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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

Table 1
Chemical composition of seed and P(3HB) production media.

Medium compositions	Shaker flask inoculum	Fed-batch bioreactors	
		Start	Feed solution
Glucose or OPF juice	20 g/l	50% OPF juice (21 g/l glucose, 11 g/l sucrose, 0.5 g/l fructose)	325 g/l (210 g/l glucose, 110 g/l sucrose, 5 g/l fructose)
$(NH_4)_2SO_4$	1 g/l	4 g/l	–
$MgSO_4 \cdot 7H_2O$	0.2 g/l	1.2 g/l	–
KH_2PO_4	1.5 g/l	5.5 g/l	–
$Na_2HPO_4 \cdot 12H_2O$	9 g/l	–	–
Citric acid	–	1.7 g/l	–
Trace element solution	–	10 ml/l	–

5 h for the determination of CDW, P(3HB) content and residual sugars concentration.

2.4. Ethanol production from OPF juice

Production medium used in this study was prepared based on Ogbonna et al. (2001) and Kosugi et al. (2010) with some modification. *S. cerevisiae* culture was used as inoculum at 10% v/v. Effect of nitrogen supplementation was studied by using two different production media: i) Medium A: OPF juice supplemented with 4 g/l of peptone and yeast extract, respectively; and ii) Medium B: OPF juice without nitrogen supplementation. Control medium was prepared to mimic medium A by using mixture of commercial sugars: glucose, sucrose and fructose, at the same concentration as in OPF juice. pH and sugar content in all OPF juice medium were not adjusted. All the flasks were cultivated on a rotary shaker (150 rpm) at 30 °C for 48 h. Samples were withdrawn at time intervals for analyses of ethanol, residual sugars and pH.

2.5. Saccharification of OPF fiber

Saccharification of OPF fiber was carried out in 250 ml conical flasks with caps. One gram (dry weight) of wet-disc-milled OPF fiber was added to 20 FPU of cellulase (Meiji Seika) in 40 ml of 0.05 M acetate buffer. Saccharification was carried out for 48 h. Sampling was done at 12 h intervals, and the amount of sugars released were analyzed by HPLC. Moisture content of OPF fiber paste was measured prior to saccharification in order to compare saccharification results with that obtained from hammer-milled OPF.

2.6. Analytical procedures

2.6.1. P(3HB) and sugars concentration analysis

P(3HB) content in the cells and sugars concentration were conducted as previously described (Zahari et al., 2012a,b).

2.6.2. TEM analysis

Sample preparation for Transmission Electron Microscopy (TEM) analysis was based on the methods described by Mumtaz et al. (2011). Specimens were examined with a TEM (Hitachi H7100 TEM) at an intensity of 10–15 kV, using 5000–40,000 magnifications.

2.6.3. Molecular mass determination

Gel permeation chromatography (GPC) was carried out in order to determine weight average molecular weight (M_w), number average molecular weight (M_n) and polydispersity index (M_w/M_n) of the biopolymer following the methods described earlier (Zahari et al., 2012a).

2.6.4. Ethanol formation

Ethanol formation was determined using a gas chromatograph, GC (Shimadzu GC-17A) equipped with BP-20 capillary column and flame ionization detector (FID). The temperature of the injector and detector were set at 150 and 200 °C, respectively. The chromatography was conducted at 185 °C with helium as the carrier gas at a flow rate of 0.5 ml/min. The standard curve for ethanol was plotted using standard ethanol sample.

2.6.5. Lignin, hemicellulose and cellulose

Lignin, hemicellulose and cellulose were analyzed according to the method proposed by Fahma et al. (2010). Fiber initial weight (a) was first taken prior to lignin removal by soaking the sample in 5% (w/w) sodium chlorite ($NaClO_2$) solution (pH 4–5) and stirred for 1.5 h at 70 °C. Filtered sample was then washed using deionized water. The residue was dried overnight at 70 °C prior to weight measurement (b). The same sample was then used for hemicellulose removal, where it was soaked and stirred in 6% (w/w) potassium hydroxide (KOH) for 24 h at room temperature, followed by filtration and rinse using deionized water. The residue was allowed to dry overnight at 70 °C, prior to weight measurement (c). Lignin, hemicellulose and cellulose content (%) were then measured according to the equations below:

$$\text{Cellulose}(\%) = c \quad (1)$$

$$\text{Lignin}(\%) = a - b \quad (2)$$

$$\text{Hemicellulose}(\%) = c - \text{lignin} \quad (3)$$

3. Results and discussion

3.1. P(3HB) production from OPF juice

3.1.1. P(3HB) production in shake flasks

In order to study the ability of *C. necator* NCIMB 11599 to utilize OPF juice as the sole renewable carbon source, we examined the effect of various OPF juice concentration in the range of 10–60% (v/v) dilution for P(3HB) production in shake flask experiment and the results are shown in Table 2. It was observed that the cell dry weight and P(3HB) production was substrate-dependent. Both cell growth and P(3HB) production was increased with the increase in initial total sugars concentration, up to 32.5 g/l in the OPF juice (50% v/v dilution). Further increased of sugars concentration inhibited the growth of *C. necator* and reduced the P(3HB) accumulation in the cells. This can be due to the increase in osmotic pressure and imbalance between glycolysis and metabolic oxidation in bacterial cells (Tabandeh and Vasheghani-Farahani, 2003).

