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Raw used cooking oil valorization into polyhydroxyalkanoates by mixed microbial cultures: evaluation of one- and two-unit configuration☆

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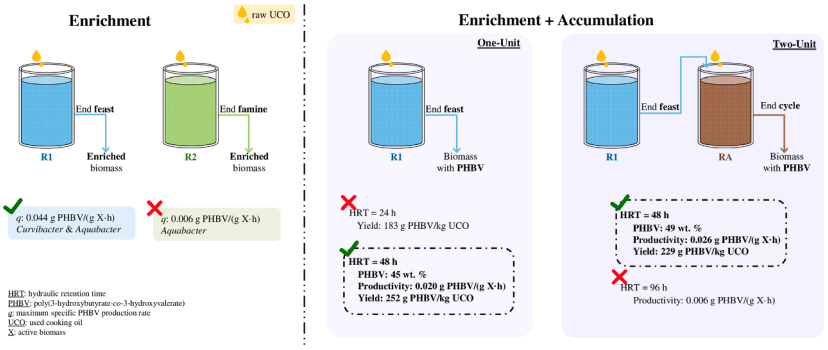
HIGHLIGHTS

- Raw used cooking oil was successfully valorized into PHBV by MMCs.
- MMC enrichment was achieved using a feast and famine strategy.
- Urea as nitrogen source improved pH stability and enhanced PHBV accumulation.
- Optimal PHBV yield and productivity were achieved at 48 h HRT in one and two units.
- One-unit approach enables enrichment and accumulation, simplifying PHBV production.

GRAPHICAL ABSTRACT



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ABSTRACT

The production of polyhydroxyalkanoates with untreated used cooking oil (UCO) as substrate represents an interesting strategy to valorize this residue into a value-added product. Three sequencing batch reactors (R1, R2, and RA) were operated, using mixed microbial cultures (MMCs) fed with raw UCO. R1 and R2 operated as enrichment units, with withdrawal at the end of the feast and famine phases, respectively. Enrichment was achieved in both within 30 days, reaching similar accumulations of the copolymer poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) (17.76 wt. % in R1 and 12.47 wt. % in R2). To improve process stability and reduce chemical consumption for pH control, urea was evaluated as nitrogen source, resulting in a reactor less dependent on pH control and increasing PHBV content from 16.7 to 25.7 wt. %. Then, the accumulation unit (RA) was operated in series with R1 to evaluate the maximum PHBV production of the biomass and compare the one-unit (R1) and two-unit (R1 + RA) configurations. Different hydraulic retention times (HRTs) were studied for the one-unit (24 and 48 h) and the two-unit (48 and 96 h) configurations. The best overall performance was observed at an HRT of 48 h in both cases, with similar accumulations (44.8–49.1 wt. % PHBV), yields (230–250 g PHBV/kg UCO) and productivities (0.010–0.013 g PHBV/(L·h)), showing that the one-unit was the best strategy for its operational simplicity. These results demonstrate the feasibility of enriching MMCs to produce PHBV using raw UCO, highlighting the potential of one-unit configuration to perform enrichment and accumulation steps in the same reactor.

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1. Introduction

The production and environmental impact of petrochemical plastics have attracted growing interest in recent decades, driving research into renewable and environmentally friendly alternatives. Among these, polyhydroxyalkanoates (PHAs) stand out as bio-based materials with the potential to replace conventional petrochemical plastics.

The PHAs are biodegradable, biocompatible, and non-toxic products with plastic-like properties, making them possible for multiple applications, both medical (tissue engineering, drug delivery, medical devices, etc.) and industrial (smart gels, packaging, adhesives, textiles, etc.) (Możejko-Ciesielska and Kiewisz, 2016; Pakalapati et al., 2018). PHAs are polyesters synthesized by various microorganisms using a range of metabolic pathways, as a reservoir of carbon and energy sources when fed with an organic carbon substrate in excess, particularly when a nutrient limitation also exists. Once the organic carbon becomes scarce in the medium, the microorganism can use the PHA as a substitute (Satoh et al., 1999; Valentin et al., 1999). Among the different PHAs, poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) has been extensively studied (Guho et al., 2020; Hori et al., 2009). This copolymer is composed of 3-hydroxybutyrate (3HB) and 3-hydroxyvalerate (3HV) monomers.

PHA production is already carried out using pure cultures (Hori et al., 2009; Martino et al., 2014; Obruca et al., 2013; Ruiz et al., 2019). However, with the aim of more economic, robust, and simple processes, its production using mixed microbial cultures (MMCs) has been extensively explored in the last years (Chen et al., 2017b; Valentino et al., 2020), along with the use of waste streams like wastewater effluents, sewage sludge, crude glycerol, animal waste, food waste, lignocellulosic waste and cheese whey as substrate (Argiz et al., 2022; Campanari et al., 2014; Din et al., 2008; Rodriguez-Perez et al., 2018). These feedstocks are usually pretreated to facilitate the carbon assimilation and PHA accumulation, consequently increasing the PHA production costs, so it needs to be considered when outline the process (Rodriguez-Perez et al., 2018). For a competitive process, the use of MMC and untreated waste streams would be ideal to reduce production costs and simplify operation. Therefore, the adequate selection of the waste and the correct enrichment of the MMC are critical for process success.

Among possible untreated waste streams suitable for PHA production, waste oil has great potential, particularly used cooking oil (UCO). UCO is a widely generated residue, for example, Europe produces around 1 million tons per year (Supple et al., 2002). One advantage of UCO is that it is collected separately, meaning that an established collection infrastructure already exists. Although UCO is primarily valorized for biodiesel production, other potential applications include hydrogen production and biosurfactant synthesis (Beghetto, 2025; Panadare and Rathod, 2015). Up-to-date PHA production from oily wastes has mainly been carried out using pure or engineered cultures (Kourmentza et al., 2018; Martino et al., 2014; Ruiz et al., 2019). In contrast, PHA production from untreated UCO using MMCs remains challenging, with only a few studies available at the laboratory scale (Din et al., 2008; Tamang and Nogueira, 2021). In these studies, the enrichment was not achieved when untreated UCO was used, due to the proliferation of filamentous bacteria, and PHA accumulation did not surpass 34 %.

Another aspect to consider when working with untreated UCO as substrate is the possible accumulation of triacylglycerides (TAGs). Previous studies on the aerobic production of PHA have shown that when TAG accumulation occurs, the yield and efficiency of the PHA accumulation process decrease (Argiz et al., 2022; Hori et al., 2009). TAGs are energy-storage lipids for microorganisms and are synthesized intracellularly under stress conditions like nutrient starvation or carbon excess (Chen et al., 2017a), similarly to PHAs. Furthermore, TAGs may also be present extracellularly as residual oil adsorbed onto the biomass surface (Garay et al., 2014). Therefore, avoiding the accumulation of these TAGs when feeding untreated UCO is key for the effective enrichment of PHA storing microorganisms and for maximizing PHA productivity.

The conventional configuration for PHA production using MMCs typically involves two reactors, an initial sequencing batch reactor (SBR) for enriching PHA-accumulating microorganisms, followed by a second fed batch reactor (FBR) dedicated to maximizing biopolymer accumulation. Nevertheless, this approach can be simplified into a one-unit system if biomass discharge from the enrichment reactor is carried out when intracellular PHA content is at its peak. Under these conditions, a separated accumulation reactor may become unnecessary if the accumulation in the enrichment one is adequate, with the corresponding simplification of the process and cost reduction (Lorini et al., 2020; Zeng et al., 2018). In this regard, Cruz et al. (2022) pointed that parameters such as solids retention time (SRT) need to be evaluated. Nevertheless, further studies are still needed to optimize PHA production with MMC in a one-unit configuration.

Furthermore, studies at pilot scale with MMC-based processes are available for substrates such as manure, slurry, or wastewater sludge, which are typically pre-fermented into volatile fatty acids (VFAs) (Chakravarty et al., 2010; Guho et al., 2020; Lorini et al., 2021). However, literature addressing oily waste streams such as untreated UCO are still limited, remarking the lack of knowledge in this field.

This study aims to fill all these gaps by enriching an MMC with untreated UCO for PHBV production at pilot scale. Furthermore, one- and two-unit configurations were investigated at different SRTs and compared in terms biopolymer accumulation in the biomass and the system's production capacity.

