Menara Perkebunan 2021, 89(2), 146-155
p-ISSN: 0125-9318/ e-ISSN: 1858-3768
DOI: http://dx.doi.org/10.22302/iribb.jur.mp.v89i2.452
Accreditation Number: 21/E/KPT/2018

Harnessing molasses as a low-cost carbon source for production of poly-hydroxy butyrate (PHB) using *Burkholderia* sp. B73 bacteria

Pemanfaatan molase sebagai sumber karbon murah untuk produksi poli hidroksi butirat (PHB) dengan menggunakan bakteri Burkholderia *sp. B73*

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Received 1 May 2021/ Accepted 25 October 2021

Abstrak

Burkholderia *sp. telah dilaporkan sebagai penghasil poli-hidroksi butirat (PHB). PHB adalah poliester alami yang telah dimanfaatkan secara luas untuk pangan, obat dan biomedis. Akan tetapi, biaya produksi PHB yang tinggi menyebabkan PHB kurang dimanfaatkan. Molase, produk samping industri gula tebu yang tersedia dalam jumlah banyak, dapat dimanfaatkan sebagai pengganti sumber karbon untuk produksi PHB. Penelitian ini bertujuan untuk mengevaluasi produksi PHB dengan menggunakan bakteri Burkholderia sp. B73 dalam media fermentasi yang mengandung molase sebagai sumber karbon alternatif. Eksperimen skala laboratorium dilakukan dengan menggunakan labu Erlenmeyer pada shaker kecepatan 150 rpm dan suhu 30°C untuk mengetahui rasio C/N terbaik dalam mengakumulasi biomassa dan produksi PHB. Parameter yang diamati adalah pertumbuhan mikroba, berat sel kering dan rendemen PHB, serta spektrum FTIR. Hasil penelitian menunjukkan bahwa molase dapat dimanfaatkan untuk menumbuhkan* Burkholderia *sp. B73 dan kadar PHB tertinggi diperoleh dengan menggunakan molase dalam medium fermentasi pada rasio C/N 20:1. Selain itu, dengan mengatur pH menjadi 7.0 sebelum fermentasi, produksi PHB tertinggi juga dicapai. Lebih penting lagi, dengan menggunakan molase sebagai sumber karbon, rendemen PHB yang diperoleh 2 kali lipat lebih tinggi dibandingkan dengan menggunakan medium sintetik Ramsay pada penelitian kami sebelumnya. Sebagai kesimpulan, pada penelitian ini dibuktikan bahwa molase dapat digunakan sebagai sumber karbon yang murah untuk produksi PHB dengan menggunakan bakteri* Burkholderia *sp. B73.*

[Kata kunci: C/N, rasio, gula tebu]

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Abstract

Burkholderia sp. has been reported as a poly-hydroxy-butyrate (PHB) producer. PHB is a natural polyester class with a wide range of applications in foods, medicines, and biomedicines. However, the high production cost of PHB may limit its potential. Molasses, a by-product of the sugarcane industry available abundantly, may be used as an alternative carbon source of PHB production. In this research, we aimed to evaluate PHB production by Burkholderia sp. B73 in fermentation media using molasses as an alternative carbon source. Small-scale experiments were performed in Erlenmeyer flasks on a shaker at 150 rpm and 30 °C to evaluate the best initial C/N ratio for biomass accumulation and PHB production. A set of parameters including bacterial growth, dry cell weight, yield, and FTIR spectrum of PHB were observed. The results showed that molasses could be used to grow *Burkholderia* sp. B73 and the highest PHB production was obtained when a 20:1 C/N ratio of molasses was applied in the fermentation medium. In addition, when the initial pH was adjusted to 7.0, the highest PHB yield was also produced. More importantly, the use of molasses as a carbon source improved the PHB yield by nearly 2-fold compared with our previous report using a synthetic Ramsay's minimal medium. In conclusion, the experiment results showed that molasses could be used as a low-cost carbon source for PHB production by *Burkholderia* sp. B73 bacteria.

[Keywords: C/N; ratio; sugarcane]

Introduction

Plastics are essential for food packaging (Accorsi *et al.,* 2014). Currently, the plastics used for food packaging are generally derived from petrochemicals, which are very difficult to degrade (Webb *et al.,* 2013). Poly β-hydroxybutyric acid

(PHB) is a natural polyester produced and accumulated intracellularly in Gram-positive and Gram-negative bacteria (Sudesh *et al.,* 2000). The physical characteristics of PHB, including molecular weight, brittleness, melting point, and glass temperature, are similar to those of some of the common petrochemical-derived plastic (Sudesh *et al.,* 2000; Luo *et al.,* 2014). The major advantage of PHB is that it is fully degradable (Sudesh *et al.,* 2000; Koller, 2017). Accordingly, PHB has promising application potential in materials science, food, biomedicine, medicine, etc., due to its eco-friendly nature.

