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Production of polyhydroxybutyrate from oil palm empty fruit bunch (OPEFB) hydrolysates by *Bacillus cereus suaeda* B-001

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ABSTRACT

Polyhydroxybutyrate (PHB) is a biodegradable polymer accumulated in intracellular granules by numerous bacteria. Its physical and chemical characteristics are like those of petrochemical plastics. PHB is produced mainly by gram-negative bacteria such as *Ralstonia eutropha*, which have lipopolysaccharides that co-purify with the PHB and cause immunogenic reactions, limiting their use for biomedical applications. PHB produced from gram-positive bacteria such as *Bacillus spp.* do not have lipopolysaccharides, which makes it suitable for biomedical application. The aim of this work was to evaluate the ability of *Bacillus cereus suaeda* B-001 to accumulate PHB using oil palm empty fruit bunch (OPEFB) hydrolysate as the sole carbon source, comparing it to commercial glucose as the control. OPEFB was chemically pre-treated using an acid-hydrolysed process by sulphuric acid and neutralized by a NaOH solution to obtain reducing sugars. PHB biopolyester accumulated to 43.1% of cell dry weight with glucose at 15 g/L as the sole carbon source, and PHB accumulated to 55.4% of cell dry weight using OPEFB hydrolysates at 20 g/L. The conversion of OPEFB acid hydrolysates to PHB using the gram-positive bacteria *Bacillus cereus suaeda* B-001 has not been reported.

1. Introduction

Plastic waste hasa great pollutant effect on the environment because it is difficult to degrade naturally. Many attempts have been devoted to developing plastic materials that are easily destroyed by nature, i.e., biodegradable plastics (Albuquerque and Malafaia, 2017; Brodin et al., 2017; Emadian et al., 2017; Horodytska et al., 2018; Zheng et al., 2005).

Polyhydroxyalkanoates (PHAs) are polyesters that are synthesized by bacteria in cells as energy reserves and cork-shaped food reserves in the cell cytoplasm. Generally, bacteria produce polyester with excessive carbon that limits the concentration of nutrients for growth, such as nitrogen, phosphorus, sulphur and oxygen (Anderson and Dawes, 1990; Tanamool et al., 2013). The properties of PHAs are insolubility in water, non-toxicity, biodegradability, biocompatibility and thermoplasticity, making them ideal to replace conventional plastics (Sudesh et al., 2000). The properties of these polymers depend on their constituent monomers, so they can be sources of various biomaterials. Polyhydroxybutyrate (PHB) is the most common and most widely produced homopolymer of bacteria. Current applications of PHB include its use as a polymer or composite in the packaging industry, medicine, pharmacy, agriculture, the food industry and the paint industry and as a raw material for pure chemicals (Anderson and Dawes, 1990; Tanamool et al., 2013).

Generally, PHAs are produced on an industrial scale using gram-negative bacteria such as *Cupriavidus necator* (Vandamme and Coenye, 2004). However, PHAs that are isolated from gram-negative organisms contain an outer layer of membrane lipopolysaccharide (LPS) endotoxins that produce pyrogens, contaminating the PHAs. The presence of LPS in PHAs causes a strong immunogenic reaction and is undesirable in biomedical use (Chen and Wu, 2005).

Gram-positive bacteria, such as *Bacillus spp.*, may also produce PHAs (Chen and Wu, 2005; Mizuno et al., 2010). Gram-positive bacteria do not contain LPS and are potential PHA sources for use in biomedical applications (Valappil et al., 2007a; Lopez et al., 2012). Production of PHB from *Bacillus sp. JMa5* using molasses as a medium can achieve a PHB content of 25–35% of cell dry weight (CDW). It is also reported that a higher carbon-to-nitrogen ratio will increase PHB production

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(Wu et al., 2001).

The initial sugar concentration affects the result of high CDW and PHB content. Zhang et al. (2013), worked with *Bacillus megaterium R11* microbes and a source of carbon glucose concentrations of 100 g/L. The research received 32.16% CDW content and 58.6% PHB. Lamani (2008) study with *Bacillus cereus CFR06* microbes at glucose concentration 2% obtained PHA yield 50% CDW. This yield is higher than that of glucose concentration 1%, proving that excess carbon will produce maximum PHB.

