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Research article

Production of Polyhydroxyalkanoates (PHAs) from Oil Palm Waste Using *Paraburkholderia* sp. PFN29

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Abstract

The increasing accumulation of plastic waste has intensified efforts to identify sustainable biodegradable alternatives. Polyhydroxyalkanoates (PHAs) are microbial polyesters with properties similar to conventional plastics; however, their commercial feasibility is limited by high production costs. This study investigated the use of oil palm waste, specifically palm kernel meal (PKM) and empty fruit bunch (EFB), as feedstocks for PHA production by *Paraburkholderia* sp. PFN29. Biomass pretreatment used sodium hydroxide (1-5% w/v) followed by hydrogen peroxide (3% v/v), and enzymatic hydrolysis with pectinase, xylanase, and cellulase. Reducing sugars were quantified using the dinitrosalicylic acid (DNS) method, and morphological changes were examined via scanning electron microscopy (SEM). Optimal saccharification with 3% (w/v) NaOH at 50°C for 48 h yielded 10.53±0.33 and 21.02±0.38 mg/mL reducing sugars from PKM and EFB, respectively. Glucose was the predominant sugar in both hydrolysates. PHA biosynthesis was assessed under various carbon- and nitrogen-supplementation conditions. The EFB hydrolysate at 100 ml supplemented with 0.1 g NH_4Cl produced the highest PHA concentration (1.12±0.02 g/L), PHA content (59.02%), and productivity (0.01 g/L/h), which was comparable to that of the mineral medium control. The PKM hydrolysate supported optimal PHA production without supplementation (PHA content, 39.02%). Fourier-transform infrared (FTIR) and nuclear magnetic resonance (NMR) analyses confirmed PHB-type PHA production. These findings indicate that palm oil residues, particularly EFB hydrolysates under optimized nitrogen conditions, are promising substrates for sustainable PHA production.

Keywords: polyhydroxyalkanoate; bioplastic; palm kernel meal; empty fruit bunch; poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate)

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1. Introduction

The global consumption of petroleum-based plastics has significantly increased over the past few decades, resulting in substantial accumulation of plastic waste in terrestrial and aquatic ecosystems. Owing to their resistance to degradation, synthetic plastics can remain in the environment for decades to centuries, posing serious environmental and health concerns (IUCN, 2024). Inadequate end-of-life management further exacerbates their environmental impact, as landfilling contributes to long-term contamination, whereas incineration produces greenhouse gases and releases toxic compounds, such as dioxins and furans, which pose significant risks to human and ecosystem health (Chae & An, 2018; Fayshal, 2024). Consequently, the development and implementation of biodegradable plastics derived from renewable resources have emerged as a crucial strategy for mitigating environmental pollution and facilitating the transition to a circular bioeconomy (Sun et al., 2025).

Among the various classes of bio-based and biodegradable polymers, polyhydroxyalkanoates (PHAs) have attracted considerable attention as promising alternatives to conventional plastics. PHAs are intracellular polyesters synthesized by a wide range of bacteria as carbon and energy-storage materials. They exhibit physicochemical and mechanical properties that are comparable to those of commodity thermoplastics. Importantly, PHAs are fully biodegradable under diverse environmental conditions, including soil, freshwater, and marine habitats, thereby reducing their long-term ecological impacts. However, the large-scale commercialization of PHAs is still constrained by high production costs, primarily associated with the use of refined carbon sources and downstream processing (Sun et al., 2025). PHAs are a structurally diverse group of microbial polyesters, and their properties are predominantly influenced by the composition and chain length of their monomers. Over 150 distinct 3-hydroxyalkanoate monomers have been identified, facilitating the production of homopolymers and copolymers that exhibit a broad spectrum of thermal and mechanical properties (Sehgal & Gupta, 2020). PHAs are typically categorized into three subclasses based on the number of carbon atoms in their repeating units: short-chain-length (scl-PHA; 3-5 carbons), medium-chain-length (mcl-PHA; 6-14 carbons), and long-chain-length (lcl-PHA; ≥15 carbons) PHAs. Scl-PHAs, such as poly(3-hydroxybutyrate) (PHB), poly(3-hydroxyvalerate) (PHV), and their copolymer poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV), are generally recognized for their high crystallinity, rigidity, and thermal stability, although they can be relatively brittle. Conversely, mcl-PHAs, which include monomers such as 3-hydroxyhexanoate or 3-hydroxyoctanoate, are characterized by reduced crystallinity and melting points, coupled with enhanced elasticity and toughness, making them suitable for applications that require flexible or elastomeric materials. Lcl-PHAs, composed of monomers with more than 14 carbon atoms, have been less extensively explored but offer potential for developing materials with soft and rubber-like properties (Samrot et al., 2021; Zytner et al., 2023). The structural diversity of PHAs allows for the tailoring of materials for specific applications by selecting appropriate monomer types and ratios, which can be achieved through metabolic pathway engineering or co-feeding strategies during fermentation. For instance, the integration of monomers such as 4-hydroxybutyrate, 3-hydroxyvalerate, or medium-chain-length 3-hydroxyalkanoates into PHB backbones can significantly mitigate brittleness and enhance processability, thereby broadening the applicability of PHAs in packaging, biomedical devices, and other high-value sectors (Li et al., 2016; Gao et al., 2022).

Recent research has increasingly focused on the utilization of low-cost, renewable, and locally available agro-industrial residues as substrates for PHA production (Zytner et

al., 2023). The processing of oil palm generates significant quantities of lignocellulosic by-products, such as palm kernel meal (PKM) and empty fruit bunch (EFB), which are frequently underutilized and may present environmental management challenges (Wahyudin & Oge, 2025). These residues are rich in structural carbohydrates that can be converted into fermentable sugars through appropriate pretreatment and enzymatic hydrolysis, rendering them promising feedstocks for microbial fermentation (Díez et al., 2020; Zytner et al., 2023). Numerous studies have demonstrated the potential of oil palm residues for PHA biosynthesis. For instance, hydrolysates derived from oil palm empty fruit bunches (OPEFB) were successfully employed for the production of PHB by bacterial strains such as *Bacillus megaterium* R11, with PHB content and production reaching 58.5% and 9.32 g/L, respectively (Zhang et al., 2013), and *Bacillus cereus suaeda* B-001, achieving PHB contents exceeding 55.40% of cell dry weight using OPEFB hydrolysates at 20 g/L (Yustinah et al., 2019). Additionally, palm oil mill effluent (POME) has been investigated as a carbon-rich substrate for PHA production in mixed microbial cultures, demonstrating the feasibility of converting palm oil-processing wastewater into value-added biopolymers. Similarly, by-products generated during palm oil refining have been evaluated as alternative carbon sources for PHB synthesis by *Cupriavidus necator* and its engineered strains (Zhang et al., 2022), and *Pseudomonas aeruginosa* (Ulia et al., 2025).