The main challenge for the commercialization of PHB is its high production cost compared to petroleum-derived plastics (Choi & Lee, 1999). To date, much effort has been focused on reducing the production cost of PHB by using different strategies, including screening high potential bacterial strains and optimization of the fermentation procedure and the recovery process (Mostafa *et al.,* 2018). Studies regarding the production of PHB suggest that the main contributor to the high production cost of PHB is the high cost of carbon substrate, making it 5-fold more costly than the production of petroleum-derived plastics. In essence, the carbon source selection is the critical aspect in reducing the total cost of the PHB final product (Kamravamanesh *et al.*, 2018). Thus, the optimal approach is to select a sustainable, economical, and readily available carbon substrate for bacterial growth and efficient PHB production.

Molasses, a by-product of the sugarcane industry, is a potential carbon source (Chauhan *et al.,* 2011). One kg of sugar produces nearly 0.3 kg of molasses, making this by-product abundantly available (Botha & von Blottnitz, 2006). As a by-product, molasses is responsible for water and air pollutions, i.e., brown water, bad smell, forming sludge, and breeding site for mosquitos and flies if not taken advantage of and properly processed (Chauhan *et al.,* 2011). However, molasses contains high concentrations of sucrose, fructose, glucose, raffinose, reducing sugar as well as carbohydrates, making it a promising alternative carbon source for fermentation (Yan *et al.*, 2011), where the content of sugar and organic materials in it are the main target in production of PHB. Studies have reported successful production of PHB by *Bacillus* sp Jma5 (Wu *et al.,* 2001), *Bacillus subtilis*, *Escherichia coli* (Gomaa, 2014), *Alcaligens eutrophus* (Beaulieu *et al*., 1995), and *Enterobacter* sp. SEL2 (Naheed & Jamil, 2014) used molasses as a carbon source; the resulting PHB yield ranged from 17 to 88% (w/w). Hence, the utilization of molasses has benefits that are not limited to its potency as a carbon source but can also to help solving environmental problems.

Burkholderia sp. has been reported for the first time to produce a relatively high amount of PHB in synthetic media compared with other polyhydroxyalkanoates (PHA)-producing bacteria (Ratnaningrum *et al.,* 2019). However, the effect of utilizing molasses for the production of PHB by *Burkholderia* sp. B73 has not been reported yet. In this paper, we observed the potency of molasses for the growth of *Burkholderia* sp. B73 and production of PHB using various C/N ratios. In addition, the effect of initial pH in the fermentation medium containing molasses for PHB production was also evaluated.

Materials and Methods

Microorganism and sub-culture conditions

Burkholderia sp B73 used in this study was obtained from the collection of the Research Center for Biotechnology, Indonesian Institute of Sciences, Cibinong-Indonesia. The strain was regenerated in a nutrient agar (NA) slant (30 ºC, 24 h). The fresh inoculum was then transferred to nutrient broth (NB) medium containing beef extract 0.3% w/v and peptone 0.5% w/v with the addition of sodium chloride 0.8% w/v and incubated in a shaker incubator (150 rpm, 30 °C, 24 h). After 24 h of cultivation, the starter culture containing 1.5 x 10^6 viable cells/mL of *Burkholderia* sp. B73 was inoculated in the fermentation medium containing molasses.

Confirmation of Burkholderia *sp. B73 as PHB producing bacteria*

The confirmation of *Burkholderia* sp. B73 as PHB-producing bacteria has been done in previous research (Ratnaningrum *et al.*, 2019). PHB-producing bacteria were observed based on Bhuwal *et al.* (2013) and Spiekermann *et al.* (1999) methods. *Burkholderi*a sp. B73 was inoculated in the nutrient agar (NA) containing Nile Red. After Nile Red staining showed bright pink to orange fluorescence under irradiation with UV light, and the fluorescent was observed under a fluorescence microscope at λ 540 nm.

Pre-treatment of molasses

About 500 g locally collected molasses (Cirebon, West Java, Indonesia) were mixed with 500 mL of distilled water and added 3 mL K4Fe(CN)6.3H2O to remove heavy metals and other inhibitors. The mixture was heated at 70 °C for 30 min and stored overnight. The mixture was then centrifuged (7000 rpm, 15 min), and the supernatant was collected and used as a carbon source/substrate for further process (Ashraf *et al.,* 2015).

