

## Research Article

# Factors Affecting Poly(3-hydroxybutyrate) Production from Oil Palm Frond Juice by *Cupriavidus necator* (CCUG52238<sup>T</sup>)

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Factors influencing poly(3-hydroxybutyrate) P(3HB) production by *Cupriavidus necator* CCUG52238<sup>T</sup> utilizing oil palm frond (OPF) juice were clarified in this study. Effects of initial medium pH, agitation speed, and ammonium sulfate (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> concentration on the production of P(3HB) were investigated in shake flasks experiments using OPF juice as the sole carbon source. The highest P(3HB) content was recorded at pH 7.0, agitation speed of 220 rpm, and (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> concentration at 0.5 g/L. By culturing the wild-type strain of *C. necator* under the aforementioned conditions, the cell dry weight (CDW) and P(3HB) content obtained were 9.31 ± 0.13 g/L and 45 ± 1.5 wt.%, respectively. This accounted for 40% increment of P(3HB) content compared to the nonoptimized condition. In the meanwhile, the effect of dissolved oxygen tension (DOT) on P(3HB) production was investigated in a 2-L bioreactor. Highest CDW (11.37 g/L) and P(3HB) content (44 wt.%) were achieved when DOT level was set at 30%. P(3HB) produced from OPF juice had a tensile strength of 40 MPa and elongation at break of 8% demonstrated that P(3HB) produced from renewable and cheap carbon source is comparable to those produced from commercial substrate.

## 1. Introduction

Poly(3-hydroxybutyrate), P(3HB) is a biodegradable thermoplastic polyester accumulated intracellularly by many microorganisms under unfavorable growth conditions [1]. The high production cost of P(3HB) can be decreased by strain development, improving fermentation and separation processes [2–4], and/or using a cheap carbon source [5]. In P(3HB) production, about 40% of the total production cost is contributed by the raw material, whereby the cost of carbon feedstock alone accounts for 70 to 80% of the total raw material cost [6, 7]. Therefore, the utilization of renewable and sustainable substrates for the production of P(3HB) has

become an important objective for the commercialization of bioplastics. A lot of research have been carried out to discuss and propose the utilization of renewable biomass to replace commercial sugars as carbon source in order to reduce the production cost of P(3HB) [8–12].

Recently, we reported on the use of oil palm frond (OPF) juice as the novel and renewable feedstock for the production of P(3HB) [13]. We demonstrated that OPF juice is a good substrate for the production of P(3HB) from wild-type *Cupriavidus necator* (CCUG52238<sup>T</sup>), with better yield of product formation in comparison to technical grade sugars. This can be explained by the presence of minerals and nutrients in the OPF juice which are essential for bacterial growth

during fermentation. Apart from contributing to higher product formation and microbial growth, the use of OPF juice is advantageous compared to the other lignocellulose-based sugars due to the ease in its processing wherein no harsh pretreatment steps and enzymatic treatment will be needed in order to obtain the sugars.

In our report, 32 wt.% of P(3HB) accumulation was successfully obtained under nonoptimized fermentation condition [13]. *C. necator* is well known as polyhydroxyalkanoate (PHA) producer and its ability to accumulate PHA more than 50 wt.% has been previously reported [8, 14]. In general, P(3HB) accumulation is favored by an excess carbon source and inadequate supply of macrocomponents such as nitrogen, phosphate, and dissolved oxygen or micro-components such as magnesium, sulphate, iron, potassium, manganese, copper, sodium, cobalt, tin, and calcium [7, 15]. Moreover, it was also reported that the accumulation of P(3HB) in microorganisms were influenced by several physical parameters including pH and agitation speed [16–19].

In order to make the production of P(3HB) feasible for industrial application, it is crucial to have high P(3HB) production yield. In this study, we investigated the effect of initial medium pH, agitation speed, and ammonium sulfate  $(\text{NH}_4)_2\text{SO}_4$  concentration on P(3HB) production from *C. necator* (CCUG52238<sup>T</sup>) utilizing OPF juice in shake flasks fermentation with the aim to clarify the effect of each fermentation parameter on the microbial growth and P(3HB) formation. The effect of dissolved oxygen tension (DOT) level on cell growth and P(3HB) production was investigated by conducting batch fermentation in 2-L-bioreactor. P(3HB) produced from this study was then characterized for its thermal and mechanical properties.

## 2. Materials and Methods

**2.1. Bacterial Strain.** In this study, *C. necator* (CCUG52238<sup>T</sup>) was obtained from the Culture Collection, University of Goteborg, Sweden and used for the production of P(3HB). The culture was maintained on slants of nutrient agar at 4°C. The inoculum preparation, media, and cultivation conditions for *C. necator* (CCUG52238<sup>T</sup>) are similar to those reported by Zahari et al. [13], unless otherwise stated.