Despite these advances, information on the utilization of PKM and EFB hydrolysates for PHA production by *Paraburkholderia* species remains limited. Previous studies have demonstrated that *Paraburkholderia* sp. PFN29 can accumulate PHA when cultivated in defined synthetic media (Sriyapai et al., 2022); however, its ability to utilize lignocellulosic hydrolysates derived from oil palm residues has not been comprehensively investigated. In this study, we applied an integrated approach combining biomass pretreatment, enzymatic hydrolysis, and microbial fermentation to evaluate PHA production from PKM and EFB hydrolysates using *Paraburkholderia* sp. PFN29. This study provides a practical and sustainable strategy for the valorization of oil palm waste into value-added biopolymers and contributes to the development of renewable feedstocks for biodegradable plastic production.

2. Materials and Methods

2.1 Preparation and pretreatment of PKM and EFB

PKM and EFB were obtained from a palm oil mill in Thailand. PKM was dried and ground to obtain a coarse powder. EFB was cut into small pieces, oven-dried at 50°C for 24 h, ground, and sieved through a 40-mesh screen. Biomass (20 g) was treated with 200 mL of sodium hydroxide (NaOH) solution at concentrations of 1%, 3%, and 5% (w/v) and autoclaved at 121°C for 30 min (Thanapimmetha et al., 2023; Premjet & Premjet, 2025). The samples were then washed with distilled water until their pH reached approximately 10. Subsequently, 200 mL of 3% (v/v) hydrogen peroxide (H_2O_2) was added, and the mixture was incubated at 60°C for 24 h (da Silva et al., 2025). After pretreatment, the biomass was washed thoroughly with distilled water until a neutral pH was achieved and then dried at 60°C to a constant weight. Untreated and pretreated PKM and EFB samples were analyzed for surface morphology and structural changes using a scanning electron microscope (JSM-7610F, JEOL Ltd., Tokyo, Japan) at the Scientific and Technological Research Equipment Center, Chulalongkorn University, Thailand.

2.2 Enzymatic hydrolysis of alkali-pretreated PKM and EFB

PKM and EFB pretreated with NaOH solution, as described in Section 2.1, were subjected to enzymatic hydrolysis using three commercial enzymes obtained from REACH Biotechnology Co., Ltd. (Bangkok, Thailand): pectinase (60,000 IU/mL), xylanase (100,000 IU/mL), and cellulase (13,000 IU/mL). The enzymes were added at volumes of 200 µL (pectinase), 500 µL (xylanase), and 500 µL (cellulase), corresponding to a total enzyme loading of approximately 1.2 mL/g biomass. Briefly, 1 g of pretreated PKM or EFB was transferred to a 250 mL Erlenmeyer flask containing 50 mL of 50 mM phosphate buffer (pH 6.0). Enzymatic hydrolysis was carried out at different temperatures (40, 50, and 60°C) for 24 and 48 h to determine the optimal saccharification conditions (Thanapimmetha et al., 2023; Premjet & Premjet, 2025). After incubation, the hydrolysates were filtered to remove residual solids, and the pH of the filtrate was adjusted to 7.0 to obtain conditioned media for subsequent microbial cultivation. The chemical composition and sugar profile of the hydrolysates were analyzed at the Food Research and Testing Laboratory, Faculty of Science, Chulalongkorn University. Individual sugar components were determined using high-performance liquid chromatography (HPLC). Reducing sugars were quantified using the 3,5-dinitrosalicylic acid (DNS) method according to Miller (1959). Briefly, 1 mL of sample was mixed with 1 mL of DNS reagent (16 g/L NaOH, 10 g/L DNS, and 300 g/L potassium sodium tartrate tetrahydrate) and heated in a boiling water bath for 10 min. After cooling to room temperature, absorbance was measured at 540 nm using a UV-Vis spectrophotometer (UNICO UV-2100, USA). Glucose solutions (0-1.0 mg/mL) were used to generate a standard calibration curve for quantifying glucose. All experiments were performed in triplicate.

2.3 PHA production and extraction

2.3.1 Screening of PHA accumulation by Nile red staining

Paraburkholderia sp. PFN29 was originally isolated from soil samples collected in Thailand (Sriyapai et al., 2022) and was maintained on nutrient agar at 30°C for 72 h. To screen for PHA accumulation, a single colony was inoculated onto a mineral medium supplemented with 2% (w/v) glucose and Nile red (0.5 mg dissolved in dimethyl sulfoxide). The plates were incubated at 30°C for 72 h. Intracellular PHA granule accumulation was examined by observing fluorescence under ultraviolet (UV) illumination. *Escherichia coli* was used as the negative control.

2.3.2 Starter preparation and PHA production

Paraburkholderia sp. PFN29 was streaked onto nutrient agar (NA) and incubated at 30°C for 72 h. A single colony was transferred to 10 mL of nutrient broth (NB) and cultured at 30°C for 48 h with shaking at 180 rpm. Cell growth was monitored by measuring the optical density at 600 nm (OD_{600}) using a spectrophotometer (UNICO UV-2100). The resulting culture (OD_{600} = 1.9) was used as the stock culture. Subsequently, 10 mL of the stock culture was inoculated into 100 mL of PHA mineral medium containing 2% (w/v) glucose and 0.1% (w/v) NH_4Cl in a 250 mL Erlenmeyer flask. The mineral medium consisted of (per liter) $Na_2HPO_4 \cdot 12H_2O$ (3.0 g), KH_2PO_4 (0.5 g), $MgSO_4 \cdot 7H_2O$ (0.1 g), $CaCl_2$ (0.1 g), and 10 mL trace element solution. The trace element solution (per liter) contained $ZnSO_4 \cdot 7H_2O$ (0.01 g), $MnCl_2 \cdot 4H_2O$ (0.003 g), H_3BO_3 (0.03 g), $CoCl_2 \cdot 6H_2O$ (0.02 g), $CuSO_4 \cdot 5H_2O$ (0.001

g), $NiCl_2 \cdot 6H_2O$ (0.002 g), $NaMoO_4 \cdot 2H_2O$ (0.003 g), and $FeCl_3$ (0.01 g). This mineral medium was used as a defined basal medium for PHA production and served as a control to evaluate the performance of waste-derived hydrolysates. The culture was incubated at 30°C for 96 h with agitation at 180 rpm. After incubation, the cells were harvested by centrifugation at 9,100 × g for 10 min. The cell pellets were washed twice with sterile 0.85% (w/v) NaCl solution and subsequently used for PHA extraction. The dry weight of the cells was determined as follows: cell pellets were washed twice with sterile 0.85% (w/v) sodium chloride solution to remove residual medium components. The washed cells were then dried in a hot air oven at 60°C for 24 h until a constant weight was obtained. The dried biomass was weighed to determine the cell dry weight, which was subsequently used to calculate the percentage of PHA content.

