VNU Journal of Science: Natural Sciences and Technology, Vol. 42, No. 4 (2026) 71-79

VNU Journal of Science: Natural Sciences and Technology

Journal homepage: https://js.vnu.edu.vn/NST

Original Article

# Biosynthesis of Polyhydroxyalkanoate Copolymer by *Cupriavidus necator* H16 using γ-butyrolactone and Sodium Propionate as Precursors

Tran Thi Loan[1], Bui Thi Viet Ha[1,*], Doan Van Thuoc[2]

[1]*VNU University of Science, 334 Nguyen Trai, Thanh Xuan, Hanoi, Vietnam*
[2]*Hanoi National University of Education, Cau giay, Hanoi, Vietnam*

Received 27th November 2025
Revised 28th September 2026; Accepted 08th October 2026

**Abstract:** Polyhydroxyalkanoate (PHA) copolymers offer tunable properties that extend beyond those of poly(3-hydroxybutyrate) (PHB), but precise control of monomer composition in waste lipid-based systems remains challenging. This study investigates precursor-directed biosynthesis of PHA copolymers by *Cupriavidus necator* H16 using waste cooking oil (WCO) and crude fish oil (CFO) as carbon sources, with γ-butyrolactone and sodium propionate supplied as precursors for 4-hydroxybutyrate (4HB) and 3-hydroxyvalerate (3HV), respectively. γ-butyrolactone (GBL) supplementation resulted in limited 4HB incorporation (≤2.8 mol%), whereas propionate enabled efficient and tunable 3HV incorporation, increasing from 1.2–1.6 mol% to 10.9–11.5 mol% across both systems as precursor concentration increased from 1 to 5 g/L. We establish a clear dose–response trade-off between precursor concentration, copolymer composition (4HB/3HV), and productivity (CDW and PHA content), defining a practical operating window for composition-tunable PHA production from waste lipids.

*Keywords: C. necator* H16, P(3HB-*co*-4HB), P(3HB-*co*-3HV), copolymer.

## 1. Introduction

Polyhydroxyalkanoates are a class of biodegradable polymers synthesized by numerous bacterial strains as intracellular carbon and energy storage materials under conditions of excess carbon and limitation of essential nutrients such as oxygen, nitrogen, phosphorus, or sulfur. Within the PHA family, poly(3-hydroxybutyrate) is the most commonly produced homopolymer; however, its high crystallinity and brittleness significantly restrict practical applications. To overcome these limitations, PHB-based copolymers have been developed, among which poly(3-hydroxybutyrate-co-3-hydroxyvalerate) [P(3HB-co-3HV)] and poly(3-hydroxybutyrate-co-4-hydroxybutyrate) [P(3HB-co-4HB)] are the most widely studied due to their improved flexibility and toughness [1, 2].

* Corresponding author.
*E-mail address:* buithivietha@hus.edu.vn

https://doi.org/10.25073/2588-1140/vnunst.5942

In recent years, increasing attention has been given to monomer composition control as a key strategy for tailoring PHA properties, shifting research focus from maximizing polymer yield toward regulating monomer incorporation [3]. Among available approaches, precursor supplementation represents a simple and effective method to influence copolymer composition during fermentation. While most previous studies have focused on carbohydrate-based substrates such as glucose, sucrose, or glycerol (Table 3), the role of precursor concentration in waste lipid-based systems remains insufficiently understood. In particular, waste cooking oil (WCO) and crude fish oil (CFO) have been widely investigated as low-cost carbon sources [4]; however, most reports have emphasized biomass formation and total PHA accumulation rather than controlled regulation of copolymer composition.

Therefore, this study investigates the effects of sodium propionate and γ-butyrolactone supplementation on cell growth, PHA accumulation, and monomer composition in *Cupriavidus necator* H16 cultivated on WCO and CFO. This work focuses on precursor concentration–dependent regulation of copolymer composition and demonstrates the feasibility of producing composition-tunable P(3HB-co-3HV) and P(3HB-co-4HB) materials from renewable low-cost feedstocks. The results provide insight into process-level control of PHA composition in lipid-based systems and support the development of application-oriented biopolyesters.

