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RESEARCH ARTICLE

Microbiota structure shift under varying organic loading rates and their impact on polyhydroxyalkanoate production in wastewater treatment plants

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Abstract The conversion of "linear" conventional wastewater treatment plants (WWTP) into "circular" biorefineries offers an eco-friendly and cost-effective method of extracting valuable resources from waste. Microbiome of sewage sludge (SS) enable the fermentation of organic contaminants, producing volatile fatty acids that can be further used to produce polyhydroxyalkanoates (PHAs)—essential bioplastic precursors that can replace petroleum-based plastics. Optimizing PHA production requires understanding the relationships between SS microbiota structure and the operational parameters. Thus, this study examines how organic loading rate (OLR) influences PHA production in a selection sequencing batch reactor (S-SBR) within a pilot-scale WWTP collecting wastewater from various facilities at the University of Palermo campus. The S-SBR, enriched with PHA-producing microorganisms, was fed by a synthetic VFA mixture under two OLR conditions and subjected to a feast-famine (F/F) cycle. The results demonstrated that high OLR level increased PHA yield and promoted the selection of bacteria involved in organic matter degradation and PHA production. Specifically, a OLR of 1.3 g COD L^{-1} d^{-1} resulted in PHA productivity reaching 20% of biomass content, compared to 15% at a lower OLR of 0.8 g COD L^{-1} d^{-1}. Indeed, metaxonomics revealed structural changes in the SS microbiota, with higher OLR driving a selection for PHA-producing bacteria belonging to Proteobacteria phylum, mainly the Rhodocyclaceae family—whose members are known for PHA production capabilities—showing increased abundance in comparison to the starting and the lower OLR conditions. Thus, shifts in microbiota structure linked to OLR variation may account for the differences in PHA yields observed under realistic conditions, thereby supporting future research toward the development of full-scale biorefinery systems.

Keywords Metataxonomic analyses · Polyhydroxyalkanoates · Rhodocyclaceae · Sewage sludge microbiota

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Introduction

One of the most serious environmental problems is pollution from petroleum-derived plastics, compromising soil productivity, water quality, and ecosystems (Galitskaya et al. 2021). In recent decades the world has produced as much plastic as in the previous half-century, derived mainly from crude oil, used as chemical raw materials and a fuel source, causing health problems for humans, animals, and the environment (Rhodes 2018). Plastic in the environment can be fragmented into smaller microplastics, enhancing its ability to absorb and concentrate organic pollutants. The direct human ingestion of microplastics through drinking water is a real possibility; even food can be contaminated by microplastics in the air. It is also surprising that microplastics have been detected in the soil, most likely transported by the wind (Wang et al. 2021). For these reasons, today, there is a growing emphasis on replacing petrochemical-derived plastics with bioplastics. Among the promising materials are thermoplastic biodegradable polyesters known as polyhydroxyalkanoates (PHAs).

PHAs have recently gained attention as innovative biopolymers and are considered renewable alternatives to traditional petrochemical polymers. PHAs are natural polyesters that microorganisms accumulate as insoluble granules under specific environmental conditions. PHA production is promoted during unbalanced growth when a carbon source is proficient while other essential nutrients, such as nitrogen and phosphorus, become limiting. This survival mechanism allows bacteria to store excess carbon for future energy use when primary carbon sources become scarce. In addition, by applying cycles of carbon substrate availability/unavailability (feast/famine), it is possible to promote the selection and enrichment of PHA producer microorganisms within a mixed microbial culture (MMC). Volatile fatty acids (VFAs) can be excellent precursors to feed the production of granules of PHAs that can reach up to 90% of the dry weight of the microbial mass (Shahid et al. 2021). The VFA conversion process into PHAs typically is facilitated under conditions where other nutrients, such as nitrogen and phosphorus, are provided in sufficient amounts to support bacterial growth and metabolism. VFAs are intermediate products in the anaerobic digestion process of organic waste. MMC and axenic cultures of microorganism can produce VFAs (Di Leto et al. 2022; Mineo et al. 2024). Since the current production costs of PHAs are not competitive with those of petroleum-based plastics due to the high costs of the starting organic material (Al Battashi et al. 2021), literature has focused on adopting raw waste material to produce PHAs. In this regard, the sewage sludge (SS) produced in wastewater treatment plants (WWTPs) could become a resource rather than a waste, according to the circular bioeconomy principles, mainly because of the high value-added chemicals that can be recovered from it. Nowadays, SS accumulated in WWTP is typically disposed of in solid waste landfills or incinerators.

During the last ten years, a fundamental shift in wastewater treatment plants WWTPs has become imperative for promoting environmentally sustainable development according to circular bioeconomy principles. In this context, applying the PHA production process within the WWTP operation may lead towards sustainable and efficient waste valorization. However, this integration implies several challenges. Indeed, an efficient PHA production process has to follow the legislation limits for discharge applied to wastewater treatment while applying favouring conditions for PHA-producer microorganisms. To date, various operating process parameters have been investigated to optimize PHA production. For instance, Samrot et al. (2021) emphasized the importance of selecting an appropriate carbon-rich substrate for efficient PHA production, noting that an excessive carbon supply can promote PHA accumulation but reduce overall biomass growth. Similarly, Liang et al. (2024) highlighted those factors such as pH, the type of fermented feedstock, and the organic load significantly, impact PHA yields. A deep comprehension of the operational parameters influencing PHA production by MMC cultivations is crucial for process optimisation. This understanding can also provide valuable insights into the role of SS microbiota in the PHA production process, enabling the development of strategies to enhance efficiency and adhere to regulatory requirements. While bacterial and archaeal species have a pivotal impact on PHA production, current research focuses on understanding how the operational conditions affect microbiota and PHA production. Several studies to date have investigated the effect of organic loading rate (OLR) on PHA production. For example, Campanari et al. (2014) evaluated the impact of OLR starting from

mill wastewater exploiting the mixed microbial community. However, a metataxonomic analysis was not conducted to identify the microorganisms involved and their relative percentage abundances. Lagoa-Costa et al. (2022) evaluated the effect of OLR on brewery wastewater by focusing on the microorganisms involved and identifying possible PHA-producing bacteria.

In this present study, a SS MMC was fed using a synthetic mixture of VFAs and subjected to a feast-famine (F/F) cycle in a selection sequencing batch reactor (S-SBR) of a pilot-scale WWTP to enhance PHA production. In particular, the pilot-scale WWTP set in the work is placed at the University of Palermo campus thanks to a deviation line that collects wastewater from various facilities within the campus (Mannina et al. 2023). A proficient carbon substrate is provided in the feast phase, allowing the microorganisms to grow and accumulate PHAs as storage granules. In the famine phase, the carbon source is limited, restricting cell growth and forcing the microorganisms to use the stored PHAs as an energy source. The experimental campaign was conducted at two different OLR aiming at highlighting the possible connection between three key aspects in the WWTP processes: (i) the reuse of wastewater-derived compounds as bioplastic precursors for PHA production, (ii) the variation of OLR, and (iii) the SS microbiota members putatively responsible for organic matter degradation and PHA synthesis. To elucidate this possible relationship, a metataxonomic analysis of SS samples was performed before and after the enrichment selection process for investigating how the microbiota structure changes in response to OLR variations. Although the effect of OLR on PHA production is already well-established in the literature, this study stands out primarily for the direct use of SS produced within an university campus, making it one of the first real-world examples of local reuse of domestic SS in an academic context. Indeed, the results are innovative not only from a technical standpoint but also in terms of sustainability and practical applicability. The initiative implemented on the Palermo campus represents a pioneering example of circular economy in action: it converts internal waste into a valuable resource, integrates wastewater treatment with bioplastic production, and offers a scalable model for other university or domestic environments.