2.3.3 PHA extraction

PHAs were extracted using the modified sodium hypochlorite–chloroform method described by Hahn et al. (1994). Briefly, 10 mL of chloroform and 10 mL of sodium hypochlorite solution were added to 1 g of dried-cell biomass. The mixture was incubated at 30°C for 5 h with agitation at 180 rpm to promote cell lysis and polymer extraction. Following incubation, the mixture was centrifuged at 9,100 × g for 10 min, resulting in the formation of three distinct layers: an upper aqueous sodium hypochlorite phase, a middle layer containing cell debris, and a lower chloroform phase containing dissolved PHA. The chloroform layer was carefully collected using a Pasteur pipette and transferred to a clean glass tube. To precipitate the polymer, two volumes of 70% methanol were added to the chloroform extract and gently mixed. The upper methanol layer was discarded, and the remaining chloroform was evaporated in a hot water bath at 70°C. The resultant polymer precipitate was subsequently dried in an oven at 60°C until completely dry. The dried polymer was weighed to determine the PHA yield relative to the dry cell weight using the following equation (1):

$$\text{PHA content (\%)} = \frac{\textit{Weight of extracted PHA}}{\textit{Dry cell weight}} \times 100 \quad (1)$$

2.4 PHA production by *Paraburkholderia* sp. PFN29 using conditioned media derived from alkali-pretreated and enzymatically hydrolyzed PKM and EFB

The starter culture of *Paraburkholderia* sp. PFN29 was prepared as described in Section 2.3.2. The cells were then cultured in conditioned medium. In this study, the conditioned medium refers to hydrolysates obtained from alkali-pretreated (3% (w/v) NaOH and 3% (v/v) H_2O_2) and enzymatically hydrolyzed PKM and EFB. The hydrolysates were used directly as basal fermentation media without dilution. The resulting hydrolysates were supplemented with various carbon and nitrogen sources. Four experimental conditions were established: (1) conditioned medium without additional supplementation, (2) conditioned medium supplemented with NH_4Cl (0.1%, w/v), (3) conditioned medium supplemented with glucose (2%, w/v), and (4) conditioned medium supplemented with both glucose (2%, w/v) and NH_4Cl (0.1%, w/v). Each treatment was prepared in a total volume of 100 mL in 250 mL Erlenmeyer flasks and sterilized by autoclaving at 110°C for 10 min. The entire hydrolysate volume (100 mL) was used for each experimental condition. The cultures were incubated at 30°C with shaking at 180 rpm for 96 h. The mineral medium described in Section 2.3.2 was used as a control. Following incubation, PHA was extracted as described in Section 2.3.3. All experiments were performed in triplicate.

2.5 Polymer characterization

2.5.1 Fourier transform infrared spectroscopy (FT-IR)

The functional groups of the extracted PHA were analyzed using a Fourier-transform infrared (FT-IR) spectrometer (Spectrum Two, PerkinElmer, USA). The extracted polymer was dried after solvent evaporation and cast into a PHB film, which was then analyzed directly using FT-IR. Infrared spectra were recorded over the wavenumber range of 4000-400 cm^{-1}. This analysis was conducted to identify the characteristic absorption bands associated with PHA polymers (Etxabide et al., 2022).

2.5.2 Nuclear magnetic resonance (NMR)

The chemical structure of the PHA polymer produced by PFN29 was further examined using proton nuclear magnetic resonance (^{1}H NMR) spectroscopy. Purified PHA samples (20–80 mg) were dissolved in deuterated chloroform ($CDCl_3$) and analyzed using a Fourier transform NMR spectrometer (Bruker Avance III, 400 MHz, Germany) at room temperature. Tetramethylsilane (TMS) was used as an internal standard. NMR measurements were conducted at the Scientific and Technological Research Equipment Center of Chulalongkorn University, Thailand. The resulting spectra were interpreted to verify the presence of characteristic proton signals corresponding to the PHB polymer structure (Trakunjae et al., 2021; Etxabide et al., 2022).

2.6 Statistical analysis

Statistical differences among the treatments were evaluated using one-way analysis of variance (ANOVA). When significant differences were observed, Tukey's honestly significant difference (HSD) test was applied for multiple comparisons at a 95% confidence level ($p<0.05$). Differences were considered statistically significant at $p<0.05$. All experiments were conducted in triplicate ($n = 3$). All statistical analyses were performed using R software (R Core Team, 2016).

3. Results and Discussion

3.1 Effect of alkaline pretreatment on the structure of PKM and EFB

PKM typically contains moderate levels of crude protein (approximately 14-19%) and relatively high crude fiber content (approximately 15-22%), indicating the presence of a substantial fraction of non-starch polysaccharides (Iyayi et al., 2015; Tang, 2021; Zubaidah et al., 2024). The raw physical appearances of PKM and EFB are shown in Figures 1a and 1b, respectively. PKM appears as a fine granular material, whereas EFB exhibits a fibrous, lignocellulosic structure. The cell wall structure of PKM is dominated by mannan-rich hemicellulose, which can account for more than 35% of the biomass, accompanied by smaller proportions of cellulose and minor amounts of xylan (Zubaidah et al., 2024; Shen et al., 2025). This compositional profile favors the use of enzymatic systems containing mannanase and cellulase, often supplemented with accessory hemicellulases, for the effective hydrolysis and saccharification of PKM-derived substrates (Iyayi et al., 2015; Zubaidah et al., 2024). The precise composition of PKM may vary depending on the oil