## 2. Experimental

### *2.1. Bacterial Strain and Maintenance*

*C. necator* H16 (DSM 428 was provided by the Laboratory of Biotechnology-Microbiology, Faculty of Biology, Hanoi National University of Education) was streaked onto nutrient-rich (NR) agar medium containing (g/L): peptone, 10; meat extract, 10; yeast extract, 2; and granulated agar, 20. After 24 hours of incubation at 30 $^{o}$C, the agar plates were stored at 4 $^{o}$C for short-term maintenance. For long-term preservation, the strain was maintained as 20% (v/v) glycerol stocks at −80 °C or preserved by lyophilization.

### *2.2. PHA Production in Shake Flasks with Different Carbon Sources*

A loopful of bacterial cells grown on NR agar plates was inoculated into 20 mL of NR liquid medium in a 100-mL flask. After 13 h of cultivation at 30 $^{o}$C on a rotary shaker (OD600 = 6.0), 2.5 mL of each culture broth was inoculated in 250-mL flasks containing 50 mL of mineral medium with (g/L): $NaH_2PO_4{\cdot}2H_2O$, 5.0; $Na_2HPO_4{\cdot}12H_2O$, 11.6; $K_2SO_4$, 0.45; $MgSO_4{\cdot}7H_2O$, 0.39; $CaCl_2{\cdot}2H_2O$, 0.06; $(NH_2)_2CO$, 0.54; peptone, 1; yeast extract, 0.4; meat extract, 1.0; and 20 g of crude fish oil (CFO from Basa fish (*Pangasius bocourti*) was collected from Ho Chi Minh City (Vietnam)) or waste cooking oil (WCO was derived from soybean oil, which was provided by a restaurant in Hai Phong City (Vietnam). The PHA precursors (γ-butyrolactone and propionate) were added into the culture medium to achieve final concentrations of 0, 1, 2, 3, 4, and 5 g/L. The pH of this medium was initially adjusted to 7.0 using 1 M NaOH. The cultures were incubated in the shaker at 30 °C with rotary shaking at 180 rpm. After 48 hours of cultivation, cells were harvested for analysis of cell dry weight (CDW) and PHA content. All experiments were performed in independent biological batches.

### *2.3. Polymer Extraction*

Approximately 2 g of lyophilized cells were mixed with 200 mL of chloroform and stirred for 3 days at room temperature. Cell debris was removed using 0.45 µm filter paper (Whatman, Sigma-Aldrich, USA). The filtrate was then concentrated using a rotary evaporator (Biobase, China). After that, 10 volumes of cold methanol were added to the concentrated extract. The precipitated polymer was collected by filtering and air dried in the fume hood [5].

### *2.4. Quantitative Analysis*

The CDW was determined by centrifuging 4.5 mL of the culture samples at 10,000 rpm for

5 min in a pre-weighed centrifuge tube ($M_0$). The pellet was washed once with 1 mL of hexane and once with 1 mL of distilled water, centrifuged, and dried at 105 $^oC$ in the oven until a constant weight was obtained. The centrifuged tube was weighed again ($M_1$) to calculate the CDW according to the formula: CDW (g/L) = ($M_1 – M_0$)/4.5 *1000.

The dry biomass of cells in each experimental sample was retained to analyze the PHA content in the cells. PHA content (wt%) in dried cell mass and its composition were determined by gas chromatography (GC). Approximately 10 mg of dried cell mass was mixed with 1 mL chloroform and 1mL methanolysis containing 85% (v/v) methanol, 15% (v/v) sulfuric acid and 0.4% (w/v) benzoic acid. The mixture was incubated at 100 $^oC$ for 3 hours to convert the intracellular polymer into their methyl esters. After cooling down to room temperature, 0.5 mL MiliQ water was added to the mixture and vortexed for 30 seconds and to allow the phase separate. The bottom layer containing methyl ester of PHA monomers was transferred to a vial containing sodium sulfate anhydrous to remove the remaining water and then analyzed by gas chromatography (GC) (TRACE 1310, Thermo Scientific, USA) [6]. Purified (P(3HB-co-3HV)) and (P(3HB-co-4HB)) were used as standard for calibration. All the analyses were performed in triplicate. The PHA content (weight, wt%) was calculated as the percentage of the ratio of PHA concentration to CDW.

