Journal of Environmental Chemical Engineering 14 (2026) 122460

Contents lists available at ScienceDirect

Journal of Environmental Chemical Engineering

journal homepage: www.elsevier.com/locate/jece

High and tailored long-term accumulation of PHA enabled by a versatile mixed microbial culture

Yolanda Lopez-Garabato [a], David Correa-Galeote [b,c], Alba Pedrouso [a], Anuska Mosquera-Corral [a,*]

[a] CRETUS, Department of Chemical Engineering, School of Engineering, Universidade de Santiago de Compostela, Santiago de Compostela, Galicia 15782, Spain
[b] Microbiology Department, Faculty of Pharmacy, University of Granada, Granada 18011, Spain
[c] Microbiology and Environmental Technology Section, Water Institute, University of Granada, Granada 18011, Spain

ARTICLE INFO

Keywords:
Microbial enrichment
Organic loading rate
PHA monomer composition
Pre-acidified wastewater
Resource recovery
Volatile fatty acids

ABSTRACT

Polyhydroxyalkanoates (PHAs), such as poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) copolymers, are biopolymers that can serve as substitutes for conventional plastics as they have similar properties. The objective of this study was to develop a robust mixed microbial culture (MMC) enriched in microorganisms capable of accumulating high intracellular PHBV content. The MMC was successfully enriched and operated stably over the long term (521 days) in a sequencing batch reactor (SBR) in which the carbon and nitrogen feeding were uncoupled. Its maximum PHBV accumulation capacity and biopolymer production yield were 62 wt% and 0.59 $Cmol_{PHBV}/Cmol_{VFA}$, respectively, and treated OLRs of up to 6 g COD/(L·d), which influenced the microbial community composition. The maximal accumulation capacity of the MMC, evaluated in batch experiments, was 83 wt% PHBV and yields of 0.63 $Cmol_{PHBV}/Cmol_{VFA}$. Similar results were obtained with the two synthetic volatile fatty acid (VFA) compositions tested. When the potential of this culture to use pre-acidified (VFA-rich) wastewater from fish canning residues as a substrate was evaluated, the highest obtained values were 56 wt% PHBV and 0.36 $Cmol_{PHBV}/Cmol_{VFA}$. The results demonstrate that PHBV composition can be adjusted by VFA feed composition (either synthetic or residual), highlighting the process versatility.

1. Introduction

The growing demand for sustainable materials has intensified interest in polyhydroxyalkanoates (PHAs), a family of biobased, biodegradable, and biocompatible biopolymers characterized by having properties comparable to those of conventional petrochemical plastics [1]. Microbial mixed cultures (MMCs) enriched in PHA-accumulating organisms offer economic and environmental advantages over pure cultures used at the industrial scale for PHA production. This is because MMCs do not require sterile conditions and can utilize residues as feedstocks [2]. MMC enrichment can be achieved by applying feast-famine regimes combined with carbon and nitrogen uncoupled feeding, in which nitrogen is supplied only in the famine phase, favoring the selection of microorganisms capable of synthesizing PHA [3]. Thus, to ensure the economic viability of MMC-based PHA production, it is crucial to identify an inexpensive and abundant organic substrate.

In the region of Galicia (northwest Spain), the canned fish production industry generates substantial wastewater volumes (more than 2.1 million m^3 in 2021), requiring effective management to ensure the sector's sustainability and to protect surrounding ecosystems [4]. These liquid streams are characterized by variable compositions and large organic content (1–90 g/L as chemical oxygen demand, COD) [5]. Previous research has shown that wastewater from the fish canning industry can be efficiently fermented under anaerobic conditions to produce volatile fatty acids (VFAs). For example, when using wastewater from mussel cooking, effluents with COD concentrations up to 12.6 g COD_{VFA}/L have been produced [4]. The produced VFAs, such as acetate, propionate, butyrate, and valerate, are widely used as substrates for PHA synthesis by MMCs [6,7]. However, when these highly concentrated streams are used as substrate, during MMC enrichment, they often require dilution to limit the applied organic loading rate (OLR) and maintain stable reactor operation, highlighting a significant operational barrier [6]. Attempts to operate MMC enrichment reactors at high OLRs have either been limited to short-term experiments [8,9] or have

* Corresponding author.
E-mail addresses: yolandalopez.garabato@usc.es (Y. Lopez-Garabato), dcorrea@ugr.es (D. Correa-Galeote), alba.pedrouso@usc.es (A. Pedrouso), anuska.mosquera@usc.es (A. Mosquera-Corral).

https://doi.org/10.1016/j.jece.2026.122460
Received 11 February 2026; Received in revised form 20 March 2026; Accepted 29 March 2026
Available online 30 March 2026

reached over 200 days of operation but resulted in a low maximum PHA accumulation capacity of 19 wt% [10]. Therefore, the long-term enrichment of robust, high-performing MMCs under elevated OLRs remains an open challenge.

Beyond achieving high PHA accumulation yields, controlling polymer composition is essential for tailoring its properties to specific applications [11]. While polyhydroxybutyrate (PHB) is the most common PHA and recognized by its polypropylene (PP)-like properties, its practical application is limited by its brittleness compared to PP [12]. The presence of 3-hydroxyvalerate (3HV) in addition to 3-hydroxybutyrate (3HB) monomers results in the copolymer poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) and reduces crystallinity and melting temperature, enhancing flexibility and processability [12].

In general, the parameter most affecting the HB:HV ratio is the composition of the VFA mixture in the substrate fed to the MMC, which ultimately determines the biopolymer's properties [11]. By selecting mixtures of VFAs as feedstock, it is possible to obtain PHBV copolymers containing 12 wt% of 3HV, which exhibit physical properties similar to those of PP, as well as PHBV copolymers containing 23 wt% of 3HV, which show physical properties closer to those of low-density polyethylene (LDPE) [1,12]. These results highlight the importance of controlling the 3HB:3HV ratio to define the polymeŕs properties. Although Lorini et al. [8] suggested that the applied OLR may also influence PHA composition, even when using the same VFA mixture, the mechanisms linking operating conditions to the resulting polymer's composition remain poorly understood. Therefore, further research is needed to determine whether increasing OLR to high levels can enable stable culture performance and maintain the desired polymer composition.

This study aims to develop and characterize a high-yield MMC for PHA accumulation, capable of producing PHBV biopolymers with defined compositions, exhibiting robust long-term operation, and treating OLRs of up to 6 g COD/(L·d). Additionally, the potential of this enriched MMC to use wastewater from the fish-canning industry, which is pre-acidified and rich in VFAs, as substrate will be evaluated.

2. Materials and methods

2.1. Reaction systems description

A sequencing batch reactor (SBR) was used to select the MMC with PHA-accumulating capacity, and accumulation experiments were performed to determine the MMC's maximum PHA-accumulation capacity.

2.1.1. Microbial mixed culture enrichment procedure

A jacketed glass SBR with a 4-L working volume was inoculated with activated sludge collected from an urban wastewater treatment plant. The reactor was operated for 521 days, maintaining a hydraulic retention time (HRT) and solid retention time (SRT) of 24 h each. The operational cycles had a duration of 12 h, divided into 5 phases: feeding of nutrients (without nitrogen) and VFAs mixture (5 min), aerobic reaction (290 min), feeding of ammonium chloride solution as nitrogenous source (1 min), aerobic reaction (419 min), and effluent withdrawal (5 min). The feast-famine regime was imposed, and carbon and nitrogen were supplied uncoupled to enable rapid culture enrichment and efficient selection of PHA-storing culture under high organic loading rates [3]. The temperature inside the reactor was controlled at 30 °C using a thermostatic bath (Tectron Bio-100, J.P. Selecta, Spain), and the pH was not controlled. The reactor was continuously aerated by means of a ceramic bubble diffuser connected to a pump (Laboport N 86 KTP, KNF Neuberger, USA), which provided mixture and dissolved oxygen (DO) for biological reactions. From day 275 onward, a mechanical stirrer (RW 20 digital, IKA, Germany) was also used to ensure the complete mixture. DO concentration was monitored online every five minutes using a luminescence electrode (LDO101, Hach Company, USA).

The fed carbon source was a synthetic mixture of VFAs with a fixed composition of 44% acetic acid, 44% butyric acid, 8% propionic acid, and 4% valeric acid (% weight of the COD) (F1) (Table 1). The F1-specific VFA composition was defined to achieve an expected 3HB:3HV ratio of 88:12 g/g, based on literature [13]. The effect of the applied OLR (Table 1) was investigated by increasing it in six operational stages (ranging from 2.5 g COD/(L·d) to 6.0 g COD/(L·d)). VFA concentrations in this fed mixture were progressively increased throughout the operational period, with rises of 25% of the initial load (2.5 g COD/(L·d)) for the first five operational stages and of 40% in the final stage. In each operational cycle, 1 L of VFA solution was fed along with 1 L of nutrient solution. The VFA solution was partially neutralized with NaOH to a pH of 5.5–6.0, resulting in a Na^+ concentration of 2.5 g/L. From day 286 (Stage V) onward, nutrient concentrations were adjusted according to VFA concentration increases, varying in the following ranges: $MgSO_4$ from 16.75 to 30.25 mg/L, KH_2PO_4 from 87.25 to 157.00 mg/L, KCl from 13.25 to 23.75 mg/L, and trace elements [14] from 0.38 to 0.68 mL/L. In all stages, 0.04 mL/L of allylthiourea (33 g/L) were added to prevent nitrification.

2.1.2. Monitoring of PHA accumulation capacity

A fed-batch reactor with a working volume ranging from 0.2 to 3.2 L, depending on the experiment, was used to evaluate the PHA accumulation capacity of the MMC. The biomass inoculated was collected from the SBR at the end of an operational cycle. A solution containing a mixture of VFAs was supplied in pulses, added once an increase in DO concentration was observed, indicating VFA depletion. The temperature inside the jacketed reactor was maintained at 30 °C with a thermostatic bath (Tectron Bio-100, J.P. Selecta, Spain).

Accumulation experiments were performed using six media containing VFAs (F1-F6). F1 and F2 were synthetic VFA mixtures (composed of acetic acid, butyric acid, propionic acid, and valeric acid, as shown in Table 1). F1 was defined to obtain an expected 3HB:3HV ratio of 88:12 g/g as in the enrichment, targeting properties like those of PP, and F2 was formulated for a 77:23 g/g 3HB:3HV ratio to achieve properties comparable to those of low-density polyethylene, based on literature data [13]. Feeding media F3 to F6 were VFA mixtures collected from a pilot plant of acidification of fish canning effluents with variable composition containing acetic acid, butyric acid, propionic acid, valeric acid, and caproic acid (Table 1). For the VFA recovery at pilot scale (F4, F5, and F6), commercial polyelectrolytes (FLOPAM EM 640 and FLOQUAT FL4820, SNF Floerger Iberica SLU) were used in the solid-liquid separation step.