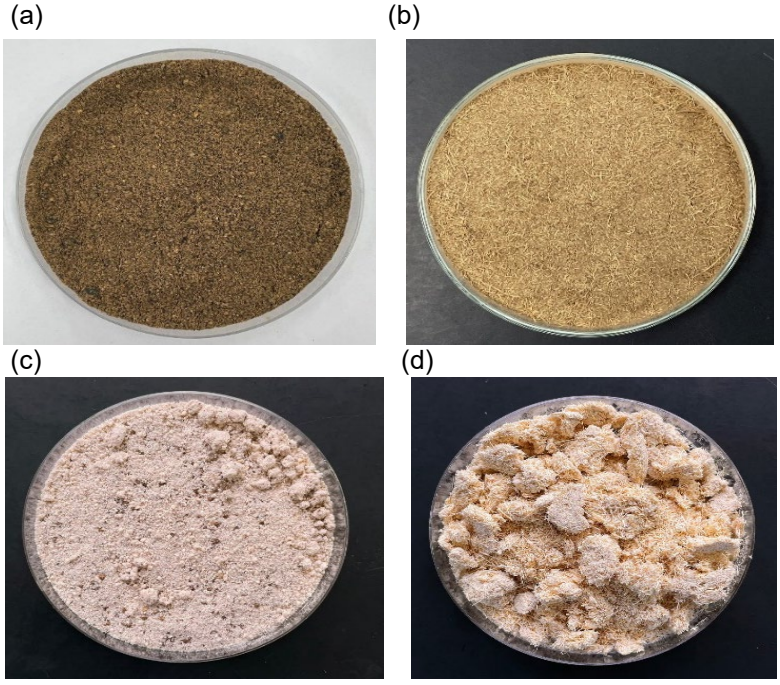

Figure 1. Physical appearance of PKM (a) and EFB (b) before pretreatment, and PKM (c) and EFB (d) after pretreatment with 3% (w/v) NaOH and 3% (v/v) H_2O_2

extraction process and the presence of residual shell material (Iyayi et al., 2015; Tang, 2021; Zubaidah et al., 2024). In general, cell wall fractions have been reported to consist of approximately 4% xylan, 12% cellulose, and up to 58% mannan, while shell or hull fractions contain high lignin contents, which may contribute to the recalcitrant structure of PKM-derived biomass (Zubaidah et al., 2024). In contrast, EFB is a lignocellulosic residue generated during palm oil processing and is characterized by a fibrous structure dominated by cellulose. On a dry weight basis, EFB commonly contains approximately 38-65% cellulose, 17-33% hemicellulose, and 13-37% lignin content. Several studies have reported cellulose content of approximately 55-56%, hemicellulose levels near 28-30%, and lignin content ranging from 15% to 28%, although these values vary depending on the cultivar, processing conditions, and analytical methods. The relatively high cellulose content and moderate hemicellulose fraction make EFB a suitable substrate for generating fermentable sugars following alkaline or oxidative pretreatment combined with enzymatic hydrolysis (Said et al., 2021; Dimawarnita et al., 2023).

Alkaline–oxidative pretreatment using 3% (w/v) NaOH and 3% (v/v) H_2O_2 resulted in a substantial reduction in the biomass weight of both PKM and EFB. The initial mass of each sample was 120 g, obtained from six independent replicates (20 g per replicate). After pretreatment, the remaining mass of PKM was 45.5 g, corresponding to approximately 38% of the original weight, whereas EFB retained 72.3 g (approximately 60%) (Figures 1c and 1d). The observed reduction in biomass weight is likely associated with the partial solubilization and removal of lignin and hemicellulose during alkaline–oxidative pretreatment, leading to the disruption of the compact lignocellulosic matrix and increased cellulose accessibility. Alkaline hydrogen peroxide pretreatment has been reported to

promote delignification under relatively mild conditions, thereby enhancing enzymatic accessibility and improving the efficiency of subsequent saccharification processes (Tareen et al., 2020). The greater mass loss observed in PKM compared to EFB may be related to differences in their chemical composition and structural characteristics, particularly the higher proportion of hemicellulosic polysaccharides and residual shell-associated components present in PKM.

Scanning electron microscopy analysis revealed clear structural modifications in PKM and EFB following alkaline–oxidative pretreatment with 3% (w/v) NaOH at 50°C for 48 h. Untreated samples exhibited compact fiber structures with relatively smooth surfaces and limited porosity (Figures 2a and 2c). In contrast, the pretreated samples displayed disrupted fiber bundles, roughened surfaces, and the formation of cracks and pores (Figures 2b and 2d). The morphological changes observed suggest partial removal of lignin and hemicellulose during alkaline–oxidative pretreatment, which disrupts the compact lignocellulosic matrix and exposes cellulose-rich regions (Radzi et al., 2020). This increases the surface roughness and porosity, thereby enhancing enzyme accessibility and facilitating the hydrolysis of structural polysaccharides (Elgharbawy et al., 2018). Consequently, the observed microstructural alterations confirm the efficacy of the NaOH–H_2O_2 pretreatment in modifying the biomass structure and increasing its susceptibility to enzymatic degradation. Such enhancements in enzymatic accessibility are expected to improve the release of fermentable sugars during hydrolysis, thereby supporting downstream microbial fermentation processes for PHA biosynthesis.

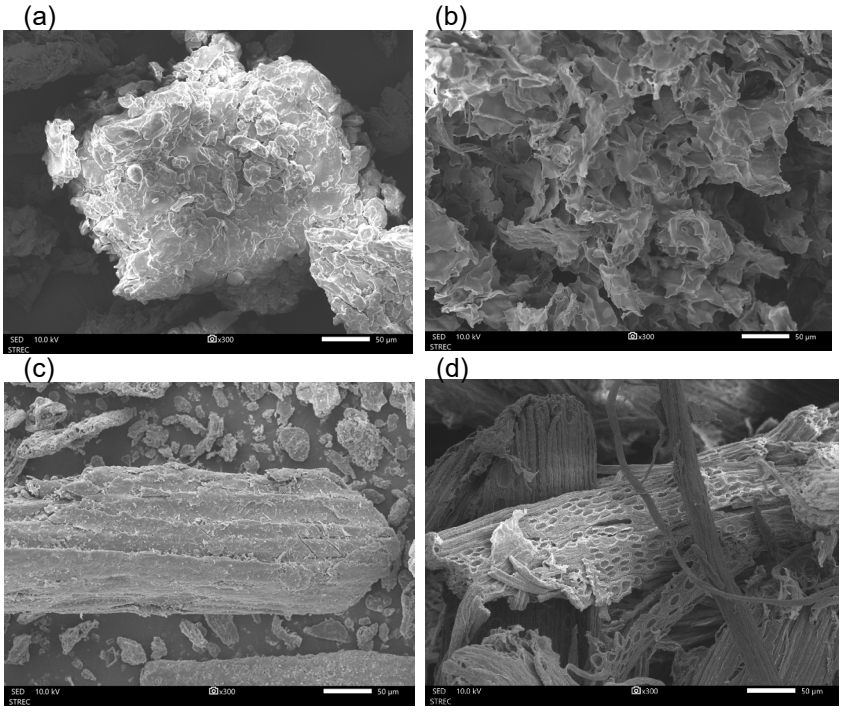

Figure 2. Scanning electron micrographs of PKM and EFB before and after alkaline–oxidative pretreatment: untreated PKM (a) and EFB (c); PKM (b) and EFB (d) after treatment with 3% (w/v) NaOH at 50°C for 48 h.

