum, 10% of the first stage inoculum was transferred into 100 ml of YPD medium and cultivated on a rotary shaker (150 rpm) at 30 °C for 12 h.

2.3. P(3HB) fermentation

2.3.1. Cultivation conditions in shake flask

Biosynthesis of P(3HB) in shake flask experiment by *C. necator* NCIMB 11599 from OPF juice was conducted by transferring the pre-grown cells (10% v/v) into a 180 ml mineral salt medium (MSM) in 500 ml flasks containing (per liter of distilled water) KH_2PO_4, 1.5 g; Na_2HPO_4, 9.0 g; $(NH_4)_2SO_4$, 1.0 g; $MgSO_4$, 0.2 g, and 1 ml microelements solution (Hassan et al., 1997). The MSM was prepared based on Ryu et al. (1997). In order to study the effect of OPF juice concentration on P(3HB) production, OPF juice with an initial total sugars concentration of 65 g/l comprising of 42 g/l glucose, 22 g/l sucrose and 1 g/l fructose was diluted to several different concentrations in the range of 10–60% (v/v). The OPF juice was added into the MSM after autoclaving and the culture medium was incubated at 31 °C and shaking at 200 rpm. The samples were harvested after 48 h for the determination of residual sugars concentration, cell dry mass and P(3HB) content in the cells and all experiments were conducted in duplicates.

2.3.2. Batch fermentation in 2 L bioreactor

MSM was used as production medium for batch fermentation in a 2 L bioreactor (Biostat A, Sartorius, Germany) with 1 L working volume. 100 ml of pre-grown cells were transferred into 900 ml MSM medium supplemented with OPF juice at 50% (v/v) dilution, containing 32.5 g/l of total initial sugars comprising of 21 g/l glucose, 11 g/l sucrose and 0.5 g/l fructose. Temperature was maintained at 31 °C and pH was controlled at 6.80 ± 0.05 with 10% H_2SO_4 solution and 25% NH_4OH solution, while DOT level was maintained at 20% of saturation throughout the fermentation using cascade mode at air flow rate of 1.0 vvm. Samples were withdrawn at 5 h intervals for the determination of CDW, P(3HB) content and residual sugars concentration.

2.3.3. Fed-batch fermentation in 2 L bioreactor

Concentrated OPF juice (80% water removal) was used as feed in fed-batch fermentation. Total initial sugars concentration in the concentrated OPF juice was 325 g/l, comprising of 210 g/l glucose, 110 g/l sucrose and 5 g/l fructose. Fed-batch culture was conducted in a 2 L bioreactor (Biostat A, Sartorius, Germany). Seed and production media were modified from Ryu et al. (1997) and Haas et al. (2008), as tabulated in Table 1. Trace element solution was as previously described in Zahari et al. (2012a). Total sugars concentration was maintained at between 10 and 30 g/l. Oxygen control is similar as previously described. Temperature was maintained at 31 °C and pH was controlled at 6.80 ± 0.05 with 10% H_2SO_4 solution and 25% NH_4OH solution. Sugars concentration in the bioreactor during fed-batch fermentation was estimated using glucose analyzer (Labo-TRACE, Trace Analytics, Germany). Samples were withdrawn every