2.2. Sampling and analytical methods

Liquor media samples were collected for analysis of the liquid and solid fractions. Total and volatile suspended solids (TSS and VSS) were measured in duplicate according to the Standard Methods for the Examination of Water and Wastewater [15] and the pH was measured with a pH meter (GLP 22, CRISON, Spain).

The liquid fraction was obtained after the sample was filtered through a cellulose-ester filter with a 0.45 μm pore size (Advantec, Japan). It was subjected to analyses to determine the concentrations of soluble COD [15], ammonium using the Bower and Holm–Hansen methodology [16], and total organic carbon (TOC) and total nitrogen (TN) (TOC-L analyzer with the TNM-module, TOC-5000 Shimadzu, Japan). VFA concentrations were determined by gas chromatography (GC) (Agilent Technologies 6850 Series II, Agilent, USA) with a DB-Wax column (30 m x 0.25 mm×0.25 μm) using pre-acidified samples (10 μL of H_3PO_4 (85%) per mL of sample), nitrogen gas as the carrier (1.5 mL/min), and a flame ionization detector (FID) (300 °C) with a H_2/air mixture at 40:400 mL/min.

For solid-fraction analysis, media samples were collected to quantify the PHA content within cells by propanolysis. Immediately after sampling, 100 μL of a 37% v/v formaldehyde solution was added to 50 mL of the broth sample to stop microbial activity [11,13]. Then, the samples

Table 1
Operating conditions of the enrichment sequencing batch reactor at the beginning of the cycle, excluding the ammonium supplied at the start of the famine phase, and characteristics of the volatile fatty acids (VFAs) mixtures used as substrate in the accumulation experiments. F6 was modified by adding synthetic VFAs, and the data shown corresponds to the sample after these VFAs were added. SBR: sequencing batch reactor, OLR: organic loading rate, COD: chemical oxygen demand, Ac: acetic acid, Pr: propionic acid, Bu: butyric acid, Va: valeric acid, Ca: caproic acid, SM: synthetic medium, AW: pre-acidified wastewater.

	Enrichment SBR						Accumulation experiments						
Stage	**I**	**II**	**III**	**IV**	**V**	**VI**	**Feeding**	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**
Days of operation	1–42	43–142	143–199	200–231	232–329	330 – 521	**Days of sampling**	66, 134, 168, 211, 282, 296	329, 505	367	387	407	505
VFAs (g COD/L)	1.3	1.6	1.9	2.2	2.5	3.0	**Substrate media**	SM	SM	AW	AW	AW	AW+SM
OLR (g COD-VFA/(L·d))	2.5	3.1	3.8	4.4	5.0	6.0	**Soluble COD (g COD/L)**	1391.6[a]	1415.8[a]	25.5	16.4	8.2	29.6
NH_4^+ (mg N/L)	43.2	54.0	64.8	75.6	75.6/ 81.0/ 86.4	99.3/ 103.7	**VFAs (g COD/L)**	1391.6[a]	1415.8[a]	23.2	13.7	5.3	53.2
							VFA composition (Ac:Pr:Bu :Va:Ca wt%)	57:33: 7:3:0	47:27: 22:4:0	66:9: 23:2:0	70:10: 13:3:4	68:8: 12:9:3	57:33: 7:3:0
							NH_4^+ (mg N/L)	0	0	765	811	556	807
							pH	5.5[b]	5.5[b]	5.5[b]	6.23	9.47	9.96

[a] Theoretically calculated as a mixture of pure VFAs in the prepared synthetic solutions.
[b] Adjusted to this pH with NaOH addition (being the Na^+ concentration in the F1 VFA solution of 2.5 g/L).

were centrifuged at 7500 rpm (Centrifuge 5430, Eppendorf, Germany), and the separated solid fraction was dried in an oven (Wulkex C-80) at 50 °C for 48 h. The extraction protocol, adapted from Smolders et al. [17], was then applied. Each sample was digested at 100 °C for 3 h after adding 1.5 mL of 1,2-dichloroethane, 1.5 mL of a solution of propanol (with 25% v/v of hydrochloric acid), and 50 µL of a solution of benzoic acid (1 mg of benzoic acid in 100 mL of propanol) as an internal standard. Then water was added, and the organic phase was transferred to a vial for quantification of PHA by GC (Agilent Technologies 6850 Series II, Agilent, USA). This instrument was equipped with an automatic injector (6850 Series II, Hewlett Packard, USA), a chromatographic column (HP-INNOWAX 30 m x 0.25 mm×0.25 µm, Agilent, USA) with Helium as the carrier gas (1 mL/min) and a flame ionization detector (FID) (275 °C) with a H_2/air mixture at 40:400 mL/min. A commercial PHA standard (Sigma) containing 92% mol of 3HB and 8% mol of 3HV was used for calibration. 3HB and 3HV were distinguished by their different retention times in the obtained chromatogram.

Mixed liquor samples collected at the beginning of an enrichment cycle, at the end of the feast phase (feeding as stage VI), and at the end of an accumulation experiment (after the addition of 0.39 Cmol of VFAs) were observed under transmission electron microscopy (TEM Jeol JEM 1011). Inductively Coupled Plasma combined with Optical Emission Spectroscopy (ICP-OES) was used to measure concentrations of 18 chemical elements (Table S3) in pre-acidified wastewater samples, previously centrifuged and filtered (0.45 µm).

Microbiological analyses were performed with mixed liquor samples. DNA was extracted using the FastDNA SPIN Kit and the FastPrep 24-Instrument (MP Biomedicals, Germany) according to the manufacturer's protocol. DNA extracts were subjected to Illumina analysis for partial bacterial 16S rRNA sequencing, using the primers Pro341F and Pro805R [18]. Raw sequencing data were processed through Mothur V1.44.3 using default settings for quality control, primer trimming, filtering, pre-clustering, and chimera detection [19]. Operational taxonomic units (OTUs) at a 97% cut-off level of homology were grouped, and the OTUs with an abundance of fewer than 10 sequences were removed before further analysis. After quality filtering and processing, the average number of sequences per sample was 59,611 ± 8145. Taxonomic classification was made using the blast tool of the Geneious Prime v.2019 software (Geneious, U.S.) and the 16S ribosomal database from the National Center for Biotechnology Information (NCBI, U.S.).

In addition to collecting periodic samples, single operational cycles were monitored at each operational stage to evaluate MMC enrichment performance, with at least 10 samples collected throughout the 12-h cycle. In the fed-batch accumulation experiments, media samples were collected before each substrate pulse.

2.3. Calculations

The PHBV content inside the cells was determined as a percentage of the dried weight of the cells and calculated as follows:

$$\text{PHBV (wt\%)} = \frac{\text{PHBV}}{\text{VSS}} \cdot 100$$

Then, the 3HB:3HV ratio was calculated as a percentage by dividing the measured mass of each polymer by the total mass of PHBV. The concentration of active biomass (X) was estimated by subtracting the mass of the accumulated PHBV from the mass of VSS measured. PHBV, 3HB, 3HV, and X yields ($Y_{PHBV/VFA}$, $Y_{3HB/VFA}$, $Y_{3HV/VFA,}$ and $Y_{X/PHBV}$) were calculated by dividing the maximum production rate of PHBV, 3HB, 3HV, or X by the consumption rate of substrate (sum of all VFAs or PHBV) in Cmmol units. The VFA mixture composition and the specific carbon number of each VFA were used in the conversion.

Volumetric PHBV productivity (g $_{PHBV}$/(L·d)) of the enrichment refers to the entire cycle duration. Still, it was calculated as if the biomass had been harvested from the reactor at the end of the feast phase. It was determined as the amount of PHBV produced per unit of reactor volume

at the end of the feast phase, from VFA feeding to depletion (including PHBV already stored within the biomass at the beginning of the cycle). For the accumulation experiments, volumetric productivity was calculated using data at the end of the experiments. Specific accumulation and consumption rates ($Cmol_{PHBV}/(Cmol_X \cdot h)$ and $Cmol_{VFA}/(Cmol_X \cdot h)$) were obtained by dividing the slope of the linear regression of the PHBV or VFA concentrations over time, respectively, by the active biomass concentration. The specific consumption rates of each VFA were estimated from the slope of the linear regression of VFA concentration divided by the active biomass concentration and represented over time. To convert PHBV to COD concentration, the following factors were used: 1.67 g COD/g PHB and 1.92 g COD/g PHV [20].

3. Results and discussion

3.1. Enhanced PHBV-accumulating MMC enrichment

In the enrichment SBR, the feast-and-famine regime was established almost after inoculation, and the length of the feast phase went below 20% of the total cycle length after just 7 days of operation (Figure S1), indicating that the selective pressure for PHA-storing populations development was already imposed in the system [21]. Notably, by day 17, MMC enrichment was confirmed, as the PHBV accumulated at the end of the feast phase already reached 51 wt%. This rapid enrichment is likely attributed to the carbon and nitrogen uncoupled feeding regime applied. In this way, the imposed nitrogen limitation directly favored the metabolism of PHBV-accumulating microorganisms over non-storing heterotrophic bacteria from the first operational cycle onward. In contrast, relying solely on the conventional feast-and-famine regime requires multiple cycles for these specialized organisms to establish a competitive advantage over non-accumulating heterotrophs [3].

The robust stability of the enriched MMC was demonstrated by its reproducible performance over 521 days of long-term operation. At all stages, TN and COD were almost consumed, with concentrations at the end of the cycle averaging 14 ± 11 mg TN/L and 96 ± 27 mg COD/L, respectively (Figure S2). The DO concentration over time during the feast phase was very similar across the different stages, consistently exceeding 2.5 mg O_2/L (Figure S3), despite the progressive increase in applied OLR (Table 1), indicating that oxygen limitation did not occur at any stage. Finally, the MMC consistently exhibited a high PHBV accumulation capacity of 59 ± 5 wt% on average (Fig. 1), which is a value in the highest range of percentages obtained in previous studies with MMC fed with VFAs (Table S1). Stable enrichment was verified by monitoring single operational cycles at each stage; the one corresponding to day 64 of operation is described in the supplementary material (Figure S4) as an illustrative example.