2. Materials and methods

2.1. Operational strategies

Three identical SBRs, designated R1, R2, and RA, each with a working volume of 24 L, were operated under aerobic conditions. R1 was operated for 196 days following the patented PRETENACC process (PCT/ES2022/070806, Mosquera-Corral et al. (2022)). R2 was operated solely as an enrichment reactor, coinciding with days 10 to 50 of the operation of R1. RA served as an accumulation reactor and received the effluent withdrawn from R1 between days 51 and 156 for dedicated PHBV accumulation.

The cycle configurations for the three reactors are described in Table S1 (see supplementary material). The volume exchange ratio was 50 % in all three reactors. The SRT was equal to the hydraulic retention time (HRT). Aeration was continuously supplied via air pumps (Laboport N 86 KTP, KNF Neuberger, USA) through diffusers at the bottom of the reactors supplying 18 L/min, to provide oxygen for biological reactions and ensure the mixing of the liquor media within the reactors. A mechanical stirrer (RW 20 digital, IKA Works Inc., Germany) was used to assist in mixing the biomass and the raw UCO in the liquor media with a speed of 80 rpm. The temperature was maintained at 30 °C using a thermostatic bath (Tectron Bio-100, JP Selecta, Spain) connected to a thermal jacket.

The raw UCO used as substrate in the three SBRs was obtained from a waste oil management company (PMA Nutrigrás S.A., Galicia, Spain) and characterized in terms of fatty acid percentage (93.61 %), with oleic (52.89 %) and linoleic (33.48 %) acids as the dominant components, elemental composition ($CH_{1.9}O_{0.1}$), and organic matter content as total chemical oxygen demand (2.8 ± 0.4 g tCOD/g UCO) (Table S2) (see supplementary material). This UCO was fed without any previous treatment.

The nutrient solution used to feed R1 and R2 after withdrawal was prepared according to Argiz et al., (2022), with the buffering effect of the phosphate salts which fixes the pH value at 7.0 ± 0.3 in the solution. The composition includes KH_2PO_4, $MgSO_4$, KCl, K_2HPO_4, allylthiourea, a trace solution (Vishniac and Santer, 1957) and NH_4Cl or $CO(NH_2)_2$ in R1, the NH_4Cl in R2 was supplied separately, more details provided in

Table S3 (see supplementary material).

2.1.1. One-unit configuration for enrichment and accumulation (R1)

To start the operation of the enrichment and accumulation unit using the PRETENACC process, R1 was inoculated with activated sludge from a municipal wastewater treatment plant of Santiago de Compostela (Northwest Spain) at a volatile suspended solids (VSS) concentration of 2.53 ± 0.02 g VSS/L. R1 was operated under aerobic dynamic feeding (ADF) and double growth limitation (DGL) strategies to enrich a PHBV-accumulating MMC. A feast/famine regime was imposed, with each phase representing half of the total cycle duration. During the feast phase, raw UCO was supplied as the substrate in a single pulse (12.0 mL) for the first 30 days, and in pulses of between 4.0 mL or 3.5 mL every 2 h thereafter (Table 1). After the feast phase, the withdrawn reactor content contains the maximum percent of PHBV accumulated. At the beginning of the famine phase, the reactor was refilled with the nutrient solution containing nitrogen. The pH in R1 was set to 7.0 ± 0.5 by a controller (46 Series, Chemitec Srl, Italy) with two pumps that supplied HCl (1 M) or NaOH (1 M), as needed. The reactor operated for 196 days in three stages (R1-I, R1-II, and R1-III) (Table 1).

During the first stage (R1-I, days 0–84), ammonium chloride (NH_4Cl) was used as the nitrogen source, and the SBR cycle lasted 12 h (6 h feast and 6 h famine) (Table S1a, b) (see supplementary material). In stage R1-II (days 85–134), urea ($CO(NH_2)_2$) replaced ammonium chloride as the nitrogen source, while maintaining a 12-h cycle (Table S1b) (see supplementary material). In the final stage (R1-III, days 135–196), the cycle length was doubled to 24 h (12 h feast and 12 h famine), with urea as the nitrogen source (Table S1d) (see supplementary material). In this last stage, with the duplication of the cycle length the raw UCO and the nitrogen amounts added were also duplicated to maintain the organic loading rate (OLR) and the ratio COD/N, respectively (Table 1). Furthermore, the concentration of the nutrient solution was increased, to maintain the ratio of carbon to nitrogen.

2.1.2. Enrichment unit (R2)

The enrichment unit R2 was inoculated with biomass collected on day 10 from R1. R2 was operated in parallel to R1 for 40 days. The operational conditions of R2 were similar to R1-I (Table 1): the OLR was 2.4 g tCOD/(L·d); the nitrogen source used was NH_4Cl; the cycle length was 12 h, and the addition of the raw UCO changed from one to three pulses the same day as in R1 keeping the total added volume constant. The difference between the two reactors was the timing of biomass withdrawal and nutrient filling. In R2, it occurred at the end of the famine phase and the nutrient filled do not have nitrogen, whereas in R1, the withdrawal and nutrient filling was at the end of the feast phase (Table S1a, b) (see supplementary material). The nitrogen solution prepared for R2 had a concentration of 45.25 g NH_4Cl/L, and 44 mL of it was added separately each cycle at the end of the feast phase (Table S3) (see supplementary material). Furthermore, the pH inside R2 was not controlled, but maintained around 6.5 ± 0.5 by the buffering effect of the phosphate salts in the nutrients.

2.1.3. Accumulation unit (RA)

The accumulation unit, RA, was inoculated with biomass from R1 (day 51) and operated for a total of 105 days. RA received the effluent withdrawn from R1 in subsequent operational cycles between days 51 and 156. The operational cycle of RA was configured to expand the accumulation time for the biomass of R1, so raw UCO was fed at the same pulse volume and frequency as in R1 (every 2 h) (Table S1c) (see supplementary material). In this reactor, no pH control was used. The operation of RA was divided into two stages depending on the cycle length (12 or 24 h) (Table 1). Stage RA-I/II (days 51–134) was operated with a 12-h cycle. When the cycle length of R1 was subsequently doubled (Table S1e) (see supplementary material), the operating cycle of RA was also increased to 24 h (stage RA-III, days 135–156).

2.2. Batch accumulation assays

On day 29 of the operation, batch assays were performed to assess the MMC's maximal PHA accumulation capacity in R1 and R2. Two fed-batch reactors with a working volume of 4 L were inoculated with the biomass withdrawn from each reactor (at the end of the feast and famine phases for R1 and R2, respectively). The temperature was maintained at 30 °C using a thermostatic bath (Tectron Bio-100, JP Selecta, Spain) connected to a thermal jacket. Aeration was continuously supplied using air pumps (Laboport N 86 KTP, KNF Neuberger, USA) with diffusers at the bottom of the reactor. Substrate pulses of 1.25 mL/pulse were added every three hours (3 pulses in total, corresponding to 1800 mg tCOD/L inside the reactor), when the dissolved oxygen concentration had recovered after the previous carbon addition. Liquid and solid samples were taken before each pulse to track the process.

2.3. Sampling and analytical methods

Dissolved oxygen (DO) concentration was monitored inside the reactors every 5 min using a DO luminescence probe connected to a portable multimeter (LDO101 and HQ40d, Hach-Lange, USA). In all reactors, two samples were collected and analyzed by duplicate periodically. In R1 and R2, samples were collected at the end of the feast and the famine phases. In the case of RA, samples were collected at the midpoint (hours 6 and 12 in 12- and 24-h cycles, respectively) and at the end of the cycle. In each sample, the pH was measured with a pH & Ion-Meter model GLP 22 (Crison, Spain), and both the liquid and solid fractions were then characterized.

For the liquid fraction, the samples were centrifuged and filtered (0.45 μm pore size, mixed cellulose ester membrane, Advantec, Japan). With this, the soluble chemical oxygen demand (sCOD) (APHA-AWWA-WPCF, 2017) and the total nitrogen (TN) (TOC-L analyser with the TNM-

Table 1
Summary of operational parameters and feeding strategies applied in R1 and R2 throughout the mixed microbial culture (MMC) enrichment process.