**2.2. Biosynthesis of P(3HB) in Shake Flask.** P(3HB) biosynthesis was carried out through one-stage cultivation fermentation in shake flasks. OPF juice in this study was obtained by pressing fresh OPF following the method described earlier [13]. OPF juice which comprises fructose, glucose and sucrose was diluted from stock (55 g/L) to 16–17 g/L of total initial sugars and used as carbon sources throughout the study period. In order to study the effect of culture medium initial pH on biosynthesis of P(3HB), the initial pH value of each MSM and OPF juice was adjusted to pH 6.0–8.0 using 2 M NaOH prior to autoclaving. Another set of experiment was conducted to study the effect of agitation on P(3HB) production by testing several agitation speed at 180, 200, 220, 240, and 260 rpm. For the effect of ammonium sulfate

concentration, various concentrations of  $(\text{NH}_4)_2\text{SO}_4$  in the range of 0–2.0 g/L were tested. The cultures were incubated at 30°C under aerobic condition, and all experiments were conducted in duplicates.

**2.3. Biosynthesis of P(3HB) in 2-L-Bioreactor.** In order to study the effect of dissolved oxygen tension (DOT) on cell growth and P(3HB) production profile under the optimized condition obtained from the shake flask study, batch experiment was conducted in 2-L-bioreactor (1 L working volume) at different DOT levels of 20, 30, 40, and 50%. 100 mL of pregrown cells from growing stage were transferred into 900 mL MSM in 2L bioreactors (Sartorius, Germany) supplemented with OPF juice at 30% (v/v) dilution. The stock of OPF juice with 55 g/L of initial total sugars concentration was autoclaved separately prior to addition with the MSM medium. The MSM compositions were prepared as previously reported by Zahari et al. [13], except that 0.5 g/L of  $(\text{NH}_4)_2\text{SO}_4$  was used in this study. The temperature inside the bioreactor was set at 30°C, while DOT level was set at various concentrations of saturation throughout the fermentation using cascade mode and supplied with air at 1.0 vvm. The pH value during fermentation was controlled at  $\text{pH } 7.0 \pm 0.05$  by 2 M NaOH/ $\text{H}_2\text{SO}_4$ . Samples were withdrawn every 5 h for the period of 50 h for the determination of CDW, P(3HB) concentration, residual sugars, and ammoniacal nitrogen ( $\text{NH}_3\text{-N}$ ) content.

## 2.4. Analytical Procedures

**2.4.1. Biomass and Culture Medium Separation.** Residual sugars concentration, cell dry weight measurement, and P(3HB) analysis were done as previously described by Zahari et al. [13]. The samples from the bacterial fermentations were taken at the end of the cultivation period to measure the total dry weight and P(3HB) content. Each sample was centrifuged at  $11,000 \times g$  for 5 min at 4°C (Thermo Fisher Scientific, NC, USA) and the solids were washed with distilled water and centrifuged for two consecutive times.

**2.4.2. Determination of Cell Dry Weight and P(3HB) Content.** Dry weight measurements were carried out by drying the solids at 50°C and cooling in a desiccator to constant weight. The P(3HB) content and composition in the lyophilized cell were determined using the gas chromatography (Shimadzu GC-2014). Approximately, 20 mg of lyophilized cells were subjected to methanolysis in the presence of methanol and sulfuric acid [85% : 15% (v/v)]. The organic layer containing the reaction products was separated, dried over  $\text{Na}_2\text{SO}_4$ , and analyzed by GC according to the standard method [20] using an ID-BP1 capillary column, 30 m  $\times$  0.25 mm  $\times$  0.25  $\mu\text{m}$  film thickness (SGE).

**2.4.3. Determination of Residual Ammoniacal Nitrogen ( $\text{NH}_3\text{-N}$ ) Content.** The supernatant was then analyzed for residual sugars and ammoniacal nitrogen content. Residual ammoniacal nitrogen ( $\text{NH}_3\text{-N}$ ) content analysis was done using

TABLE 1: Effect of initial pH value on the biosynthesis of P(3HB)<sup>a</sup>.

Initial pH	CDW (g/L)	Total P(3HB) (g/L)	P(3HB) content (wt.%) <sup>b</sup>
6.0	6.42	1.28	20
6.5	7.12	1.99	28
7.0	8.57	2.91	34
7.5	6.89	1.72	25
8.0	4.02	0.40	10

<sup>a</sup>MSM containing 16 g/L of total sugars in OPF juice and supplied with 1.0 g/L of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, incubated at 30°C for 48 h with agitation at 200 rpm.

<sup>b</sup>Determination by GC from freeze dried samples.

\*Values obtained herewith are means of two independent experiments.

