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The Production of Polyhydroxyalkanoate (PHA) Bioplastic from Palm Oil Mill Effluent (POME) using *Pseudomonas aeruginosa*

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Abstract

Biodegradable plastics were continuously developed to replace non-degradable plastics. One type of bioplastic that was used was poly(hydroxyalkanoate) (PHA). In this study, PHA was synthesized using Palm Oil Mill Effluent (POME) and the bacterium *Pseudomonas aeruginosa* through a fed-batch (aerobic–anaerobic) fermentation process over six days. The study aimed to evaluate the use of POME as the sole carbon source for PHA synthesis by *Pseudomonas aeruginosa* through a fed-batch aerobic–anaerobic fermentation process. The total substrate volumes used in this study were 30, 60, and 90% (v/v), which were gradually added (fed-batch) at rates of 5, 10, and 15% (v/v)/day over the six-day fermentation period. The analytical results showed significant reductions in BOD (Biological Oxygen Demand), COD (Chemical Oxygen Demand), ammonia (NH_3), and total suspended solids (TSS) after the fermentation process, indicating that organic and chemical compounds were degraded during fermentation. PHA production from POME showed that the maximum yield occurred on the third day, with 16.89 g/kg dry biomass and a PHA concentration of 9.089 wt% (1.53 g/kg) from a total substrate volume of 9 L. The fermentation process reached the exponential phase on the third day, declined on the fourth day, and stabilized by the sixth day, with an average yield of 5.248 wt%. Characterization using X-ray diffraction (XRD) confirmed that the produced PHA had a structure consistent with standard PHA. Further analysis of water absorption and biodegradability showed fluctuating results, with the best performance observed at a 5% concentration, indicating low water absorption and good biodegradability. In addition, the bioplastic produced had a tensile strength of 554.32 MPa and a modulus of elasticity of 5834.95 MPa.

1. Introduction

Currently, non-degradable plastics remain a serious environmental issue. These types of plastics accumulate in nature and take a very long time to decompose, making them one of the main sources of pollution. Therefore, the use of non-degradable plastics should be avoided, and alternative raw materials that can degrade naturally are needed [1]. Biodegradable plastics are continuously being developed to replace non-degradable ones. One type of bioplastic that can be used is poly(hydroxyalkanoate) (PHA). PHA is an environmentally friendly plastic product that can be broken down by microorganisms [2]. PHA is a biodegradable plastic polymer that can decompose in the environment. This compound is synthesized by microorganisms from various substrates, including fatty acids and sugars [3].

The isolate *Pseudomonas aeruginosa* is known to produce up to 52.3% PHA, while *Bacillus subtilis* produces up to 38.4% when grown on used cooking oil as the substrate [4]. Cultivated *Sphingobacterium* sp. in a molasses-based medium and found that at a certain concentration, the PHA content remained constant [5]. Previous studies have also reported PHA production from volatile fatty acids (VFAs) derived from palm oil mill effluent (POME) using *Ralstonia eutropha* JMP 134. The PHA concentration and cell dry weight (CDW) obtained with batch addition of VFAs from palm oil mill wastewater at 20 and 40 hours were 0.014 g/L·h, 2.76 g/L, and 3.66

g/L, respectively. The results also showed that the timing of VFA addition significantly influenced cell growth, with the best addition time being at the 20th hour [6]. PHA production from POME using *Pseudomonas aeruginosa* has also been conducted. The results showed that a 50% POME concentration produced 0.3913 wt% PHA, and fermentation for six days yielded more PHA than fermentation for five days [2]. PHA cell growth requires a carbon source and a substrate as an energy source [7]. An alternative low-cost and abundantly available carbon source in Indonesia is POME. POME is a liquid waste generated from the palm oil extraction process in palm oil mills. It has a high BOD level and low nitrogen content (less than 200 ppm as ammonium nitrogen and less than 500 ppm as total nitrogen). These characteristics make POME a potential substrate and suitable carbon source for PHA production [8].

In 2023, Indonesia produced approximately 45.5 million tons of palm oil. Palm oil mills generate 0.7–1 m^3 of POME for every ton of Fresh Fruit Bunches (FFB) processed. A palm oil mill with a processing capacity of 30 tons of FFB per hour is estimated to produce about 21 m^3 of POME per hour (around 400 m^3/day), equivalent to 1.1 MWe. POME from the mill has a temperature of 60–80°C, a pH of 3.3–4.6, a dark brown color, and contains solids, oil, and grease (190–14,720 mg/L), COD (15,103–65,100 mg/L), and BOD (8,200–35,000 mg/L) in high amounts [9]. POME must be properly treated because it contains high levels of greenhouse gases that contribute to global warming [2].

In this study, PHA bioplastic was produced using POME as the raw material. The synthesis was conducted through fermentation with *Pseudomonas aeruginosa* employing a hybrid fed-batch method under anaerobic–aerobic conditions. The fed-batch method involves the repeated addition of substrate (carbon source) during fermentation, which can enhance bioplastic production compared to the batch method, as reported in a previous study [10]. This increase is due to the sufficient availability of carbon sources during fermentation, allowing for higher conversion. The fermentation process was carried out with total accumulated substrate levels of 30%, 60%, and 90% (v/v). Dry cells were extracted using acetone by maceration for 24 hours, and the resulting PHA was subsequently used to produce bioplastic sheets with varying concentrations. The PHA was characterized using Fourier Transform Infrared Spectroscopy (FTIR) and X-ray Diffraction (XRD). The bioplastics were then analyzed for water absorption, biodegradability, tensile strength, and modulus of elasticity. This study is expected to produce PHA with a high yield and bioplastics with optimal characteristics.