The chemical structure of the purified polymer was determined using proton nuclear magnetic resonance (NMR). The purified polymer was dissolved in $CDCl_3$ at a concentration of 10 mg/mL and analyzed with a ARX500 spectrometer (Bruker, USA) operating at 500 MHz.

## 3. Results and Discussion

### *3.1. P(3HB-co-4HB) Synthesis by C. necator H16 from Crude Fish Oil and Waste Cooking Oil Feeding with γ-butyrolactone*

The ability of *C. necator* H16 to grow and synthesize copolymer using crude fish oil or waste cooking oil as the main carbon source, together with different concentrations of γ-butyrolactone as a PHA precursor, was investigated. As shown in Table 1, PHB was synthesized only when crude fish oil or waste cooking oil was used as the carbon substrate. A high CDW of 18.7 g/L, PHB content of 86.3 wt%, and PHB concentration of 16.1 g/L were obtained using 20 g/L crude fish oil after 48 hours of cultivation, with a conversion rate of 0.81 g/g. In the case of waste cooking oil, slightly lower values were observed, including a CDW of 17.4 g/L, PHB content of 85.7 wt%, PHB concentration of 14.9 g/L, and a conversion rate of 0.75 g/g under the same cultivation conditions.

The $^1H$ NMR spectrum of the purified polymer isolated from the bacterial cells demonstrated that the copolymer P(3HB-co-4HB) was synthesized when γ-butyrolactone was added into the culture medium. A peak at 1.28 ppm, two peaks at 2.45 and 2.62 ppm, and a peak at 5.25 ppm correspond to the methyl, methylene, and methine groups of the 3HB monomer, respectively. In contrast, three small peaks at 1.95, 2.35, and 4.11 ppm correspond to the methylene groups of the 4HB monomer (Figure 1).

The results in Table 1 further demonstrate that the 4HB fraction increased in a concentration-dependent manner with increasing γ-butyrolactone supplementation. The highest 4HB content of 2.8 mol% was obtained using 20 g/L crude fish oil and 5 g/L γ-butyrolactone, while a maximum of 2.6 mol% was achieved using 20 g/L waste cooking oil and 4 g/L γ-butyrolactone. Although these values remain relatively low compared with carbohydrate-based production systems (Table 3), the observed trend clearly confirms the feasibility of producing P(3HB-co-4HB) directly from waste lipid substrates via precursor supplementation. The limited 4HB incorporation is likely due to dominant acetyl-CoA flux generated through *β*-oxidation of lipids, which preferentially supports 3HB biosynthesis and competes with the metabolic route leading to 4HB-CoA formation.

In contrast, increasing γ-butyrolactone concentration resulted in a gradual decrease in both CDW and PHA content. For crude fish oil, CDW decreased from 18.6 to 14.4 g/L, while PHA content decreased from 86.9 to 78.1 wt% as γ-butyrolactone increased from 1 to 5 g/L. A similar trend was observed for waste cooking oil, where PHA content decreased from 85.4 to 77.9 wt% over the same precursor range. CDW decreased from 17.1 to 14.5 g/L when γ-butyrolactone increased from 1 to 3 g/L, while remaining relatively stable at higher concentrations (4–5 g/L), suggesting a partial saturation of inhibitory effects on cell growth.

These results are consistent with previous reports showing that elevated γ-butyrolactone concentrations can negatively affect both biomass formation and PHA accumulation [7]. Similar inhibitory effects have also been reported for other precursors such as propionate and propanol [7-9]. These phenomena are generally attributed to metabolic burdens associated with precursor uptake and activation, increased CoA-dependent conversion steps, elevated redox and energy demands, and potential perturbation of central carbon metabolism.

Statistical analysis confirmed that γ-butyrolactone supplementation significantly influenced biomass formation, polymer accumulation, and copolymer composition. Concentrations up to 2 g/L did not significantly affect CDW, whereas higher concentrations resulted in a significant reduction in both CDW and PHA content. More importantly, increasing precursor concentration induced statistically significant shifts in monomer composition, characterized by a decrease in the 3HB fraction and an increase in 4HB incorporation. Although the absolute 4HB content remained limited, these findings demonstrate that waste-oil-based fermentation systems enable compositional tuning of PHA copolymers without genetic modification of the production strain.