Nessler method according to standard procedures (HACH, USA) which was previously described by Zakaria [21]. Samples with appropriate dilution factor were filled to 25 mL in the sampling bottles. Three drops of mineral stabilizer were added into solution and the bottle was inverted for several times. Three drops of polyvinyl alcohol also were added into the solution and mixed well with inversion several times. Lastly, 1.0 mL of Nessler reagent was added to the mixtures and mix thoroughly by inversion. The standard solution was prepared by replacing the samples with deionised water as blank sample. The sample solution was determined at the wavelength ( $\lambda$ ) 425 nm using DR/4000 spectrophotometer by following the manufacturer, instructions (HACH, USA).

2.4.4. *Determination of Residual Sugars.* Residual sugars were determined by a high performance liquid chromatography (HPLC) (Agilent Series 1200, USA) using the Supelcosil LC-NH<sub>2</sub> column (Sigma Aldrich) (25 cm  $\times$  4.6 mm ID, 5  $\mu$ m particles) with a RI detector operated at 30°C. The mobile phase was acetonitrile: water (75%:25%) at a flow rate of 1.0 mL/min. The components were identified by comparing their retention times with those of authentic standards under analytical conditions and quantified by external standard method [22].

2.5. *Extraction of P(3HB).* Solvent extraction method as described by Zakaria et al. [23] was carried out in order to extract the P(3HB) produced from fermentation. P(3HB) film was then prepared by solvent casting using chloroform.

2.6. *Characterization of P(3HB).* Thermal properties of the polymer were determined by differential scanning calorimetry (DSC) (TA Instruments). For DSC analysis, 5–7 mg of homopolymer samples were weighed and heated from 20 to 200°C at heating rates 10°C/min and held for 1 min. The first scan was conducted to eliminate the polymer history. The samples were then fast cooled from 200°C to –30°C. The second scan was used in reheating the samples at the same heating rates and was used in evaluating the thermal properties of the biopolymer. The tensile strength, Young's modulus and elongation to break were determined by using Instron Universal Testing Machine (Model 4301) at 5 mm/min of crosshead speed [21]. Mechanical tensile data were calculated from the stress-strain curves on average of five specimens.

### 3. Results and Discussion

#### 3.1. Biosynthesis of P(3HB) in Shake Flask Experiment

3.1.1. *Effect of Initial Medium pH.* The effect of initial medium pH on biosynthesis of P(3HB) from OPF juice was studied by varying the pH between pH 6.0 and 8.0 due to the fact that *C. necator* can tolerate and produce PHA at the aforementioned pH range [24]. Suitable initial medium pH is crucial for the cell growth and P(3HB) accumulation by *C. necator* (CCUG52238<sup>T</sup>). As shown in Table 1, increasing the initial medium pH value at intervals of 0.5 units affected both the cell growth and P(3HB) production. Both the cell growth and P(3HB) content were increased when the initial medium pH was increased from pH 6.0 to pH 7.0, that is, from 6.42 g/L to 8.57 g/L for CDW and 20 wt.% to 34 wt.% for P(3HB) content, respectively. However, further increase of initial medium pH above pH 7.0 decreased both the CDW and P(3HB) content. From the results, it can be concluded that pH 7.0 was the optimum initial medium pH for the growth and biosynthesis of P(3HB) by *C. necator* (CCUG52238<sup>T</sup>) in which, 8.57 g/L of CDW and 34 wt.% of P(3HB) accumulation was recorded. The optimal pH for the cell growth and P(3HB) accumulation in this study was similar to those reported in the literature. It was reported that the optimum pH for growth and P(3HB) production by *A. eutrophus* was pH 6.9 and that a pH of 5.4 inhibited its growth [16].

On the other hand, lowest CDW and P(3HB) content, 4.02 g/L and 10 wt.%, respectively, were obtained at pH 8.0. Lowest cell growth and P(3HB) accumulation at this initial pH value were obtained might due to alkaline condition which could affect the P(3HB) production. These results corroborate with other be previous findings. For instance, N. J. Palleroni and A. V Palleroni. [25], recommended a pH range of between 6.0 to 7.5 for microbial growth and P(3HB) production. Although P(3HB) production can be controlled by precisely manipulating the medium pH, it has been reported that pH values other than 7.0 affected P(3HB) production [26]. These results suggested that P(3HB) production is sensitive to the pH of cultivation.

3.1.2. *Effect of Agitation Speed.* Table 2 displays the effect of agitation speed on biosynthesis of P(3HB) using OPF juice as substrate in shake flasks experiment. It is interesting to note that both cell growth and P(3HB) production

TABLE 2: Effect of agitation speed on the biosynthesis of P(3HB)<sup>a</sup>.

Agitation speed (rpm)	CDW (g/L)	Total P(3HB) (g/L)	P(3HB) content (wt.%) <sup>b</sup>
180	7.37	1.62	22
200	8.30	2.66	32
220	9.42	3.77	40
240	6.37	1.72	27
260	5.19	1.09	21

<sup>a</sup>MSM containing 16 g/L of total sugars in OPF juice and supplied with 1.0 g/L of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, incubated at 30°C for 48 h (initial pH medium adjusted at 7.0 ± 0.1).