2. Experimental

2.1. Equipment and Materials

The equipment used in this study included glassware, an autoclave, a centrifuge, a magnetic stirrer, a hotplate, a vacuum filtration setup, a desiccator, and an oven. PHA characterization was carried out using FTIR and XRD. The bioplastic was analyzed for water absorption, biodegradability, tensile strength, and elastic modulus. The main materials used in this study were pure *Pseudomonas aeruginosa* culture, Nutrient Broth (Merck), POME (from a palm oil industry in Padang), distilled water, phosphate buffer (Merck), 5% NaOCl (Merck), and acetone (Merck).

2.2. Substrate Preparation

First, the POME sample was sterilized. A total of 18 L of POME was placed into a beaker and sterilized using an autoclave at 121°C for 15 minutes to eliminate unwanted microorganisms. The pH was then adjusted to approximately 7.0 using KOH, and a buffer solution was added to maintain a stable environment. The POME was subsequently stirred for 2 hours at 35°C and stored in a sealed container at room temperature for 2 days under anaerobic conditions. These anaerobic conditions promote acid fermentation, resulting in the production of short-chain fatty acids (such as acetic, butyric, propionic, and valeric acids), which serve as essential carbon substrates for PHA production [11].

Samples of POME, collected before and after fermentation, were analyzed to evaluate the reduction of biodegradable organic pollutants, specifically BOD, COD, total ammonia (NH_3-N), and ammonium ions (NH_4^+). BOD was determined following the APHA Standard Method 5210B, which involves a 5-day incubation at 20°C in the dark. COD was measured using the Closed Reflux Colorimetric Method in accordance with APHA 5220D, employing dichromate digestion under acidic conditions. The concentration of NH_3-N was quantified using the phenate method (APHA 4500-NH_3 F) with spectrophotometric detection at 640 nm, while NH_4^+ ions were specifically analyzed using ion chromatography based on APHA 4110B. All analytical procedures were performed in accordance with the Standard Methods for the Examination of Water and Wastewater (APHA, 2017) and supported by related findings in Osman *et al.* [12].

2.3. Activation of *Pseudomonas aeruginosa*

The pure culture of *Pseudomonas aeruginosa* was activated by inoculating it into 10 mL of sterile Nutrient Broth, which had been autoclaved at 121°C for 15 minutes. It was incubated in a water bath shaker at 120 rpm and 30°C for 24 hours. The active culture was then grown on slanted Nutrient Agar as a stock culture. The *Pseudomonas aeruginosa* strain used in this study was FNCC 0063, a standard Gram-negative strain obtained from the Food and Nutrition Culture Collection (FNCC). This strain originated from the ATCC standard culture and had been officially registered. A starter for PHA production was prepared by inoculating 2% (1 mL) of the active culture into 50 mL of liquid Nutrient Broth. This was incubated in a water bath shaker at 100 rpm and 30°C for 20 hours.

2.4. Fermentation Process

Fermentation was carried out in a fed-batch system for six days using a 10 L fermenter with a working volume of 6 L. A total of 5 mL (2%) of the starter was added to the fermentation medium to initiate the process. The fermentation medium consisted solely of POME that had undergone acidogenic fermentation to produce VFAs, such as acetic acid, propionic acid, and butyric acid [13].

The substrate, POME, was added every 24 hours at increasing concentrations of 5%, 10%, and 15% (v/v), resulting in total accumulated substrate levels of 30%, 60%, and 90% (v/v), equivalent to 3 L, 6 L, and 9 L, respectively. This strategy aimed to maintain a continuous carbon supply, prevent the buildup of toxic compounds such as VFAs, and support microbial growth and PHA accumulation [14].

Fermentation was conducted in a water bath shaker at a constant temperature of 30°C and a shaking speed of 180 rpm to maintain homogeneity and a stable temperature. The process alternated between an aerobic phase of 8 hours and an anaerobic phase of 16 hours. During the aerobic phase, aeration was supplied via an external air pump connected through a sterile filter and air stone to ensure efficient oxygen transfer. In the anaerobic phase, aeration was stopped, and the fermenter was tightly sealed to create oxygen-free conditions. This two-phase approach created a feast–famine pattern in the fed-batch system, which could increase PHA accumulation by controlling carbon supply and oxygen availability [15]. During fermentation, parameters were monitored every 24 hours, including culture pH, biomass concentration (g/L), and PHA content (% dry weight) to evaluate microbial growth and the efficiency of PHA biosynthesis.

2.5. PHA Extraction

After incubation, 50 mL of the bacterial culture was centrifuged at 4000 rpm for 15 minutes to separate the biomass from the liquid medium. The supernatant was discarded, and the resulting pellet was washed twice with n-hexane to remove residual oil and undegraded substrate, considering that the substrate used was complex POME. The pellet was then washed again with sterile distilled water to ensure the cleanliness of the biomass. Subsequently, the pellet was resuspended in 5 mL of distilled water, placed into an aluminum foil molded into a container shape, and dried in an oven at 70°C for 24 hours until a constant weight was achieved. This dry weight was used to determine the total biomass. Additionally, 1 mL of the cell suspension was dried under the same conditions for further dry weight measurement, if needed.

The next step was cell disruption and PHA purification. A total of 3 mL of phosphate buffer (pH 7.0) and 1 mL of 5% NaOCl solution were added to the cell suspension. The mixture was then incubated at 25°C with shaking at 180 rpm for 24 hours to degrade non-PHA organic matter. The remaining pellet containing PHA was recovered by centrifugation at 4000 rpm for 15 minutes and dried in an oven at 70°C for 24 hours. The PHA contained in the dry biomass was then extracted using acetone at a 1:2 ratio (dry cell weight: solvent volume) through a 24-hour maceration process [16]. The resulting solution was distilled to remove the solvent, and the PHA-rich fraction was washed with cold methanol to precipitate the polymer. The PHA precipitate was then dried at 70°C for 8 hours to obtain purified PHA. The purified PHA was subsequently characterized and used to fabricate bioplastic sheets.