Almost similar results were obtained in our previous study whereby different concentrations of OPF juice affected P(3HB) accumulation in the wild type *C. necator* CCUG52238^T (Zahari et al., 2012a). However, in the current finding, higher P(3HB) content and yield were obtained. As shown in Table 2, P(3HB) content obtained was in the range of 30–65 wt.%, meanwhile P(3HB) yield was in the range of 0.24–0.40 g P(3HB)/g sugars consumed. The highest P(3HB) content and yield, *i.e.* 65 wt.% and 0.40 g P(3HB)/g sugars consumed, respectively, was obtained when 50% (v/v) of OPF juice with an initial total sugars of 32.5 g/l was supplied in the shake flask. As comparison, lower P(3HB) content and yield were obtained in our previous study (Zahari et al., 2012a). This can be explained by the nature of wild type *C. necator* which does not assimilate glucose, and prefers fructose (Orita et al., 2012; Balkwill, 2005). It was reported that most of the wild type *C. necator*, for instance *C. necator* H16 shows lack of glucose-utilization ability

Table 2
Biosynthesis of P(3HB) by *Cupriavidus necator* NCIMB 11599 on various concentrations of OPF juice.[a]

OPF juice concentration (v/v %)	Total initial sugars concentration (g/l)[b]	Residual sugars (g/l)[b]	Cell dry mass (g/l)	$Y_{x/S}$ (g biomass/g sugars consumed)	PHB concentration		$Y_{P/S}$ (g PHB/g sugars consumed)
					(G/L)	(%)[c]	
10	6.5	ND	3.9	0.60	1.56	40	0.24
20	13.0	ND	8.2	0.63	3.44	42	0.26
30	19.5	ND	12.6	0.65	6.17	49	0.32
40	26.0	ND	16.7	0.64	10.02	60	0.39
50	32.5	ND	19.8	0.61	12.87	65	0.40
60	39.0	2.5	19.1	0.52	11.46	60	0.31

ND = Not detected.
[a] Fermentation was conducted at 31 °C, 200 rpm for 48 h and all experiment were conducted in duplicates.
[b] Determined by HPLC analysis.
[c] % of PHB content in cells, determined by GC.

which is caused by the deficiency of both glucose uptake and phosphorylation abilities (Orita et al., 2012). On the other hand, glucose-utilization ability of mutant strain *C. necator* NCIMB 11599 is attributed to extended sugar specificity of the mutated *NagE* and derepression of *nagFE* expression by inactivation of *NagR* (Orita et al., 2012). Since OPF juice contained mainly glucose, hence *C. necator* NCIMB 11599 had better ability to utilize OPF juice compared to *C. necator* CCUG52238T. The results indicate that mutant strain of *C. necator* NCIMB 11599 is more suitable to be used in the production of P(3HB) from OPF juice compared to the wild type *C. necator*.

Furthermore, results obtained herewith are better as compared to those reported previously using synthetic media with glucose. For instance, Doi et al. (1988) reported lower P(3HB) yield of 0.25 g P(3HB)/g glucose from shake flasks experiment by *Alcaligenes eutrophus* NCIMB 11599 when synthetic media with glucose was used. In addition to that, Beaulieu et al. (1995) also reported a lower P(3HB) yield at 0.17–0.26 g P(3HB)/g glucose from *A. eutrophus* DSM 545 using synthetic media with glucose, various ammonium sources and cane molasses. Higher P(3HB) yield achieved in this study could be attributed to the fact that glucose was not the only carbon source in the OPF juice. Other carbon sources, i.e. sucrose and fructose and other organic compounds such as amino acids, carbohydrates and other minerals which were previously characterized in the OPF juice (Zahari et al., 2012a), may increase *C. necator* NCIMB 11599 growth and P(3HB) yield in respect to total sugars leading to improvements in P(3HB) accumulation.

3.1.2. Cell growth, P(3HB) production and sugars consumption profile in 2 L bioreactor by batch and fed-batch cultivation processes

Fig. 1 shows the profile of CDW, P(3HB) content and residual sugars concentration during batch fermentation of P(3HB) by *C. necator* NCIMB 11599. As can be observed in Fig. 1, the cells grew steadily and reached their stationary phase at 35th h. Sugars concentration in the media dropped down from 32.5 g/l and completely exhausted after 35 h of fermentation period. Glucose was completely utilized after 25 h of cultivation period at higher consumption rate, i.e. 0.84 g/l/h compared with sucrose at 0.31 g/l/h. In overall, the total sugars consumption rate was 0.93 g/l/h indicating a good level of utilization of the carbon source by *C. necator* NCIMB 11599. A maximum CDW of 19.8 g/l and P(3HB) content of 65.5 wt.% was achieved at this point. The maximum P(3HB) productivity was 0.37 g/l/h.

As the bacterium can only withstand up to 50% (v/v) of OPF juice, fed-batch mode was applied in order to study the ability of the cells to grow at higher cells concentration. Time course of P(3HB) fermentation by fed-batch mode is shown in Fig. 2. Overall, it was found that at the end of the fermentation period (60 h), CDW and P(3HB) content were recorded at 40.3 g/l and 75 wt.%, respectively. The P(3HB) productivity obtained was 0.51 g/l/h. Based on the result, it is shown that the cell growth, P(3HB) production and productivity were improved when fed-batch fermentation was conducted. CDW was doubled compared to that obtained from batch fermentation. Higher CDW and P(3HB) accumulation was observed in fed-batch bioreactor was due to the excess supply of carbon source for microbial growth as well as for P(3HB) accumulation. Fig. 3 shows TEM micrographs of PHB accumulation in *C. necator* NCIMB 11599 during fed-batch fermentation. According to Kim et al. (1994), fed-batch culture has been found to be the most effective strategy to obtain high cell density as well as high productivity and yield of the desired product. In overall, the P(3HB) content (75 wt.%) and yield (0.33 g P(3HB)/g sugars consumed)

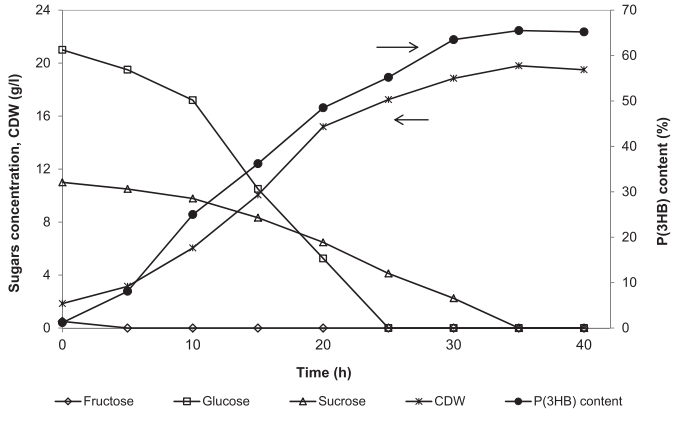

Fig. 1. Time course for batch fermentation of *C. necator* NCIMB 11599 using OPF juice in 2-L bioreactor.