Materials and methods

Experimental setup and analytical methods

The experimental activities were carried out at the Water Resource Recovery Facility of the Palermo University Campus (Mannina et al. 2021a, 2021b, 2023). Specifically, at the PHA production line of the pilot plant hall, the excess sludge produced from the wastewater treatment pilot plant is used to produce the biopolymer (Mannina and Mineo 2023). The PHA production was carried out by adopting an enriched accumulation strategy by aerobic dynamic feeding (Isern-Cazorla et al. 2023). As described in Fig. 1, the system was composed of an enrichment reactor (S-SBR, operating volume 30 L) and a batch accumulation reactor (operating volume 1.5 L). The influence of the OLR was studied in the enrichment process by applying two OLRs: 1.3 g COD L^{-1} in S-SBR A and 0.8 g COD L^{-1} in S-SBR B. The OLR difference is related to the different substrate concentration in the feeding. The S-SBR cycle lasted 12 h and consisted

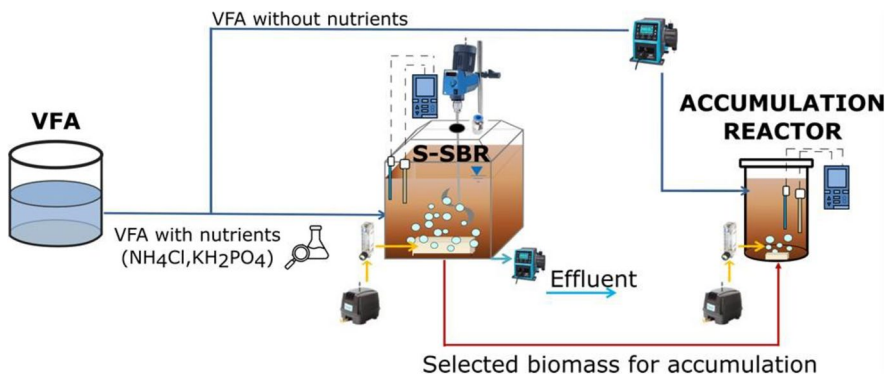

Fig. 1 Schematic representation of the system adopted

of feeding (15 min), aerobic feast-famine (660 min), settling (30 min), and effluent withdrawal (15 min). The S-SBR monitoring started after the feast/famine ratio did not change significantly (<2% deviation) for around 10 days. After 30 days of monitoring, the enriched sludge was used to accumulate PHA in the batch reactors. The biomass (around 3 L) was withdrawn from the S-SBR, washed with tap water and left to settle to remove 1 L of supernatant. Synthetic substrate without NH_4Cl (1 L) was added and the biomass was left aerated overnight. This step is required as the biomass has to reach endogenous conditions before starting the accumulation test. The accumulation was performed by adopting a feed-on-demand approach by means of software (Mineo et al. 2023). More detailed information about the process can be found in the literature, while the operating conditions of the two S-SBRs are reported in Table 1 (Isern-Cazorla et al. 2023; Mineo et al. 2023).

The system was fed a synthetic VFA mixture that mimicked the fermented sludge liquid. According to the literature, the substrate was composed of 70% acetic acid and 30% propionic acid (Frison et al. 2021). Ammonium (NH_4^+) and phosphate (PO_4^{3-}) were added as nutrients by dosing NH_4Cl and K_2HPO_4 in the VFA mixture. The average composition of the substrate is reported in Table 2. The synthetic substrate adoption is used to ensure experimental reproducibility and to isolate the effect of the operational parameters without interference from variable influent composition.

Table 2 Average characteristics of the adopted substrate

Parameter	U.M	S-SBR A		S-SBR B	
		Average	Standard deviation	Average	Standard deviation
sCOD	mg/L	2488	556	1305	248
NH_4-N	mg N/L	121	21	68	6
PO_4-P	mg P/L	18	2	12	2

Prokaryotic microbiota structure analysis

The prokaryotic microbiota structure in the SS was analysed through metataxonomic analysis using next-generation sequencing (NGS) of 16S rDNA gene amplicons derived from metagenomic DNA. Specifically, four SS samples were examined before and after the enrichment. T0-A and T0-B represent the initial SS used for the two S-SBR experiments, A and B, respectively. At the same time, TF-A and TF-B are samples collected before the accumulation tests. The analyses were conducted on unfiltered and uncentrifuged samples following established protocols (Presti et al. 2021; Di Leto et al. 2024). DNA extractions were verified through 1% (w/v) agarose gel electrophoresis, with ethidium bromide (0.5 µg/mL) aiding visualisation under UV light. DNA concentrations extracted from 1 g of SS samples and corresponding tenfold serial dilutions were measured by NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) absorbance readings at 260 nm. Purity assessments of extracted DNA were determined by absorbance ratios (260/280 and 260/230 nm) to individuate contamination due to protein, organic compound, or chaotropic agent contamination. PCR targeting the 16S rDNA gene utilised parameters and primers detailed in Di Leto et al. 2024. Amplification products underwent sequencing via one 300 bp paired end run on an Illumina MiSeq platform at BMR Genomics (Padova, Italy). Raw

Table 1 Average operational parameter of the two enrichment reactors

Parameter	U.M	S-SBR A		S-SBR B	
		Average	Standard deviation	Average	Standard deviation
Organic Loading Rate—OLR	g COD L^{-1} d^{-1}	1.3	0.3	0.8	0.2
Hydraulic Retention Time—HRT	hours	2	0.1	2	0.1
Sludge Retention Time—SRT	days	3	0.5	3	0.5
Food to microrganisms ratio—F/M	kg BOD kg^{-1} VSS·d^{-1}	0.4	0.2	0.3	0.1
Mixed Liquor Suspended Solids	g L^{-1}	4.5	0.1	4.8	0.9
Feast to famine ratio—F/F	min min^{-1}	0.16	0.05	0.23	0.11

16S rDNA data were processed using QIIME2 software (https://qiime2.org/) as paired-end sequences. DADA2 plugin was employed for denoising and processing overlapping paired end reads. Sequence data are deposited in the The National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/) with the accession code PRJNA1268185.

Unique amplicon sequence variants (ASVs) were assigned and aligned to the Greengenes reference database at 99% sequence similarity (https://greengenes.secondgenome.com/). ASV numbers and relative abundances of phyla, orders, classes, and families were determined for each sample. HeatMap generation utilised the online web server (http://heatmapper.ca/expression/) with a "complete linkage" calculation based on Pearson correlation, focusing on families. METAGENassist (http://www.metagenassist.ca) distinguished microbial species based on metabolic activity.

Enrichment and accumulation analysis

The substrate and S-SBR effluent were sampled twice per week and analysed for sCOD, NH_4^+-N and PO_4^{3-}-P while the S-SBR mixed liquor was sampled and analysed once per week for total suspended solids. The mixed liquor in the S-SBR and the accumulation reactor was sampled at the end of the feast phase and the end of the accumulation process (30th hour). sCOD, NH_4^+-N and PO_4^{3-}-P analysis was performed in filtered (0.45 µm) samples by UV–VIS spectroscopy. The suspended solids content was analysed according to standard methods (Baird et al. 2017). Samples for PHA analysis were mixed with 10 mL formaldeyde solution (37%w/w) to stop the biomass' biological activity. The pellet was lyophilised after centrifugation (8000 rpm, 40 min). A fixed amount of lyophilized sample was weighted in test tube, mixed with butanol (1.5 mL) and chloridric acid (0.5 mL) and incubated at 100 °C for 8 h, as reported in the literature (Werker et al. 2008). Hexane (2.5 mL) is then used to extract the organic phase, which is later washed with milli Q grade water (4 mL). The liquor is centrifuged (8000 rpm, 10 min), and the organic phase is filtered (0.22 µm) for gas chromatography analysis. A gas chromatographer equipped with a Restek stabilwax column (30 m × 0.53 mm × 1.00 µm film thickness) and a flame ionization detector are used to detect hydroxybutyrate (HB) and hydroxyvalerate (HV) concentrations according to the protocol described by Montiel-Jarillo et al. (2017). The PHA concentrations are calculated as the sum of poly HB and poly HV and expressed as the weight percentage on volatile suspended solids (VSS) (Conca et al. 2020). The PHA storage yield was expressed as g COD_{PHA}/g COD_{VFA}, considering the conversion factors for PHB and PHV.

