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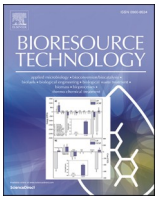

Mesophilic fermentation explorations for anaerobic carboxylates production from commercial bioplastic products: PHA-based cups & PLA-based lids

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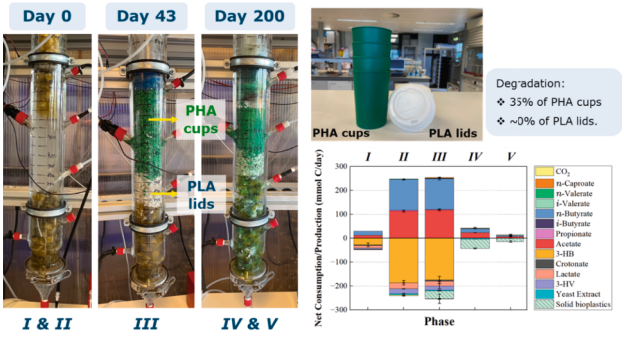
HIGHLIGHTS

- PHA cups converted up to 35 % into acetate and *n*-butyrate in 157 days fermentation.
- PLA lids showed negligible conversion under mesophilic fermentation.
- Several microbial species likely contributed to direct solid PHA degradation.

GRAPHICAL ABSTRACT



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ABSTRACT

Biodegradable plastic polymers such as polyhydroxyalkanoates (PHA) and polylactic acid (PLA) are increasingly applied in commercial products, but their end-of-life (EoL) processing remains inefficient in terms of carbon and energy recovery. This study investigated mesophilic (35℃) open-culture fermentation to convert PHA and PLA raw materials and commercial bioplastic products into carboxylates. A gas-lift anaerobic filter bioreactor was inoculated with a mixed culture sourced from rumen liquid and lab-scale open-culture fermenters, and operated over 200 days under controlled pH (5.9) and hydraulic retention times (2–18 days). Hydrolysates obtained from hydrothermally pretreated (150℃, 15 h) PHA (10 g/L) and PLA (1.4 g/L) pellets were continuously fermented, yielding up to 6.6 g/L acetate and 4.8 g/L *n*-butyrate. Subsequent co-fermentation with shredded commercial bioplastics (PHA-based cups and PLA-based lids) further increased acetate and *n*-butyrate to 7.2 g/L and 5.5 g/L, respectively. Approximately 35 % of the PHA-based cups were converted into carboxylates, while PLA-based lids showed negligible degradation despite an operation time of 157 days. Full-length 16S sRNA sequencing revealed that *Clostridium tyrobutyricum* was likely a dominant species during hydrolysate fermentation of PHA and PLA pellets, while a broader microbial consortium contributed to solid bioplastic product conversion. These findings indicate that mesophilic open-culture fermentation offers a viable route for the conversion of real PHA-based products into carboxylates, whereas PLA-based products may remain more recalcitrant under the same conditions.

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1. Introduction

Fossil-based plastics, due to their recalcitrant nature, pose a growing threat to the environment and human health (Thompson et al., 2009). In contrast, biobased and biodegradable plastics—particularly polyhydroxyalkanoates (PHA) and polylactic acid (PLA)—are receiving increasing attention as alternative (Europe Bioplastics, 2025). However, innovation is still required to improve both material safety and end-of-life (EoL) management through effective recycling technologies (Crippa et al., 2019). Although biodegradable plastic products and their raw materials are often certified for a certain biodegradability under specific conditions, the real-world degradation behaviors may vary. Nevertheless, consumer adoption of biodegradable items—such as PHA-based cups, food containers, and packaging, as well as PLA-based coffee lids, mulch films, and *T*-shirt—is steadily increasing (Boey et al., 2021). Conventional EoL options for these materials include industrial composting, in-field degradation, and incineration (Afshar et al., 2024; Moshood et al., 2022). All these approaches have a limited carbon recovery due to the release of CO_2 during the treatments.

To support the circular economy, more effective recycling routes are needed—ones that retain carbon in the value chain and facilitate high-value reuse, as envisioned by the Dutch Biobased Circular growth fund initiative (BioBased-Circular, 2025). In this context, the carboxylate platform emerges as a promising approach. This platform focuses on the fermentative production of volatile fatty acids (VFA) from various microbially convertible biomass and waste streams, including biodegradable plastics (Holtzapple et al., 2022). The VFA generated can be directly valorized in chemical or biological processes or used as precursors for resynthesizing PHA, thereby closing the loop for bioplastic materials (Al Battashi et al., 2021; Jin et al., 2025b; Jin et al., 2023).

Several studies have examined the anaerobic fermentation of biodegradable plastics for VFA production. Various raw PHA materials have shown potential for partial conversion into VFA under mesophilic conditions, with or without prior pretreatment (García-Depraect et al., 2022; Jin et al., 2023). For PLA, however, VFA production has not been reported without pretreatment. When a mild thermal pretreatment was employed on PLA-based packaging material, microbial fermentation of the resulting hydrolysates successfully yielded VFA (Strik and Heusschen, 2023). Similarly, thermo-alkaline pretreatment has been shown to enhance the anaerobic digestion of PHA and PLA, improving biogas production (Benn and Zitomer, 2018). More recent work demonstrated that combining PHA and PLA raw materials during hydrothermal pretreatment (150℃ for 15 h) enhanced hydrolysis efficiency (Jin et al., 2025a). Fermentation of the resulting hydrolysates achieved up to 90 % COD conversion into carboxylates—a significant improvement over earlier work which only reached ~ 10 % carboxylate recovery due to insufficient pretreatment (120℃, 4 h, 0.1 M KOH) (Jin et al., 2025a; Jin et al., 2023). These findings clearly show that pretreatment improves the microbial availability of PHA and PLA for carboxylate production. However, such pretreatments also require significant external energy and chemical inputs, which may negatively affect process economics and life cycle performance (Jin et al., 2023; Samorì et al., 2022). Avoiding the need for pretreatment could reduce costs and simplify the process. Moreover, real-world bioplastic products typically consist of blends or compounds and often contain additives, which can influence biodegradability and microbial accessibility compared to pure raw materials (Cazaudehore, 2022). Yet, to date, no studies have investigated the anaerobic fermentation of mixed PHA and PLA-based consumer products under mesophilic conditions for carboxylate production.

