



Case study

A novel sustainable transesterification of polyhydroxyalkanoate-rich microbial biomass into methyl esters using acetic acid as a green catalyst

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ABSTRACT

A novel green process was developed for the direct transesterification of microbial polyhydroxyalkanoate (PHA) to hydroxyalkanoate methyl esters (HAME) using acetic acid as a sustainable catalyst. PHA-rich microbial biomass (PMB) was converted to HAME under optimal conditions without prior extraction. Process parameters, including acid concentration, temperature, and reaction time, were systematically optimized. The maximum HAME yield (56.1 ± 0.3 %) was achieved at 20 % acetic acid, 70 °C, and 70 h. Fourier Transform Infrared spectroscopy (FTIR) analysis confirmed ester formation by the characteristic absorption band at 1742 cm^{-1} . Partial evaluation of fuel properties indicated that the product's density and viscosity are comparable to those of commercial biodiesel, as determined by standard methods. This study demonstrates the feasibility of using acetic acid as a green catalyst for the direct microbial conversion of microbial biomass into biofuel precursors, offering a promising strategy for sustainable bioprocessing.

1. Introduction

Fossil fuels have long served as the dominant global energy source, supporting industrial growth, transportation, and power generation (Javed et al., 2019). However, their depletion and adverse environmental effects, such as greenhouse gas emissions, toxic byproducts, and global warming, have raised serious sustainability concerns (Amulya et al., 2016; Qari et al., 2017). According to DNV's Energy Transition Outlook 2025, the global energy transition remains too slow to meet the Paris Agreement targets, with projected warming of about 2.2 °C by 2100, underscoring the urgent need for renewable and sustainable alternatives to fossil fuels. Among renewable options, biofuels derived from microbial (Choonut et al., 2022; Junpadit et al., 2014; Sangkharak et al., 2021; Wang et al., 2010; Zhang et al., 2009) and agricultural (Kant et al., 2025; Rial, 2024) have emerged as promising substitutes due to their biodegradability, renewability, and reduced carbon footprint.

Biofuel development has evolved through several generations, from food crops to lignocellulosic biomass (Padder et al., 2024) and, more recently, to microbial polyhydroxyalkanoates (PHAs) (Akinwumi et al., 2022). PHAs are microbial polyesters synthesized under nutrient-limited conditions and recognized as renewable, biodegradable feedstocks for value-added products, including biofuels (Riaz et al., 2021; Zhou et al.,

2023). Among their derivatives, hydroxyalkanoate methyl esters (HAME) and hydroxybutyrate methyl ester (HBME, derived from polyhydroxybutyrate, PHB) (Wang et al., 2010) have gained attention for their favorable physicochemical properties, high oxygen content, absence of nitrogen and sulfur compounds (Zhang et al., 2009), high cetane number, good oxidative stability, and suitable viscosity and flash points (Wang et al., 2010). In addition, the esterification of PHB to form HBME reduces polarity and acidity, thereby improving volatility and physicochemical stability, properties advantageous for biofuel applications (Zhang et al., 2013). These features contribute to efficient combustion, reduced emissions, and stable storage. Moreover, β -hydroxy esters, structurally related to HAME/HBME, exhibit superior ignition quality and oxidative stability compared to conventional fatty acid methyl esters (FAMES) (McCormick et al., 2024), positioning HAME/HBME as sustainable alternatives to petroleum diesel and biodiesel.

In this study, the term HAME refers broadly to methyl esters derived from PHAs, regardless of their monomer composition. Following the pioneering studies on HAME production reported around 2009 (Zhang et al., 2009), research in this area remained limited until recent years, during which significant contributions to PHA-based biofuel development have been made, as reflected by multiple publications cited in recent reviews (Akinwumi et al., 2022; Riaz et al., 2021).

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Conventionally, HAMEs are synthesized *via* acid (Sangkharak et al., 2021; Wang et al., 2010; Zhang et al., 2009) or enzyme (Choonut et al., 2022) catalyzed transesterification. Enzymatic transesterification, for instance, employs lipases to cleave PHA ester bonds and esterify them with methanol under mild conditions, and the acid process involves replacing the hydroxyl group (–OH) of the PHA monomer's carboxylic group with a methoxy group (–OCH₃) from the alcohol (Tanneru et al., 2014). However, large-scale HAME production remains limited by high PHA costs and reliance on hazardous catalysts such as concentrated mineral acids or expensive enzymes.

To address these limitations, this study introduces an environmentally friendly and cost-effective catalytic strategy for the direct conversion of PHA-rich microbial biomass (PMB) into HAME using acetic acid under mild conditions. Acetic acid acts as a weak organic catalyst, donating a proton to activate the carbonyl group of PHA monomers (Go et al., 2014), facilitating nucleophilic attack by methanol and promoting transesterification without the need for strong mineral acids. This approach eliminates the need for PHA extraction steps, reduces chemical hazards, and aligns with green chemistry principles.

