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Integrated thermophilic single-strain conversion of cellulose from coconut meal waste to PHB by *Caldibacillus thermoamylovorans* PHA005

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ABSTRACT

This proof-of-concept study evaluates the thermophilic bacterium *Caldibacillus thermoamylovorans* PHA005 as a single-strain platform for the sequential conversion of coconut meal cellulose, a major by-product of coconut milk production, into polyhydroxybutyrate (PHB) without external hydrolytic enzymes. The strain exhibited pronounced endoglucanase activity, with CMCase peaking at 36 h and releasing up to 0.814 g/L reducing sugars within 72 h at 45 °C. Statistical optimization using Response Surface Methodology (RSM), employing the in situ–generated hydrolysate as the sole carbon source, increased PHB concentration from 0.12 to 0.16 g/L at 48 h, while PHB content rose from 17 to 26.4% of cell dry weight (CDW). Kinetic analysis showed that the specific growth rate (μ) increased from 0.0289 to 0.051 h^{-1} and the specific PHB production rate (q_P) from 0.00385 to 0.00575 h^{-1} under optimized conditions (pH 7.4, 45 °C, and 160 rpm). FTIR and DSC analyses confirmed the chemical identity and thermal properties of the extracted polymer, consistent with commercial PHB. A preliminary laboratory-scale assessment suggested an estimated unit production cost reduction of approximately 25%. By integrating biomass saccharification and PHB biosynthesis within a single thermophilic strain, this approach simplifies process configuration and eliminates the need for external enzyme supplementation. The thermotolerant nature of PHA005 may further reduce cooling demand and contamination risk, supporting the development of a streamlined and potentially scalable bioplastic production strategy based on coconut residues.

1. Introduction

Plastics are widely used for their lightweight and excellent mechanical properties, with global production projected to reach 760 million tons by 2025 (Gross and Enck, 2021). Conventional disposal through incineration or landfilling causes long-term ecological damage, driving growing interest in biodegradable alternatives.

Biopolymers such as polyhydroxybutyrate (PHB), a member of the polyhydroxyalkanoate (PHA) family, are microbially synthesized storage polymers that readily degrade into water and carbon dioxide, offering a sustainable alternative to petrochemical plastics (De Donno Novelli et al., 2021). However, the high production cost of PHB remains a major barrier to industrial-scale implementation. This cost is largely driven by the use of refined carbon substrates, the need for chemical or enzymatic pretreatment of complex feedstocks, high energy inputs for sterilization and temperature control, and downstream recovery processes (Pérez et al., 2020). Consequently, the utilization of low-cost agricultural and agro-industrial residues rich in polysaccharides has emerged as a key strategy to improve the economic feasibility of PHA production while reducing environmental burdens.

Microorganisms of the genus *Caldibacillus*, commonly found in thermophilic and mesophilic composting environments, play important roles in cellulose and lignin degradation. *Caldibacillus thermoamylovorans* is a thermophilic, spore-forming bacterium capable of degrading diverse organic substrates under both aerobic and anaerobic conditions (Flint et al., 2017; Wushke et al., 2015), and certain strains have also

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been reported to produce PHA (Choonut et al., 2020). Nevertheless, most reported cellulolytic or thermophilic PHA production systems rely on chemical and/or enzymatic hydrolysis of lignocellulosic substrates followed by separate fermentation steps (Hassan et al., 2025; Rehakova et al., 2023). In these systems, commercial cellulase cocktails are typically required to generate fermentable sugars, substantially increasing process cost and complexity. As a result, truly integrated single-strain thermophilic systems that combine biomass depolymerization and PHA biosynthesis within a single organism remain scarce.

In this proof-of-concept study, a newly isolated strain, *C. thermoamylovorans* PHA005 (Choonut et al., 2020), was evaluated as a unified thermophilic biocatalyst for direct conversion of coconut meal cellulose into PHB through a single-strain, sequential process. Although the strain itself has been previously identified, its ability to couple in situ enzymatic saccharification of lignocellulosic biomass with intracellular PHB accumulation within a single thermophilic process has not been demonstrated. Accordingly, the novelty of this work lies in establishing *C. thermoamylovorans* PHA005 as an integrated thermophilic platform for biomass hydrolysis and PHB biosynthesis without the addition of commercial enzymes or multi-stage processing.

Although integrated thermophilic systems offer promising advantages in reducing process complexity and cost, production efficiency remains strongly influenced by environmental parameters such as temperature, pH, and aeration (Bolla et al., 2025). Statistical optimization tools, particularly Response Surface Methodology (RSM), have been widely applied to enhance PHA production by identifying optimal cultivation conditions while minimizing experimental runs (Khamkong et al., 2022; Kumar Sachan et al., 2024). However, such systematic optimization has not yet been applied to thermophilic single-strain systems integrating biomass hydrolysis and PHB biosynthesis. Therefore, incorporating RSM into this study provides a rational approach to maximize PHB accumulation under thermophilic conditions. In addition, the physicochemical properties of the produced polymer were characterized to confirm its identity and thermal behavior, including analysis by Fourier transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC).

This study systematically assessed cellulase production, in situ saccharification of coconut meal to release fermentable sugars, and subsequent intracellular PHB accumulation. Notably, PHA005 exhibits a dual functional capability by sequentially hydrolyzing lignocellulosic biomass and converting the released sugars into PHB using the same strain. By employing coconut meal, a regionally abundant residue from coconut milk processing and a major agro-industrial waste in Asia and Thailand (Sangkharak et al., 2020), this study demonstrates the potential for sustainable conversion of coconut-processing residues into value-added biopolymers. This work provides a preliminary framework for sustainable thermophilic bioprocessing, supporting responsible production and consumption (SDG 12), climate mitigation (SDG 13), and sustainable terrestrial ecosystems (SDG 15).

2. Materials and methods

2.1. Bacterial strain

C. thermoamylovorans PHA005 used in this study was obtained from a previous investigation, in which thermotolerant PHA-producing bacteria were isolated from palm oil mill effluent (Choonut et al., 2020). According to the original report, PHA005 grows optimally at 45 °C and remains viable up to 60 °C, classifying it as a thermotolerant bacterium. In the present study, this strain was re-evaluated for its ability to function under higher-temperature bioprocessing conditions (45 °C), focusing on cellulase production, in situ saccharification of coconut-meal cellulose, and subsequent PHB biosynthesis. This represents the first assessment of PHA005 for combined cellulolytic activity and PHB production from lignocellulosic biomass.

2.2. Preparation of coconut meal waste-derived cellulose

Coconut meal waste remaining after oil extraction was used as the lignocellulosic substrate. The solid coconut waste was first dried at 50 °C until a constant weight was reached. For alkaline pretreatment, 5 g of dried biomass was mixed with 100 mL of 50% (*w/v*) NaOH in a 500-mL Erlenmeyer flask and autoclaved at 121 °C for 40 min. After treatment, the mixture was filtered to separate the liquid fraction from the solid residue. The solid fraction was repeatedly washed with distilled water at room temperature until the pH became neutral, and then dried at 50 °C to constant weight (Sangkharak et al., 2020). The pretreated cellulose-rich material was subsequently used as the substrate for enzymatic saccharification by PHA005.

2.3. Bacterial cultivation and enzyme preparation

2.3.1. Cultivation for enzyme production

C. thermoamylovorans PHA005 was inoculated into a basal medium containing (g/L): yeast extract 3, peptone 5, NaCl 5, and carboxymethyl cellulose (CMC) 10 as the inducer substrate. The culture was incubated at 45 °C with shaking at 150 rpm for 36 h (previously published, 2020), based on preliminary tests showing peak CMCase activity at this time. After incubation, the culture was centrifuged at 8000 ×*g* for 10 min at 4 °C. The resulting supernatant was collected as the crude enzyme preparation and used immediately for cellulase activity assays.

2.3.2. Endoglucanase (CMCase) activity assay

CMCase activity was determined using the DNS method. Briefly, 0.5 mL of crude enzyme was mixed with 0.5 mL of 1% (*w/v*) CMC in 50 mM phosphate buffer (pH 7.0) and incubated at 50 °C for 30 min. The reaction was stopped by adding DNS reagent, boiled for 5 min, cooled, and then diluted with distilled water. Reducing sugars released were quantified spectrophotometrically at 540 nm using a glucose standard curve. One unit (U) of CMCase activity was defined as the amount of enzyme releasing 1 μmol of glucose equivalents per minute under the assay conditions.

2.3.3. Total cellulase (FPase) activity assay

FPase activity was determined using a Whatman No. 1 filter paper strip (1 × 6 cm) as the substrate. The reaction mixture contained 0.5 mL of crude enzyme and 1 mL of 50 mM citrate buffer (pH 4.8) with the filter paper strip. After incubation at 50 °C for 60 min, the reaction was stopped by adding 3 mL of DNS reagent, boiled for 5 min, cooled, and diluted with distilled water. Reducing sugars released were measured at 540 nm. One unit (U) of FPase activity was defined as the amount of enzyme releasing 1 μmol of glucose equivalents per minute from the filter paper under the assay conditions (Kadoguchi et al., 2024; Triola, 2008).

2.4. In situ saccharification of coconut-derived cellulose

For enzymatic saccharification, *C. thermoamylovorans* PHA005 was first cultivated in basal medium at 45 °C and 150 rpm for 48 h. The culture was then centrifuged at 8000 ×*g* for 10 min at 4 °C, and the cell pellet was washed twice with sterile distilled water and resuspended to an optical density at 600 nm (OD_{600}) of 1.0, measured using a UV–Vis spectrophotometer (Model V-5100, Metash Instruments Co., Ltd.).