3.2 Enzymatic hydrolysis of pretreated PKM and EFB for reducing sugar production

PKM and EFB were pretreated with NaOH solutions at concentrations of 1%, 3%, and 5% (w/v) and subsequently subjected to enzymatic hydrolysis using a pectinase, xylanase, and cellulase cocktail at 40, 50, and 60°C for 24 and 48 h to determine the optimal saccharification conditions (Figure 3). The highest reducing sugar concentrations were obtained at 50°C after 48 h of hydrolysis following pretreatment with 3% (w/v) NaOH, reaching 10.53±0.33 mg/mL for PKM hydrolysate (Figure 3a) and 21.02 ±0.38 mg/mL for EFB hydrolysate (Figure 3b). Increasing the temperature to 60°C resulted in a decline in sugar yield, which may be attributed to the partial thermal inactivation of hydrolytic enzymes and/or degradation of the released sugars under harsher conditions. Hydrolysis temperatures of 40, 50, and 60°C were selected to represent low, moderate, and high ranges, respectively, within the reported activity profiles of the cellulase–xylanase–pectinase cocktail. Cellulases generally exhibit optimal activity at 40-50°C and may

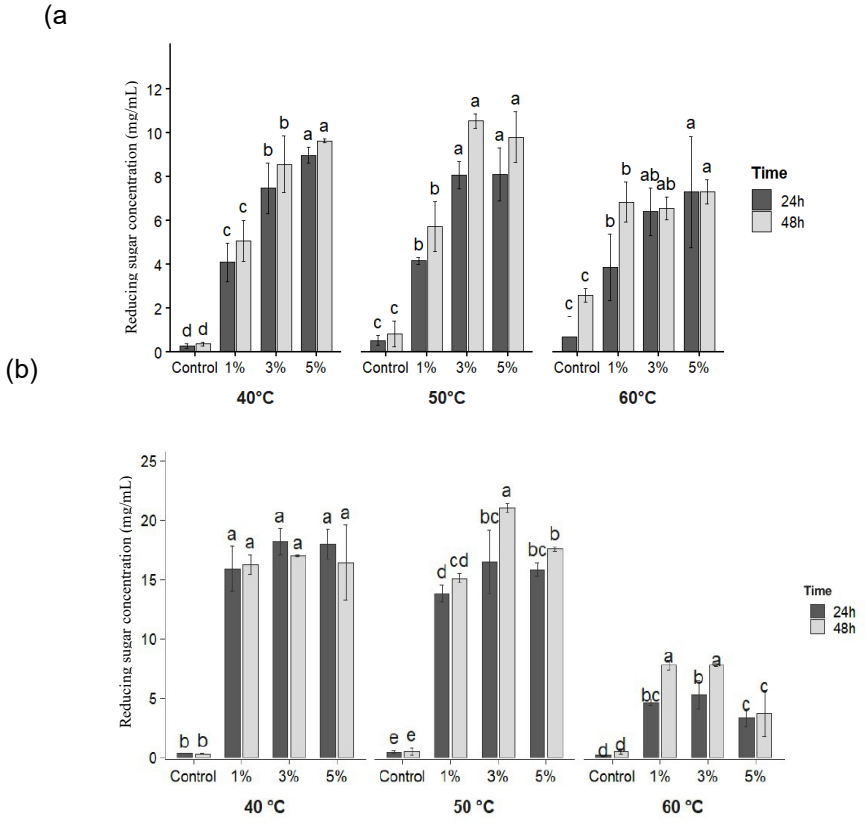

Figure 3. Effects of NaOH concentration (1%, 3%, and 5% w/v), pretreatment temperature (40-60°C), and enzymatic hydrolysis time (24 and 48 h) on reducing sugar concentration released from PKM (a) and EFB (b). The untreated sample served as the control. Data are expressed as mean ± standard deviation. Different letters above the bars indicate significant differences among treatments ($p<0.05$).

experience thermal denaturation at higher temperatures (Sartori et al., 2015). Similarly, xylanase and pectinase from various microbial sources typically show optimal performance within the 30-50°C range (Irfan et al., 2016; Satapathy et al., 2020). In this study, the highest reducing sugar yield at 50°C indicates that this temperature provided the most suitable balance between enzyme activity and stability. The selected temperature range (40-60°C) is consistent with the optimal temperature ranges reported for the commercial enzymes used in this study, where pectinase (40-60°C), xylanase (35-70°C), and cellulase (25-55°C) exhibit effective activity according to the manufacturer's specifications. In contrast, the untreated controls produced only minimal amounts of reducing sugars, indicating that the native lignocellulosic structures of PKM and EFB remained largely intact, limiting enzymatic access to the cellulose fibers (Pant et al., 2021). A comparison between the PKM and EFB hydrolysates revealed that EFB consistently generated higher reducing sugar concentrations under similar pretreatment and hydrolysis conditions. The maximum sugar yield obtained from EFB was approximately two-fold higher than that obtained from PKM. This difference was primarily associated with variations in the chemical composition and structural characteristics of the two biomass types. EFB generally contains a higher proportion of cellulose and a more accessible fibrous structure, which facilitates enzymatic hydrolysis after pretreatment. In contrast, PKM contains a greater fraction of mannan-rich hemicellulose and residual shell components, which may limit enzyme accessibility and reduces the hydrolysis efficiency (Wang et al., 2020).

Enzymatic hydrolysis was performed using a cocktail of pectinase, xylanase, and cellulase. The application of a multi-enzyme system was consistent with previous studies demonstrating that combinations of cellulolytic and hemicellulolytic enzymes could effectively depolymerize lignocellulosic biomass, including palm kernel cake and oil palm empty fruit bunches, thereby enhancing the release of fermentable sugars (Ghazali & Makhtar, 2018). Similarly, mixed enzyme systems have been reported to significantly improve cellulose conversion from alkaline-pretreated biomass. For example, a cellulase–pectinase–xylanase mixture (8:1:1) achieved a cellulose conversion efficiency of up to 96.2% from NaOH-pretreated sweet potato residue (Xia et al., 2020). In addition, sorghum grains treated with an enzyme cocktail containing a higher proportion of xylanase and equal proportions of cellulase and pectinase exhibited a more eroded surface structure than other treatment combinations, indicating enhanced cell wall degradation (Sruthi et al., 2023). These enzyme systems act synergistically by targeting multiple structural components of plant cell walls, including cellulose, hemicellulose, and pectin, thereby facilitating the deconstruction of the complex lignocellulosic matrices. Specifically, cellulases hydrolyze cellulose fibers, whereas accessory enzymes, such as xylanases and pectinases, assist in the removal of hemicellulose and pectin surrounding cellulose microfibrils, thereby improving enzyme accessibility to polysaccharide substrates (Østby et al., 2020). The chemical compositions of PKM and EFB hydrolysates obtained after NaOH pretreatment (3%, w/v; 50°C; 48 h) followed by enzymatic hydrolysis under optimized conditions are summarized in Table 1.