Therefore, this study aims to optimize the conversion of PMB to HAME *via* acetic-acid-catalyzed transesterification by systematically evaluating the effects of acid concentration, temperature, and reaction time on HAME yield, methyl ester content, density, viscosity, and key fuel properties, and benchmarking against international biodiesel standards. This work provides a sustainable pathway for microbial biofuel production and advances HAME as a next-generation renewable fuel aligned with global clean energy transitions.

2. Materials and methods

2.1. Chemicals and reagents

Methanol, acetic acid, and culture media components (peptone, yeast extract, sodium octanoate, and ammonium chloride) used throughout this study were obtained from Merck (Darmstadt, Germany) and were of analytical grade. The standard methyl esters used to prepare calibration curves, and the HAME standard (HAME-Std; C6:0 to C18:3) used as an internal standard were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All chemicals and reagents were used without further purification.

2.2. Substrate for HAME

The PMB derived from *Caldibacillus thermoamylovorans* PHA005 was utilized as the substrate for HAME production in this study.

2.2.1. Microbial strains and background

The thermotolerant bacterium *C. thermoamylovorans* PHA005 was previously isolated from palm oil mill effluent and characterized for its ability to synthesize medium-chain-length polyhydroxyalkanoates (mcl-PHA) (Choonut et al., 2020a). This strain was selected due to its distinctive physiological and metabolic advantages, including efficient growth at elevated temperatures (up to 50 °C) (Flint et al., 2017; Moriyadee and Pathom-Aree, 2008), ability to utilize a broad range of inexpensive carbon sources (e.g., xylose, cellobiose, glycerol), and high intracellular PHA accumulation. These characteristics render the strain suitable for cost-effective, sustainable, and thermotolerant bioprocess applications. Relevant cultivation conditions and PHA characteristics from previous studies are summarized here to provide background for the present HAME synthesis experiments.

2.2.2. PHA production

C. thermoamylovorans PHA005 was cultured in nutrient broth (NB) medium supplemented with 3.0 g/L sodium octanoate as the sole carbon source, maintaining a C:N ratio of 5:1 (equivalent to 0.02 g/L ammonium chloride). Actively growing seed cultures (10 %, v/v) were

inoculated into fresh NB medium and incubated at 45 °C with continuous agitation at 150 rpm for up to 120 h (Choonut et al., 2020b).

2.2.3. GC-FID analysis of PHA

PHA composition were determined by methanolysis of lyophilized cells or extracted PHA in a chloroform–methanol–sulfuric acid mixture. The resulting hydroxyacyl methyl esters were analyzed by gas chromatography equipped with a flame ionization detector (GC-FID; Hewlett Packard GC-6890, HP-INNOWAX capillary column, 30 m × 0.25 mm × 0.25 µm film thickness) (Timm et al., 1990).

2.2.4. Preparation of PHA-rich microbial biomass (PMB) for HAME synthesis

Harvested microbial biomass (PMB) was washed repeatedly with distilled water to remove residual medium components, air-dried at room temperature, and used directly as the feedstock for HAME production *via* organic acid-catalyzed transesterification.

2.3. The production of HAME from PHA-rich microbial biomass (PMB) *via* acid-catalyzed transesterification

HAME were produced *via* an acid-catalyzed transesterification reaction using PMB as the feedstock. The reaction was performed in a 125 mL closed flask containing PMB, acetic acid, and methanol, following the method described by Sangkharak et al. (2021) with modifications. Preliminary investigation and optimization of HAME production were conducted using the one-factor-at-a-time (OFAT) approach.

The reaction mixture was prepared at a fixed PMB-to-methanol ratio of 1:3 (% w/v) (Tangsathitkulchai et al., 2012). To ensure methodological clarity and reproducibility, molar ratios of methanol to PHA and acetic acid to PHA were calculated based on the PHA content of the biomass used. From 3.0 g of PMB containing 63.27 % PHA (equivalent to 1.90 g PHA) and assuming an average molecular weight of 286 g/mol for PHA monomers, the methanol-to-PHA molar ratio was calculated to be approximately **149:1 (mol/mol)**. Acetic acid was added at concentrations ranging from 5.0 to 25 % (v/v), corresponding to molar ratios of approximately **5.3:1 to 26.5:1 (mol/mol)** relative to PHA, with an average of about **16:1 at 15 % (v/v) acetic acid**. These calculated molar ratios were used to ensure accuracy and reproducibility in the transesterification process.

Typically, 3 g of PMB and 40 mL of methanol were placed in a closed reaction flask and maintained at 60 °C with continuous stirring at 150 rpm. The effect of acetic acid concentration (5–25 % v/v per 3.0 g of PMB) on HAME yield was first examined. Subsequently, other key reaction parameters, including reaction temperature (50–100 °C) and reaction time (0–100h), were systematically optimized while maintaining the fixed biomass-to-methanol ratio. All reactions were performed in triplicate under controlled shaking conditions (NB-205LF, N-BIOTEK, Korea) at 150 rpm to ensure reproducibility.