<sup>b</sup>Determination by GC from freeze dried samples.

\*Values obtained herewith are means of two independent experiments.

TABLE 3: Effect of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> concentration on biosynthesis of P(3HB)<sup>a</sup>.

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> concentration (g/L)	CDW (g/L)	Total P(3HB) (g/L)	P(3HB) content (wt.%) <sup>b</sup>
0.0	5.25	2.31	44
0.5	8.31	3.49	42
1.0	8.65	2.94	34
1.5	9.05	2.62	29
2.0	10.15	2.33	23

<sup>a</sup>MSM containing 16 g/L of total sugars in OPF juice, incubated at 30°C for 48 h with agitation at 200 rpm (initial pH medium adjusted at 7.0 ± 0.1).

<sup>b</sup>Determination by GC from freeze dried samples.

\*Values obtained herewith are means of two independent experiments.

showed an increasing trend with the agitation speed up to 220 rpm. For the agitation speed of more than 220 rpm, the cell biomass and P(3HB) content was decreased. This result suggests that agitation speed plays an important role in the fermentation process. Agitation not only provides mixing and homogeneous cell and heat dispersion in the fermentation broth, but also better aeration for the cells by increasing the oxygen transfer rate throughout the fermentation medium. Generally, slower agitation speed may cause the possibilities of cells aggregation, making the culture medium more heterogeneous. This may cause the cell growth to be decreased and thus affecting the production of P(3HB). On the other hand, increasing agitation speed higher than its optimal level may reduce the P(3HB) formation, and hence, the CDW. This is due to the fact that PHA is only produced and stored as granules in the cell cytoplasm by microorganisms when they are under stress conditions, for example when there is limitation of nutrient or electron acceptor such as oxygen [27].

In our study, the best condition for the biosynthesis of P(3HB) is at moderate agitation speed which is at 220 rpm with the highest CDW and P(3HB) content reaching up to 9.42 g/L and 40 wt.%, respectively.

**3.1.3. Effect of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> Concentration.** Nitrogen is an essential element for cell growth and P(3HB) accumulation. (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> has been widely used as the inorganic nitrogen source for the biosynthesis of P(3HB) by *C. necator*. It is important to optimize nitrogen content in fermentation medium as P(3HB) accumulation in the microorganisms can be triggered when one of the nutrients (N, P, Mg, and O<sub>2</sub>) in the mineral salt is limited in the presence of excess carbon source [14, 15].

The effect of different (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> concentrations on biosynthesis of P(3HB) by *C. necator* (CCUG52338<sup>T</sup>) from OPF juice is summarized in Table 3. In overall, it was observed that CDW was increased when (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> concentration increased from 0 to 2.0 g/L. On the other hand, P(3HB) accumulation decreased with the increase of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> concentration. Highest P(3HB) accumulation at 44 wt.% was achieved when there was no addition of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, in the culture medium. However, unsatisfactory cell growth that is, 5.25 g/L of CDW was observed in the experiment.

Based on the results, it was also found that (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> concentration at 0.5 g/L was the optimal concentration for P(3HB) accumulation and CDW formation, giving 42 wt.% and 8.31 g/L, respectively. Further increasing nitrogen concentration slightly improved the cells growth; however the accumulation of P(3HB) was restricted. This may be due to excess nitrogen concentration that limited the P(3HB) accumulation. These results corroborate to the literature, which reported that P(3HB) formation predominantly occurs under-nitrogen and oxygen-limited conditions [14, 15, 28, 29]. It was discussed that excess nitrogen source may restrict acetyl-CoA from entering P(3HB) production pathways and otherwise channelling into TCA cycle for biomass production [15, 21, 28].

**3.1.4. Biosynthesis of P(3HB) under Optimized Condition in Shake Flask.** Biosynthesis of P(3HB) was then carried out in shake flask under the optimized conditions: initial pH medium, 7.0; agitation speed, 220 rpm and (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> concentration, 0.5 g/L. Under these conditions, the maximum cell dry weight obtained was 9.31 ± 0.13 g/L with 45 ± 1.5 wt.% of P(3HB) accumulation. The P(3HB) produced from this