This study aims to study the feasibility of anaerobic fermentation of mixed PHA and PLA consumer products into carboxylates under mesophilic conditions. The specific objectives were to: (i) establish a microbial community capable of converting hydrolyzed PHA and PLA into carboxylates, (ii) evaluate the fermentation performance of solid biodegradable plastic products, and (iii) understand the possible functional role of key taxa in the degradation and conversion processes. To achieve these goals, a sequential bioprocess in a gas-lift anaerobic filter bioreactor was employed and microbial community analyses on fermentation broth, bioplastic surfaces, and inert polyurethane (PU) biofilm carriers were conducted. Several experimental phases were studied whereby pre-hydrolyzed PHA and PLA and/or the sole consumer bioplastic products were supplied. This integrated approach provides insights on bioprocess-level and microbial contributions to bioplastic valorization.

2. Materials and methods

2.1. Materials

PHA pellets were obtained from ENMAT™ Thermoplastics PHBV (poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate), Resin Y1000P, Ningbo, China), consisting of 98 % 3-hydroxybutyrate (3-HB) and 2 % 3-hydroxyvalerate (3-HV). PLA pellets were sourced from Ingeo™ Biopolymer 4043D (Natureworks, USA). Commercial PHA-based cups were ordered from Happy Cups™ (Oosterwolde, the Netherlands), containing the same ENMAT™ PHBV Resin Y1000P pellets and green-color masterbatch (HappyCups, 2025). PLA-based lids (cPLA: crystallized polylactic acid) were ordered from Green Box GmbH & Co. KG (Bremen, Germany) (cPLA-lid, 2025).

To inhibit methanogenesis, 2-bromoethanesulfonate sodium (BES, 98 %, Alfa) was added at a concentration of 5 g/L. The microbial growth medium used for open-culture fermentation was based on a previously established formulation (see supplementary materials). A 4 mol/L KOH solution was used to adjust the pH before fermentation, and a 1 mol/L KOH was applied for pH control during the process. The inoculum was sourced from rumen liquid (collected from the Animal Science Department, Wageningen University & Research) and effluents from ethanol-, lactate-, and glucose-based fermentation (Jin et al., 2025b).

2.2. Bioplastics pretreatment

PHA and PLA pellets were hydrothermally pretreated in a 1 L benchtop stirred reactor (Parr 4857, Parr Instruments Moline, Illinois, USA), as described in a previous study (Jin et al., 2025a). A total of 35 g PHA pellets and 5 g PLA pellets were mixed and processed at 150℃ for 15 h in 700 mL of demineralized water. After pretreatment, the resulting hydrolysates were filtered using a 0.45 μm membrane (CHROMAFIL Xtra, Machinerey-Nagel, Germany) and subsequently diluted fivefold. The final concentrations were approximately 10 g/L for PHA and 1.4 g/L for PLA. The diluted hydrolysates were used as fermentation substrates in the first three experimental phases. PHA cups and PLA lids were shredded into pieces using a shredder (Felfil Model 500) (see supplementary materials) and added in Phase III.

2.3. Experimental setup and design

Open-culture fermentation was performed in a continuous gas-lift anaerobic filter bioreactor—a double-walled glass column (80 cm in height) with a working volume of 1 L and a headspace of 0.8 L (see supplementary materials). A sampling port was located at the midpoint of the reactor. The pH was maintained at 5.9 ± 0.1 by automatic titration using 1 M KOH as needed. The fermentation temperature was controlled at 35℃ via a water bath (Julabo F25, the Netherlands). A continuous gas flow of N_2 (9 NmL/min) and CO_2 (0.8 NmL/min) was regulated via the LabVIEW program (the Netherlands) and delivered through gas flow controllers (Bronkhorst, the Netherlands). In this experiment, the gas composition and flow rates were selected in the same order of magnitude as reported in earlier studies, to ensure conditions favorable for fermentation and carboxylate formation (Roghair et al., 2018).The effluent gas flow rate was monitored with a flow meter (BPC Instruments AB, Sweden). A gas recirculation loop transported headspace gases back to the reactor base using a peristaltic pump

Table 1
Overview of experimental phases during open-culture fermentation of bioplastic-derived substrates.

Phase	Duration (days)	Medium composition	Bioplastics added	HRT (days)	Inoculation
I	0–6	PHA (10 g/L) + PLA (1.4 g/L) hydrolysates [a]	–	–	20 mL
II	6–43	PHA (10 g/L) + PLA (1.4 g/L) hydrolysates [a]	–	2	–
III	43–95	PHA (10 g/L) + PLA (1.4 g/L) hydrolysates [a]	'Happy Cups' & Lids [b]	2	20 mL
IV	95–123	Nutrients only	'Happy Cups' & Lids [b]	2	–
V	123–200	Nutrients only	'Happy Cups' & Lids [b]	18	–

Notes: **a.** Hydrolysates were prepared by hydrothermal pretreatment of 35 g PHA pellets and 5 g PLA pellets in 700 mL demineralized water, followed by diluted fivefold. **b.** 'Happy Cups' & Lids refer to commercial solid bioplastic composed of PHA-based cups and PLA-based coffee lids.

(Watson Marlow P500). The hydraulic retention time (HRT) was adjusted using a peristaltic pump (Watson-Marlow, Falmouth, UK).

The experiment was conducted in five distinct phases (Table 1). In Phase I, batch fermentation was initiated using hydrolysates derived from mixed PHA and PLA pellets (raw materials) at a 7:1 wt ratio, with the reactor inoculated with 20 mL (2 % v/v) of microbial culture. Phase II transitioned to continuous fermentation using the same hydrolysates-based medium, operated at an HRT of 2 days. This allowed the establishment of a microbial community specialized in fermenting bioplastic intermediates before introducing solid bioplastic products. In Phase III, shredded commercial bioplastic products—230.24 g of PHA cups and 32.84 g of PLA lids, with the same 7:1 wt ratio—were introduced and co-fermented with the hydroxylates (see supplementary materials). An additional 20 mL of inoculum was supplied to enrich microorganisms potentially capable of degrading solid biodegradable plastic materials. During Phase IV, only nutrients (without hydrolyzed PHA or PLA pellets) were supplied to enable the continued fermentation of the remaining solid bioplastics. Finally, in Phase V, the same conditions as Phase IV were maintained, but the HRT was extended from 2 days to 18 days to stimulate further carboxylate accumulation.