Preparation of fermentation medium containing molasses

Fermentation media containing molasses at C/N ratios of 5:1, 15:1, and 20:1 were prepared by adding molasses at 5, 15, and 20 g L^{-1} into a mixture

of Ramsay minimal media of 17 g L^{-1} $(NH_4)_2HPO_4$, 10 mL $MgSO_4.7H_2O$ 0.1 M, 3.7 g L^{-1} $KH_2PO_4.7H_2O$, 5.8 g L^{-1} K_2HPO_4, trace element solution containing 0.0278 g $L^{-1}FeO_4.7H_2O$, 0.0198 g L^{-1} $MnCl_2.4H_2O$, 0.0281 g L^{-1} $CoSo_4.7H_2O$, 0.0167 g L^{-1} $CaCl_2.7H_2O$, 0.0017 g L^{-1} $CuCl_2.2H_2O$ and 0.0029 g L^{-1} $ZnSO_4.7H_2O$. The mixture was sterilized in an autoclave (at 121 °C, 1 atm for 15 min) and kept at ambient temperature before fermentation.

Production of PHB

The production of PHB was conducted at a lab-scale by using a 500 mL Erlenmeyer flask containing 100 mL of fermentation medium. The fermentation process of PHB production was conducted in a shaker incubator (150 rpm, 30 °C for 72 h).

Effect of molasses as a carbon source on the growth of Burkholderia sp. B73

Molasses at various C/N ratios 5:1, 15:1 and 20:1 were added into the fermentation medium for *Burkholderia s*p. B73 cultivation. The supernatant of the fermentation medium was collected for reducing sugar analysis, whereas the bacterial cells were collected for dry cell weight determination.

Dry cell weight determination

The dry cell weight (DCW) of *Burkholderia* sp. B73 was determined by gravimetry analysis. Ten mL of culture sample was centrifuged at 7000 rpm for 15 min to separate cells and supernatant. The pellets obtained were washed twice with 5 mL of distilled water. This procedure was repeated two times. After washing, the cell pellets were suspended in 10 mL of distilled water. About 1 mL of cell suspension was transferred into a falcon tube for gravimetric analysis (Aramvash *et al.*, 2018). Three independent replications were performed.

Extraction and quantification of PHB yield

The extraction of the PHB produced was conducted according to Kresnawaty *et al.* (2016) with a slight modification. Ten mL of cell culture suspension was centrifugated at 7000 rpm for 15 min. The supernatant was collected for reducing sugar analysis, and the cell pellets were suspended in 10 mL distilled water. One mL of cell suspension was processed for cell lysis. The cell suspension was mixed with 1 mL of natrium hypochlorite 5% v/v and 3 mL of 0.1 M pH 7.0 phosphate buffer for the cell lysis. The mixture was shaken at 180 rpm for 24 h (RT). The cell lysis was stopped after 24 h, and the supernatant was removed by centrifugation at 7000 rpm for 15 min. The pellets obtained were washed with 5 mL of distilled water, 3 mL of acetone, and 3 mL of diethyl ether, respectively. The PHB obtained was then dried in an oven blower (70 °C) until constant weight.

The yield of PHB was calculated with the following formula:

$$\text{The yield of PHB (\%)} = \frac{\text{dry weight of extracted PHB (g/L)}}{\text{dry cell weight (g/L)}} \text{ x } 100\%$$

Reducing sugar analysis

The concentration of reducing sugar in the fermentation medium was analyzed according to the Nelson Somogy method. One mL of sample was added to a test tube and mixed with 1 mL Nelson-Somogy reagent (25 mL of Nelson A and 1 mL of Nelson B solution). The mixture was incubated for 20 min at 100°C in a water bath. Finally, the sample solution was added with 1 mL arsenomolibdat reagent and 7 mL of aquadest. The absorbance was recorded with a Hitachi U-2800 spectrophotometer at 520 nm. The reducing sugar concentration was calculated based on the standard glucose curve (Wen *et al.,* 2004).

Effect of C/N ratio in fermentation medium containing molasses on PHB yield

The effects of various C/N ratios (5:1, 15:1 and 20:1) in the fermentation medium containing molasses on PHB production by *Burkholderia* sp B73 were observed during an incubation period of 72 h. Molasses at various C/N ratios (5:1, 15:1 and 20:1) was added into fermentation medium for *Burkholderia* sp. B73 cultivation. The bacterial cells were collected for determination of PHB yield.

Effect of initial pH of fermentation media on PHB yield

The effect of initial pH on PHB production by *Burkholderia* sp B73 was evaluated in a fermentation medium containing molasses at a C/N ratio of 20:1. The fermentation medium was adjusted to an initial pH of 7, 8 and 9. The inoculum of *Burkholderia* sp. B73 was transferred to each Erlenmeyer flask and incubated at 30 °C on a shaker incubator at 150 rpm for 72 h. The cells were collected for PHB yield.