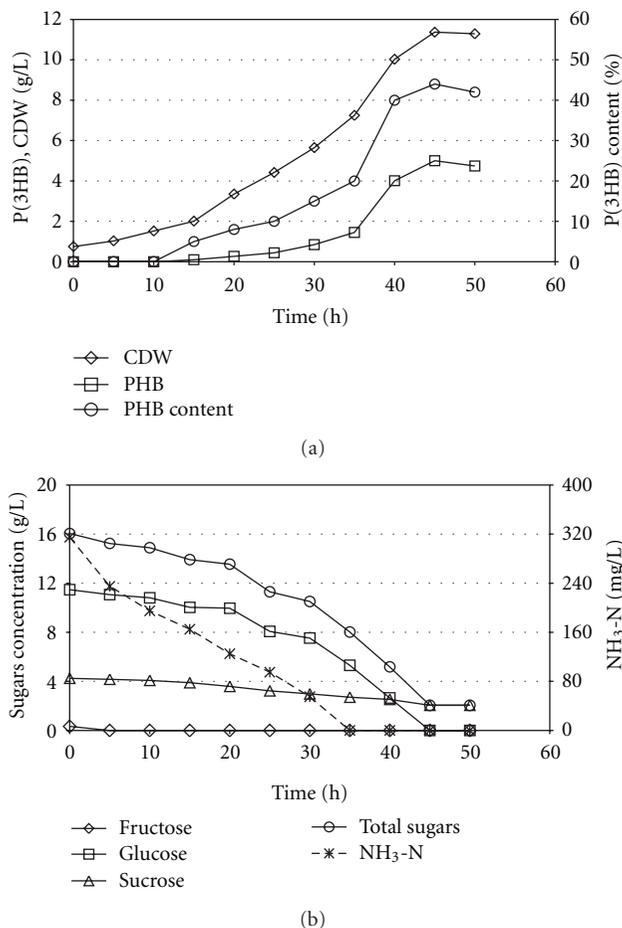


FIGURE 1: (a) Time profile of cell growth and P(3HB) production by *C. necator* (CCUG52238<sup>T</sup>) using OPF juice in 2-L bioreactor at 30% DOT level. (b) Time profile of sugars and ammonical nitrogen utilization by *C. necator* (CCUG52238<sup>T</sup>) using OPF juice in 2-L bioreactor at 30% DOT level.

study is 40% higher compared to the P(3HB) produced under nonoptimized condition as shown in our previous study [13]. The results presented herewith demonstrated that pH, agitation speed, and nitrogen concentration indeed plays an important role in the P(3HB) production by *C. necator* (CCUG52338<sup>T</sup>) utilizing OPF juice.

### 3.2. Biosynthesis of P(3HB) in 2-L Bioreactor

**3.2.1. Effect of Dissolved Oxygen Tension (DOT).** Biosynthesis of P(3HB) from OPF juice by *C. necator* (CCUG52338<sup>T</sup>) was carried out through batch cultivation process in 2-L bioreactor. The effect of DOT level in the bioreactor was studied for DO concentrations of 20 to 50% and the results are shown in Table 4. It was observed that CDW was increased when DOT level increased from 20 to 50%. On the other hand, P(3HB) accumulation was decreased with the increase in DOT level. Highest CDW (12.81 g/L) and P(3HB) content (46 wt.%) were achieved at 50 and 20% DOT level, respectively. Based on the

result, it was found that dissolved oxygen concentration in the fermentation medium improved the cell growth; however, P(3HB) accumulation was found to be increased towards oxygen limitation. This result suggested that appropriate level of oxygen is needed for cell development, and oxygen depletion was favorable for P(3HB) accumulation.

As shown in Table 4, P(3HB) accumulation was tripled at lower dissolved oxygen concentration (20%) compared to the higher ones (50%). This might be due to the fact that insufficient supply of oxygen to the bacteria may decrease oxidation of NADH and lead to P(3HB) biosynthesis [15, 21, 28]. A similar observation was obtained in our previous study on the effect of different (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> concentration on P(3HB) production using OPF juice in shake flask experiment. These results indicate that both nitrogen and oxygen limitation do not improve cell biomass development, but markedly improve the P(3HB) accumulation. Therefore, it can be suggested that besides nitrogen depletion, oxygen limitation is also important in getting the optimal level of P(3HB) accumulation.

**3.2.2. Cell Biomass and P(3HB) Production Profile.** In order to study cell biomass and P(3HB) production profile by *C. necator* (CCUG52238<sup>T</sup>), batch cultivation process was carried out using OPF juice in 2-L bioreactor with aeration supplied at 30% DOT level and the results were depicted in Figures 1(a) and 1(b). It was observed that the culture entered the exponential phase after a lag of 15 h, and nitrogen was completely consumed within 35 h. Highest CDW (11.37 g/L) and P(3HB) content (44 wt.%) were achieved at 45 hr cultivation period. The biomass yield ( $Y_{x/s}$ ) and P(3HB) yield ( $Y_{p/s}$ ) were 0.81 g biomass/g sugars consumed and 0.36 g P(3HB)/g sugars consumed, respectively. The maximum P(3HB) productivity was 0.11 g/L/h.