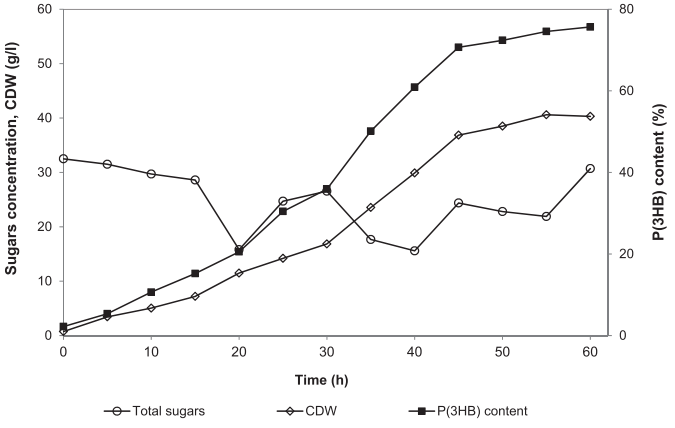

Fig. 2. Time course for cell dry weight (CDW), P (3HB) content (wt.%) and total sugars concentration of *C. necator* NCIMB 11599 during fed-batch fermentation using OPF juice.

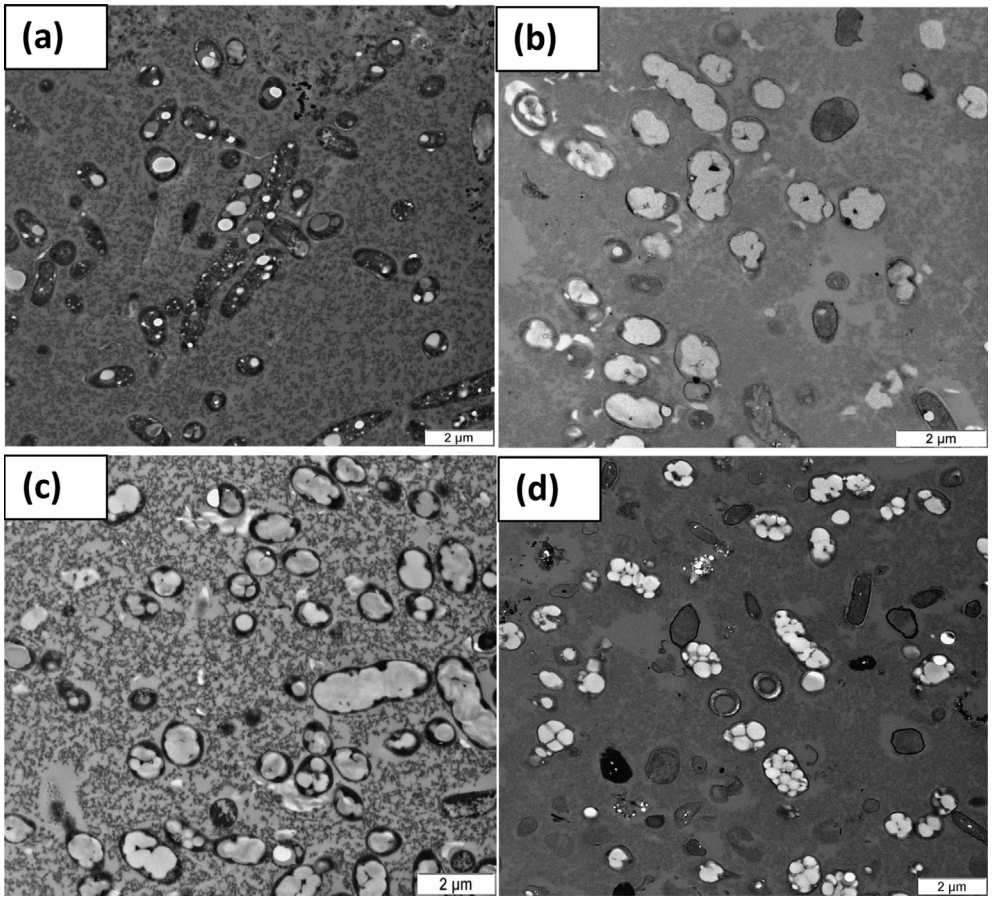

Fig. 3. TEM images of *C. necator* NCIMB 11599 in fed-batch fermentation (a) 10 h (b) 30 h, (c) 45 h (d) 60 h (Magnification a and d 8,000×; b and c 10,000×; bar = 2 μm).

obtained in this study was slightly higher compared to those reported by Haas et al. (2008) indicating that OPF juice can be an alternative and renewable cheap carbon source in P(3HB) production.

3.1.3. Characterization of P(3HB) produced from OPF juice

Table 3 shows the comparison of M_w and M_n of P(3HB) produced from OPF juice with P(3HB) produced from other renewable substrates. It can be observed that the molecular mass and polydispersity index (M_w/M_n) obtained from this study were comparable to the P(3HB) obtained from OPF juice by *C. necator* CCUG52238^T (Zahari et al., 2012a). Meanwhile, the values were slightly higher compared to those produced from renewable substrates such as waste glycerol and maple sap. This is due to the fact *A. eutrophus* or *C. necator* is able to accumulate P(3HB) with molecular masses of 600 kDa to more than 1000 kDa (Anderson and Dawes, 1990). Moreover, it has been demonstrated that P(3HB) fermentation using glucose, fructose and sucrose managed to produce polymers with an average molecular mass higher than or very close to 1000 kDa (Cavalheiro et al., 2009). It is important to highlight that the molecular mass of polymers can be an important factor affecting their physicochemical and mechanical properties, and thus, their applications (Yezza et al., 2007). The relatively high molecular mass measured for the P(3HB) obtained in this study suggested that the biopolymer produced from OPF juice has a sufficient degree of polymerization that is suitable for commercial utilization.