The Fourier transform infrared (FTIR) spectroscopy analysis

The FT-IR spectrum was measured using Nicolet iS5 FTIR spectrometer (ThermoScientific Fisher, United States). The sample was scanned between a wavenumber range of 4000 to 400 cm^{-1} with 64 scans at a resolution of 2 cm^{-1}.

Statistical analysis

The data were calculated as mean ± SD (n=3) using the Microsoft Excel 2019 software. The treatment factors were molasses substrate at various C/N ratios 5:1; 15:1; 20:1 (A1) and incubation time at 24, 48 and 72 h (B1). Each treatment was repeated three times to minimize experimental error.

Results and Discussion

The by-product of agroindustry is a potential carbon source due to its high carbohydrate contents for microbial PHB production. Species of *Burkholderia* have been investigated for PHB production (Zhu *et al.,* 2010; Pan *et al*., 2012). For the rapid confirmation of the potential *Burkholderia* sp. B73 as PHB-producing bacteria, Nile red staining was used in this experiment. We confirmed that *Burkholderia* sp. B73 was a PHB producer as suggested by pink color fluorescence under UV-light (Fig. 1A). Bacterial cells stained with Nile red staining were from the carbon-rich nutrient agar after 24 hours grown with colonies method. Then, the cells were observed under UV light (235 nm) showed pink colonies. As for PHB granules would be seen purple spots in the bacterial cells under a fluorescence microscope (400x) (Fig. 1B). The data have been accomplished in the previous research (Ratnaningrum *et a*l., 2019). Therefore, *Burkholderia* sp. B73 was selected for further optimization of PHB production.

Prior to the investigation of the potential utilization of molasses as a carbon source for the growth of *Burkholderia* sp. B73, we analyzed the growth profiles of *Burkholderia* sp. B73 in NB medium. The *Burkholderia* sp. B73 growth profile was observed based on its optical density (OD) of 600 nm as a function of time. As can be seen in Figure 2, the growth of *Burkholderia* sp. B73 increased rapidly during the first 24 h. The maximum OD_{600nm} value was reached after 72 hours of incubation (OD_{600nm} of 2.095). It seems that the *Burkholderia* sp. B73 entered the stationary phase at 72 to 96 h, the graph tending to decrease during this incubation period. A previous study reported *Burkholderia* sp. entered the stationary phase after ten h of incubation in NB medium (Khleifat *et al.,* 2007). Our results were completely different from those of Khleifat *et al*. (2007); this may be due to the different species used during the study. Based on the growth curve profile of *Burkholderia* sp. B73, we, therefore, suggest that production of PHB began after 24 h of incubation.

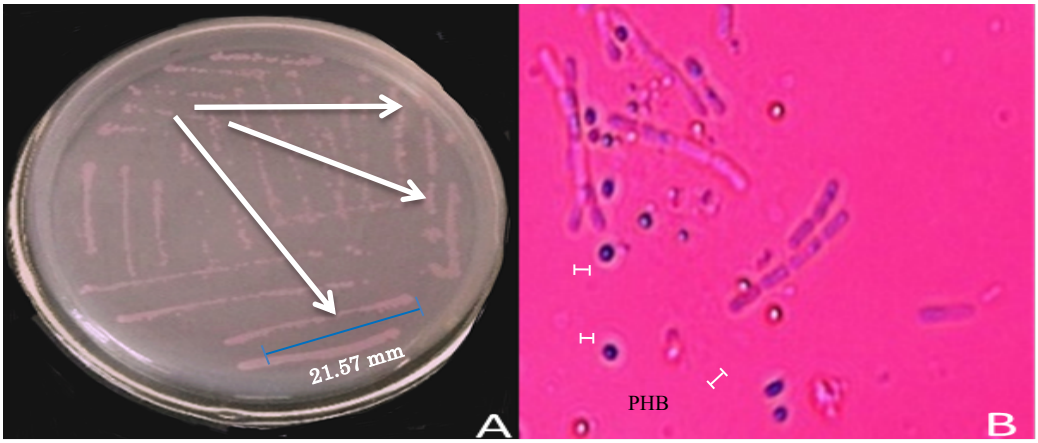

Figure 1. *Burkholderia* sp B73 cell stained with Nile red staining under UV light (A), PHB granule with stained Nile red (purple) under fluorescence microscope (400x)

Gambar 1. Burkholderia *sp B73 diwarnai dengan pewarna Nile red di bawah lampu UV(A), Granul PHB dengan pewarna Nile red (ungu) di bawah mikroskop floresen (400x)*