Results and discussion

Enrichment process of PHA production

The performance of the enrichment process was assessed by calculating the sCOD, NH_4^+ and PO_4^{3-} removal efficiencies, reported in Table 3. At both OLRs, the S-SBR achieved a high sCOD removal, always higher than 91%, while a slight worsening was noticed in NH_4^+ removal. S-SBR A NH_4^+ removal accounted for 83.7 ± 12.1% while it reached 75.6 ± 11.9% in S-SBR B. The small difference may be related to the different concentrations

Table 3 Trend of removal efficiency for the S-SBRs

Days	S-SBR A removal efficiency [%]			S-SBR B removal efficiency [%]		
	sCOD	NH_4^+	PO_4^{3-}	sCOD	NH_4^+	PO_4^{3-}
3	96.70 ± 2.82	92.20 ± 2.69	79.60 ± 2.06	97.10 ± 1.97	91.10 ± 2.14	64.20 ± 1.53
6	98.00 ± 2.80	98.50 ± 2.62	75.70 ± 1.93	96.30 ± 2.06	84.80 ± 1.99	80.80 ± 2.05
9	98.60 ± 2.73	96.00 ± 2.77	78.70 ± 1.95	97.00 ± 2.06	64.70 ± 1.92	96.90 ± 2.07
13	99.50 ± 2.22	79.80 ± 2.22	76.20 ± 1.59	94.50 ± 2.39	72.10 ± 1.49	83.70 ± 2.48
16	99.40 ± 2.66	74.60 ± 2.01	73.70 ± 1.61	91.90 ± 2.13	73.30 ± 2.13	91.10 ± 2.54
20	98.20 ± 2.67	83.00 ± 2.17	74.90 ± 2.10	95.00 ± 2.70	55.10 ± 1.49	92.00 ± 1.96
25	99.70 ± 2.32	61.80 ± 1.30	70.90 ± 1.73	98.50 ± 2.16	77.20 ± 2.14	88.60 ± 1.94
30	98.60 ± 2.45	83.70 ± 2.39	74.90 ± 1.93	98.00 ± 2.86	86.30 ± 1.82	81.00 ± 2.03

of influent NH_4^+ (Table 2) between the two S-SBRs rather than a better performance. As also proved by the PO_4^{3-} removal efficiency, there is a non-direct correlation between OLR and process performance.

On the contrary, OLR of the enrichment affected the PHA production in enrichment and accumulation. As reported in Fig. 2, the OLR increment had a positive effect on PHA production, reaching 6.6% w/w at the end of the feast phase and 19.6% w/w at the end of the accumulation process for S-SBR A. Adopting 0.8 g COD L^{-1} as OLR (S-SBR B) resulted in a lower PHA production during the enrichment (6% w/w) and the accumulation (15.1% w/w). A higher PHV fraction (6.9% w/w) was produced in accumulation test A compared to accumulation test B, which achieved 4.5% w/w. The effect was mainly related to the better-performing enrichment process, as the same substrate with the same propionic acid share was used in both systems for the accumulation. Previous studies, such as Abate et al. (2024), have already addressed PHA recovery strategies, polymer properties, and economic evaluations under similar operating conditions. In this work, at an OLR of 1.3 g $CODL^{-1}$ d^{-1}, PHA productivity reached 20% of the biomass content, compared to 15% at an OLR of 0.8g$CODL^{-1}$ d^{-1}, demonstrating an increase in the efficiency of converting carbon into biopolymers. With an improvement of the polymer quality. Additionally, the OLR adopted in the enrichment (within 1.3 g COD L^{-1}) doesn't significantly affect the contaminants removal efficiency but rather increases the amount of PHA accumulated

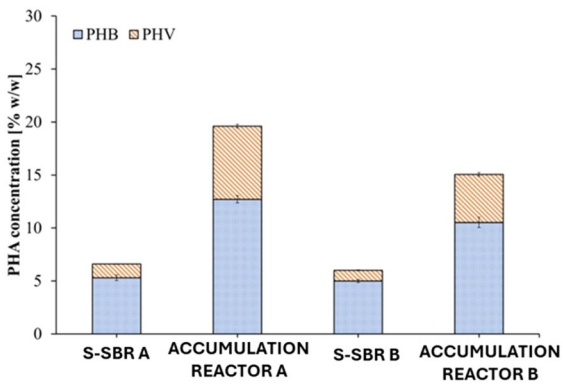

Fig. 2 Amount of PHA produced at the end of the feast phase and at the end of the accumulation process for the two conditions (**A** and **B**) analysed. **A** 1.3 g COD L^{-1} OLR; **B** 0.8 g COD L^{-1} OLR

(Estévez-Alonso et al. 2022). However, the PHA storage yield was low for both systems, achieving 0.18 $gCOD_{PHA}/gCOD_{VFA}$ and 0.14 $gCOD_{PHA}/gCOD_{VFA}$ in systems A and B, respectively. Despite the slightly higher amount of PHA produced in system A, the low storage yield highlights that the OLR applied during the selection was too low to achieve the threshold of 0.4 $gCOD_{PHA}/gCOD_{VFA}$ (Werker et al. 2018).

Structure at phylum level of SS prokaryotic microbiota residing in the S-SBR

Prokaryotic member abundance in the SS was determined by performing NGS analysis of 16S rDNA gene amplicons obtained from the metagenomic DNA using the SS of two PHA production experiments at the pilot plant different only for the vORL. All ASVs detected in this work have been assigned to the bacteria domain (100%) and no microorganisms belonging to the archaea domain have been identified. The absence of Archaea in the initial samples can be attributed to the characteristics of the sewage sludge. This observation is consistent with previous studies (Di Leto et al. 2025), in which the prokaryotic microbiota structure was exclusively constituted by Bacteria, with no detectable presence of Archaea. Furthermore, even if Archaea had been present in the initial samples, the modulation of the OLR to enhance PHA production would have selectively favored heterotrophic bacterial populations capable of accumulating intracellular polymers under conditions of carbon excess or environmental stress. Consequently, in these conditions, Archaea would have not survived and their abundance would have been drastically reduced. Several previous studies on SS produced similar results, indicating that bacteria made up nearly the entire prokaryotic population in SS (98.8%), while archaea represented only 1.2% (Yu et al. 2012; Zhang et al. 2012; Xia et al. 2010; McLellan et al. 2010; Mathur et al. 2023). Our previous studies focused on metataxonomic analyses of SS exploited for VFA production, but we revealed only bacteria (Di Leto et al. 2024). For each experiment, two samples were collected and analysed, the starting samples, T0-A and T0-B, and the final samples, TF-A and TF-B. Although the samples were collected in two different seasons (winter and spring, respectively), and despite the variations in temperature conditions, the T0 samples exhibited a similar

microbial composition. The most represented phyla in both experiments were Proteobacteria, Bacteroidetes followed by Verrucomicrobia. These three bacterial phyla constitute the core prokaryotic community of SS, consisting of microbial species always found, regardless of seasonal, geographical, or environmental changes. On the other hand, each SS features variable microbial components. The existence of both a core and a variable community is crucial for the resilience and efficiency of SS treatment processes. The core community ensures the continuity of essential functions, while the variable community provides an adaptive response to variations in treatment conditions, as suggested by Saunders et al. 2016. In previous studies, such as Yu and Zhang (2012) and Scaccia et al. (2020), it has been confirmed that the core community of SS typically includes Proteobacteria, Bacteroidetes, and Verrucomicrobia, as well as Firmicutes and Actinobacteria. In our study, Proteobacteria, Bacteroidetes, and Verrucomicrobia were found at 54.52%, 37.36%, and 4.52% in T0-A, and at 48.64%, 41.47%, and 3.59% in T0-B, respectively (Fig. 3). Firmicutes and Actinobacteria were present in low percentages, at 0.42% and 0.01% in T0-A, and 0.81% and 0.29% in T0-B. Compared to our previous studies, the microbial community consistently includes a core of Proteobacteria and Bacteroidetes, with some variations: in Di Leto et al. (2022), the initial SSs also featured Firmicutes and Chloroflexi; in Mineo et al. (2024), Chlamydiae were also present; in Di Leto et al. (2024), along with Proteobacteria and Bacteroidetes, Firmicutes and Verrucomicrobia were also identified, similar to the current findings. Therefore, the fluctuations in the core community could be due to environmental and anthropic conditions, microbial competitions and interactions (Zeng et al. 2022; Peces et al. 2022) or technical reasons (Crognale et al. 2022). Phyla not deeply studied were found in T0-A and T0-B; for example, the phylum GN02 was present at 1.45% in T0-A and 1.07% in T0-B. The phylum Acidobacteria was found at 0.42% in T0-A and 0.79% in T0-B.