	R1			R2	RA	
Stage	R1-I	R1-II	R1-III		RA-I/II	RA-III
Operational days	0–84	85–134	135–196	10–50	51–134	135–156
N source	NH_4Cl	$CO(NH_2)_2$	$CO(NH_2)_2$	NH_4Cl	–	–
Cycle length (h)	12	12	24	12	12	24
HRT (h)	24	24	48	24	24	48
UCO pulses/cycle	1–3	3	6	1–3	6	12
UCO added (mL/pulse)	12.0–4.0	4.0	4.0–3.5	11.5–3.8	3.5	3.5
OLR (g tCOD/(L·d))	2.5	2.5	2.5–2.2	2.4	4.4	4.4
tCOD (mg/L) [a]	1263	1263	2525–2321	1213	2183	4367
N added (mg N/L) [a]	20.0	20.0–22.5	42.5–46.7	20.0	–	–
COD/N (g/g)	63.1	63.1–56.1	59.4–54.6	60.6	–	–

HRT: hydraulic retention time; UCO: used cooking oil; OLR: organic loading rate; COD: chemical oxygen demand; tCOD: total COD.
[a] Values inside the reactor after feeding the UCO (feast) or the nitrogen source (famine).

module, TOC-5000 Shimadzu, Japan) were determined. With the solid phase, the concentrations of total COD (tCOD), total and volatile suspended solids (TSS and VSS) (APHA-AWWA-WPCF, 2017), as well as the content of PHBV and TAG, were measured. All measurements were performed in duplicate and the results are reported as mean ± standard deviation (SD).

PHBV and TAG quantification were performed by gas chromatography using the method described by Smolders et al. (1994), with minor modifications. Biomass samples were dried at 50 °C in an oven (Memmert BE300, Memmert, Germany) for 24 h without pre-freezing. Between 15 and 20 mg of the dried sample was weighed into glass tubes. A mixture of HCl:1-propanol for acid digestion, 1,2-dichloroethane for extraction, and 1-propanol:benzoic acid as the internal standard was added. The samples were then digested at 100 °C for 3 h (Conterm, JP Selecta, Spain). After digestion, 3 mL of distilled water were added to facilitate liquid–liquid extraction. Following vortex mixing and phase separation, the organic phase was transferred to a vial for bioproduct quantification. Then, a flame ionization detector (GC-FID) was used for analysis, and PHBV and TAG were separated on the HP-INNOVAX (Agilent, USA) capillary column. Calibration curves were obtained using commercial standards of PHBV copolymer (3HB, 90.82 % w/w, and 3HV, 9.18 % w/w) and TAG (palmitic, stearic, oleic, and linoleic acids).

2.4. Microbiological analysis

DNA from the biomass of both enrichment reactors (R1 and R2) was extracted using the FastDNA SPIN Kit and the FastPrep 24 Instrument (MP Biomedicals, Germany) according to the manufacturer's protocol. Subsequently, partial bacterial 16S rRNA DNA sequencing was performed by using the primers Pro341F and Pro805R (Takahashi et al., 2014) in a NextSeq 500 sequencer (Illumina, USA). The Mothur V1.44.3 software was used to process the raw sequencing data (default settings for quality control, primer trimming, filtering, pre-clustering, and chimaera detection were employed). Operational taxonomic units (OTUs) at 97 % homology with an abundance of fewer than 100 sequences were removed for later analysis. Taxonomic classification was performed using the BLAST tool in Geneious Prime v.2019 (Geneious, New Zealand) against the 16S rRNA database at the National Centre for Biotechnology Information (USA).

2.5. Calculations

The active biomass concentration, X (g VSS/L), was calculated by subtracting the storage compounds accumulated (PHBV + TAG, in g/L) from the VSS concentration, supposing these as the only products accumulated. The maximum specific PHBV production rate (q, g PHBV/(g X·h)) during the feast or batch accumulation assays was determined using the maximum slope of the experimental data (g PHBV/(L·h)) collected in single cycle measurements divided by the active biomass concentration. For the values in Cmmol of the productivities, the individual slopes for 3HB and 3HV were used, along with their respective conversion factors to Cmmol, and the elemental composition of the biomass was assumed as $CH_{1.8}O_{0.5}N_{0.2}$ (Argiz et al., 2022), using its corresponding conversion. The productivity of PHBV (g PHBV/(L·d)) for one- and two-unit configurations was determined considering the characteristics of the effluent obtained (TSS concentration and wt. % PHBV) divided by the HRT of the whole configuration. The PHBV yield was calculated as the g of PHBV produced per kg of raw UCO fed. For statistical analysis, a one-factor ANOVA was performed by R statistics for the R1 and R2 performance (day 10 to 50), with a significance level set at p-value < 0.01.

3. Results and discussion

3.1. Enrichment configuration: R1 vs R2

The enrichment of a PHBV-accumulating MMC was studied with two different reactor configurations: R1 with the withdrawal at the end of the feast phase and R2 with the withdrawal at the end of the famine phase. Therefore, in R1 the famine phase begins with half of the active biomass containing the PHBV accumulated during the feast phase, resulting in a lower PHBV-to-nitrogen (PHBV/N) ratio at the onset of famine compared to R2. Conversely, the feast phase in R1 starts with all the active biomass, whereas in R2 it begins with only half, due to the prior withdrawal.

To verify the level of enrichment achieved, the performance of the biomass during the feast phase was monitored on specific days to study the PHBV accumulation (Fig. S1a, c) (see supplementary material). The results showed that both reactors exhibited similar behavior, increasing their PHBV content at the end of the feast phase, from 3.19 ± 0.01 (day 11) to 7.07 ± 0.02 wt. % (day 30) in R1, and from 5.42 ± 0.02 (day 15) to 6.78 ± 0.02 wt. % (day 23) in R2, independently of the moment of the effluent removal (end of the feast or famine phase, respectively). Fed-batch accumulation assays performed on day 29 to determine the maximal PHBV accumulation capacity of the biomass from each reactor confirmed the potential of the MMCs, reaching PHBV contents of 17.76 ± 0.03 wt. % (R1) and 12.47 ± 0.01 wt. % (R2) after 9 h (Fig. S1b, d) (see supplementary material), with a 8.2 mol % and a 11.1 mol % of 3HV, respectively (Table 2). The PHBV accumulation values obtained in this study were moderate compared to other studies using pure cultures and UCO as substrate and extended batch accumulation assays (30–50 h), where values between 37–63 wt. % PHA have been reported (Cruz et al., 2022; Martino et al., 2014). However, these results confirm that both operational configurations are suitable for achieving MMC

Table 2
Comparison of the main results obtained in the enrichment configurations in R1 and R2.

	R1	R2
Reactor performance	*Days 10–50*	*Days 10–50*
X (g VSS/L)	0.32 ± 0.13°	0.37 ± 0.09°
wt. % PHBV	15.9 ± 5.9°	17.6 ± 5.8°
mol % 3HB	92.3	92.6
mol % 3HV	7.7	7.4
sCOD end feast phase (mg/L)	270 ± 110**	1501 ± 80**
N end famine phase (mg/L)	9.0 ± 4.3*	5.9 ± 1.5*
Feast (1 pulse, 6 h)	*Day 30*	*Day 23*
X (g VSS/L) [a]	0.24 ± 0.01	0.41 ± 0.01
wt. % PHBV	7.07 ± 0.02	6.78 ± 0.02
mol % 3HB	85.7	79.1
mol % 3HV	14.3	20.9
q (g PHBV/(g X·h))	0.044 ± 0.001	0.006 ± 0.001
q (Cmmol PHBV/(Cmmol X·h))	0.048 ± 0.001	0.008 ± 0.001
Fed-batch Assay (3 pulses, 9 h)	*Day 29*	*Day 29*
X (g VSS/L) [a]	0.35 ± 0.01	0.34 ± 0.01
wt. % PHBV	17.76 ± 0.03	12.47 ± 0.01
mol % 3HB	91.8	88.9
mol % 3HV	8.2	11.1
q (g PHBV/(g X·h))	0.040 ± 0.001	0.029 ± 0.001
q (Cmmol PHBV/(Cmmol X·h))	0.043 ± 0.001	0.031 ± 0.001

One-factor ANOVA performed for the *Reactor performance*: *p-value < 0.01, **p-value < 0.001, °p-value > 0.01 (no significant differences).
Values are expressed as mean of the operational period ± standard deviation (SD) in case of the *Reactor performance*, and as the mean of the duplicates of the sample point ± SD in the *Feast* and *Fed-batch Assay*.
X: active biomass concentration at the end of the famine; VSS: volatile suspended solids; PHBV: poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) at the end of the feast; 3HB: 3-hydroxybutyrate; 3HV: 3-hydroxyvalerate; sCOD: soluble chemical oxygen demand; N: total nitrogen; q: specific production rate.
[a] Active biomass before the assay starts.

enrichment using raw UCO.