The reactor bottom was filled with 1 cm glass beads (Hecht-Assistant, Germany) to facilitate gas dispersion. To promote initial biomass attachment and growth, polyurethane (PU) sponge carrier materials in the form of 15 × 15 mm cubes (Recticel, Belgium) was added at the start of the experiment. This type of non-biodegradable sponge materials was used in anaerobic fermentation bioreactors designed to study microbial chain elongation processes (de Leeuw et al., 2021; Grootscholten et al., 2013). At Phase III, the suspended PU sponges were removed and replaced with fragmented pieces of PHA cups and PLA lids, which were directly loaded into the reactor. A part of the PU sponges was removed to create space for the addition of solid PHA and PLA materials. Some PU sponge cubes were retained at the bottom of the reactor to preserve part of the microbial community, while the removed sponges were used for microbial community analysis. To prevent washout of the bioplastic solids, a PU sponge (9.5 × 9.5 × 4.8 cm, Juwel Aquarium, Rotenburg, Germany) was placed above the bioplastics as a physical barrier (see supplementary materials).

2.4. Sampling and measurements

Reactor check-ups were performed three times a week. At each check, both headspace gas and reactor medium were sampled. Operational parameters such as gas flow rate, pH, temperature, influent and effluent weights, and base consumption were recorded to assess reactor performance and stability.

Headspace gases (N_2, CO_2, O_2) were analyzed using gas chromatography (Shimadzu GC-2010, Japan) equipped with Porabond Q and Molsieve 5A column and H_2 as carrier gas. H_2 and CH_4 were determined using a separate GC system (HP-5890, Agilent, USA) with an HP Molsieve 5A column and argon carrier gas. Approximately 8.5 mL of reactor medium was sampled each time. 0.5 mL of this was used for pH measurement, and the remaining liquid was filtrated using a 0.45 μm membrane filter (CHROMAFIL Xtra, Machinerey-Nagel, Germany), aliquoted into 2.0 mL vials, and stored at −20℃ for further analysis. Carboxylates and alcohols were quantified by gas chromatography (Agilent 7890B, USA) with a HP-FFAP column and FID detector, nitrogen was used as the carrier gas. Hydrolysates intermediates (3-HB, crotonate, lactate, and 3-HV) were measured using high-performance liquid chromatography (HPLC, Thermo Dionex Ultimate 3000 RS), equipped with a UV-RI detector (254 nm) and Astec CLC-L Chiral column. The column temperature was maintained at 45℃, and 5 mM H_2SO_4 was used as the eluent at a continuous flow of 0.15 mL/min. The injection volume was 10 μL. Specific technical details can be found in supplementary materials. Data were processed using Chromeleon software (version 7.3). Error bars indicate the standard deviation of the average values calculated for each fermentation phase.

2.5. Microbial community analysis

Biomass samples were collected from both reactor broth and biofilms throughout the experiment, at the end of each phase except phase I (see supplementary materials). For broth samples, the fermentation medium was homogenized by pipetting ten times using a 10-mL syringe (Gemeinhardt et al., 2024). Approximately 120 mL was sampled at each sampling point, centrifuged at 4000 RPM for 5 min, and the supernatant was discarded. Biomass pellets were frozen using liquid N_2 for ~ 30 s and stored at −80℃ for further DNA extraction. Biofilm samples were collected at the end of Phase II using a 40 cm metal clamp inserted through the reactor headplate, while N_2 was sparged through the headspace at ~ 50 NmL/min. Polyurethane cubes from the upper reactor region were collected. At the end of Phase V, PHA cup residues and PU cubes from the reactor were also sampled. All biomass samples were immediately stored at −80℃.

DNA extraction and 16S rRNA amplicon analysis were performed following standard protocols. Microbial cells were lysed, genomic DNA (gDNA) was extracted, and concentrations were quantified using a Qubit fluorometer. For each sample, 10 ng of gDNA was used as input for polymerase chain reaction (PCR) amplification with the 16S Barcoding Kit 24 V14 (SQK-16S114.24; Oxford Nanopore Technologies) and LongAmp Hot Start Taq 2X Master Mix. The PCR program consisted of an initial denaturation at 95℃ for 1 min, followed by 25 cycles of 95℃ for 20 s, 55℃ for 30 s, and 65℃ for 2 min, with a final extension at 65℃ for 5 min. Barcoded PCR products were pooled in equimolar ratios, purified with AMPure XP beads, and ligated with a Rapid Adapter (RA) to prepare the sequencing library.

The library was loaded onto an R10.4.1 Flow Cell (FLO-MIN114) after priming with Flow Cell Flush (FCF) supplemented with bovine serum albumin (BSA) to enhance sequencing performance. Sequencing was conducted on a GridION platform (Oxford Nanopore Technologies) using MinKNOW for data acquisition. Basecalling was performed with Dorado using the dna_r10.4.1_e8.2_400bps_5khz_sup.cfg model. The sequencing library strategy was amplicon-based metagenomics, generated with primers 27F-1492R. Post-sequencing analysis was performed using the EPI2ME 16S amplicons workflow (Curry et al., 2022), which

classified full-length 16S rRNA rads and generated species-level taxonomic abundance profiles.

3. Results and Discussion

3.1. Efficient production of acetate and n-butyrate from hydrolysates of PHA and PLA pellets pretreated by hydrothermal pretreatment (Phases I & II)

Hydrothermal pretreatment combined with open-culture fermentation enabled near-complete conversion of biodegradable plastic hydrolysates into carboxylates, predominantly acetate and *n*-butyrate. During continuous fermentation of pretreated PHA and PLA hydrolysates, the carboxylate concentration reached 6.6 g/L acetate and 4.8 g/L *n*-butyrate, corresponding to ~ 220 mM C of each. The applied pretreatment solubilized about 89 % of the carbon, and all soluble intermediates were subsequently converted into carboxylate (see supplementary materials for the calculations). These results demonstrate the effectiveness of this strategy for carboxylate production from mixed biodegradable plastic raw materials.

The bioprocess started with batch fermentation using hydrolysates derived from 10 g/L PHA and 1.4 g/L PLA pellets, thermally pretreated at 150℃ for 15 h. After a 6-day acclimation period (Phase I), the reactor was switched to continuous mode (Phase II). The pretreated hydrolysates mainly contained 3-hydroxybutyrate (3-HB) from PHA and L-lactate from PLA (Fig. 1). These substrates were almost completely consumed, as no residual concentrations were detected in the reactor effluent (see supplementary materials). Acetate and *n*-butyrate were the dominant products (Fig. 1A), derived from 330 mM C 3-HB (8.6 g/L) and 45 mM C L-lactate (1.4 g/L) initially supplied. Only trace amounts of crotonate—an intermediate of PHA hydrolysis or 3-HB oxidation—were detected, likely due to the relatively mild pretreatment temperature of 150℃. This contrasts with earlier studies that applied thermolytic distillation at 170℃ and 150 mbar, achieving up to 98 % crotonic acid yield (Samorì et al., 2022).