Almost similar P(3HB) content with some improvement in cell growth was obtained in this study compared to the shake flask experiment under optimal condition. Higher CDW (11.37 g/L) and biomass yield (0.81 g biomass/g sugars consumed) obtained in fermentor compared to shake flask were due to different conditions which prevail in the shake flasks and fermentor; some of these conditions include aeration, agitation, and temperature. In fermentor, aeration was supplied via air sparging, and agitation is provided by an impeller or by the motion imparted to the broth (liquid phase) by rising gas bubbles [30]. Temperature is maintained at a constant and uniform value by circulation of cooling water through coils in the vessel or in a jacket surrounding the vessel [31]. Compared to our previous studies in shake flasks using technical grade sugars [13], batch studies in 2-L bioreactor using renewable sugars from OPF juice showed superior results in *C. necator* CCUG52238<sup>T</sup> probably due to the additional components in the OPF juice that improve the fermentation performance. An almost similar observation was reported by Koutinas et al. [12] when WH and FE were used as renewable feedstock for P(3HB) production. It was reported that the consumption of various carbon sources (carbohydrates,

TABLE 4: Effect of DOT (%) level on biosynthesis of P(3HB)<sup>a</sup>.

DOT (%)	Maximum CDW (g/L)	Maximum total P(3HB) (g/L)	Maximum P(3HB) content (wt.%) <sup>b</sup>
20	9.55	3.93	46
30	11.37	4.78	44
40	12.52	3.38	25
50	12.81	2.37	15

<sup>a</sup>Experiments were conducted in 2-L bioreactor (1 L working volume) by batch mode using OPF juice with initial total sugars of 16 g/L as substrates.

<sup>b</sup>Determination by GC from freeze dried samples.

\*Values obtained herewith are means of duplicate sample.

TABLE 5: Comparison of thermal and mechanical properties of P(3HB) obtained in this study with literature.

Microorganisms	Carbon sources	$T_m$	Tensile strength (MPa)	Elongation to break (%)	References
<i>C. necator</i> CCUG52238 <sup>T</sup>	OPF juice	162.2	40	8	This study
<i>R. eutropha</i>	Fructose	177	43	5	Doi, 1990 [28]
<i>A. latus</i>	Maple sap	177	—	—	Yezza et al., 2007 [10]

amino acids, peptides) presented in the feedstock resulted in high growth yields (up to 1.07 g cells/g glucose) as related to glucose.

As shown in Figures 1(a) and 1(b), the microbial growth is mainly associated with ammoniacal nitrogen consumption. For the first 35 h, lower sugars consumption by *C. necator* was observed. The sugars consumption within the time range was only 8.03 g/L which is half of the total sugars in the culture broth. On the other hand, the NH<sub>3</sub>-N was found to be decreased drastically from initial and completely exhausted after 35 h of cultivation period. This result indicates that at initial, the microbial growth was mainly attributed by the consumption of nitrogen sources from (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> supplied earlier as one of the medium composition in bioreactor. In addition to that, other organic compounds such as amino acids, carbohydrates, and other minerals which were previously characterized in the OPF juice could be used as supplementary growth substrates by the bacterium [13]. After that, the cell growth was mainly contributed by the cell expansion due to P(3HB) accumulation inside the cells. It can be seen that the P(3HB) accumulation was doubled that is, 20 wt.% to 40 wt.%, from 35 h to 40 h of cultivation period. From sugars consumption and P(3HB) profiles, it can be observed that the detectable depletion of sugars in the medium from 35 h onwards can be associated with P(3HB) accumulation. These results are in agreement with the findings of other researchers that reported P(3HB) accumulation is favored by an excess of carbon source and limited supply of macrocomponents such as nitrogen and dissolved oxygen [7, 16, 29]. It is interesting to note that *C. necator* CCUG52238<sup>T</sup> completely utilized the glucose in the OPF juice. Regardless of fructose (due to too low concentration of fructose in the medium), it seems like the bacterium preferred to consume glucose compared to sucrose. Glucose consumption rate by *C. necator* CCUG52238<sup>T</sup> was much higher at 0.33 g/L/h, compared to that of sucrose that is, 0.049 g/L/h. This shows that *C. necator* CCUG52238<sup>T</sup> prefers monosaccharide than disaccharide as its carbon source.

**3.2.3. Characterization of Homopolymer P(3HB).** The mechanical and thermal properties of the homopolymer produced in 2-L bioreactor are shown in Table 5. The mechanical and thermal properties of P(3HB) obtained in this study showed an almost similar properties to those reported in the literature. For instance, the tensile strength and elongation to break for P(3HB) produced in this study were 40 MPa and 8%, respectively, and it was comparable to the P(3HB) produced from pure fructose [28]. The melting temperature,  $T_m$  of P(3HB) obtained from OPF juice ( $T_m = 162.2^\circ\text{C}$ ), was slightly lower compared to the melting point  $177^\circ\text{C}$  reported for P(3HB) produced from pure fructose [28] and other renewable sugars such as maple sap [10]. This could be influenced by other properties of the P(3HB) such as molecular weight. It has been reported that the molecular weight of P(3HB) produced is mainly influenced by the type of bacterial strain, substrate, growth rate, and production temperature [15, 29, 32].

