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ORIGINAL PAPER

Valorization of Waste Coconut Oil into Polyhydroxyalkanoates via Thermophilic Bioconversion

Nassareeya Binlateh[1] · Aophat Choonut[2] · Narisa Binhayeeding[3] · Nisa Paichid[1] · Benjamas Cheirsilp[4] · Kanokphorn Sangkharak[2]

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Abstract
The conversion of agro-industrial residues into biodegradable polymers represents an approach for waste valorization and resource utilization. In this study, residual coconut solids from coconut milk processing were investigated as a feedstock for polyhydroxyalkanoate (PHA) production via thermophilic bioconversion. Waste coconut oil (WCO), recovered from the residues, was employed as the sole carbon source for PHA biosynthesis by *Caldibacillus thermoamylovorans* PHA005. Fatty acid profiling of WCO using Fourier Transform Infrared Spectroscopy (FTIR) and Gas Chromatography–Mass Spectrometry (GC–MS) revealed a heterogeneous composition comprising medium- and long-chain fatty acids (C12–C18). FT-IR was further used to confirm the functional groups of the extracted PHA, while GC–MS was employed for the analysis of hydroxyalkanoate monomers derived from the produced polymer. Under optimized cultivation conditions (4% w/v WCO, 45 °C, 250 rpm, 60 h), a maximum PHA accumulation of 89.7 ± 0.65% of dry cell weight was achieved, demonstrating substantial intracellular biopolymer accumulation from a waste-derived lipid substrate. Monomer composition analysis of methanolysed PHA revealed hydroxyalkanoates ranging from C8 to C18, including the odd-chain monomers C15 and C17, indicating a broad monomer composition. Thermal characterization revealed a melting temperature (T_m) of 75.0 °C, a glass transition temperature (T_g) of 5.1 °C, and a crystallization temperature (T_{cc}) of 56 °C, indicating distinct thermal characteristics of the produced polymer. The results demonstrate that waste coconut oil can serve as a carbon source for PHA production by *C. thermoamylovorans* PHA005 and provide further information on the composition and thermal properties of the resulting polymer. To the best of our knowledge, this is the first report describing PHA production from waste coconut oil by *C. thermoamylovorans* PHA005, expanding the range of waste-derived substrates evaluated for thermophilic PHA production.

Keywords Polyhydroxyalkanoates (PHA) · Waste coconut oil · Thermophilic bioconversion · Waste valorization · Circular bioeconomy

Introduction

The rapid growth of global population and industrialization has intensified environmental challenges, particularly the accumulation of persistent plastic waste. Conventional plastics such as polypropylene, polyethylene, and polyvinyl chloride are extensively used due to their durability, chemical resistance, and low production cost. However, their resistance to degradation and dependence on fossil resources have led to severe environmental pollution and increased greenhouse gas emissions throughout their life cycle [7, 18]. These concerns have driven increasing interest in biodegradable and bio-based polymers as sustainable

✉ Kanokphorn Sangkharak
kanokphorn.s@pkru.ac.th

1 Innovative Materials Chemistry for Environment Center, Faculty of Science and Digital Innovation, Thaksin University, Phatthalung 93210, Thailand

2 Faculty of Agricultural Technology, Phuket Rajabhat University, Phuket 83000, Thailand

3 Faculty of Science and Technology, Princess of Naradhiwas University, Narathiwat 96000, Thailand

4 Faculty of Agro-Industry, Prince of Songkla University, Songkhla 90110, Thailand

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alternatives that can mitigate plastic pollution and support circular material systems.

Among these alternatives, polyhydroxyalkanoates (PHAs) have attracted considerable attention as microbially synthesized polyesters that are fully biodegradable under various environmental conditions, including soil and marine environments [50]. The physicochemical properties of PHAs depend strongly on their monomer composition and carbon chain length. Based on monomer structure, PHAs are classified into short-chain-length (scl-PHAs, C3–C5), medium-chain-length (mcl-PHAs, C6–C14), and long-chain-length (lcl-PHAs, > C14) polymers [16, 21]. While scl-PHAs typically exhibit high crystallinity and brittleness, mcl- and lcl-PHAs show lower crystallinity, enhanced flexibility, and elastomeric properties, making them suitable for a wide range of applications. In particular, PHAs have been widely investigated for biodegradable food packaging films, agricultural mulch films, controlled drug delivery systems, tissue engineering scaffolds, and disposable medical products [6, 21]. Recent studies have also highlighted their potential in speciality applications such as bio-based adhesives, coatings, and flexible elastomeric materials, depending on monomer composition and crystallinity [2]. Importantly, the monomer composition of PHAs can be tailored by the type and composition of carbon substrates, particularly lipid-based feedstocks, which provide diverse metabolic precursors influencing polymer architecture and functional properties [19]. However, PHAs containing a broad range of medium- and long-chain-length monomers remain relatively underexplored.In parallel, the valorization of agro-industrial residues has attracted increasing attention as an approach for waste utilization and resource recovery. Coconut (Cocos nucifera), a major crop in tropical regions, generates approximately 55 million tons annually worldwide [8]. Coconut milk processing produces substantial quantities of residual pulp (coconut meal), which is often discarded or underutilized. This by-product contains 10–20% oil, primarily composed of medium-chain fatty acids (C8–C12), commonly referred to as waste coconut oil (WCO) [20, 38]. The high lipid content of WCO makes it a promising carbon source for microbial biopolymer production and provides an opportunity for the utilization of agro-industrial residues.

Although several studies have reported PHA production from coconut-derived oils using mesophilic bacteria such as *Pseudomonas* spp. and *Burkholderia cepacia* [4, 6, 13, 43], limited information is available regarding the utilization of waste coconut oil by thermophilic microorganisms for PHA production. Furthermore, information regarding the monomer composition of PHAs produced from waste coconut oil by thermophilic bacteria remains scarce. Thermophilic microorganisms have been reported to offer potential advantages for industrial bioprocessing, including operation at elevated temperatures and reduced susceptibility to contamination. However, comparative evaluations between thermophilic and mesophilic PHA production systems remain limited [15].

Therefore, this study investigates, for the first time, the utilization of waste coconut oil recovered from coconut milk processing residues as a carbon substrate for PHA biosynthesis by the thermophilic bacterium *Caldibacillus thermoamylovorans* PHA005. The resulting polymer was characterized in terms of monomer composition and thermal properties to evaluate its structural characteristics. This work expands the range of waste-derived substrates investigated for thermophilic PHA production and provides additional information on the characteristics of PHAs produced from waste coconut oil by *C. thermoamylovorans* PHA005.

Materials and Methods

Collection and Preparation of Waste Coconut Oil (WCO)

WCO was recovered from residual coconut solids obtained after coconut milk extraction at local fresh markets in Pa Phayom District, Phatthalung Province, Thailand. The residual coconut pulp was collected and allowed to undergo mild natural fermentation at room temperature for 72 h to promote slight acidification and phase separation. Following fermentation, the mixture was centrifuged at 5000 × g for 30 min to separate the lipid-rich upper layer from aqueous and solid residues [38]. The recovered lipid fraction was carefully collected and stored at 4 °C for subsequent experiments. The WCO was characterized for acid value (AV), free fatty acid (FFA) content, and fatty acid composition using FT-IR and GC–MS, identifying the major fatty acids that serve as precursors for substrate-driven monomer incorporation in PHA biosynthesis.

Microorganism, Medium, and Growth Conditions

The PHA-producing bacterium was classified as *Caldibacillus thermoamylovorans* PHA005 (formerly *Bacillus thermoamylovorans* PHA005). The strain was originally isolated from palm oil mill effluent and identified via 16S rRNA gene sequencing and biochemical profiling using Hi Carbohydrate™ kits (Himedia), showing 99% sequence identity to *C. thermoamylovorans*. The partial 16S rRNA gene sequence has been deposited in NCBI under accession number MK622837 [14]. The strain is maintained in 25% glycerol at − 20 °C and recultured monthly.

Routine cultivation was performed in 25 mL nutrient-rich (NR) medium (3 g/L yeast extract, 5 g/L peptone, pH 7.0) in 100 mL conical flasks, incubated at 45 °C on a rotary shaker at 150 rpm for 48 h. All experiments were conducted in triplicate.

Inoculum Preparation for PHA Production

Two loops of cells from NR agar plates (NR medium with 15 g/L agar) were transferred into 50 mL NR medium and incubated at 45 °C, 150 rpm for 48 h. Cells were used for inoculation when the optical density at 600 nm reached 1. Main fermentation was carried out in 500 mL conical flasks containing 250 mL NR medium (pH 7), inoculated with 25 mL of prepared culture (10% v/v), and incubated at 45 °C with continuous shaking [15].