When feeding a VFA mixture, distinct phases in VFA consumption driven by substrate preferences are often observed [22]. In the present study, all VFAs were consumed simultaneously, and only one phase was observed (Figure S5). However, the fastest uptake was observed for the butyric acid (Table S2), independently of the stage of operation, as previously reported in other studies [10,23]. Compared with acetic acid, which in this study was fed at the same COD proportion, the preference for butyric acid can be explained by the lower overall energy requirements for its conversion to PHB. While the conversion of acetic acid to PHB requires both ATP and NADH, the metabolism of butyric acid consumes ATP but generates NADH, thereby providing the reducing equivalents necessary for PHB synthesis [24].

This achieved PHBV accumulation percentage together with already demonstrated effective extraction protocols, purity (89 ± 2%), and recovery (78 ± 3%), for PHA values above 32 wt% [25], proved the feasibility of directly processing this end-of-feast biomass. This would simplify the MMC PHA production process, as the direct biomass harvested at the end of the feast phase is appropriate for downstream processing, eliminating the need for a separate subsequent accumulation stage [26]. Furthermore, the composition of the accumulated PHBV, in terms of the 3HB:3HV ratio, also remained stable at approximately 87:13 g/g (excluding Stage III), close to the target ratio of 88:12 g/g (Fig. 1).

The uncoupled feeding applied in this study resulted in PHBV accumulation values (Table 2) similar to those reported in studies that used only a feast-famine regime (coupled). This is the case of Tamis et al. [27], who used fermented paper mill wastewater, and Korkakaki et al. [28], who used a mixture of synthetic VFAs and leachate from composting the separated organic fraction of municipal solid waste (OFMSW) (75:25% by volume), achieving in both cases slightly over 50 wt% accumulation (Table S1). Lower accumulation values were obtained in studies using fermented cheese whey (17 wt%) [3] and fermented molasses (25 wt%) [29], in reactors operated at SRTs of 4 and 10 days, respectively, longer than the 1-day SRT applied in the present study. This is consistent with Matos et al. [2], who observed higher accumulation percentages in the enrichment reactor operated at lower SRT. The average PHBV yield of 0.56 $Cmol_{PHBV}/Cmol_{VFA}$ obtained in the present study falls within the range reported in other studies (0.39 [3] and 0.7 $Cmol_{PHA}/Cmol_{VFA}$ [29]). Burniol Figols et al. [22], operating at

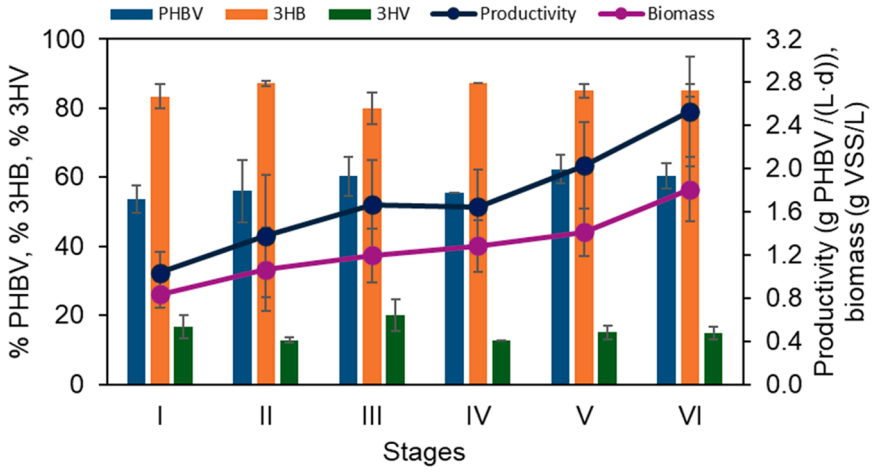

Fig. 1. Evolution over time of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) productivity (g PHBV/(L·d)), biomass concentration at the end of the famine phase expressed as volatile suspended solids (VSS) (g VSS/L), wt% of accumulated PHBV, and within this PHBV the wt% of 3HB monomer and 3HV monomer. The data represent the average results from each operational stage of the enrichment sequencing batch reactor, I corresponds to an applied organic loading rate of 2.50 g COD/(L·d), II to 3.13 g COD/(L·d), III to 3.75 g COD/(L·d), IV to 4.38 g COD/(L·d), V to 5.00 g COD/(L·d) and VI to 6.00 g COD/(L·d).

Table 2
Active biomass (X) concentration, maximum intracellular accumulation percentage (ACC_{max}), chemical oxygen demand consumed (COD_{cons}), poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) production yield over volatile fatty acids (VFA) ($Y_{PHBV/VFA}$), 3HB:3HV ratio, and specific accumulation rate (q_{PHBV}) were determined in different cycles monitored in the enrichment sequencing batch reactor throughout the operational stages and in different accumulation experiments testing six different feeding compositions: F1 and F2 (synthetic media) and F3, F4, F5, and F6 (pre-acidified fish canning wastewater).

	Feed	Stage	Day	X (g/L)	ACC_{max} (wt% PHBV)	COD_{cons} (g COD/L)	$Y_{PHBV/VFA}$ (Cmol/Cmol)	3HB:3HV g/g	q_{PHBV} ($Cmol_{PHBV}$/($Cmol_X$·h))
Enrichment	F1	I	29	0.48 ± 0.04	53	1.25	0.57 ± 0.03	87:13	0.48 ± 0.06
	F1	II	64	0.56 ± 0.08	58	1.56	0.62 ± 0.06	88:12	0.77 ± 0.04
	F1	III	168	0.57 ± 0.05	62	1.88	0.57 ± 0.11	82:18	0.68 ± 0.11
	F1	IV	211	0.84 ± 0.08	55	2.19	0.54 ± 0.11	87:13	0.45 ± 0.08
	F1	V	308	0.81 ± 0.05	52	2.50	0.51 ± 0.19	87:13	0.53 ± 0.07
Accumulation	F1	II	66	1.12 ± 0.20	65	5.83	0.55	90:10	0.18
	F1	II	134	1.25 ± 0.11	75	7.40	0.63 ± 0.07	88:12	0.41 ± 0.01
	F1	III	168	0.99 ± 0.14	83	7.90	0.63 ± 0.02	81:19	0.48 ± 0.01
	F1	IV	211	2.01 ± 0.18	68	8.90	0.63 ± 0.02	90:10	0.15 ± 0.04
	F1	V	282	1.24 ± 0.09	75	7.20	0.54 ± 0.21	85:15	0.28 ± 0.10
	F1	V	296	1.22 ± 0.04	75	7.00	0.49 ± 0.04	89:11	0.29 ± 0.02
	F2	V	329	1.60 ± 0.08	76	8.10	0.56 ± 0.04	75:25	0.28 ± 0.01
	F2	VI	505	1.89 ± 0.13	75	10.10	0.58 ± 0.04	75:25	0.25 ± 0.03
	F3	V	367	1.60–2.61[a]	33	7.07	0.32 ± 0.01	90:10	0.04 ± 0.01
	F4	V	387	1.44–1.96[a]	40	8.52	0.33 ± 0.08	84:16	0.05 ± 0.04
	F5	V	407	1.57–0.53[b]	56	8.34	0.36 ± 0.07	80:20	0.08 ± 0.01
	F6	VI	505	1.78–3.00[a]	16	6.01	0.16 ± 0.03	94:06	0.01 ± 0.01

[a] There is an interval due to the biomass growth during the experiment.
[b] The active biomass concentration decreased due to dilution associated with the high volume of substrate added.

equal HRT and SRT of 1 day, using a synthetic VFA medium supplemented with crude glycerol, achieved a yield of 0.78 $Cmol_{PHA}/Cmol_{substrate}$. However, they reported an accumulation percentage (39 wt%) lower than in the present study. Regarding studies with uncoupled nitrogen and carbon feeding, Giovanella et al. [23] achieved an accumulation percentage of 22 wt% and a yield of 0.65 Cmol/Cmol feeding a salted synthetic VFA mixture, and Cruz et al. [30] reported an accumulation of 19–33 wt% and a yield of 0.44–0.59 Cmol/Cmol feeding a synthetic mixture of acetic and propionic acids. Although the yields are similar, the PHA accumulation percentages are much lower than those reported in the present study, probably due to their larger SRTs. These relatively low PHA accumulation percentages might be a drawback for downstream processing. Therefore, the combination of the high yield and percentage of PHA accumulated achieved in the present study, according to the authors' knowledge, is among the highest reported with MMCs (Table S1). The trade-off between the two parameters is crucial for achieving high productivity and favoring broader application.

3.2. Impact of applied organic loading rate on enrichment performance

The applied OLR significantly influenced overall reactor productivity. As OLR increased, biomass concentration rose proportionally, reaching 1.81 g VSS/L at the end of the famine phase in stage VI. Thus, the substrate-to-biomass ratio remained nearly constant, and no substrate inhibition was observed. Moreover, it was found in previous studies that when applying the uncoupled feeding strategy, the lower the SRT, the lower the sensitivity of cultures to high loads [2]. In the present study, the applied SRT of 1 day appears beneficial. Given that the pre-acidified wastewater tested contained no salt, the most likely limiting effect would be ammonia concentration or the potential inhibitory effects of metals (as discussed further below).

Reactor productivity more than doubled, from 1.03 g PHBV/(L·d) in Stage I to 2.53 g PHBV/(L·d) in Stage VI (Fig. 1). In that last stage, the load applied (6 g COD/(L·d)) may be considered high for PHBV-accumulating MMC enrichment processes [31]. The applied uncoupled feeding regime enabled sustained high OLRs without compromising MMC selection efficiency or process stability [3]. These high-treated OLRs highlight the potential of the selected operational strategy to effectively valorize waste streams with relatively high organic content as VFAs, without the need for dilution with clean process water to prevent oxygen limitation at high COD concentrations [6]. This high-load approach offers a clear path toward reducing bioreactor footprints and improving the volumetric productivity of PHA at an industrial scale.