Upon completion of the reaction, samples were centrifuged at 8000 rpm for 10 min to collect the upper organic layer containing the HAME. The obtained organic phase was repeatedly washed with distilled water to remove residual catalyst, acetic acid, and impurities. The purified organic phase was oven-dried at 105 °C for 24 h to remove residual moisture and unreacted alcohol before further characterization.

2.4. Methyl ester content, chemical structure and fuel property analysis

The methyl ester content of the synthesized HAME was determined using gas chromatography equipped with a flame ionization detector (GC-FID; Hewlett Packard GC-1450, HP-INNOWAX capillary column: 30 m × 0.25 mm i.d., 0.25 µm film thickness). Quantification was performed using a mixed methyl ester standard solution (C6:0–C18:3) as an internal standard (Kuepethkaew et al., 2017). Calibration curves were prepared using five concentrations of standard methyl esters to ensure accuracy, and the methyl ester content (% w/w) was calculated based on

relative peak areas obtained from chromatograms.

FTIR analysis was performed over a wavenumber range of 4000–400 cm^{-1} to characterize the chemical structure of HAME. The presence of characteristic absorption bands corresponding to ester carbonyl ($\text{C}=\text{O}$) and alkoxy ($\text{C}-\text{O}$) stretching vibrations confirmed successful transesterification of PHA into methyl esters (Mathimani et al., 2015).

The fuel properties of HAME were analyzed using key biodiesel quality indicators, including density at 15 °C, dynamic viscosity at 40 °C, and methyl ester content (% w/w). All analyses followed standardized procedures established by ASTM International (2008) and the Thai Ministry of Energy biodiesel specifications. Specifically, density at 15 °C was determined according to ASTM D1298, dynamic viscosity at 40 °C was determined according to ASTM D445, and methyl ester content was quantified by GC-FID as described above. Results were compared with biodiesel quality standards defined by ASTM D6751 (ASTM) and the Thai Ministry of Energy (Thai) to assess the suitability of HAME as a potential biofuel (Patchimpet et al., 2020).

2.5. Statistical analysis

All experiments were conducted in triplicate ($n = 3$) to ensure reproducibility and reliability of results. All experimental data were analyzed using a completely randomized design (CRD) and subjected to one-way analysis of variance (ANOVA). Differences among treatment means were evaluated using Duncan's multiple range test (DMRT) at a significance level of $P < 0.05$. Statistical analyses were performed using SPSS software (SPSS Inc., Chicago, IL, USA). Data are expressed as mean \pm standard deviation.

3. Results and discussion

3.1. The production of biofuel-HAME

The PMB used as substrate was obtained from *C. thermoamylovorans* PHA005. Its biomass yield, PHA accumulation, and monomer composition have been previously reported (Choonut et al., 2020b) and are summarized here for context. Under reference cultivation conditions, PHA005 produced 2.75 ± 0.03 g/L of biomass and 1.74 ± 0.02 g/L of PHA, corresponding to 63.27 % of dry cell weight (DCW). GC-FID analysis revealed C8–C18 monomers, predominantly 3-hydroxyhexadecanoic acid (3HHD, 39.25 mol%), representing a novel composition for this species. FTIR confirmed characteristic ester and hydroxyl groups, while DSC indicated a melting temperature (T_m) of 68 °C, a glass transition temperature (T_g) of 1.3 °C, and a crystallinity of 43 %, suggesting elastomeric behavior.

The intrinsic physicochemical properties of mcl-PHA, particularly its moderate crystallinity and low T_g , facilitate polymer processing and chemical modification (Reddy et al., 2022). These properties may also enhance methanol diffusion, promoting acid-catalyzed transesterification during HAME production. Previous observations consistently show that mcl-PHA exhibits superior properties compared to short-chain PHAs, such as PHB, as chemical modification of ester groups generally preserves the side-chain carbon length (Knothe and Razon, 2017). Longer, more branched chains result in higher octane and cetane numbers (Chukwuezie et al., 2017).

Given these favorable properties, this study aimed to develop a method for the direct transesterification of microbial PHAs, particularly mcl-PHA, into HAME using acetic acid as a sustainable catalyst. PMB was converted to HAME under optimal conditions without prior extraction. Key process parameters, including acid concentration, temperature, and reaction time, were systematically optimized.

3.1.1. Acid concentration

Acetic acid concentration was identified as a critical factor influencing HAME yield. An increase in acetic acid concentration from 5 % to 25 % (v/v) resulted in a continuous improvement in methyl ester

content, reaching a maximum yield of 38.4 ± 0.9 % at 20 % (Fig. 1). This enhancement is attributed to improved protonation of ester bonds within the PHA matrix, which increases the electrophilicity of the carbonyl carbon and facilitates nucleophilic attack by methanol, forming tetrahedral intermediates that undergo subsequent proton transfer and chain cleavage (Chaber et al., 2024). Similar to observations in microalgal biomass, disruption of microbial biomass structures can enhance the accessibility of ester bonds, facilitating acid-catalyzed transesterification (Huang et al., 2019).