Minor products included propionate (~9 mM C) and *n*-valerate (3.5 mM C), with their accumulation increasing slightly during continuous operation. These may have originated from the oxidation of 3-hydroxyvalerate (3-HV, 40 mM C)—a minor component in the PHA hydrolysates, as these acids are typically precursors for 3-HV synthesis (Chang et al., 2012; García-Depraect et al., 2022; Reischwitz et al., 1997). Gas analysis indicated net CO_2 consumption (~3 mmol/day, see supplementary materials), and H_2 remained below 0.2 % in the headspace (see supplementary materials). Total carboxylates productivity reached ~ 240 mmol C/day, closely matching the carbon input, confirming the high conversion efficiency of the hydrolysates (Fig. 1D).

Comparison with previous studies underscores the importance of substrate composition, reactor type, and concentration (Table 2). A parallel study using ~ 5 g/L PLA hydrolysates achieved higher lactate supply, resulting in promoted *n*-butyrate production (8.0 g/L) and detectable *n*-caproate formation (0.3 g/L) (Jin et al., 2025a). This increased carboxylate productivity to 526 mmol C/L/d, compared to 438 mmol C/L/d in the present study. These results highlight the role of PLA-derived lactate as an electron donor that supports chain elongation and enhances product diversification (Jin et al., 2025b). Moreover, fermentation of hydrolyzed organic waste in anaerobic filters has achieved peak carboxylate productivities (C_4-C_8) of up to 1,675 mmol C/L/d, indicating further optimization potential for biodegradable plastic conversion (Grootscholten et al., 2014). In contrast, studies employing thermal and/or alkaline pretreatment for batch fermentation showed substantially lower performance. For instance, thermo-alkaline hydrolysis of PHA (0.1 M KOH, 120℃, 4 h) followed by batch fermentation produced only 1.7 g/L acetate and 1.2 g/L *n*-butyrate (22.2 mmol C/L/d) (Jin et al., 2023). Similarly, thermal treatment of PLA packaging (70℃, 21 days, 60 g/L) yielded diverse but low-concentrations of carboxylates (Strik and Heusschen, 2023), while mesophilic digestion of PHA (35℃, 12 h) mainly produced methane with a productivity of just 18.5 mmol C/L/d (Benn and Zitomer, 2018). These comparisons further emphasize the effectiveness of the combined hydrothermal pretreatment and continuous fermentation strategy employed in this study.

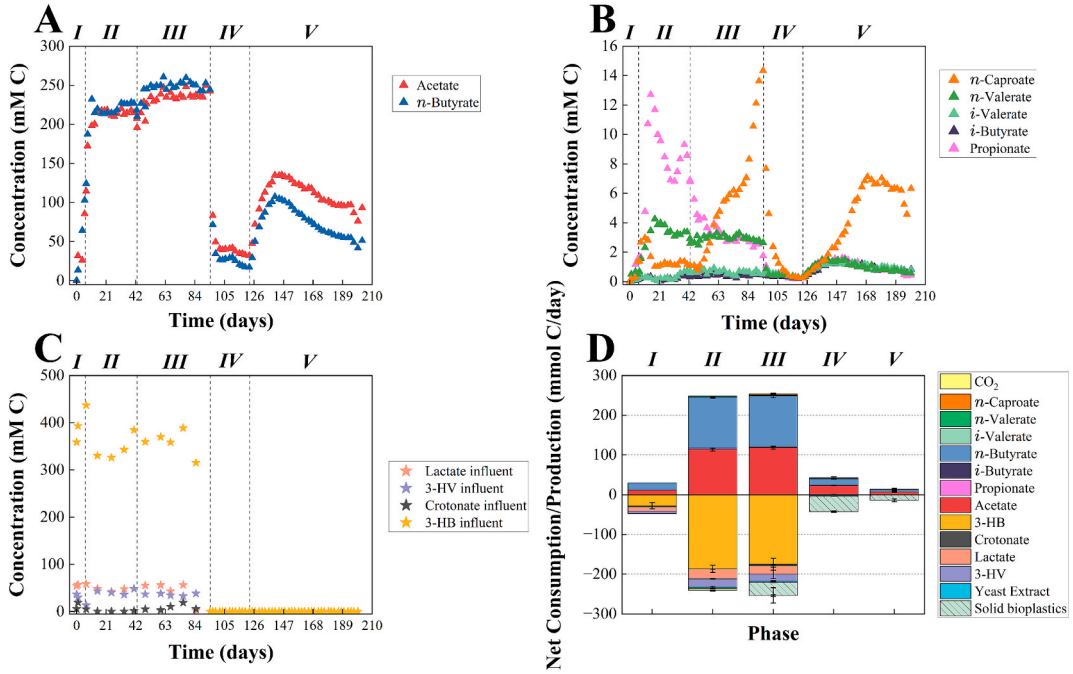

Fig. 1. Carbon fluxes and metabolite dynamics during open-culture fermentation of bioplastics across different phases. **A.** Variations in acetate and *n*-butyrate bioreactor concentrations (mM C) across different fermentation phases (limited carbon source other than the provided bioplastic solids, CO_2, and yeast extract in Phases IV and V). **B.** Changes in propionate, *i*-butyrate, *i*-valerate, *n*-valerate, and *n*-caproate bioreactor concentrations (mM C) throughout fermentation. **C.** Composition variations in 3-HB, crotonate, lactate, and 3-HV concentrations (mM C) of the influent. **D.** Averaged consumption rates and productivities (mmol C/day) of each fermentation phase. '3-HB' means 3-hydroxybutyrate, and '3-HV' means 3-hydroxyvalerate.

Table 2
Comparison of carboxylates/methane production from organic waste or pretreated PHA/PLA under different conditions.