While biomass concentration and productivity increased with OLR, the PHBV production yield remained within the range of 0.53–0.62 $Cmol_{PHBV}/Cmol_{VFA}$ across the different operational stages. Under these conditions, the larger VFA fraction was converted to PHA. Farghaly et al. [32] observed a decrease from 0.50 ± 0.39 $Cmol_{PHA}/Cmol_S$ to 0.39 ± 0.22 $Cmol_{PHA}/Cmol_S$ when the applied OLR increased from 1.5 to 2.0 g COD/(L·d) in an SBR fed with acetate and operated at an SRT of 10 days. In their case, enrichments subjected to OLRs above 2.0 g COD/(L·d) exhibited no clear feast and famine profiles, probably caused by DO limitation. In contrast, the present study consistently maintained feast and famine phases, even at the high applied OLRs of 6 g COD/(L·d) in stage VI (Figure S3), indicating a stable selection regime. Morgan-Sagastume et al. [33] found that lower OLR promoted higher PHA accumulation capacity at HRTs of 4 and 8 h, using residual VFAs as substrates. Their PHA-accumulation yield increased from 0.14–0.26 $Cmol_{PHA}/Cmol_{VFA}$ at 12 g COD/(L·d) to 0.22–0.43 $Cmol_{PHA}/Cmol_{VFA}$ at 6 g COD/(L·d) because of the lowered feast-to-cycle-length ratio.

However, other studies have shown that higher OLRs can be tolerated in short-term experiments with systems in which carbon and nitrogen feeding are uncoupled, yielding accumulation percentages similar to those of the present study (Table S1). Lorini et al. [8] obtained a PHA content of 0.53 g PHA/g VSS at an OLR of 12.7 g COD/(L·d), and Crognale et al. [9] achieved 0.62 g PHA/g VSS even at an OLR as high as 12.75 g COD/(L·d). This suggests that once adequate enrichment is established, high OLRs can be treated while maintaining a high percentage of PHA accumulated. Lorini et al. [8] also obtained a PHA productivity of 1.64 g PHA/(L·d) at an OLR of 8.5 g COD/(L·d). It is noteworthy that the present study achieved a higher productivity of 2.53 g PHBV/(L·d) at a lower applied OLR of 6 g COD/(L·d). Similarly, the active biomass concentration of 1.64 g X/L achieved at an applied OLR of 6 g COD/(L·d) is comparable to the 1.68 g X/L achieved by Crognale et al. [9] working with an OLR of 8.5 g COD/(L·d).

Matos et al. [10] also achieved a PHA-accumulating culture and increased the OLR applied, reaching a value of 8.7 g COD/(L·d) at a SRT of 4 days (HRT of 1 day), but the PHA accumulated during the feast phase accounted for 19 wt%. In that case, the gradual increases in the

fed OLR led to higher yields and greater accumulation percentages. In another study conducted by Matos et al. [2], who worked with OLR values between 2.7 and 14.5 g COD/(L·d) at a SRT of 2 days (HRT of 1 day), they also found that the yields increased as the OLR fed was increased, ranging from 0.41 to 0.77 Cmol/Cmol (Table S1). In that case, the PHA accumulation percentages showed no clear trend and averaged 26 ± 4 wt%. According to the literature, the OLR applied could continue to increase, potentially enhancing system performance or at least not impairing it. In the present study, it was possible to operate at a high OLR and maintain long-term enrichment, being able to apply an OLR of 6 g COD/(L·d) for 191 days, achieving an average accumulation percentage of 60 ± 4 wt%. The novelty of this research work lies in combining long-term enrichment with high accumulation values.

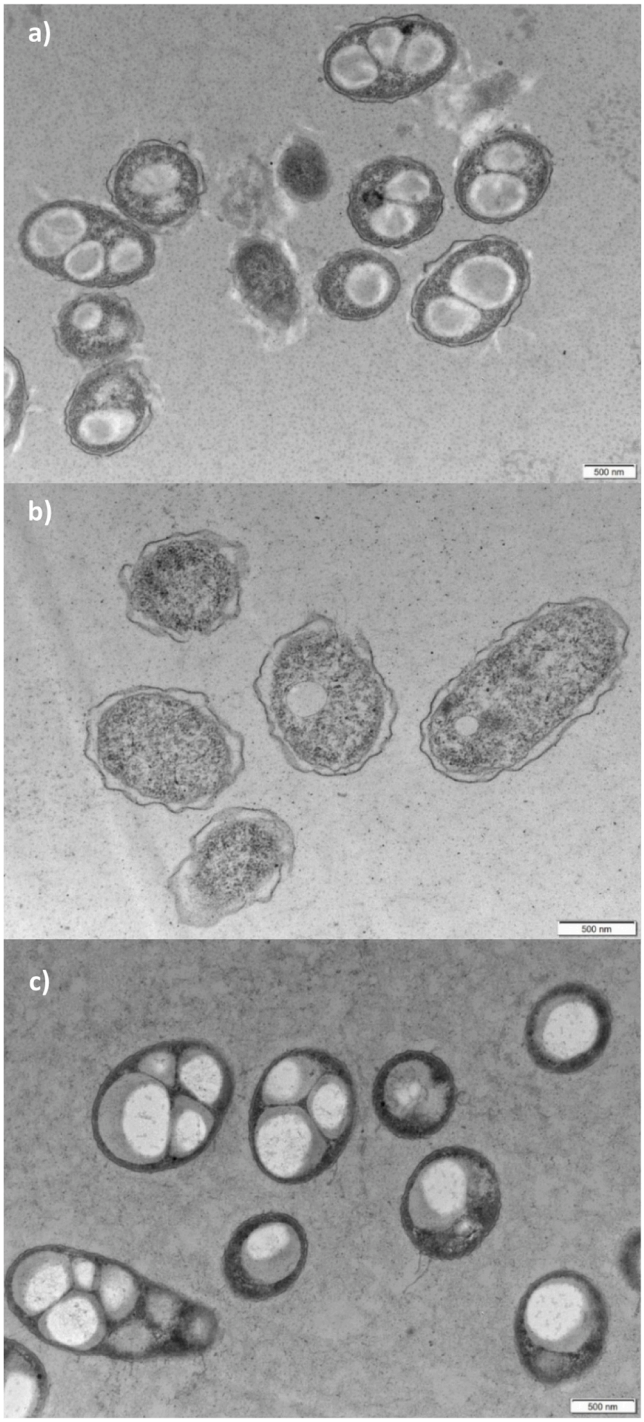

Fig. 2. Transmission electron microscopy (TEM) images from biomass samples collected at the end of the feast phase during an enrichment cycle (a), at the end of an enrichment cycle (b), and at the end of an accumulation experiment (c). Size bar of 500 nm.

3.3. Enriched MMC characterization: microbial structure and accumulation performance

3.3.1. Microstructural characterization of PHBV accumulation and microbial heterogeneity

Intracellular PHBV accumulation of the MMC was indicated by the presence of spherical inclusions (PHBV granules) inside the bacterial cells (Fig. 2). These granules varied in size and number, indicating a certain degree of heterogeneity across the culture. At the end of the feast phase (Fig. 2a), most cells contained one or more PHBV granules, supporting the high PHBV content (57 wt%). However, not all cells exhibited visible PHBV granules, indicating differences in the capacity to accumulate within the microbial community. After the famine phase (Fig. 2b), the intracellular PHBV content decreased to support cell growth and maintenance, resulting in a PHBV accumulation of 10 wt%. PHBV granules became smaller, fewer in number, or, in some cases, nearly absent.

To evaluate the coexistence of microbial species with varying PHBV accumulation capacities, microbiological analyses were performed on samples collected at each operational stage (excluding stage I). In stage V, three samples were collected, one from the bottom and one from the top of the SBR on day 273, and from the discharge after the implementation of a mechanical stirrer on day 328.

The bacterial community comprised 21 different phyla (Fig. 3a). *Pseudomonadota* (average relative abundance (RA) 33.65%) and *Actinomycetota* (average RA 8.32%) were the main dominant bacterial phyla. The ability of several members of the phylum *Pseudomonadota* to produce PHAs has been amply described, a fact attributed to their highly flexible metabolic capabilities, allowing the members of this phylum to utilize various waste substrates for that purpose [34–37]. While the RA of *Pseudomonadota* was broadly stable across the distinct operational periods, that of *Actinomycetota* was highly variable, being non-dominant on days 230 (stage III) and 273 (stage IV). In these samples, *Actinomycetota*, was replaced by *Bacteroidota* and *Verrucomicrobiota*, respectively. On the other hand, while the phylum *Actinomyceota* and *Verrucomicrobiota* harbor different taxa able to accumulate PHA [35,38], genera within the phylum *Bacteroidota* with this capability have not yet been described. Despite fluctuations in the relative abundance of the main phyla, PHA accumulation remained stable over time. This reinforces functional redundancy as a key advantage of working with MMCs since the biomass is composed of a consortium of genera from different bacterial phyla, which can adjust their structure by themselves to cope with operational changes without major impacts on PHA accumulation performance.

The dominant bacterial OTUs (average RA > 1%) were OtuB0001 (*Amaricoccus*, average RA 12.80%), OtuB0002 (*Pseudofulvimonas*, 11.64%), OtuB0003 (*Luteimonas*, 10.02%), OtuB0004 (*Stenotrophobacter*, 4.81%), OtuB0005 (*Luteolibacter*, 4.29%), OtuB0006 (*Paenimyroide*, 4.18%), OtuB0007 (*Aromatoleum*, 3.92%), OtuB0008 (*Gordonia*, 3.83%), OtuB0009 (*Paracoccus*, 3.05%), OtuB0010 (*Millisia*, 3.04%), OtuB0011 (*Streptomyces*, 2.89%), OtuB0012 (*Brevundimonas*, 2.69%), OtuB0013 (*Roseibacillus*, 2.53%), OtuB0014 (*Pseudoxanthomonas*, 1.33%), OtuB0015 (*Mesorhizobium*, 1.29%), OtuB0016 (*Terrimicrobium*, 1.10%), OtuB0017 (*Bosea*, 1.05%), OtuB0018 (*Pararhodobacter*, 1.04%) and OtuB0019 (*Arenimonas*, 1.02%). Also, 24.49% of the OTUs had an overall RA < 1% and were classified as miscellaneous minority bacterial OTUs. It should be noted that *Amaricoccus*, the most abundant bacterial OTU, showed a high accumulation capacity of PHB using acetate [34], organic acid mixtures [36], or agroindustrial by-products [35] as carbon sources.