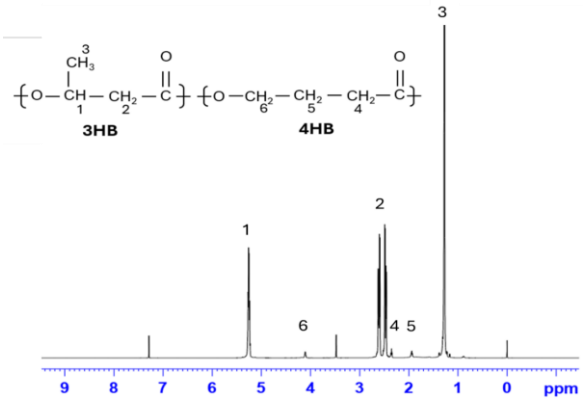



Figure 1. $^{1}$H NMR spectrum confirming the presence of 4HB monomer in P(3HB-*co*-4HB) copolymer produced by *C. necator* H16 using γ-butyrolactone as precursor.

Table 1. P(3HB-*co*-4HB) synthesis by *C. necator* H16 in the presence of crude fish oil and waste cooking oil (20 g/L) and different concentrations of γ-butyrolactone

| **γ-butyrolactone conc.** | **Crude fish oil** | | | **Waste cooking oil** | | |
|---|---|---|---|---|---|---|
| **(g/L)** | **CDW (g/L)** | **PHA content (%)** | **4HB (mol%)** | **CDW (g/L)** | **PHA content (%)** | **4HB (mol%)** |
| **0** | 18.7 ± 0.5a | 86.3 ± 1.1a | 0.0 ± 0.0d | 17.4 ± 0.0a | 85.7 ± 2.0a | 0.0 ± 0.0d |
| **1** | 18.6 ±0.3a | 86.9 ± 0.2a | 1.2 ± 0.2c | 17.1 ± 0.3a | 85.4 ± 0.9a | 1.2 ± 0.1c |
| **2** | 18.6 ±0.3a | 84.4 ± 0.3ab | 1.8 ± 0.1bc | 17.0 ± 0.1a | 83.9 ± 0.6ab | 1.6 ± 0.1b |

| | | | | | | |
|---|---|---|---|---|---|---|
| **3** | 17.4 ± 0.4b | 80.3 ± 1.0bc | 2.3 ± 0.1ab | 14.5 ± 0.3b | 80.3 ± 1.5ab | 2.4 ± 0.2a |
| **4** | 15.9 ± 0.3c | 79.3 ± 1.7c | 2.4 ± 0.1a | 14.3 ± 0.3b | 79.6 ± 1.1ab | 2.6 ± 0.1a |
| **5** | 14.4 ± 0.1d | 78.1 ± 1.4c | 2.8 ± 0.1a | 14.5 ± 0.1b | 77.9 ± 2.4b | 2.6 ± 0.0a |

*Note:* Values are presented as mean ± SD (n = 3). Different superscript letters within the same column indicate significant differences according to Tukey's HSD test ($p < 0.05$).

### *3.2. P(3HB-co-3HV) Synthesis by C. necator H16 from Crude Fish Oil and Waste Cooking Oil Feeding with Propionate*

The synthesis of P(3HB-co-3HV) requires simultaneous supply of 3HB and 3HV repeating units. In *Cupriavidus necator*, the 3HV unit can be generated intracellularly from short-chain precursors such as propionate (used here) or valerate via CoA-dependent activation and conversion. Consistent with this route, $^1$H-NMR spectra (Figure 2) of polymers isolated from propionate-fed cultures displayed the characteristic PHB signals at δ 1.28 ppm (–$CH_3$), 2.45 and 2.62 ppm (–$CH_2$– next to the carbonyl), and 5.25 ppm (methine –CH–). Introduction of 3HV units was evidenced by the appearance of a new terminal methyl resonance at ~δ 0.90 ppm and an additional methylene envelope near δ 1.6 ppm**,** together with increased intensity in the carbonyl-adjacent methylenes at δ 2.45–2.62 ppm-features diagnostic of P(3HB-co-3HV) rather than a physical mixture of homopolymers.