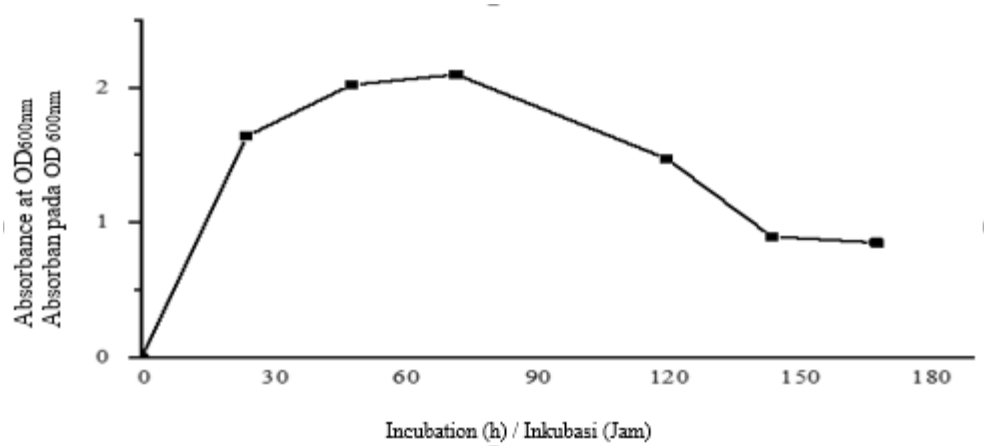

Figure 2. The growth curve of *Burkholderia* sp B37 in NB medium. The curve represents the absorbance and incubation period (h) at wave length 600 nm

Gambar 2. Kurva pertumbuhan Burkholderia *sp B73 dalam medium NB. Kurva menggambarkan absorbansi dan waktu inkubasi (jam) pada panjang gelombang 600 nm*

Effect of molasses on the growth of Burkholderia sp. B73

Considering the sugar-based materials of by-product agroindustry that could be possibly used for microbial growth, molasses is a potential substrate for the growth of *Burkholderia* sp. B73. According to the growth profile of *Burkholderia* sp. B73 in the NB medium, we observed the effect of molasses as a carbon source for the growth of *Burkholderia* sp. B73 at 24, 36, and 72 h of incubation and C/N ratios of 5:1, 15:1 and 20:1 with the initial concentration of carbon in molasses used for the growth of *Burkholderia* sp B73 with each C/N ratios were 2, 6 and 8 g L^{-1}.

As shown in Figure 3A, at a C/N ratio of 5:1, the DCW of *Burkholderia* sp B73 did not increase. A considerable increment of DCW was observed when the C/N ratio of molasses was increased to 15:1 and 20:1 during 48 to 72 h of incubation. It seems that at a molasses C/N ratio of 5:1, *Burkholderia* sp. B73 failed to grow due to the limited carbon concentration. A similar result was found when *Ralstonia eutropha* ATCC 17699 was fermented in corn step liquor at a C/N ratio of 5:1.

Furthermore, it is noted that the critical glucose concentration for cell growth is 10 g L^{-1} (Marangoni *et al.,* 2001). Hence, we suggest that the minimum C/N ratio of molasses for the growth of *Burkholderia* sp. B73 is 15:1. Atifah *et al.* (2007) reported that in a glucose concentration range of 10 to 40 g L^{-1}, in general, the higher sugar concentration also higher of concentration *Ralstonia eutropha* cell during fermentation. Meanwhile, at a certain limit, a higher concentration of carbon and nitrogen with the same ratio will be more material that can be converted into the material building and cell reproduction. According to *Gouda et al.* (2007), the best of *B. megaterium* growth was obtained with 3% molasses, while the maximum got 2% and decreased when 5% molasses. According to the several research previously reported, the finding strain *Burkholderia* sp B73 as PHA producer and using molasses as a carbon source is for the first time reported to date.

In this experiment, it appeared that the maximum value of DCW was observed after 72 h of incubation. By considering the DCW of *Burkholderia sp.* B73, the molasses as a carbon source should have been consumed. To confirm this, we evaluated the reducing sugar concentration of the fermentation medium containing molasses after inoculating with *Burkholderia* sp. B73. As shown in Figure 3B at a C/N ratio of 20:1 and 15:1, the reducing sugar concentration of the fermentation medium containing molasses showed a decreasing trend and entered the stationary phase after 48 h.

In contrast, the fermentation medium containing molasses at a C/N ratio of 5:1 had a flat curve. The calculations of sugar consumption of *Burkholderia* sp. B73 suggests that at a C/N ratio of 15:1 and 20:1, *Burkholderia* sp. B73 consumed about 55% of the sugar content, leaving about 6.68 and 8.85 g L^{-1} reducing sugar concentration after 72 h of incubation. These results are nearly similar to those from the study of Oliveira (1999), which showed that about 10 g L^{-1} of glucose still remains in the fermentation medium after inoculation with PHB-producing bacteria. Our study indicated that *Burkholderia* sp. B73 can utilize molasses for its growth.