The PKM hydrolysate contained 1.49 g/100 g total carbohydrates and 0.86 g/100 g glucose, whereas the EFB hydrolysate contained 2.05 g/100 g total carbohydrates and 1.14 g/100 g glucose. The higher glucose concentration observed in the EFB hydrolysate indicates that EFB was more readily hydrolyzed than PKM under identical pretreatment and hydrolysis conditions. This observation is consistent with the generally higher cellulose content and more accessible fibrous structure reported for oil palm EFB, which facilitates the enzymatic hydrolysis of lignocellulosic biomass (Kim, 2018; Sundalian et al., 2021).

Table 1 Chemical composition and mineral contents of PKM and EFB hydrolysates after NaOH pretreatment (3%, w/v; 50°C; 48 h) and subsequent enzymatic hydrolysis under optimal conditions

Parameter	PKM Hydrolysate	EFB Hydrolysate
Total carbohydrate (%)	1.49	2.05
Moisture (%)	97.66	97.09
Ash (%)	0.62	0.63
Total fat (%)	0.00	0.00
Protein (%)	0.23	0.23
Glucose (%)	0.86	1.14
Na (mg/100g)	49.91	54.06
Ca (mg/100g)	4.70	4.81
Fe (mg/100g)	1.50	1.87
Cu (mg/100g)	0.048	0.033
Mg (mg/100g)	0.94	4.26
Mn (mg/100g)	0.062	0.057
K (mg/100g)	117.88	118.11
Zn (mg/100g)	0.114	0.130
pH	5.56	5.10

Glucose was identified as the predominant reducing sugar in both hydrolysates, indicating that the combined alkaline pretreatment and enzymatic hydrolysis effectively released cellulose-derived sugars from the lignocellulosic matrix (Palamae et al., 2017). In addition to soluble carbohydrates, both hydrolysates contained residual mineral components, with potassium being the most abundant element, while the moisture content remained relatively high and the fat content was negligible. These compositional characteristics are consistent with those of previous analyses of hydrolysates derived from oil palm residues. The presence of substantial amounts of glucose and other soluble carbohydrates suggests that these hydrolysates can serve as suitable carbon sources for microbial growth and metabolism. Consequently, hydrolysates derived from PKM and EFB represent promising renewable feedstocks for subsequent biotechnological applications, including microbial fermentation processes for PHA production (Yustinah et al., 2019). Sugar profile analysis indicated that glucose was the sole detectable monosaccharide in the PKM and EFB hydrolysates. While lignocellulosic biomass typically yields a mixture of sugars, including xylose and mannose (Chen et al., 2013; Jung et al., 2022), their absence here may be attributed to the alkaline–oxidative pretreatment, which likely solubilized and removed hemicellulose fractions during the washing steps. Alternatively, the residual levels of xylose, mannose, and other minor sugars may have been below the HPLC detection limit. These findings suggest that the applied conditions favored cellulose conversion, resulting in the predominant release of glucose.

3.3 PHA production by *Paraburkholderia* sp. PFN29 using conditioned media derived from alkali-pretreated and enzymatically hydrolyzed PKM and EFB

The production of PHAs from EFB and PKM was evaluated using hydrolysates derived from sodium hydroxide pretreatment followed by enzymatic hydrolysis of *Paraburkholderia* sp. PFN29 cultivated under various carbon and nitrogen supplementation conditions (Figure 4). For the EFB hydrolysate, co-supplementation with NH_4Cl and glucose resulted in the highest cell dry weight (2.4±0.05 g/L), which was significantly higher than that under most other conditions ($p<0.05$), indicating that the simultaneous availability of carbon and nitrogen facilitated robust growth. In contrast, NH_4Cl alone yielded the highest PHA concentration (1.12±0.02 g/L) and a high PHA content (59.02%), which were not significantly different from those obtained in the mineral medium (60.13%) ($p>0.05$). The estimated endpoint volumetric productivity under this condition was approximately 0.01 g/L/h. Conversely, among the PKM-based conditions, the PKM hydrolysate demonstrated optimal performance without additional glucose or NH_4Cl, achieving the highest cell dry weight (2.4±0.05 g/L), PHA concentration (0.94±0.02 g/L), and PHA content (39.02%), which were significantly higher than those obtained under other PKM-based conditions ($p<0.05$). This suggests that the PKM hydrolysate provided sufficient utilizable carbon and residual nitrogen to support both growth and polymer biosynthesis, which was consistent with the relatively high protein and nitrogen contents reported for PKM. Although the mineral medium exhibited the highest PHA content (60.13%), which was greater than that of most experimental conditions, this did not imply that waste hydrolysates were unsuitable for PHA production. The mineral medium was formulated with an optimized carbon-to-nitrogen (C/N) ratio specifically favoring polymer accumulation (Cui et al., 2017), whereas EFB and PKM hydrolysates had more complex compositions (Lyu et al., 2025). Notably, when supplemented with NH_4Cl, the EFB hydrolysate achieved a PHA content of 59.02%, which was not significantly different from that of the mineral medium ($p>0.05$). Moreover, the PKM hydrolysate alone yielded 39.02% PHA without any external supplementation. These results confirm that both waste-derived hydrolysates remain promising feedstocks for PHA production, particularly when considering economic feasibility and process sustainability. Overall, both hydrolysates supported microbial growth and PHA production; however, the extent of biomass formation and polymer accumulation was strongly dependent on the substrate type and the supplementation strategy. These findings highlight the role of feedstock composition and carbon/nitrogen balance in regulating PHA biosynthesis (Cui et al., 2017). The observed differences in PHA accumulation can be explained by the carbon-to-nitrogen (C/N) balance. Under nitrogen-sufficient conditions, carbon is primarily utilized for biomass formation, whereas under nitrogen-limited conditions, microbial growth is restricted, and excess carbon is redirected toward intracellular storage metabolism. This is consistent with the higher PHA content observed in the NH_4Cl-supplemented EFB hydrolysate, where a high C/N ratio resulted in nitrogen limitation. From a bioenergetic perspective, nitrogen limitation reduces carbon use efficiency (CUE), favoring carbon allocation to storage polymers (Chakrawal et al., 2022). These results confirm that the C/N ratio plays a key role in regulating the metabolic allocation between growth and PHA accumulation (Justes et al., 2009; Chakrawal et al., 2022). At the metabolic level, nitrogen limitation reduces biomass synthesis, leading to the redirection of excess carbon toward acetyl-CoA. The increased availability of NADPH further promotes PHA biosynthesis (Cui et al., 2017). Although the maximum PHA concentration (1.12±0.02 g/L) was lower than the values reported in literature using