Approximately 45% of total PHB production expense is the cost of carbon sources, such as glucose or sucrose (Du et al., 2004). Some research has attempted to minimize production costs, for example by the use of alternative carbon sources for the growth of low-cost bacteria. Efforts have been made to reduce costs by using renewable and inexpensive carbon sources (Khanna and Srivastava, 2005). Silva et al. (2004) cultivated *Burkholderia sacchari* and *Burkholderia cepacia* using xylose in hydrolysate bagasse sugar cane. Both strains' cell dry weight (CDW) levels reached 4.4 g/L, and PHA content reached 62% and 53% CDW, respectively. In nutritional conditions limited to high cell densities, PHA content from *Ralstonia eutropha* can reach 65% using bagasse hydrolysates as a carbon source (Yu and Stahl, 2008). PHA production by *B. cepacia* ATCC 17759, using a detoxified sugar maple hemicellulosic hydrolysate, can reach 8.72 g/L (Pan et al., 2012).

In 2010, Indonesia produced around 23.2 million tonnes of crude palm oil (CPO) (Amraini et al., 2017). The process of extracting CPO from oil-palm fruit produces the lignocellulosic bundle of oil palm empty fruit bunches (OPEFB) as solid waste. In 2010, around 25.52 million tonnes of OPEFB were produced in Indonesia by the palm-oil industry (Amraini et al., 2017). OPEFB's content comprises cellulose (50.4%), hemicellulose (21.9%), lignin (10%) and ash (17.7%) (Umi Kalsom et al., 1997). Cellulose and hemicellulose can be hydrolysed into sugars such as glucose and xylose, potentially as a carbon source for PHB (Chen et al., 2007; Rahman et al., 2007).

The aim of this research was to convert OPEFB acid hydrolysates into a biodegradable plastic, polyhydroxybutyrate (PHB), a common type of PHA. The conversion of OPEFB acid hydrolysates to PHB using the gram-positive bacteria *Bacillus cereus suaeda* B-001 has not been reported.

2. Materials and methods

2.1. Media preparation

Luria-Bertani (LB) broth was obtained from Merch Indonesia. Trace elements in the solution included: 10 g/L $FeSO_4.7H_2O$; 2.25 g/L $ZnSO_4.7H_2O$; 2.25 g/L $CuSO_4.5H_2O$; 0.5 g/L $MnSO_4.5H_2O$; 2 g/L $CaCl.2H_2O$; 0.3 g/L H_3BO_4; and 0.1 g/L $(NH_4)\ 6Mo_7O_{24}.H_2O$. The mineral salts medium (MSM) contained 0.5 g/L $MgSO_4.7H_2O$; 1.5 g/L $citrate.H_2O$; 11.8 g/L KH_2PO_4; and 10 mL/L of trace-elements solution with a pH of 6.8.

Glucose and OPEFB acid hydrolysates were used as carbon sources and $(NH4)_2SO_4$ was used as a nitrogen source at specified concentrations. The carbon sources, MSM and nitrogen sources were sterilized separately at 121 °C for 15 min and mixed prior to inoculation.

2.2. Bacterial strain

A PHB-producing *Bacillus cereus* microorganism was obtained from one of the state research institutions in West Java, Indonesia, School of Biological Sciences and Technology, *Institut Teknologi Bandung* (SITH ITB). Strains were identified by 16S rRNA sequencing. Isolation of genomic DNA, PCR amplification of 16S rRNA and sequencing were carried out at Biotechnology Laboratory, Agency for The Assessment and Application of Technology (BPPT), Ministry of Research, Technology and Higher Education of The Republic of Indonesia.

Isolation of genomic DNA was carried out using a Geneaid Gel/PCR DNA fragments extraction kit. Full-length 16S rRNA sequences were amplified by PCR using forward primers 8F (5′ AGA GTT TGA TCC TGG CTC AG-3′) and reverse primer 1492R (5′-TAC GGY TAC CTT GTTACG ACT T-3′). The PCR products were then separated by agarose gel electrophoresis and purified using InstaGene Matrix (Bio-Rad).

Bacteria were incubated in nutrient agar media at 30 °C for 24 h. The pre-culture was prepared by cultivating a single colony of *Bacillus cereus* in LB liquid medium at 30 °C for 24 h. Cells were then collected by centrifuge at 5000 rpm for 10 min, re-suspended in sterile 9 g/L NaCl solution and stored at 4 °C until use.

2.3. Delignification and acid hydrolysis OPEFB

OPEFB were obtained from a palm-oil refinery in Sumatra. The raw materials were washed, air dried and milled to a size of less than 1 mm. The dried materials were stored at room temperature until further use. The OPEFB fibres were treated by a delignification process with 5% NH_4OH at 121 °C and15 lb pressure for 60 min in an autoclave, washed with deionized water to pH 7 and dried at 105 °C.