2.6. PHA Characterization

The extracted PHA was characterized to identify its functional groups and crystalline structure using Fourier Transform Infrared Spectroscopy (FTIR) and X-ray Diffraction (XRD). The FTIR spectra were recorded using an FTIR spectrometer (Shimadzu IRSpirit-T) in the range of 4000–400 cm^{-1} with a resolution of 4 cm^{-1} and 32 scans per sample. The polymer crystallinity was observed using an XRD (Shimadzu XRD-7000) with Cu-Kα radiation (λ = 1.5406 Å), operated at 40 kV and 30 mA. Data were collected in the 2θ range of 5° to 60° with a scan rate of 2°/min [17].

2.7. Bioplastic Production from PHA

Bioplastic films were made using the solvent casting method with PHA concentrations of 4, 5, and 6% w/v. PHA was dissolved in acetone with a solvent-to-polymer ratio of 2:1. The mixture was stirred for 8 hours at 30°C to ensure the PHA was completely dissolved. Sonication was then carried out for 3 hours to improve the homogeneity of the solution, reduce polymer aggregation, and enhance the uniformity of the film. Sonication also helped to partially modify the polymer surface to support better film formation [18]. The homogeneous polymer solution was then poured onto clean glass plates and dried at room temperature to evaporate the solvent [19]. After drying, the films were carefully removed and stored in a desiccator until further analysis. The resulting films were then characterized to measure water absorption, biodegradability, tensile strength, and elastic modulus.

2.7.1. Measure Water Absorption

Bioplastic films, each measuring 3 × 3 cm, were initially cleaned and weighed (W_1). They were then submerged in 20 mL of distilled water in petri dishes for 48 hours. After soaking, the films were gently blotted to remove surface moisture without complete drying and weighed again (W_2). Water absorption was calculated using Equation 1.

$$\%Water\ absorption = \frac{W_2 - W_1}{W_1} \times 100\% \qquad (1)$$

2.7.2. Biodegradability

PHA films were cut into 3 × 3 cm pieces and weighed (W_1), then buried in soil at a depth of approximately 10 cm (pH ~7.3, 80% moisture, and temperature of ~30°C). The films were buried for seven days, then retrieved, gently cleaned, and reweighed (W_2). The biodegradability of the PHA films was calculated using Equation 2.

$$\%Biodegradability = \frac{W_2 - W_1}{W_1} \times 100\% \qquad (2)$$

Where, W_1 was the initial dry weight, and W_2 was the final dry weight after burial.

2.7.3. Tensile Strength and Elastic Modulus

Tensile strength and elastic modulus of the PHA film were measured according to ASTM D882-12 using a universal testing machine (Model 5543A, Instron Engineering Corp., Norwood, MA, USA). Film strips (approximately 1 × 8 cm^2) were placed between grips with an initial gauge length of 40 mm and pulled at a constant crosshead speed of 5 mm/min. Tensile strength (the maximum stress at break) and elastic modulus (the initial slope of the stress–strain curve) were recorded [20].

3. Results and Discussion

3.1. Characterization of POME Before and After Fermentation

The pollutant content in the substrate can be used to evaluate the effectiveness of the fermentation process [21]. The values of BOD, COD, NH_3, and TSS before and after fermentation are shown in Table 1.

Table 1 shows changes in POME characteristics before and after fermentation. There was a significant decrease in BOD level from 46,250 mg/L to 2,553 mg/L. COD level also decreased from 12,250 mg/L to 5,550 mg/L. The NH_3 concentration dropped from 18.3 mg/L to 8.51 mg/L, and the TSS level decreased from 3.034 mg/L to 2.524 mg/L after fermentation. These results indicate that the majority of biodegradable organic matter and other oxidizable compounds were degraded during the fermentation process [22].

Furthermore, the findings suggest that bacteria reduced the oxygen demand required for organic matter decomposition. The reduction in ammonia concentration also implies that nitrogen in the form of ammonia was utilized by the microbes as a nitrogen source, contributing to cell growth and PHA synthesis. The results indicate that PHA was produced as a carbon and energy storage material under nutrient stress conditions, specifically, when excess carbon sources were present while nitrogen was limited [23]. The decrease in NH_3 supports the idea that nitrogen limitation triggered PHA production by the bacteria. The data show a significant reduction in all parameters after fermentation. The average BOD decreased from 46,249.3 mg/L to 2,548 mg/L, COD from 12,249 mg/L to 5,550 mg/L, NH_3 from 18.326 mg/L to 8.5 mg/L, and TSS from 3,032.6 mg/L to 2,522.6 mg/L. These reductions were accompanied by low standard deviations, indicating consistent and accurate measurements. Statistically, these results confirm that the fermentation process was effective in reducing the pollutant content in the substrate.

3.2. PHA Formation from POME Fermentation

The PHA synthesis process using *Pseudomonas aeruginosa* was carried out for six days using a fed-batch method under aerobic conditions, with aeration applied for 8 hours per day. During the fermentation process, biomass levels and relative PHA production were monitored, as shown in Figure 1.

Figure 1a shows biomass production over a 6-day fed-batch fermentation under alternating aerobic and anaerobic conditions. On the first day, biomass remained low as the microorganisms were in the lag phase, adapting to the fermentation environment. The initial sub-phase (Lag1) reflects metabolic adaptation, whereas the subsequent sub-phase (Lag2) involves slow cell growth before entering the exponential phase [24]. Following the addition of POME as a substrate and daily aeration for 8 hours, a significant increase in biomass was observed on day 3 across all substrate concentrations (5%, 10%, and 15% v/v), reaching approximately 12.08, 15.12, and 16.89 g/kg, respectively. These results indicate that the culture had entered the exponential growth phase and was actively synthesizing PHA. A decline in biomass was recorded on day 4, and fermentation was terminated on day 6, yielding final biomass concentrations of 9.78, 10.55, and 10.95 g/kg for each respective substrate concentration.