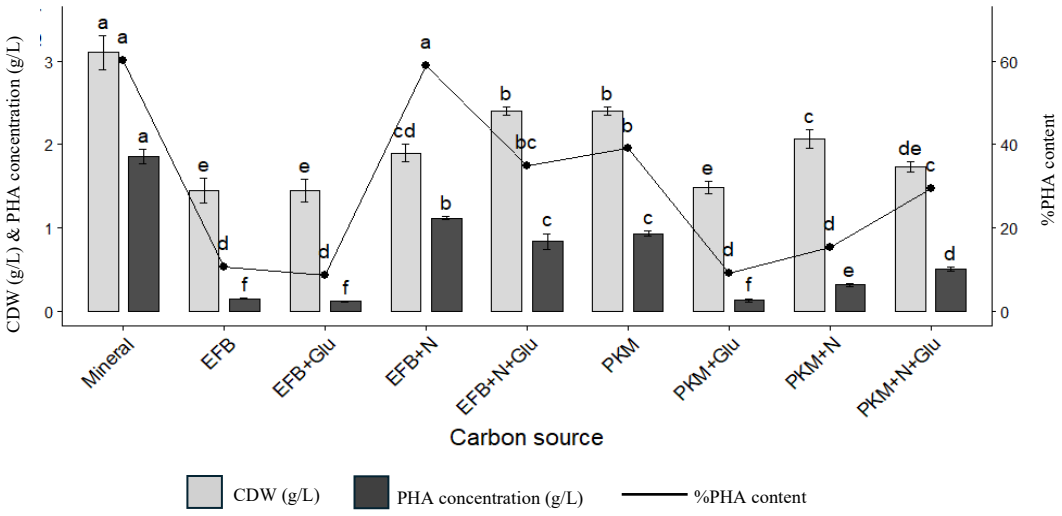

Figure 4. PHA production from conditioned media by *Paraburkholderia* sp. PFN29. Cell dry weight (CDW), PHA concentration, and PHA content (%) obtained for strain PFN29 cultivated on EFB and PKM hydrolysates after NaOH pretreatment and enzymatic hydrolysis, with NH_4Cl (N; 0.1%, w/v) and glucose (Glu; 2%, w/v) supplementation, compared with the mineral medium as control. Data are presented as mean±standard deviation (n = 3). Different letters above the bars indicate statistically significant differences among treatments based on one-way ANOVA followed by Tukey's HSD test ($p<0.05$).

optimized synthetic media, it represents a promising outcome given the use of unrefined, non-concentrated waste hydrolysates. The presence of pretreatment-derived inhibitors, such as furfural and phenolic compounds (Chen et al., 2013), alongside a restricted sugar profile, potentially constrains both cell growth and polymer accumulation. Future optimization, including hydrolysate detoxification and sugar concentration, could significantly enhance the volumetric productivity.

3.4 Characterization of PHA

The FT-IR spectra of PHA synthesized from EFB hydrolysate supplemented with NH_4Cl and PKM hydrolysate exhibited major absorption band characteristics of PHB (Figure 5). The polymer derived from the EFB hydrolysate showed prominent peaks at approximately 2980 cm^{-1}, attributed to aliphatic C–H stretching of CH_3 groups, 1721 cm^{-1} corresponding to ester carbonyl (C=O) stretching, 1380 cm^{-1} associated with CH_3 bending, and 1228, 1180, and 1057 cm^{-1} related to ester-region C–O–C and C–O stretching vibrations (Figure 5a). Similarly, the polymer obtained from the PKM hydrolysate displayed band characteristics at approximately 2930, 1721, 1453, 1380, 1278, 1180, and 1057 cm^{-1}, corresponding to aliphatic C–H stretching, ester carbonyl stretching, CH_3/CH_2 bending, CH_3 bending, and C–O–C/C–O stretching vibrations, respectively (Figure 5b). These absorption bands aligned with the FT-IR features previously reported for PHB, indicating that the polymers produced from both hydrolysates possess functional groups typical of PHB. Minor shifts in the peak position between the two samples may reflect differences in

polymer purity and crystallinity or the presence of residual matrix components originating from each hydrolysate (Lu et al., 2021; Etxabide et al., 2022).

The ^{1}H NMR spectra of the polymers produced by *Paraburkholderia* sp. PFN29 cultivated in EFB hydrolysate supplemented with NH_4Cl and PKM hydrolysate without NH_4Cl supplementation exhibited three characteristic signals at approximately 1.28, 2.45-2.60, and 5.25 ppm (Figure 6). These signals correspond to the methyl ($–CH_3$), methylene ($–CH_2–$), and methine (–CH–) protons of the 3-hydroxybutyrate repeating unit, respectively, and similar spectral patterns were observed for both polymer samples. These chemical shifts are consistent with those reported by Shin et al. (2024).

The observed ^{1}H NMR chemical shifts are also consistent with previously reported PHB spectra, which typically exhibited resonances at approximately 1.2-1.3 ppm for the methyl group, 2.4-2.7 ppm for the methylene group adjacent to the carbonyl carbon, and 5.1-5.3 ppm for the methine proton attached to the oxygen-bearing carbon (Trakunjae et al., 2021; Etxabide et al., 2022). Together with the FT-IR results, these findings confirm that the intracellular polyester synthesized by PFN29 from EFB and PKM hydrolysates is structurally consistent with PHB.