Comparing the results at the end of the feast phase (single pulse) with those obtained at the end of the fed-batch accumulation assays (3 pulses) (Table 2), it was observed that when the effluent is withdrawn at the end of the feast phase (R1) the specific production rate (q) values were very similar in both cases (0.043–0.048 Cmmol PHBV/(Cmmol X·h)), suggesting that the maximum q was already achieved during the continuous operation of the reactor. However, when the effluent is removed after the famine phase (R2), the maximum q value was obtained in the fed-batch accumulation assay (0.031 Cmmol PHBV/(Cmmol X·h)), indicating that the R2 configuration did not enable the MMC to reach its full accumulation potential during the feast phase. Similar values have been reported by other authors under 12–18-h cycles using MMCs, but with acetate as a synthetic carbon source (0.01–0.07 Cmmol PHA/(Cmmol X·h)) (Moita Fidalgo et al., 2014; Montiel-Jarillo et al., 2017; Zeng et al., 2018), even though in the present study, a complex substrate such as raw UCO is used as substrate. The results obtained comparing R1 and R2 are consistent with those reported by Cruz et al. (2022), who also found better performance in the reactor operated with biomass withdrawal at the end of the feast phase compared to that operated with withdrawal at the end of the famine phase. However, unlike the present work, Cruz et al. (2022), reported notably higher active biomass concentrations in the reactor with withdrawal after the famine phase. In contrast, in this study, active biomass concentrations remained similar in both reactor configurations (Table 2).

Based on the results of the fed-batch accumulation assays and to improve the accumulation capacity of the MMC in R2, the feeding pattern in both reactors was changed from 1 to 3 pulses on day 30, maintaining the total raw UCO volume added per cycle. Argiz et al. (2022) also followed a similar strategy, feeding an MMC with fish waste oil as a carbon source, starting with a single pulse and switching to multiple pulses after 20 days of operation. In their study single-pulse feeding was identified as necessary at the beginning for proper enrichment, which caused stress in the culture that helped with selection. Then, switching to pulsed feeding resulted in higher PHBV accumulation at the end of the feast phase as well as a higher active biomass concentration. Furthermore, the pulse-feeding strategy helps prevent substrate inhibition (Moita Fidalgo et al., 2014), which is common when VFA mixtures are used as substrates. Moreover, several authors have reported the greater suitability of pulse feeding compared to other feeding modes (Argiz et al., 2022; Zeng et al., 2018), such as single-pulse or continuous feeding, for PHA accumulation.

Average values from days 10–50 (Table 2) show lower sCOD concentration at the end of the feast and lower nitrogen concentration at the end of the famine phase in R2, which favors MMC enrichment. However, the overall performance metrics in terms of active biomass (0.37 ± 0.09 g VSS/L for R2 and 0.32 ± 0.13 g VSS/L for R1) and PHBV accumulated at the end of the feast (17.6 ± 5.8 wt. %) showed non-statistically significant differences (p-value > 0.01, Table 2). Given this similarity and the practical advantage of coupling enrichment and accumulation in one unit (since biomass is withdrawn after the feast phase, when PHBV content is maximal), R1 was selected as the most suitable configuration.

Samples were taken in R1 and R2 to study the microbial diversity and the evolution of the MMC enrichment. Relative abundance of bacterial OTUs for days 25 and 46 are represented in Fig. 1. The bacterial community comprised 19 dominant bacterial OTUs (average relative abundance > 0.5 %). These dominant OTUs were: OtuB001 (*Aquabacter*), OtuB0002 (*Curvibacter*), OtuB0003 (*Telluria*), OtuB0004 (*Dulcicalothrix*) and OtuB0005 (*Pseudacidovorax*). Also, 21.3 % of the OTUs had an overall relative abundance < 0.5 % and were clustered into the miscellaneous minority bacterial OTUs. Although in R2 the community stays diverse compared to days 25 and 46, in R1 could be observed a main dominant OTU (OtuB002 *Curvibacter*). *Curvibacter* has not been described as a PHA-accumulating microorganism except for some authors that observed accumulation capacity in a few species (Nielsen et al., 2009). By the end of the operation, OtuB001 (*Aquabacter*) emerged as the dominant OTU in both reactors. To the best of the authors knowledge, the PHA-accumulation capacity of the genus *Aquabacter* has not been previously demonstrated, although it was found in other enriched PHA-accumulating MMC, but in a significant lower relative abundance (Correa-Galeote et al., 2022). On the other hand, OTUs belonging to *Telluria* (Diard et al., 2002), *Pseudacidovorax* (Bruland et al., 2009), and other less abundant OTUs have been related to bacteria able to bioaccumulate PHA (Alaux et al., 2023; Correa-Galeote et al., 2022; Liang and Faucher, 2024).

The enrichment achieved in R1 and R2 represents one of the few successful MMC enrichment processes using raw UCO as substrate. Previous studies mainly relied on pure cultures (Cruz et al., 2016; Kourmentza et al., 2018) or on pretreated UCO (Din et al., 2008; Obruca et al., 2013; Ruiz et al., 2019). In earlier laboratory-scale MMC studies, enrichment was achieved but typically required more than 100 days. In contrast, in the present research work, enrichment was achieved in approximately 30 days, reaching 17.76 wt. % PHBV (Table 2) without any pretreatment of the waste or prior adaptation of the culture to a

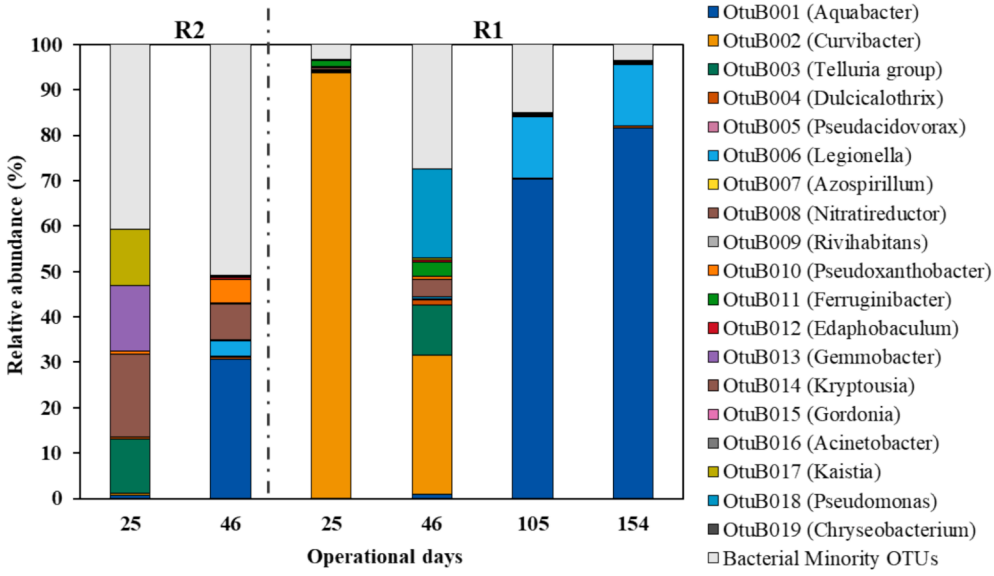

Fig. 1. Distribution of the main bacterial OTUs (relative abundance > 0.5 %) in the mixed microbial culture of R1 and R2 on specific days.

synthetic carbon source. Kourmentza et al. (2018) worked with a pure strain of *Burkholderia thailandensis* fed with UCO, achieving up to 60 wt. % PHA. Similarly, Cruz et al. (2016) tested several pure strains with UCO and reported that some species were unable to grow on oil, while others could grow but did not accumulate PHA; the highest PHA content obtained was 60 wt. %. Ruiz et al. (2019) hydrolyzed UCO before feeding a pure culture of *Pseudomonas putida*, obtaining a maximum of 36 wt. % PHA in batch accumulation. Although pure cultures can achieve higher PHA accumulation levels, the use of MMCs with raw UCO, as in the present study, represents a more straightforward and sustainable approach.