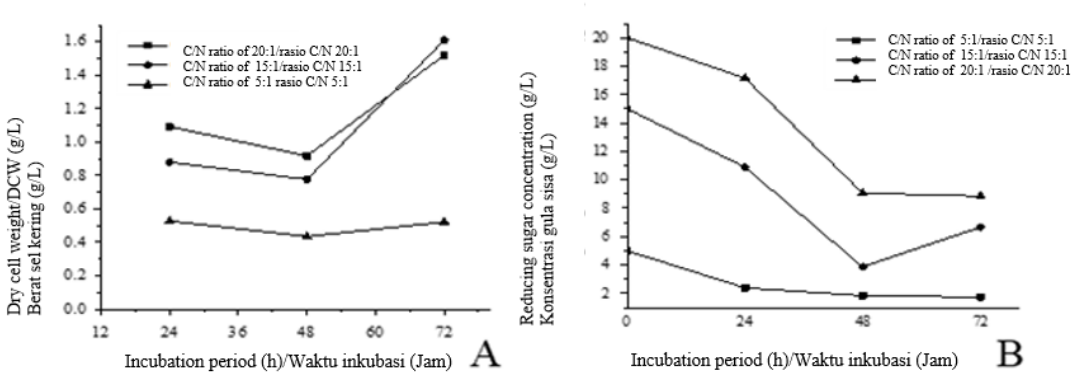

Figure 3. (A) The effect of different C/N ratios (5:1; 15:1 and 20:1) and incubation period (h) on dry cell weight of *Burkholderia* sp B73 (g L^{-1}) and, (B) The effect of different C/N ratio and incubation period (h) on reducing sugar concentration (g L^{-1})

Gambar 3. (A) Pengaruh perbedaan rasio C/N (5:1; 15:1 dan 20:1) dan waktu inkubasi (jam) terhadap berat sel kering Burkholderia *sp B73 (g L^{-1}) dan, (B) Pengaruh perbedaan rasio C/N (5:1; 15:1 dan 20:1) dan waktu inkubasi (jam) terhadap konsentrasi gula sisa (g L^{-1})*

Effect of C/N ratio of molasses in fermentation medium on PHB yield

The key to PHB production lies on the availability and concentration of carbon. As a by-product of the sugar industry, molasses is lucrative for PHB production. We have confirmed that the minimum C/N ratio of molasses is required for the growth of *Burkholderia* sp. B73 is 15:1. However, this result did not reflect on the PHB yield. Thus, it is important to evaluate the PHB yield after extraction since PHB is accumulated intracellularly. As depicted in Figure 4A, at a 20:1 C/N ratio of molasses in the fermentation medium, *Burkholderia* sp. B73 produced about 85% PHB but less at C/N ratios of 5:1 and 15:1. It seems that at C/N ratios of 5:1 and 15:1, *Burkholderia* sp. B73 uses molasses as a carbon source as a nutrient and energy, but not for PHB production (Sudesh *et al.,* 2000). Therefore, we strongly suggest applying molasses at a C/N ratio of 20:1 for PHB production.

Effect of initial pH value on PHB yield

Another important parameter that influences PHB production is the initial pH of the fermentation medium. In this research, we observed the effect of the initial pH value in the range of 7.0 to 9.0 towards PHB production by *Burkholderia* sp. B73 using molasses at a C/N ratio of 20:1. As shown in Figure 4B, when the pH value of the molasses fermentation medium was adjusted to 8 and 9, the PHB yield tended to decrease. It is noted that the pH medium plays an important role in cell metabolism hence influencing the growth of bacteria. Saleem *et al.* (2014) recommended applying an initial pH in the range of 6.0 to 7.5 for microbial growth and PHB production (Saleem *et al.,* 2014). Whereas, Irwandi *et al.* (2018) reported that at pH above 8.5 PHB producing bacteria did not grow well (Irwandi *et al.,* 2018). Furthermore, PHB is decomposed in alkaline conditions (Yu *et al.,* 2005). The data observed in this study were in agreement with these findings. Based on the maximum PHB produced, we suggest applying an initial pH of 7.0 for the optimum growth of *Burkholderia* sp B73 and PHB production.

It is important to note that the production of PHB by *Burkholderia* sp. B73 was 52.9% in Ramsay's minimal medium (Ratnaningrum *et al.,* 2019). In this study, we reveal that the yield of PHB obtained by using molasses as substrate was two times higher. Molasses has been reported to contain high concentrations of carbohydrates, proteins, as well as micronutrients. The presence of carbohydrates and micronutrients supports the biosynthesis of PHB (Yüksekdağ *et al.,* 2004). Therefore, molasses is very promising as a low-cost carbon source for enhancing the production of PHB.