## 4. Conclusions

This study demonstrated that higher cell growth and P(3HB) accumulation can be obtained by culturing *Cupriavidus necator* strain CCUG52238<sup>T</sup> at optimized condition using OPF juice as the sole renewable carbon source. Under the optimal conditions, the highest cell weight was  $9.31 \pm 0.13$  g/L with  $45 \pm 1.5$  wt.% of P(3HB) contained in the cells, accounts of 40% increment for P(3HB) content compared to the nonoptimized condition. Cultivation in a 2-L bioreactor with 30% DOT yielded CDW of 11.37 g/L and P(3HB) content of 44 wt.%. In the meanwhile, thermal and mechanical characterization of the P(3HB) obtained from OPF juice showed almost similar properties to those reported in the literature. It is worth to mention that this study may contribute to the process development for P(3HB) production from renewable OPF juice in pilot and industrial scale. Furthermore, since OPF is an abundant solid waste at oil palm plantation and is currently underutilized, it has a great potential to be used as sustainable, renewable, and cheap fermentation feedstock for the production of P(3HB).

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## References

- [1] S. Y. Lee, "Review bacterial polyhydroxyalkanoates," *Biotechnology and Bioengineering*, vol. 49, pp. 1–14, 1996.
- [2] Beom Soo Kim, Seung Chul Lee, Sang Yup Lee, Ho Nam Chang, Yong Keun Chang, and Seong Ihl Woo, "Production of poly(3-hydroxybutyric acid) by fed-batch culture of *Alcaligenes eutrophus* with glucose concentration control," *Biotechnology and Bioengineering*, vol. 43, no. 9, pp. 892–898, 1994.
- [3] B. S. Kim and H. N. Chang, "Production of poly(3-hydroxybutyrate) from starch by *Azotobacter chroococcum*," *Biotechnology Letters*, vol. 20, no. 2, pp. 109–112, 1998.
- [4] Sei Kwang Hahn, Yong Keun Chang, Beom Soo Kim, and Ho Nam Chang, "Communication to the editor optimization of microbial poly(3-hydroxybutyrate) recovery using dispersions of sodium hypochlorite solution and chloroform," *Biotechnology and Bioengineering*, vol. 44, no. 2, pp. 256–261, 1994.
- [5] W. J. Page, "Production of poly- $\beta$ -hydroxybutyrate by *Azotobacter vinelandii* strain UWD during growth on molasses and other complex carbon sources," *Applied Microbiology and Biotechnology*, vol. 31, no. 4, pp. 329–333, 1989.
- [6] J. M. B. T. Cavalheiro, M. C. M. D. de Almeida, C. Grandfils, and M. M. R. da Fonseca, "Poly(3-hydroxybutyrate) production by *Cupriavidus necator* using waste glycerol," *Process Biochemistry*, vol. 44, no. 5, pp. 509–515, 2009.
- [7] M. Koller, A. Atlıć, M. Dias, A. Reiterer, and G. Brauneegg, "Microbial production from waste raw materials," *Microbiology Monograph*, vol. 14, pp. 85–119, 2010.
- [8] D. Rusendi and J. D. Sheppard, "Hydrolysis of potato processing waste for the production of poly- $\beta$ -hydroxybutyrate," *Bioresource Technology*, vol. 54, no. 2, pp. 191–196, 1995.
- [9] B. S. Kim, "Production of poly(3-hydroxybutyrate) from inexpensive substrates," *Enzyme and Microbial Technology*, vol. 27, no. 10, pp. 774–777, 2000.
- [10] A. Yezza, A. Halasz, W. Levadoux, and J. Hawari, "Production of poly- $\beta$ -hydroxybutyrate (PHB) by *Alcaligenes latus* from maple sap," *Applied Microbiology and Biotechnology*, vol. 77, no. 2, pp. 269–274, 2007.
- [11] R. Haas, B. Jin, and F. T. Zepf, "Production of poly(3-hydroxybutyrate) from waste potato starch," *Bioscience, Biotechnology and Biochemistry*, vol. 72, no. 1, pp. 253–256, 2008.
- [12] A. A. Koutinas, Y. Xu, R. Wang, and C. Webb, "Polyhydroxybutyrate production from a novel feedstock derived from a wheat-based biorefinery," *Enzyme and Microbial Technology*, vol. 40, no. 5, pp. 1035–1044, 2007.
- [13] M. A. K. M. Zahari, M. R. Zakaria, H. Ariffin et al., "Renewable sugars from oil palm frond juice as an alternative novel fermentation feedstock for value-added products," *Bioresource Technology*, vol. 110, pp. 566–571, 2012.