Acid hydrolysis of OPEFB biomass was carried out in a laboratory autoclave at 121 °C and15 lb pressure for 60 min. The medium consisted of 1–5 g H_2SO/100 g liquor using a charge of 1 g OPEFB fibre/20 g liquor on a dry basis. The samples were diluted with deionized water, and insoluble solids were separated from the aqueous solution by filtration. The filtrate was analysed for xylose, glucose, acetic acid and furfural. The hydrolysate solution was neutralized by adding 1N NaOH and stored in a refrigerator at 4 °C for later use.

2.4. PHB production

PHB production from glucose was conducted in 300 mL conical flasks containing 150 mL of MSM supplemented with a specified amount of sugar and nitrogen source at 30 ^{O}C on a rotary shaker at 150 rpm. Samples were taken every 12 h for 96 h. OPEFB hydrolysates were then supplemented with MSM stock solution and $(NH4)_2SO_4$ as the nitrogen source at a specified concentration and then used directly for PHB production without detoxification. Fermentation was done at 30 ^{O}C on a rotary shaker at 150 rpm. These process conditions followed the experiment by Zhang et al. (2013).

2.5. Optimization of PHB production

Central Composite Design (CCD) of Response Surface Methodology (Design Expert 9.0, Stat Ease, Inc., Minneapolis, USA) was employed to determine the effect of independent variables on response and factor interactions with different combinations of variables. Two different independent variables, amount $(NH4)_2SO_4$ (A) and Temperature (B) were evaluated for PHB production from *Bacillus cereus suaeda* B-001. The maximum and minimum range of independent variables as follows: $(NH4)_2SO_4$ (0.02–0.5 gr), Temperature (30–70 °C). Two process variables were chosen at five levels by the software tool (Table 1). A total of 13 experiments were designed and response (PHB yield) was determined. The results were analyzed using design expert software. The regression equation resulted in an empirical model that relates the measured response to the independent variables of the experiments.

Table 1
Coding levels involved in the optimizing PHB production.

Independent variables	Symbol	Coded levels				
		-α	−1	0	+1	+α
$(NH4)_2SO_4$ (gr)	A	0.02	0.1	0.3	0.5	0.58
Temperature (^{O} C)	B	30	40	50	60	70

2.6. Analytical methods

To determine cell dry weight (CDW), cells were centrifuged at 5000 rpm for 10 min, washed twice with deionized water and dried at 70 °C to constant weight. The reducing sugars in the fermentation solution were measured based on a colorimetric reaction between the sugars and dinitro salicylic acid and calibrated with a glucose standard (Yu and Stahl, 2008).

PHB content was determined by gas chromatography (GC), and cells were subjected to methanolysis with sulfuric acid and methanol. Dry cells (30 mg) were added into a screw-cap test tube with 2 mL chloroform and 2 mL acidic methanol (2.8 M H2SO4 in methanol). A benzoic acid solution of 1 g/L was dissolved in methanol and used as an internal standard. A methanolysis process was performed at 100 °C for 2 h in a water bath with shaking every 30 min. After samples were cooled to room temperature, 1 mL of deionised water was added into the test tubes; the mixture was shaken vigorously for 1 min and remained for about 30 min for phase separation. After phase separation, the organic phase was collected and dried over anhydrous sodium sulphate. One mL of the organic phase was then filtered into glass vials for GC (Zhang et al., 2013).

The analysis was performed using a gas chromatograph (Perkin-Elmer, USA) equipped with an Agilent J & W DB-FFAP capillary column (30 m × 0.25 mm x 0.25 µm). The sample (1 µL), in chloroform, was injected with hydrogen (1 mL/min) as the carrier gas. The injector temperature was 220 °C and the column temperature was increased from 40 to 320 °C at 20 °C/min and held at the final temperature for 6 min.

2.7. Extraction of PHB

Cells were harvested by centrifugation at 5000 rpm for 10 min and dried at 70 °C to constant weight. PHB was extracted by treating 1 g of dried cells with a dispersion containing 50 mL of chloroform and 50 mL of a diluted (30%) sodium hypochlorite solution in water. The mixture was shaken at 150 rpm for 2 h and sonicated for 20 min, and the cells were treated at 38 °C for 1 h. The mixture obtained was then centrifuged at 5000 rpm for 10 min, which resulted in three separate phases. PHB was recovered from the bottom phase, that of enriched chloroform. The PHB-enriched chloroform was then condensed in a rotary evaporator and mixed with chilled methanol (methanol:chloroform = 9:1, v/v) to precipitate PHB polymers. The polymer granules were then collected by filtration using filter paper and dried at room temperature to constant weight (Zhang et al., 2013).

2.8. FTIR analysis

Produced PHB by extraction was confirmed using FTIR spectroscopy for the1 mg sample. The relative intensity of transmitting light energy was measured against the wavelength of absorption, 4000–650 cm^{-1}, using a Cary 630 FTIR spectrometer (Agilent, USA).