3.2. Effect of nitrogen source change on the accumulation performance

During the operation of R1-I, the reactor experienced several instability episodes associated with pH probe failures due to fouling by oil, despite having a pH control system, which resulted in medium acidification and increased chemical consumption. Acidification occurred mainly during the famine phase, due to consumption of the nitrogen source (NH_4Cl). To enhance operational stability and mitigate pH drops, the nitrogen source was changed from NH_4Cl to urea ($CO(NH_2)_2$) in stage R1-II. Urea hydrolysis produces ammonia, which buffers the system by releasing OH^- ions at equilibrium with water, thereby stabilizing pH. This modification helped reduce reagent consumption for pH control, as observed in the daily NaOH demand by the pH controller, which decreased from 2.9 g/d to 0.2 g/d (Fig. S2a) (see supplementary material).

In stage R1-II, the average active biomass decreased (to 0.25 ± 0.09 g VSS/L) compared with the previous stage (0.32 ± 0.12 g VSS/L) (Fig. 2), but the PHBV accumulation clearly increased (Fig. 3a), reaching 40.1 wt. % (96.8 mol % of 3HB and a 3.2 mol % of 3HV) on day 105 of operation and stabilized thereafter around 25.7 ± 9.7 wt. %. Therefore, the volumetric PHBV productivity was not negatively affected, and it doubled to 0.12 g PHBV/(L·d) in comparison with R1-I (0.06 g PHBV/(L·d)) using ammonium chloride (Fig. 2). Residual nitrogen measured at the end of the famine phase (5.2 ± 0.4 mg N/L) was within the same range as previously observed with NH_4Cl (8.0 ± 3.7 mg N/L) (Fig. S2b) (supplementary material), indicating that the biomass effectively assimilated urea. Nevertheless, a lower TAG content was observed after this change (Fig. 3a).

Regarding the microbial population of the reactor, the nitrogen source change had a strong impact on the relative abundance of the bacteria OTUs (Fig. 1). Before this change, in R1 a clear dominance of *Curvibacter* could be observed (day 25). After 20 days of operation under urea as nitrogen source (day 105) the population changed completely for a predominance of *Aquabacter*, suggesting that the use of urea instead ammonium chloride acted as a driving force of the microbial community structure, favoring the development of an MMC amply dominated by this bacterium. In this regard, it was reported that urea is a more energetically efficient nitrogen source for promoting bacterial growth than ammonium salts (Yao et al., 2024) as assimilation from ammonium has a higher cost (1 ATP per ammonia) compared to urea (½ ATP per ammonia) (Konzock et al., 2022), enhancing a selective enrichment of this bacterium.

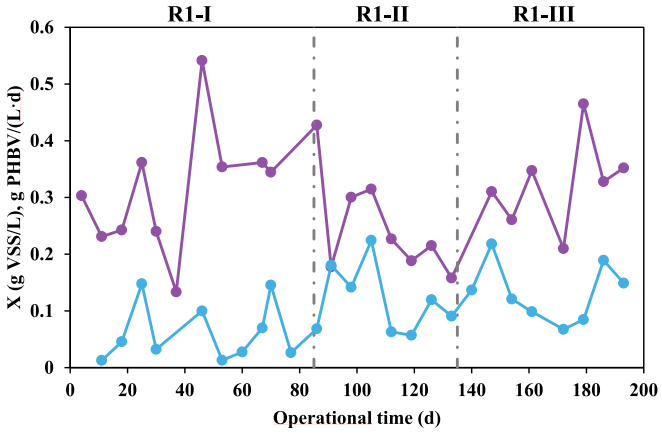

Fig. 2. Evolution of the concentration of active biomass (X) at the end of the famine phase (●), and poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) productivity (●) measured in R1 throughout the operational time. All the values are the mean of the duplicate replicas taken. The different operational stages are indicated with a pointed vertical line (⁞). VSS: volatile suspended solids.

The improved performance of R1 in terms of PHBV accumulated following the nitrogen source change also had a direct impact on the subsequent accumulation reactor (RA), where the PHBV content increased from 29.3 wt. % on day 77 (84.0 mol % of 3HB and 16.0 mol % of 3HV) to 61.0 wt. % on day 91 (98.3 mol % of 3HB and 1.7 mol % of 3HV) (Fig. 3b). Then, the PHBV content in RA stabilized around 46.1 wt. % between days 95 and 135. Regarding TAG, the values in RA fluctuated but consistently remained below 20.0 wt. %.

Most PHA accumulation studies use NH_4Cl or $(NH_4)_2SO_4$ as nitrogen sources (Campanari et al., 2014; Chen et al., 2017b; Moita Fidalgo et al., 2014; Montiel-Jarillo et al., 2017; Zeng et al., 2018). However, when NH_4Cl is compared to the urea, the latter shows better results in terms of PHA accumulation, as demonstrated by Fazielawanie et al. (2021), and in agreement with the present study.

3.3. Effect of the hydraulic retention time increase by cycle length extension

The use of 24 h HRT values in PHA enrichment reactors is common (Campanari et al., 2014; Chen et al., 2017b; Valentino et al., 2020), although the cycle length may vary depending on the volume exchanged in the withdrawal phase. However, some authors have suggested that significantly longer HRTs (up to 9 days) can enhance PHA accumulation (Din et al., 2008). Therefore, HRT in R1 was increased from 24 h to 48 h on day 135 (R1-III) (Table S1d) (see supplementary material). This was achieved by doubling the cycle length and simultaneously doubling the tCOD and nitrogen feed concentrations. As a result, the sCOD and the nitrogen concentrations remained at the end of the feast and famine phases, respectively, increased in R1-III (Fig. S2b) (see supplementary material).

With the extension in the HRT, and consequently the SRT, the active biomass progressively rose from 0.25 ± 0.09 to 0.32 ± 0.08 g VSS/L (Fig. 2), and the PHBV content also increased, from 25.7 ± 9.7 wt. % to 35.3 ± 10.6 wt. %, reaching a maximum of 54.3 wt. % on day 154 (97.5 mol % of 3HB and 2.5 mol % of 3HV) (Fig. 3a), while TAG levels remained low and stable, below 20.0 wt. % (Fig. 3a). Contrary to this study, Moretto et al. (2020) reported higher PHA accumulation at an HRT of 24 h (13–20 wt. %) compared to 48 h (7–14 wt. %). In their study, the increase in HRT was achieved by reducing the volume exchange ratio, a strategy that may be detrimental due to decreased biomass renewal per cycle. In the present study the volume exchange ratio was maintained and the cycle time extended, so the renewal stays the same value per cycle.

The parameter that best reflects the overall process improvement is the PHBV productivity, which increased from 0.10 g PHBV/(L·d) in stage R1-II to 0.14 g PHBV/(L·d) in stage R1-III (Fig. 2). This improvement can be attributed to the longer reaction time available for the culture to metabolize the raw UCO and synthesized PHBV during the feast phase, as well as to the greater amount of carbon supplied for accumulation. In addition, during the famine phase, the extended reaction time combined with higher nitrogen availability supported greater biomass growth compared to the previous stage. In terms of relative bacterial abundance, this change does not result in an important modification of the OTUs present on the MMC of R1. In this period, the most abundant OTU was still the *Aquabacter* (day 154), with a clear dominance over the others (Fig. 1).