Enzymatic hydrolysis of coconut-derived cellulose was performed following the method of Liu et al. (2012). Briefly, 2% (*w/v*) pretreated cellulose was suspended in 50 mL of 50 mM citric acid/sodium citrate buffer (pH 4.8) in 100-mL Erlenmeyer flasks. The resuspended PHA005 cells were added at 10% (*v/v*) inoculum, and the mixture was incubated at 45 °C with shaking at 150 rpm for 120 h (Liu et al., 2012). Aliquots were withdrawn at 24-h intervals, centrifuged at 10,000 ×*g* for 10 min, and the supernatant was stored at 4 °C. Reducing sugars released during saccharification were measured using the DNS method. Since the

inoculum was prepared from CMC-induced cultures as described in Section 2.3, a basal level of extracellular cellulase activity may have been present at the beginning of the saccharification experiment due to enzyme carryover. Therefore, the activity detected at 0 h represents pre-existing enzymes rather than de novo enzyme synthesis during the saccharification period.

2.5. Baseline PHB production

The hydrolysate obtained after 72 h of enzymatic saccharification (section 2.4) was adjusted to pH 7 and sterilized at 121 °C for 10 min prior to use as the sole carbon source for PHB production. PHA005 was inoculated at 10% (*v*/v) into 100 mL of hydrolysate medium in 250-mL Erlenmeyer flasks and cultivated at 45 °C with agitation at 150 rpm for 120 h.

Samples were collected every 24 h to determine cell dry weight (CDW), PHB concentration (g/L), and PHB content (%CDW). The time-course data were used to evaluate biomass formation and intracellular polymer accumulation under non-optimized conditions. These results served as baseline information for subsequent process optimization.

2.6. Process optimization by response surface methodology (RSM)

Based on the baseline production results, process optimization was performed to enhance PHB accumulation using RSM. A Central Composite Design (CCD) with three independent variables was employed. The variables selected were incubation temperature (25–50 °C), initial pH (5–9), and agitation rate (50–250 rpm) (Table 1) (Choonut et al., 2020).

The selection of these variables was based on their strong influence on microbial growth and PHB biosynthesis. Temperature affects metabolic activity and enzyme stability. Since PHA005 is a thermotolerant strain with optimal growth around 45 °C, a broad range was evaluated to assess production flexibility. Initial pH influences enzyme activity, nutrient availability, and overall cellular metabolism. Agitation rate was included due to its effect on oxygen transfer, which may influence intracellular PHB accumulation (Bolla et al., 2025).

A total of 17 experimental runs, including five center points, were generated using Design-Expert software (v13, StatEase Inc., USA). Each experimental unit consisted of hydrolysate medium inoculated with 10% (*v*/v) active culture and incubated under the specified experimental conditions for 48 h. The cultivation time for RSM experiments was fixed at 48 h based on preliminary kinetic analysis indicating that significant PHB accumulation occurred within this period.

Table 1
Central composite design (CCD) matrix for optimization of PHB production.

Run	Temperature (°C)	pH	Agitation (rpm)	PHB content (% CDW)
1	50.0	9.0	150	18.46
2	37.5	9.0	250	15.71
3	37.5	7.5	150	24.00
4	37.5	6.0	50	11.02
5	37.5	7.5	150	26.67
6	25.0	7.5	50	8.46
7	50.0	6.0	150	17.14
8	37.5	6.0	250	12.22
9	25.0	9.0	150	6.32
10	25.0	6.0	150	7.91
11	37.5	9.0	50	14.12
12	37.5	7.5	150	27.50
13	25.0	7.5	250	9.92
14	37.5	7.5	150	24.44
15	50.0	7.5	250	27.50
16	50.0	7.5	50	21.82
17	37.5	7.5	150	24.41

Note: The experimental design consisted of a three-factor CCD including factorial, axial, and center points. The center point (37.5°C, pH 7.5, 150 rpm) was replicated five times to estimate pure experimental error. PHB content is expressed as percentage of CDW.

PHB content (%CDW) was selected as the primary response variable for model development. Experimental data were fitted to a second-order polynomial model, and the statistical significance of linear, quadratic, and interaction terms was evaluated using analysis of variance (ANOVA) at a 95% confidence level. Model adequacy was assessed by the coefficient of determination (R^2), adjusted R^2, and the lack-of-fit test.

The predicted optimal conditions were experimentally validated in triplicate. In confirmation experiments, CDW, PHB concentration (g/L), and PHB content (%) were determined and compared with model-predicted values within the 95% prediction interval.

2.7. Analytical methods

2.7.1. Determination of biomass (CDW)

Cell biomass was harvested by centrifugation at 8000 ×*g* for 10 min, washed twice with distilled water, and dried at 60 °C to constant weight. The CDW was recorded, and the true cell concentration was calculated by subtracting the PHB content from the total dry biomass.

2.7.2. PHB extraction and quantification

Dried cell biomass was extracted with chloroform under agitation at 60 °C for 12 h to dissolve intracellular PHB. The chloroform phase was separated by filtration and concentrated by evaporation. PHB was precipitated by the addition of cold methanol (1:3, *v*/v), collected by filtration, and washed with methanol to remove residual impurities. The purified polymer was dried to constant weight and quantified gravimetrically. PHB content was expressed as percentage of cell dry weight (PHB, % CDW).

Polymer identity was confirmed by FTIR using samples obtained under baseline cultivation conditions to verify the presence of characteristic PHB functional groups. As the carbon source and biosynthetic pathway remained unchanged throughout the optimization experiments, additional FTIR analyses were not performed for all RSM runs. Instead, the thermal properties of PHB produced under optimized conditions were evaluated by DSC (Rigaku Thermo Plus EVO2). The sample was heated from room temperature to 200 °C at 10 °C min^{-1} under a nitrogen atmosphere to determine the melting temperature and thermal stability.

2.7.3. Kinetic parameter calculation

The kinetics of PHB production were analyzed using time-course data obtained under both baseline and optimized conditions to evaluate the impact of process optimization on growth and polymer accumulation behavior.

2.7.3.1. Specific growth rate (μ, h^{-1}). The specific growth rate represents the exponential growth rate of the biomass during the cultivation period and was calculated as:

$$\mathbf{\mu} = [\mathbf{lnX} - \mathbf{lnX0}]/\Delta t$$

where: X0 = initial biomass concentration (g/L), X = final biomass concentration (g/L) and Δt = time interval (h) between the two measurements.

2.7.3.2. Steady PHB production rate (q_p, h^{-1}). The steady PHB production rate indicates the rate of polymer accumulation per hour during the cultivation period and was calculated using the linear portion of the PHB accumulation curve:

$$\mathbf{q_p} = \mathbf{\Delta P} \big/ \mathbf{\Delta t}$$

where: ΔP = P − P0 is the change in PHB concentration (g/L), and Δt is the corresponding time interval (h).

2.7.3.3. Substrate-normalized yield ($Y_{P/S}$, g/g). The substrate-normalized yield reflects the efficiency of sugar conversion into PHB:

$$Y_{P/S} = P/S$$

where: P = PHB concentration (g/L) and S = total sugar consumed during the cultivation (g/L). The yield in this study was 0.23 g PHB per g sugar, indicating efficient conversion of coconut hydrolysate sugars into PHB by PHA005.

2.8. Statistical analysis

All experiments were performed in triplicate, and results are presented as mean ± standard deviation. For the RSM experiments, statistical analysis was conducted using Design-Expert software (version 13, Stat-Ease Inc., USA). The significance of the regression model and individual terms was evaluated by analysis of variance (ANOVA) at a 95% confidence level ($p < 0.05$). Differences between baseline and optimized conditions were assessed using one-way ANOVA, with statistical significance accepted at $p < 0.05$.

3. Results and discussion

3.1. Hydrolytic capacity and cellulase production

The cellulolytic capacity of *C. thermoamylovorans* PHA005 was evaluated on CMC, revealing a hydrolytic capacity (HC) of 5.71 ± 0.23. FPase activity, representing total cellulase, initially reached 0.565 ± 0.020 U/mL and gradually declined to 0.314 ± 0.006 U/mL by 96 h. In contrast, CMCase activity, indicative of *endo*-acting cellulase, increased from 0.771 ± 0.009 U/mL at 0 h to a peak of 1.530 ± 0.066 U/mL at 36 h before decreasing to 0.753 ± 0.028 U/mL at 96 h. The detectable activity at 0 h reflects baseline extracellular enzymes carried over from the CMC-induced preculture (Section 2.3), rather than newly synthesized enzymes during the monitored incubation period (Sulyman et al., 2020). Consequently, the CMCase/FPase ratio increased from 1.365 to 3.367 at 72 h (Fig. 1), indicating a predominance of endoglucanase activity during the later stages of cultivation.

The different temporal profiles of FPase and CMCase indicate that endoglucanase activity was more sustained than total cellulase activity during cultivation. While FPase continuously declined after reaching its maximum, CMCase activity remained relatively high until 36 h before decreasing at later time points. This suggests that strain PHA005 preferentially maintains endoglucanase activity under thermophilic conditions, which is particularly important for the hydrolysis of amorphous regions of cellulose. Similar trends have been reported for thermophilic *Bacillus*-related cellulolytic strains, where endoglucanase plays a dominant role in initial cellulose depolymerization (Shyaula et al., 2023).

3.2. Cellulase production using coconut meal cellulose

The cellulase production of *C. thermoamylovorans* PHA005 was evaluated on cellulose extracted from coconut meal biomass, reflecting its ability to hydrolyze lignocellulosic residues under thermophilic conditions (Fig. 2). FPase activity started at 0.203 ± 0.010 U/mL and peaked at 0.350 ± 0.010 U/mL at 12 h, before gradually declining to 0.112 ± 0.012 U/mL at 96 h. CMCase activity followed a similar pattern, peaking at 0.528 ± 0.039 U/mL at 12 h and decreasing to 0.090 ± 0.011 U/mL at 96 h. The CMCase/FPase ratio fluctuated from 1.717 at 0 h to a maximum of 2.253 at 36 h, indicating a predominance of endoglucanase activity during the mid-phase of cultivation, important for breaking down amorphous cellulose.