- [14] S. Khanna and A. K. Srivastava, "Statistical media optimization studies for growth and PHB production by *Ralstonia eutropha*," *Process Biochemistry*, vol. 40, no. 6, pp. 2173–2182, 2005.
- [15] A. J. Anderson and E. A. Dawes, "Occurrence, metabolism, metabolic role, and industrial uses of bacterial polyhydroxyalkanoates," *Microbiological Reviews*, vol. 54, no. 4, pp. 450–472, 1990.
- [16] M. Beaulieu, Y. Beaulieu, J. Melinard, S. Pandian, and J. Goulet, "Influence of ammonium salts and cane molasses on growth of *Alcaligenes eutrophus* and production of polyhydroxybutyrate," *Applied and Environmental Microbiology*, vol. 61, no. 1, pp. 165–169, 1995.
- [17] F. Tabandeh and E. Vasheghani-Farahani, "Biosynthesis of poly- $\beta$ -hydroxybutyrate as a biodegradable polymer," *Iranian Polymer Journal*, vol. 12, no. 1, pp. 37–42, 2003.
- [18] M. S. Baei, G. D. Najafpour, H. Younesi, F. Tabandeh, and H. Eisazadeh, "Poly(3-hydroxybutyrate) synthesis by *Cupriavidus necator* DSMZ 545 utilizing various carbon sources," *World Applied Science Journal*, vol. 7, no. 2, pp. 157–161, 2009.
- [19] S. Philip, S. Sengupta, T. Keshavarz, and I. Roy, "Effect of impeller speed and pH on the production of poly(3-hydroxybutyrate) using *Bacillus cereus* SPV," *Biomacromolecules*, vol. 10, no. 4, pp. 691–699, 2009.
- [20] G. Brauneegg, B. Sonnleitner, and R. M. Lafferty, "A rapid gas chromatographic method for the determination of poly  $\beta$  hydroxybutyric acid in microbial biomass," *European Journal of Applied Microbiology and Biotechnology*, vol. 6, no. 1, pp. 29–37, 1978.
- [21] M. R. Zakaria, *Biosynthesis of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) copolymer from organic acids using Comamonas sp. EB172 [Ph.D. thesis]*, Faculty of Biotechnology & Biomolecular Sciences, Universiti Putra Malaysia, 2011.
- [22] E. Kafkas, M. Koşar, N. Türemiş, and K. H. C. Başer, "Analysis of sugars, organic acids and vitamin C contents of blackberry genotypes from Turkey," *Food Chemistry*, vol. 97, no. 4, pp. 732–736, 2006.
- [23] M. R. Zakaria, H. Ariffin, N. A. Mohd Johar et al., "Biosynthesis and characterization of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) copolymer from wild-type *Comamonas* sp. EB172," *Polymer Degradation and Stability*, vol. 95, no. 8, pp. 1382–1386, 2010.
- [24] L. P. A. Paladino, *Screening, optimization and extraction of polyhydroxyalkanoates and peptidoglycan from Bacillus megaterium [M.S. dissertation]*, Michigan Technological University, 2009.
- [25] N. J. Palleroni and A. V. Palleroni, "*Alcaligenes latus*, a new species of hydrogen-utilizing bacteria," *International Journal of Systematic Bacteriology*, vol. 28, no. 3, pp. 416–424, 1978.
- [26] Y. H. Wei, W. C. Chen, C. K. Huang et al., "Screening and evaluation of polyhydroxybutyrate-producing strains from indigenous isolate *Cupriavidus taiwanensis* strains," *International Journal of Molecular Sciences*, vol. 12, no. 1, pp. 252–265, 2011.
- [27] L. S. Serafim, P. C. Lemos, M. G. E. Albuquerque, and M. A. M. Reis, "Strategies for PHA production by mixed cultures and renewable waste materials," *Applied Microbiology and Biotechnology*, vol. 81, no. 4, pp. 615–628, 2008.
- [28] Y. Doi, "Structure and properties of poly(3-hydroxybutyrate)," in *Microbial Polyesters*, VCH, New York, NY, USA, 1990.
- [29] L. L. Madison and G. W. Huisman, "Metabolic engineering of poly(3-hydroxyalkanoates): from DNA to plastic,"

*Microbiology and Molecular Biology Reviews*, vol. 63, no. 1, pp. 21–53, 1999.

- [30] H. S. Fogler, *Elements of Chemical Reaction Engineering*, Prentice-Hall, Upper Saddle River, NJ, USA, 2nd edition, 1992.
- [31] H. W. Blanch and D. S. Clark, *Biochemical Engineering*, Marcel Dekker, New York, NY, USA, 1997.
- [32] M. A. Hassan, Y. Shirai, H. Umeki et al., “Acetic acid separation from anaerobically treated palm oil mill effluent by ion exchange resins for the production of polyhydroxyalkanoate by *Alcaligenes eutrophus*,” *Bioscience, Biotechnology and Biochemistry*, vol. 61, no. 9, pp. 1465–1468, 1997.



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