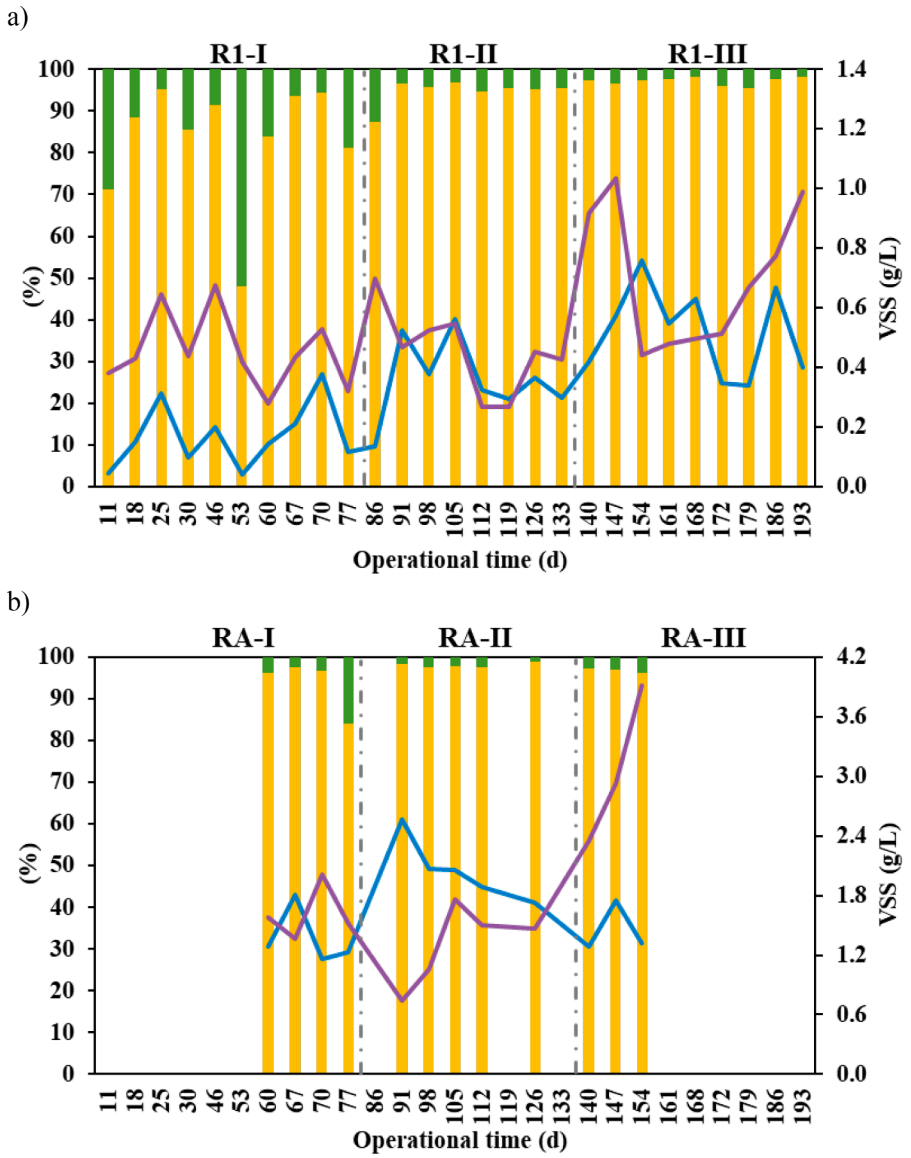

Fig. 3. Evolution throughout the operational time of poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) (▬, wt. %), with the amount of 3-hydroxybutyrate (3HB) (■, mol %) and 3-hydroxyvalerate (3HV) (■, mol %), and volatile suspended solids concentrations (▬, g VSS/L) in the effluent of a) R1 and b) RA. All the values are the mean of the duplicate replicas taken. The different stages are indicated with a pointed vertical line (⁞).

When the HRT was doubled in R1, it was also doubled in RA from 24 to 48 h (Stage RA-III, Table 1). However, the HRT change in RA did not enhance the intracellular PHBV content, which even decreased from values of 41.8 ± 7.5 wt. % in Stage RA-I/II to values of 34.6 ± 6.3 wt. % in Stage RA-III (Fig. 3b). Several authors have attributed this behavior to the existence of an adequate HRT that favors biomass performance, whereas further increases may become detrimental due to the excessive residence time of the biomass (Lai et al., 2025; Liu et al., 2011). In the present study, the operation of the accumulation unit (RA) with an HRT of 48 days appears to be unfavorable for the proper accumulation of PHBV while in R1 it was beneficial. However, it should be noted that the biomass fed to RA had already undergone a residence time in R1.

3.4. *One-unit vs two-unit system for PHBV production*

The results obtained from R1 and RA allowed the comparison of the two configurations for PHBV production under different HRTs: a one-unit system (R1) operated at 24 h and 48 h HRT, and a two-unit system (R1 + RA) operated at 48 h and 96 h HRT (Table 3). Unlike most studies based on independent batch accumulation assays (Chakravarty et al., 2010; Guho et al., 2020; Martino et al., 2014; Tamang and Nogueira, 2021), the two-unit (R1 + RA) configuration enabled the continuous coupling of enrichment and accumulation units to be investigated.

The results summarized in Table 3 show that the scenarios of the one-unit at 24 h HRT and the two-unit at 96 h HRT delivered the lower values of PHBV content (around 35.0 wt. % for both) and yield (180 and 210 g PHBV/kg UCO, respectively).

Comparing the one-unit and two-unit systems at the same HRT (48 h), the results were very similar, with no significant differences. However, the one-unit configuration showed a slightly higher yield (250 vs 230 g PHBV/kg UCO), whereas the two-unit configuration was a little better in terms of PHBV content (49.1 ± 7.5 wt. %) and productivity (0.026 ± 0.007 g PHBV/(g X·h)) (Table 3). As suggested by several studies, a minimum PHA content of approximately 40 wt. % is required for efficient extraction, making it a critical factor for the overall economic feasibility of the process (Bengtsson et al., 2017; Estévez-Alonso et al., 2022). In the present study, configurations operated with an HRT of 48 h proved to be the most suitable, as the average accumulation percentage exceeded this value. Moreover, as indicated by other authors

Table 3
Comparison of one-unit (R1) and two-unit (R1 + RA) configurations for PHBV production at different values of HRT.

Configuration	HRT (h)	PHBV (wt. %)	3HB (mol %)	3HV (mol %)	g PHBV/(L·h)	g PHBV/(g X·h)	g PHBV/kg UCO
One-unit	24	34.9 ± 7.0	96.3	3.7	0.008 ± 0.002	0.024 ± 0.007	180 ± 40
(R1)	48	44.8 ± 6.8	97.4	2.6	0.010 ± 0.008	0.020 ± 0.005	250 ± 60
Two-units	48 (24 + 24)	49.1 ± 7.5	98.1	1.9	0.013 ± 0.003	0.026 ± 0.007	230 ± 60
(R1 + RA)	96 (48 + 48)	34.6 ± 6.3	97.0	3.0	0.012 ± 0.002	0.006 ± 0.001	210 ± 30

Values are expressed as mean of the operational period ± standard deviation (SD).
HRT: hydraulic retention time; PHBV: poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate); 3HB: 3-hydroxybutyrate; 3HV: 3-hydroxyvalerate; UCO: used cooking oil.

the one-unit system reduces the PHA production costs, due to the lower capital investment compared to the two-unit system and the reduction of equipment and energetic costs (Ahuja et al., 2024; Cruz et al., 2022). These production costs are a bottleneck when trying to compete with petroleum-based plastics, so its reduction is important to an economically feasible process.

The results of the present study are comparable to those obtained in lab-scale studies (2–10 L) for pure cultures of *Cupriavidus necator* using UCO, in terms of productivity (0.001 to 0.020 g PHA/(g X·h)) (Martino et al., 2014; Obruca et al., 2013) and in terms of yield (290 g PHA/kg UCO) (Martino et al., 2014). Kourmentza et al. (2018) achieved better results with 350 g PHBV/kg UCO using a pure strain of *Burkholderia thailandensis*, which reached 60 wt. % of PHA with a productivity of 0.063 g PHA/(L·h) in a batch assay of 120 h. When MMC is used, yield can decrease. Tamang and Nogueira (2021), for example, obtained 75 g PHA/kg UCO in batch assays, although the enrichment was previously made in synthetic nonanoic acid after the enrichment with UCO was unsuccessful.

4. Conclusions

This study demonstrates the feasibility of enriching an MMC and accumulating PHBV at pilot scale using untreated UCO as the sole carbon source. Enrichment was successfully achieved within 30 operational days under two different cycle configurations, regardless of whether biomass withdrawal occurred at the end of feast (R1) or famine phase (R2). The strategy of starting with a single raw UCO pulse and the subsequent distribution in pulses promoted an increase in active biomass in both reactors. The replacement of ammonium chloride by urea as the nitrogen source reduced chemical consumption for pH control, improved the overall process stability and increased the PHBV accumulation, achieving values of 40.1 wt. %. Furthermore, the nitrogen source change acted as a driving force for bacterial community shifts, leading to the selection of the genus *Aquabacter*. These results indicate that, while the withdrawal moment does not critically influence enrichment, carbon feeding strategy and nitrogen source are key factors for the effective culture selection and growth.

The comparison between one-unit and two-unit systems at different HRTs identified 48 h as the optimal value in both cases, where similar accumulation (44.8 and 49.1 wt. %), yields (250 and 230 g PHBV/kg UCO) and productivities (0.010 and 0.013 g PHBV/(L·h)) were obtained. Considering its operational simplicity and potential cost reduction, the one-unit system represents an efficient and practical strategy, as it integrates enrichment and accumulation within a single reactor.