Effect of Nutrient and Environmental Conditions

NR medium was supplemented with WCO as the sole carbon source. Cultivation conditions were optimized using a one-factor-at-a-time (OFAT) approach, in which a single variable was varied while all other parameters were maintained constant. The effect of WCO concentration (0.5–4% w/v) on PHA production was initially evaluated while maintaining other parameters constant (pH 7, 45 °C, 150 rpm, 48 h). The concentration yielding the highest PHA accumulation was subsequently selected for further optimization of environmental conditions. Incubation temperature was then evaluated from room temperature (28–32 °C) to 55 °C, followed by agitation speed ranging from 100 to 250 rpm. Cellular growth (dry cell weight, DCW) and PHA content (% of DCW) were measured to determine suitable conditions for PHA accumulation.

Because WCO is a lipid-rich and hydrophobic substrate, temperature and agitation were considered important operational parameters affecting substrate dispersion and oxygen transfer during cultivation [24]. Elevated temperatures may improve substrate fluidity and reduce oil viscosity [22], while agitation may enhance homogenization and microbial accessibility to hydrophobic carbon sources [36]. Therefore, these parameters were investigated to evaluate the suitability of thermophilic cultivation for lipid-based mcl-PHA production. In contrast, the initial medium pH (7.0) was maintained constant based on previous studies reporting optimal growth and PHA production of *C. thermoamylovorans* under neutral conditions [15]. The OFAT strategy was employed to systematically evaluate the effect of each selected operational parameter on PHA production under controlled and reproducible conditions.

Time-Course Study of PHA Production

Time-course experiments were conducted under optimal conditions in a 3-L glass fermenter equipped with three six-bladed Rushton turbine impellers (40 mm diameter). The pH was maintained at 7.0 and monitored via probes. Samples were collected at 12 h intervals over 72 h to determine DCW and PHA content.

Kinetic parameters, including specific growth rate (μ), product-to-cell yield coefficient ($Y_{p/x}$), and volumetric productivity (R_m), were calculated to evaluate fermentation performance according to Eq. (1)–(3) [15].

$$\mu = \mathrm{In}X_2 - \mathrm{In}X_1 / t_2 - t \quad (1)$$

where X_1 and X_2 are the biomass concentrations (g L^{-1}) at times t_1 and t_2, respectively.

$$Y_{p/x} = P / X \quad (2)$$

where P is the PHA concentration (g L^{-1}), and X is the biomass concentration (g L^{-1}).

$$R_m = P / t \quad (3)$$

where P is the PHA concentration (g L^{-1}) at cultivation time t (h).

Analytical Methods

Determination of Cell Concentration

Cellular biomass was determined gravimetrically. Ten milliliters of culture were centrifuged at 13,000 rpm (12,846 × g) for 15 min at 4 °C. Pellets were washed with 10 mL of distilled water and centrifuged again. Washed cells were dried at 105 °C for 24 h and cooled in a desiccator. Drying was repeated until a constant weight was reached. True cell concentration was calculated by subtracting PHA content from total biomass [14].

Determination of PHA Content and Composition

The isolated PHA was purified by re-precipitation in methanol and subsequently dried under vacuum. To cleave the polymer ester bonds, 1 mL of a sulfuric acid (H_2SO_4)–methanol mixture (containing 15% v/v H_2SO_4) and 1 mL of chloroform were added to the extracted polymer, and the mixture was heated at 100 °C for 3 h. The resulting hydroxyalkanoate methyl esters were recovered from the chloroform phase after the addition of distilled water [45].

The monomer composition of the purified PHA was analyzed using GC–MS. The analytical procedure was adapted from Choonut et al. [15] with minor modifications to optimize detection of medium- and long-chain hydroxyalkanoate methyl esters (C8–C18). GC analysis was performed using a Hewlett-Packard GC-6890 system equipped with a 5973-mass spectrometer and an HP-INNOWax capillary column (30 m × 0.25 mm × 0.25 µm). Samples (1 µL) were injected in splitless mode at 230 °C. The oven temperature program was set as follows: initial temperature 40 °C (hold 8 min), increased to 240 °C at 20 °C min^{-1}, and held for 10 min. Helium was used as the carrier gas at a flow rate of 3 mL min^{-1}. The mass spectrometer was operated in scan mode (m/z 40–360). Monomers were identified by comparison with the NIST library, and relative composition was calculated from GC peak areas. Intracellular PHA accumulation (%DCW) was calculated as:

$$\text{PHA accumulation (\%DCW)} = (\text{Weight of purified PHA / Dry cell weight}) \times 100 \quad (4)$$

Purified PHA yield (g L^{-1}) was calculated as the mass of purified polymer recovered after extraction divided by the culture volume.

Fourier Transform Infrared Spectroscopy (FTIR)

Approximately 5 mg of PHA were analyzed by FTIR over the spectral range of 4000–400 cm^{-1} (2.5–25 µm) to identify characteristic functional groups and confirm the polymer type.

Table 1 Chemical characteristics and fatty acid composition of waste coconut oil (WCO)

Parameter	Value / Result
Acid Value	1.46 ± 0.81 mg KOH/g
Free Fatty Acid (FFA)	0.67 ± 0.38%
Moisture Content	0.2 ± 0.61%
Functional Groups (FTIR)	2852–2923 cm^{-1} (Aliphatic C–H stretching (CH_2/CH_3)) 1741 cm^{-1} (Ester carbonyl (C = O) stretching) 1464 cm^{-1} (CH_2 bending vibration) 1376 cm^{-1} (CH_3 bending vibration) 1110 cm^{-1} (C–O stretching of ester groups)
Fatty Acid Composition (GC–MS)	Lauric acid (C12): 45.1% Myristic acid (C14): 27.4% Stearic acid (C18): 15.7% Palmitic acid (C16): 1.6% Elaidic (C18:1): 5.2% Linoleic (C18:2): > 2% Caprylic acid (C8): > 1% Others: < 1%

Differential Scanning Calorimetry (DSC)

The thermal properties of PHA were evaluated using a DSC 8500 (PerkinElmer). Approximately 5 mg of polymer was sealed in aluminum pans. Samples were first equilibrated at 40 °C, then heated from 40 to 200 °C at a rate of 10 °C/min under a nitrogen atmosphere (flow rate 20 mL/min). An empty aluminum pan was used as a reference. Key thermal parameters, including melting temperature (T_m), glass transition temperature (T_g) and crystallization temperature (T_{cc}), were determined from the thermograms [15].

Determination of Acid Value, Free Fatty Acids and Moisture Content

The AV and FFA content of WCO was determined according to standard AOAC titration principles using potassium hydroxide (KOH). Briefly, approximately 5 g of oil sample was dissolved in a neutralized ethanol–diethyl ether mixture (1:1 v/v). The solution was titrated with standardized 0.1 N KOH using phenolphthalein as an indicator until a persistent pink endpoint was observed. The acid value was expressed as mg KOH per g of oil. The free fatty acid content was calculated as oleic acid equivalent using standard conversion factors.

Moisture content of WCO was determined by the oven-drying method according to AOAC International [5]. Approximately 2 g of the oil sample was heated at 105 °C until constant weight was achieved. Moisture content was calculated as the percentage weight loss after drying.

Statistical Analysis

All experiments were conducted in triplicate with independent biological replicates. Differences between treatments were assessed using one-way ANOVA (SPSS Inc.). Differences with $P < 0.05$ were considered statistically significant. Results are presented as mean ± standard deviation. Batch variability was included in the analysis to ensure robustness.

Results and Discussion

Fatty acid Composition of Waste Coconut Oil (WCO) and Its Relevance to PHA Monomer Formation

WCO derived from coconut milk processing was evaluated as a potential carbon substrate for the production of PHAs. Key physicochemical properties, including acidity, functional groups, and fatty acid composition, were analyzed to assess its suitability as a metabolic precursor for PHA biosynthesis (Table 1).

The extracted WCO exhibited an acid value of 1.46 ± 0.81 mg KOH g^{-1} and a FFA content of 0.67 ± 0.38%, indicating relatively low acidity and good substrate stability. Such characteristics are generally favorable for microbial lipid assimilation and downstream bioconversion processes. The relatively high standard deviations observed for acid value and FFA content may reflect the heterogeneous nature of waste coconut oil recovered from coconut milk processing residues. In addition, the oil recovery process involved a 72-h natural fermentation step prior to lipid separation, during which variations in microbial activity and the extent of triglyceride hydrolysis may have influenced free fatty acid formation. Previous studies have reported that fermentation can promote lipid hydrolysis and increase acid value and FFA levels through the breakdown of triglycerides into free fatty acids [48]. Therefore, some variability in these parameters is expected for waste-derived lipid feedstocks.