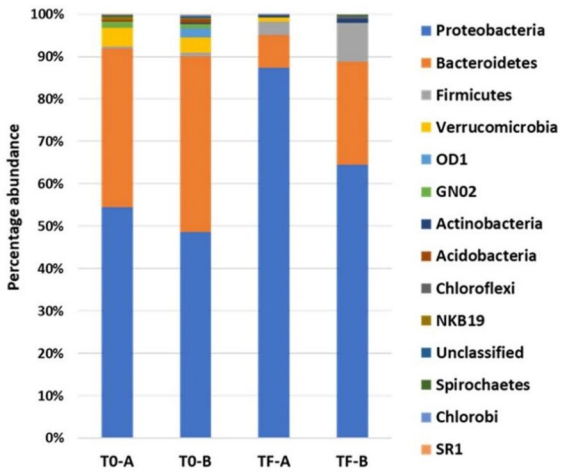

Fig. 3 Histograms reporting the relative abundances of prokaryotic microbiota taxa of phyla of T0-A, TF-A, T0-B, and TF-B. The relative abundance indicated the percentage of each phylum compared to all the identified phyla

By analysing the capabilities of these bacterial phyla in PHA production, Proteobacteria emerged as the best PHA producers. In a study on PHA production from acetate (Sruamsiri et al. 2020), metataxonomic analysis revealed the predominance of Proteobacteria, specifically Betaproteobacteria (51.37% of total sequences), Gammaproteobacteria (23.44% of total sequences) and Alphaproteobacteria (13.26% of total sequences). In our study, in T0-A Proteobacteria accounted for 54.52%, with 19.79% Betaproteobacteria, 15.32% Gammaproteobacteria, 10.52% Deltaproteobacteria, 6.88% Alphaproteobacteria, and 2.02% Epsilonproteobacteria (Fig. S1). In T0-B, Proteobacteria accounted for 48.64% with 18.47% Betaproteobacteria, 12.76% Gammaproteobacteria, 10.92% Alphaproteobacteria, 4.09% Deltaproteobacteria and finally 2.40% Epsilonproteobacteria.

In experiment A, where the highest yield of PHAs was achieved (20% w/w), Proteobacteria increased from 54.52% at T0-A to 87.41% at TF-A. Specifically, this population was constituted by 97.06% Betaproteobacteria, 1.28% Alphaproteobacteria and Gammaproteobacteria, Deltaproteobacteria, and Epsilonproteobacteria disappeared. In experiment B, where the yield of PHAs was 18% w/w, Proteobacteria also increased, but to a lesser extent than in experiment A. Their population grew from 48.64% at T0-B to 64.55% at TF-B, showing a 1.33-fold increase. This population of TF-B consisted of 34.10% Betaproteobaceria, 23.17% Alphaproteobacteria, 5.99% Gammaproteobacteria, 1.22% Deltaproteobacteria and finally, 0.07% Epsilonproteobacteria. Ultimately, a significant increase in Betaproteobacteria was observed in TF-A (4.91-fold increase), accompanied by a reduction in all other classes of Proteobacteria.

In TF-B, there was an increase in both Alphaproteobacteria (2.13-fold increase) and Betaproteobacteria (1.85-fold increase), while all other classes of Proteobacteria declined. As noted by Sruamsiri et al. (2020), Betaproteobacteria are primarily responsible for the production and accumulation of PHAs. Therefore, Betaproteobacteria make up 97.06% of all bacterial classes in the TF-A sample, which had the highest PHA yield, which can justify the higher PHA yield in experiment A compared to experiment B. For the other bacterial phyla observed, Bacteroidetes decreased from 37.36% at T0-A to 7.60% at TF-A, and from 41.47% at T0-B to 24.18% at TF-B. Verrucomicrobia declined from 4.52% at T0-A to 1.07% at TF-A, and from 3.59% at T0-B to undetectable in TF-B. The capabilities of Bacteroidetes and Verrucomicrobia in producing PHAs are not well-documented in the literature. While some species within these phyla may have the ability to produce PHAs (Sabapathy et al. 2020), they are not major producers compared to groups like Betaproteobacteria. Thus, the reduction in their percentages during experiments A and B is unlikely to significantly affect the overall PHA production. Actinobacteria slightly increased from 0.01% at T0-A to 0.36% at TF-A, and from 0.29% at T0-B to 0.98% at TF-B. In contrast, Acidobacteria and GN02 decreased from 0.42% and 1.45% at T0-A to 0.09% and 0.00% at TF-A and 0.79% and 1.07% at T0-B to 0.00% at TF-B. Given the low percentages, it is not likely that they contribute significantly to PHA production. An interesting increase in the percentage abundance was given by Firmicutes which increased from T0-A (0.42%) to TF-A (3.17%) and from T0-B (0.81%) to TF-B (9.25%), increasing 7.93 times in A and 11.57 times in B. Firmicutes, which are good VFA producers (Di Leto et al. 2022, 2024; Mineo et al. 2024), can also produce PHAs, although they are not among the major producers. Some members of the Firmicutes are capable of degrading PHAs, as this group includes species with PHA depolymerase enzymes that hydrolyze PHAs into monomers, making them usable as a source of carbon and energy (Vogel et al. 2021). Therefore, considering these points, we surmise that most of the PHA production was carried out by Proteobacteria, particularly Betaproteobacteria, especially in TF-A where the maximum PHA yield was achieved and where Betaproteobacteria were 97.06% of total sequences. The other bacterial phyla contributed minimally to PHA production, and sometimes, as in the case of Firmicutes in TF-B, they may have contributed to their degradation.

Structure at family level of the SS prokaryotic microbiota residing in the S-SBR and bacterial signatures

The SS microbiota was also studied at the taxonomic level of families (Fig. 4A) to better understand the changes and dynamics within the microbial community before and after the production. At family level, the primary families in the T0-A and T0-B samples were Rhodocyclaceae (6.36 and 5.78% respectively), Saprospiraceae 9.26 and 17.92%), Rhodobacteraceae (5.02 and 2.72%), Comamonadaceae (9.68 and 8.15%), Xanthomonadaceae (5.94 and 4.03%), Cytophagaceae (3.24 and 3.11%), Chitinophagaceae (7.93 and 2.63%), and Moraxellaceae (6.79 and 2.25%). Consistent with the abundances observed at the phyla level, the predominant component at the family level is proteobacteria. Specifically, Rhodobacteraceae belong to the Alphaproteobacteria class, Rhodocyclaceae and Comamonadaceae belong to the Betaproteobacteria class, and Xanthomonadaceae and Moraxellaceae belong to the Gammaproteobacteria. Following the PHAs production process, the bacteria were selected inside the reactor. While the starting samples presented a bacterial signature with a Proteobacteria/Bacteroidetes ratio (Fig. 4B) equal to 1.66 in the T0-A sample and with a Bacteroidetes/Proteobacteria ratio equal to 1.04 in T0-B, in the final samples, a radical change occurred. In TF-A, the Proteobacteria/Bacteroidetes ratio significantly increased, reaching 546.06. In TF-B, the microbial composition was inverted as the Proteobacteria increased compared to the Bacteroidetes, unlike the respective sample starting point, reaching a Proteobacteria/ Bacteroidetes ratio of 4.54. In TF-A, the only bacterial family that increased notably was the Rhodocyclaceae, which increased from 6.36% in T0-A to 96.62%; in the experiment B, Rhodobacteraceae rose from 2.72% in T0-B to 13.95%. In TF-B and Cytophagaceae, from 3.11% in T0-B reached 4.47%. All other bacterial families decreased. It is likely that in the TF-A sample, almost all of the PHAs were produced by Rhodocyclaceae (96.62% in TF-A compared to 1.97% in TF-B). Recent studies have confirmed that, during the production of PHAs from industrial waste, 60–70% of