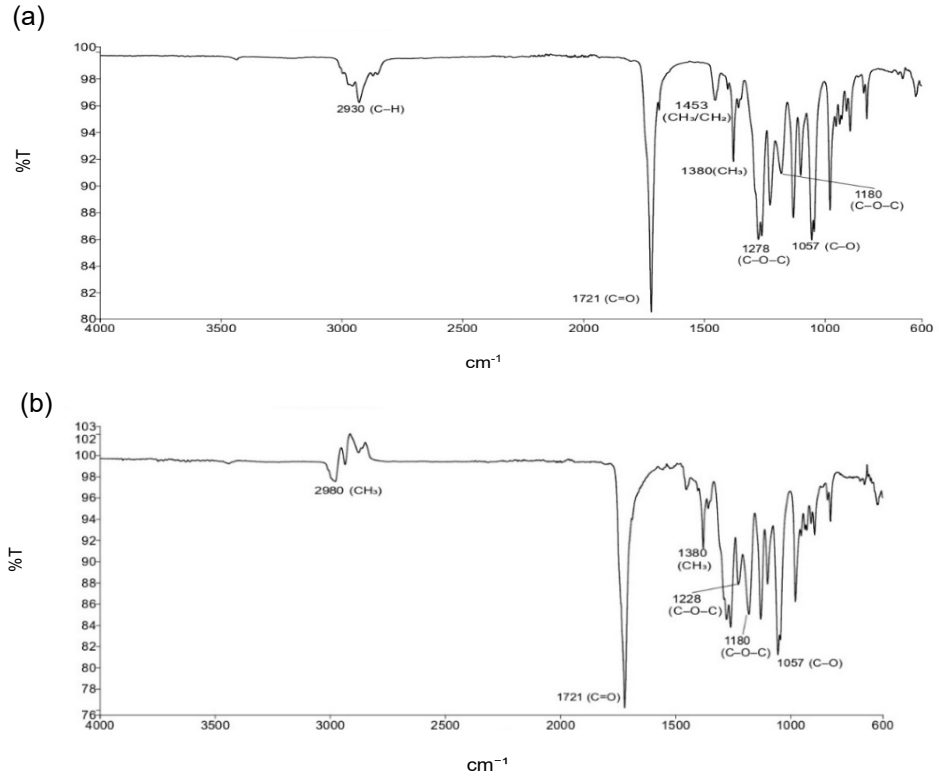

Figure 5. FT-IR spectra of PHA extracted from cultures grown in EFB hydrolysate supplemented with NH_4Cl (a) and PKM hydrolysate (b). Both spectra exhibited the characteristic absorption bands of PHB, including ester carbonyl stretching at approximately 1721 cm^{-1}, aliphatic C–H stretching in the 2930–2980 cm^{-1} region, and bands in the fingerprint region corresponding to the C–O–C and C–O stretching vibrations of the polyester backbone.

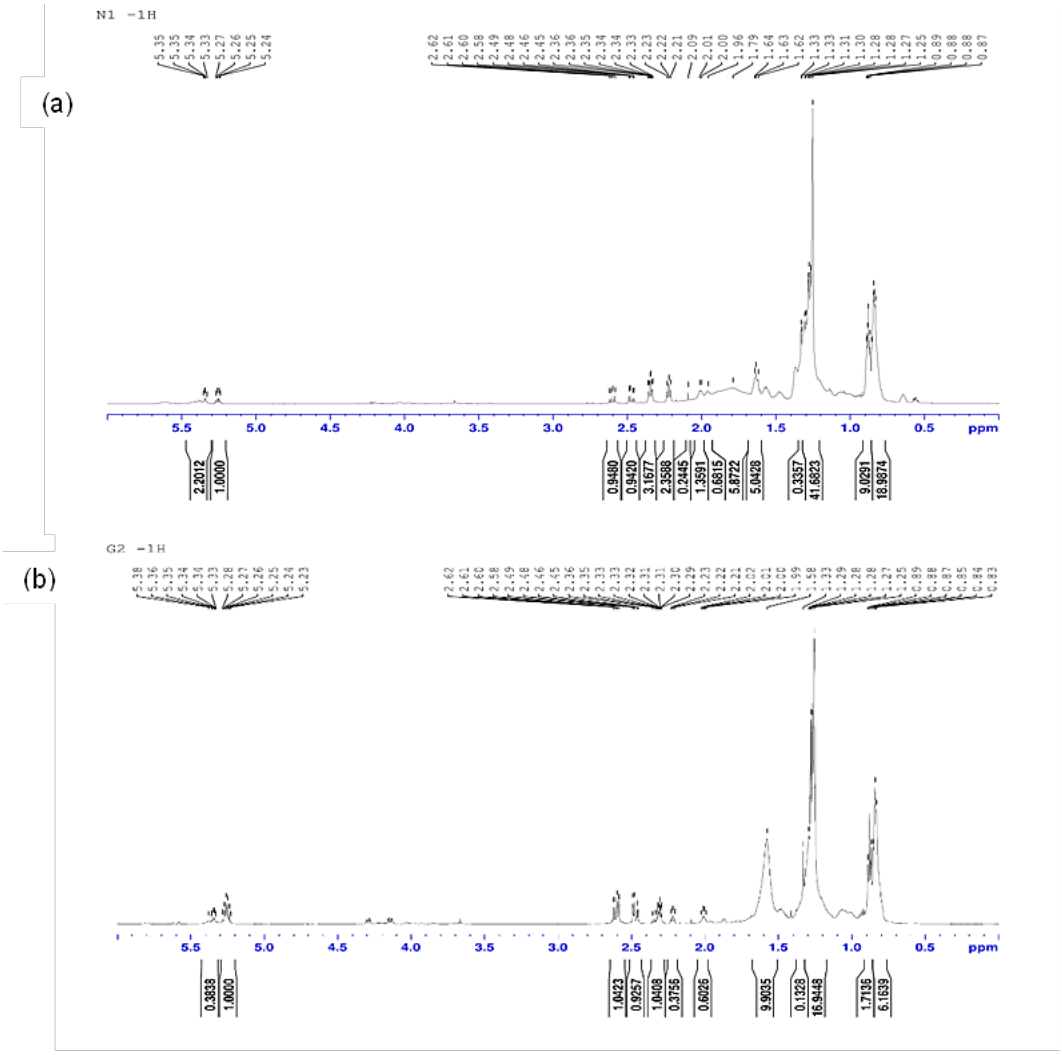

Figure 6. ^{1}H NMR spectra of PHB produced by *Paraburkholderia* sp. PFN29 cultivated in (a) EFB hydrolysate supplemented with NH_4Cl and (b) PKM hydrolysate without NH_4Cl supplementation.

4. Conclusions

This study demonstrates that oil palm residues can be effectively valorized as substrates for the production of PHB-type PHA by *Paraburkholderia* sp. PFN29. Under optimized alkaline–oxidative pretreatment and enzymatic hydrolysis, both PKM and EFB hydrolysates provided sufficient fermentable sugars to support microbial growth and intracellular polyester accumulation, respectively. EFB hydrolysate supplemented with ammonium chloride yielded PHB levels comparable to those obtained using a defined mineral medium, whereas PKM hydrolysate required no additional nutrients. FT-IR and NMR spectroscopic analyses confirmed that the accumulated polymer was predominantly PHB, underscoring the ability of PFN29 to convert lignocellulosic side streams into a

well-characterized biodegradable polyester. Overall, the results highlight EFB and PKM as promising, low-cost feedstocks for PHB production and provide a foundation for future studies on process intensification, life cycle assessment, and scale-up for industrial applications within a palm oil-based circular bioeconomy.

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6. Authors' Contributions

Sumintra Chaimongkol: conducted the experiments, analyzed the data, and prepared the manuscript; Thayat Sriyapai: designed the experiments and performed the data analysis; and Pichapak Sriyapai: designed the experiments, interpreted the results, coordinated the research, and contributed to the writing of the manuscript.

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7. Conflicts of Interest

The authors declare no conflicts of interest related to the publication of this paper.

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