Previous studies using Pseudomonas fluorescens for PHA production from waste cooking oil under fed-batch conditions have reported significantly higher dry cell densities compared to batch cultures. Moreover, both 3 L and 10 L-scale fermenters demonstrated high productivity within a relatively short duration (48–60 hours), with dry biomass reaching up to 0.64 g/L [25]. These findings support the effectiveness of the fed-batch strategy in enhancing cell density and PHA accumulation due to its gradual carbon feeding mechanism, which mitigates substrate toxicity and maintains optimal growth conditions [26].

Figure 1b presents the relative PHA yield, which aligns with the corresponding biomass levels across different substrate concentrations. On day 3, 5%, 10%, and 15% (v/v) resulted in relative PHA contents of 7.208, 8.616, and 9.089 wt%, respectively—corresponding to an average of 8.304% PHA based on biomass. These data indicate that the dry weights of PHA were 0.87, 1.302, and 1.535 g, respectively. This observation aligns with earlier studies reporting that, under fed-batch conditions with excess carbon and limited oxygen availability, microorganisms such as *Pseudomonas aeruginosa* tend to shift their metabolism toward PHA accumulation as an energy storage strategy [25].

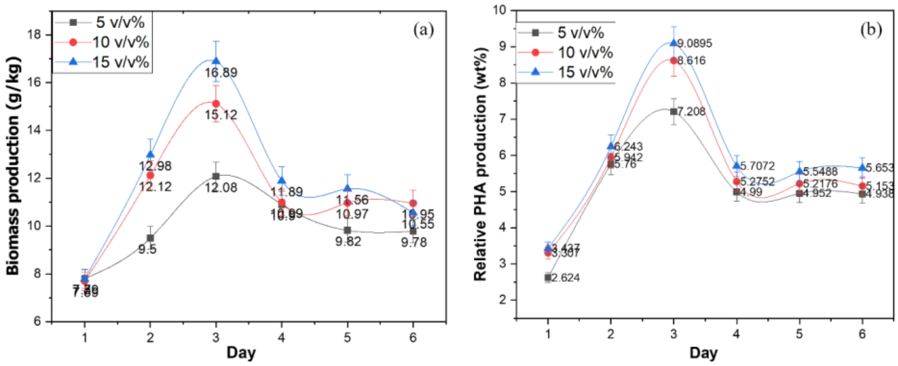

Figure 1. a) biomass production and b) relative PHA production

Table 1. POME specifications before and after fermentation

Parameter	Before fermentation (mg/L)	Error	Mean	Standard deviation	After fermentation (mg/L)	Error	Mean	Standard deviation
BOD	46,250	±1.333	46,249	0.577	2,553	±12.3	2,548	5.686
COD	12,250	±4	12,249	1	5,550	±4	5,550	1
NH_3	18.3	±0.0025	18.33	0.025	8.51	±0.004	8.5	0.01
TSS	3,034	±5.333	3,033	1.154	2,524	±9.333	2,523	1.527

3.3. Characterization of PHA

XRD is commonly used to verify the crystalline structure of PHA, as its degree of crystallinity significantly affects its mechanical properties and biodegradability [27]. The XRD characterization results of the synthesized PHA and standard PHA (poly(3-hydroxybutyrate)) (PHB) are shown in Figure 2. Figure 2 presents the XRD characterization results of the synthesized PHA and the standard PHA (poly(3-hydroxybutyrate)) (PHB). The standard PHA exhibits low peak intensity and a broad diffraction pattern in the 2θ range of 20°–30°, indicating a predominantly amorphous structure with characteristic crystalline features of PHB.

The ratio between the crystalline and amorphous regions in PHA significantly affects its physical and mechanical properties [28]. In contrast, the synthesized PHA exhibits increased peak intensity compared to the standard. Sharper diffraction peaks around a 2θ angle of 20°–30° indicate the development of a more crystalline structure, although not yet reaching optimal crystallinity. The sharper and higher intensity peaks suggest that the molecular structure of PHA became more ordered after synthesis. This increase in crystallinity could potentially slow down the degradation rate, which is beneficial for certain applications [29].

Sharper diffraction peaks in the 2θ range of 20°–30° indicate that the synthesized PHA is a short-chain-length PHA (SCL-PHA), such as poly(3-hydroxybutyrate) (PHB). This observation aligns with previous reports stating that SCL-PHAs generally possess a highly crystalline structure, which gives rise to distinct XRD patterns in this region [28]. The XRD profile of the sample also displays characteristic peaks at approximately 2θ = 13.5°, 16.9°, 20.0°, and 25.5°, corresponding to the (020), (110), (101), and (121) crystal planes of PHB, a well-known SCL-PHA [27]. These peaks are indicative of the semicrystalline nature of PHB, reflecting its organized molecular structure. In contrast, medium-chain-length PHAs (MCL-PHAs) typically exhibit broader and less defined diffraction patterns due to their more amorphous structure [30].

To verify the presence of specific functional groups in the synthesized PHA, FTIR spectroscopy analysis was conducted. The resulting FTIR spectra are shown in Figure 3. Based on Figure 3, the FTIR spectra of the standard PHA (poly(3-hydroxybutyrate)) and the PHA synthesized from POME exhibit significant similarities. The main absorption band in the standard PHA appears around 2900 cm^{-1}, corresponding to C–H stretching, while another prominent peak at approximately 1700 cm^{-1} is attributed to C=O stretching of ester groups. These findings are consistent with previous studies reporting that FTIR peaks at 1720–1730 cm^{-1} (C=O of esters), 2920–2935 cm^{-1} and 2850 cm^{-1} (asymmetric and symmetric C–H stretching of $-CH_2-$ and $-CH_3-$), along with bands at 1270–1280 cm^{-1} and 1375–1460 cm^{-1}, indicate the presence of SCL-PHA, such as PHB [31]. SCL-PHAs typically show strong ester C=O absorption around 1720–1730 cm^{-1}, and sharp methylene and methyl peaks in the 2800–3000 cm^{-1} range [27]. In contrast, MCL-PHAs tend to exhibit broader spectra and slightly shifted carbonyl peaks due to longer side chains, which influence crystallinity and molecular mobility [32].