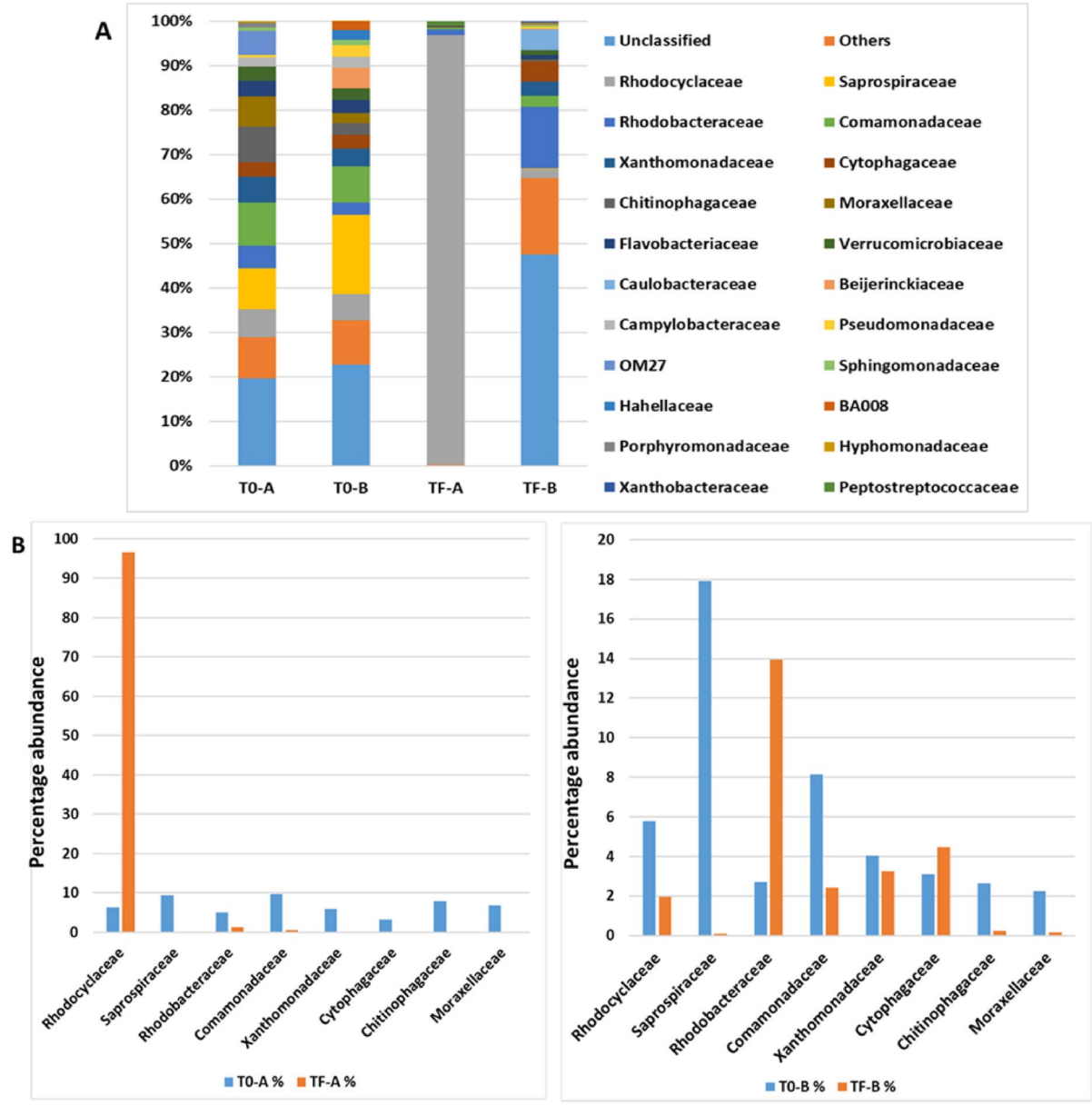

Fig. 4 **A** Histograms reporting the relative abundances of families of sewage sludge samples T0-A, TF-A, T0-B, and TF-B. The relative abundance indicated the percentage of each family compared to all the identified families. Taxa having percentage abundance lower than 1 were grouped as "Others". **B** Histograms reporting the percentage abundances of the leading bacterial families (Proteobacteria-Bacteroidetes) showing notable variations in percentage abundance between T0 and TF in the two different A and B experimental set-up. The percentage value indicates the relative abundance of each family in comparison to the overall abundance of the identified families

the microbial community was made up of Rhodocyclaceae, which are positively correlated to the production of PHAs after the depletion of the carbon source (De Donno Novelli et al. 2021). A previous study dealing with the modulation of carbon-to-nitrogen ratios reported an increase the relative abundance of Rhodocyclaceae family members which paralleled the observed PHA yield increase (Di Leto et al. 2025). Almeida and collaborators (2022) also confirmed that the increase in Rhodocyclaceae was concomitant with

the increase in OLR, which aligns perfectly with the results of this study. The Rhodobacteraceae (1.23% in TF-A and 13.95% in TF-B), being Proteobacteria, have good PHA production capabilities, accumulating over 84% of their weight in PHAs (Carvalho et al. 2022). There is little knowledge about the ability of Cytophagaceae to produce PHA. However, recent reports indicated a positive role of Cytophagaceae in PHA production (Perez-Zabaleta and collaborators 2021). Therefore, we surmise that the PHAs produced in TF-A can be attributed to Rhodocyclaceae, which represents the most abundant family after the production and selection process.

As previously discussed, the microbial composition of the two initial samples, T0-A and T0-B, was similar. This was confirmed by the heatmap analysis (Fig. 5). The heatmap was generated using hierarchical clustering based on the Pearson correlation distance metric and the average linkage algorithm. The data shown in the heatmap were normalized using row-wise Z-score transformation (i.e., each taxon was standardized across samples to have a mean of 0 and standard deviation of 1). This transformation highlights relative differences in abundance patterns among taxa. Heatmap showed the two T0 samples clustering into a single group. The TF-B sample was similar to the T0 samples. At the same time, the TF-A, which yielded the highest PHAs, exhibited a distinct microbial composition from the other samples and formed a separate cluster.

Structure at genus level of SS prokaryotic microbiota residing in the S-SBR

The most striking feature seen at the genera level was the predominance of *Azoarcus,* which, from 0.01% in T0-A, was present at 94.16% in TF-A (Fig. 6; Table S1). *Azoarcus* belongs to the Rhodocyclaceae family (Proteobacteria), the predominant family in the TF-A sample. Although *Azoarcus* genus represents 94.16% of the microbial community in TF-A (Fig. 6), suggesting a near monoculture, Fig. 4A reports a 96.62% presence of the Rhodocyclaceae family. The 2.46% discrepancy can be attributed to the presence of other genera belonging to the same family, including Thauera, Dechloromonas, and Candidatus Accumulibacter, which were detected in smaller percentages. These genera, while not predominant, still

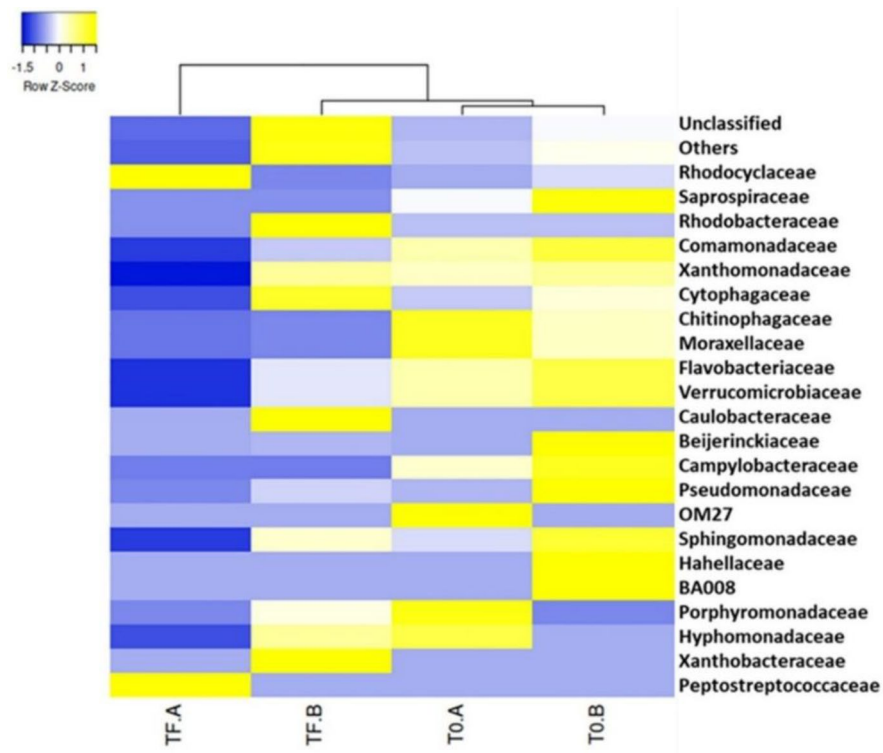

Fig. 5 Heatmap of 24 most abundant prokaryotic family composition profiles in T0-A, TF-A, T0-B and TF-B samples as inferred by HeatMapper bioinformatic analysis. The heatmap was generated using hierarchical clustering based on the Pearson correlation distance metric and the average linkage algorithm. The colour intensity in each sample was normalized using row-wise Z-score transformation in the two groups. Colours from blue to yellow indicated the relative values of microbiota