The selected range of acetic acid concentrations (5–25 % v/v) was based on preliminary experiments and literature precedent for acid-catalyzed transesterification of PHAs. Concentrations below 5 % typically resulted in incomplete conversion due to insufficient catalytic activity, while concentrations above 20 % did not significantly increase HAME yield and could complicate reaction work-up. This range is consistent with prior studies using strong acids (e.g., H_2SO_4 at 10–15 % v/v), which reported optimal methyl ester yields under similar conditions (Zhang et al., 2009; Wang et al., 2010).

Although sulfuric acid (10–15 %) remains the most commonly used catalyst for PHA transesterification, with reported yields exceeding 44 % under harsher reaction conditions (Junpadit et al., 2014; Sangkharak et al., 2021; Wang et al., 2010; Zhang et al., 2009). Its drawbacks, including equipment corrosion, post-reaction neutralization requirements, and environmental risks, limit its application, particularly at larger scales (Lam et al., 2010; Leung et al., 2010; Talha et al., 2016). In contrast, although it yields slightly lower product quantities, acetic acid offers substantial benefits in terms of sustainability, safety, and operational simplicity. Consequently, 20 % (v/v) acetic acid was selected as the optimal acid concentration for subsequent experiments.

3.1.2. Reaction temperature

Reaction temperature significantly influenced HAME yield, with the highest yield (53.5 ± 1.0 %) obtained at 70 °C (Fig. 2). Elevated temperatures are known to accelerate reaction kinetics by increasing the frequency and energy of molecular collisions, thereby enhancing the rate of ester bond cleavage and methyl ester formation (Chen et al., 2002; El-Malek and Steinbüchel, 2021). This principle has been demonstrated in biodiesel transesterification systems, where methyl ester yields have improved markedly within the temperature range of 55–65 °C (Esonye et al., 2019).

However, exceeding the boiling point of methanol (~ 65 °C) introduces risks of methanol evaporation (Sangkharak et al., 2021) and undesirable side reactions such as saponification (Silva and Andrade, 2020). Previous studies using sulfuric acid as a catalyst have reported optimal methyl ester production within 65–100 °C (Junpadit et al.,

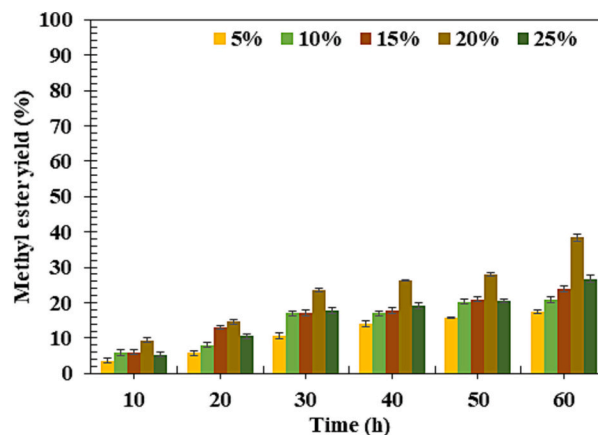


Fig. 1. Effect of acetic acid concentration (5–25 % v/v) on hydroxyalkanoate methyl esters (HAME) yield at 60 °C and 150 rpm (60 h). Bars represent the standard deviation from triplicate determinations.

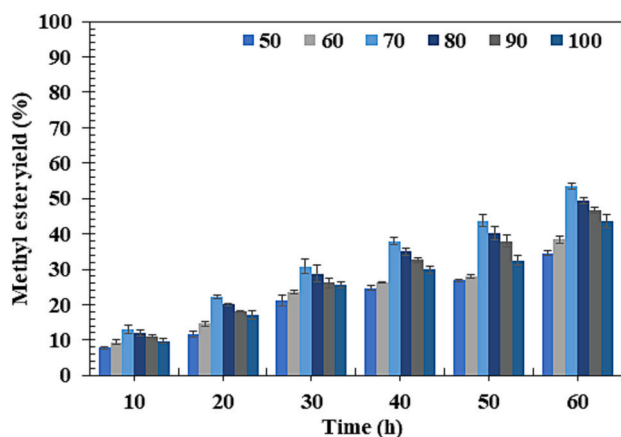


Fig. 2. Effect of reaction temperature (50–100 °C) on hydroxyalkanoate methyl esters (HAME) yield using 20 % acetic acid at 150 rpm for 60 h. Bars represent the standard deviation from triplicate determinations.

2014; Sangkharak et al., 2021; Wang et al., 2010; Zhang et al., 2009). Similar trends have been observed in acid-catalyzed transesterification of other feedstocks, including microalgal lipids (Kasirajan, 2021), castor kernel oil (Mamuye and Reshad, 2022), and mixed crude palm oil (Saetiao et al., 2023), demonstrating that optimal methyl ester formation generally occurs around 60–70 °C, and reaction efficiency is highly dependent on catalyst concentration and feedstock accessibility (Naseef and Tulaimat, 2025), although these systems often require stringent control due to high reactivity, volatility losses, and potential degradation. In contrast, the present study achieved high HAME yields at 70 °C without noticeable methanol loss, likely due to the sealed reaction system (Duran bottles), which minimized volatility, improved safety, and reduced environmental hazards. Operating at 70 °C offered a balance between energy efficiency and conversion, aligning with green chemistry principles. The yield decline at temperatures above 70 °C further supports the notion that moderate heating under controlled conditions is optimal for biomass-to-HAME conversion. Thus, 70 °C was selected as the standardized temperature for further optimization.