3.2. OPF juice as substrate for ethanol fermentation

The potential of OPF juice as fermentation feedstock was further evaluated by testing the substrate for ethanol production by *S. cerevisiae* (Baker's yeast). Effect of nitrogen supplementation on ethanol production from OPF juice was tested by supplementing peptone and yeast extract to the fermentation medium. The results are shown in Fig. 4a–c. Overall, sugars in OPF juice were completely consumed by the yeast at the end of fermentation period (Fig. 4a–b), including sucrose. Since sucrose is not simple sugar like glucose and fructose, hence the observed condition can be explained by the

Table 3
Comparison of molecular mass and polydispersity index of purified P(3HB) obtained from this study with other P(3HB)s produced from renewable resources.

Strain	Substrate	M_n[a] × 10^{-3} (Da)	M_w[a] × 10^{-3} (Da)	M_w/M_n[a]	Reference
C. necator NCIMB 11599	OPF juice	485	848	1.75	This study
C. necator CCUG52238^T	OPF juice	515	812	1.61	Zahari et al. (2012a)
C. necator DSM 545	Waste glycerol	215	786	3.66	Cavalheiro et al. (2009)
A. latus ATCC 29174	Maple sap	300	507	1.69	Yezza et al. (2007)

[a] Determined by GPC analysis.

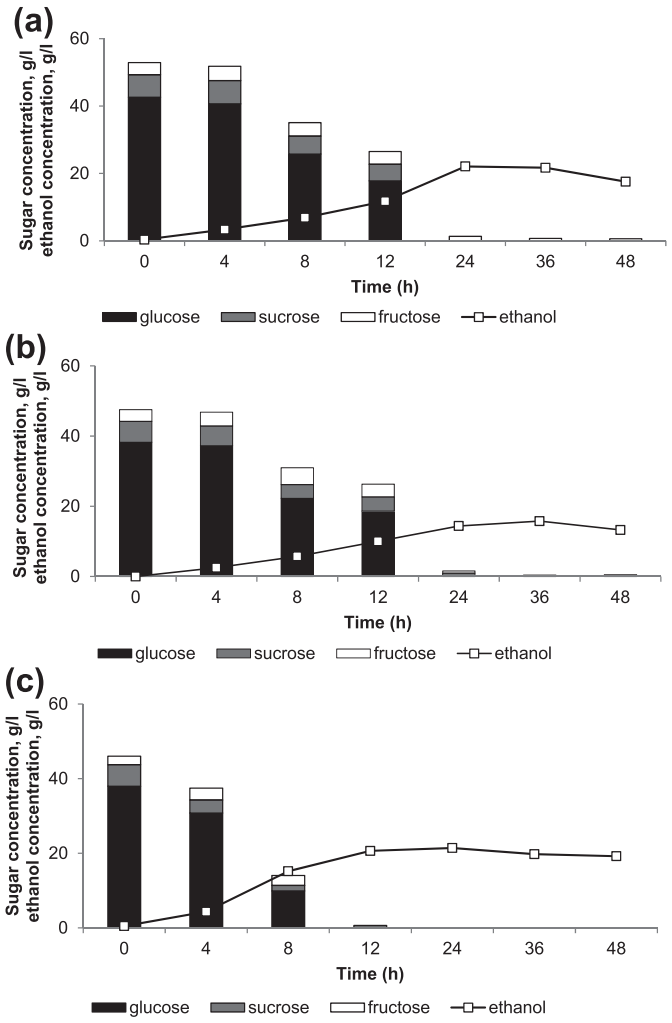

Fig. 4. Ethanol production, sugars consumption and pH profile in (a) medium A (heat sterilized, nitrogen source supplemented OPF juice), (b) medium B (heat sterilized, no nitrogen source supplemented OPF juice), (c) control medium.

presence of invertase that breaks down sucrose during the fermentation. This is supported by previous studies which reported that *S. cerevisiae* has the ability to produce invertase enzyme (Badotti et al., 2008; Dodić et al., 2009; Wu et al., 2010).

The presence of nitrogen source in medium A brought positive effect on ethanol production, whereby higher ethanol concentration was recorded compared to that in medium B. Maximum ethanol production was obtained at 24 h and 36 h of fermentation in media A and B, respectively. Slower ethanol production in Medium B is attributed to the absence of nitrogen source. It has been reported that deficient in nitrogen source during fermentation resulted in slow rate of sugars utilization and ethanol productivity (Nuanpeng et al., 2011). This explains the low ethanol production in medium B.

Overall, OPF juice supplemented with peptone and yeast extract (medium A) yielded 0.49 g ethanol/g sugars. This value is comparable to that of ethanol produced from oil palm trunk sap (Kosugi et al., 2010) and other renewable resources as reported previously (Table 4). The promising yield of ethanol obtained in this study suggests that OPF juice is suitable as fermentation feedstock for ethanol production.

3.3. Fermentable sugars from saccharified OPF fiber

Apart from OPF juice, fibrous part of OPF can as well be utilized as fermentation feedstock. Chemical composition analysis of the OPF fiber showed that OPF fiber contained 13.45, 35.56 and 45.02% of lignin, hemicellulose and cellulose, respectively. Lignin content of OPF used in this study is relatively low compared to the other oil palm biomass such as oil palm trunk (OPT), oil palm empty fruit bunch (OPEFB) and oil palm mesocarp fiber (OPMF), which have lignin content in the range of 15–28% (Kelly-Yong et al., 2007; Ariffin et al., 2008). Lignin acts as glue that binds cellulose and hemicellulose to give plants the strong structure. Therefore, low lignin content of lignocellulose fiber is good for saccharification since the low lignin composition may suggest a relatively easier saccharification process as enzymes can penetrate into cellulose and hemicellulose structure easier.