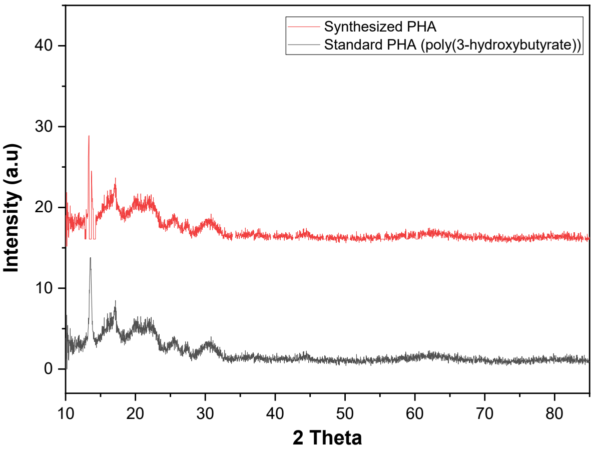

Figure 2. XRD analysis of PHA

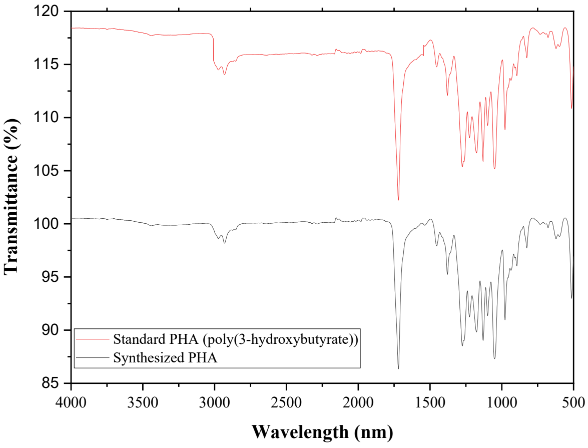

Figure 3. FTIR spectra of PHA

The FTIR spectrum of the synthesized PHA shows slight variations in peak intensity and position, suggesting that its main chemical structure is comparable to that of the standard PHA. Minor differences may result from the production method or the characteristics of the substrate used. The identification of ester (C=O) and methylene (CH_2) groups in both spectra confirms that the PHA produced from POME fermentation has a chemical composition consistent with standard PHA. These results indicate that POME is a promising alternative carbon source for the environmentally friendly and biodegradable production of PHA.

3.4. PHA Film Analysis

Water absorption is an important factor in determining the mechanical stability and environmental resistance of PHA-based materials. Biodegradability refers to the ability of a material to break down naturally by microorganisms into simpler substances, such as water, carbon dioxide, and biomass, without leaving harmful residues in the environment [20]. Figure 4 shows the water absorption and biodegradability of PHA bioplastics prepared at concentrations of 4, 5, and 6% w/v. At 4%, the water absorption was 5.842%, which decreased to 5.357% at a concentration of 5%, indicating improved water resistance.

However, at 6%, the absorption slightly increased to 6.212%. The decrease at 5% is likely due to a more even distribution of the PHA polymer within the matrix, creating a more effective barrier against water penetration. This is consistent with previous studies, showing PHA's good water resistance, with absorption below 5% after 30 days, due to its semi-crystalline structure that slows water diffusion [33]. Lower water absorption indicates higher stability and durability, which is critical for industrial applications such as packaging and medical devices made from biopolymers.

The slight increase in water absorption at 6% is attributed to microstructural changes that increase porosity and facilitate water uptake [34]. Biodegradability also fluctuated with varying PHA concentrations. At 4%, biodegradability reached 28.314%, but dropped to 25.591% at 5%. A significant increase was observed at 6%, reaching 36.029%, indicating that higher polymer concentrations promote microbial degradation. This increase is due to the higher availability of PHA polymer chains that are more susceptible to enzymatic decomposition [35]. However, the decrease of 5% suggests that there may be optimal interactions within the material matrix that reduce its environmental degradation potential. The 5% concentration presents the best compromise between water resistance and biodegradability, making it the preferred formulation for further modification. This outcome aligns with reports that optimal polymer composition yields more balanced material performance for specific applications [36].

To further evaluate the material performance, the mechanical properties of PHA, particularly tensile strength and elastic modulus, were analyzed, as presented in Figure 5. At a concentration of 4%, PHA exhibited a tensile strength of 449.12 MPa and an elastic modulus of 5,443.88 MPa. Increasing the concentration to 5% resulted in a tensile strength of 554.32 MPa and a modulus of 5,834.95 MPa. At 6% concentration, the tensile strength further increased to 587.23 MPa, accompanied by a slight increase in the elastic modulus to 5,872.30 MPa. These findings indicate that tensile strength improves with higher polymer concentration, possibly due to a denser and more crystalline polymer structure, which contributes to greater mechanical integrity [37]. The increase in tensile strength suggests that higher PHA concentrations produce materials that are more resistant to mechanical stress and deformation.

The increase in PHA concentration from 4% to 5% produced a substantial rise in the elastic modulus (from 5,443.88 MPa to 5,834.95 MPa), with a difference of 391.07 MPa. However, at 6%, the modulus only slightly increased to 5,872.30 MPa, representing a relatively small gain of 37.35 MPa compared to 5%. This result indicates the occurrence of a plateau, where further increases in polymer concentration no longer proportionally enhance stiffness. It also demonstrates that at higher monomer concentrations, the elastic modulus may reach saturation due to excessive entanglement and pre-strain within the polymer network. Moreover, studies on PHA have shown that while an increase in the crystalline phase enhances stiffness, it simultaneously reduces chain flexibility, leading to a balance between modulus and elasticity [38].