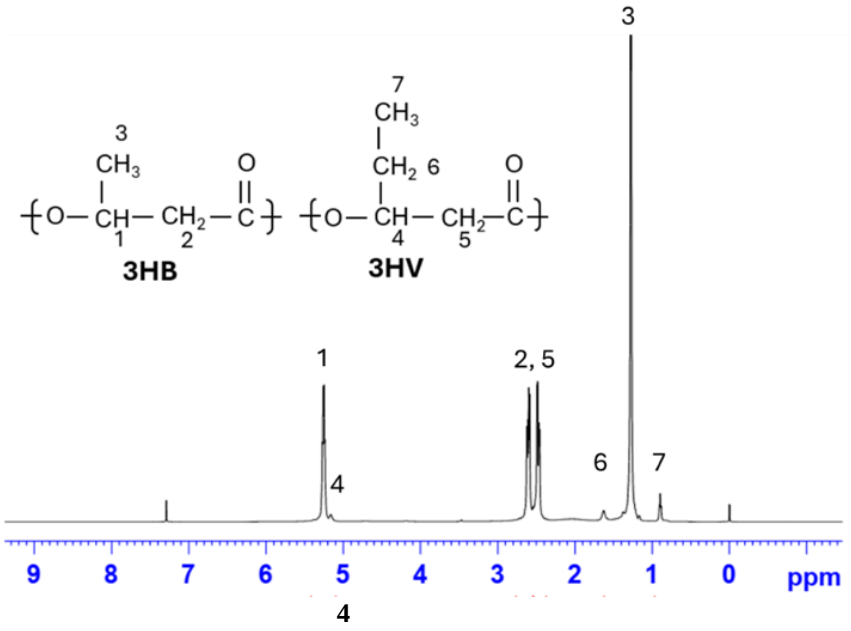



Figure 2. $^1$H NMR spectrum confirming the presence of 3HV monomer in P(3HB-*co*-3HV) copolymer produced by *C. necator* H16 using sodium propionate as precursor.

The results in Table 2 showed that propionate supplementation significantly influenced biomass formation, PHA accumulation, and 3HV incorporation in cultures grown on both CFO and WCO. Increasing propionate concentration led to a reduction in CDW and PHA accumulation, while enhancing 3HV incorporation into the

copolymer. For CFO, CDW decreased from 17.1 to 7.4 g/L, and PHA content decreased from 87.2% to 56.1% as propionate increased from 0 to 5 g/L. A similar trend was observed for WCO, where CDW decreased from 17.6 to 6.3 g/L and PHA content from 85.0% to 59.0%. In contrast, 3HV content increased in a concentration-dependent manner, reaching 10.9 and 11.5 mol% at 5 g/L propionate in CFO- and WCO-grown cultures, respectively.

Although both carbon sources achieved comparable maximum 3HV contents, WCO maintained higher polymer accumulation at intermediate precursor concentrations. Notably, at 4 g/L propionate, WCO supported 69.7% PHA with 6.6 mol% 3HV, compared with 61.5% PHA and 7.8 mol% 3HV for CFO. These results indicate that WCO provides a more favorable balance between copolymer compositional control and polymer productivity. At the highest propionate concentration, although 3HV incorporation was maximized, CDW and PHA accumulation were minimized, indicating a clear trade-off between polymer yield and monomer modification. Therefore, the optimal propionate concentration should be selected according to the intended application. High propionate concentrations may be advantageous when maximizing 3HV content is the primary objective, whereas intermediate concentrations are more suitable when a balance among biomass production, PHA accumulation, and copolymer composition is required.

The 3HV contents achieved in this study (10.9 mol% for CFO and 11.5 mol% for WCO under sodium propionate supplementation) are comparable to or higher than previously reported values for propionate-based PHA production systems (Table 3). In particular, these levels are similar to those reported for *C. necator* DSM 545 (10.7 mol%) [10] and exceed those of *Methylocystis parvus* OBPP (8.0 mol%) [11] and *Salinivibrio* sp. TGB11 (8.6 mol%) [12], demonstrating the effectiveness of lipid-based waste substrates in promoting efficient monomer incorporation. Although recombinant *E. coli* systems have achieved higher 3HV contents (up to 20.2 mol%) [13], this was accompanied by substantially reduced biomass and PHA accumulation