CRediT authorship contribution statement

C. Ucha: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **S. Martínez-Rey:** Methodology, Investigation, Formal analysis, Data curation. **D. Correa-Galeote:** Visualization, Methodology, Investigation, Formal analysis. **A. Pedrouso:** Writing – review & editing, Supervision. **A. Mosquera-Corral:** Writing – review & editing, Supervision, Resources, Funding acquisition. **A.Val del Río:** Writing – review & editing, Writing – original draft, Supervision, Resources, Funding acquisition, Conceptualization.

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.2026.134291.

Data availability

Data will be made available on request.

References

Ahuja, V., Singh, P.K., Mahata, C., Jeon, J.M., Kumar, G., Yang, Y.H., Bhatia, S.K., 2024. A review on microbes mediated resource recovery and bioplastic (polyhydroxyalkanoates) production from wastewater. Microbial Cell Factories 2024 23:1 23, 187-. doi:10.1186/S12934-024-02430-0.

Alaux, E., Marie, B., Couvreur, M., Bounouba, M., Hernandez-Raquet, G., 2023. Impact of phosphorus limitation on medium-chain-length polyhydroxyalkanoate production by activated sludge. Applied Microbiology and Biotechnology 2023 107:11 107, 3509–3522. doi:10.1007/S00253-023-12528-X.

APHA-AWWA-WPCF, 2017. Standard methods for the examination of water and wastewater. American Public Health Assoc.(APHA): Washington, DC, USA 23.

Argiz, L., Val del Río, Á., Correa-Galeote, D., Rodelas, B., Mosquera-Corral, A., 2022. Simplified engineering design towards a competitive lipid-rich effluents valorization. J. Environ. Manage. 317. https://doi.org/10.1016/j.jenvman.2022.115433.

Beghetto, V., 2025. Waste Cooking oils into High-Value Products: where is the Industry going? Polymers (basel). 17, 887. https://doi.org/10.3390/polym17070887.

Bengtsson, S., Werker, A., Visser, C., Korving, L., 2017. PHARIO: stepping stone to a sustainable value chain for PHA bioplastic using municipal activated sludge. Stowa 93.

Bruland, N., Bathe, S., Willems, A., Steinbüchel, A., 2009. Pseudorhodoferax soli gen. nov., sp. nov. and Pseudorhodoferax caeni sp. nov., two members of the class