Compared to CMC, overall enzyme activities were lower on coconut meal cellulose, likely due to its complex lignocellulosic structure in which cellulose is embedded within lignin and hemicellulose, limiting enzyme accessibility, as the chemical and physical properties of cellulose strongly influence enzymatic degradability (Erdal and Hakkarainen, 2022). Nevertheless, PHA005 efficiently hydrolyzed the biomass during the first 36–48 h, indicating its capacity to initiate saccharification of recalcitrant substrates.

Notably, the earlier peak of CMCase activity observed on coconut meal–derived cellulose, relative to CMC, may be attributed to the presence of low-molecular-weight soluble sugars and oligosaccharides released during alkaline pretreatment and early hydrolysis, which can act as inducers of cellulase expression. Cellulase synthesis is known to be inducible by cellulose-derived compounds such as cellobiose and sophorose, whereas CMC requires initial enzymatic depolymerization before sufficient inducers accumulate, resulting in a delayed maximum in CMCase activity (Kaur et al., 2006).

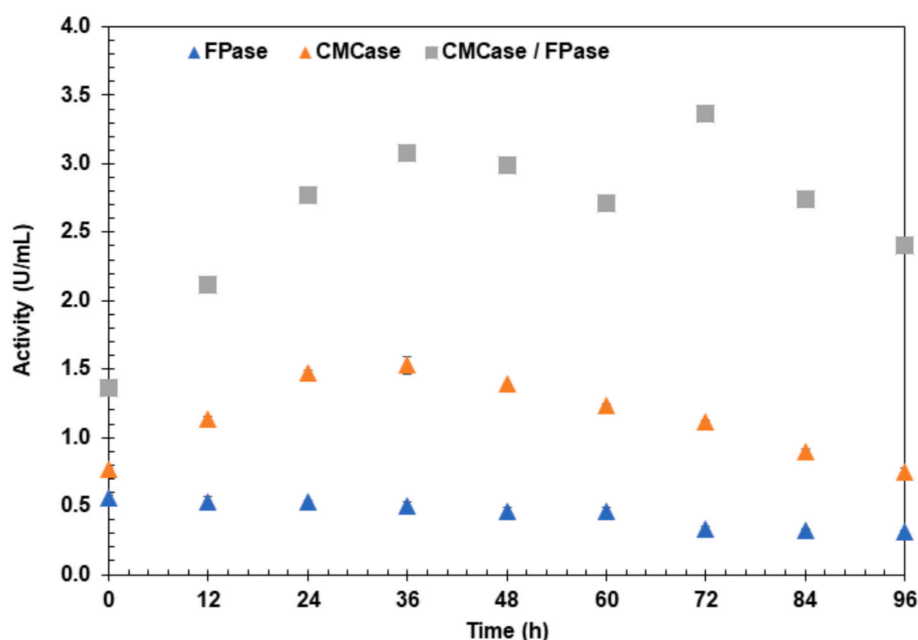

Fig. 1. Time profiles of FPase and CMCase activities of strain PHA005 during cultivation on carboxymethyl cellulose. Error bars represent standard deviation ($n = 3$).

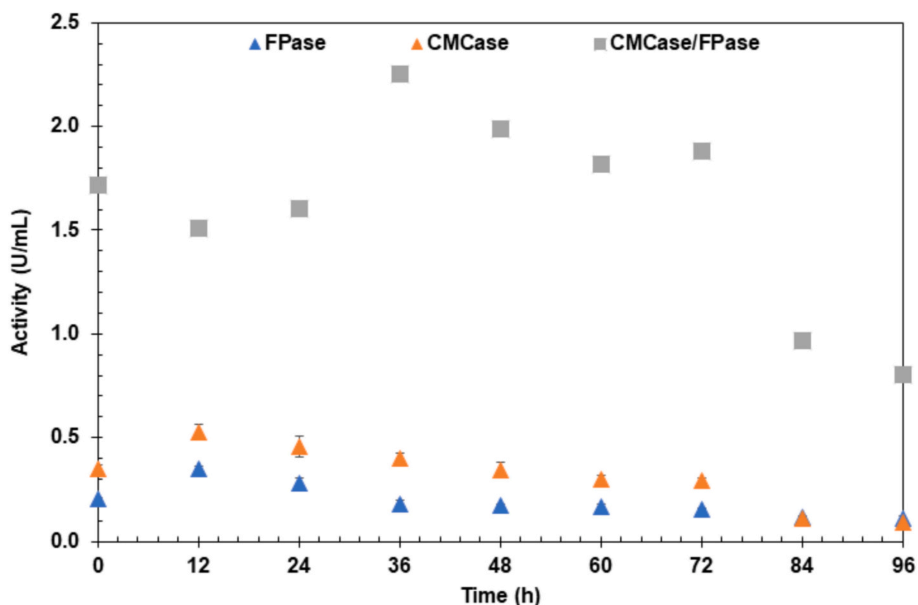

Fig. 2. Time profiles of FPase and CMCase activities of strain PHA005 during cultivation on coconut meal cellulose. Error bars represent standard deviation ($n = 3$).

3.3. Reducing sugar release from coconut meal cellulose

The enzymatic activity of *C. thermoamylovorans* PHA005 was reflected in measurable reducing sugar accumulation from coconut meal cellulose (Fig. 3). The reducing sugar concentration gradually increased from 0.693 ± 0.010 g/L at 0 h to a peak of 0.814 ± 0.012 g/L at 72 h, corresponding to a yield of 14.284 ± 0.209 mg-RS/g-cellulose. After the peak, sugar levels slightly decreased to 0.741 ± 0.005 g/L at 120 h, suggesting partial utilization of the released sugars or minor degradation over time.

The accumulation of reducing sugars correlated with the measured FPase and CMCase activities, confirming that PHA005 effectively hydrolyzed accessible cellulose fractions. The slower sugar release during the early phase reflects the recalcitrant nature of lignocellulosic biomass, where cellulose is embedded within lignin and hemicellulose matrices (Troiano and Studer, 2025). The peak sugar concentration at 72 h indicates the optimal harvesting time for maximal recovery. It highlights the strain's potential for enzymatic saccharification of agricultural residues in a single-strain, thermophilic process, supporting the proof-of-concept for biomass-to-bioplastic conversion.

3.4. Baseline PHB production

The PHB production by *C. thermoamylovorans* PHA005 using coconut meal cellulose–derived hydrolysate demonstrated that the strain can effectively convert lignocellulosic sugars into intracellular biopolymer. Biomass accumulation (CDW) reached a maximum of 1.30 ± 0.01 g/L at 96 h. PHB content peaked earlier at 48 h (17 ± 0.04%) and slightly decreased to 15.00 ± 0.08% at 96 h (Fig. 4). Correspondingly, PHB concentration increased from 0.12 g/L at 48 h to a maximum of 0.19 ± 0.01 g/L at 96 h, primarily due to continued biomass accumulation. These results suggest that PHB synthesis was predominantly growth-

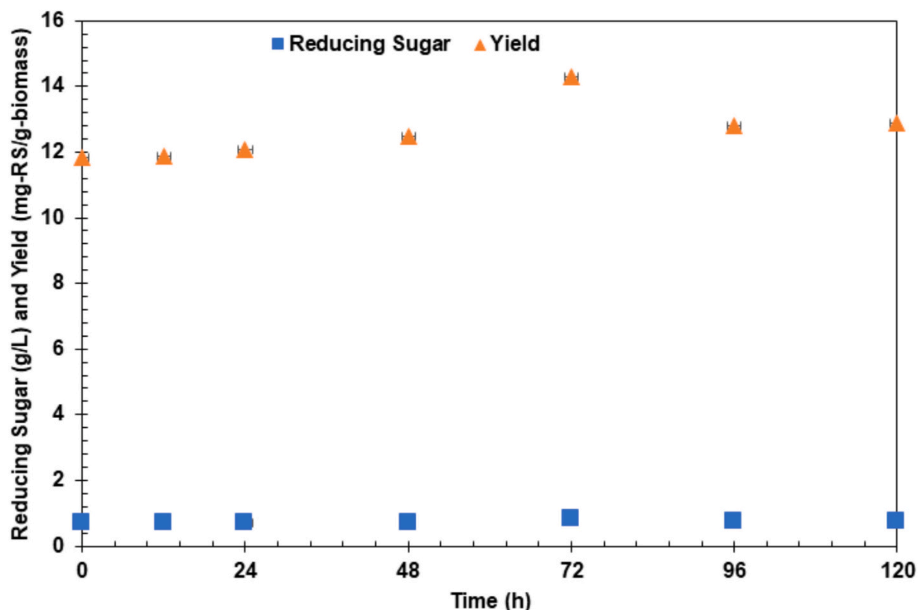

Fig. 3. Time profile of reducing sugar production from coconut meal cellulose hydrolyzed by strain PHA005. Error bars represent standard deviation ($n = 3$).