Reactor mode	Feedstock	Pretreatment	Substrates	Products	Conversion rate (mmol C/L/d) [c]	Reference
Gas-lift anaerobic filter [a]	PHA & PLA pellets	150℃, 15 h	~10 g/L PHA and ~ 1.4 g/L PLA hydrolysates	6.6 g/L C_2 4.8 g/L n-C_4	438	This study
CSTR [b]			~10 g/L PHA and ~ 5 g/L PLA hydrolysates	4.4 g/L C_2 8.0 g/L n-C_4 0.3 g/L n-C_6	526	(Jin et al., 2025a)
Anaerobic filter	MSW & ethanol	Acidification at 35℃	15.6 g/L ethanol 2.0 g/L acetate 1.3 g/L propionate	1.3 g/L n-C_4 0.9 g/L n-C_5 11.9 g/L n-C_6 0.5 g/L n-C_7 0.4 g/L n-C_8	1,675	(Grootscholten et al., 2014)
Batch	PHA pellets	0.1 M KOH, 120℃, 4 h	4 g COD/L PHA hydrolysates	1.7 g/L C_2 1.2 g/L n-C_4	22	(Jin et al., 2023)
	PLA packaging materials	70℃, 21 d, 2 x 2 cm	60 g/L PLA hydrolysates	1.2 g/L C_2 2.1 g/L C_3 6.5 g/L n-C_4 0.8 i-C_4 0.3 g/L n-C_5 0.2 g/L n-C_6	13	(Strik and Heusschen, 2023)
	PHA pellets	35℃, pH 12, 24 h, milled to 0.15 mm	25 g/L pretreated PHB pellets	CH_4	19	(Benn and Zitomer, 2018)

Note: **a.** Continuous gas-lift anaerobic filter reactor. **b.** Continuous stirred tank reactor. **c.** Conversion rate was calculated as the total amount of carboxylate produced per reactor volume per day, expressed in mmol C/L/d. 'MSW' means municipal solid waste. C_2, C_3, n-C_4, i-C_4, n-C_5, n-C_6, n-C_7, n-C_8, and CH_4 represent acetate, propionate, n-butyrate, isobutyrate, n-valerate, n-caproate, n-heptylate, n-caprylate, and methane, respectively.

3.2. Addition of commercial biodegradable plastics boosted carboxylate production (Phase III)

The co-fermentation of hydrolyzed PHA/PLA pellets with commercial biodegradable plastics—PHA-based cups and PLA-based lids—enhanced acetate, *n*-butyrate, and *n*-caproate production, demonstrating the reactor's capacity to convert real biodegradable plastic products into carboxylates under mesophilic conditions.

In Phase III, the bioreactor was supplemented with solid commercial bioplastics, including shredded 230.24 g of PHA-based cups and 32.84 g of PLA-based lids (see supplementary materials), alongside continued feeding with pretreated PHA and PLA hydrolysates from pellets (see supplementary materials). This co-feeding strategy slightly enhanced the carboxylate production, reaching ~ 240 mM C acetate (7.2 g/L) and ~ 250 mM C *n*-butyrate (5.5 g/L) from 370 mM C 3-HB (9.6 g/L) and 45 mM C *L*-lactate (1.4 g/L) (see Fig. 1A). Notably, *n*-caproate concentrations increased to ~ 14 mM C under these conditions (Fig. 1B), likely driven by lactate-based chain elongation (Candry et al., 2020). Propionate and *n*-valerate concentrations remained at low concentrations (~3 mM C) (Fig. 1B).

The observed improvement in carboxylate production suggests that the microbial community was possibly well-acclimated to the fermentation environment and capable of partially degrading the added solid biodegradable plastics. Based on steady-state volumetric productivity estimates, a portion of the commercial bioplastics contributed ~ 32.5 ± 19.4 mmol C/L/d to carboxylate production (see supplementary materials, Fig. 1D). As examined at the end of the experiment, PLA was negligible converted. Assuming this rate remains constant under the applied operational conditions (35℃, HRT of 2 days), complete conversion of the PHA based cups would require over 330 days of continuous operation (see supplementary materials). Operational monitoring further supported these observations. H_2 remained below 0.2 % in the headspace, and hydroxide dosing increased to 600 mmol OH^-/L/day to maintain a pH of 5.9 (see supplementary materials), reflecting increased acidification due to enhanced fermentation activity. Microbial community analysis revealed a 90 % relative abundance of *Clostridium tyrobutyricum* suggesting enrichment of this species during co-fermentation of hydrolyzed raw materials and shredded commercial bioplastics (Fig. 2).

3.3. Fermentation with commercial bioplastic solids as primary carbon source: Acetate and n-butyrate remained dominant (Phases IV & V)

Under substrate-limited conditions using commercial biodegradable plastics as the main carbon source, fermentation activity declined, but partial degradation of PHA-based cups still enabled acetate and *n*-butyrate production, whereas PLA remained negligibly degraded. In Phases IV and V, the feed of hydrolyzed PHA and PLA pellets were removed, leaving the solid shredded bioplastics (PHA-based cups and PLA-based lids) as the primary carbon sources, along with CO_2 and yeast extract. The solids were retained within the reactor throughout the entire operation. This transition resulted in reduced fermentation activity compared to earlier phases.

During Phase IV, fermentation continued at low levels, with acetate (~40 mM C) and *n*-butyrate (~25 mM C) remaining the dominant products (Fig. 1), while other carboxylates such as propionate, *n*-valerate, and *n*-caproate declined to near-zero levels. No H_2 production was detected in the gas phase, and hydroxide consumption dropped to ~ 250 mmol OH^-/L/day, consistent with reduced microbial activity (see supplementary materials). In Phase V, the hydraulic retention time (HRT) was increased from 2 to 18 days to stimulate further carboxylate accumulation under these substrate-limited conditions (see supplementary materials). This strategy led to a transient increase in acetate and *n*-butyrate concentrations, peaking at 135 mM C and 103 mM C, respectively, after 21 days (Fig. 1A). However, their concentrations gradually declined thereafter, likely due to depletion of readily biodegradable carbon source. The accumulation of non-biodegradable components, such as the green-color masterbatch in the PHA cups, may have further prevented microbial access to degradable fractions. *n*-caproate slightly increased to ~ 7 mM C, whereas propionate and *n*-valerate concentrations were below 2 mM C (Fig. 1B). Correspondingly, hydroxide consumption further declined to ~ 60 mmol OH^-/L/day, and CO_2 production stabilized at ~ 3 mmol/day (see supplementary materials), reflecting low overall fermentative activity.