Betaproteobacteria belonging to the family Comamonadaceae. Int. J. Syst. Evol. Microbiol. 59, 2702–2707. https://doi.org/10.1099/ijs.0.006791-0.
Campanari, S., Silva, E., F.A., Bertin, L., Villano, M., Majone, M., 2014. Effect of the organic loading rate on the production of polyhydroxyalkanoates in a multi-stage process aimed at the valorization of olive oil mill wastewater. Int. J. Biol. Macromol. 71, 34–41. https://doi.org/10.1016/j.ijbiomac.2014.06.006.
Chakravarty, P., Mhaisalkar, V., Chakrabarti, T., 2010. Study on poly-hydroxyalkanoate (PHA) production in pilot scale continuous mode wastewater treatment system. Bioresour. Technol. 101, 2896–2899. https://doi.org/10.1016/j.biortech.2009.11.097.
Chen, B., Wan, C., Mehmood, M.A., Chang, J.S., Bai, F., Zhao, X., 2017a. Manipulating environmental stresses and stress tolerance of microalgae for enhanced production of lipids and value-added products–a review. Bioresour. Technol. https://doi.org/10.1016/j.biortech.2017.05.170.
Chen, Z., Huang, L., Wen, Q., Zhang, H., Guo, Z., 2017b. Effects of sludge retention time, carbon and initial biomass concentrations on selection process: from activated sludge to polyhydroxyalkanoate accumulating cultures. J. Environ. Sci. 52, 76–84. https://doi.org/10.1016/J.JES.2016.03.014.
Correa-Galeote, D., Argiz, L., Val Del Rio, A., Mosquera-Corral, A., Juarez-Jimenez, B., Gonzalez-Lopez, J., Rodelas, B., 2022. Dynamics of PHA-accumulating bacterial communities fed with lipid-rich liquid effluents from fish-canning industries. Polymers (basel). 14, 1396. https://doi.org/10.3390/polym14071396.
Cruz, R.A.P., Oehmen, A., Reis, M.A.M., 2022. The impact of biomass withdrawal strategy on the biomass selection and polyhydroxyalkanoates accumulation of mixed microbial cultures. N. Biotechnol. 66, 8–15. https://doi.org/10.1016/j.nbt.2021.08.004.
Cruz, M.V., Freitas, F., Paiva, A., Mano, F., Dionísio, M., Ramos, A.M., Reis, M.A.M., 2016. Valorization of fatty acids-containing wastes and byproducts into short- and medium-chain length polyhydroxyalkanoates. N. Biotechnol. 33, 206–215. https://doi.org/10.1016/j.nbt.2015.05.005.
Diard, S., Carlier, J.P., Ageron, E., Grimont, P.A.D., Langlois, V., Guérin, P., Bouvet, O.M. M., 2002. Accumulation of Poly(3-hydroxybutyrate) from octanoate in different pseudomonas belonging to the rRNA homology group I. Syst. Appl. Microbiol. 25, 183–188. https://doi.org/10.1078/0723-2020-00114.
Estévez-Alonso, Á., Altamira-Algarra, B., Arnau-Segarra, C., van Loosdrecht, M.C.M., Kleerebezem, R., Werker, A., 2022. Process conditions affect properties and outcomes of polyhydroxyalkanoate accumulation in municipal activated sludge. Bioresour. Technol. 364, 128035. https://doi.org/10.1016/j.biortech.2022.128035.
Din, F.M., M., Ujang, Z., Loosdrecht, M. Van, Mohd, &, Ahmad, A., 2008. Polyhydroxyalkanoates (PHAs) production from saponified sunflower oil in mixed cultures under aerobic condition. Jurnal Teknologi.
Fazielawanie, N., Rashid, M., Suet, T., Amelia, M., Amirul, A.-A.-A., Bhubalan, K., 2021. Dual-production of polyhydroxyalkanoate and rhamnolipid by *Pseudomonas aeruginosa* UMTKB-5 using industrial by-products. Malaysian J. Anal. Sci.
Garay, L.A., Boundy-Mills, K.L., German, J.B., 2014. Accumulation of high-value lipids in single-cell microorganisms: a mechanistic approach and future perspectives. J. Agric. Food Chem. 62, 2709–2727. https://doi.org/10.1021/jf4042134.
Guho, N.M., Pokhrel, D., Abbasi, M., McDonald, A.G., Alfaro, M., Brinkman, C.K., Coats, E.R., 2020. Pilot-scale production of poly-3-hydroxybutyrate-co-3-hydroxyvalerate from fermented dairy manure: Process performance, polymer characterization, and scale-up implications. Bioresour. Technol. Rep. 12, 100588. https://doi.org/10.1016/J.BITEB.2020.100588.
Hori, K., Abe, M., Unno, H., 2009. Production of triacylglycerol and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) by the toluene-degrading bacterium Rhodococcus aetherivorans IAR1. J. Biosci. Bioeng. 108, 319–324. https://doi.org/10.1016/J.JBIOSC.2009.04.020.
Konzock, O., Zaghen, S., Fu, J., Kerkhoven, E.J., 2022. Urea Is a Drop-in Nitrogen Source Alternative to Ammonium Sulphate in Yarrowia Lipolytica. iScience 25, 105703. https://doi.org/10.1016/j.isci.2022.105703.
Kourmentza, C., Costa, J., Azevedo, Z., Servin, C., Grandfils, C., De Freitas, V., Reis, M.A. M., 2018. Burkholderia thailandensis as a microbial cell factory for the bioconversion of used cooking oil to polyhydroxyalkanoates and rhamnolipids. Bioresour. Technol. 247, 829–837. https://doi.org/10.1016/j.biortech.2017.09.138.
Lai, P.J., Shen, M.Y., Rani, A., Chu, C.Y., Petracchini, F., Wu, H.C., 2025. Advanced Bioconversion of sugary wastewater: Integrated polyhydroxybutyrate and hydrogen productions in a continuous airlift two-chamber bioreactor. Int. J. Hydrogen Energy 138, 1239–1248. https://doi.org/10.1016/j.ijhydene.2024.09.293.
Liang, J., Faucher, S.P., 2024. Interactions between chaperone and energy storage networks during the evolution of *Legionella pneumophila* under heat shock. PeerJ 12, e17197. https://doi.org/10.7717/PEERJ.17197/SUPP-20.
Liu, H., VanderGheynst, J.S., Darby, J.L., Thompson, D.E., Green, P.G., Loge, F.J., 2011. Factorial experimental designs for enhancement of concurrent Poly (Hydroxyalkanoate) production and brewery wastewater treatment. Water Environ. Res 83, 36–43. https://doi.org/10.2175/106143010X12681059116932.
Lorini, L., di Re, F., Majone, M., Valentino, F., 2020. High rate selection of PHA accumulating mixed cultures in sequencing batch reactors with uncoupled carbon and nitrogen feeding. N. Biotechnol. 56, 140–148. https://doi.org/10.1016/J.NBT.2020.01.006.
Lorini, L., Martinelli, A., Capuani, G., Frison, N., Reis, M., Sommer Ferreira, B., Villano, M., Majone, M., Valentino, F., 2021. Characterization of polyhydroxyalkanoates produced at pilot scale from different organic wastes. Front. Bioeng. Biotechnol. 9, 628719. https://doi.org/10.3389/fbioe.2021.628719.
Martino, L., Cruz, M.V., Scoma, A., Freitas, F., Bertin, L., Scandola, M., Reis, M.A.M., 2014. Recovery of amorphous polyhydroxybutyrate granules from Cupriavidus necator cells grown on used cooking oil. Int. J. Biol. Macromol. 71, 117–123. https://doi.org/10.1016/J.IJBIOMAC.2014.04.016.
Moita Fidalgo, R., Ortigueira, J., Freches, A., Pelica, J., Gonçalves, M., Mendes, B., Lemos, P.C., 2014. Bio-oil upgrading strategies to improve PHA production from selected aerobic mixed cultures. N. Biotechnol. 31, 297–307. https://doi.org/10.1016/J.NBT.2013.10.009.
Montiel-Jarillo, G., Carrera, J., Suárez-Ojeda, M.E., 2017. Enrichment of a mixed microbial culture for polyhydroxyalkanoates production: effect of pH and N and P concentrations. Sci. Total Environ. 583, 300–307. https://doi.org/10.1016/J.SCITOTENV.2017.01.069.
Moretto, G., Lorini, L., Pavan, P., Crognale, S., Tonanzi, B., Rossetti, S., Majone, M., Valentino, F., 2020. Biopolymers from urban organic waste: influence of the solid retention time to cycle Length ratio in the enrichment of a mixed microbial culture (MMC). ACS Sustain. Chem. Eng. 8, 14531–14539. https://doi.org/10.1021/ACSSUSCHEMENG.0C04980.
Mosquera-Corral, A., Val del Rio, A., Argiz Montes, L., 2022. Method for producing polyhydroxyalkanoates (PHAs) and triacylglycerides (TAGs).
Możejko-Ciesielska, J., Kiewisz, R., 2016. Bacterial polyhydroxyalkanoates: still fabulous? Microbiol. Res. 192, 271–282. https://doi.org/10.1016/j.micres.2016.07.010.
Nielsen, P.H., Kragelund, C., Seviour, R.J., Nielsen, J.L., 2009. Identity and ecophysiology of filamentous bacteria in activated sludge. FEMS Microbiol. Rev. 33, 969–998. https://doi.org/10.1111/J.1574-6976.2009.00186.X.
Obruca, S., Benesova, P., Oborna, J., Marova, I., 2013. Application of protease-hydrolyzed whey as a complex nitrogen source to increase poly(3-hydroxybutyrate) production from oils by Cupriavidus necator. Biotechnol. Lett. 2013 36:4 36, 775–781. doi:10.1007/S10529-013-1407-Z.
Pakalapati, H., Chang, C.K., Show, P.L., Arumugasamy, S.K., Lan, J.C.W., 2018. Development of polyhydroxyalkanoates production from waste feedstocks and applications. J. Biosci. Bioeng. 126, 282–292. https://doi.org/10.1016/j.jbiosc.2018.03.016.
Panadare, D.C., Rathod, V.K., 2015. Applications of Waste Cooking Oil other than biodiesel: a Review, iranian. J. Chem. Eng.
Rodriguez-Perez, S., Serrano, A., Pantión, A.A., Alonso-Fariñas, B., 2018. Challenges of scaling-up PHA production from waste streams. A review. J. Environ. Manage. 205, 215–230. https://doi.org/10.1016/j.jenvman.2017.09.083.
Ruiz, C., Kenny, S.T., Ramesh Babu, P., Walsh, M., Narancic, T., O'Connor, K.E., 2019. High Cell Density Conversion of Hydrolysed Waste Cooking Oil Fatty Acids Into Medium Chain Length Polyhydroxyalkanoate Using *Pseudomonas putida* KT2440. Catalysts 2019, Vol. 9, Page 468 9, 468. doi:10.3390/CATAL9050468.
Satoh, H., Mino, T., Matsuo, T., 1999. PHA production by activated sludge. Int. J. Biol. Macromol. https://doi.org/10.1016/S0141-8130(99)00021-5.
Smolders, G.J.F., van der Meij, J., van Loosdrecht, M.C.M., Heijnen, J.J., 1994. Stoichiometric model of the aerobic metabolism of the biological phosphorus removal process. Biotechnol. Bioeng. 44, 837–848. https://doi.org/10.1002/bit.260440709.
Supple, B., Howard-Hildige, R., Gonzalez-Gomez, E., Leahy, J.J., 2002. The effect of steam treating waste cooking oil on the yield of methyl ester. Journal of the American Oil Chemists' Society 2002 79:2 79, 175–178. doi:10.1007/S11746-002-0454-1.
Takahashi, S., Tomita, J., Nishioka, K., Hisada, T., Nishijima, M., 2014. Development of a prokaryotic universal primer for simultaneous analysis of Bacteria and Archaea using next-generation sequencing. PLoS One 9. https://doi.org/10.1371/journal.pone.0105592.
Tamang, P., Nogueira, R., 2021. Valorisation of waste cooking oil using mixed culture into short- and medium-chain length polyhydroxyalkanoates: effect of concentration, temperature and ammonium. J. Biotechnol. 342, 92–101. https://doi.org/10.1016/j.jbiotec.2021.10.006.
Valentin, H.E., Broyles, D.L., Casagrande, L.A., Colburn, S.M., Creely, W.L., Delaquil, P. A., Felton, H.M., Gonzalez, K.A., Houmiel, K.L., Lutke, K., Mahadeo, D.A., Mitsky, T. A., Padgette, S.R., Reiser, S.E., Slater, S., Stark, D.M., Stock, R.T., Stone, D.A., Taylor, N.B., Thorne, G.M., Tran, M., Gruys, K.J., 1999. PHA production, from bacteria to plants. Int. J. Biol. Macromol. https://doi.org/10.1016/S0141-8130(99)00045-8.
Valentino, F., Lorini, L., Gottardo, M., Pavan, P., Majone, M., 2020. Effect of the temperature in a mixed culture pilot scale aerobic process for food waste and sewage sludge conversion into polyhydroxyalkanoates. J. Biotechnol. 323, 54–61. https://doi.org/10.1016/J.JBIOTEC.2020.07.022.
Vishniac, W., Santer, M., 1957. The thiobacilli. Bacteriol. Rev. 21, 195–213. https://doi.org/10.1128/mmbr.21.3.195-213.1957.
Yao, F., Yuan, K., Zhou, W., Tang, W., Tang, T., Yang, X., Liu, H., Li, F., Xu, Q., Peng, C., 2024. Unlocking growth potential in Halomonas bluephagenesis for enhanced PHA production with sulfate ions. J. Ind. Microbiol. Biotechnol. 51, 13. https://doi.org/10.1093/jimb/kuae013.
Zeng, S., Song, F., Lu, P., He, Q., Zhang, D., 2018. Improving PHA production in a SBR of coupling PHA-storing microorganism enrichment and PHA accumulation by feed-on-demand control. AMB Express 2018 8:1 8, 1–12. doi:10.1186/S13568-018-0628-X.