FTIR spectroscopy revealed characteristic absorption bands typical of triglyceride-based lipids, including C–H stretching vibrations at 2852 and 2923 cm^{-1} corresponding to aliphatic CH_2 and CH_3 groups, a strong ester carbonyl (C = O) stretching band at 1741 cm^{-1}, CH_2 bending at 1464 cm^{-1}, CH_3 bending at 1376 cm^{-1}, and C–O stretching of ester groups around 1110 cm^{-1} (Table 1 and Supporting Information). These spectral features confirm the presence of fatty acid ester bonds and long hydrocarbon chains characteristic of waste coconut oil, which can serve as carbon-rich substrates for microbial PHA biosynthesis.

GC–MS analysis further revealed that WCO was predominantly composed of lauric acid (C12, 45.1%), myristic acid (C14, 27.4%), and stearic acid (C18, 15.7%), with smaller fractions of palmitic acid (C16, 1.6%), elaidic acid (C18:1, 5.2%), linoleic acid (C18:2, > 2%), and caprylic acid (C8, > 1%) (Table 1). The remaining fraction (< 1%) consisted of other fatty acids and triglycerides that were not individually quantified. The dominance of medium- and long-chain fatty acids indicates that WCO contains a diverse range of fatty acid components that may be associated with the monomer composition of the resulting PHA polymer.

The FTIR and GC–MS profiles obtained in this study are consistent with previously reported fatty acid compositions of coconut oil and other vegetable oils [9]. Similar feedstocks rich in fatty acids have been shown to support microbial PHA synthesis and influence the structural characteristics of the resulting polymers [34, 37].

Our previous studies demonstrated that *C. thermoamylovorans* PHA005 is capable of synthesizing PHAs when cultivated with lipid-based substrates such as used vegetable oil (UVO). While the wild-type strain predominantly produced short-chain-length PHAs [40], genetically modified variants [27] or supplementation with sodium octanoate enabled the formation of polymers containing longer-chain monomers [15]. However, PHA production containing both medium- and long-chain-length monomers using WCO as the sole carbon source has not yet been reported.

Given its high fatty acid content and favorable chemical composition, WCO represents a promising waste-derived feedstock for sustainable PHA production. The presence of diverse fatty acid chains in WCO may contribute to the diversity of monomers detected in the produced PHA. The resulting polymer exhibited distinct thermal characteristics, which were further evaluated in subsequent analyses. Therefore, in the subsequent sections, cultivation conditions were optimized to enhance polymer production from WCO using the thermophilic bacterium *C. thermoamylovorans* PHA005. The relationship between the fatty acid composition of WCO and the monomer composition of the resulting PHA is further discussed in Sect. "Characterisation of PHA".

Cultivation parameters influencing PHA biosynthesis from waste coconut oil (WCO)

To enable efficient conversion of waste coconut oil into PHAs, key cultivation parameters affecting microbial growth and polymer biosynthesis were investigated. In particular, the concentration of WCO, incubation temperature, and agitation speed were evaluated due to their influence on carbon availability, oxygen transfer, and intracellular PHA accumulation. Understanding these parameters is important not only for maximizing polymer yield but also for establishing suitable conditions for producing PHA biomaterials from lipid-rich waste substrates.

Effect of Waste Coconut Oil (WCO) Concentration

The influence of WCO concentration on PHA biosynthesis by *C. thermoamylovorans* PHA005 was investigated using 0, 0.5, 1, 2, and 4% (w/v) WCO in NR medium (pH 7). Cultures were incubated at 45 °C with agitation at 150 rpm for 48 h. As shown in Fig. 1, the concentration of WCO strongly affected intracellular polymer accumulation. In the absence of WCO, cultures produced only 10.3 ± 0.42% PHA of DCW, indicating minimal background polymer synthesis. Increasing the WCO concentration gradually enhanced PHA accumulation to 16.1 ± 0.11%, 17.3 ± 0.34%, and 22.0 ± 0.92% DCW at 0.5%, 1%, and 2% WCO, respectively, with the highest accumulation of 41.2 ± 0.18% DCW observed at 4% WCO.

These findings highlight the importance of sufficient carbon availability for efficient PHA biosynthesis, as fatty-acid-derived substrates are recognized as suitable carbon sources for microbial PHA production [12]. Lipid-based carbon sources such as vegetable oils are known to enhance PHA

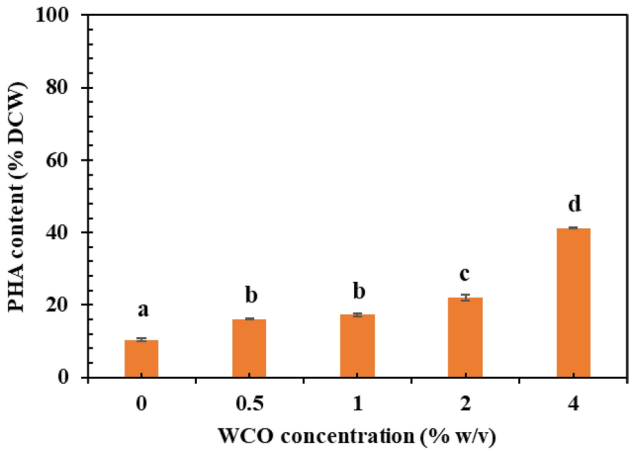

Fig. 1 Effect of waste coconut oil (WCO) concentration on PHA accumulation by *Caldibacillus thermoamylovorans* PHA005 after 48 h. Values with different letters are significantly different ($p < 0.05$)

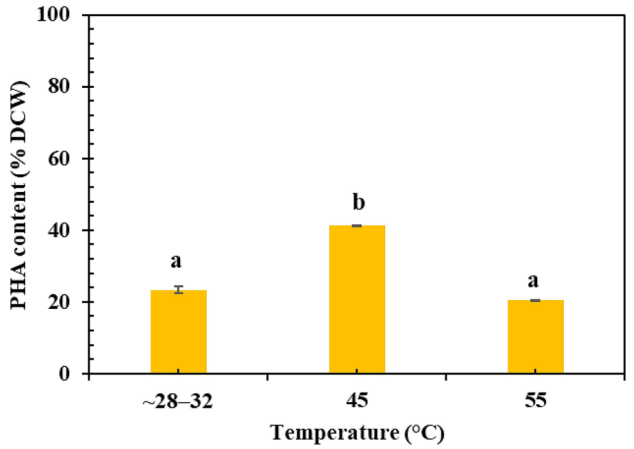

Fig. 2 Effect of incubation temperature on PHA accumulation by *Caldibacillus thermoamylovorans* PHA005 after 48 h. Values with different letters are significantly different ($p < 0.05$)

production due to their high energy density and the availability of fatty acid intermediates that can enter β-oxidation pathways involved in PHA biosynthesis. Although higher WCO concentrations were not evaluated in the present study, excessive lipid levels may lead to substrate inhibition, oxygen transfer limitations, or emulsification effects that negatively impact microbial activity [11]. Previous studies similarly reported that increasing UVO concentrations beyond 4% (w/v) did not further enhance PHA accumulation [39]. Additionally, elevated fatty acid levels may exert antimicrobial effects, destabilizing bacterial membranes and inhibiting microbial growth [49]. Lauric acid, a major component of coconut oil, has also been reported to inhibit the growth of certain Gram-positive bacteria [26]. Concentrations higher than 4% (w/v) were not further investigated in this study due to the observed optimal performance at 4% (w/v) and the known limitations of lipid-rich substrates at elevated concentrations, including reduced oxygen transfer efficiency and potential substrate inhibition. The 4% (w/v) concentration was identified as the practical upper performance limit under the present experimental system. Therefore, 4% (w/v) WCO was selected as the optimal substrate concentration for subsequent experiments.

Effect of Incubation Temperature

Temperature is an important parameter influencing microbial metabolism, enzyme activity, and intracellular polymer biosynthesis. The effect of incubation temperature on PHA accumulation by *C. thermoamylovorans* PHA005 was evaluated at room temperature (~28–32 °C), 45 °C, and 55 °C in NR medium supplemented with 4% (w/v) WCO, with agitation at 150 rpm for 48 h. As shown in Fig. 2, the highest PHA accumulation (41.2 ± 0.18% DCW) was observed at 45 °C, whereas cultures incubated at room temperature and 55 °C produced lower PHA contents of 23.3 ± 0.91% and 20.4 ± 0.08% DCW, respectively.

C. thermoamylovorans is classified as a moderately thermophilic bacterium capable of growth at temperatures up to approximately 58 °C, with an optimal growth range near 50 °C [17]. However, 50 °C was not included as an experimental condition, as preliminary screening and literature comparison indicated that no clear improvement in PHA accumulation was observed beyond 45 °C under sodium octanoate-based cultivation conditions [15]. Maximal polymer accumulation does not always coincide with maximal growth temperature. The reduced PHA accumulation observed at elevated temperatures may be associated with physiological stress responses and altered cellular metabolism [12]. Similar observations have been reported in other thermophilic microorganisms. For instance, *Haloferax mediterranei* exhibited maximum PHA biosynthesis at temperatures different from those supporting optimal cell growth [32], while *Cupriavidus* sp. S-6 isolated from a hot spring produced maximal PHA at 50 °C [42]. Based on these findings, 45 °C was selected as the optimal incubation temperature for further experiments and was used as the representative condition for subsequent studies. This selection is consistent with our previous work on *C. thermoamylovorans* PHA005, where 45 °C was identified as the optimal temperature for mcl-PHA production [15].