3.1.3. Reaction time

Reaction time played a crucial role in determining HAME yield. A maximum yield of 56.1 ± 0.3 % was obtained after 70 h (Fig. 3), demonstrating the time-dependent kinetics of biomass-based acid-catalyzed transesterification. A slight decrease in HAME yield was observed after 70 h, which may be attributed to reversible transesterification or

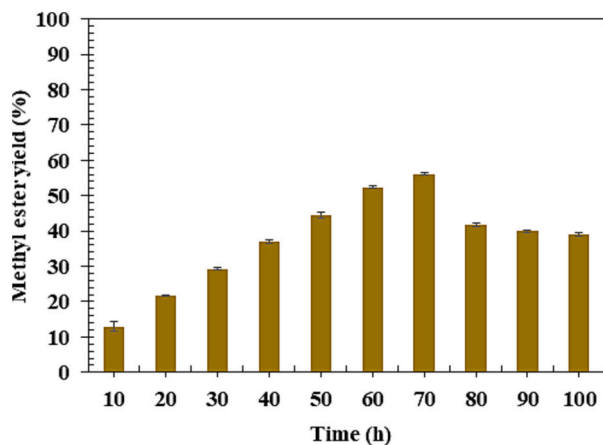


Fig. 3. Effect of reaction time (10–100 h) on hydroxyalkanoate methyl esters (HAME) yield using 20 % acetic acid at 70 °C and 150 rpm. Bars represent the standard deviation from triplicate determinations.

hydrolysis reactions under prolonged acidic and high-temperature conditions. Direct esterification is a dynamic, reversible reaction in which esters form in the forward direction, while ester hydrolysis occurs in the reverse direction (Ahmed et al., 2010).

Acid catalysts effectively promote both esterification and transesterification but generally exhibit slower reaction kinetics and require longer reaction times than alkaline methods, as observed with FFA-rich plant oil feedstocks (Goff et al., 2004; Marchetti et al., 2007). Similar behavior is observed in biomass-based acid-catalyzed transesterification, where substrate accessibility and reaction time critically influence conversion efficiency. Nevertheless, the reaction rate can increase as mixing progresses and esterification advances (Silva and Andrade, 2020). In general, the HAME yield reaches a maximum within a defined reaction period, after which further reaction times does not significantly increase the yield. Similar trends have been observed in acid-catalyzed biodiesel production from low-free fatty acid feedstocks (Buasri et al., 2013; Chen et al., 2002; Okwundu et al., 2019; Saputro et al., 2021), highlighting the importance of optimizing reaction time to maximum conversion efficiency. For example, Okwundu et al. (2019) reported that biodiesel yield from beef tallow increased with reaction time, reaching a maximum within 1–4 h, while Saputro et al. (2021) demonstrated that waste cooking oil achieved maximum methyl ester conversion within 40–60 min before reverse reactions became significant. Chen et al. (2002) also showed that prolonged reaction times in acid-catalyzed transesterification of vegetable oils initially increased methyl ester yield, but further extension led to equilibrium saturation.

In the present study, HAME yield plateaued after 70 h, indicating that the system had reached equilibrium. Hence, 70 h was selected as the optimal reaction time.

3.1.4. Implications

Taken together, this study demonstrates that organic acid-catalyzed transesterification, although generally less efficient than conventional strong acid systems, offers a viable and sustainable alternative for HAME production from microbial biomass. The optimized conditions (20 % acetic acid, 70 °C, and 70 h) yielded the highest HAME under moderate reaction conditions, avoiding the use of hazardous catalysts and additional polymer extraction steps. Compared with conventional acid-catalyzed transesterification processes that typically require 55–80 °C and reaction durations exceeding 50 h (Marchetti et al., 2007) or up to 8 h at higher acid concentrations (Wang et al., 2006), the present method operates efficiently at similar or lower temperatures, indicating a less energy-intensive reaction environment. The PHA content in the microbial biomass was determined to be 0.6327 g PHA/g dry biomass (63.27 % w/w), indicating that the obtained HAME yield of 56.1 ± 0.3 % corresponds to approximately 0.355 g HAME per g dry biomass. This quantitative relationship highlights that the acetic acid-mediated process efficiently converts the majority of intracellular PHA into HAME without requiring polymer purification.

3.2. Characterization of HAME

The FTIR spectrum of HAME exhibited a prominent absorption band at 1742 cm^{-1} , corresponding to the C=O stretching vibrations characteristic of ester functional groups (Fig. 4). This confirms the successful formation of methyl ester in the product. Similar C=O stretching vibrations have been observed in other methyl esters, including biodiesel derived from plant oils (Nosal et al., 2021), supporting the general consistency of ester functional group formation.