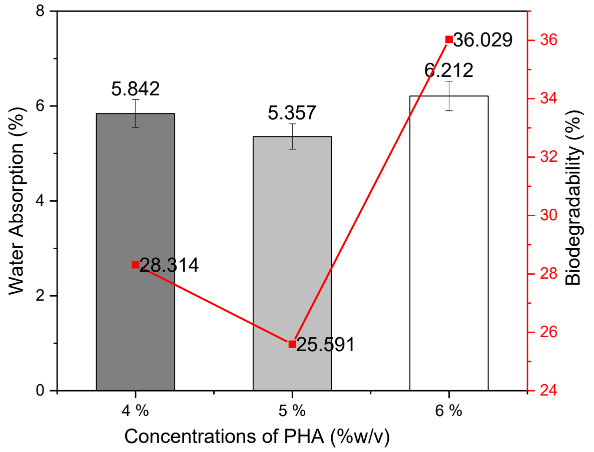

Figure 4. Water absorption and biodegradability of PHA bioplastic film

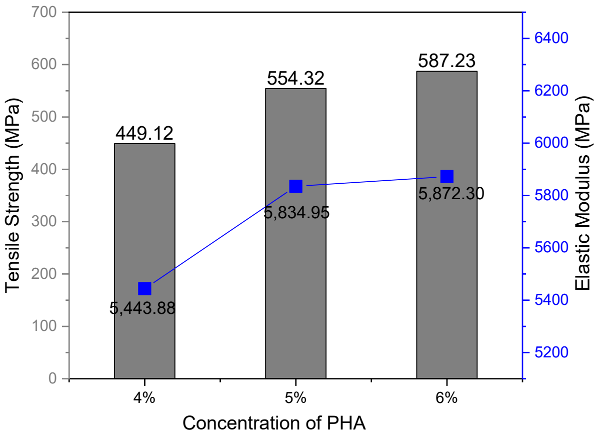

Figure 5. Tensile strength and elastic modulus values of PHA at 4%, 5%, and 6%

4. Conclusion

Based on the results obtained, the fed-batch fermentation of palm oil mill effluent (POME) using *Pseudomonas aeruginosa* proved effective in reducing major pollutants such as BOD, COD, NH_3, and TSS. The process was able to significantly degrade organic and chemical substances. Fermentation also supported biomass formation and PHA production, with a significant increase in biomass observed on the third day at all substrate concentrations (5%, 10%, and 15% v/v), reaching approximately 12.08, 15.12, and 16.89 g/kg, respectively. Relative PHA stability was achieved by the sixth day. Characterization of PHA using XRD and FTIR confirmed that the product obtained from POME fermentation was indeed PHA. Furthermore, water absorption and biodegradability analyses showed that the 5% concentration provided the best balance between water resistance and biodegradability. Although higher concentrations improved biodegradability, they reduced water stability. At the 5% concentration, PHA exhibited a tensile strength of 554.32 MPa and an elastic modulus of 5834.95 MPa. Therefore, the 5% concentration is considered the optimal condition for producing stable PHA, and POME can be regarded as a promising carbon source for environmentally friendly PHA production.

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References

[1] Min Liu, Tianrui Zhang, Liangkun Long, Rui Zhang, Shaojun Ding, Efficient enzymatic degradation of poly (ε-caprolactone) by an engineered bifunctional lipase-cutinase, *Polymer Degradation and Stability*, 160, (2019), 120-125 https://doi.org/10.1016/j.polymdegradstab.2018.12.020

[2] Irma Kresnawaty, Agustin Sri Mulyatni, Deden Dewantara Eris, Haryo Tejo Prakoso, Karakterisasi PHA yang dihasilkan oleh *Pseudomonas aeruginosa* dan *Bacillus subtilis* yang ditumbuhkan dalam media limbah cair pabrik kelapa sawit, *Menara Perkebunan*, 82, 2, (2016), 57-63 https://doi.org/10.22302/iribb.jur.mp.v82i2.20

[3] Viviana Urtuvia, Pamela Villegas, Myriam González, Michael Seeger, Bacterial production of the biodegradable plastics polyhydroxyalkanoates, *International Journal of Biological Macromolecules*, 70, (2014), 208-213 https://doi.org/10.1016/j.ijbiomac.2014.06.001

[4] Saiqa Tufail, Sajida Munir, Nazia Jamil, Variation analysis of bacterial polyhydroxyalkanoates production using saturated and unsaturated hydrocarbons, *Brazilian Journal of Microbiology*, 48, 4, (2017), 629-636 https://doi.org/10.1016/j.bjm.2017.02.008

[5] Dhawal P. Tamboli, Mayur B. Kurade, Tatoba R. Waghmode, Swati M. Joshi, Sanjay P. Govindwar, Exploring the ability of *Sphingobacterium* sp. ATM to degrade textile dye Direct Blue GLL, mixture of dyes and textile effluent and production of polyhydroxyhexadecanoic acid using waste biomass generated after dye degradation, *Journal of Hazardous Materials*, 182, 1, (2010), 169-176 https://doi.org/10.1016/j.jhazmat.2010.06.011

[6] Aznury Martha, Setiadi Tjandra, Identifikasi Senyawa Asam Lemak Volatil dari Air Limbah Industri Minyak Kelapa Sawit untuk Produksi Polihidroksialkanoat oleh *Ralstonia eutropha* JMP 134, *Jurnal Selulosa*, 11, 01, (2021), 49-58

[7] Guilherme H. D. Oliveira, Marcelo Zaiat, José Alberto D. Rodrigues, Juliana A. Ramsay, Bruce A. Ramsay, Towards the Production of mcl-PHA with Enriched Dominant Monomer Content: Process Development for the Sugarcane Biorefinery Context, *Journal of Polymers and the Environment*, 28, 3, (2020), 844-853 https://doi.org/10.1007/s10924-019-01637-2