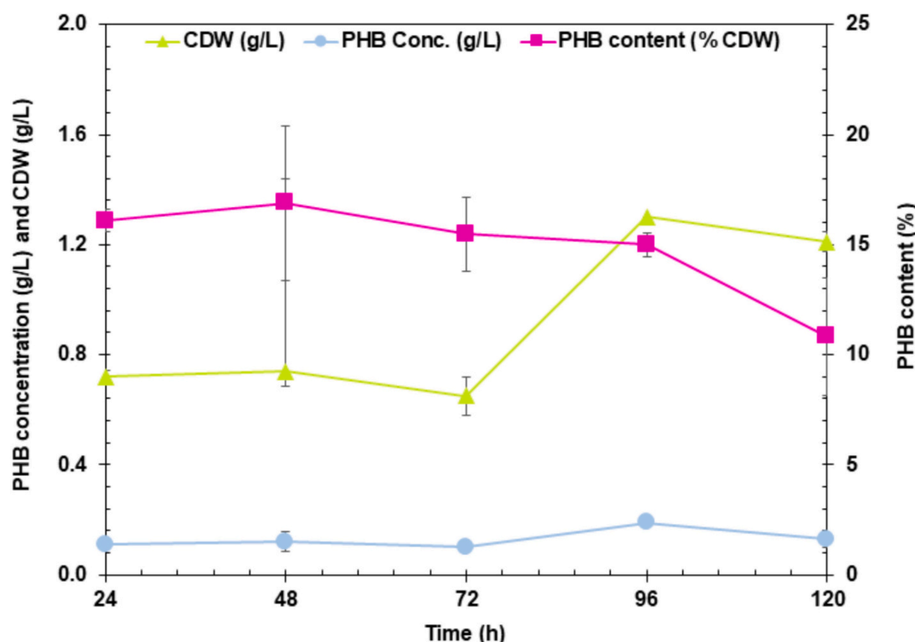

Fig. 4. Time profiles of cell dry weight (CDW), PHB concentration, and PHB content of strain PHA005 cultivated on coconut meal cellulose–derived hydrolysate. CDW and PHB concentration (g/L) are plotted on the left Y-axis, while PHB content (%) is plotted on the right Y-axis. Error bars represent standard deviation ($n = 3$).

associated, with intracellular polymer accumulation occurring during the active growth phase. Although the volumetric PHB concentration was highest at 96 h, 48 h was selected as the optimal production time because it provided higher intracellular PHB content together with a substantially shorter cultivation period, thereby improving overall process productivity and operational efficiency.

The moderate specific growth rate ($\mu = 0.0289\ h^{-1}$) and consistent PHB production rate ($q_P = 0.00385\ h^{-1}$) suggest that although the strain actively metabolizes mixed sugars from the hydrolysate, minor inhibitory compounds or substrate complexity may limit rapid growth, a behavior widely reported in PHA/PHB-producing strains grown on lignocellulosic substrates, including thermophilic and mesophilic organisms (Allegue et al., 2021; Hassan et al., 2025; Zhou et al., 2025). The relatively stable biomass-based PHB yield (0.146–0.162 g/g CDW) and substrate-normalized yield ($Y_{P/S} = 0.23$ g/g sugar) highlight that coconut meal cellulose hydrolysate serves as a viable carbon source for polymer biosynthesis, comparable to yields reported for thermophilic *Aneurinibacillus* spp. and *Caldimonas thermodepolymerans* cultivated on complex polysaccharides (Rehakova et al., 2023; Zhou et al., 2025).

FTIR spectroscopy further supported the identification of the polymer as PHB, showing characteristic functional groups of PHB, including a strong ester carbonyl band near 1720 cm^{-1} and C–O–C stretching vibrations in the range of 1050–1250 cm^{-1} (Fig. 5A). As shown in Fig. 5B, the absorbance intensity of the C═O peak of the polymer extracted from PHA005 (0.228) was lower than that of commercial PHB (0.444), which is consistent with the lower intracellular polymer content obtained from the culture (17% CDW). However, the absorbance intensities of the C–O–C stretching peaks at 1279 cm^{-1} (0.154) and 1100 cm^{-1} (0.124)

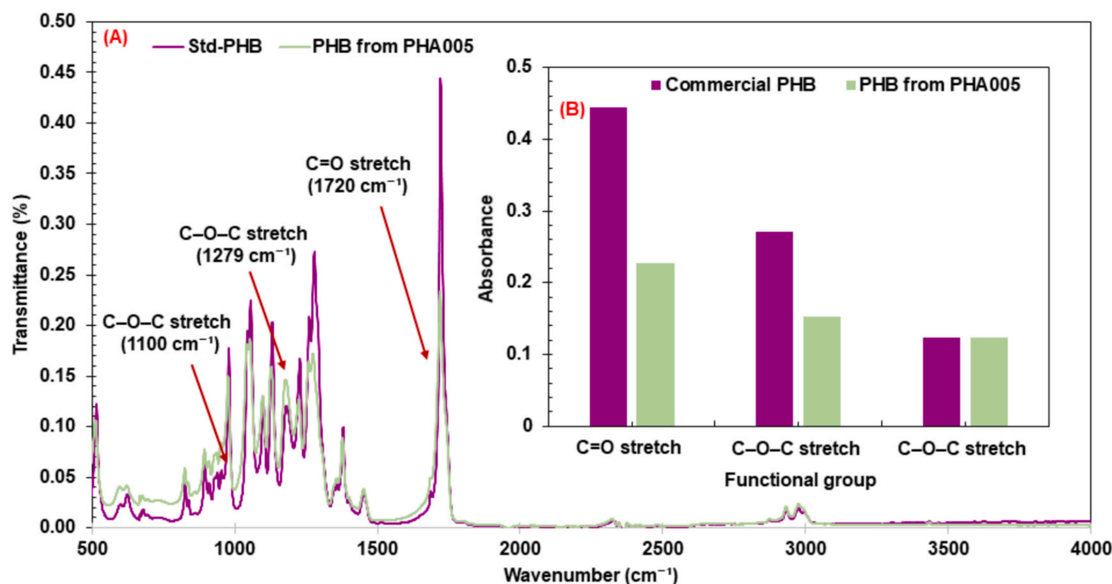

Fig. 5. FTIR spectra (A) and normalized absorbance intensities of key PHB functional groups (C═O at 1720 cm^{-1}, C–O–C at 1279 and 1100 cm^{-1}) comparing PHA005-produced polymer and commercial PHB (B).

were comparable to those of the PHB standard (0.271 and 0.123, respectively), indicating that the ester backbone structure of PHB was correctly formed. No additional peaks associated with medium-chain-length or copolymer PHA structures were observed, suggesting that the accumulated polymer was predominantly a PHB homopolymer.

In addition, PHB is the most commonly reported PHA type produced by members of the *Bacillaceae* and related thermophilic genera when grown on sugars or carbohydrate-rich substrates, as their PHA biosynthesis is typically dominated by the β-ketothiolase (*PhaA*), acetoacetyl-CoA reductase (*PhaB*), and PHB synthase (*PhaC*) (*PhaA*–*PhaB*–*PhaC*) pathway leading to PHB (Sooksawat et al., 2025). The use of cellulose-derived sugars as the main carbon source in this study, therefore, further supports that PHB, rather than other PHA types, was preferentially accumulated by *C. thermoamylovorans* PHA005.

These spectral features closely matched those of a commercial PHB standard, confirming that the polymer produced from coconut meal cellulose–derived sugars exhibits the characteristic functional groups of PHB. Moreover, the FTIR profiles and relative band intensities are consistent with previous reports identifying PHB as the dominant intracellular polymer produced from lignocellulosic hydrolysates (Hassan et al., 2025), supporting the biological feasibility of PHB biosynthesis from coconut meal–derived sugars under thermophilic conditions. These results demonstrate the feasibility of integrating enzymatic saccharification and PHB biosynthesis in a single thermophilic strain as a proof-of-concept strategy for valorizing coconut-processing residues into biopolymer precursors. This approach aligns with recent strategies for valorizing lignocellulosic agro-forestry residues (Li et al., 2025).

Despite successful PHB production under baseline conditions, the moderate accumulation level indicates that further optimization of cultivation parameters is required to enhance polymer yield. Therefore, RSM was applied to systematically evaluate the interactive effects of temperature, pH, and agitation rate on PHB production.

3.5. Process optimization by RSM

3.5.1. Model adequacy and statistical validation

To enhance PHB accumulation, RSM based on a CCD was employed to evaluate the combined effects of temperature (A), pH (B), and agitation rate (C) on PHB content (% of CDW). A second-order quadratic polynomial model was fitted to the experimental data. The ANOVA results demonstrated that the model was highly significant ($F = 22.51$, $p = 0.0002$), confirming that the regression adequately explained the variability in PHB production (Supplementary Table 1). The coefficient of determination ($R^2 = 0.9666$) indicated that 96.66% of the variation in PHB content was explained by the model, while the adjusted R^2 (0.9237) showed good agreement, confirming model reliability. Although the predicted R^2 (0.6259) was lower than the adjusted R^2, this discrepancy may be attributed to the limited number of experimental runs inherent to the CCD design and possible experimental variability (Supplementary Table 2). Nevertheless, the model remained statistically acceptable for optimization within the studied design space within the studied design space, particularly for process navigation rather than precise prediction outside the experimental domain.

The lack-of-fit test was not significant ($p = 0.1824$), indicating that the model adequately represented the experimental data. Furthermore, the adequate precision value (12.64), which exceeded the threshold value of 4, confirmed an adequate signal-to-noise ratio and the suitability of the model for navigation within the experimental domain. Collectively, these statistical indicators demonstrate that the quadratic model is robust and reliable for predicting PHB accumulation under the investigated conditions.