At the end of Phase V, the remaining solid bioplastic materials were retrieved from the reactor for a mass balance assessment (see supplementary materials). Approximately 35 % of the initial PHA cup mass was degraded over the 157-day operation, as determined by weight loss measurements. This closely aligns with the theoretical estimate of 36 %

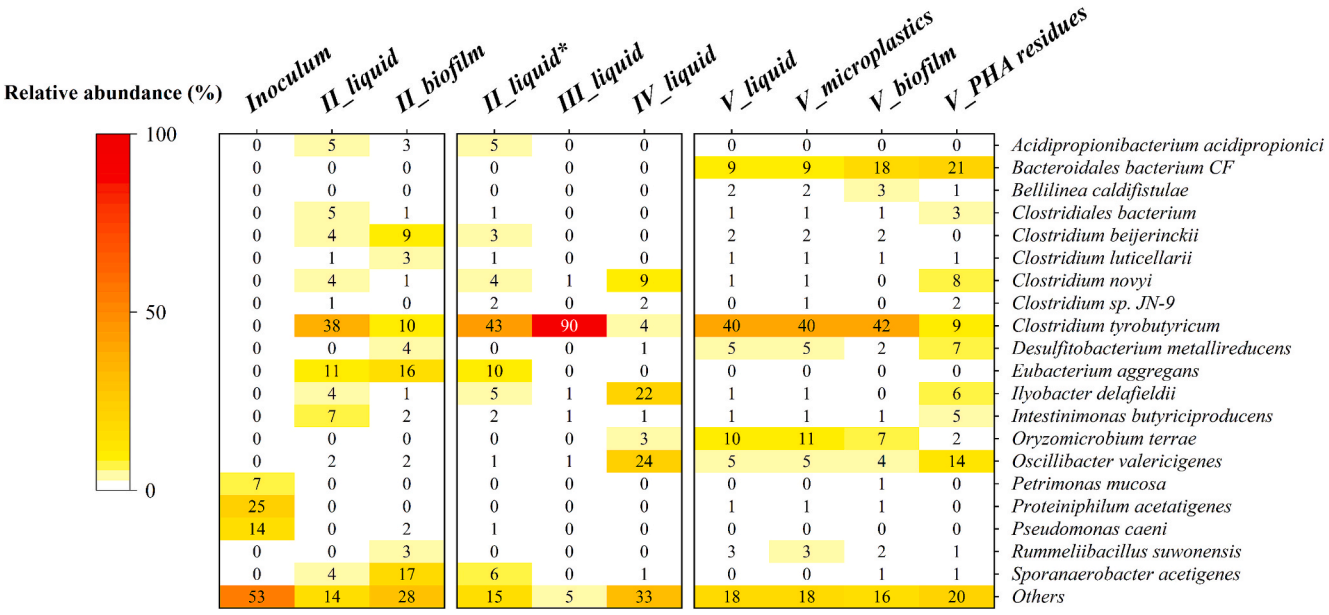

Fig. 2. Heatmap of microbial community composition (species level) based on relative abundance determined from 16S rRNA sequencing. Samples were taken at the end of each phase. The sample labeled '***II_liquid****' represents suspended fermentation broth collected after reinoculation but before Phase III. '***V_microplastics***' refers to pigments removed from the surface of PHA cups. '***II_biofilm***' denotes biofilm from PU cubes collected from the top of the reactor at the end of Phase II, whereas '***V_biofilm***' indicates biofilm from PU cubes sampled from the bottom of the reactor at the end of Phase V.

bioplastic conversion during Phases III-V, based on acetate and *n*-butyrate production rates under steady-state conditions (supplementary material). The degradation of PHA predominantly contributed to acetate and *n*-butyrate formation, with a peak conversion rate of 39 ± 1.8 mmol C/L/d observed during Phase IV. In contrast, the PLA lids showed negligible mass loss. Instead, a slight mass gain was recorded (~2.7 g, see supplementary materials), likely due to biofilm formation and/or adsorption of PHA-derived compounds, supported by the observed

Table 3
Comparison of anaerobic conversion of non-chemical pretreated bioplastics to carboxylates (fermentation)/biogas (digestion) under mesophilic and thermophilic conditions.

Feedstock	Trade name	Plastic size	Inoculum	Temp (°C)	Products	Retention time (days)	Biodegradability (%)	Conversion rate (mmol C/L/d)	Ref
PHA cups	ENMAT™ Y1000P	Pieces	Open culture	35	Carboxylates	157	35	39.0 ± 1.8 [a]	This study
PLA lids	cPLA						0	0	
PHA & PLA pellets	ENMAT™ Y1000P, 2 % HV & Biopolymer-4043D	Hydrolysates	Open culture	35	Carboxylates	3	100	526	(Jin et al., 2025a)
PLA/PHB (80/20)	Biopolymer-4043D & ENMAT™ Y1000	20 × 20 × 0.2 cm specimens	Active inoculum [b]	52	Methane	80	100	3.5	Fig. 2C in (Narancic et al., 2018)
			Anaerobic sludge [c]	35	Methane	56	0	0	Fig. 5C in (Narancic et al., 2018)
PLA	Biopolymer-4043D	Pellets	Active inoculum [b]	52	Methane	75	100	3.6	Fig. 2C in (Narancic et al., 2018)
		Pellets	Anaerobic sludge [c]	35	Methane	56	0	0	Fig. 5C in (Narancic et al., 2018)
PHB	ENMAT™ Y1000	Pellets	Active inoculum [b]	52	Methane	45	100	7.1	Fig. 2C in (Narancic et al., 2018)
		Pellets	Anaerobic sludge [c]	35	Methane	56	100	–	Fig. 5C in (Narancic et al., 2018)
PHB	Sigma-Aldrich	125–250 μm	Anaerobic sludge	37	Methane	9	90	0.7	(Yagi et al., 2014)
PLA	Unitika					90	7	0.03	
PHBV	ENMAT™ Y1000P, 3 % HV	100–250 μm	Anaerobic sludge	36	Carboxylates	43	35	2.0	(García-Depraect et al., 2022)
PHB	ENMAT™ Y3000P					43	10	2.5	
PLA	LUMINY® L105					56	0	0	

Notes: **a.** Conversion rate calculate for Phase III of this study. **b.** 'Active inoculum' prepared according to ISO 15985. **c.** 'Anaerobic sludge' prepared according to ISO14853. Conversion rate was calculated as the total amount of carboxylate produced per reactor volume per day, expressed in mmol C/L/d. '-' indicates data not reported.

discoloration of the PLA surface from white to light green (see supplementary materials).

These results highlight the distinct biodegradability profiles of PHA and PLA under mesophilic conditions. While PHA was partially degraded and converted into carboxylates, PLA remained largely recalcitrant. This result is consistent with previous findings (Table 3), where unpretreated PHA demonstrated moderate to high biodegradation efficiencies under mesophilic (10–100 %) and thermophilic (100 %) conditions. In contrast, PLA consistently exhibited low to zero biodegradability under mesophilic conditions, even when incubated with active or acclimated inocula (Narancic et al., 2018; Yagi et al., 2014). This discrepancy may be attributed to the chemical and structure differences between the two polymers, as well as to formulation-specific additives present in the PHA cups and PLA lids. The ENMAT^{TM} PHA pellets used in this study are known to undergo microbial conversion into carboxylates under anaerobic conditions (García-Depraect et al., 2022). Conversely, the PLA lid is a crystallized PLA product—an aliphatic polyester synthesized chemically—which typically requires abiotic hydrolysis and oxidation for initial degradation. These processes are highly temperature-dependent and are generally limited under anaerobic mesophilic conditions (Aarsen et al., 2024; Teixeira et al., 2021). Although enzymatic hydrolysis of PLA by lipase, cutinase, esterase, or protease has been reported (Jannatiha and Gutiérrez, 2025), likely contributed little here. Remarkably, the conversion rate of PHA to carboxylates observed here was 5–10 times higher than typical methane production reported in standard anaerobic digestion tests (García-Depraect et al., 2022; Narancic et al., 2018). These findings support the potential of tailored bioprocess development, as explored in this study, to enhance the efficiency and rate of biodegradable plastic conversion into value-added products.