Fig. 5 shows the time course for saccharification of OPF fiber paste (wet disc-milled OPF, WDM-OPF) and hammer-milled OPF (HM-OPF). Overall, higher sugars release was found from saccharification of OPF fiber paste. Upon saccharification, HM-OPF fiber released a maximum concentration of 0.273 g glucose and 0.213 g xylose, respectively per g of OPF petiole; while OPF fiber paste (WDM-OPF) gave a maximum glucose and xylose concentrations of 0.469 g and 0.298 g, respectively per g of OPF petiole.

It is interesting to highlight the difference of sugars recovery from hammer-milled OPF fiber and OPF fiber paste. A marked different in sugars concentration was observed from the two OPF fibers. Since OPF petioles used in this study were from the same batch, hence it is postulated that the difference in sugars concentration was contributed by the method of milling. Hammer-milling mode of action mainly involves cutting to shorten the length of the fiber. On the other hand, mechanism of fiber size reduction by disc-milling involves the use of two milling stones which results in cutting to short length, imparting and shearing the OPF fiber. This produces small and thin lignocellulose microfibrils. Moreover, the use of water is very useful to assist the disc-milling process so that the gap between the two stones can be reduced to almost zero

Table 4
Comparison of ethanol production from various renewable carbon sources.

Strain	Substrate	Sugar concentration (g/l)	Ethanol yield, $Y_{p/s}$ (g/g)	Reference
Saccharomyces cerevisiae Kyokai no.7.	Oil palm trunk sap	55	0.48	Kosugi et al., 2010
Saccharomyces cerevisiae (*commercial Bakers yeas, Mauripant*)	Glucose from residual starch of sago hampas	80	0.48	Awang Adeni et al., 2013
Saccharomyces cerevisiae (strain DTN)	Sugar beet molasses	100	0.41	Razmovski and Vucurovic, 2012
Saccharomyces cerevisiae (strain DTN)	Sugar beet thick juice	100	0.43	Razmovski and Vucurovic, 2012
Saccharomyces cerevisiae (dry baking yeast, Fleischmann, Montevideo, Uruguay)	Sweet sorghum stalk juice (M81)	110	0.39	Mairan et al., 2011
Saccharomyces cerevisiae (*commercial Bakers yeast, Mauripan*)	OPF juice	53	0.49	This study

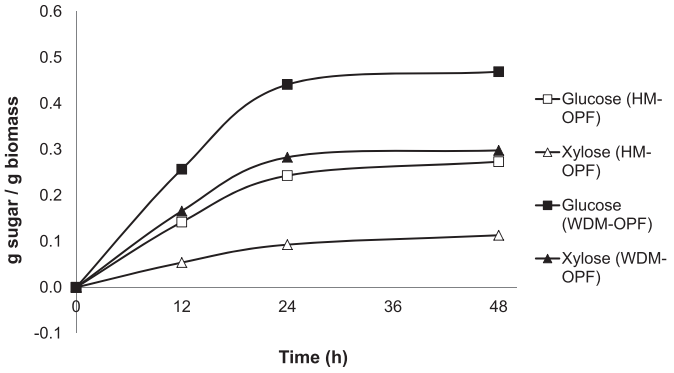

Fig. 5. Time course for saccharification of hammer-milled OPF (HM-OPF) and OPF fiber paste (wet disc-milled OPF, WDM-OPF).

Table 5
Total OPF processed per mill per year. Calculation was made based on the current practice at the plantation— for every 1 FFB processed, 2 OPF will be pruned.

Average capacity of the mill	200,000,000 kg/yr of FFB processed
Average weight of FFB	26 kg/FFB
Amount of FFB processed (a)	7,692,307.69 FFB/yr
During pruning, 1 FFB will generate 2 OPF. Hence:	
Amount of OPF generated = (a) × 2	15, 384, 615.38 OPF/yr
Average weight of OPF	2 kg/OPF
Total weight of OPF generated from 1 mill	30,000 tonne/yr/mill
Total weight of OPF generated from 10 mill	300,000 tonne/yr

without heating effect. The close gap between the two milling stones resulted in a very fine OPF fiber (in paste form), which is advantageous for saccharification.

Considering hemicellulose and cellulose content in OPF fiber which accounted to 80.58% of the OPF fiber weight, percentage of holocellulose conversion into reducing sugars was calculated. It was found that about 95% of holocellulose in OPF fiber paste was being bio-converted into reducing sugars during the saccharification. This is 1.5 fold higher compared to HM-OPF, which recorded only 60% bioconversion. This value is also higher compared to the bioconversion of oil palm empty fruit bunch (OPEFB), which was recorded at 65% as reported earlier (Ariffin et al., 2008).

3.4. Overview on the efficient utilization of OPF for bio-based products and biorefinery

It has been demonstrated herewith and in our previous reports (Zahari et al., 2012a, 2012b) that OPF juice can be used for the production of bioplastics and biofuel, due to its high sugars content which is favorable for fermentation. The advantage of OPF juice compared to lignocellulose materials and other biomass is the easiness of getting the sugars, whereby only simple pressing is needed. We chose PHB and bioethanol as example of fermentation products from OPF juice since the two products are currently at high demand worldwide. Furthermore, bioethanol produced from this proposed integrated process can be used as a source of energy for PHB production. It has been reported that high energy is required to produce PHA, estimated at 40–49 MJ/kg PHA produced (Sharma and Mudhoo, 2011). Therefore, it will be appropriate to make use of bioethanol produced from this process as a source of energy for PHB production.