Effect of Agitation Speed

Agitation plays a critical role in aerobic microbial cultivation by influencing oxygen transfer, substrate dispersion, and nutrient availability. The effect of agitation speed on PHA biosynthesis by *C. thermoamylovorans* PHA005 was evaluated at 100, 150, 200, and 250 rpm under incubation at

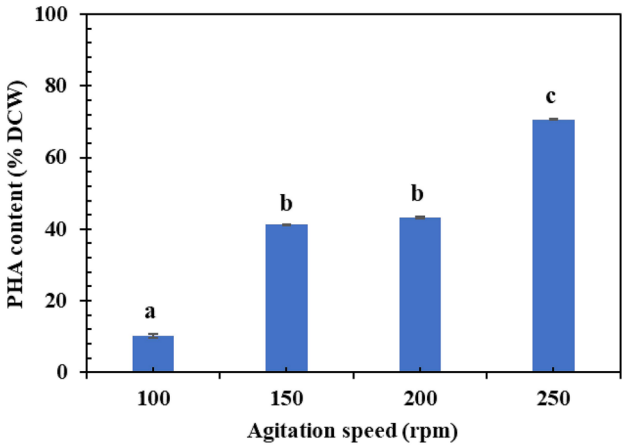

Fig. 3 Effect of agitation speed on PHA accumulation by *Caldibacillus thermoamylovorans* PHA005 after 48 h. Values with different letters are significantly different ($p<0.05$)

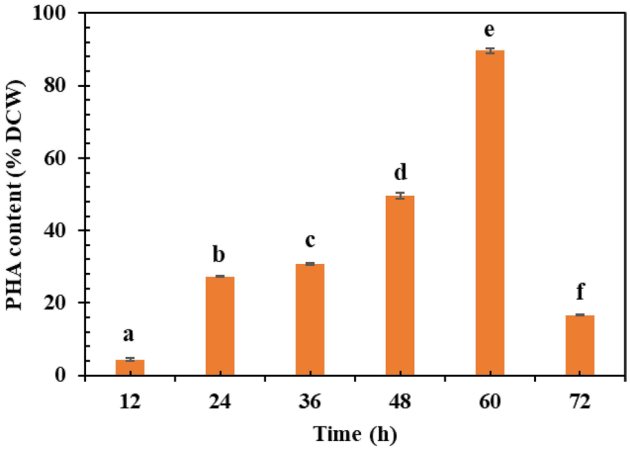

Fig. 4 Time-course of PHA accumulation by *Caldibacillus thermoamylovorans* PHA005 under optimized conditions (45 °C, 4% WCO, 250 rpm) over 72 h. Values with different letters are significantly different ($p<0.05$)

45 °C using NR medium supplemented with 4% (w/v) WCO for 48 h. As shown in Fig. 3, PHA accumulation increased with agitation speed, reaching 10.1 ± 0.56%, 41.2 ± 0.18%, 43.2 ± 0.33%, and 70.6 ± 0.10% DCW at 100, 150, 200, and 250 rpm, respectively.

The increase in PHA accumulation at higher agitation speeds may be attributed to improved mixing and oxygen transfer, which enhance substrate utilization and microbial activity during cultivation. At lower agitation speeds, limited oxygen transfer and insufficient mass transport likely restrict cellular metabolism, resulting in reduced polymer accumulation. These findings are consistent with previous reports demonstrating that agitation significantly affects microbial growth and PHA biosynthesis through its impact on oxygen transfer and mixing efficiency [40, 42]. Agitation speeds higher than 250 rpm were not investigated due to the operational limitations of the shake flask system and potential issues such as increased shear stress, foaming, and higher energy consumption at elevated agitation rates commonly reported in aerobic fermentation processes [25, 36]. Within the tested range, no decline in PHA accumulation was observed, indicating that 250 rpm represents the optimal balance between oxygen transfer efficiency and cellular stability. Based on these results, the optimized cultivation conditions for PHA production from WCO using *C. thermoamylovorans* PHA005 were 4% (w/v) WCO, 45 °C, and 250 rpm.

Time-Course Study

The temporal profile of PHA accumulation by *C. thermoamylovorans* PHA005 was monitored for 72 h under the optimized cultivation conditions (45 °C, 4% WCO, 250 rpm) in a 3-L fermenter. Samples were collected at 12, 24, 36, 48, 60, and 72 h to determine DCW and intracellular PHA content (Fig. 4).

PHA synthesis initiated slowly during the early cultivation stage, reaching 4.3 ± 0.42% of DCW at 12 h, followed by a rapid increase after 24 h. Polymer accumulation continued to rise throughout the exponential phase and reached a maximum of 89.7 ± 0.65% of DCW at 60 h. After this point, PHA content declined sharply to 16.6 ± 0.13% at 72 h, which may be attributed to intracellular depolymerase activity or the reutilization of stored polymer as an endogenous carbon and energy source under nutrient-limited conditions [10, 28, 46]. The delayed onset of polymer accumulation during the first 24 h may reflect an adaptation phase before substantial polymer accumulation was observed. Previous studies have demonstrated that fatty-acid substrates can support PHA biosynthesis through β-oxidation-associated pathways in various microorganisms (Kruaejun et al. 2025). However, the metabolic mechanisms responsible for PHA accumulation were not investigated in the present study.

Residual WCO concentrations fluctuated slightly between approximately 0.10–0.15% (w/v) throughout the cultivation period, indicating continuous substrate utilization linked to microbial growth dynamics and lipid metabolism. Such behavior is typical in oil-based fermentation systems, where substrate uptake depends on emulsification, enzyme activity, and transport processes associated with fatty acid degradation.

A comparison with previous studies further highlights the influence of substrate composition on PHA accumulation. When sodium octanoate (SO) was used as a defined carbon source, *C. thermoamylovorans* PHA005 accumulated 63.9% PHA at 96 h, with a lower specific growth rate and product yield [15]. In contrast, the present study achieved a higher polymer accumulation (89.7% of DCW) within a

shorter cultivation time (60 h) using WCO (Table 2). The higher PHA accumulation observed with WCO compared with sodium octanoate indicates that waste coconut oil can serve as an effective substrate for PHA production by *C. thermoamylovorans* PHA005. However, the mechanisms underlying these differences were not investigated in the present study.

Similar patterns have been observed in other thermophilic PHA-producing microorganisms, where lipid-based substrates support high intracellular polymer accumulation but also lead to rapid polymer turnover once external nutrients become depleted [3, 10, 28]. Our previous work also demonstrated that PHA005 accumulated 87.5% PHA after 48 h when cultivated with UVO, although the resulting polymer consisted mainly of scl-PHA [40]. In contrast, the present study demonstrates enhanced PHA accumulation when WCO was used as the carbon source, suggesting that differences in substrate composition may be associated with variations in PHA production performance.

These findings demonstrate that WCO can support substantial PHA accumulation under the cultivation conditions employed and provide a basis for further characterization of the resulting polymer. The polymer obtained under these optimized conditions was therefore subjected to further analysis to determine its monomer composition and physicochemical properties, as discussed in the following section.

Characterisation of PHA

FTIR Analysis

The FTIR spectra revealed characteristic absorption bands associated with PHA polymers. A weak broad band around 3300 cm^{-1} may be attributed to O–H stretching vibrations associated with terminal hydroxyl groups or residual moisture. The aliphatic C–H stretching region observed at 2852–2921 cm^{-1} corresponds to methyl and methylene groups within the polymer backbone. A strong absorption band at approximately 1741 cm^{-1} was assigned to ester carbonyl (C=O) stretching, representing the characteristic ester linkage of PHA polymers. Additional bands at 1464 and 1376 cm^{-1} correspond to CH_2 and CH_3 bending vibrations, respectively, while the absorption region around 1050–1188 cm^{-1} was attributed to C–O stretching of ester groups (Table 3 and Supporting Information).

These spectral features are consistent with previously reported FTIR profiles of PHAs, including commercially available short-chain-length PHAs such as polyhydroxybutyrate (PHB) and PHB-co-hydroxyvalerate (PHB-co-PHV) [35, 41, 44]. Slight variations in peak position and intensity between polymers derived from different carbon sources may reflect differences in monomer composition and chain packing within the polymer matrix. Overall, the FT-IR results confirm that the polymer produced from waste coconut oil retains the essential functional groups characteristic of structurally intact PHA.