Under thermophilic conditions, both PHA and PLA can be hydrolyzed and anaerobically converted to biogas, as demonstrated in studies using PHA/PLA blends (20/80) that were fully digested over 80 days (Narancic et al., 2018). Furthermore, hyperthermophilic pretreatment (≥70°C) has been shown to facilitate PLA hydrolysis (Benn and Zitomer, 2018; Strik and Heusschen, 2023), suggesting that hyperthermophilic fermentation or digestion could enhance the anaerobic conversion of PHA/PLA blends. However, the high temperature required for effective PLA depolymerization may inhibit the activity of functional acidogenic and chain-elongating microorganisms responsible for carboxylate production (Ren et al., 2024). Optimizing temperature regimes is necessary to balance effective depolymerization with microbial activity for carboxylate production. In parallel, coupling pretreatment with microbial fermentation offers another promising strategy to improve conversion efficiency. For instance, non-thermal approaches such as cold plasma or UV radiation have been reported as effective pretreatment methods for bioplastic depolymerization (Mat Yasin et al., 2022).

3.4. Microbial community analysis

Microbial community dynamics reflected substrate shifts and operational changes across fermentation phases. *Clostridium tyrobutyricum* likely dominated during hydrolysate fermentation, driving acetate and *n*-butyrate production from PHA and PLA hydrolysates. In contrast as explained below, solid biodegradable plastic fermentation enriched a more diverse community, including species related to *Bacteroidales bacterium CF*, *Ilyobacter delafieldii*, *Oscillibacter valericigenes*, and *Clostridium novyi*, indicating adaptation to slower-degrading carbon sources.

To describe the related species of the microbial community, biomass samples from different fermentation stages were analyzed using full-length 16S rRNA sequencing. Over 98.3 % of the obtained sequence reads were taxonomically classified using the reference database and the applied bioinformatics pipeline (see supplementary materials), indicating high classification efficiency. However, this high classification rate may not fully reflect the native microbial diversity, as certain taxa might be underrepresented or absent in the reference database. The metabolic characteristics of species with ≥ 9 % relative abundance are summarized in Table 4.

In the inoculum, *Petrimonas mucosa* (7 %), *Pseudomonas caeni* (25 %), and *Proteiniphilum acetatigenes* (14 %) were among the dominant taxa. During continuous fermentation of hydrolysates derived from PHA and PLA pellets, *Clostridium tyrobutyricum* was consistently abundant in the fermentation broth (Fig. 2). This species is known to ferment cellulose and sugars into acetate, *n*-butyrate, H_2 and CO_2 (Wu and Yang, 2003), and was likely involved in the metabolism of monomers such as 3-HB, 3-HV, crotonate, and lactate (Jin et al., 2025a). In Phase II ('*II_liquid*'), *C. tyrobutyricum* (38 %) and *Eubacterium aggregans* (11 %) became more dominant. A similar microbial distribution was observed after reinoculation ('*II_liquid**', Fig. 2). These two species likely utilized monomers derived from PHA and PLA, contributing to acetate and *n*-butyrate production (Table 4) (Mechichi et al., 1998; Jin et al., 2025a). Interestingly, a distinct microbial community was detected in the biofilm attached to PU cubes floating at the liquid surface in Phase II ('*II_biofilm*'). In addition to *C. tyrobutyricum* (10 %), dominant species included *Clostridium beijerinckii* (9 %), *E. aggregans* (16 %), and *Sporanaerobacter acetigenes* (17 %). These species are also likely involved in the production of acetate, *n*-butyrate (Hernandez-Eugenio et al., 2002; Mechichi et al., 1998; Reddy et al., 2020; Survase et al., 2011; Zou et al., 2021).

In Phase III, when mixed PHA and PLA hydrolysates and commercial bioplastic pieces were co-fermented, *C. tyrobutyricum* dominated the community, comprising approximately 90 % of the population present in the liquid fermentation broth. This finding underscores its competitive advantage in utilizing soluble monomers derived from hydrolyzed PHA/PLA (Jin et al., 2025a). However, a shift in microbial composition was observed in Phase IV, where the feedstock was changed to predominantly commercial solid bioplastics (Fig. 2). The relative

Table 4
Characteristics of mostly related species (relative abundance ≥ 9 %) during the open-culture fermentation of hydrolysates and/or bioplastics.

Species	Substrates	Products	Ref
Clostridium tyrobutyricum	Cellulose, xylose, glucose, sucrose	Acetate, *n*-butyrate, H_2, CO_2	(Wu and Yang, 2003; Jin et al., 2025a)
Clostridium beijerinckii	Glucose, lignocellulose	Acetone, butanol, ethanol, acetate, butyrate, isopropanol, lactate, CO_2, H_2	(Reddy et al., 2020; Survase et al., 2011; Zou et al., 2021)
Clostridium novyi	Glucose, maltose	Fatty acids, toxin	(Jeong et al., 2020)
Bacteroidales bacterium CF	Ethanol, lactate	Acetate, propionate, butyrate	(Tang and Edwards, 2013)
Eubacterium aggregans	Glucose, fructose, sucrose, lactate, crotonate, formate, betaine, methanol	Acetate, butyrate, H_2, and CO_2	(Mechichi et al., 1998)
Ilyobacter delafieldii	PHB, 3-HB, crotonate, lactate, pyruvate	Acetate, propionate, butyrate, CO_2, and H_2	(Janssen and Harfoot, 1990)
Oryzomicrobium terrae	3-HB, lactate, propionate, succinate	Acetate, butyrate	(Liu et al., 2017)
Oscillibacter valericigenes	Glucose, pentoses	Valerate, butyrate	(Iino et al., 2007; Joshi et al., 2021)
Proteiniphilum acetatigenes	Yeast extract, peptone, pyruvate, glycine, and L-arginine	Acetate, propionate, CO_2	(Chen and Dong, 2005)
Pseudomonas caeni	Caprate, malate	Fatty acids	(Xiao et al., 2009)
Sporanaerobacter acetigenes	Glucose, peptone	Acetate, *i*-butyrate, *i*-valerate	(Hernandez-Eugenio et al., 2002)

abundance of *C. tyrobutyricum* dropped sharply to 4 %, likely due to a reduced availability of easily fermentable monomers in the liquid phase such as 3-HB and L-lactate.