[8] Mohd Fadhil Md. Din, Ponraj Mohanadoss, Zaini Ujang, Mark van Loosdrecht, Salmiati Muhd Yunus, Shreeshivadasan Chelliapan, Vasudeo Zambare, Gustaf Olsson, Development of Bio-PORec® system for polyhydroxyalkanoates (PHA) production and its storage in mixed cultures of palm oil mill effluent (POME), *Bioresource Technology*, 124, (2012), 208-216 https://doi.org/10.1016/j.biortech.2012.08.036

[9] Noreen Iftikhar, Faiza Quddus, Navera Nadeem, Iftikhar Ali, Muhammad Usama Raza, Muhammad Zaid, Production of Polyhydroxyalkanoates (pha) by *bacillus* and *pseudomonas* on Cheap Carbon Substrates, *Brazilian Archives of Biology and Technology*, 67, (2024), https://doi.org/10.1590/1678-4324-2024230082

[10] Maciej W. Guzik, Gearóid F. Duane, Shane T. Kenny, Eoin Casey, Paweł Mielcarek, Magdalena Wojnarowska, Kevin E. O'Connor, A polyhydroxyalkanoates bioprocess improvement case study based on four fed-batch feeding strategies, *Microbial Biotechnology*, 15, 3, (2022), 996-1006 https://doi.org/10.1111/1751-7915.13879

[11] Sharifah Mohammad, Siti Baidurah, Takaomi Kobayashi, Norli Ismail, Cheu Peng Leh, Palm Oil Mill Effluent Treatment Processes—A Review, *Processes*, 9, 5, (2021), 739 https://doi.org/10.3390/pr9050739

[12] Nurul Atiqah Osman, Farhana Aziz Ujang, Ahmad Muhaimin Roslan, Mohamad Faizal Ibrahim, Mohd Ali Hassan, The effect of Palm Oil Mill Effluent Final Discharge on the Characteristics of *Pennisetum purpureum*, *Scientific Reports*, 10, 1, (2020), 6613 https://doi.org/10.1038/s41598-020-62815-0

[13] Salmiati, Mohd Razman Salim, Zaini Ujang, Farrah Aini Dahalan, Gustaf Olsson, Polyhydroxyalkanoates (PHAs) production from complex polymer organic waste using anaerobic and aerobic sequence batch reactor, *Journal of Biochemistry, Microbiology and Biotechnology*, 2, 2, (2014), 61-66 https://doi.org/10.54987/jobimb.v2i2.152

[14] Isabel Thiele, Lara Santolin, Svea Detels, Riccardo Osele, Peter Neubauer, Sebastian L. Riedel, High-cell-density fed-batch strategy to manufacture tailor-made P(HB-co-HHx) by engineered *Ralstonia eutropha* at laboratory scale and pilot scale, *Microbial Biotechnology*, 17, 6, (2024), e14488 https://doi.org/10.1111/1751-7915.14488

[15] Rijuta Ganesh Saratale, Si-Kyung Cho, Ganesh Dattatraya Saratale, Manu Kumar, Ram Naresh Bharagava, Sunita Varjani, Avinash A. Kadam, Gajanan S. Ghodake, Ramasubba Reddy Palem, Sikandar I. Mulla, Dong-Su Kim, Han-Seung Shin, An Overview of Recent Advancements in Microbial Polyhydroxyalkanoates (PHA) Production from Dark Fermentation Acidogenic Effluents: A Path to an Integrated Bio-Refinery, *Polymers*, 13, 24, (2021), 4297 https://doi.org/10.3390/polym13244297

[16] Giorgia Pagliano, Paola Galletti, Chiara Samorì, Agnese Zaghini, Cristian Torri, Recovery of Polyhydroxyalkanoates From Single and Mixed Microbial Cultures: A Review, *Frontiers in Bioengineering and Biotechnology*, Volume 9 - 2021, (2021), https://doi.org/10.3389/fbioe.2021.624021

[17] Gabriela J. Pedroso, Desirée M. S. Costa, Lucas T. Felipe Kokuszi, Eduardo B. V. da Silva, Marcos F. O. Cavalcante, Eduardo Junca, Cassio A. O. Moraes, Claus T. Pich, Vânia R. de Lima, Sumbal Saba, Jamal Rafique, Tiago E. A. Frizon, Selenylated indoles: synthesis, effects on lipid membrane properties and DNA cleavage, *New Journal of Chemistry*, 47, 6, (2023), 2719-2726 https://doi.org/10.1039/d2nj04330k

[18] Luis Getino, Irene García, Alfonso Cornejo, Raúl Mateos, Luisa M. Ariza-Carmona, Natalia Sánchez-Castro, José F. Moran, Elías R. Olivera, Alejandro Chamizo-Ampudia, The Effectiveness of Polyhydroxyalkanoate (PHA) Extraction Methods in Gram-Negative *Pseudomonas putida* U, *Polymers*, 17, 2, (2025), 150 https://doi.org/10.3390/polym17020150

[19] Lorena Atarés, Amparo Chiralt, Chelo González-Martínez, Maria Vargas, Production of Polyhydroxyalkanoates for Biodegradable Food Packaging Applications Using *Haloferax mediterranei* and Agrifood Wastes, *Foods*, 13, 6, (2024), 950 https://doi.org/10.3390/foods13060950

[20] Nadia Altaee, Gamal A. El-Hiti, Ayad Fahdil, Kumar Sudesh, Emad Yousif, Biodegradation of different formulations of polyhydroxybutyrate films in soil, *SpringerPlus*, 5, 1, (2016), 762 https://doi.org/10.1186/s40064-016-2480-2

[21] Haithem Aib, Ildiko Czegeny, Ramzi Benhizia, Herta Mária Czédli, Evaluating the Efficiency of Wastewater Treatment Plants in the Northern Hungarian Plains Using Physicochemical and Microbiological Parameters, *Water*, 16, 24, (2024), 3590 https://doi.org/10.3390/w16243590

[22] Nezha Tahri Joutey, Wifak Bahafid, Hanane Sayel, Naima El Ghachtouli, Biodegradation: Involved Microorganisms and Genetically Engineered Microorganisms, in: R. Chamy (Ed.) *Biodegradation - Life of Science*, IntechOpen, Rijeka, 2013, https://doi.org/10.5772/56194