Table 2 Comparison of PHA production kinetics and yields of *Caldibacillus thermoamylovorans* PHA005 on different carbon sources: waste coconut oil (WCO) and sodium octanoate (SO)

Carbon Source	Time (h)	PHA (% DCW)	μ, h^{-1}	Yp/x, g/g	Rm, g/L·h
WCO	60	89.7	0.022	1.37	0.00633
SO	96	63.9	0.018	0.68	0.05

Table 3 Key FTIR absorption bands of PHA derived from different carbon sources, including sodium octanoate (SO) and used vegetable oil (UVO), and compares them with the current study (waste coconut oil; WCO)

Functional Group	[a]SO	[b]UVO	[c]WCO
O–H stretching	-	3300 cm^{-1}	3300 cm^{-1}
C–H stretching	2978–2932 cm^{-1}	2800–3000 cm^{-1}	2852–2921 cm^{-1}
C=O stretching	1721 cm^{-1}	1650 cm^{-1}	1741 cm^{-1}
C–O stretching	1054 cm^{-1}	1050 cm^{-1}	1050 cm^{-1}

[a]Choonut et al. [15]

[b]Petpheng et al. (2023)

[c]This study

GC–MS Analysis

The monomer composition of the PHA produced by *C. thermoamylovorans* PHA005 grown on WCO was determined using GC–MS analysis (Table 4). The polymer consisted of hydroxyalkanoate monomers ranging from C8 to C18, indicating the formation of a medium- to long-chain-length PHA copolymer. Minor fractions of 3-hydroxyoctanoate (3HO, < 1 mol%) and 3-hydroxydecanoate (3HD, < 1 mol%) were detected as representative mcl units, whereas the polymer was dominated by long-chain monomers including 3-hydroxypentadecanoate (3HPD, 53.9 mol%), 3-hydroxyheptadecanoate (3HHeD, 20.3 mol%), and 3-hydroxyoctadecanoate (3HOD, 24.6 mol%).

The predominance of long-chain monomers may be associated with the lipid-rich nature of WCO. Similar monomer distributions have been reported in PHA-producing microorganisms cultivated on fatty-acid-based substrates [30]. Fatty-acid utilization in many PHA-producing bacteria is generally associated with β-oxidation pathways that generate hydroxyacyl-CoA intermediates for polymer synthesis. However, the metabolic pathways involved were not investigated in the present study.

A notable feature of the present study is the substantial incorporation of odd-chain hydroxyalkanoate monomers (C15 and C17). Although waste coconut oil predominantly contains even-chain fatty acids, previous studies have

Table 4 Monomer composition of PHA produced from different carbon sources: sodium octanoate (SO), coconut oil (CO), used vegetable oil (UVO), and waste coconut oil (WCO)

Monomer	Abbrev	C#	[a]SO	[b]UVO	[c]UVO	[d]CO	[e]WCO
3-Hydroxyoctanoate	3HO	8	24.1	–	–	–	< 1
3-Hydroxydecanoate	3HD	10	15.5	–	–	–	< 1
3-Hydroxydodecanoate	3HDD	12	–	–	–	–	–
3-Hydroxytetradecanoate	3HTD	14	18.4	30.5	32.4	–	–
3-Hydroxypentadecanoate	3HPD	15	–	–	–	–	53.9
3-Hydroxyhexadecanoate	3HHD	16	19.7	25.5	32.9	13	–
3-Hydroxyheptadecanoate	3HHeD	17	–	–	–	–	20.3
3-Hydroxyoctadecanoate	3HOD	18	20.9	44.3	43.7	18.1	24.6

[a]Choonut et al. [14, 15]

[b]Kruaejun et al. (2025)

[c]Petpheng et al. (2023)

[d]Singh and Mallick study [43]

[e]This study

Table 5 Comparison of thermal properties of PHAs produced from different carbon sources: sodium octanoate (SO), used vegetable oil (UVO), and waste coconut oil (WCO)

PHA Type	Source	Tm (°C)	Tg (°C)	ΔHm (J/g)	Tcc (°C)	ΔHc (J/g)
[a]mcl-co-lcl	WCO	75.0	5.1	70.3	56.0	31.4
[b]mcl-co-lcl	SO	68.0	1.3	62.9	47.3	26.5
[c]scl-co-lcl	UVO	115–120	−13 to −14	n.d	n.d	n.d

[a]This study

[b]Choonut et al. [14, 15]

[c]Singh and Mallick study (2009)

reported trace amounts of odd-chain fatty acids in coconut-derived oils [31]. In addition, odd-chain hydroxyalkanoates may arise through metabolic transformations associated with fatty acid degradation and biosynthesis. A plausible explanation is that β-oxidation and related fatty acid metabolic pathways generate acyl-CoA intermediates of different chain lengths, some of which may subsequently be incorporated into the growing PHA polymer. Similar observations have been reported in engineered and natural PHA-producing microorganisms, where alterations in precursor pools and carbon fluxes resulted in the formation of odd-chain monomers [51]. However, the specific metabolic mechanisms responsible for C15 and C17 monomer formation in *C. thermoamylovorans* PHA005 were not investigated in the present study and therefore require further examination.

Comparisons with previous studies further highlight the influence of substrate composition on polymer architecture (Table 4). When sodium octanoate was used as a carbon source, *C. thermoamylovorans* PHA005 produced mcl-co-lcl PHA containing monomers such as 3HO, 3HD, 3-Hydroxytetradecanoate (3HTD), and 3HOD, with 3-Hydroxyhexadecanoate (3HHD) as the dominant monomer (39.25%) [15]. Similarly, gene knockout studies confirmed the formation of mcl-co-lcl PHA from used vegetable oil through β-oxidation-mediated pathways (Kruaejun et al. 2025). In contrast, the present work demonstrates a broader monomer distribution, including both mcl (C8–C10) and lcl (C16–C18) units together with a high proportion of odd-chain monomers. These results demonstrate that WCO can support the biosynthesis of PHAs containing a broad range of hydroxyalkanoate monomers. The observed monomer diversity highlights the potential of waste-derived lipid substrates for producing structurally diverse biodegradable polymers.

DSC Analysis

The thermal properties of the PHA produced from WCO were analyzed using DSC. The polymer exhibited a melting temperature (T_m) of 75.0 °C, a glass transition temperature (T_g) of 5.1 °C, and a melting enthalpy (Δ_{Hm}) of 70.3 J g^{-1} (Table 5). During the cooling cycle, a crystallization temperature (T_{cc}) of 56 °C with a crystallization enthalpy (Δ_{Hc}) of 31.4 J g^{-1} was observed. These values are slightly higher than those reported for mcl-co-lcl PHA produced from sodium octanoate (T_m = 68 °C, T_g = 1.3 °C) [15], suggesting that differences in monomer composition may influence the crystallinity and thermal stability of the polymer. The presence of long-chain hydroxyalkanoate units can enhance intermolecular interactions and improve packing within crystalline domains, thereby increasing the melting temperature and enthalpy [1, 23].

Table 6 Comparison of PHA production, monomer composition, and carbon sources among different microbial strains: coconut oil (CO), sodium octanoate (SO), used vegetable oil (UVO), and waste coconut oil (WCO)

Carbon Source/Conc	Strain	PHA (%)	Detected hydroxyalkanoate monomers	Ref
0.3% CO	MTCC 7925	25.3	scl-co-lcl PHA C4, C5, C16, C18	[43]
0.3% SO	PHA005	63.3	PHA (C8–C18) C8, C10, C14, C16, C18	[15]
2% UVO	TS1L	54	PHA (C8–C18) C14, C16, C18	[37]
2% CO	CH50	58	mcl PHA C8, C10, C12	[6]
1% CO	JC-1	18.8	scl PHA C4	[13]
4% WCO	PHA005	89.7	PHA containing C8, C10, C15, C17, C18	This study

Direct studies on WCO-derived PHA are scarce; comparative data include different oils and microbial strains

At the same time, the incorporation of odd-chain monomers (C15 and C17) may partially disrupt regular chain packing, contributing to moderate crystallinity and maintaining chain mobility within amorphous regions. Such structural features often result in polymers that exhibit a balance between flexibility and thermal resistance. Similar structure–property relationships have been reported for copolymers containing mixed chain-length hydroxyalkanoates [29, 33]. Compared with scl-lcl PHA derived from coconut oil, which exhibits much higher melting temperatures (115–120 °C) and extremely low glass transition temperatures (−13 to−14 °C) [43], the polymer produced in this study displays a more moderate thermal profile. The combination of T_m = 75 °C and T_g = 5.1 °C suggests that the material retains sufficient rigidity while still allowing limited chain mobility at ambient temperatures.

The observed thermal characteristics indicate that the polymer possesses distinct thermal behavior compared with previously reported PHAs. However, additional characterization, including molecular weight determination and mechanical property evaluation, would be required to fully assess its suitability for specific material applications. More broadly, these findings suggest that variations in PHA monomer composition are associated with differences in thermal behavior. Further studies investigating polymer microstructure and biosynthetic mechanisms would be valuable for understanding the relationships between composition and material properties.