We propose herewith a biorefinery concept for PHA and bioethanol production from OPF juice. An average size of palm oil mill in Malaysia has the capacity to process 200,000 tonne of oil palm fresh fruit bunch (FFB) per year. Considering that 2 OPF will be pruned for each 1 FFB harvested, a total of 300,000 tonne OPF will be obtained from 10 mills per year (Table 5). Subsequently, sugars will be obtained from both OPF juice and fiber for the production of

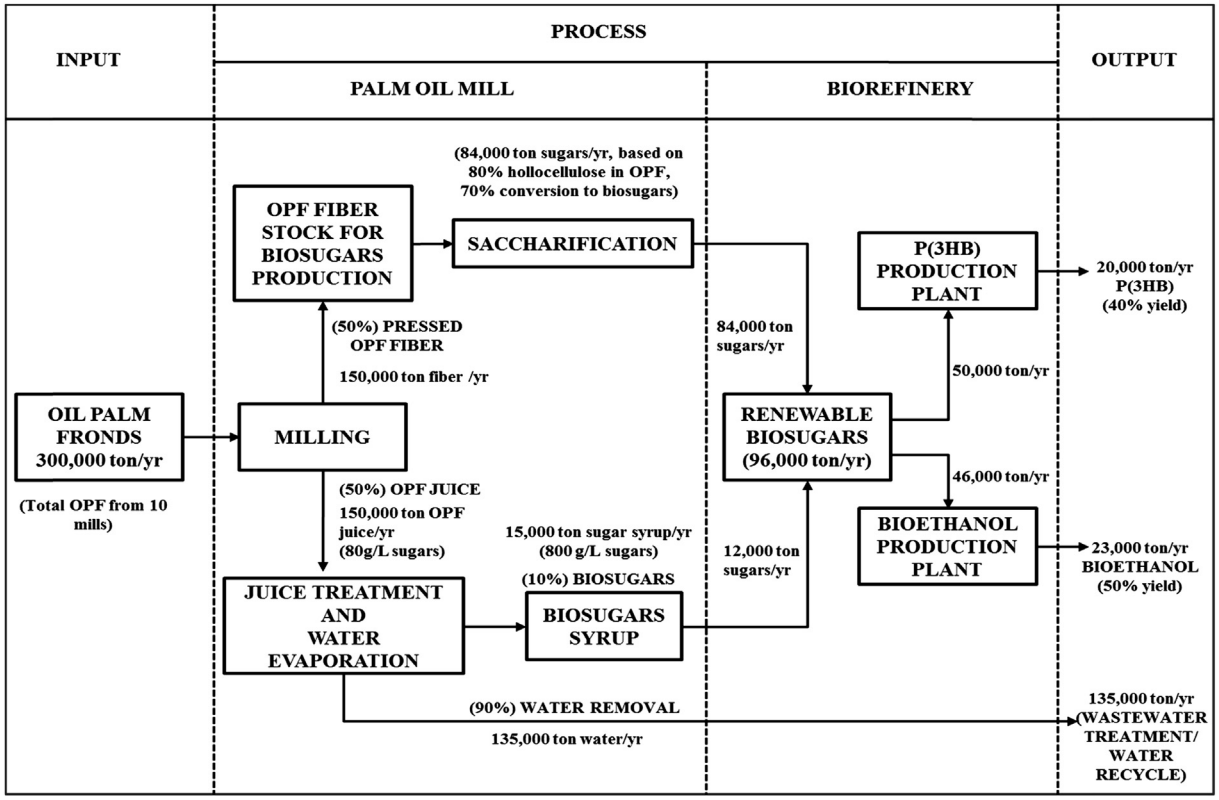

Fig. 6. Example of PHA and bioethanol biorefinery at palm oil mill.

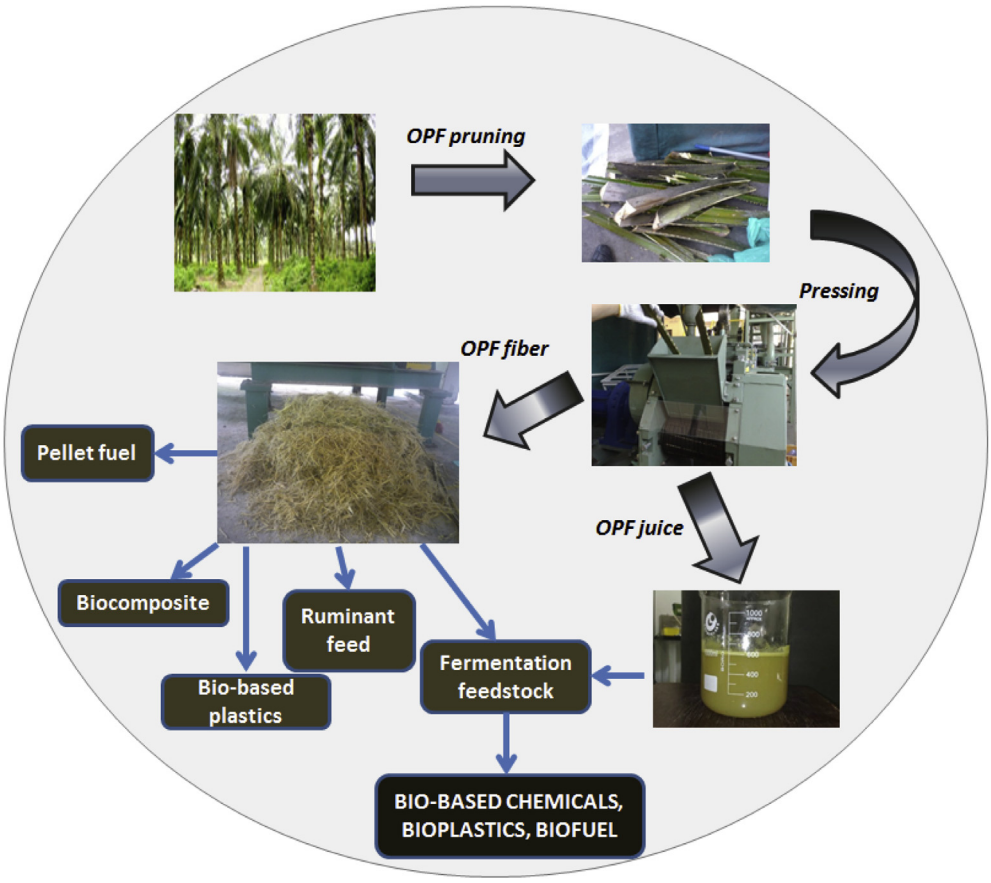

Fig. 7. Efficient utilization of oil palm fronds.