Characterization of PHB

Finally, we characterized the polymer powder obtained by FTIR analysis to confirm PHB production. As depicted in Figure 5a, the FTIR spectrum of PHB extracted from *Burkholderia* sp. B73 shows peaks at 1722 cm^{-1} and 1288 cm^{-1} to 973 cm^{-1}, corresponding to different functional groups in PHB. The peak at 1722 cm^{-1} corresponds C=O stretch of the ester group (Hassan *et al.,* 2016; Ratnaningrum *et al.,* 2019). Whereas the peaks at 1288-973 cm^{-1} correspond to C-C, C-O, and C-H groups. Absorption bands recorded at 2956 and 2925 cm^{-1} indicate aliphatic -CH_3 and -CH_2 groups (Ratnaningrum *et al.,* 2020). Bhagowati *et al.* (2015) reported that the FTIR spectrum of standard PHB shows peaks at a wavenumber of 1725 and 1288 cm^{-1}, and it is strengthened by Pan *et al.* (2012) using *Burkholderia cepacia* ATCC17759 with sugar maple hemicellulose hydrolysate which analysis by NMR and physical-chemical characterization showed that PHA produced was identified as poly-hydroxybutyrate (PHB). These all peaks were similar to the characteristics of standard poly-hydroxybutyrate, as shown in Figure 5b.

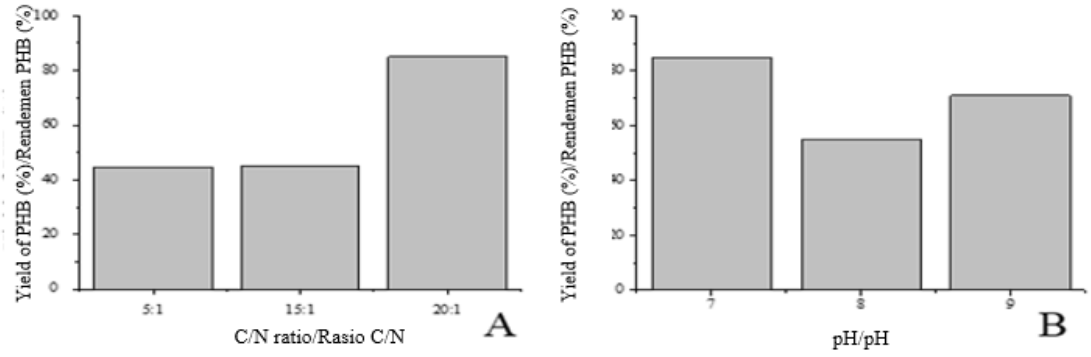

Figure 4. (A) PHB yield at different C/N ratios after 72 h of incubation and (B) effect of initial pH value on PHB yield at C/N ratio of 20:1 after 72 h of incubation

Gambar 4. (A) Rendemen PHB pada rasio C/N yang berbeda setelah inkubasi selama 72 jam dan (B) pengaruh pH awal terhadap rendemen PHB pada rasio C/N 20:1 setelah inkubasi selama 72 jam

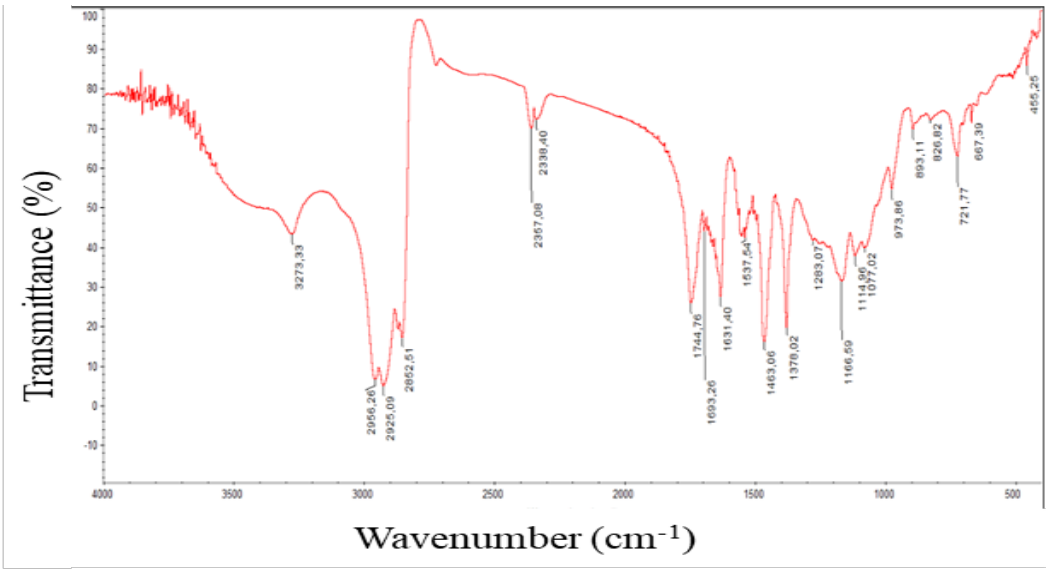

Figure 5a. FTIR spectrum of PHB extracted from *Burkholderia* sp. B73. The curve represents the transmittance (%) and wavenumber (cm^{-1})