The density at 15 °C and the dynamic viscosity at 40 °C were found to be 854 kg/m^3 and $3.9 \text{ mm}^2/\text{s}$, respectively (Table 1), which are comparable to conventional biodiesel. These values are consistent with previous work on PHA-based biofuels (Sangkharak et al., 2021; Wang et al., 2010) and with reported properties of biodiesel–gasoline blends from waste vegetable oils (Kassem et al., 2020). However, the methyl ester content was 80.4 ± 0.8 %, which is below the ASTM D6751

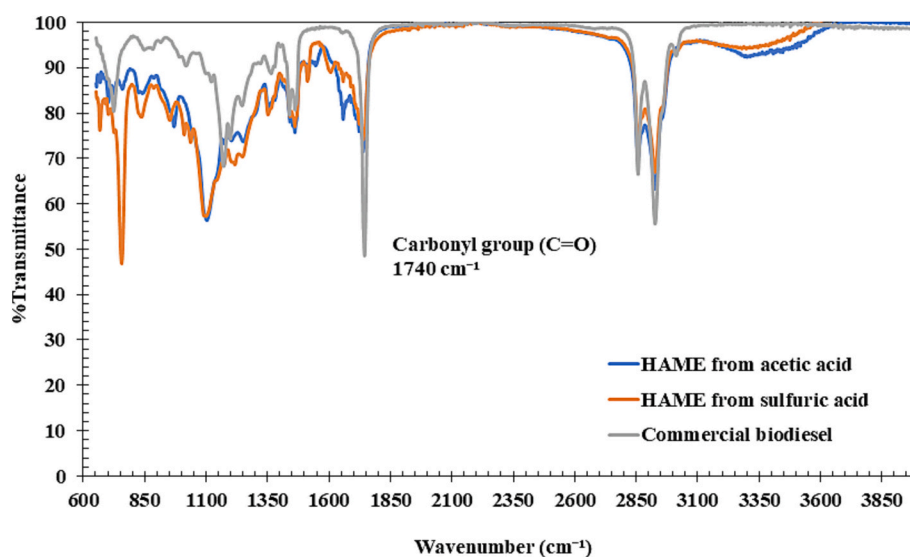


Fig. 4. FTIR spectra of polyhydroxyalkanoate-rich microbial biomass (PMB), hydroxyalkanoate methyl esters (HAME) produced using acetic acid (blue), HAME produced using sulfuric acid (orange), and commercial biodiesel (grey).

Table 1
Partial fuel characterization for HAME.

Properties	^a HAME	^b HAME	^c Biodiesel	^d Thai	^e ASTM
Density at 15 °C (kg/m ³)	854 ± 1.2	860	890	860–900	-ND
Dynamic Viscosity at 40 °C (mm ² /s)	3.9 ± 0.7	4	4	3.5–5	-ND
Methyl Ester Content (%)	80.4 ± 0.8	97	96.5	96.5	96.5

^a HAME (hydroxyalkanoate methyl esters) produced in this study.

^b Data from Sangkharak et al. (2021).

^c Commercial biodiesel.

^d Thai is the Thai Ministry of Energy biodiesel standard.

^e ASTM is ASTM D6751 standard, and -ND is not defined in the corresponding standard.

minimum requirement of 96.5 %. This lower methyl ester content may be attributed to the use of whole-cell biomass without prior polymer purification and the milder catalytic system (acetic acid) employed in this study. In contrast, studies that achieved higher ester content often employed stronger acid catalysts and more extensive purification steps.

Based on the GC-derived methyl ester profile of HAME obtained in a previous enzymatic transesterification study (Choonut et al., 2022), fuel-related parameters were predicted using established empirical equations (Nayak and Behera, 2025). The HAME fraction consisted primarily of methyl 3-hydroxyhexadecanoate (56.1 ± 0.3 %), followed by methyl 3-hydroxytetradecanoate (19.2 %), methyl 3-hydroxydecanoate (12.3 %), methyl 3-hydroxyoctadecanoate (10.2 %), and methyl 3-hydroxyoctanoate (2.2 %). The calculated average degree of unsaturation (ADU) was zero, consistent with a saturated structure. The predicted physicochemical properties were: Kinematic viscosity (KV) = 5.21 mm²/s, Specific gravity (SG) = 0.8726, Cetane number (CN) = 62.9, Cloud point (CP) = 19.99 °C, Iodine value (IV) = 12.71 g I₂/100 g and Higher heating value (HHV) = 38.53 MJ/kg.

These values indicate that HAME has superior ignition quality and oxidative stability compared to typical polyunsaturated FAMES, consistent with the findings of previous studies that emphasized the importance of fatty acid chain length and degree of unsaturation on biodiesel performance (Hoekman et al., 2012; Singh et al., 2018; Veríssimo and Gomes, 2011). However, due to the hydroxyl substitution on the alkyl chain, actual viscosity and stability may deviate from the model predictions. These predictions are preliminary and require

experimental validation. The detailed calculation equations and results are provided in **Supplementary Table S1**.