[23] Martin Koller, Chapter 1 - Production, properties, and processing of microbial polyhydroxyalkanoate (PHA) biopolyesters, in: S. Das, H.R. Dash (Eds.) *Microbial and Natural Macromolecules*, Academic Press, 2021, https://doi.org/10.1016/B978-0-12-820084-1.00001-6

[24] Beom-Jung Kang, Jong-Min Jeon, Shashi Kant Bhatia, Do-Hyung Kim, Yung-Hun Yang, Sangwon Jung, Jeong-Jun Yoon, Two-Stage Bio-Hydrogen and Polyhydroxyalkanoate Production: Upcycling of Spent Coffee Grounds, *Polymers*, 15, 3, (2023), 681 https://doi.org/10.3390/polym15030681

[25] Rawia F. Gamal, Hemmat M. Abdelhady, Taha A. Khodair, Tarek S. El-Tayeb, Enas A. Hassan, Khadiga A. Aboutaleb, Semi-scale production of PHAs from waste frying oil by *Pseudomonas fluorescens* S48, *Brazilian Journal of Microbiology*, 44, 2, (2013), https://doi.org/10.1590/S1517-83822013000200034

[26] Manaswini Gundlapalli, Sunantha Ganesan, Polyhydroxyalkanoates (PHAs): Key Challenges in production and sustainable strategies for cost reduction within a circular economy framework, *Results in Engineering*, 26, (2025), 105345 https://doi.org/10.1016/j.rineng.2025.105345

[27] K. Sudesh, H. Abe, Y. Doi, Synthesis, structure and properties of polyhydroxyalkanoates: biological polyesters, *Progress in Polymer Science*, 25, 10, (2000), 1503-1555 https://doi.org/10.1016/S0079-6700(00)00035-6

[28] Bronwyn Laycock, Peter Halley, Steven Pratt, Alan Werker, Paul Lant, The chemomechanical properties of microbial polyhydroxyalkanoates, *Progress in Polymer Science*, 39, 2, (2014), 397-442 https://doi.org/10.1016/j.progpolymsci.2013.06.008

[29] Harshini Pakalapati, Chih-Kai Chang, Pau Loke Show, Senthil Kumar Arumugasamy, John Chi-Wei Lan, Development of polyhydroxyalkanoates production from waste feedstocks and applications, *Journal of Bioscience and Bioengineering*, 126, 3, (2018), 282-292 https://doi.org/10.1016/j.jbiosc.2018.03.016

[30] Patricia Feijoo, Kerly Samaniego-Aguilar, Estefanía Sánchez-Safont, Sergio Torres-Giner, Jose M. Lagaron, Jose Gamez-Perez, Luis Cabedo, Development and Characterization of Fully Renewable and Biodegradable Polyhydroxyalkanoate Blends with Improved Thermoformability, *Polymers*, 14, 13, (2022), 2527 https://doi.org/10.3390/polym14132527

[31] Karlo Grgurević, Dora Bramberger, Martina Miloloža, Krešimir Stublić, Vesna Ocelić Bulatović, Jasmina Ranilović, Šime Ukić, Tomislav Bolanča, Matija Cvetnić, Marinko Markić, Dajana Kučić Grgić, Producing and Characterizing Polyhydroxyalkanoates from Starch and Chickpea Waste Using Mixed Microbial Cultures in Solid-State Fermentation, *Polymers*, 16, 23, (2024), 3407 https://doi.org/10.3390/polym16233407

[32] Pinanong Tanikkul, Geraint L. Sullivan, Sarper Sarp, Nipon Pisutpaisal, Biosynthesis of medium chain length polyhydroxyalkanoates (mcl-PHAs) from palm oil, *Case Studies in Chemical and Environmental Engineering*, 2, (2020), 100045 https://doi.org/10.1016/j.cscee.2020.100045

[33] Ana Rita C. Duarte, Simone S. Silva, João F. Mano, Rui L. Reis, Ionic liquids as foaming agents of semi-crystalline natural-based polymers, *Green Chemistry*, 14, 7, (2012), 1949-1955 https://doi.org/10.1039/C2GC16652F

[34] Veena Paul, Saloni Rai, Urvashi Vikranta, Alisha Nandan, Aparna Agarwal, Abhishek Dutt Tripathi, Starch-PHA Blend-Based Biopolymers with Potential Food Applications, *Starch - Stärke*, 76, 11-12, (2024), 2300131 https://doi.org/10.1002/star.202300131

[35] Kjeld W. Meereboer, Manjusri Misra, Amar K. Mohanty, Review of recent advances in the biodegradability of polyhydroxyalkanoate (PHA) bioplastics and their composites, *Green Chemistry*, 22, 17, (2020), 5519-5558 https://doi.org/10.1039/d0gc01647k

[36] Martin Koller, Rodolfo Bona, Gerhart Braunegg, Carmen Hermann, Predrag Horvat, Markus Kroutil, Julia Martinz, Jose Neto, Luis Pereira, Paula Varila, Production of Polyhydroxyalkanoates from Agricultural Waste and Surplus Materials, *Biomacromolecules*, 6, 2, (2005), 561-565 https://doi.org/10.1021/bm049478b

[37] Ailifeire Fulati, Koichiro Uto, Mitsuhiro Ebara, Influences of Crystallinity and Crosslinking Density on the Shape Recovery Force in Poly(ε-Caprolactone)-Based Shape-Memory Polymer Blends, *Polymers*, 14, 21, (2022), 4740 https://doi.org/10.3390/polym14214740

[38] Guo-Qiang Chen, Qiong Wu, The application of polyhydroxyalkanoates as tissue engineering materials, *Biomaterials*, 26, 33, (2005), 6565-6578 https://doi.org/10.1016/j.biomaterials.2005.04.036