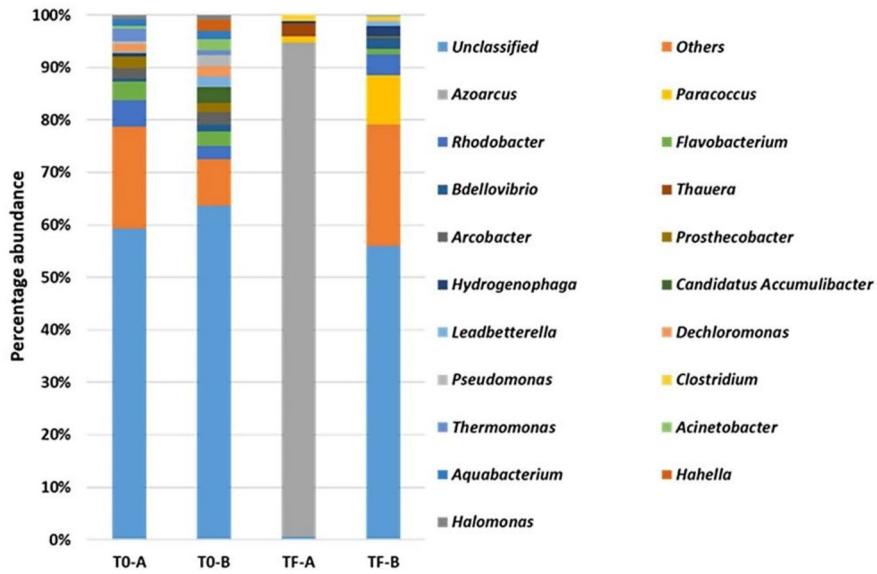

Fig. 6 Histograms reporting the relative abundances of prokaryotic microbiota taxa of sewage sludge samples T0-A, TF-A, T0-B, and TF-B at the level of genera. The relative abundance indicated the percentage of each genus compared to all the identified genera. Taxa having percentage abundance lower than 1 are grouped as "Others"

contribute to the composition of the microbial community to a marginal extent compared to the genus *Azoarcus*. It is known to accumulate PHA as a carbon and energy reserve, especially under stressful conditions (Carvalho et al. 2022).

Even the *Thauera* genus belonging to the Rhodocyclaceae family (Proteobacteria) was found to increase from 0.01% in T0-A to 2.46% in TF-A (Fig. 6). Previous studies showed that the relative abundances of both *Thauera* and *Azoarcus* genera are positively associated with bioreactor removal processes for quinoline and petroleum hydrocarbons, as revealed by microbial community structure comparisons. (Liu et al. 2006; Di Bella et al. 2023). Interestingly, it was recently demonstrated that *Thauera* is a good PHA producer (Andreolli et al. 2022). In this study, *Azoarcus* and *Thauera* comprise approximately 96% of the genera in this sample, strongly suggesting their predominant role in PHA production. On the contrary, in TF-A, some bacterial genera were present in a lower percentage concerning TF-B, like *Paracoccus* (1.23% in TF-A and 9.49% in TF-B), *Bdellovibrio* (0.00% in TF-A and 2.12% in TF-B), *Rhodobacter* (0.00% in TF-A and 3.85% in TF-B) and *Hydrogenophaga* (0.44% in TF-A and 1.95% in TF-B). Their implication in PHA production was previously investigated with *Paracoccus* able to use synthetic short and medium-chain carboxylic acids as the principal carbon sources in the growth medium, producing a higher PHA rate, about yields of 26% of cell dry mass (Szacherska et al. 2022). However, some genera of *Paracoccus* can activate pathways that reduce the production of PHAs. In gene regulation, phasins (PhaPs) are predominantly proteins associated with PHA granules that positively influence PHA synthesis. However, the *phaR* gene has been identified in *Paracoccus*, which codes for a negative regulator involved in the expression of *phaP*. The latter plays a role in regulating the expression of PhaP and, therefore, indirectly modulating the production of PHA (Maehara et al. 2002). *Bdellovibrio* has a very delicate role in the final yield of PHAs. Some species of *Bdellovibrio* genus are included among the "predatory bacteria", i.e., bacteria capable of preying on gram-negative species that produce and accumulate PHAs. After having lysed the cell of the prey bacterium, the predator hydrolyzes thanks to a depolymerase (*pha*Z) and consumes part of the PHA released into the extracellular environment. This process allows the hydrolysis of significant quantities of PHA granules. In genetic engineering, *pha*Z-inactivated strains have been created to improve the recovery of PHA but not hydrolysis (Martinez et al. 2016). The genus *Hydrogenophaga* is capable of producing PHA; in particular, previous studies (Povolo et al. 2013) have shown that it is not only capable of producing PHA-containing comonomers such as 3-hydroxybutyrate, 3-hydroxyvalerate, and 4-hydroxybutyrate but also is capable of producing PHAs from waste resources. Even *Rhodobacter,* being a

Protobacterium, is capable of producing PHAs. The photosynthetic bacterium *Rhodobacter* produces high PHA levels when an adequate carbon source is available. The three essential genes used in the PHA biosynthetic pathway are *phaA*, which encodes beta-keto thiolase; *phaB*, which encodes acetoacetyl coenzyme A reductase; and *phaC*, which encodes PHA synthase (Kranz et al. 1997). Therefore, although *Rhodobacter* and *Hydrogenophaga* are PHA producers, the TF-B sample shows the highest percentages at the genus level for *Paracoccus* and *Bdellovibrio*. These genera, which can reduce production or degrade PHA, likely contributed to the lower yield observed in TF-B.

Predicting metabolism associated to SS microbiome of the S-SBR

The metabolic activities mainly represented in the starting samples (Fig. 7) were dehalogenation (18.2% in T0-A and 15.5% in T0-B), sulfate reduction (15.5% in T0-A and 12.7% in T0-B) and ammonia oxidization (19.1% in T0-A and 15.8% in T0-B). These metabolic functions may be connected to the production of PHAs. Dehalogenating strains, for instance, remove halogen atoms (such as chlorine, bromine, or iodine) from organic compounds and use halogenated compounds as electron acceptors. These bacteria can indirectly support the production of substrates for PHA synthesis by generating dehalogenated organic acids. However, toxic intermediate compounds can be formed during dehalogenation, inhibiting the growth of PHA-producing bacteria or disrupting the metabolic pathways involved in PHA synthesis. Additionally, these toxic products can negatively impact essential cofactors for PHA biosynthesis, such as NADH and ATP, thereby reducing production yields (Janssen et al. 2001). Sulfate-reducing bacteria can contribute to producing metabolites, such as volatile fatty acids like acetate and propionate, during the sulfate reduction process. These metabolites serve as precursors for the synthesis of PHAs by PHA-producing bacteria (Liu et al. 2015). Finally, some ammonia-oxidizing bacteria can also accumulate carbon intermediates that could be used for PHA synthesis. Fra-Vázquez and colleagues (2019) found that, in batch reactors, exposing a MMC cultivation to a feast/famine regime resulted in PHA yields reaching 60% of the bacterial mass by weight. Additionally, they observed the development of partial nitridation activity (oxidation of ammonia to nitrite), likely due to the inhibition by free ammonia or the selected sludge retention time. After the PHA production process, the selection of the microbial community members caused a reduction in the A experiment of dehalogenating bacteria (from 18.2% in T0-A to 1.4% in TF-A), sulfate reducers (from 15.5% in T0-A to 1.6% in TF-A) and ammonia-oxidizers (from 19.1% in T0-A to 1.6% in TF-A). In the B experiment, however, there was an increase in dehalogenating bacteria (from 15.5% in T0-B to 72.3% in TF-B), sulfate reducers (from 12.7% in T0-B to 68.8% in TF-B), and ammonia oxidizers (from 15.8% in T0-B to 27.3% in TF-B).