3.3. Advancements over previous approaches

Previous research has extensively explored HAME production via chemical and enzymatic transesterification, predominantly using purified PHAs. Sulfuric acid-catalyzed transesterification under optimized conditions (10–15 % H₂SO₄, 65–100 °C, 50–60 h) yields up to 96.5–97 % methyl esters (Junpadit et al., 2014; Sangkharak et al., 2021; Wang et al., 2010; Zhang et al., 2009) (Table 2). Although effective, this approach requires polymer extraction, harsh reagents, and extensive post-processing steps that increase costs, environmental risks, and complexity (Lam et al., 2010; Leung et al., 2010; Talha et al., 2016). Enzymatic catalysis using lipases offers milder conditions and environmental advantages (Kalita et al., 2022), achieving yields similar to those under harsher conditions (e.g., 96.5 % at 45 °C, 30 h) (Choonut et al., 2022). However, scale-up is limited by high enzyme costs, operational sensitivity, and limited reusability (Krisdiyanto et al., 2025; Zheng and Cho, 2025).

In contrast, this study presents a one-pot, extraction-free method using acetic acid to directly convert unrefined PMB into HAME. Despite a lower ester content (80.4 ± 0.8 %), the process achieved a high methyl ester yield (56.1 ± 0.3 %) while significantly reducing in energy, chemical use, and processing complexity. To date, this is the first report to demonstrate direct HAME synthesis from intact PMB using an organic acid catalyst, enabling scalable, decentralized biofuel production

Table 2
Evaluation of catalytic strategies for HAME synthesis.

Parameter	^a Sulfuric acid	^b Enzyme	^c Acetic acid
Substrate	PHA	PHA	PMB
Catalyst conc.	10–15 %	0.5 g	20 %
Optimal temp. (°C)	65–100	45	70
Reaction time (h)	50–60	30	70
Methyl Ester (%)	Up to 97	96.5	80.4 ± 0.8 (n = 3)
Pre-treatment	Yes	Yes	None
Environmental impact	High	Low	Low
Reusability	No	Yes	No

^a Data from Sangkharak et al. (2021), Wang et al. (2010) and Zhang et al. (2009).

^b Data from Choonut et al. (2022).

^c HAME (hydroxyalkanoate methyl esters) produced in this study.

aligned with circular bioeconomy principles.

Importantly, the comparative analysis underscores that while traditional acid or enzymatic routes maximize yield, they may compromise economic and environmental feasibility. Conversely, the proposed acetic acid-mediated pathway achieves a favorable balance between yield efficiency, operational simplicity, and environmental impact. Although actual scale-up experiments were not conducted, the method's simplicity, one-pot operation, and lack of pre-treatment steps suggest potential applicability in broader or decentralized biorefinery settings. These trade-offs highlight the feasibility of this approach for valorizing microbial biomass into renewable fuels under resource-limited conditions.

3.4. Application and potential of HAME

Despite the lower methyl ester content (80.4 ± 0.8 %) relative to ASTM D6751 standards, the HAME produced here holds significant promise. In the energy sector, it can serve as a blend component with higher-purity biodiesel or fossil diesel, particularly in decentralized systems where extensive purification infrastructure is unavailable (McCormick et al., 2024) (Table 3). Its high calorific value (~ 30 kJ/g), exceeding that of ethanol (27 kJ/g), also makes it a valuable additive for ethanol-diesel blends (Zhang et al., 2009). Beyond energy, HAME and its monomers possess biomedical potential, including antibacterial activity (against *Salmonella*, *Listeria*, *E. coli* (Kalia et al., 2021), support for cell proliferation (Pramanik et al., 2016), and therapeutic applications for osteoporosis, hypoglycemia (Cao et al., 2014; Kalia et al., 2021), and neurodegenerative diseases (Zhang et al., 2013). This multifunctionality enhances HAME value proposition and positions it as a bridge between the energy and health sectors within a sustainable biorefinery framework.

3.5. Environmental and economic implications

Catalyst selection critically impacts both the environmental profile and economic feasibility of biofuel production. While H_2SO_4 and enzyme-based systems provide high ester content, they are burdened with corrosivity, high cost, and scale-up limitations. By contrast, acetic acid enables a mild, low-cost, extraction-free process that aligns with green chemistry and circular economy principles.

This method simplifies operations, minimizes methanol evaporation (via sealed reactors), and valorizes microbial biomass from low-cost feedstocks such as wastewater or agro-industrial residues (Table 4). Economic estimates suggest a production cost of \$600–700/ton, significantly lower than that of sulfuric acid \$1400/ton (Zhang et al., 2009).

This strategy offers a practical route for low-tech, low-cost, decentralized biofuel production, especially in rural or developing regions. Although actual scale-up experiments were not conducted, the method's simplicity, one-pot operation, and lack of pre-treatment steps suggest

Table 3
Applications and potential of HAME.