Gambar 5a. Spektrum FTIR PHB yang diekstraksi dari Burkholderia *sp. B73. Kurva yang menggambarkan transmisi (%) dan angka gelombang (cm^{-1})*

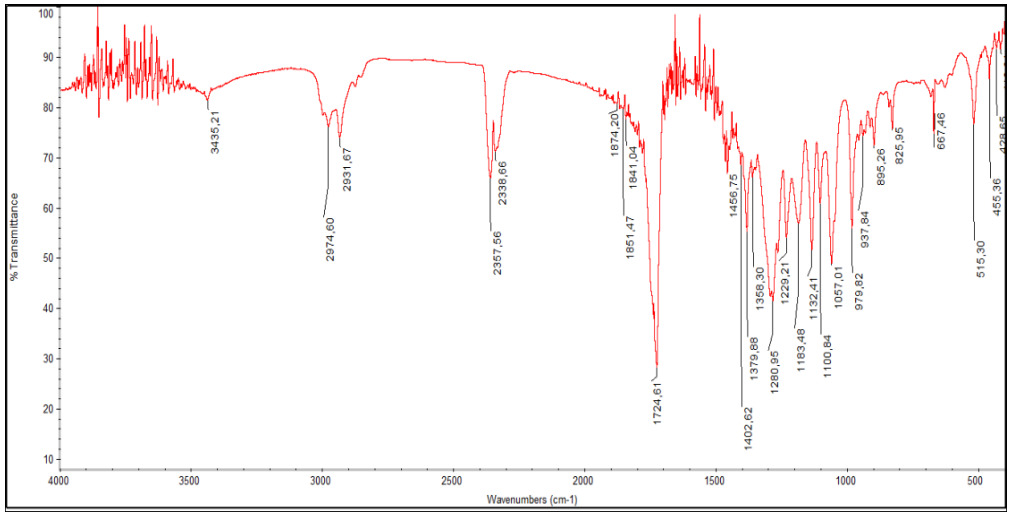

Figure 5b. FTIR standard spectrum of PHB. The curve represents the transmittance (%) and wavenumber (cm^{-1})

Gambar 5b. Spektrum standar FTIR PHB. Kurva menggambarkan transmisi (%) dan angka gelombang (cm^{-1})

In this study, the FTIR spectrum of PHB produced by *Burkholderia* sp. B73 in fermentation medium containing molasses is very similar to previous reports, including PHB extracted from *Bacillus drentensis* BP17 (Jiun *et al.,* 2010; Yuanzhen *et al.*, 2014; Matias *et al*., 2016; Yamada *et al.,* 2018; Zapata *et al.*, 2019; Penkhrue *et al.*, 2020). It has also been carried out from previous studies, thermal characteristics on the purified PHB granules extracted from *Burkholderia* sp B73 with TGA analysis (Ratnaningrum *et al.,* 2019). The result obtained shows that at temperature of 63.5°C and 88.1°C there was a small weight loss, indicating the presence of volatile compounds and water and at temperature 293.3°C, residual mass obtained below 0% were similar with standard PHB (Lee *et al.,* 2002). Hopefully, the purified PHB can be fully degraded without leaving any residues.

Conclusion

In summary, it was confirmed that molasses, an agro-industrial by-product easily available substrate, are cheap and can cut down the cost of PHB production. Based on the characteristics and the potency, these molasses can be used as a low-cost carbon source for PHB production by *Burkholderia* sp. B73. It is suggested to use molasses in fermentation medium at C/N ratio 20:1, initial pH 7, and 72 h of incubation for optimum PHB production by *Burkholderia* sp. B73 with PHB yield was 85%.

Acknowledgements

This work was supported by Mandiri Project. Authors thank to the Research Unit for Clean Technology, Indonesian Institutes Sciences for research facilities, e-layanan sains (e-ELSA), Indonesian Institute of Sciences for sample analysis, Research Center for Biotechnology, Indonesian Institute of Sciences for providing the bacteria, Mrs. Sri Pudjiraharti for delivering materials and Mr. Zybrand for proofreading the manuscript.

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