According to Crognale et al. [39], the main gene involved in PHA

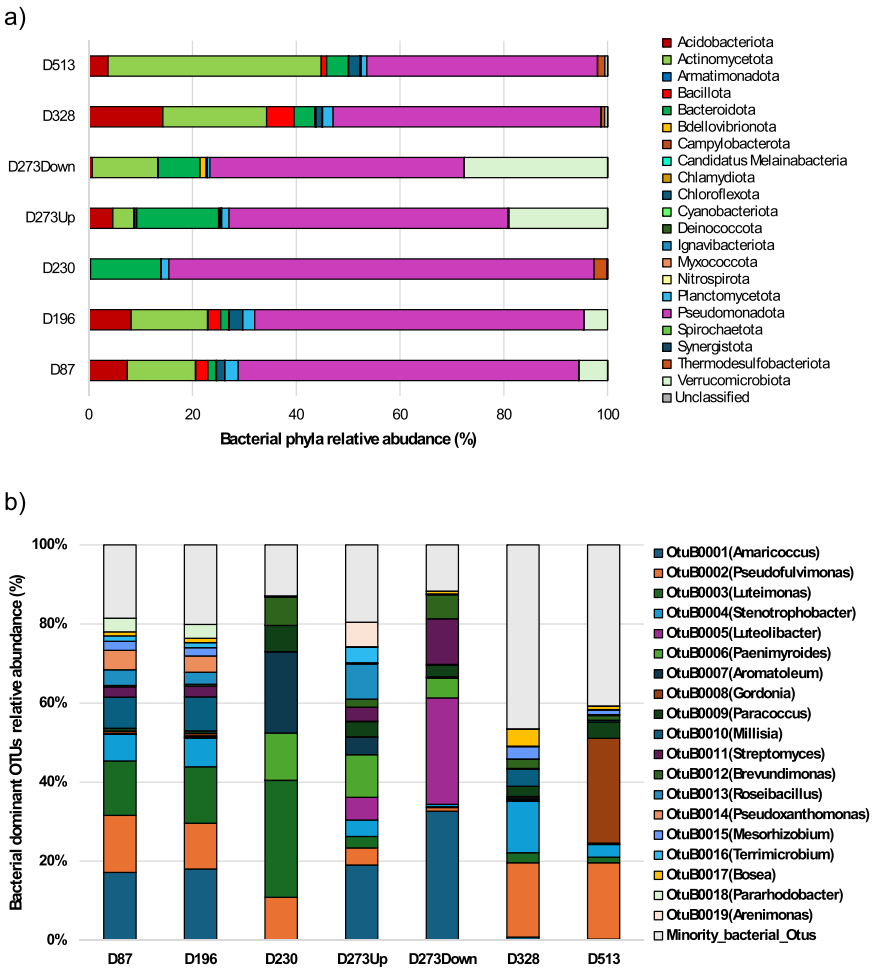

Fig. 3. (a) Relative abundance of the bacterial phyla in the bioreactor. (b) Dominant bacterial OTUs (relative abundance > 1%) in the bioreactor. Days of sample collection are indicated by a D followed by the corresponding number. On day 273, one from the top and one from the bottom of the reactor were taken.

accumulation is *phaC*, which encodes the poly(R)-hydroxyalkanoic acid synthase, the last enzyme in the PHA biosynthesis pathway. In this regard, to the best of the author's knowledge, the PhaC enzyme of *Amaricoccus* has not been isolated and characterized; nevertheless, a protein containing 607 amino acids has been predicted as a class I poly (R)-hydroxyalkanoic acid synthase (NCBI Reference Sequence: WP_233713022.1), linking the observed dominance of this genus to the biological capacity of the accumulated PHA. Similarly, *Pseudofulvimonas* [37], *Luteolibacter* [36], *Gordonia* [38], *Paracoccus* [37], *Streptomyces* [40], *Brevundimonas* [41], *Mesorhizobium* [42], *Bosea* [39], and *Pararhodobacter* [43] have all been previously described in PHA-accumulating enriched MMCs. In addition, different proteins have been annotated in the UniProt database (https://www.uniprot.org/) as PhaC enzymes for *Pseudofulvimonas* (A0A4V3UU), *Mesorhizobium* (A0ABY5QR50), *Bosea* (A0ABW0P7U9), and *Pararhodobacter* (A0ABT3GY56). On the other hand, the biosynthetic pathways for PHA accumulation have been well described for *Gordonia* [44], *Paracoccus* [45], *Streptomyces* [46], and *Brevundimonas* [47]. Conversely, genetic evidence of PHA accumulation biosynthetic capacity in *Luteolibacter* has not yet been described. Considering the broad diversity of bacteria potentially harboring *phaC*-like genes, the redundancy of PHA-accumulating bacteria within the MMC likely contributed to the consistently high PHA accumulation observed; however, strong successional patterns among the genera were observed across the different sampling times analyzed. It should be noted that the metabolic capacities of *Amaricoccus* [48] are more ample than those of *Pseudofulvimonas* [49], as the latter can only grow using a few compounds as sole carbon sources (L-arabinose, cellobiose, D-fructose, D-galactose, and dl-3-hydroxybutyrate). Hence, the replacement of the genus *Amaricoccus* by *Pseudofulvimonas* at the end of phase V suggests that it could be linked to the increase in the carbon availability mediated by an increased OLR, promoting the growth of this bacterium, as previously observed with different carbon substrate concentrations [37].

Therefore, the high variance in community structure across operational stages suggests that the increasing OLR is a driving force in the selection of the main bacterial OTUs, acting as a selective pressure for the settlement of putative PHA-accumulating bacteria within the MMC [9].

3.3.2. Dynamics of PHBV accumulation with synthetic VFA mixtures

Accumulation batch experiments were conducted to determine the maximum PHBV accumulation capacity of the enriched MMC (Figure S6) using two different VFA mixtures (F1 and F2) (Table 1) and as inoculum biomass from the enrichment SBR collected in the different operational periods.

For both tested synthetic VFA mixtures (F1 and F2), the PHBV accumulation percentage ranged from 65 to 83 wt% with an average value of 74 wt% (Table 2). The slight differences in PHBV accumulation did not follow a defined trend, indicating a stable capacity for PHBV accumulation, with maximum values of 83 and 76 wt% with F1 and F2, respectively. From the results obtained (Figure S6), it can be inferred that the biomass would accumulate more PHBV if the experiment had been prolonged. However, it must be considered that accumulation is always limited by the physical constraints of the existing cell wall

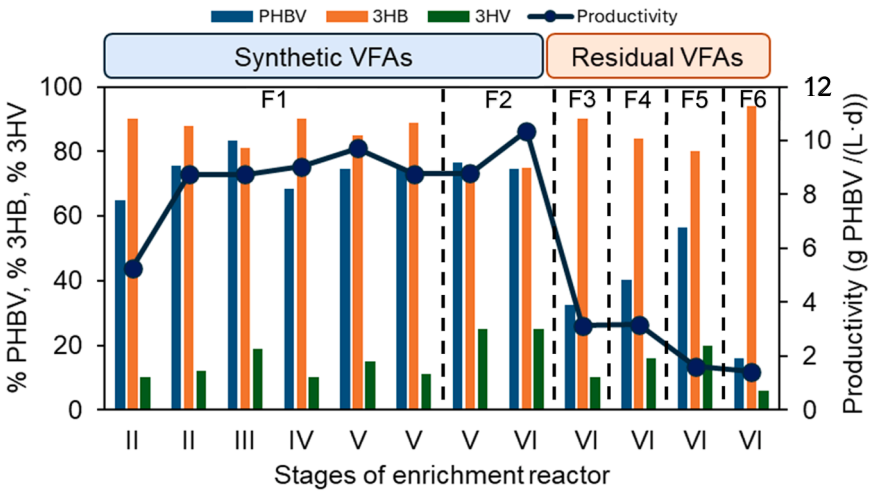

Figure 4. Results for each batch experiment of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) accumulation using different feedings and biomass from different stages of the reactor: productivity (g PHBV/(L·d)), wt% of accumulated PHBV and within this PHBV the wt% of 3HB and 3HV monomers. Stage I corresponds to an applied organic loading rate of 2.50 g COD/(L·d) in the enrichment sequencing batch reactor, II to 3.13 g COD/(L·d), III to 3.75 g COD/(L·d), IV to 4.38 g COD/(L·d), V to 5.00 g COD/(L·d) and VI to 6.00 g COD/(L·d).

material, as observed in pure cultures, which reached a maximum of 90% [50]. In this sense, TEM images (Fig. 2c) confirmed that at a high PHBV content (87 wt%), PHBV granules occupied most of the cell volume and cells became more spherical.

PHBV productivity varied significantly across experiments (5.27 – 10.36 g PHBV/(L·d)) (Figure 4), showing no clear dependence on the organic load fed. The PHBV accumulation and VFA consumption rates were also estimated in experiments with F1 and F2, respectively, using as inoculum biomass collected from the SBR at stage V: days 296 and 329 (Figure S6). They resulted in values of 0.31 $Cmol_{PHBV}/(Cmol_X \cdot h)$ and 0.63 $Cmol_{VFA}/(Cmol_X \cdot h)$ using F1 and 0.26 $Cmol_{PHBV}/(Cmol_X \cdot h)$ and 0.46 $Cmol_{VFA}/(Cmol_X \cdot h)$ using F2.

The maximum PHBV accumulation achieved in the accumulation experiments (83 wt%, Table 2) falls within the highest range reported by other authors working with PHA production systems using MMC, 78 wt % [28] with synthetic mixtures and 59 wt% [32] and 54 wt% [51] with VFA mixtures from fermentation. Pedrouso et al. [52] obtained lower PHA accumulated (41 wt%) but a comparable PHA production yield of 0.48 $Cmmol_{PHA}/Cmmol_{VFA}$ using a synthetic VFA mixture.

3.3.3. *Accumulation potential with pre-acidified fish canning residues*

The PHBV production capacity of the MMC was evaluated in accumulation experiments performed using VFA mixtures obtained from the acidification of fish canning wastes (F3-F6, Table 1 and Figure S7) that already contained significant nitrogen concentrations. The highest PHBV accumulation (56 wt%, Table 2) was achieved in the experiment conducted with F5. However, process scalability was limited by low VFA concentrations in F5, requiring large feeding volumes, thereby diluting the biomass and reducing overall process productivity. The observed variability in 3HB:3HV ratios of the produced PHBV across these experiments corresponded to differences in VFA composition among F3 - F6 feeding. The presence of nitrogen in the feeding increased active biomass concentration during the experiments (Figure S7). It is well established that nitrogen availability hinders PHA accumulation, as biomass preferentially utilizes substrates for growth rather than accumulation [53]. The change from synthetic media in the enrichment to pre-acidified wastewater in the accumulation does not necessarily cause the lowered accumulation value. In the study conducted by Burniol-Figols et al. [22], in which the culture was enriched with synthetic media and then the clarified effluent from crude glycerol fermentation was fed during the accumulation batch tests with ammonia present at negligible concentrations, they obtained an average PHA content of 76 wt%. Other studies using complex nitrogen-deficient feedstocks yielded PHA contents of 69 wt% and 81 wt% using pH-stat mode, where the acidified stream was continuously supplied as an acid solution to control the reactor-setpoint pH [2,10]. The lower PHA accumulation using acidified wastewater compared to synthetic wastewater (Table 2) was previously reported by Pedrouso et al. [52], who achieved 25 wt% with acidified wastewater and 41 wt% with synthetic media. This was related to the presence of ammonium nitrogen and to salinity from the acidified mussel cooker wastewater. However, Mulders et al. [54], working with wastewater with a high total ammonia nitrogen content, achieved a PHA accumulation of 0.77 g PHA/g VSS, demonstrating that higher accumulation percentages can be achieved in well-enriched MMCs even when ammonium is present. These results suggest that, in addition to nitrogen, the wastewater streams used in the present study may contain specific compounds that hinder PHBV accumulation.