3.5.2. Effect of temperature (A)

Among the linear terms, temperature exerted the most significant influence on PHB production ($p < 0.0001$) with the highest F-value (81.18), identifying it as the dominant factor. This finding aligns with previous studies demonstrating that temperature strongly affects microbial metabolic activity and PHA accumulation kinetics. Mohanrasu et al. reported maximal PHB production by *Bacillus megaterium* at 37 °C, whereas higher temperatures reduced polymerase enzyme activity (Mohanrasu et al., 2020). Similarly, Fernández et al. observed significantly higher PHA productivity at 30 °C compared to 18 °C in *Pseudomonas aeruginosa* (Fernández et al., 2005). However, optimal temperature is highly strain-dependent. Mascarenhas reported maximum PHA yield at 28 °C for *B. megaterium* JHA (Mascarenhas, 2021), whereas *Ensifer* sp. HD34 showed reduced growth and PHA accumulation at 45 °C due to possible thermal inactivation of key biosynthetic enzymes (Khamkong et al., 2022). In contrast, thermophilic bacteria such as *Bacillus thermoamylovorans* can grow optimally at 50 °C and tolerate up to 58 °C (Coorevits et al., 2011), emphasizing the species-specific nature of thermal tolerance. From a biochemical perspective, moderate temperature elevation enhances substrate solubility, diffusion coefficients, and enzymatic reaction rates, potentially improving metabolic flux toward PHB synthesis. However, exceeding the optimal threshold can disrupt membrane stability, protein conformation, and enzymatic integrity, leading to reduced biomass formation and polymer accumulation (Asad-ur-Rehman et al., 2016). Therefore, the significant temperature effect observed in this study reflects a balance between enhanced metabolic kinetics and thermal stress on cellular physiology, as further illustrated by the response surface plots (Fig. 6A and 6B).

3.5.3. Effect of pH (B)

Although the linear effect of pH was not statistically significant, the quadratic term (B^2) was highly significant ($p < 0.0001$), indicating that PHB production is strongly dependent on a narrow optimal pH range rather than following a simple linear relationship. This curvature suggests that even small deviations from the optimum substantially alter metabolic activity and carbon partitioning toward PHB biosynthesis. Such behavior is consistent with enzyme-mediated processes, where catalytic efficiency is highly sensitive to proton concentration. Previous studies frequently report neutral pH as optimal for PHA production. Mohammed et al. demonstrated maximum PHA production by *Bacillus cereus* at pH 7.0 (Mohammed et al., 2020), while *Bacillus thuringiensis* also exhibited peak productivity near neutral conditions (Odeniyi and Adeola, 2017). Mechanistically, pH influences enzyme conformation, catalytic activity, membrane transport systems, redox balance, and intracellular homeostasis (Wei et al., 2011). Deviation from the optimal pH may activate degradative enzymes or depolymerases, resulting in simultaneous PHB synthesis and consumption. Additionally, pH fluctuations can alter metabolic flux distribution and polymer composition (Villano et al., 2010). The pronounced quadratic response observed here confirms that precise pH control is critical for maximizing PHB production and that optimal pH is strain-specific rather than universally neutral. The curvature of the response is also evident from the elliptical contour patterns observed in the interaction plots (Fig. 6A and 6C), supporting the existence of a narrow optimal pH range.

3.5.4. Effect of agitation rate (C)

The linear effect of agitation rate was not statistically significant; however, its quadratic term was significant, suggesting the presence of an optimal region. Although agitation is a mechanical parameter, it plays a fundamental physiological role through its influence on oxygen transfer and dissolved oxygen (DO) levels. Oxygen availability is a critical determinant of microbial growth and PHA biosynthesis. Previous studies have demonstrated that oxygen limitation may enhance PHA accumulation, whereas higher aeration primarily promotes biomass formation (Madhusoodanan et al., 2022; Shantini et al., 2015). In aerobic PHA-producing bacteria, oxygen serves as the terminal electron acceptor, directly influencing ATP generation and carbon flux distribution. Under oxygen-sufficient conditions, carbon substrates are

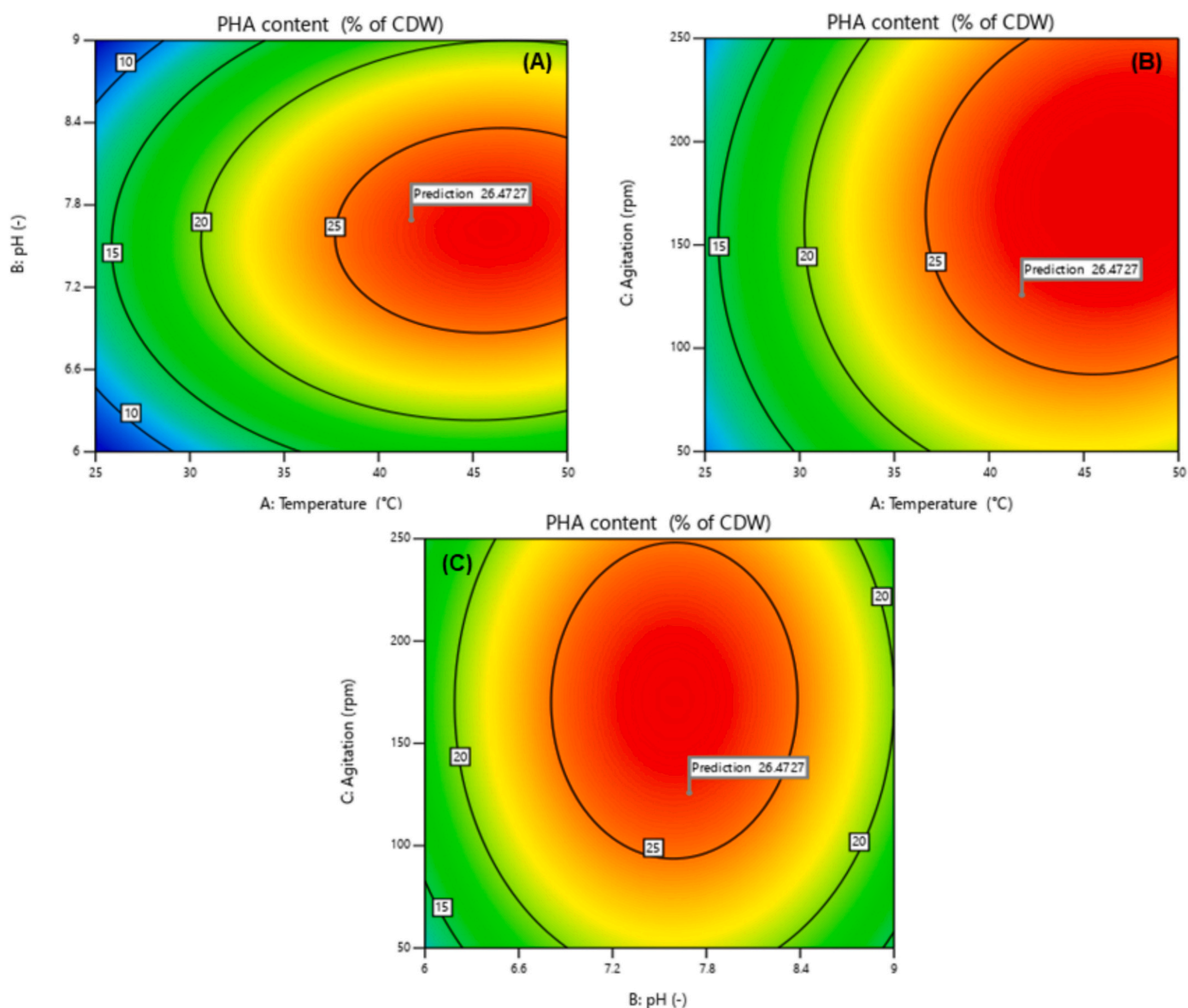

Fig. 6. Response surface and contour plots showing the effects of process variables on PHB content (% of CDW): (A) interaction between temperature and pH; (B) interaction between temperature and agitation rate; and (C) interaction between pH and agitation rate. The red regions indicate the predicted maximum PHB accumulation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

preferentially directed toward cell growth. In contrast, moderate oxygen limitation can redirect excess carbon toward intracellular PHB storage to maintain redox balance. Excessively high agitation may also introduce shear stress and elevated oxygen transfer rates that reduce metabolic stress conditions necessary for polymer accumulation. Conversely, insufficient agitation can create oxygen gradients, nutrient heterogeneity, and cell aggregation, limiting both growth and polymer synthesis (Serafim et al., 2008). The quadratic trend observed in this study, therefore, reflects the balance between adequate oxygen supply for cell viability and controlled metabolic stress to stimulate PHB accumulation. This behavior is consistent with the contour profiles shown in Fig. 6B and 6C, where PHB accumulation increased within an intermediate agitation range before declining at higher levels.

3.5.5. *Response surface analysis and optimal region*

All quadratic terms (A^2, B^2, and C^2) were statistically significant ($p < 0.01$), indicating curvature in the response surface and the presence of a well-defined optimal region. In contrast, the interaction terms (AB, AC, and BC) were not significant, suggesting that individual quadratic contributions exerted greater influence than factor interactions within the studied domain. The final quadratic regression model in terms of coded factors was expressed as:

$$\text{PHB content} = 25.41 + 6.54A + 0.79B + 1.24C + 0.73AB + 1.06AC + 0.09BC - 4.65A^2 - 8.31B^2 - 3.84C^2.$$

The positive linear coefficient of temperature indicates enhanced PHB accumulation within the experimental range, whereas the negative quadratic coefficients reflect the existence of a maximum response within the investigated design space. Three-dimensional response surface and contour plots (Fig. 6) exhibited elliptical patterns, further supporting the quadratic nature of the model.

Based on the model prediction, the highest PHB content (approximately 26–27% of CDW) was obtained at a temperature of 45 °C, pH around 7.4, and agitation between 160 rpm. From a bioprocess engineering perspective, these conditions indicate that PHB accumulation is favored under moderately elevated temperature, near-neutral pH, and controlled aeration. This observation is consistent with previous reports

emphasizing the critical roles of temperature, pH stability, and oxygen availability in regulating PHA biosynthesis (Madhusoodanan et al., 2022; Mohammed et al., 2020; Mohanrasu et al., 2020). The optimization outcome highlights the importance of balancing metabolic activity and environmental stress to strategically decouple biomass formation from polymer storage, a phenomenon widely observed under controlled oxygen limitation strategies (Serafim et al., 2008).