Comparison of PHA Production, Monomer Composition, and Carbon Sources Among Different Microbial Strains

A comparison of PHA production by different microbial strains using various carbon sources reveals substantial variation in both polymer yield and monomer composition (Table 6). These differences may be associated with the characteristics of the producing microorganisms and the carbon substrates employed.

For example, *Pseudomonas aeruginosa* MTCC 7925 produced PHA from CO with a relatively low yield of 25.3% of DCW and monomers corresponding to C4, C5, C16, and C18 hydroxyalkanoates [43]. In contrast, *C. thermoamylovorans* PHA005, grown on SO, accumulated 63.3% PHA of DCW and produced polymers containing monomers ranging from C8 to C18 [15]. Similarly, *Enterobacter* sp. TS1L cultivated on UVO accumulated 54% PHA of DCW, with monomers primarily corresponding to C14, C16, and C18 hydroxyalkanoates [37]. Comparable observations have also been reported for other microorganisms. For instance, *Pseudomonas mendocina* CH50 cultivated on CO accumulated 58% mcl-PHA consisting mainly of C8–C12 monomers [6]. In contrast, *Burkholderia cepacia* JC-1 synthesized a scl-PHA dominated by C4 monomers with a relatively low polymer content of 18.8% DCW [13].

Among the strains compared, the polymer synthesized in the present study from WCO by *C. thermoamylovorans* PHA005 exhibited the highest intracellular PHA accumulation (89.7% DCW) together with a broad monomer spectrum including C8, C10, C15, C17, and C18 hydroxyalkanoates. Compared with previously reported waste-oil-based PHA production systems, these findings highlight both a quantitative advantage in polymer accumulation and an expanded monomer composition. To the best of our knowledge, this is the first report describing PHA production from waste coconut oil by *C. thermoamylovorans* PHA005.

The occurrence of odd-chain hydroxyalkanoates (C15 and C17) contributes to the overall monomer diversity observed in the polymer. Similar observations have been reported in PHAs synthesized from lipid-based substrates, although the metabolic pathways responsible for monomer incorporation may vary among microorganisms [30, 47]. Although waste coconut oil is predominantly composed of even-chain fatty acids, a plausible explanation is that β-oxidation and related fatty acid metabolic pathways generate acyl-CoA intermediates of different chain lengths during fatty acid degradation. Some of these intermediates may subsequently be converted into hydroxyacyl-CoA precursors and incorporated into the growing PHA polymer. Therefore, the occurrence of C15 and C17 monomers observed in the present study may be associated with metabolic transformations occurring

during fatty acid utilization rather than direct incorporation from the feedstock alone. However, the specific metabolic mechanisms underlying the formation of odd-chain monomers in PHA005 were not investigated in the present study and therefore require further examination. From a materials perspective, the coexistence of medium- and long-chain hydroxyalkanoates together with odd-chain monomers may influence polymer structure and thermal behavior. Previous studies have suggested that variations in monomer composition can affect crystallinity, chain mobility, and flexibility [33]. Accordingly, the broad monomer composition observed in the present study may contribute to the distinctive thermal characteristics of the resulting polymer.

Overall, the present findings demonstrate the feasibility of utilizing waste coconut oil as a substrate for thermophilic PHA production. The combination of high intracellular PHA accumulation and broad monomer diversity expands the current knowledge of waste-oil-based PHA biosynthesis by thermophilic microorganisms and provides a basis for future investigations into polymer structure–property relationships and process development.

Monomer Composition and Thermal Characteristics of Waste Coconut Oil (WCO)-Derived PHA

The fatty acid analysis of WCO revealed the presence of medium- and long-chain fatty acids, including lauric, myristic, palmitic, stearic, elaidic, and linoleic acids. Such lipid-rich substrates have previously been reported as suitable carbon sources for microbial PHA production [11].

GC–MS analysis of methanolysed PHA produced by *C. thermoamylovorans* PHA005 revealed hydroxyalkanoate monomers corresponding to C8, C10, C15, C17, and C18, indicating the formation of an mcl-lcl PHA copolymer. The occurrence of odd-chain monomers (C15 and C17) contributed to the overall diversity of the polymer composition. Similar observations have been reported in PHAs synthesized from lipid-based substrates [30]. However, the metabolic pathways responsible for monomer incorporation were not investigated in the present study. Variations in monomer composition have been reported to influence polymer packing, crystallinity, and thermal behavior. Polymers containing mixed chain-length hydroxyalkanoates generally exhibit lower crystallinity and greater flexibility than conventional scl PHAs [29]. In the present study, differential scanning calorimetry revealed a T_m of 75 °C and a T_g of 5.1 °C, suggesting moderate crystallinity and elastomer-like behavior. These thermal characteristics are consistent with previous reports describing mcl and lcl PHA copolymers that exhibit enhanced flexibility and distinctive thermal properties [33]. Although the polymer produced in this study displayed a broad monomer composition together with moderate thermal stability, further investigations involving molecular-weight analysis, polymer microstructure characterization, and metabolic studies would be required to establish direct relationships between substrate composition, monomer distribution, and material properties.

Overall, the results demonstrate that waste coconut oil can support the production of PHAs containing a broad range of hydroxyalkanoate monomers while exhibiting distinct thermal characteristics. The combination of high polymer accumulation, diverse monomer composition, and moderate thermal stability highlights the potential of WCO as a renewable substrate for thermophilic PHA production. Further investigations into polymer microstructure, molecular-weight distribution, and biosynthetic mechanisms would provide a more comprehensive understanding of the factors influencing polymer composition and material properties.

Waste Valorization Potential of Waste Coconut Oil (WCO) for PHA Production

The utilization of WCO as a carbon source for PHA production demonstrates the feasibility of converting an agro-industrial by-product into a value-added biopolymer. Coconut milk processing generates considerable quantities of residual coconut solids containing recoverable lipids, which may represent an underutilized resource. The recovery and utilization of WCO for microbial fermentation therefore provide an alternative approach for the use of agro-industrial residues.

Previous studies have shown that lipid-rich waste materials, including waste cooking oil and industrial oil residues, can serve as substrates for microbial PHA biosynthesis [37]. In the present study, WCO supported substantial intracellular PHA accumulation by *C. thermoamylovorans* PHA005, indicating that this waste-derived lipid source can be utilized for biopolymer production under thermophilic cultivation conditions.

PHAs are biodegradable polyesters produced by numerous microorganisms and have been widely investigated as alternatives to conventional petroleum-derived plastics [6]. In addition, variations in monomer composition have been associated with differences in polymer characteristics [21, 33]. The polymer produced in this study exhibited a diverse monomer composition together with distinct thermal characteristics, demonstrating that WCO can serve as a suitable substrate for generating PHAs with measurable physicochemical properties.

It should be noted that environmental and economic assessments, including life-cycle assessment (LCA), techno-economic analysis (TEA), and sustainability metrics, were beyond the scope of the present study. Therefore, the environmental benefits and economic viability of the

proposed process cannot be quantitatively evaluated based on the current results.

Potential Implications for Thermophilic PHA Production

Another notable aspect of the present study is the use of the thermophilic bacterium *C. thermoamylovorans* PHA005 for PHA biosynthesis. Thermophilic microorganisms have been reported to offer potential advantages for industrial bioprocesses, including improved process stability and reduced contamination risks during fermentation. Operating at elevated temperatures may suppress the growth of mesophilic contaminants, potentially reducing sterilization requirements and improving process robustness. In addition, thermophilic conditions may facilitate the handling of lipid-based feedstocks, as oils and fatty acids generally exhibit increased fluidity at elevated temperatures, which may improve substrate dispersion within the culture medium.

Although the present study was conducted under laboratory-scale batch cultivation, *C. thermoamylovorans* PHA005 achieved a high intracellular PHA accumulation of 89.7% of DCW under the cultivation conditions employed. These findings demonstrate the ability of this thermophilic strain to utilize waste coconut oil as a substrate for PHA production. However, further evaluation of biomass concentration, polymer titer, volumetric productivity, substrate utilization, and carbon conversion efficiency will be required before industrial applicability can be assessed.

Future studies should therefore investigate fed-batch and continuous cultivation strategies together with optimization of oxygen transfer, nutrient balance, and substrate feeding regimes to evaluate process scalability and overall production performance. In addition, the present study did not include direct comparisons with mesophilic microorganisms or alternative carbon sources. Therefore, the observed PHA accumulation cannot be attributed solely to thermophilic cultivation conditions, and comparative studies will be required to evaluate the specific contribution of thermophilic cultivation to process performance.

Limitations, Future Perspectives, and Implications

Although the present study demonstrates promising PHA production from waste coconut oil using a thermophilic bacterium, several limitations should be acknowledged.