The accumulation experiment with F6 was deemed invalid due to poor PHBV accumulation performance (16 wt%). Even though this feeding was supplemented with synthetic VFAs, the culture failed to use them to accumulate PHBV. This strongly suggested the presence of inhibitory substances, such as metals, originating from the coagulants and/or flocculants used during the solid-liquid separation process following the acidification of the fish canning waste. Specifically, F6 had been treated with commercial high-molecular-weight polyacrylamide-based coagulants and flocculants, and anionic polymers present in these chemical products can affect enzyme activity involved in PHA synthesis by binding essential cations. Moreover, certain inhibitory compounds may persist in some agro-industrial by-products after acidification, affecting the selection stage and, consequently, reducing the final PHA yield [55].

Hence, analyses of various chemical elements were performed on the different VFA batches (Table S3). For instance, F6 showed the highest concentrations of metals such as Al, Ti, Cr, Ni, As, Se, and Hg. Additionally, sodium concentration, one of the most widely studied inhibitors of biological processes, was the highest in F6. However, considering the feed dilution, the sodium concentration in the accumulation experiment carried out feeding F4 (where 40 wt% PHBV was achieved) was equivalent to that in the experiment carried out feeding F6, suggesting that sodium itself was not inhibitory. Moreover, since the MMC was enriched with a VFA mixture neutralized with NaOH, a basal sodium concentration was already present.

Minimal differences in the composition of the medium regarding

trace metals can significantly affect microbial competition and, thereby, influence PHA production capacity [56]. For example, Stouten et al. [56] showed that increasing iron bioavailability can shift bacterial populations and increase PHA accumulation from 30 wt% to 52 wt% by the end of the accumulation phase. In the present study, iron was always present in excess compared to the synthetic media composition. Thus, a significant role of iron in the poor performance of F6 is not anticipated. Besides iron, other metals, such as calcium, were also found to be beneficial for PHA accumulation in waste activated sludge [57], and in F6, the concentration of this ion was the lowest. Other metals (K, Mn, Cu, Zn), often needed in trace amounts for enzyme function or antioxidant defense, were found at lower concentrations in all pre-acidified wastewater batches (F3-F6) than in the mineral nutrient medium (Table S3), suggesting that they could be limiting factors for PHA activity. However, their concentrations in F6 were comparable to, or even higher than, those in the higher-performing F4. Therefore, while these differences in trace element profiles may explain the performance gap between synthetic and pre-acidified wastewater experiments, they are unlikely to account for the specific decline observed in F6.

Overall, the failed performance in the F6 experiment highlights a critical consideration for full-scale applications: the compatibility of upstream wastewater treatment chemicals with downstream biological processes to avoid cross-process interference and ensure system robustness.

The proposed PHBV-producing system operates by uncoupling carbon and nitrogen feeding in the enrichment unit, while maintaining a low nitrogen concentration in the feeding to the accumulation unit. To implement this system at full scale, using pre-acidified fish-canning wastewater, some operational adjustments will be necessary. To produce PHBV from complex or inhibitory wastewater, such as the one tested in the present study, coming from the fish canning industry, a strategy to cope with these characteristics consists of enriching the PHBV-accumulating biomass using a substrate stream without nitrogen and mainly composed of VFAs (fermented molasses, for example), supplemented with a small amount of the complex wastewater. Once enriched, biomass can be directly exposed to the complex substrate during accumulation experiments since the PHA producers maintain their advantage even when switching to a more complex VFA source [28]. Additionally, given the feedstock's C/N ratio, the acidification step should provide a stream with a higher percentage of VFAs than those produced in the previous acidifying reactor, which is outside the scope of this paper. Increasing this pretreatment efficiency will improve the feasibility of the proposed system. Regarding the metal content, the high accumulation percentage observed with F5 demonstrates that effective upstream treatment without inhibitory metal concentrations is possible. To use these streams of pre-acidified wastewater, a strategy that tests the specific MMC with the VFA-rich stream obtained after adding different amounts of coagulants and flocculants is proposed. When a stream exceeds the inhibition limit, various strategies can be used, such as dilution or purification using membrane or ion-exchange resins. If the amount of coagulant or flocculant added is always inhibitory, it would be best to use natural substances that do not interfere with biological processes.

3.3.4. Predictive relationship between VFA composition and PHBV monomers

With respect to the biopolymer composition, previous studies have shown that even when the same VFA substrate composition is used, different MMCs can yield markedly different 3HB:3HV ratios depending on their specific characteristics [58,59]. In this study, increasing the OLR from 2.5 to 6 g COD/(L·d), the 3HB:3HV ratio achieved during enrichment remained constant (except for stage III) despite fluctuations in the community across stages (Fig. 1). This stability is notable considering that Lorini et al. [8] reported that increases in OLR could also shift the HB:HV ratio, even under constant feed composition, presenting a HV content of 0.24 g HV/g PHA treating 8.5 g COD/(L·d) and 0.14 g HV/g PHA treating 12.73 g COD/(L·d). This suggests that the selected MMC in the present study has a robust, functionally stable metabolic response, enabling consistent PHBV monomer distribution and aligning with the predicted value based on the VFA composition.

In the accumulation experiments, different results were obtained with F1 and F2. The 3HB:3HV ratio remained close to the target values (88:12 g/g for F1 and 75:25 g/g for F2), reflecting the distinct VFA mixture compositions of the two feeds. This is consistent with well-established metabolic pathways in which odd-carbon VFAs (e.g., propionate and valerate) serve as precursors for HV incorporation, thereby promoting PHBV polymerization [7]. In contrast, even-carbon VFAs result solely in HB [7]. According to known metabolic pathways, following VFAs transport into the cell, VFAs are activated into their respective acyl-CoA molecules [60]. While acetic acid and propionic acid can be converted directly to acetyl-CoA and propionyl-CoA, longer-chain VFAs undergo β-oxidation [60]. In this pathway, butyric and caproic acids are cleaved into acetyl-CoA, whereas valeric acid is converted to acetyl-CoA molecules and a terminal propionyl-CoA molecule that remains after the last turn of the β-oxidation cycle [61]. In addition, a fraction of the propionyl-CoA produced may be converted to acetyl-CoA [60]. Acetyl-CoA and propionyl-CoA are then reduced and condensed to form the biopolymer. The combination of two acetyl-CoA ultimately leads to the formation of 3HB, and the combination of one acetyl-CoA with one propionyl-CoA forms 3HV [62]. Taking this into account and considering literature correlations linking individual VFAs to resulting HB:HV ratios [13], a direct relationship between the VFA feed composition and the resulting 3HB:3HV ratio was proposed and assessed with the VFA mixtures (F1-F6) used (Table S4). In all valid experiments (excluding F6), the proportion of valeric and propionic acids in the feed directly influenced 3HV content, confirming their role as precursors. This ability to predict polymer composition from feed characteristics (synthetic or residual) highlights the technological potential. Accumulation yields were of 0.43 $Cmol_{3HB}/Cmol_{VFA}$ and 0.06 $Cmol_{3HV}/Cmol_{VFA}$ feeding F1 and 0.40 $Cmol_{3HB}/Cmol_{VFA}$ and 0.16 $Cmol_{3HV}/Cmol_{VFA}$ feeding F2. This capacity to tailor biopolymers for high-yield production is made possible by the MMC's selection from a feed comprising several VFAs [13].

The stable and predictable biopolymer composition achieved underscores the robustness of the enrichment process. This versatility would greatly simplify the potential implementation of this biotechnological process, enabling the production of tailored biopolymer compositions without the need for separate enrichment strategies.

4. Conclusions

The enriched MMC exhibited a high capacity for PHBV accumulation (average of 59 wt% at the end of the feast phase in the enrichment reactor) and remained stable for 521 days without decay, despite broad differences in bacterial community structure. Under these conditions, PHBV production could be achieved in the same enrichment reactor, removing the effluent at the end of the feast phase.

Increasing the fed OLRs from 2.5 to 6.0 g COD/(L·d) did not significantly affect the biopolymer accumulation, while the reactor productivity doubled, reaching up to 2.53 g PHBV/(L·d).

Maximum accumulation of PHBV of 83 wt%, comparable to those of pure cultures, was achieved with conversion yields as high as 0.63 $Cmol_{PHBV}/Cmol_{VFA}$.

A maximum percentage of PHBV accumulated of 56 wt% was reached with pre-acidified fish-canning effluents; however, this substrate complexity requires careful consideration. It was observed that the monomer composition was predictable and depended on the VFA mixture. The 3HB:3HV ratio becomes predictable, whether treating synthetic or residual streams, demonstrating the versatility of the MMC.

CRediT authorship contribution statement

Alba Pedrouso: Writing – review & editing, Visualization,

Validation, Supervision, Methodology, Conceptualization. **David Correa-Galeote:** Visualization, Formal analysis. **Anuska Mosquera-Corral:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **López-Garabato Yolanda:** Writing – original draft, Visualization, Methodology, Investigation.

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.

Acknowledgements

This research was granted through the BIOCENPLAS project from the Ministry of Agriculture, Fisheries and Food (Government of Spain) (2021-PN070) and by Grant POLYGO1 (TED2021–130164B-I00) funded by MICIU/AEI/ 10.13039/501100011033 and by the "European Union NextGenerationEU/PRTR". Authors also acknowledge Ramon y Cajal (RYC2023–044762-I) funded by MICIU/AEI/10.13039/501100011033 and ESF+ . The authors would like to thank the RIAIDT-USC analytical facilities for their support.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jece.2026.122460.

Data availability

Data will be made available on request.

References

[1] M. Avella, E. Martuscelli, M. Raimo, Review Properties of blends and composites based on poly(3-hydroxy)butyrate (PHB) and poly(3-hydroxybutyrate-hydroxyvalerate) (PHBV) copolymers, Mater. Sci. 35 (2000) 523–545, https://doi.org/10.1023/A:1004740522751.