PHB and bioethanol. Fig. 6 shows the overall picture of the proposed integrated process.

Apart from being used as material for saccharification, pressed OPF fiber has a lot of other potential uses, for example as feed for ruminants, bio-briquette for energy generation, reinforce material for the production of biocomposites and also for the production of bio-based plastics, such as cellulose acetate and lignin-based plastics. Due to its various potential applications, OPF petiole is foreseen to contribute to the development of sustainable products as the production of value-added products will not only help in reducing the amount of biomass generated at the plantation, but at the same time it may create new business potential for the oil palm industry which will contribute to new profit and creation of new jobs. Fig. 7 shows the example of efficient utilization of the OPF, which will in turn benefiting the 3P's – Profit, People and Planet.

4. Conclusions

It can be concluded that sugars derived from OPF juice can be used as sustainable and renewable carbon source for P(3HB) and bioethanol production. The production of P(3HB) from OPF juice was improved by using *C. necator* NCIMB 11599 compared to the wild type strain, *i.e. C. necator* CCUG52238^{T}. Cultivation in fed-batch bioreactor yielded final P(3HB) concentration of 30.5 g/l, comprising of 75 wt.% of the biomass dry weight. In addition to that, ethanol was successfully produced from OPF juice by the *S. cerevisiae*, with the yield of 0.49 g ethanol/g sugars consumed. Meanwhile, pressing the OPF petiole yielded pressed OPF fiber, which is also useful for fermentation. Low lignin content of OPF is an advantage. It was found that bioconversion of OPF pressed fiber treated with wet disc-milling gave about 95% of holocellulose conversion, suggesting OPF pressed fiber as a promising new fermentation feedstock. Moreover, OPF pressed fiber is also advantageous for the production of bio-based plastics such as cellulose acetate, used as filler for biocomposites, applied as ruminant feeds and also as bio-briquettes for energy generation. All these may create new business potential to the oil palm industry which in turns contributing to the generation of profit and new jobs. Most importantly, efficient utilization of OPF will reduce the amount of biomass generated at the plantation which could help the industry in achieving zero emission targets.

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References

Awang Adeni, D.S., Bujang, K.B., Hassan, M.A., Abd-Aziz, S., 2013. Recovery of glucose from residual starch of sago hampas for bioethanol production. BioMed. Res. Int. 2013, 8. Article ID 935852.

Anderson, A.J., Dawes, E.A., 1990. Occurrence, metabolism, metabolic role and industrial uses of bacterial polyhydroxyalkanoates. Microbiol. Rev. 54, 450–472.

Ariffin, H., Hassan, M.A., Umi Kalsom, M.S., Abdullah, N., Shirai, Y., 2008. Effect of physical, chemical and thermal pretreatments on the enzymatic hydrolysis of oil palm empty fruit bunch (OPEFB). J. Trop. Agric. Fd. Sc. 36, 259–268.

Badotti, F., Dário, M.G., Alves, S.L., Cordioli, M. L. a, Miletti, L.C., de Araujo, P.S., Stambuk, B.U., 2008. Switching the mode of sucrose utilization by Saccharomyces cerevisiae. Microb. Cell Fact. 7, 4. http://dx.doi.org/10.1186/1475-2859-7-4.

Balkwill, D.L., 2005. Genus ii. *Cupriavidus* Makkar and Casida 1987a, 325^{VP}. In: Don, J.B., David, R.B., James, T.S., George, M.G., Noel, R.K., Paul, De-V., Michael, G., Fred, A.R., Karl-Heinz, S. (Eds.), Bergey's Manual of Systematic Bacteriology, The Proteobacteria (Part C), vol. 2. Springer, New York, pp. 600–604.

Beaulieu, M., Beaulieu, Y., Mélinard, J., Pandian, S., Goulet, J., 1995. Influence of ammonium salts and cane molasses on growth of *Alcaligenese utrophus* and production of polyhydroxybutyrate. Appl. Microbiol. Biot. 61, 165–169.

Cavalheiro, J.M.B.T., de Almeida, M.C.M.D., Grandfils, C., da Fonseca, M.M.R., 2009. Poly(3-hydroxybutyrate) production by *Cupriavidus necator* using waste glycerol. Process. Biochem. 44, 509–515.

Dodić, S., Popov, S., Dodić, J., Ranković, J., Zavargo, Z., Jevtić Mučibabić, R., 2009. Bioethanol production from thick juice as intermediate of sugar beet processing. Biomass Bioenergy 33 (5), 822–827.

Doi, Y., Tamaki, A., Kunioka, M., Soga, K., 1988. Production of copolyesters of 3-hydroxybutyrate and 3-hydroxyvalerate by *Alcaligenes eutrophus* from butyric and pentanoic acids. Appl. Microbiol. Biot. 28, 330–334.

Fahma, F., Iwamoto, S., Hori, N., Iwata, T., Takemura, A., 2010. Isolation, preparation and characterization of nanofibers from oil palm empty fruit bunch (OPEFB). Cellulose 17, 977–985.

Haas, R., Jin, B., Zepp, F.T., 2008. Production of poly(3-hydroxybutyrate) from waste potato starch. Biosci. Biotech. Bioch. 72 (1), 253–256.

Hassan, M.A., Shirai, Y., Umeki, H., Yamazumi, H., Jin, S., Yamamoto, S., 1997. Acetic acid separation from anaerobically treated palm oil mill effluent by ion exchange resins for the production of polyhydroxyalkanoate by *Alcaligenes eutrophus*. Biosci. Biotech. Bioch. 61, 1465–1468.