3.5.6. Confirmation experiment

To validate the adequacy of the developed RSM model, a confirmation experiment was performed under the optimal conditions predicted within the experimental design space. The selected solution corresponded to the highest predicted PHB production. The predicted optimal conditions were incubation time of 48 h, temperature of 45 °C, pH 7.4, and agitation rate of 160 rpm. Under these conditions, the model predicted a PHB content of 27.71% of CDW. The validation experiment was conducted in triplicate ($n = 3$), yielding an experimental PHB content of 26.4 ± 1.05% of CDW. This value was in close agreement with the predicted response. The 95% prediction interval (PI) ranged from 24.15% to 31.27%, and the experimentally obtained value fell well within this interval, confirming the reliability and predictive capability of the quadratic model. The relative error between predicted and experimental values was approximately 3.3%, indicating good model accuracy and suitability of RSM for process optimization. Overall, the confirmation test verified that the developed model can reliably predict PHB production within the studied range of process variables. The close agreement between predicted and experimental values demonstrates the robustness of the model and confirms its applicability for scale-up and further process development.

To further characterize culture performance under the optimized condition, key kinetic parameters were determined. The specific growth rate (μ) reached 0.051 h^{-1} compared with 0.0289 h^{-1} under the non-optimized condition. Similarly, the specific PHB production rate (q_P) increased to 0.00575 h^{-1}. In contrast, the yield coefficient ($Y_{P/S}$) decreased slightly to 0.196 g/g, suggesting that although polymer accumulation accelerated, substrate conversion efficiency toward PHB formation was marginally reduced (Ali et al., 2024). These kinetic trends indicate enhanced metabolic activity under the optimized environmental conditions.

DSC analysis was performed to evaluate the thermal properties of the extracted polymer (Supplementary Fig. 1). The thermogram exhibited a single sharp endothermic peak corresponding to the melting transition of PHB. The onset, peak melting temperature (Tm), and endset temperatures were 167.1 °C, 175.6 °C, and 180.1 °C, respectively. The melting enthalpy (ΔHm) was 74.01 J/g. The observed melting temperature falls within the typical range reported for PHB (170–180 °C) (Mandragutti et al., 2024; Pradhan et al., 2018), confirming the formation of a highly crystalline polymer. Based on the theoretical enthalpy of fusion of 146 J/g for 100% crystalline PHB (Pradhan et al., 2018), the degree of crystallinity was estimated to be approximately 51%. These results indicate that the produced PHB possessed good thermal stability and crystallinity comparable to previously reported PHB materials.

3.6. Comparison of PHB production before and after optimization

To evaluate the effectiveness of the optimization strategy, PHB production performance under baseline and optimized conditions was compared (Table 2). Under the initial condition (45 °C, pH 7.0, 150 rpm), PHB content reached 17% of CDW with a PHB concentration of 0.12 g/L after 48 h. Following optimization (45 °C, pH 7.4, 160 rpm), PHB content increased markedly to 26.4% of CDW, while PHB concentration increased to 0.16 g/L. This represents an approximately 59% increase in intracellular PHB content and a 33% increase in PHB concentration. Kinetic analysis further revealed that the specific growth rate (μ) increased from 0.0289 h^{-1} to 0.051 h^{-1}, and the specific PHB production rate (q_P) increased from 0.00385 h^{-1} to 0.00575 h^{-1} under optimized conditions. Although the yield coefficient ($Y_{P/S}$) slightly decreased from 0.23 g/g to 0.196 g/g, the enhanced growth and production rates indicate improved metabolic activity and carbon flux toward PHB biosynthesis.

Table 2
Comparison of PHB production and kinetic parameters before and after optimization.

Parameter	Before optimization	After optimization	Improvement (%)
Temperature (°C)	45	45	–
Initial pH	7.0	7.4	–
Agitation rate (rpm)	150	160	–
Cultivation time (h)	48	48	–
CDW (g/L)	0.74	0.58	−21.6
PHB concentration (g/L)	0.12	0.16	+33.3
PHB content (% CDW)	17	26.4	+59.2
Specific growth rate, μ (h^{-1})	0.0289	0.051	+76.5
Specific PHB production rate, q_P (h^{-1})	0.00385	0.00575	+49.4
Yield coefficient, $Y_{P/S}$ (g/g)	0.23	0.196	−14.8

Note: Values were obtained after 48 h of cultivation under both baseline and optimized conditions. μ, specific growth rate; q_P, specific PHB production rate; $Y_{P/S}$, PHB yield coefficient based on substrate consumption; CDW, cell dry weight.

Overall, these results demonstrate that RSM-based optimization significantly enhanced PHB accumulation and overall process performance. The slight reduction in CDW and $Y_{P/S}$ after optimization may be attributed to a metabolic shift favoring intracellular PHB accumulation rather than biomass formation under the optimized conditions. Such redistribution of carbon flux toward polymer storage has been commonly reported in PHA-producing systems (Anderson and Dawes, 1990; Khanna and Srivastava, 2005) and does not necessarily indicate reduced process efficiency.

3.7. Relationship between cellulase activity, sugar utilization, and PHB biosynthesis

3.7.1. Logical explanation: enzyme → sugar → PHB

The temporal patterns of cellulase activity, reducing sugar release, and PHB accumulation indicate a sequential relationship between biomass hydrolysis and polymer biosynthesis. Elevated CMCase and FPase activities during the early cultivation phase promoted cellulose depolymerization, resulting in the gradual accumulation of soluble sugars in the hydrolysate. These sugars were subsequently assimilated by *C. thermoamylovorans* PHA005, supporting biomass growth and intracellular PHB synthesis, as commonly observed in cellulolytic PHA/PHB-producing systems utilizing lignocellulosic substrates (Allegue et al., 2021; Hassan et al., 2025; Rehakova et al., 2023; Zhou et al., 2025).

The peak PHB content observed at 48 h coincided with active cell growth and sufficient carbon availability, suggesting that PHB production was predominantly growth-associated rather than a strictly stationary-phase storage response. This behavior is consistent with reports indicating that many members of the family *Bacillaceae*, including *Bacillus* and related genera within the order *Bacillales*, are capable of PHA accumulation during exponential growth under balanced or carbon-excess conditions (Mohapatra et al., 2017).

As cultivation progressed, partial depletion of fermentable sugars and the accumulation of inhibitory by-products derived from lignocellulosic hydrolysates may have constrained further polymer accumulation, leading to a slight decline in PHB content at later stages. This trend is consistent with previous findings showing that PHA accumulation is closely linked to intracellular carbon flux and the availability of key

precursors, such as acetyl-CoA and reducing equivalents (NADPH), during active metabolism rather than being exclusively triggered by nutrient limitation in the stationary phase (Kalia et al., 2007).

3.7.2. Hypothetical metabolic pathway of PHB production

A hypothetical metabolic pathway for PHB biosynthesis from coconut meal cellulose–derived sugars is proposed to explain the observed relationship between cellulase activity, sugar utilization, and intracellular PHB accumulation. Cellulolytic enzymes secreted by *C. thermoamylovorans* PHA005 hydrolyze cellulose into soluble oligosaccharides and glucose, which are subsequently transported into the cells and metabolized via glycolysis and the pentose phosphate pathway to generate pyruvate and, ultimately, acetyl-CoA. Under conditions of excess carbon relative to other essential nutrients, surplus acetyl-CoA is redirected toward PHB biosynthesis through the classical three-step pathway involving *PhaA*, *PhaB*, and *PhaC*, leading to intracellular accumulation of PHB granules (Sagong et al., 2018).

This canonical route, widely recognized as the primary pathway for PHB formation from sugar substrates in bacteria, can be conceptually summarized as: (1) extracellular cellulose depolymerization, (2) sugar uptake and intracellular availability, (3) central carbon metabolism, (4) acetyl-CoA formation, (5) carbon flux redirection under carbon-excess conditions, (6) PHB biosynthesis via the *PhaA*–*PhaB*–*PhaC* pathway, and (7) intracellular PHB accumulation.

This PHB biosynthetic pathway is highly conserved among PHB-producing members of the family *Bacillaceae* (Senila et al., 2023), including mesophilic and thermophilic genera closely related to *Bacillus* and *Caldibacillus* that utilize lignocellulosic or biomass-derived substrates for PHB production. Therefore, the observed PHB accumulation from coconut meal cellulose–derived sugars in this study are biochemically plausible and supports the feasibility of integrating biomass depolymerization and PHB biosynthesis within a single thermophilic strain.

3.8. Comparison with previous PHA production studies using lignocellulosic substrates

Several studies have investigated microbial PHA production from lignocellulosic and agro-industrial residues using both mesophilic and thermophilic microorganisms (Allegue et al., 2021; Hassan et al., 2025; Rehakova et al., 2023; Zhou et al., 2025). Reported PHA yields generally range from 16 to 30% CDW, depending on the strain, substrate, and PHA type. For instance, the mesophilic bacterium *Mycolicibacterium smegmatis* accumulated 22–28% CDW PHB when cultivated on sugarcane bagasse hydrolysate (Hassan et al., 2025), while purple phototrophic bacteria produced up to 21 wt% PHA from lignocellulosic hydrolysates (Allegue et al., 2021). Among thermophiles, *C. thermodepolymerans* achieved 16–30% CDW when grown on beechwood xylan or wheat arabinoxylan (Zhou et al., 2025). However, most of these processes require prior enzymatic or chemical hydrolysis of lignocellulosic substrates, which increases cost, complexity, and waste disposal burdens due to the use of commercial enzymes or chemical reagents.