In Phase IV, other taxa likely involved in solid bioplastic degradation and fermentation became enriched. These included *Clostridium novyi* (9 %), *Ilyobacter delafieldii* (22 %), and *Oscillibacter valericigenes* (24 %), along with a diverse group of other bacteria (33 %) (Fig. 2). *C. novyi*, although associated with toxin production, can also ferment sugars into fatty acids (Jeong et al., 2020). *I. delafieldii* is capable of converting PHA, crotonate, 3-HB, and lactate into acetate, propionate and *n*-butyrate and biogases (Janssen and Harfoot, 1990). *O. valericigenes* may explain the occasional detection of valerate during this phase, as it is known to produce valerate and butyrate from glucose and pentoses (Iino et al., 2007; Joshi et al., 2021). This species may also utilize 3-HV as a substrate for valerate production.

In Phase V, the HRT was extended from 2 to 18 days to allow more time for bioplastic hydrolysis and microbial enrichment. This modification led to renewed dominance of *C. tyrobutyricum*, with its relative abundance reached at 40 % across the suspended biomass ('*V_liquid*'), biofilm on PU cubes (located at the reactor bottom, '*V_biofilm*'), and microbial communities attached to PHA-derived microplastics ('*V_microplastics*') (Fig. 2). This enrichment likely resulted from the gradual release of 3-HB as a consequence of PHA cups biodegradation through HRT extension. *Bacteroidales bacterium CF*, capable of converting ethanol and lactate into carboxylates, also enriched to around 9 % (Tang and Edwards, 2013). Additionally, *Oryzomicrobium terrae*, which can convert 3-HB, lactate into acetate, and *n*-butyrate, was likely among the dominant species in the reactor bulk (Liu et al., 2017). On the surface of PHA cup pieces ('*V_PHA residues*'), at least 7 species were detected with $\geq$ 5 % relative abundance, suggesting a diverse microbial consortium contributed to PHA hydrolysis and carboxylate production (Fig. 2). Further analyses, such as species isolation and detailed characterization, are required to better understand the biochemical pathways and microbial interactions involved in bioplastic conversion.

3.5. What still needs to be investigated?

Advancing the technical feasibility on the recycling of bioplastics through anaerobic fermentation as part of the carboxylate platform requires a multidisciplinary effort. This includes refining pretreatment technologies, advancing microbial and enzymatic engineering, and designing plastic formulations that are fully compatible with fermentation-based recovery. Firstly, the microbial and enzymatic pathways responsible for PLA degradation under anaerobic conditions remain poorly understood, even though several microorganisms have demonstrated the ability to metabolize PLA materials (Qi et al., 2017). In the present study, the microbial conversion of PHA materials proceeds at a relatively slow rate compared to comparable PHA hydrolysates, while a complete fermentation was estimated to take up to 330 days. Further research should focus on identifying rate-limiting steps (like enzymatic hydrolysis) and enhancing biodegradability of these biopolymers, potentially through the use of synthetically adapted or genetically engineered strains, as has been demonstrated for polyethylene terephthalate (PET) depolymerization (Yip et al., 2024). Secondly, the optimization of hydrothermal pretreatment and subsequent fermentation steps requires further attention. Although pretreatment improves solubilization and fermentability of both PHA and PLA, commercial plastic products generally contain additives, such as plasticizers, fillers, and colorants, that may inhibit microbial activity (Maddela et al., 2023). These compounds are frequently non-biodegradable and may persist or accumulate during fermentation. Investigating the impact of biodegradable additives on microbial metabolism could facilitate the development of more fermentation-compatible plastic formulations (Lenzi et al., 2023). Thirdly, the formation of microplastics during hydrothermal pretreatment raises environmental and process-related concerns. Microplastics may resist degradation and accumulate in the system, counteracting the goal of complete bioconversion (Bao et al., 2025; Thompson et al., 2024). For example, PHA-derived microplastics have been reported to inhibit methane production (Zhang et al., 2025), whereas other studies have shown that anaerobic digestion of kitchen waste can enrich microbial populations capable of degrading PLA microplastic (Lu et al., 2022). Future work can focus on quantifying microplastic formation, identifying their chemical composition, and assessing the potential of specific microbial taxa to degrade them (Wang and Zhou, 2024).

4. Conclusion

This study demonstrates the partial bioconversion of a commercial PHA-based solid product into carboxylates via mesophilic anaerobic fermentation. In contrast, near-complete conversion of hydrolysates derived from PHA and PLA raw material pellets was achieved, primarily yielding acetate and *n*-butyrate. After 157 days of operation, approximately 35 % of the shredded PHA-based cup materials were degraded, whereas the PLA-based lid pieces conversion remained negligible, even under extended hydraulic retention time from 2 days to 18 days. Microbial community analysis revealed that *Clostridium tyrobutyricum* was likely the dominant functional species during hydrolysates fermentation, while a more diverse consortium emerged during solid PHA-based cups degradation. These findings highlight that there is a variation on mesophilic bioplastic fermentability. Nonetheless, integrating hydrothermal pretreatment with open-culture fermentation shows already an efficient conversion of biodegradable plastics into carboxylates.

Declaration of AI assistance in writing

The authors utilized ChatGPT 4.0 to enhance the English language quality of this manuscript, with a particular emphasis on refining grammar, readability, and clarity. All AI-generated suggestions were rigorously reviewed and further edited by the authors, who retain full responsibility for the accuracy and integrity of the final content presented in this publication.

CRediT authorship contribution statement

Yong Jin: Writing – original draft, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Roel van den Enden:** Investigation, Data curation. **Elvis Castrikum:** Investigation, Data curation. **Kasper D. de Leeuw:** Writing – review & editing, Supervision, Conceptualization. **David P.B.T.B. Strik:** Writing – review & editing, Supervision, Project administration, Methodology, 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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2025.133512.

Data availability

The original data presented in this paper is available at the 4TU. Research Database via this site: https://doi.org/10.4121/7be1be62-529a-4918-a298-27dfc85b83c0. Microbiota raw sequencing data is available at the ENA (European Nucleotide Archive) database under access number PRJEB98625 via this site: https://www.ebi.ac.uk/ena/browser/view/PRJEB98625.

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