3. Results and discussion

3.1. Species identification

Genomic DNA isolated from *Bacillus cereus*, obtained from one of the state research institutions in West Java, Indonesia, was used for amplification of the 16S rRNA gene by PCR. A BLAST (NCBI) analysis of the partial nucleotide sequence (GenBank accession no. KT981877.1) resulted in a highest identical homology of 99%, with a maximum score of 2582 and a 100% query to *Bacillus cereus suaeda* B-001.

The phylogeny tree as shown in Fig. 1 was built using MEGA Version 7.0.26. This phylogeny tree shows that the *Bacillus cereus suaeda* B-001 has very close relationship with *Bacillus sp.* NG06, *Bacillus Thuringiensis strain WB27, Bacillus pseudomycoides* strain XZPGS4, *Bacillus*

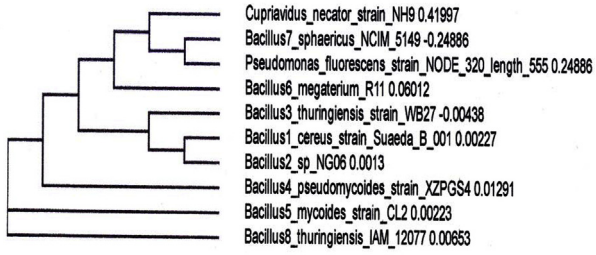


Fig. 1. The phylogenic tree.

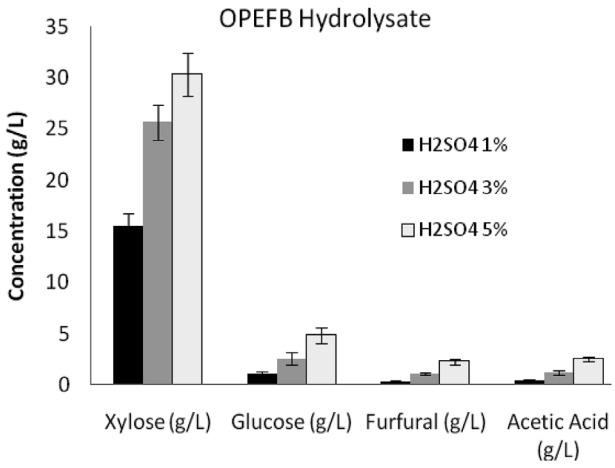


Fig. 2. Diluted acid hydrolysis of OPEFB.

mycoides strain CL2.

Although Valappil et al. (2007b) reported that *Bacillus cereu SPV* was able to produce PHB, no research to date has been found reporting the accumulation of PHB from *Bacillus cereus suaeda* B-001.

3.2. OPEFB hydrolysates

The compositions of hydrolysates derived from OPEFB under various sulphuric -acid solutions are given in Fig. 2. Analysis of acid hydrolysate showed that the highest release of xylose from hemicellulose was 30.35 g/L, when the acid concentration was 5%. Glucose was also released during acid hydrolysis of sugar, but in a low concentration. The highest amount of glucose released in the hydrolysate was 4.85 g/L, when the acid concentration was 5%. Rahman et al. (2006) reported that the release of xylose and glucose in the hydrolysate was dependent on experimental operating conditions. The maximum concentration of xylose, 30.81 g/L, was achieved when the reaction was carried out at 115 °C for 60 min with the acid concentration maintained at 4%. On the other hand, release of glucose was highest (7.61 g/L) when the operating temperature and time period were 130 °C and 90 min, respectively, with acid concentration maintained at 6%.

The furfural was generated as a decomposition product of hydrolysate (Panjaitan et al., 2017). When the sulfuric acid concentration was increased from 1% to 5%, the furfural concentration was increased in the solution. The highest concentration of furfural noticed was 2.27 g/L, when the acid concentration was 5%. During the hydrolysis process, acetic acid was generated from acetyl groups of hemicellulose. The maximum and minimum concentrations of acetic acid in the resulting hydrolysate were found to be 2.54 and 0.39 g/L, when sulfuric acid concentrations were 5% and 1%, respectively. The result is consistent with those reported by Rahman et al. (2006), who found that increasing the acid concentration will increase production of xylose and glucose. Some researchers showed that hydrolysis of POEFB mostly resulted mainly in the xylose (as the main monomer of hemicellulose in POEFB)

Table 2
Experimental and predicted yield of PHB using set of experiments design based on CCD of RSM.