In contrast, our study employing *C. thermoamylovorans* PHA005 demonstrates a single thermophilic strain capable of both enzymatic saccharification and PHB accumulation using coconut meal waste. The strain accumulated up to 17% PHB from the released sugars, highlighting its dual capacity to hydrolyze lignocellulosic biomass and synthesize PHB within an integrated biomass-to-bioplastic process. PHA005 produces cellulolytic enzymes in situ to degrade coconut meal and subsequently converts the liberated sugars into PHB, thereby minimizing the need for external enzymatic or chemical pretreatment. In addition to this integrated bioconversion capability, the application of RSM further strengthened process performance. Unlike many previous studies that reported PHA yields under non-optimized or one-factor-at-a-time conditions, the present work systematically optimized temperature, pH, and agitation rate to enhance intracellular PHB accumulation. Although the maximum PHB content (26.4% CDW) falls within the lower-to-middle range reported in literature, the combination of thermophilic operation, in situ enzymatic saccharification, and statistical process optimization provides a robust framework for future scale-up and further yield improvement. Overall, these findings provide a clear proof-of-concept for the sustainable thermophilic bioconversion of coconut-processing waste. Supplementary Table 3 summarizes previous studies and the present work, including microorganisms, substrates, cultivation temperatures, PHA types, yields, and key distinguishing features.

3.9. Preliminary cost analysis

A preliminary laboratory-scale cost assessment of PHB production from coconut meal hydrolysate estimated a total operating cost of approximately 17.7 USD per batch, corresponding to ~8.9 USD per gram of PHB based on a baseline yield of 2.0 g per batch (Supplementary Table 4). At this experimental scale, solvent consumption during polymer extraction and precipitation constituted the dominant cost fraction, which is typical of laboratory recovery protocols that rely on single-use organic solvents. In contrast, raw materials and medium components represented a comparatively minor proportion of the total cost. Therefore, these values should be interpreted strictly as laboratory-scale estimates rather than projections of industrial production cost.

Following statistical optimization using RSM, PHB concentration increased from 0.12 to 0.16 g/L. Under identical working volume and material input conditions, this corresponds to an estimated batch yield of approximately 2.67 g. Because the optimization involved only minor adjustments in cultivation parameters (pH and agitation rate) without increasing substrate loading, medium composition, solvent volume, or processing steps, the total batch operating cost was reasonably assumed to remain comparable at the laboratory scale. Under this assumption, the unit production cost decreased from ~8.9 to approximately 6.63 USD/g, representing a reduction of about 25%. These results indicate that improvements in volumetric productivity can directly improve process economics, even when absolute operating costs remain unchanged.

The analysis further identifies clear opportunities for cost reduction through solvent recycling, alternative downstream recovery strategies, and additional gains in PHB productivity. The thermotolerance of *C. thermoamylovorans* PHA005 may contribute to improved process economics by reducing cooling demand and lowering contamination risk during fermentation. Elevated operating temperatures may also facilitate efficient biomass hydrolysis due to enhanced mass transfer and the thermal stability of cellulolytic enzymes in high-solid systems. Importantly, the capability of a single strain to perform both saccharification and PHB biosynthesis eliminates the requirement for external enzyme supplementation, a major cost contributor in conventional lignocellulose-based PHA production processes.

Consequently, further scale-up, solvent recovery integration, and downstream process optimization are expected to substantially improve overall economic feasibility, as fermenter utilization efficiency, energy integration, and capital expenditure become increasingly dominant factors in large-scale production (Ozturk et al., 2025).

3.10. Limitations and future perspectives

This study demonstrates that *C. thermoamylovorans* PHA005 can hydrolyze coconut meal derived cellulose and convert the released sugars into PHB within a single thermophilic platform. Although the primary objective was to establish integrated saccharification and biopolymer biosynthesis, key cultivation parameters were subsequently optimized using RSM, leading to improved PHB productivity. A preliminary laboratory-scale cost assessment was also conducted to examine the economic impact of enhanced productivity.

Despite these advances, several limitations remain. Pretreatment

severity and hydrolysis efficiency were not systematically optimized in relation to enzyme kinetics or solid loading, and comprehensive compositional analysis of the hydrolysate was not performed. Future investigations should therefore include quantitative profiling of released sugars, potential inhibitory compounds, and carbon flux distribution to better elucidate substrate utilization efficiency and metabolic allocation toward PHB synthesis.

Although ATR-FTIR and DSC analyses confirmed the chemical identity and thermal transition behavior of the extracted polymer, additional physicochemical characterization, such as X-ray diffraction (XRD) and thermogravimetric analysis (TGA), is required to further assess crystallinity, thermal stability, and material performance. Such analyses will be important for evaluating processing feasibility and application potential.

Process performance could be further enhanced through improved enzyme expression, refined fermentation control (including temperature modulation, agitation strategy, and feeding regime), or metabolic and systems-level optimization. While the single-strain approach eliminates the need for external enzyme supplementation, comparative assessment with co-culture systems or engineered strains may help define productivity limits and scalability potential.

Finally, scale-up studies integrated with techno-economic and life cycle assessments will be essential to determine industrial viability. At larger production scales, solvent recovery integration, energy efficiency, and reactor design are expected to become dominant factors influencing overall process economics. Collectively, these directions outline a clear pathway for advancing this thermophilic, single-strain PHB production system toward practical and sustainable bioplastic manufacturing.

4. Conclusions

This study demonstrates that *C. thermoamylovorans* PHA005 exhibits dual functionality by directly hydrolyzing coconut meal–derived cellulose and converting the released sugars into PHB within a single thermophilic system. Endogenous cellulolytic enzyme production enabled effective biomass saccharification, while the resulting sugars supported bacterial growth and intracellular PHB accumulation, confirming the feasibility of integrating enzymatic hydrolysis and biopolymer biosynthesis in one microorganism. Process optimization using RSM significantly enhanced PHB accumulation, increasing intracellular PHB content from 17% to 26.4% of CDW and improving key kinetic parameters, thereby demonstrating the importance of statistical optimization in strengthening process performance. Thermal analysis by DSC further confirmed that the extracted polymer exhibited melting characteristics typical of PHB, indicating the formation of a highly crystalline biopolymer. The key novelty of this work lies in the use of a single thermophilic strain capable of simultaneous biomass depolymerization and PHB production from coconut-processing residues without the need for external enzymes or multi-step microbial consortia. Compared with conventional lignocellulose-based PHB processes that rely on commercial cellulases and separate hydrolysis–fermentation stages, this consolidated bioprocessing strategy offers a simplified and potentially more cost-effective route for agro-industrial waste valorization. Although this study establishes the technical feasibility of the proposed system, further improvements in process optimization, comprehensive polymer characterization, and scale-up assessment will be necessary to fully evaluate industrial applicability.

CRediT authorship contribution statement

Aophat Choonut: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Narisa Binhayeeding:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Nisa Paichid:** Methodology, Investigation, Formal analysis. **Benjamas Cheirsilp:** Supervision, Project administration, Funding acquisition. **Atipan Saimmai:** Methodology, Investigation, Formal analysis. **Kanokphorn Sangkharak:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

We have no conflict of interest to declare.

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Appendix A. Supplementary data

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Data availability

Data will be made available on request.

References

Ali, S., Lodhi, F.S., Ahmad, M.U., Khan, Q.F., Asad ur, R., Ahmed, A., Liaqat, I., Aftab, M. N., Shah, T.A., Salamatullah, A.M., Wondmie, G.F., Bourhia, M., 2024. Kinetics and synthesis of poly(3-hydroxybutyrate) by a putative-mutant of *Bacillus licheniformis*. Bioresour. Bioprocess. 11 (1), 41.

Allegue, L.D., Ventura, M., Melero, J.A., Puyol, D., 2021. Integrated sustainable process for polyhydroxyalkanoates production from lignocellulosic waste by purple phototrophic bacteria. GCB Bioenergy 13 (5), 862–875.

Anderson, A.J., Dawes, E.A., 1990. Occurrence, metabolism, metabolic role, and industrial uses of bacterial polyhydroxyalkanoates. Microbiol. Rev. 54 (4), 450–472.

Asad-ur-Rehman, A.-u.-R., Alia Aslam, A.A., Rushda Masood, R.M., Aftab, M.N., Rabia Ajmal, R.A., Ikram-ul-Haq, I.-u.-H., 2016. Production and Characterization of a Thermostable Bioplastic (Poly-β-hydroxybutyrate) from *Bacillus cereus* NRRL-B-3711.

Bolla, M., Pettinato, M., Ferrari, P.F., Fabiano, B., Perego, P., 2025. Polyhydroxyalkanoates production from laboratory to industrial scale: a review. Int. J. Biol. Macromol. 310, 143255.

Choonut, A., Prasertsan, P., Klomklao, S., et al., 2020. Study on mcl-PHA production by novel thermotolerant Gram-positive isolate. J. Polym. Environ. 28, 2410–2421.

Coorevits, A., Logan, N.A., Dinsdale, A.E., Halket, G., Scheldeman, P., Heyndrickx, M., Schumann, P., Van Landschoot, A., 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.

De Donno Novelli, L., Moreno Sayavedra, S., Rene, E.R., 2021. Polyhydroxyalkanoate (PHA) production via resource recovery from industrial waste streams: a review of techniques and perspectives. Bioresour. Technol. 331, 124985.

Erdal, N.B., Hakkarainen, M., 2022. Degradation of cellulose derivatives in laboratory, man-made, and natural environments. Biomacromolecules 23 (7), 2713–2729.