Table 2. P(3HB-*co*-3HV) synthesis by *C. necator* H16 in the presence of crude fish oil and waste cooking oil (20 g/L) and different concentrations of sodium propionate

| **Propionate conc.** | **Crude fish oil** | | | **Waste cooking oil** | | |
|---|---|---|---|---|---|---|
| **(g/L)** | **CDW (g/L)** | **PHA content (%)** | **3HV (mol%)** | **CDW (g/L)** | **PHA content (%)** | **3HV (mol%)** |
| **0** | 17.1 ± 0.4a | 87.2 ± 2.6a | 0.0 ± 0.0d | 17.6 ± 0.3a | 85.0 ± 1.7a | 0.0 ± 0.0e |
| **1** | 17.0 ± 0.6a | 82.3 ± 0.4ab | 1.6 ± 0.2d | 14.5 ± 0.2b | 84.3 ± 1.2a | 1.2 ± 0.0de |
| **2** | 15.0 ± 0.2b | 81.3 ± 0.4ab | 1.8 ± 0.3cd | 14.1 ± 0.1b | 83.6 ± 1.2a | 1.7 ± 0.1d |
| **3** | 10.5 ± 0.1c | 75.1 ± 2.9b | 4.1 ± 0.6c | 10.8 ± 0.1c | 81.5 ± 0.4a | 4.2 ± 0.7c |
| **4** | 10.6 ± 0.2c | 61.5 ± 3.0c | 7.8 ± 0.8b | 10.2 ± 0.2c | 69.7 ± 3.2b | 6.6 ± 1.1b |
| **5** | 7.4 ± 0.8d | 56.1 ± 3.9c | 10.9 ± 0.9a | 6.3 ± 0.2d | 59.1 ± 0.8c | 11.5 ± 0.2a |

*Note:* Values are presented as mean ± SD (n = 3). Different superscript letters within the same column indicate significant differences according to Tukey's HSD test ($p < 0.05$).

PHA synthase (PhaC) is the key enzyme in PHA production because it is responsible for catalyzing the polymerization of PHA monomers into PHA polymers. In addition, it also determines the monomer composition integrated into the PHA polymer, which will impact on the properties of the PHA produced. Based on the substrate specificities of PhaC and subunit composition, PhaCs are categorized into four main classes: class I, II, III and IV. Class I, III and IV PhaCs can only incorporate SCL-PHA monomers while class II can only incorporate MCL-PHA monomers. PhaC of *C. necator* belongs to class I, so this kind of enzyme can only synthesize short-chain PHA such as PHB and P(3HB-co-3HV) [14, 15]. For that reason, high mol% of HV fraction was synthesized when propionate was used as 3HV precursor (Table 2).

Table 3. Comparison of PHA copolymer production using γ-butyrolactone and propionate as precursor substrates

| Strain | Carbon | CDW (g/L) | PHA content (wt%) | PHA composition (mol %) | | Reference |
|---|---|---|---|---|---|---|
| | | | | 4HB | 3HV | |
| *C.necator* H16 | CFO + γ-butyrolactone | 14.4 | 78.1 | 2.8 | - | This work |
| *C.necator* H16 | WCO + γ-butyrolactone | 14.5 | 77.9 | 2.6 | - | This work |
| *C.necator DSM 545* | Glycerol + γ-butyrolactone | - | 25.9 | 11.4 | - | [16] |
| *C.necator* H16 | Fructose + γ-butyrolactone | 3.41 | 52.1 | 2.51 | - | [17] |
| *C. necator* NCIMB 11599 | Glucose + γ-butyrolactone | 5.49 | 77 | 0.41 | - | [18] |
| *Yangia* sp. ND199 | Fructose+ γ-butyrolactone | 1.75 | 18.3 | 5.8 | - | [5] |
| *C.necator* H16 | CFO + sodium propionate | 7.4 | 56.1 | - | 10.9 | This work |
| *C.necator* H16 | WCO + sodium propionate | 6.3 | 59.1 | - | 11.5 | This work |
| *C.necator* DSM 545 | Glucose + propionic acid | 24.6 | 77.6 | - | 10.7 | [10] |
| Recombinant *E. coli* | Glucose + propionic acid | 3.20 | 58.5 | - | 20.2 | [13] |
| *Methylocystis parvus* OBBP | Methane + propionic acid | 1.25 | 32.0 | - | 8.0 | [11] |
| *Salinivibrio sp. TGB11* | Glucose + propionic acid | 7.63 | 57.0 | - | 8.6 | [12] |