[2] M. Matos, R.A.P. Cruz, P. Cardoso, F. Silva, E.B. Freitas, G. Carvalho, M.A.M. Reis, Sludge retention time impacts on polyhydroxyalkanoate productivity in uncoupled storage/growth processes, Sci. Total Environ. 799 (2021), https://doi.org/10.1016/j.scitotenv.2021.149363.

[3] C.S.S. Oliveira, C.E. Silva, G. Carvalho, M.A. Reis, Strategies for efficiently selecting PHA producing mixed microbial cultures using complex feedstocks: Feast and famine regime and uncoupled carbon and nitrogen availabilities, N. Biotechnol. 37 (2017) 69–79, https://doi.org/10.1016/j.nbt.2016.10.008.

[4] T. Casero-Díaz, C. Castro-Barros, A. Taboada-Santos, L. Rodríguez-Hernández, M. Mauricio-Iglesias, M. Carballa, Turning fish canning wastewater into resources: Effluents and operational conditions selection for volatile fatty acids production, J. Water Process Eng. 64 (2024) 105738, https://doi.org/10.1016/J.JWPE.2024.105738.

[5] P. Chowdhury, T. Viraraghavan, A. Srinivasan, Biological treatment processes for fish processing wastewater – A review, Bioresour. Technol. 101 (2010) 439–449, https://doi.org/10.1016/J.BIORTECH.2009.08.065.

[6] J. Tamis, K. Lužkov, Y. Jiang, M.C.M. van Loosdrecht, R. Kleerebezem, Enrichment of Plasticicumulans acidivorans at pilot-scale for PHA production on industrial wastewater, J. Biotechnol. 192 (2014) 161–169, https://doi.org/10.1016/j.jbiotec.2014.10.022.

[7] P.C. Lemos, L.S. Serafim, M.A.M. Reis, Synthesis of polyhydroxyalkanoates from different short-chain fatty acids by mixed cultures submitted to aerobic dynamic feeding, J. Biotechnol. 122 (2006) 226–238, https://doi.org/10.1016/J.JBIOTEC.2005.09.006.

[8] L. Lorini, F. di Re, M. Majone, F. Valentino, High rate selection of PHA accumulating mixed cultures in sequencing batch reactors with uncoupled carbon and nitrogen feeding, N. Biotechnol. 56 (2020) 140–148, https://doi.org/10.1016/j.nbt.2020.01.006.

[9] S. Crognale, L. Lorini, F. Valentino, M. Villano, C. Marzo Gago, B. Tonanzi, M. Majone, S. Rosetti, Effect of the organic loading rate on the PHA-storing microbiome in sequencing batch reactors operated with uncoupled carbon and nitrogen feeding, Sci. Total Environ. 825 (2022) 153995, https://doi.org/10.1016/j.scitotenv.2022.153995.

[10] M. Matos, R.A.P. Cruz, P. Cardoso, F. Silva, E.B. Freitas, G. Carvalho, M.A.M. Reis, Combined strategies to boost polyhydroxyalkanoate production from fruit waste in a three-stage pilot plant, ACS Sustain. Chem. Eng. 9 (2021) 8270–8279, https://doi.org/10.1021/acssuschemeng.1c02432.

[11] T. Palmeiro-Sánchez, C.S.S. Oliveira, A.R. Gouveia, J.P. Noronha, A.M. Ramos, A. Mosquera-Corral, M.A.M. Reis, NaCl presence and purification affect the properties of mixed culture PHAs, Eur. Polym. J. 85 (2016) 256–265, https://doi.org/10.1016/j.eurpolymj.2016.10.035.

[12] C. Thellen, M. Coyne, D. Froio, M. Auerbach, C. Wirsen, J.A. Ratto, A processing, characterization and marine biodegradation study of melt-extruded polyhydroxyalkanoate (PHA) films, J. Polym. Environ. 16 (2008) 1–11, https://doi.org/10.1007/s10924-008-0079-6.

[13] M.T. Sánchez Palmeiro, Valorisation of saline wastewaters: a challenge for the obtainment of bioproducts, 2017.

[14] W. Vishniac, M. Santer, THE THIOBACILLI, Bacteriol. Rev. 21 (1957), https://doi.org/10.1128/br.21.3.195-213.1957.

[15] APHA-AWWA-WEF, Standard Methods for the Examination of Water and Wastewater, 1999.

[16] C.E. Bower, T. Holm-Hansen, A Salicylate–Hypochlorite Method for Determining Ammonia in Seawater, Can. J. Fish. Aquat. Sci. 37 (1980) 794–798, https://doi.org/10.1139/f80-106.

[17] G.J. Smolders, J. van der Meij, M.C. van Loosdrecht, J.J. Heijnen, Stoichiometric model of the aerobic metabolism of the biological phosphorus removal process, Biotechnol. Bioeng. 44 (1994) 837–848, https://doi.org/10.1002/bit.260440709.

[18] S. Takahashi, J. Tomita, K. Nishioka, T. Hisada, M. Nishijima, Development of a prokaryotic universal primer for simultaneous analysis of Bacteria and Archaea using next-generation sequencing, PLoS One 9 (2014), https://doi.org/10.1371/journal.pone.0105592.

[19] P.D. Schloss, S.L. Westcott, T. Ryabin, J.R. Hall, M. Hartmann, E.B. Hollister, R. A. Lesniewski, B.B. Oakley, D.H. Parks, C.J. Robinson, J.W. Sahl, B. Stres, G. G. Thallinger, D.J. Van Horn, C.F. Weber, Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities, Appl. Environ. Microbiol. 75 (2009) 7537–7541, https://doi.org/10.1128/AEM.01541-09.

[20] A. Mineo, M.M.C. van Loosdrecht, G. Mannina, Comparing two advanced selection strategies for polyhydroxyalkanoate production from domestic waste activated sludge, Chem. Eng. J. 513 (2025), https://doi.org/10.1016/j.cej.2025.163046.

[21] D. Dionisi, M. Majone, G. Vallini, S. Di Gregorio, M. Beccari, Effect of the length of the cycle on biodegradable polymer production and microbial community selection in a sequencing batch reactor, Biotechnol. Prog. 23 (2007) 1064–1073, https://doi.org/10.1021/bp060370c.

[22] A. Burniol-Figols, C. Varrone, S.B. Le, A.E. Daugaard, I.V. Skiadas, H.N. Gavala, Combined polyhydroxyalkanoates (PHA) and 1,3-propanediol production from crude glycerol: Selective conversion of volatile fatty acids into PHA by mixed microbial consortia, Water Res 136 (2018) 180–191, https://doi.org/10.1016/J.WATRES.2018.02.029.

[23] A. Giovanella, M. Carvalheira, M. Grana, M.A.M. Reis, B.C. Marreiros, Impact of saline osmotic stress on halotolerant polyhydroxyalkanoate (PHA)-Accumulating mixed microbial cultures: Boosting PHA production by osmotic downshock, J. Environ. Chem. Eng. 13 (2025), https://doi.org/10.1016/j.jece.2025.116521.

[24] L. Marang, Y. Jiang, M.C.M. van Loosdrecht, R. Kleerebezem, Butyrate as preferred substrate for polyhydroxybutyrate production, Bioresour. Technol. 142 (2013) 232–239, https://doi.org/10.1016/j.biortech.2013.05.031.

[25] A. Mineo, L. Isern-Cazorla, C. Rizzo, A.Palumbo Piccionello, M.Eugenia Suárez-Ojeda, G. Mannina, Polyhydroxyalkanoates production by an advanced food-on-demand strategy: The effect of operational conditions, Chem. Eng. J. 472 (2023) 145007, https://doi.org/10.1016/j.cej.2023.145007.

[26] F. Silva, S. Campanari, S. Matteo, F. Valentino, M. Majone, M. Villano, Impact of nitrogen feeding regulation on polyhydroxyalkanoates production by mixed microbial cultures, N. Biotechnol. 37 (2017) 90–98, https://doi.org/10.1016/j.nbt.2016.07.013.

[27] J. Tamis, M. Mulders, H. Dijkman, R. Rozendal, MarkC.M. van Loosdrecht, R. Kleerebezem, Pilot-Scale Polyhydroxyalkanoate Production from Paper Mill Wastewater: Process Characteristics and Identification of Bottlenecks for Full-Scale Implementation, J. Environ. Eng. 144 (2018), https://doi.org/10.1061/(asce)ee.1943-7870.0001444.

[28] E. Korkakaki, M. Mulders, A. Veeken, R. Rozendal, M.C.M. van Loosdrecht, R. Kleerebezem, PHA production from the organic fraction of municipal solid waste (OFMSW): Overcoming the inhibitory matrix, Water Res 96 (2016) 74–83, https://doi.org/10.1016/j.watres.2016.03.033.

[29] M.G.E. Albuquerque, C.A.V. Torres, M.A.M. Reis, Polyhydroxyalkanoate (PHA) production by a mixed microbial culture using sugar molasses: Effect of the influent substrate concentration on culture selection, Water Res 44 (2010) 3419–3433, https://doi.org/10.1016/j.watres.2010.03.021.

[30] R.A.P. Cruz, A. Oehmen, M.A.M. Reis, The impact of biomass withdrawal strategy on the biomass selection and polyhydroxyalkanoates accumulation of mixed microbial cultures, N. Biotechnol. 66 (2022) 8–15, https://doi.org/10.1016/j.nbt.2021.08.004.

[31] Z. Chen, L. Zhao, Y. Ji, Q. Wen, L. Huang, Reconsideration on the effect of nitrogen on mixed culture polyhydroxyalkanoate production toward high organic loading enrichment history, Front. Environ. Sci. Eng. 13 (2019), https://doi.org/10.1007/s11783-019-1135-2.

[32] A. Farghaly, A.M. Enitan, S. Kumari, F. Bux, A. Tawfik, Polyhydroxyalkanoates production from fermented paperboard mill wastewater using acetate-enriched bacteria, Clean. Technol. Environ. Policy 19 (2017) 935–947, https://doi.org/10.1007/s10098-016-1286-9.