Fernández, D., Rodríguez, E., Bassas, M., Viñas, M., Solanas, A.M., Llorens, J., Marqués, A.M., Manresa, A., 2005. Agro-industrial oily wastes as substrates for PHA production by the new strain Pseudomonas aeruginosa NCIB 40045: effect of culture conditions. Biochem. Eng. J. 26 (2), 159–167.

Flint, S., Gonzaga, Z.J., Good, J., Palmer, J., 2017. Bacillus thermoamylovorans – a new threat to the dairy industry – a review. Int. Dairy J. 65, 38–43.

Gross, L., Enck, J.J.P.B., 2021. Confronting Plastic Pollution to Protect Environmental and Public Health, 19. Public Library of Science, San Francisco, CA USA, p. e3001131.

Hassan, S., Khudur, L.S., Nahar, K., Dekiwadia, C., Ball, A.S., 2025. Isolation and identification of xylose-utilising, polyhydroxyalkanoate-producing bacteria from sugarcane bagasse: first report of PHA production by Mycolicibacterium smegmatis. J. Polym. Environ. 33 (7), 3144–3157.

Kadoguchi, E.A., Velasco, J., Silva, S.S.d., Ingle, A.P., Segato, F., Chandel, A.K., 2024. Optimization of cellulase production from agri-industrial residues by *Aspergillus terreus* NIH2624. Processes 12 (10), 2169.

Kalia, V.C., Lal, S., Cheema, S., 2007. Insight in to the phylogeny of polyhydroxyalkanoate biosynthesis: horizontal gene transfer. Gene 389 (1), 19–26.
Kaur, J., Chadha, B.S., Saini, H.S., 2006. Regulation of cellulase production in two thermophilic fungi Melanocarpus sp. MTCC 3922 and Scytalidium thermophilum MTCC 4520. Enzym. Microb. Technol. 38 (7), 931–936.
Khamkong, T., Penkhrue, W., Lumyong, S., 2022. Optimization of production of Polyhydroxyalkanoates (PHAs) from newly Isolated *Ensifer* sp. Strain HD34 by Response Surface Methodology. Processes 10 (8), 1632.
Khanna, S., Srivastava, A.K., 2005. Recent advances in microbial polyhydroxyalkanoates. Process Biochem. 40 (2), 607–619.
Kumar Sachan, R.S., Devgon, I., Mohammad Said Al-Tawaha, A.R., Karnwal, A., 2024. Optimizing polyhydroxyalkanoate production using a novel *Bacillus paranthracis* isolate: a response surface methodology approach. Heliyon 10 (15), e35398.
Li, D., Liu, S., Wang, Q., Ma, X., Li, J., 2025. Integrated pretreatment and microbial matching for PHA production from lignocellulosic agro-forestry residues. Fermentation 11 (10), 563.
Liu, H.Q., Feng, Y., Zhao, D.Q., Jiang, J.X., 2012. Influence of cellulose content on the enzyme activity in the saccharification digests of furfural residues. Asia Pac. J. Chem. Eng. 7 (S3), S275–S279.
Madhusoodanan, G., Hariharapura, R.C., Somashekara, D., 2022. Dissolved oxygen as a propulsive parameter for polyhydroxyalkanoate production using Bacillus endophyticus cultures. Environ. Dev. Sustain. 24 (4), 4641–4658.
Mandragutti, T., Jarso, T.S., Godi, S., Begum, S.S., K, B., 2024. Physicochemical characterization of polyhydroxybutyrate (PHB) produced by the rare halophile *Brachybacterium paraconglomeratum* MTCC 13074. Microb. Cell Factories 23 (1), 59.
Mascarenhas, J., 2021. Production and optimization of polyhydroxyalkanoate obtained from Bacillus megaterium JHA. J. Appl. Biotechnol. Rep. 8 (4), 346–360.
Mohammed, S., Behera, H.T., Dekebo, A., Ray, L., 2020. Optimization of the culture conditions for production of Polyhydroxyalkanoate and its characterization from a new Bacillus cereus sp. BNPI-92 strain, isolated from plastic waste dumping yard. Int. J. Biol. Macromol. 156, 1064–1080.
Mohanrasu, K., Rao, R.G.R., Dinesh, G.H., Zhang, K., Prakash, G.S., Song, D.-P., Muniyasamy, S., Pugazhendhi, A., Jeyakanthan, J., Arun, A., 2020. Optimization of media components and culture conditions for polyhydroxyalkanoates production by Bacillus megaterium. Fuel 271, 117522.
Mohapatra, S., Sarkar, B., Samantaray, D.P., Daware, A., Maity, S., Pattnaik, S., Bhattacharjee, S., 2017. Bioconversion of fish solid waste into PHB using Bacillus subtilis based submerged fermentation process. Environ. Technol. 38 (24), 3201–3208.
Odeniyi, O.A., Adeola, O.J., 2017. Production and characterization of polyhydroxyalkanoic acid from Bacillus thuringiensis using different carbon substrates. Int. J. Biol. Macromol. 104, 407–413.
Ozturk, A.B., Kourilova, X., Buchtikova, I., Obruca, S., 2025. Techno-economic assessment of polyhydroxyalkanoates production from lignocellulosic biomass employing halophilic and thermophilic microbial platform: effect of fermentation conditions and downstream operations. Waste Manag. 203, 114887.
Pérez, V., Mota, C.R., Muñoz, R., Lebrero, R., 2020. Polyhydroxyalkanoates (PHA) production from biogas in waste treatment facilities: assessing the potential impacts on economy, environment and society. Chemosphere 255, 126929.
Pradhan, S., Dikshit, P.K., Moholkar, V.S., 2018. Production, ultrasonic extraction, and characterization of poly (3-hydroxybutyrate) (PHB) using *Bacillus megaterium* and *Cupriavidus necator*. Polym. Adv. Technol. 29 (8), 2392–2400.
Rehakova, V., Pernicova, I., Kourilova, X., Sedlacek, P., Musilova, J., Sedlar, K., Koller, M., Kalina, M., Obruca, S., 2023. Biosynthesis of versatile PHA copolymers by thermophilic members of the genus Aneurinibacillus. Int. J. Biol. Macromol. 225, 1588–1598.
Sagong, H.-Y., Son, H.F., Choi, S.Y., Lee, S.Y., Kim, K.-J., 2018. Structural insights into polyhydroxyalkanoates biosynthesis. Trends Biochem. Sci. 43 (10), 790–805.
Sangkharak, K., Chookhun, K., Numreung, J., et al., 2020. Utilization of coconut meal, a waste product of milk processing, as a novel substrate for biodiesel and bioethanol production. Biomass Conv. Bioref. 10, 651–662.
Senila, L., Gál, E., Kovacs, E., Cadar, O., Dan, M., Senila, M., Roman, C., 2023. Poly(3-hydroxybutyrate) production from lignocellulosic wastes using *Bacillus megaterium* ATCC 14581. Polymers 15 (23), 4488.
Serafim, L.S., Lemos, P.C., Albuquerque, M.G., Reis, M.A., 2008. Strategies for PHA production by mixed cultures and renewable waste materials. Appl. Microbiol. Biotechnol. 81 (4), 615–628.
Shantini, K., Yahya, A.R.M., Amirul, A.A., 2015. Influence of feeding and controlled dissolved oxygen level on the production of Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) copolymer by Cupriavidus sp. USMAA2-4 and its characterization. Appl. Biochem. Biotechnol. 176 (5), 1315–1334.
Shyaula, M., Regmi, S., Khadka, D., Poudel, R.C., Dhakal, A., Koirala, D., Sijapati, J., Singh, A., Maharjan, J., 2023. Characterization of thermostable cellulase from Bacillus licheniformis PANG L isolated from the Himalayan soil. Int. J. Food Microbiol. 2023 (1), 3615757.
Sooksawat, T., Ngaopok, K., Siripornadulsil, S., Amnuaypanich, S., Attapong, M., Siripornadulsil, W., 2025. Sustainable production of polyhydroxybutyrate biopolymers and cellulose microfibers from sugarcane waste. Process Biochem. 150, 134–147.
Sulyman, A.O., Igunnu, A., Malomo, S.O., 2020. Isolation, purification and characterization of cellulase produced by Aspergillus niger cultured on Arachis hypogaea shells. Heliyon 6 (12), e05668.
Triola, M.F., 2008. Introdução à estatística. In: Introdução à estatística, p. xxvi, 310-xxvi, 310.
Troiano, D.T., Studer, M.H.P., 2025. Microbial consortia for the conversion of biomass into fuels and chemicals. Nat. Commun. 16 (1), 6712.
Villano, M., Beccari, M., Dionisi, D., Lampis, S., Miccheli, A., Vallini, G., Majone, M., 2010. Effect of pH on the production of bacterial polyhydroxyalkanoates by mixed cultures enriched under periodic feeding. Process Biochem. 45 (5), 714–723.
Wei, Y.-H., Chen, W.-C., Huang, C.-K., Wu, H.-S., Sun, Y.-M., Lo, C.-W., Janarthanan, O.-M., 2011. Screening and evaluation of polyhydroxybutyrate-producing strains from indigenous isolate *Cupriavidus taiwanensis* strains. Int. J. Mol. Sci. 12 (1), 252–265.
Wushke, S., Levin David, B., Cicek, N., Sparling, R., 2015. Facultative anaerobe *Caldibacillus debilis* GB1: characterization and use in a designed aerotolerant, cellulose-degrading coculture with *Clostridium thermocellum*. Appl. Environ. Microbiol. 81 (16), 5567–5573.
Zhou, W., Llorens, S.B., Deuss, P.J., Euverink, G.-J.W., Krooneman, J., 2025. Polyhydroxyalkanoate (PHA) production by thermophilic Caldimonas thermodepolymerans comb. nov. from xylan. RSC Sustainability 3 (4), 1685–1690.