## 4. Conclusion

The present study showed that the bacterial strain *C. necator* H16 is able to produce copolymers such as P(3HB-*co*-4HB) and P(3HB-*co*-3HV) when suitable precursors were added to the culture medium. The biosynthesis of P(3HB-*co*-4HB) was achieved by using crude fish oil or waste cooking oil as main carbon source, and γ-butyrolactone as 4HB precursors. P(3HB-*co*-3HV) was synthesized when sodium propionate was used as cosubstrate. The composition of copolymer varies depending on the concentrations of carbon sources supplied. Different kinds of PHA with different properties have great potential for application in many areas. In synthesis, the waste-oil platform and the quantitative trade-off maps, along with the mixture-property linkages, create a useful practical framework for manufacturing composition-tunable PHAs within established productivity boundaries.

## Acknowledgements

The authors gratefully acknowledge the University of Science-VNU, and the Hanoi National University of Education, Vietnam, for their valuable support and for providing the research infrastructure and facilities that made this study possible.

## References

[1] D. V. Thuoc, J. Quillaguamán, Improving Culture Conditions for Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) Production by Bacillus sp. ND153, Annals of Microbiology, Vol. 64, No. 3, 2014, pp. 991–997, https://doi.org/10.1007/s13213-013-0736-4.

[2] G. N. M. Huijberts, H. van der Wal, C. Wilkinson, G. Eggink, Gas-Chromatographic Analysis of Poly(3-Hydroxyalkanoates) in Bacteria, Biotechnology Techniques, Vol. 8, No. 3, 1994, pp. 187–192, https://doi.org/10.1007/BF00161588.

[3] J. Mai, K. Kockler, E. Parisi, C. M. Chan, S. Pratt, B. Laycock, Synthesis and Physical Properties of Polyhydroxyalkanoate (PHA)-based Block Copolymers: A Review, International Journal of Biological Macromolecules, Vol. 263, 2024, pp. 130204, https://doi.org/10.1016/j.ijbiomac.2024.130204.

[4] T. T. Loan, D. T. Q. Trang, P. Q. Huy, P. X. Ninh, D. V. Thuoc, A Fermentation Process for the Production of Poly(3-Hydroxybutyrate) Using Waste Cooking Oil or Waste Fish Oil as Inexpensive Carbon Substrate, Biotechnology Reports, Vol. 33, 2022, pp. e00700, https://doi.org/10.1016/j.btre.2022.e00700.

[5] T. H. Phong, D. V. Thuoc, K. Sudesh, Biosynthesis of Poly(3-Hydroxybutyrate) and Its Copolymers by *Yangia* sp. ND199 from Different Carbon Sources, International Journal of Biological Macromolecules, Vol. 84, 2016, pp. 361–366, https://doi.org/10.1016/j.ijbiomac.2015.12.037.

[6] S. Philip, T. Keshavarz, I. Roy, Polyhydroxyalkanoates: Biodegradable Polymers with a Range of Applications, Journal of Chemical Technology and Biotechnology, Vol. 82, No. 3, 2007, pp. 233–247, https://doi.org/10.1002/jctb.1667.

[7] B. H. A. Rehm, Polyester Synthases: Natural Catalysts for Plastics, Biochemical Journal, Vol. 376, No. 1, 2003, pp. 15–33, https://doi.org/10.1042/bj20031254.

[8] R. Bhati, N. Mallick, Production and Characterization of Poly(3-Hydroxybutyrate-Co-3-Hydroxyvalerate) Co-Polymer by a N2-Fixing Cyanobacterium Nostoc muscorum Agardh, Journal of Chemical Technology and Biotechnology, Vol. 87, No. 4, 2012, pp. 505–512, https://doi.org/10.1002/jctb.2737.