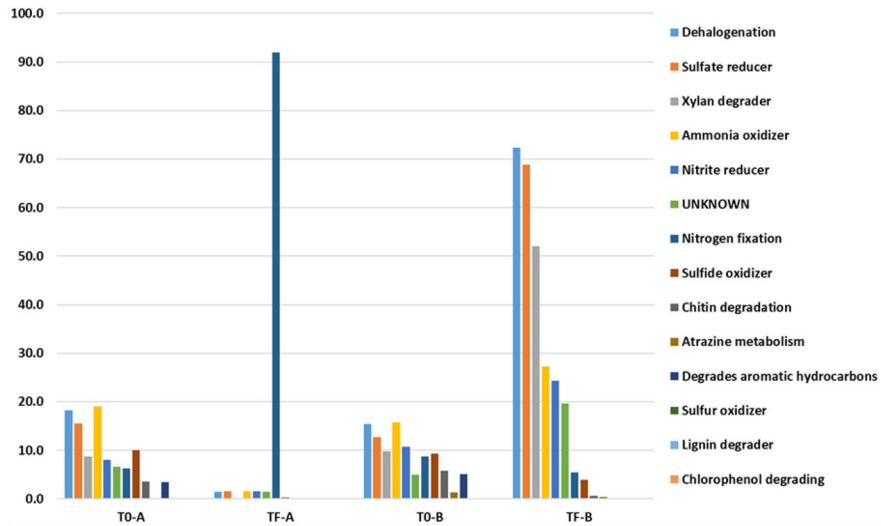

Fig. 7 Putative metabolic requirements of the bacterial communities residing in the samples T0-A, TF-A, T0-B, TF-B as inferred by METAGENassist bioinformatic analysis. The abundance percentage of bacteria having a specific metabolism is indicated

Another interesting increase was seen in experiment B with xylan-degrading bacteria that increased from 9.8% in T0-B to 52.0% in TF-B. In contrast, in experiment A, they remained at low concentrations (from 8.8% in T0-A to 0.2% in TF-A). Bacteria can degrade and hydrolyze xylan and utilise xylan-derived xylose for growth and PHA production (Salamanca-Cardona et al. 2014). Finally, in experiment A, there was a substantial increase in nitrogen-fixing bacteria, rising from 6.3% in T0-A to 91.9% in TF-A, representing the highest metabolic capacity observed. Nitrogen-fixing bacteria carry out a crucial biological process in which atmospheric nitrogen (N_2) is converted into ammonia (NH_3). Nitrogen and carbon are primary elements for PHA production within a feast/famine regime. Specifically, nitrogen imposes significant selective pressure beneficial for microorganisms engaged in PHA storage (Correa-Galeote et al. 2022). As a result, the nitrogen-fixing bacteria played a crucial role, significantly enhancing PHA production in the TF-A sample and leading to the highest yield.

In the TF-A sample, the enzymatic activity related to nitrogen fixation, as inferred from predictive functional annotation, suggests an increased microbial investment in nitrogen assimilation processes. This metabolic shift may be indicative of a nitrogen-limited environment, which typically stimulates the expression of nitrogenase enzymes and favors the proliferation of diazotrophic taxa capable of compensating for the deficit through biological nitrogen fixation.

Concomitantly, the increased abundance of Rhodocyclaceae is particularly relevant, as several genera within this family—such as *Thauera* and *Azoarcus*—are well-documented PHA producers. These microorganisms are known to synthesize PHAs as intracellular carbon and energy storage compounds, particularly under conditions of nutrient imbalance, such as an excess of carbon relative to nitrogen. The observed co-occurrence of elevated nitrogen fixation potential and Rhodocyclaceae abundance suggests a microbial community structure and functional profile conducive to enhanced PHA biosynthesis. Previous studies (Mikes et al. 2021) have reported a positive correlation between nitrogen fixation activity and the proliferation of Rhodocyclaceae, accompanied by an increase in polyhydroxyalkanoate (PHA) accumulation. This relationship has been interpreted as an adaptive microbial strategy under nutrient-limited conditions, facilitating the intracellular storage of carbon. However, the applicability of these findings to Rhodocyclaceae populations specifically derived from wastewater environments remains to be independently validated by other research groups.

Taken together, these findings support the hypothesis that the TF-A condition, characterized by limited nitrogen availability and high organic loading, fosters a selective environment that promotes the enrichment of functionally specialized microbial taxa. In particular, the simultaneous increase in nitrogen-transforming bacteria (e.g., nitrogen-fixing or nitrate-reducing genera and PHA-accumulating Rhodocyclaceae (e.g., *Thauera, Azoarcus*) suggests a community-level adaptation to nutrient imbalance. Under these conditions, taxa capable of coping with nitrogen stress while efficiently storing excess carbon as PHAs are likely favoured. Predictive functional profiling further supports this dual enrichment, indicating increased metabolic potential for both nitrogen metabolism and biopolymer synthesis. This interplay between microbial community structure, functional potential, and selective pressures highlights a plausible ecological mechanism underlying the observed PHA accumulation. In sample TF-B, the predicted nitrogen fixation function is significantly less represented compared to TF-A. At the same time, a broader range of functional activities—such as sulfate reduction, ammonia oxidation, and sulfate oxidation—predominates. These functions are generally not associated with microbial taxa known for PHA accumulation. This pattern aligns with the lower PHA yield observed in TF-B, suggesting that the increased functional diversity may reflect incresed metabolic competition among microorganisms, thereby reducing the efficiency of carbon conversion toward PHA synthesis. Thus, these data corrobarate the possible correlation between microbiota structure, functional capacity, and PHA production, in a realistic setting highlighting the crucial role of functional specialization in the valorisation of activated SS.

Conclusions

This study provides valuable insights into the effect of OLR on PHA production under realistic conditions in a pilot-scale WWTP. Metataxonomics analysis revealed that OLR variation influenced the

structure of the PHA-producing microbiota, particularly enriching Proteobacteria—especially Rhodocyclaceae family from Betaproteobacteria—at higher OLRs. While PHA yields increased with OLR, contaminant removal remained stable, confirming the system's robustness.

Importantly, this is the first demonstration of PHA production directly from wastewater collected on a University campus, converting internal waste into biopolymers on-site thereby transforming an internal waste stream into valuable biopolymers within the same facility.

Therefore, this research not only provides valuable insights into the role of bacteria in optimizing PHA production but also paves the way for future biotechnological and industrial applications, underscoring the potential of adopting waste-activated sludge from WWTPs within the framework of a circular bioeconomy. By repurposing this waste stream to produce valuable materials like PHAs, waste disposal and environmental impact can be significantly reduced while enhancing biopolymer production.

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Author contributions Y.L. and A.M. performed investigations, wrote the original draft, and prepared figures. F.C.C. conducted investigations and data curation. A.P.P. conducted data curation and provided resources. R.A. supervised research activities. G.M. managed and conceptualized the study and acquired funding. G.G. supervised research activities, provided resources, and conceptualized the study. All authors reviewed the manuscript.

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Data availability Data are provided within the manuscript or supplementary information files. Sequence data supporting the findings of this study have been deposited in the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/) under the accession code PRJNA1268185.

Declarations

Conflict of interest The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Giorgio Mannina reports financial support was provided by EU Framework Programme for Research and Innovation Euratom. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Abate T, Amabile C, Muñoz R, Chianese S, Musmarra D (2024) Polyhydroxyalkanoate recovery overview: properties, characterizations, and extraction strategies. Chemosphere. https://doi.org/10.1016/j.chemosphere.2024.141950

Al Battashi H, Al-Kindi S, Gupta VK, SIvakumar N, (2021) Polyhydroxyalkanoate (PHA) production using volatile fatty acids derived from the anaerobic digestion of waste paper. J Polym Environ 29:250–259. https://doi.org/10.1007/s10924-020-01870-0

Almeida JR, Fradinho JC, Carvalho G, Oehmen A, Reis MAM (2022) Dynamics of microbial communities in phototrophic polyhydroxyalkanoate accumulating cultures. Microorganisms 10(2):351. https://doi.org/10.3390/microorganisms10020351

Andreolli M, Scerbacov V, Frison N, Zaccone C, Lampis S (2022) *Thauera* sp. Sel9, a new bacterial strain for polyhydroxyalkanoates production from volatile fatty acids. New Biotechnol 72:71–79. https://doi.org/10.1016/j.nbt.2022.09.004

Baird R, Eaton AD, Rice EW, Bridgewater L (2017) Standard methods for the examination of water and wastewater. American Public Health Association, Washington D.C

Campanari S, Silva FA, 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

Carvalho JM, Marreiros BC, Reis MAM (2022) Polyhydroxyalkanoates production by mixed microbial culture under high salinity. Sustainability 14(3):1346. https://doi.org/10.3390/su14031346

Conca V, da Ros C, Valentino F, Eusebi AL, Frison N, Fatone F (2020) Long-term validation of polyhydroxyalkanoates production potential from the sidestream of municipal wastewater treatment plant at pilot scale. Chem Eng J 390:124627. https://doi.org/10.1016/j.cej.2020.124627

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 14(7):1396. https://doi.org/10.3390/polym14071396

Crognale S, Lorini L, Valentino F, Villano M, Marzo Gago C, Tonanzi B, Majone M, Rossetti S (2022) 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. https://doi.org/10.1016/j.scitotenv.2022.153995

De Donno Novelli L, Sayavedra SM, Rene ER (2021) Polyhydroxyalkanoate (PHA) production via resource recovery from industrial waste streams: a review of techniques and perspectives. Bioresour Technol 331:124985. https://doi.org/10.1016/j.biortech.2021.124985