Kelly-Yong, T.L., Lee, K.T., Mohamed, A.R., Bhatia, S., 2007. Potential of hydrogen from oil palm biomass as a source of renewable energy worldwide. Energy Policy 35, 5692–5701.

Kim, B.S., Lee, S.C., Lee, S.Y., Chang, H.N., Chang, Y.K., Woo, S.I., 1994. Production of poly(3-hydroxybutyric acid) by fed-batch culture of *Alcaligenes eutrophus* with glucose concentration control. Biotechnol. Bioeng. 43, 892–898.

Kosugi, A., Tanaka, R., Magara, K., Murata, Y., Arai, T., Sulaiman, O., Hashim, R., Abdul Hamid, Z.A., Azri Yahya, M.K., Mohd Yusof, M.N., Ibrahim, W.A., Mori, Y., 2010. Ethanol and lactic acid production using sap squeezed from old oil palm trunks felled for replanting. J. Biosci. Bioeng. 110, 322–325.

Lee, S.Y., Choi, J., 1998. Effect of fermentation performance on the economics of poly(3-hydroxybutyrate) production by *Alcaligenes latus*. Polym. Degrad. Stabil. 59, 387–393.

Mairan, G., Claudia, L., Leticia Veronica, P., Maria Elena, L., Daniel, V., Mario, D.F., 2011. Bioethanol production from sweet sorghum: evaluation of post-harvest treatments on sugar extraction and fermentation. Biomass Bioenerg. 35, 3058–3062.

Malaysian Innovation Agency (MIA), 2011. National Biomass Strategy 2020: New Wealth Creation for Malaysia's Palm Oil Industry. Available at: http://www.innovation.my/pdf/1mbas/National_Biomass_Strategy_Nov_2011_FINAL.pdf (accessed January 2013).

Malaysian Palm Oil Council (MPOC), 2010. Palm Oil: a Success Story in Green Technology Innovations. Available at: http://www.akademisains.gov.my/download/asmic/asmic2010/Plenary12.pdf (accessed January 2013).

Mumtaz, T., Abd-Aziz, S., Rahman, N.A., Yee, P.L., Wasoh, H., Shirai, Y., Hassan, M.A., 2011. Visualization of core-shell PHBV granules of wild type *Comamonas sp.* EB172 in vivo under transmission electron microscope. Int. J. Polym. Anal. Ch. 16, 228–238.

Nuanpeng, S., Laopaiboon, L., Srinophakun, P., Klanrit, P., Jaisil, P., Laopaiboon, P., 2011. Ethanol production from sweet sorghum juice under very high gravity conditions: batch, repeated-batch and scale up fermentation. Electron. J. Biotechnol.. ISSN: 0717-3458. http://dx.doi.org/10.2225/vol14-issue1-fulltext-2.

Ogbonna, J.C., Mashima, H., Tanaka, H., 2001. Scale up of fuel ethanol production from sugar beet juice using loofa sponge immobilized bioreactor. Bioresour. Technol. 76, 1–8.

Orita, I., Iwazawa, R., Nakamura, S., Fukui, T., 2012. Identification of mutation points in *Cupriavidus necator* NCIMB 11599 and genetic reconstitution of glucose-utilization ability in wild type strain H16 for polyhydyroxyalkanoate production. J. Biosci. Bioeng. 113 (1), 63–69.

Park, J.S., Park, H.C., Huh, T.L., Lee, Y.H., 1995. Production of poly-β-hydroxybutyrate by *Alcaligenes eutrophus* transformants harboring cloned phbCAB genes. Biotechnol. Lett. 17, 735–740.

Razmovski, R., Vučurović, V., 2012. Bioethanol production from sugar beet molasses and thick juice using *Saccharomyces cerevisiae* immobilized on maize stem ground tissue. Fuel 92 (1), 1–8.

Rusendi, D., Sheppard, D., 1995. Hydrolysis of potato processing waste for the production of poly-β-hydroxybutyrate. Bioresour. Technol. 54, 191–196.

Ryu, H.W., Hahn, S.K., Chang, Y.K., Chang, H.N., 1997. Production of poly(3-hydroxybutyrate) by high cell density fed-batch culture of *Alcaligenes eutrophus* with phosphate limitation. Biotechnol. Bioeng. 55, 28–32.

Sharma, S.K., Mudhoo, A., 2011. A Handbook of Applied Biopolymer Technology.

Tabandeh, F., Vasheghani-Farahani, E., 2003. Biosynthesis of poly-β-hydroxybutyrate as a biodegradable polymer. Iranian Polym. J. 12 (1), 37–42.

Wu, X., Staggenborg, S., Propheter, J.L., Rooney, W.L., Yu, J., Wang, D., 2010. Features of sweet sorghum juice and their performance in ethanol fermentation. Ind. Crops Prod. 31 (1), 164–170.

Yezza, A., Halasz, A., Levadoux, W., Hawari, J., 2007. Production of poly-β-hydroxybutyrate (PHB) by *Alcaligenes latus* from maple sap. Appl. Microbiol. Biot. 77, 269–274.

Zahari, M.A.K.M., Zakaria, M.R., Ariffin, H., Mokhtar, M.N., Salihon, J., Shirai, Y., Hassan, M.A., 2012a. Renewable sugars from oil palm frond juice as an alternative novel fermentation feedstock for value-added products. Bioresour. Technol. 110, 556–561.

Zahari, M.A.K.M., Ariffin, H., Mokhtar, M.N., Salihon, J., Shirai, Y., Hassan, M.A., 2012b. Factors affecting poly(3-hydroxybutyrate) production from oil palm frond juice by *Cupriavidus necator* (CCUG52238^{T}). J. Biomed. Biotechnol., 8. http://dx.doi.org/10.1155/2012/125865. Article ID 125865.