First, the study does not include mechanistic investigations of monomer incorporation. Although variations in fatty acid composition and PHA monomer distribution were observed, the metabolic pathways, precursor fluxes, and enzymatic mechanisms underlying polymer formation were not examined. In addition, no comparative experiments using pure carbon sources or non-thermophilic strains were conducted; therefore, the relative contribution of substrate type and thermophilic cultivation conditions cannot be quantitatively distinguished.

Second, the optimization of cultivation parameters was performed using an OFAT approach. While effective for identifying suitable conditions, this method does not account for interaction effects among variables. Future studies employing statistical experimental designs such as response surface methodology (RSM) would provide more robust optimization and predictive capability. Furthermore, substrate-to-product yield and carbon conversion efficiency were not quantified, limiting evaluation of process economics.

Third, polymer characterization was limited to FTIR, GC–MS, and DSC analyses. Molecular weight parameters (Mw, Mn, and PDI) were not determined due to the absence of gel permeation chromatography (GPC) analysis. As these parameters strongly influence mechanical properties and processability, future studies should incorporate molecular weight characterization together with mechanical testing.

Finally, no life cycle assessment, techno-economic analysis, or green chemistry metrics (e.g., E-factor, PMI, GWP) were performed. Therefore, claims related to sustainability and circular bioeconomy potential should be interpreted qualitatively rather than quantitatively. In addition, the study was conducted at laboratory scale, and feedstock variability may affect reproducibility at larger scales.

Despite these limitations, the present study provides important insight into thermophilic PHA production from waste lipid substrates and highlights the potential of integrating waste valorization with biopolymer synthesis. Future work integrating metabolic analysis, statistical process optimization, and sustainability assessment will be essential for advancing toward industrial application.

Conclusion

This study demonstrates the conversion of WCO into PHAs by the thermophilic bacterium *C. thermoamylovorans* PHA005. Fatty acid analysis revealed that WCO contains a mixture of medium- and long-chain fatty acids that can serve as carbon sources for microbial PHA production. Under the cultivation conditions investigated (4% WCO, 45 °C, 250 rpm, 60 h), the strain achieved a maximum intracellular PHA accumulation of 89.7% of dry cell weight. Monomer analysis revealed hydroxyalkanoate units corresponding to C8, C10, C15, C17, and C18, indicating the formation of a medium- to long-chain-length PHA copolymer. The occurrence of odd-chain monomers (C15 and C17) contributed to the overall diversity of the polymer composition, although

the metabolic mechanisms responsible for monomer incorporation were not investigated in the present study. Thermal analysis revealed a T_m of 75 °C, a T_g of 5.1 °C, and a T_{cc} of 56 °C, indicating distinct thermal characteristics of the produced polymer. To the best of our knowledge, this is the first report describing PHA production from waste coconut oil by *C. thermoamylovorans* PHA005. The findings demonstrate that waste coconut oil can serve as a substrate for thermophilic PHA production and provide additional information regarding the monomer composition and thermal characteristics of PHA synthesized from this agro-industrial residue. Further studies involving molecular-weight determination, detailed metabolic investigations, substrate conversion analyses, and sustainability assessments will be necessary to support material evaluation and potential industrial application.

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Data Availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Conflict of Interest The authors declare no competing interests.

References

1. Abetz V, Simon PF (2005) Phase behaviour and morphologies of block copolymers. Block copolymers I. Springer Berlin Heidelberg, Berlin, Heidelberg, pp 125–212
2. Alexy P, Horváth V, Plavec R, Vanovčanová Z, Tomanová K, Ďurfina M, Feranc J (2025) Fully biobased biodegradable elastomeric polymer blends based on PHAs. Polymers 17(21):2811. https://doi.org/10.3390/polym17212811
3. An J, Ha B, Lee SK (2023) Production of polyhydroxyalkanoates by the thermophile *Cupriavidus cauae* PHS1. Bioresource Technol 371:128627. https://doi.org/10.1016/j.biortech.2023.128627
4. Ashby RD, Foglia TA (1998) Poly(hydroxyalkanoate) biosynthesis from triglyceride substrates. Appl Microbiol Biotechnol 49(4):431–437. https://doi.org/10.1007/s002530051194
5. AOAC Association of Official Analytical Chemists (2016) Official Methods of Analysis of AOAC International, 20th ed.; AOAC International: Benjamin Franklin Station, Washington, DC, USA.
6. Basnett P, Marcello E, Lukasiewicz B, Panchal B, Nigmatullin R, Knowles JC, Roy I (2018) Biosynthesis and characterization of a novel, biocompatible medium chain length polyhydroxyalkanoate by *Pseudomonas mendocina* CH50 using coconut oil as the carbon source. J Mater Sci Mater Med 29(12):179. https://doi.org/10.1007/s10856-018-6183-9
7. Benavides PT, Lee U, Zarè-Mehrjerdi O (2020) Life cycle greenhouse gas emissions and energy use of polylactic acid, bio-derived polyethylene, and fossil-derived polyethylene. J Clean Prod 277:124010
8. Bolivar-Telleria M, Turbay C, Favarato L, Carneiro T, de Biasi RS, Fernandes AAR, Santos AM, Fernandes PM (2018) Second-generation bioethanol from coconut husk. Biomed Res Int 2018(1):4916497
9. Brandão RD, de Freitas Júnior AM, Oliveira SC, Suarez PAZ, Prauchner MJ (2021) The conversion of coconut oil into hydrocarbons within the chain length range of jet fuel. Biomass Convers Biorefinery 11(3):837–847. https://doi.org/10.1007/s13399-020-01046-9
10. Brigham CJ, Speth DR, Rha C, Sinskey AJ (2012) Whole-genome microarray and gene deletion studies reveal regulation of the polyhydroxyalkanoate production cycle by the stringent response in Ralstonia eutropha H16. Appl Environ Microbiol 78(22):8033–8044
11. Brocklehurst TF, Wilson PDG (2000) The role of lipids in controlling microbial growth. Grasas Aceites 51(1–2):66–73. https://doi.org/10.3989/gya.2000.v51.i1-2.408
12. Chamizo-Ampudia A, Alonso RM, Ariza-Carmona L, Sanchiz Á, San-Martín MI (2025) A review of bioelectrochemical strategies for enhanced polyhydroxyalkanoate production. Bioengineering 12(6):616
13. Chin JH-C, Samian MR, Normi YM (2022) Characterization of polyhydroxyalkanoate production capacity, composition and weight synthesized by *Burkholderia cepacia* JC-1 from various carbon sources. Heliyon 8(3):e09174. https://doi.org/10.1016/j.heliyon.2022.e09174
14. Choonut A, Prasertsan P, Klomklao S, Sangkharak K (2020) *Bacillus thermoamylovorans*-related strain isolated from high temperature sites as potential producers of medium-chain-length polyhydroxyalkanoate (mcl-PHA). Curr Microbiol 77(10):3044–3056. https://doi.org/10.1007/s00284-020-02118-9
15. Choonut A, Prasertsan P, Klomklao S, Sangkharak K (2020) Study on mcl-PHA production by novel thermotolerant gram-positive isolate. J Polym Environ 28(9):2410–2421. https://doi.org/10.1007/s10924-020-01779-8
16. Choonut A, Prasertsan P, Klomklao S, Sangkharak K (2022) A novel green process for synthesis of 3-hydroxyalkanoate methyl ester using lipase and novel mcl-co-lcl PHA as catalyst and substrate. J Polym Environ 30(4):1423–1434. https://doi.org/10.1007/s10924-021-02288-y
17. Coorevits A, Logan NA, Dinsdale AE, Halket G, Scheldeman P, Heyndrickx M, De Vos P (2011) *Bacillus thermolactis* sp. nov., isolated from dairy farms, and emended description of *Bacillus thermoamylovorans*. Int J Syst Evol Microbiol 61(8):1954–1961. https://doi.org/10.1099/ijs.0.024240-0