Sector	Application	Detail
Energy	Biofuel blending	For off-grid/localized use; high combustion value
	Ethanol blend enhancer	Adds energy content to bioethanol-based fuels
Biomedical	Antibacterial activity	Effective against foodborne pathogens
	Cell proliferation support	e.g., hydroxybutyrate derivatives
	Therapeutic potential	Osteoporosis, Alzheimer's, metabolic diseases
Process	Post-reaction enhancement	Membrane/distillation to meet biodiesel standards
	Bio-based material feedstock	Used in green chemicals and biomaterials

Table 4
Comparative techno-economic and environmental assessment.

Parameter	^a Sulfuric acid	^b Enzyme	^c Acetic acid
Catalyst cost	Low	Very High	Low
Reaction conditions	Harsh	Mild	Mild
Pre-treatment	Required	Required	None
Yield (%)	44.4–74.4	96	56.1 ± 0.3 ($n = 3$)
Methyl ester content	High (≥ 96.5 %)	High (≥ 96.5 %)	Moderate (80.4 ± 0.8 %) ($n = 3$)
Estimated cost/ton	\$1100–1200	\$1400+	\$600–700
Environmental impact	High	Low	Low
Neutralization needed	Yes	No	No

Note: Estimated values for cost and operational feasibility are based on literature reports and the intrinsic simplicity of the acetic acid-mediated process. Actual scale-up experiments were not performed. Environmental impact and pre-treatment requirements are based on process characteristics and standard chemical handling practices.

^a Data from Sangkharak et al. (2021), Wang et al. (2010) and Zhang et al. (2009).

^b Data from Choonut et al. (2022).

^c HAME (hydroxyalkanoate methyl esters) produced in this study.

potential applicability in broader contexts. Future work should focus on scale-up, waste valorization, and product upgrading to meet international fuel standards and broaden market potential.

3.6. Conceptual mechanism of acetic acid-catalyzed HAME production

This figure illustrates the proposed reaction mechanism and the physical changes that occur during the direct transesterification of PMB into HAME under mild acidic conditions (Supplementary Fig. S1).

- 1) PMB: The initial biomass matrix consists of intact microbial cells that intracellularly accumulate PHA. These cells are enclosed within semipermeable membranes that act as diffusion barriers.
- 2) Catalyst and alcohol penetration: Upon the addition of acetic acid (CH_3COOH) and methanol (CH_3OH), the acidic environment facilitates partial disruption of cell membranes, allowing methanol and protons (H^+) to access intracellular PHA granules. Acetic acid acts as both a proton donor and a mild acid catalyst, initiating the acid-catalyzed esterification.
- 3) Esterification and cell disintegration: Acidic protonation of the ester carbonyl groups on PHA increases the electrophilicity of the carbon atom, which enables nucleophilic attack by methanol. This reaction leads to the formation of tetrahedral intermediates, followed by cleavage of the polymer backbone and release of methyl ester monomers (HAME). Concurrently, cell disintegration releases intracellular contents into the reaction medium, forming a heterogeneous reaction system containing HAME, water, cell debris, and unreacted reagents.
- 4) Phase separation and recovery: After completion of the reaction, the mixture is subjected to phase separation (e.g., sedimentation, centrifugation), enabling recovery of the HAME-rich organic phase. Residual biomass and cellular debris are removed from the bottom phase. This process does not require prior PHA purification or the use of toxic solvents, reducing both energy consumption and chemical waste (Fig. 1S).

This one-pot strategy aligns with green chemistry principles, including atom economy, reduced hazard, and energy efficiency. The use of acetic acid, a bio-derived and biodegradable acid, along with whole-cell biomass, eliminates multiple downstream processing steps such as solvent extraction and polymer isolation, thereby enhancing the environmental and economic feasibility of biofuel production.

4. Conclusions

This study introduces a novel, environmentally friendly approach for the direct conversion of microbial PHA in biomass into HAME, using acetic acid as a green, sustainable catalyst. The process eliminates the need for polymer extraction and employs mild reaction conditions, achieving a maximum methyl ester yield of 56.1 ± 0.3 % under optimized conditions (20 % acetic acid, 70 °C, 70 h). FTIR analysis confirmed successful transesterification, while partial fuel property evaluation demonstrated density and viscosity comparable to those of commercial biodiesel. Although the methyl ester content (80.4 ± 0.8 %) was below ASTM D6751 specifications, the overall process significantly reduces chemical usage, environmental impact, and production costs. To the best of current knowledge, this is the first report to demonstrate the direct synthesis of HAME from unrefined microbial biomass using acetic acid. The findings highlight the feasibility of integrating this process into decentralized biofuel production systems and circular biorefinery platforms.

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CRediT authorship contribution statement

Narisa Binhayeeding: Validation, Investigation, Formal analysis, Data curation. **Aophat Choonut:** Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Atipan Siammai:** Supervision, Methodology. **Kanokphorn Sangkharak:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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