RUN	$(NH4)_2SO_4$ (gr)	Temperature (°C)	PHB yield Experimental (g/L)	PHB yield Predicted (g/L)
1	0.58	50	1.71	1.53
2	0.3	50	1.05	0.87
3	0.3	70	0.10	0.05
4	0.1	40	1.83	1.92
5	0.5	60	0.12	0.26
6	0.3	50	1.11	0.87
7	0.3	50	1.13	0.87
8	0.1	60	0.09	0.16
9	0.3	50	0.50	0.87
10	0.02	50	0.75	0.67
11	0.5	40	2.89	3.06
12	0.3	30	3.35	4.60
13	0.3	50	0.58	0.87

Table 3
Result of ANOVA for PHB production from *Bacillus cereus suaeda* B-001 using CCD of RSM.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	12.34	5	2.47	24.81	0.0003	significant
A	0.75	1	0.75	7.54	0.0287	significant
B	10.37	1	10.37	104.19	< 0.0001	significant
AB	0.27	1	0.27	2.73	0.1424	Not significant
A^2	0.095	1	0.095	0.95	0.3618	Not significant
B^2	0.92	1	0.92	9.27	0.0187	significant
Residual	0.70	7	0.099			
Lack of Fit	0.13	3	0.043	0.30	0.8228	Not significant
Pure Error	0.57	4	0.14			
Cor Total	13.04	12				

and glucose (as the main monomer of cellulose in POEFB) (Saka et al., 2008; Harish et al., 2015).

Zhang et al. (2013) reported the use of OPEFB hydrolysates in the enzymatic-hydrolysis process as carbon source in PHB production. The enzymatic-hydrolysis process requires a large expenditure. OPEFB acid hydrolysates were used as a carbon source to reduce production costs has never been reported.

3.3. Optimization of PHB production

Response surface methodology (RSM) based optimization of two variables: amounts of $(NH4)_2SO_4$ (A) and Temperature (B) was done for PHB production, while other parameters were maintained at constant level. RSM generated 13 experimental runs and the corresponding responses (PHB yield) are presented in Table 2. Experimental and predicted PHB yield was analyzed from the experimental runs. The run no 12 exhibited a maximum response of PHB yield 3.35 gr/L. The lower response was found in run no 8 with PHB yield 0.09 gr/L. From these results, *Bacillus cereus suaeda* B-001 can synthesize maximum PHB at 30 °C with concentration $(NH4)_2SO_4$ 0,3 gr/100 ml. Multiple regression analysis was used to analyze the data, and thus polynomial equation was derived from regression analysis. The regression equation for the quadratic model suggested in terms of coded factors showed the variation of PHB yield (Y) as a function of the two variables, $(NH4)_2SO_4$ (A) and Temperature (B).

$$Y = 13{,}51581 + 6{,}29712\,A - 0{,}43889\,B - 0{,}13031\,AB + 2{,}91641\,A^2 + 3{,}64156E\text{-}003\,B^2$$

ANOVA was performed to the experimental design. The calculated R^2 value of 0.85 for PHB yield, shows improved correlation between the experimental and predicted response. The R^2 value is always between 0 and 1. The closer the R^2 is to 1, the stronger the model and the better it predicts the response (Nisha et al., 2010). Values of "Prob > F" less than 0.05 indicated model terms are significant. The model F value of 24.81 and P > F value of 0.0003 indicated that the model is significant. Results of ANOVA of quadratic model are represented in Table 3. In this case A, B, and B^2 are significant and AB, A^2 are not significant for PHB yield.

3.4. FTIR result

The FTIR spectrum of PHB was recorded in the range of 4000–600 cm^{-1}. Obtained spectral peak values and the presence of broad bands responding to the groups C—O and C═O indicate structure similar to PHB. Specifically, two intense absorption bands at 1719.810–1720.771 cm^{-1} and 1277.685–1261.305 cm^{-1} correspond