[33] F. Morgan-Sagastume, A. Karlsson, P. Johansson, S. Pratt, N. Boon, P. Lant, A. Werker, Production of polyhydroxyalkanoates in open, mixed cultures from a

waste sludge stream containing high levels of soluble organics, nitrogen and phosphorus, Water Res 44 (2010) 5196–5211, https://doi.org/10.1016/j.watres.2010.06.043.
[34] A. Falvo, C. Levantesi, S. Rossetti, R.J. Seviour, V. Tandoi, Synthesis of intracellular storage polymers by Amaricoccus kaplicensis, a tetrad forming bacterium present in activated sludge, Appl. Microbiol. 91 (2001), https://doi.org/10.1046/j.1365-2672.2001.01384.x.
[35] B. Lagoa-Costa, C. Kennes, M.C. Veiga, Exploiting Cheese Whey for Efficient Selection of Polyhydroxyalkanoates-Storing Bacteria, Fermentation 9 (2023) 574, https://doi.org/10.3390/fermentation9060574.
[36] E. Clagnan, F. Adani, Influence of feedstock source on the development of polyhydroxyalkanoates-producing mixed microbial cultures in continuously stirred tank reactors, N. Biotechnol. 76 (2023) 90–97, https://doi.org/10.1016/J.NBT.2023.05.005.
[37] Z. Chen, C. Zhang, L. Shen, H. Li, Y. Peng, H. Wang, N. He, Q. Li, Y. Wang, Synthesis of Short-Chain-Length and Medium-Chain-Length Polyhydroxyalkanoate Blends from Activated Sludge by Manipulating Octanoic Acid and Nonanoic Acid as Carbon Sources, J. Agric. Food Chem. 66 (2018) 11043–11054, https://doi.org/10.1021/ACS.JAFC.8B04001/ASSET/IMAGES/LARGE/JF-2018-040015_0006.JPEG.
[38] D. Mabasa, A. Ranjan, M. Le Roes-Hill, T. Mthethwa, P.J. Welz, Polyhydroxyalkanoate Production by Actinobacterial Isolates in Lignocellulosic Hydrolysate, Processes 12 (2024) 1112, https://doi.org/10.3390/PR12061112/S1.
[39] S. Crognale, B. Tonanzi, F. Valentino, M. Majone, S. Rossetti, Microbiome dynamics and phaC synthase genes selected in a pilot plant producing polyhydroxyalkanoate from the organic fraction of urban waste, Sci. Total Environ. 689 (2019) 765–773, https://doi.org/10.1016/J.SCITOTENV.2019.06.491.
[40] S.P. Valappil, A.R. Boccaccini, C. Bucke, I. Roy, Polyhydroxyalkanoates in Gram-positive bacteria: Insights from the genera Bacillus and Streptomyces, Antonie van Leeuwenhoek, Int. J. Gen. Mol. Microbiol. 91 (2007) 1–17, https://doi.org/10.1007/S10482-006-9095-5/METRICS.
[41] J.A. Silva, L.M. Tobella, J. Becerra, F. Godoy, M.A. Martínez, Biosynthesis of poly-β-hydroxyalkanoate by Brevundimonas vesicularis LMG P-23615 and Sphingopyxis macrogoltabida LMG 17324 using acid-hydrolyzed sawdust as carbon source, J. Biosci. Bioeng. 103 (2007) 542–546, https://doi.org/10.1263/JBB.103.542.
[42] M. Marcos-García, P. García-Fraile, A. Filipová, E. Menéndez, P.F. Mateos, E. Velázquez, T. Cajthaml, R. Rivas, Mesorhizobium bacterial strains isolated from the legume Lotus corniculatus are an alternative source for the production of polyhydroxyalkanoates (PHAs) to obtain bioplastics, Environ. Sci. Pollut. Res. 24 (2017) 17436–17445, https://doi.org/10.1007/S11356-017-9319-4/TABLES/3.
[43] K.-K. Sam, N.-S. Lau, G. Furusawa, A.-A.A. Amirul, Draft Genome Sequence of the Halophilic Pararhodobacter-Like Strain CCB-MM2, Which Has Polyhydroxyalkanoate-Synthesizing Potential, Microbiol. Resour. Announc 8 (2019), https://doi.org/10.1128/MRA.01248-19.
[44] S. Xu, R. Han, L. Tao, Z. Zhang, J. Gao, X. Wang, W. Zhao, X. Zhang, Z. Huang, Newly isolated halotolerant Gordonia terrae S-LD serves as a microbial cell factory for the bioconversion of used soybean oil into polyhydroxybutyrate, 18, Biotechnol. Biofuels Bioprod. 2025 18 (1) (2025) 15, https://doi.org/10.1186/s13068-025-02613-w.
[45] A. López-Cortés, O. Rodríguez-Fernández, H. Latisnere-Barragán, H.C. Mejía-Ruíz, G. González-Gutiérrez, C. Lomelí-Ortega, Characterization of polyhydroxyalkanoate and the phaC gene of Paracoccus seriniphilus E71 strain isolated from a polluted marine microbial mat, 26, World J. Microbiol. Biotechnol. 2009 26 (1) (2009) 109–118, https://doi.org/10.1007/s11274-009-0149-5.
[46] S.P. Valappil, A.R. Boccaccini, C. Bucke, I. Roy, Polyhydroxyalkanoates in Gram-positive bacteria: insights from the genera Bacillus and Streptomyces, 91, Antonie Van. Leeuwenhoek 2006 91 (1) (2006) 1–17, https://doi.org/10.1007/s10482-006-9095-5.
[47] N.G. Assefa, H. Hansen, B. Altermark, A unique class I polyhydroxyalkanoate synthase (PhaC) from Brevundimonas sp. KH11J01 exists as a functional trimer: A comparative study with PhaC from Cupriavidus necator H16, N. Biotechnol. 70 (2022) 57–66, https://doi.org/10.1016/j.nbt.2022.05.003.
[48] F. Aulenta, D. Dionisi, M. Majone, A. Parisi, R. Ramadori, V. Tandoi, Effect of periodic feeding in sequencing batch reactor on substrate uptake and storage rates by a pure culture of Amaricoccus kaplicensis, Water Res 37 (2003) 2764–2772, https://doi.org/10.1016/S0043-1354(03)00059-9.
[49] P. Kämpfer, E. Martin, N. Lodders, S. Langer, P. Schumann, U. Jäckel, H.J. Busse, Pseudofulvimonas gallinarii gen. nov., sp. nov., a new member of the family Xanthomonadaceae, Int. J. Syst. Evol. Microbiol 60 (2010) 1427–1431, https://doi.org/10.1099/ijs.0.014548-0.
[50] J. Możejko-Ciesielska, R. Kiewisz, Bacterial polyhydroxyalkanoates: Still fabulous? Microbiol. Res. 192 (2016) 271–282, https://doi.org/10.1016/j.micres.2016.07.010.
[51] H. Chen, H. Meng, Z. Nie, M. Zhang, Polyhydroxyalkanoate production from fermented volatile fatty acids: Effect of pH and feeding regimes, Bioresour. Technol. 128 (2013) 533–538, https://doi.org/10.1016/j.biortech.2012.10.121.
[52] A. Pedrouso, A. Fra-Vazquez, A.V. Del Rio, A. Mosquera-Corral, Recovery of polyhydroxyalkanoates from cooked mussel processing wastewater at high salinity and acidic conditions, Sustain. (Switz.) 12 (2020) 1–16, https://doi.org/10.3390/su122410386.
[53] K. Johnson, R. Kleerebezem, M.C.M. van Loosdrecht, Influence of ammonium on the accumulation of polyhydroxybutyrate (PHB) in aerobic open mixed cultures, J. Biotechnol. 147 (2010) 73–79, https://doi.org/10.1016/J.JBIOTEC.2010.02.003.
[54] M. Mulders, J. Tamis, B. Abbas, J. Sousa, H. Dijkman, R. Rozendal, R. Kleerebezem, Pilot-Scale Polyhydroxyalkanoate Production from Organic Waste: Process Characteristics at High pH and High Ammonium Concentration, J. Environ. Eng. 146 (2020), https://doi.org/10.1061/(asce)ee.1943-7870.0001719.
[55] D. Queirós, A. Fonseca, P.C. Lemos, L.S. Serafim, Long-term operation of a two-stage polyhydroxyalkanoates production process from hardwood sulphite spent liquor, J. Chem. Technol. Biotechnol. 91 (2015), https://doi.org/10.1002/jctb.4841.
[56] G.R. Stouten, K. Hamers, R.J. Van Tatenhove-Pel, E. Van Der Knaap, R. Kleerebezem, Seemingly trivial secondary factors may determine microbial competition: A cautionary tale on the impact of iron supplementation through corrosion, FEMS Microbiol. Ecol. 97 (2021), https://doi.org/10.1093/femsec/fiab002.
[57] Á. Estévez-Alonso, M. Arias-Buendía, R. Pei, H.P.J. van Veelen, M.C.M. van Loosdrecht, R. Kleerebezem, A. Werker, Calcium enhances polyhydroxyalkanoate production and promotes selective growth of the polyhydroxyalkanoate-storing biomass in municipal activated sludge, Water Res 226 (2022) 119259, https://doi.org/10.1016/j.watres.2022.119259.
[58] M.G.E. Albuquerque, G. Carvalho, C. Kragelund, A.F. Silva, M.T. Barreto Crespo, M.A.M. Reis, P.H. Nielsen, Link between microbial composition and carbon substrate-uptake preferences in a PHA-storing community, 7, ISME J. 2013 7 (1) (2012) 1–12, https://doi.org/10.1038/ismej.2012.74.
[59] G. Carvalho, A. Oehmen, M.G.E. Albuquerque, M.A.M. Reis, The relationship between mixed microbial culture composition and PHA production performance from fermented molasses, N. Biotechnol. 31 (2014) 257–263, https://doi.org/10.1016/j.nbt.2013.08.010.
[60] F. Pardelha, M.G.E. Albuquerque, M.A.M. Reis, R. Oliveira, J.M.L. Dias, Dynamic metabolic modelling of volatile fatty acids conversion to polyhydroxyalkanoates by a mixed microbial culture, N. Biotechnol. 31 (2014) 335–344, https://doi.org/10.1016/j.nbt.2013.06.008.
[61] A. Steinbüchel, T. Lütke-Eversloh, Metabolic engineering and pathway construction for biotechnological production of relevant polyhydroxyalkanoates in microorganisms, Biochem. Eng. J. 16 (2003) 81–96, https://doi.org/10.1016/S1369-703X(03)00036-6.
[62] J.M.L. Dias, A. Oehmen, L.S. Serafim, P.C. Lemos, M.A.M. Reis, R. Oliveira, Metabolic modelling of polyhydroxyalkanoate copolymers production by mixed microbial cultures, 2, BMC Syst. Biol. 2008 2 (1) (2008) 59, https://doi.org/10.1186/1752-0509-2-59.