18. Dokl M, Copot A, Krajnc D, Fan YV, Vujanović A, Aviso KB et al (2024) Global projections of plastic use, end-of-life fate and potential changes in consumption, reduction, recycling and replacement with bioplastics to 2050. Sustain Prod Consum 51:498–518. https://doi.org/10.1016/j.spc.2024.09.025
19. Dong H, Yang X, Shi J, Xiao C, Zhang Y (2023) Exploring the feasibility of cell-free synthesis as a platform for polyhydroxyalkanoate (PHA) production: opportunities and challenges. Polymers 15(10):2333
20. Duranova H, Kuzelova L, Fialkova V, Simora V, Kovacikova E, Joanidis P et al (2025) Coconut-sourced MCT oil: its potential health benefits beyond traditional coconut oil. Phytochem Rev 24(1):659–700. https://doi.org/10.1007/s11101-024-09969-1
21. Hahn T, Alzate MO, Leonhardt S, Tamang P, Zibek S (2024) Current trends in medium-chain-length polyhydroxyalkanoates: microbial production, purification, and characterization. Eng Life Sci 24(6):2300211. https://doi.org/10.1002/elsc.202300211
22. Hermann-Krauss C, Koller M, Muhr A, Fasl H, Stelzer F, Braunegg G (2013) Archaeal production of polyhydroxyalkanoate (PHA) co- and terpolyesters from biodiesel industry-derived by-products. Archaea 129268. https://doi.org/10.1155/2013/129268
23. Huang S, Jiang S (2014) Structures and morphologies of biocompatible and biodegradable block copolymers. RSC Adv 4(47):24566–24583
24. Kachrimanidou V, Kopsahelis N, Vlysidis A, Papanikolaou S, Kookos IK, Martínez BM, Rondán MCE, Koutinas AA (2016) Downstream separation of poly(hydroxyalkanoates) using crude enzyme consortia produced via solid state fermentation integrated in a biorefinery concept. Food Bioprod Process 100:323–334. https://doi.org/10.1016/j.fbp.2016.08.002
25. Kamble A, Meena V, Banerjee U (2010) Effect of agitation and aeration on the production of nitrile hydratase by Rhodococcus erythropolis MTCC 1526 in a stirred tank reactor. Lett Appl Microbiol 51(4):413–420
26. Kitahara T, Koyama N, Matsuda J, Aoyama Y, Hirakata Y, Kamihira S et al (2004) Antimicrobial activity of saturated fatty acids and fatty amines against methicillin-resistant Staphylococcus aureus. Biol Pharm Bull 27(9):1321–1326
27. Kruaejun A, Rakkan T, Paichid N, Sangkharak K (2025) Engineering of β-oxidation pathway to tailor polyhydroxyalkanoate (PHA) production in Bacillus thermoamylovorans. ScienceAsia 51S:s019. https://doi.org/10.2306/scienceasia1513-1874.2025.s019
28. Lawrence AG, Schoenheit J, He A, Tian J, Liu P, Stubbe J et al (2005) Transcriptional analysis of Ralstonia eutropha genes related to poly-(R)-3-hydroxybutyrate homeostasis during batch fermentation. Appl Microbiol Biotechnol 68(5):663–672
29. Laycock B, Halley P, Pratt S, Werker A, Lant P (2013) The chemomechanical properties of microbial polyhydroxyalkanoates. Prog Polym Sci 38(3–4):536–583
30. Li M, Ma Y, Zhang X, Zhang L, Chen X, Ye J-W, Chen G-Q (2021) Tailor-made polyhydroxyalkanoates by reconstructing *Pseudomonas entomophila*. Adv Mater 33(41):2102766. https://doi.org/10.1002/adma.202102766
31. Liau KM, Lee YY, Chen CK, Rasool AHG (2011) An open-label pilot study to assess the efficacy and safety of virgin coconut oil in reducing visceral adiposity. Int Sch Res Notices 2011(1):949686. https://doi.org/10.5402/2011/949686
32. Lillo JG, Rodriguez-Valera F (1990) Effects of culture conditions on poly(β-hydroxybutyric acid) production by Haloferax mediterranei. Appl Environ Microbiol 56(8):2517–2521. https://doi.org/10.1128/aem.56.8.2517-2521.1990
33. Mai J, Kockler K, Parisi E, Chan CM, Pratt S, Laycock B (2024) Synthesis and physical properties of polyhydroxyalkanoate (PHA)-based block copolymers: a review. Int J Biol Macromol 263:130204. https://doi.org/10.1016/j.ijbiomac.2024.130204
34. Mansir N, Taufiq-Yap YH, Rashid U, Lokman IM (2017) Investigation of heterogeneous solid acid catalyst performance on low grade feedstocks for biodiesel production: a review. Energy Convers Manage 141:171–182
35. Napper IE, Bakir A, Rowland SJ, Thompson RC (2015) Characterisation, quantity and sorptive properties of microplastics extracted from cosmetics. Mar Pollut Bull 99(1–2):178–185
36. Papapostolou A, Karasavvas E, Chatzidoukas C (2019) Oxygen mass transfer limitations set the performance boundaries of microbial PHA production processes – a model-based problem investigation supporting scale-up studies. Biochem Eng J 148:224–238. https://doi.org/10.1016/j.bej.2019.04.024
37. Petpheng B, Mudtaleb B, Thongduang S, Meekhai T, Pechsiri J, Sangkharak K (2024) The production of medium-co-long chain length (mcl-co-lcl) polyhydroxyalkanoate from waste soybean oil by *Enterobacter* sp. and its application as an antibacterial agent. Biomass Convers Biorefinery 14(18):23059–23072. https://doi.org/10.1007/s13399-023-04454-9
38. Sangkharak K, Chookhun K, Numreung J, Prasertsan P (2020) Utilization of coconut meal, a waste product of milk processing, as a novel substrate for biodiesel and bioethanol production. Biomass Convers Biorefinery 10(3):651–662. https://doi.org/10.1007/s13399-019-00456-8
39. Sangkharak K, Khaithongkaeo P, Chuaikhunupakarn T, Choonut A, Prasertsan P (2021) The production of polyhydroxyalkanoate from waste cooking oil and its application in biofuel production. Biomass Convers Biorefinery 11(5):1651–1664. https://doi.org/10.1007/s13399-020-00657-6
40. Sangkharak K, Klomklao S, Paichid N, Yunu T (2023) Statistical optimization for fatty acid reduction in waste cooking oil using a biological method and the continuous process for polyhydroxyalkanoate and biodiesel production. Biomass Convers Biorefinery 13(11):9841–9854. https://doi.org/10.1007/s13399-021-01756-8
41. Sathiyanarayanan G, Bhatia SK, Song H-S, Jeon J-M, Kim J, Lee YK, Yang Y-H (2017) Production and characterization of medium-chain-length polyhydroxyalkanoate copolymer from Arctic psychrotrophic bacterium Pseudomonas sp. PAMC 28620. Int J Biol Macromol 97:710–720
42. Sheu DS, Chen WM, Lai YW, Chang RC (2012) Mutations derived from the thermophilic polyhydroxyalkanoate synthase PhaC enhance the thermostability and activity of PhaC from *Cupriavidus necator* H16. J Bacteriol 194(10):2620–2629. https://doi.org/10.1128/jb.06543-11
43. Singh AK, Mallick N (2009) Exploitation of inexpensive substrates for production of a novel SCL–LCL-PHA co-polymer by *Pseudomonas aeruginosa* MTCC 7925. J Ind Microbiol Biotechnol 36(3):347–354. https://doi.org/10.1007/s10295-008-0503-x
44. Singh S, Sithole B, Lekha P, Permaul K, Govinden R (2021) Optimization of cultivation medium and cyclic fed-batch fermentation strategy for enhanced polyhydroxyalkanoate production by *Bacillus thuringiensis* using a glucose-rich hydrolyzate. Bioresources and Bioprocessing 8(1):11
45. Song JH, Jeon CO, Choi MH, Yoon SC, Park WJ (2008) Polyhydroxyalkanoate (PHA) production using waste vegetable oil by Pseudomonas sp. strain DR2. J Microbiol Biotechnol 18(8):1408–1415
46. Tian J, He A, Lawrence AG, Liu P, Watson N, Sinskey AJ, Stubbe J (2005) Analysis of transient polyhydroxybutyrate production in *Wautersia eutropha* H16 by quantitative Western analysis and transmission electron microscopy. J Bacteriol 187(11):3825–3832. https://doi.org/10.1128/jb.187.11.3825-3832.2005
47. Vicente D, Proença DN, Morais PV (2023) The role of bacterial polyhydroalkanoate (PHA) in a sustainable future: a review on the biological diversity. Int J Environ Res Public Health 20(4):2959

48. Wang Y, Wang H, Wu Y, Xiang H, Zhao Y, Chen S, Qi B, Li L (2022) Insights into lipid oxidation and free fatty acid profiles to the development of volatile organic compounds in traditional fermented golden pomfret based on multivariate analysis. LWT 171:114112. https://doi.org/10.1016/j.lwt.2022.114112
49. Yoon BK, Jackman JA, Valle-González ER, Cho N-J (2018) Antibacterial free fatty acids and monoglycerides: biological activities, experimental testing, and therapeutic applications. Int J Mol Sci 19(4):1114
50. Zhou W, Bergsma S, Colpa DI, Euverink G-JW, Krooneman J (2023) Polyhydroxyalkanoates (PHAs) synthesis and degradation by microbes and applications towards a circular economy. J Environ Manage 341:118033. https://doi.org/10.1016/j.jenvman.2023.118033
51. Zhuang Q, Qi Q (2019) Engineering the pathway in *Escherichia coli* for the synthesis of medium-chain-length polyhydroxyalkanoates consisting of both even- and odd-chain monomers. Microb Cell Fact 18(1):135. https://doi.org/10.1186/s12934-019-1186-x