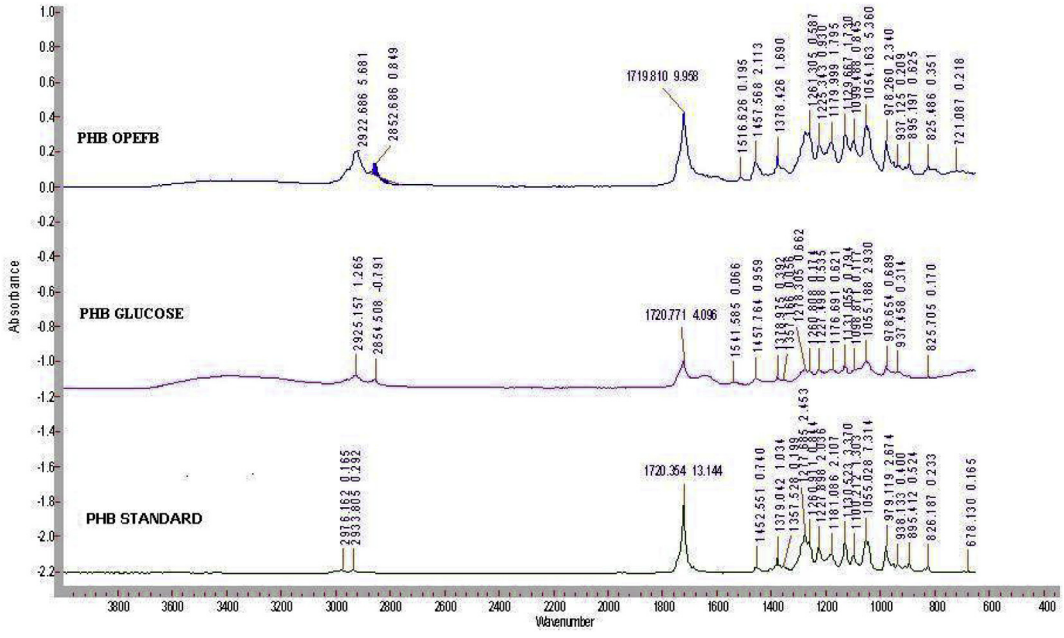
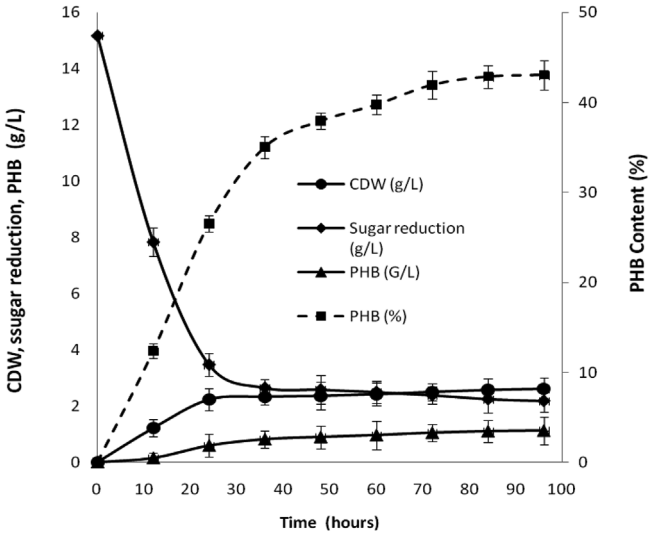
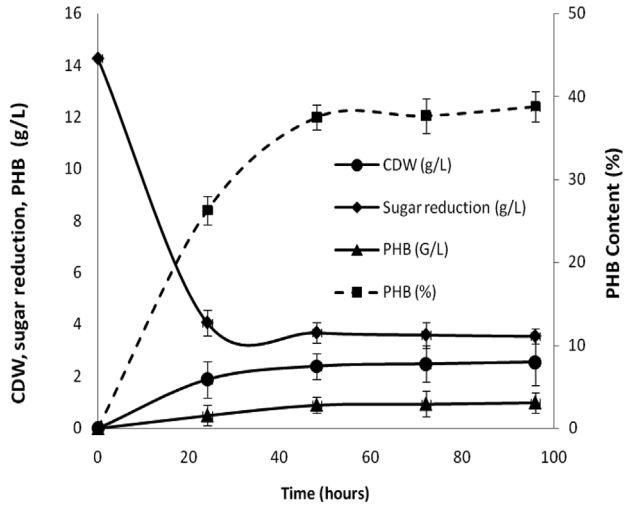


Fig. 3. FTIR Spectra obtained for PHB standard, PHB glucose, PHB OPEFB.



(a)



(b)

Fig. 4. PHB production on glucose (a) and OPEFB hydrolysate (b).

to C═O and C−O stretching groups, respectively. The C−H bond (in which the hydrogen is attached to a carbon that is singly-bonded to everything else) absorbs the range from 3000 to 2850 cm^{-1}. The peak values obtained (Fig. 3) in this study coincide with previous results (Radhika and Murugesan, 2012).

3.5. PHB production on glucose and OPEFB hydrolysates

It has been found that strain *Bacillus spp.* organisms can produce polyhydroxybutyrate (PHB) (Labuzek and Radeca, 2001). This study used the strain *Bacillus cereus suaeda* B-001, with both glucose and OPEFB hydrolysates as the carbon source. Fig. 4a shows bacterial growth in the carbon source glucose at 15 g/L. After a 96-h incubation, bacterial growth yielded CDW of 2.6 g/L and PHB content of 43.1% CDW.