[9] S. Khanna, A. K. Srivastava, Recent Advances in Microbial Polyhydroxyalkanoates, Process Biochemistry, Vol. 40, No. 2, 2005, pp. 607–619, https://doi.org/10.1016/j.procbio.2004.01.053.

[10] G. C. Du, J. Chen, J. Yu, S. Lun, Feeding Strategy of Propionic Acid for Production of Poly(3-Hydroxybutyrate-Co-3-Hydroxyvalerate) with *Ralstonia eutropha*, Biochemical Engineering Journal, Vol. 8, No. 2, 2001, pp. 103–110, https://doi.org/10.1016/S1369-703X(01)00091-2.

[11] J. Myung, J. C. A. Flanagan, R. M. Waymouth, C. S. Criddle, Methane or Methanol-Oxidation Dependent Synthesis of Poly(3-Hydroxybutyrate-Co-3-Hydroxyvalerate) by Obligate Type II Methanotrophs, Process Biochemistry, Vol. 51, No. 5, 2016, pp. 561–567, https://doi.org/10.1016/j.procbio.2016.02.005.

[12] G. B. Tao, N. Pu, M. R. Wang, Z. J. Li, Hyper Production of Polyhydroxyalkanoates by a Novel Bacterium *Salinivibrio* sp. TGB11, Biochemical Engineering Journal, Vol. 185, 2022, pp. 108538, https://doi.org/10.1016/j.bej.2022.108538.

[13] T. R. Choi, J. M. Jeon, S. K. Bhatia, R. Gurav, Y. H. Han, Y. L. Park, J. Y. Park, H. S. Song, H. Y. Park, J. J. Yoon, S. O. Seo, Y. H. Yang, Production of Low Molecular Weight P(3HB-Co-3HV) by Butyrate-Acetoacetate CoA-Transferase (cftAB) in *Escherichia coli*, Biotechnology and Bioprocess Engineering, Vol. 25, No. 2, 2020, pp. 279–286, https://doi.org/10.1007/s12257-019-0366-1.

[14] S. Obruca, I. Marova, O. Snajdar, L. Mravcova, Z. Svoboda, Production of Poly(3-Hydroxybutyrate-Co-3-Hydroxyvalerate) by Cupriavidus necator from Waste Rapeseed Oil Using Propanol as a Precursor of 3-Hydroxyvalerate, Biotechnology Letters, Vol. 32, No. 12, 2010, pp. 1925–1932, https://doi.org/10.1007/s10529-010-0376-8.

[15] S. Z. Neoh, M. F Chek, H. T. Tan, J. A. Linares-Pastén, A. Nandakumar,vT. Hakoshima, K. Sudesh, Polyhydroxyalkanoate synthase (PhaC): The Key Enzyme for Biopolyester Synthesis, Current Research in Biotechnology, Vol. 4, 2022, pp. 87–101, https://doi.org/10.1016/j.crbiot.2022.01.002.

[16] J. M. B. T. Cavalheiro, R. S. Raposo, M. C. M. D. de Almeida, M. T. Cesário, C. Sevrin, C. Grandfils, M. M. R. da Fonseca, Effect of Cultivation Parameters on the Production of Poly(3-Hydroxybutyrate-Co-4-Hydroxybutyrate) and Poly(3-Hydroxybutyrate-4-Hydroxybutyrate-3-Hydroxyvalerate) by *Cupriavidus necator* Using Waste Glycerol, Bioresource Technology, Vol. 111, 2012, pp. 391–397, https://doi.org/10.1016/j.biortech.2012.01.176.

[17] J. S. Kim, B. H. Lee, B. S. Kim, Production of Poly(3-Hydroxybutyrate-Co-4-Hydroxybutyrate) by *Ralstonia eutropha*, Biochemical Engineering Journal, Vol. 23, No. 2, 2005, pp. 169–174, https://doi.org/10.1016/j.bej.2005.01.016.

[18] J. Y. Song, B. S. Kim, Characteristics of Poly(3-Hydroxybutyrate-Co-4-Hydroxybutyrate) Production by *Ralstonia eutropha* NCIMB 11599 and ATCC 17699, Biotechnology and Bioprocess Engineering, Vol. 10, No. 6, 2005, pp. 603–606, https://doi.org/10.1007/BF02932302.