The results achieved were better than those of a previous study by Valappil et al. (2007b), in which *Bacillus cereus SPV* and 20 g/L glucose (as carbon source) yielded a CDW of 2.14 g/L and PHB content of 38% CDW after fermentation for 60 h. Labuzek and Radeca (2001) reported

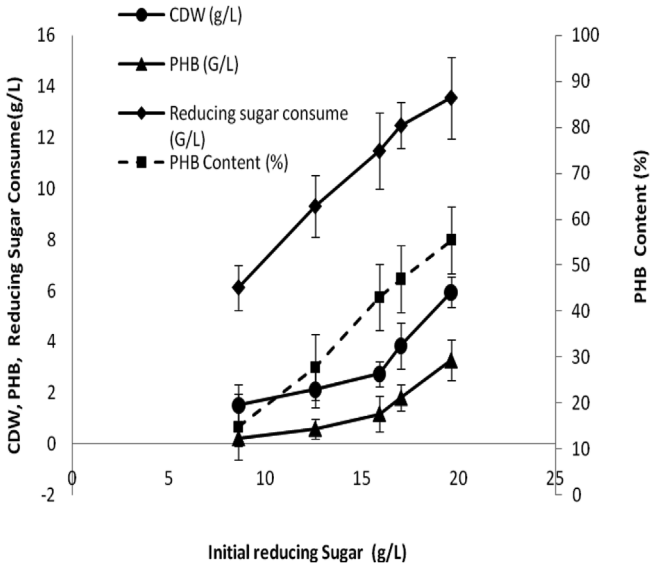


Fig. 5. Effects of initial reduced sugar concentration hydrolysate OPEFB on cell growth and PHB accumulation by *Bacillus cereussuaeda B-001*.

the results of a study with 10 g/L glucose as a carbon source, using *Bacillus cereus* UW85, which yielded a CDW of 1.8 g/L and a PHA content of 24.6% CDW. *Bacillus cereus* M5 was used by Yilmaz et al. (2005) with a nutrient-broth (NB) medium, which produced a cell concentration of 0.345 g/L and a PHB accumulation of 27.53% of CDW.

Mizuno et al. (2010) conducted a study on five strains of the *B. cereus* group in Luria-Berthani medium and glucose at 20 g/L. The strains showed a wide cell-growth range, from 0.9 to 6.7 g/L, and PHA accumulation was observed in the range of 15–44% of CDW with five different strains.

Fig. 4b shows the use of OPEFB acid hydrolysates as a carbon source with an initial reducing sugar concentration of 14.3 g/L. After incubation for 96 h, the accumulated dry weight biomass was 2.5 g/L and the PHB content was 0.99 g/L, or almost 40% of the CDW. This yield is lower than the yield using glucose as the carbon source. The acid-hydrolysis process produces furfural and acetic acid (Fig. 2), compounds which could act as inhibitors in the process of bacterial growth and accumulation of PHB (Wang et al., 2010).The low PHB yield due to inhibition was also found by Silva et al. (2007), in whose study the biomass and PHA production in the acid-hydrolysed sawdust used by *S. macrogoltabida* LMG 17324 were slightly lower than those obtained in glucose culture. The same was reported by Radhika and Murugesan (2012), who used non-detoxified acid hydrolysate water hyacinth and the bacteria *Cupriavidus necator* MTCC-1472.

3.6. The initial sugar concentration

The initial total sugar concentration is very critical for obtaining a high CDW. In order to obtain the optimum condition for PHB production, variations of the reducing sugar concentration of OPEFB hydrolysate, from 8 g/L to 20 g/L, were investigated. As it is shown in Fig. 5 when using OPEFB hydrolysates as carbon source, the higher the initial reducing sugar, the greater the cell dry weight and PHB content. The highest yield on the initial reducing sugar was 20 g/L, with CDW of 5.94 g/L and PHB content of 55.44%of CDW. This result was consistent with the previous study.

Zhang et al. (2013), reported a study using *Bacillus megaterium* R11, in which increased glucose concentration from 15 g/L to 100 g/L resulted in increased CDW from 5.33 g/L to 32.16 g/L as well as an increase in PHB content from 51.4% to 58.6%. Those results were obtained from pure glucose.

Table 4
Comparison of PHB production from lignocellulosic hydrolysate.

No	Carbon Sorce	Process	Strain	CDW (g/L)	PHB (%)	Reff
1	Grass	Enzyme hydrolysate	*Pseudomonas putida* W619	0.88	25.1	Davis et al. (2013)
2	Grass	Enzyme hydrolysate	*Pseudomonas putida* KT2440	0.81	23.4	Davis et al. (2013)
3	Grass	Enzyme hydrolysate	*Pseudomonas fluorescens* 555	0.86	34.5	Davis et al. (2013)
4	OPEFB	Acid Hydrolysate	*Bacillus cereus suaeda*	2.54	38.79	This Study
5	OPEFB	Acid Hydrolysate	*Bacillus cereus suaeda*	5.94	55.44	This study
6	OPEFB	Enzyme hydrolysate	*Bacillus megaterium R11*	15.93	58.5	Zhang et al. (2013)
7	OPEFB	Enzyme hydrolysate	*Bacillus megaterium R11*	24.19	51.6	Zhang et al. (2013)
8	Water hyacinth	Enzyme hydrolysate	*Cupriavidus nacator*	6.2	59.68	Radhika and Murugesan (2012)
9	Jackfruit seed	Enzyme hydrolysate	*Bacillus sphaericus* NCIM 5149	4.5	48.9	Ramadas et al. (2010)
10	Agro- and food waste	Acid Hydrolysate	*B.thuringiensis* IAM 12077	1.6–15.5	0–51.7	Gowda and Shivakumar (2014)

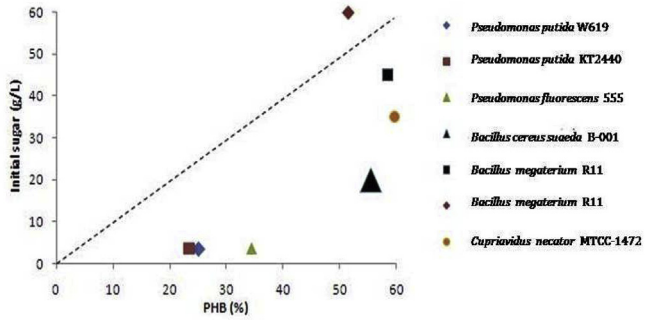


Fig. 6. Shows comparative performance of existing PHB producing bacteria.

3.7. PHB production from lignocellulosic hydrolysate

Carbon sources represent the greatest expense in bioplastic production (Salehizadeh and Van Loosdrecht, 2004). Various types of biomass can be used as carbon sources, for example, grass (Davis et al., 2013), bagasse (Yu and Stahl, 2008), water hyacinth (Radhika and Murugesan, 2012), OPEFB (Zhang et al., 2013), rice straw (Narayanan et al., 2014) and so on. This research uses OPEFB as a carbon source because OPEFB is a renewable material and is available in large quantities in Indonesia (Amraini et al., 2017).

Conversion of lignocellulose into hydrolysate solution can be done by either an enzymatic-hydrolysis process or an acid-hydrolysis process. The enzymatic-hydrolysis process is more expensive than the acid-hydrolysis process (Patel et al., 2017; Zhang et al., 2012). In this study, the carbon source comes from the OPEFB acid-hydrolysis process. The result of the acid-hydrolysis process is mostly xylose and glucose (Fig. 2).

Various strains of microorganisms can produce PHB using lignocellulose hydrolysate as a carbon source. Strain *Bacillus megaterium* R11 produces the highest CDW, 24.19 g/L, with a very high initial sugar concentration of 60 g/L, as shown in Table 4 (Zhang et al., 2013). In this study, using strain *Bacillus cereus suaeda* B-001 yielded CDW of 5.94 g/L at a concentration of initial sugar of 20 g/L.

This study (*Bacillus cereus suaeda* B-001) resulted in the most optimal PHB accumulation (%) compared to existing studies. Using a relatively low initial sugar concentration can produce PHB accumulation of 55.4%, compared to *Bacillus megaterium R11*, *Cupriavidus necator* MTCC-1472 with accumulative PHB yield of 58.5% and 59.68%, respectively. In a previous study by Zhang et al. (2013), an initial sugar concentration of 60 g/L yielded PHB content of 51.6%.

Fig. 6 is important to show, the relative position of various bacteria producing PHB. Bacteria that are located above the dash line show less effective use of sugar to produce PHB.Research in the future must develop bacteria in the position in the lower right corner, bacteria that are able to produce PHB as high as possible with the lowest possible initial sugar.

4. Conclusion

This study compared the potential of producing PHB from glucose and from OPEFB hydrolysates from the acid-hydrolysis process using *Bacillus cereus suaeda* B-001. A carbon source of glucose at 15 g/L can produce a PHB accumulation of 43.1%, while the use of OPEFB hydrolysates at 20 g/L as carbon source resulted in PHB accumulation of 55.4%. The results indicate that OPEFB hydrolysates from the acid-hydrolysis process could be utilized as a suitable and inexpensive feedstock for PHB production.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.01.057.

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