Di Bella G, Lumia L, Sutera A, Rabbeni G, Giustra MG, Gallo G (2023) Assessment on the effect of erythromycin on the microbiome of a bioslurry pilot plant for contaminated sediment bioprocessing. Bioresour Technol Rep 23:101532. https://doi.org/10.1016/j.biteb.2023.101532

Di Leto Y, Capri FC, Mineo A, Cosenza A, Gallo G, Alduina R, Mannina G (2022) Initial pH conditions shape the microbial community structure of sewage sludge in batch fermentations for the improvement of volatile fatty acid production. Microorganisms 10(10):2073. https://doi.org/10.3390/microorganisms10102073

Di Leto Y, Capri FC, Gallo G, Cosenza A, Mineo A, Mannina G, Alduina R (2024) Enhancing volatile fatty acid production in batch test reactors by modulating microbial communities with potassium permanganate. Int Biodeterior Biodegrad 191:105809. https://doi.org/10.1016/j.ibiod.2024.105809

Di Leto Y, Mineo A, Capri FC, Puccio G, Mercati F, Alduina R, Gallo G, Mannina G (2025) The role of carbon and nitrogen ratio on sewage sludge microbiota for producing polyhydroxyalkanoates. J Environ Manage. https://doi.org/10.1016/j.jenvman.2025.124240

Estévez-Alonso Á, Altamira-Algarra B, Arnau-Segarra C, van Loosdrecht MCM, 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

Fra-Vázquez A, Santorio S, Palmeiro-Sánchez T, Val del Río A, Mosquera-Corral A (2019) PHA accumulation of a mixed microbial culture co-exists with ammonia partial nitritation. Chem Eng J 360:1255–1261. https://doi.org/10.1016/j.cej.2018.10.207

Frison N, Andreolli M, Botturi A, Lampis S, Fatone F (2021) Effects of the sludge retention time and carbon source on polyhydroxyalkanoate-storing biomass selection under aerobic-feast and anoxic-famine conditions. ACS Sustain Chem Eng 9:9455–9464. https://doi.org/10.1021/acssuschemeng.1c02973

Galitskaya P, Biktasheva L, Blagodatsky S, Selivanovskaya S (2021) Response of bacterial and fungal communities to high petroleum pollution in different soils. Sci Rep 11:164. https://doi.org/10.1038/s41598-020-80631-4

Isern-Cazorla L, Mineo A, Suárez-Ojeda ME, Mannina G (2023) Effect of organic loading rate on the production of polyhydroxyalkanoates from sewage sludge. J Environ Manage 343:118272. https://doi.org/10.1016/j.jenvman.2023.118272

Janssen DB, Oppentocht JE, Poelarends GJ (2001) Microbial dehalogenation. Curr Opin Biotechnol 12(3):254–258. https://doi.org/10.1016/S0958-1669(00)00208-1

Kranz RG, Gabbert KK, Locke TA, Madigan MT (1997) Polyhydroxyalkanoate production in *Rhodobacter capsulatus*: genes, mutants, expression, and physiology. Appl Environ Microbiol 63(8):3003–3009. https://doi.org/10.1128/aem.63.8.3003-3009.1997

Lagoa-Costa B, Kennes C, Veiga MC (2022) Influence of feedstock mix ratio on microbial dynamics during acidogenic fermentation for polyhydroxyalkanoates production. J Environ Manage. https://doi.org/10.1016/j.jenvman.2021.114132

Liang X, Cha DK, Xie Q (2024) Properties, production, and modification of polyhydroxyalkanoates. Resour Conserv Recycl Adv. https://doi.org/10.1016/j.rcradv.2024.200206

Liu B, Zhang Z, Feng X, Liu Y, Yan X, Zhang X, Wang L, Zhao L (2006) *Thauera* and *Azoarcus* as functionally important genera in a denitrifying quinoline-removal bioreactor as revealed by microbial community structure comparison. FEMS Microbiol Ecol 55(2):274–286. https://doi.org/10.1111/j.1574-6941.2005.00033.x

Liu Y, Zhang Y, Ni BJ (2015) Evaluating enhanced sulfate reduction and optimized volatile fatty acids (VFA) composition in anaerobic reactor by Fe (III) addition. Environ Sci Technol 49(4):2123–2131. https://doi.org/10.1021/es504200j

Maehara A, Taguchi S, Nishiyama T, Yamane T, Doi Y (2002) A repressor protein, PhaR, regulates polyhydroxyalkanoate (PHA) synthesis via its direct interaction with PHA. J Bacteriol 184(14):3992–4002.

Mannina G, Alduina R, Badalucco L, Barbara L, Capri FC, Cosenza A, Di Trapani D, Gallo G, Laudicina VA, Muscarella SM, Presti D (2021a) Water resource recovery facilities (Wrrfs): The case study of Palermo university (Italy). Water. https://doi.org/10.3390/w13233413

Mannina G, Badalucco L, Barbara L, Cosenza A, Di Trapani D, Gallo G, Laudicina VA, Marino G, Muscarella SM, Presti D, Helness H (2021b) Enhancing a transition to a circular economy in the water sector: the EU project wider uptake. Water 13:946. https://doi.org/10.3390/w13070946

Mannina G, Mineo A (2023) Polyhydroxyalkanoate production from fermentation of domestic sewage sludge monitoring greenhouse gas emissions: a pilot plant case study at the WRRF of Palermo University (Italy). J Environ Manage 348:119423. https://doi.org/10.1016/j.jenvman.2023.119423

Martínez V, Herencias C, Jurkevitch E, Prieto MA (2016) Engineering a predatory bacterium as a proficient killer agent for intracellular bio-products recovery: the case of the polyhydroxyalkanoates. Sci Rep 18(6):24381. https://doi.org/10.1038/srep24381

Mathur H, Joshi N, Khaturia S (2023) Microbial community profiling in wastewater of effluent treatment plant. Genom Approach Biorem Princ Tool Emerg Techno. https://doi.org/10.1002/9781119852131.ch9

McLellan SL, Huse SM, Mueller-Spitz SR, Andreishcheva EN, Sogin ML (2010) Diversity and population structure of sewage-derived microorganisms in wastewater treatment plant influent. Environ Microbiol 12(2):378–392. https://doi.org/10.1111/j.1462-2920.2009.02075.x

Mikes MC, Martin TK, Moe WM (2021) *Azospira inquinata* sp. nov., a nitrate-reducing bacterium of the family *Rhodocyclaceae* isolated from contaminated groundwater. Int J Syst Evol Microbiol. https://doi.org/10.1099/ijsem.0.005172

Mineo A, Di Leto Y, Cosenza A, Capri FC, Gallo G, Alduina R, Ni BJ, Mannina G (2024) Enhancing volatile fatty acid production from sewage sludge in batch fermentation

tests. Chemosphere 349:140859. https://doi.org/10.1016/j.chemosphere.2023.140859

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

Montiel-Jarillo G, Carrera J, Su´arez-Ojeda ME (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

Peces M, Dottorini G, Nierychlo M, Andersen KS, Dueholm MKD, Nielsen PH (2022) Microbial communities across activated sludge plants show recurring species-level seasonal patterns. ISME Commun 2:18. https://doi.org/10.1038/s43705-022-00098-4

Perez-Zabaleta M, Atasoy M, Khatami K, Eriksson E, Cetecioglu Z (2021) Bio-based conversion of volatile fatty acids from waste streams to polyhydroxyalkanoates using mixed microbial cultures. Bioresour Technol 323:124604. https://doi.org/10.1016/j.biortech.2020.124604

Povolo S, Romanelli MG, Basaglia M, Ilieva VI, Corti A, Morelli A, Chiellini E, Casella S (2013) Polyhydroxyalkanoate biosynthesis by *Hydrogenophaga pseudoflava* DSM1034 from structurally unrelated carbon sources. New Biotechnol 30(6):629–634. https://doi.org/10.1016/j.nbt.2012.11.019

Presti D, Cosenza A, Capri FC, Gallo G, Alduina R, Mannina G (2021) Influence of volatile solids and pH for the production of volatile fatty acids: batch fermentation tests using sewage sludge. Bioresour Technol 342:125853. https://doi.org/10.1016/j.biortech.2021.125853

Rhodes CJ (2018) Plastic pollution and potential solutions. Sci Prog 101(3):207–260. https://doi.org/10.3184/003685018X1529487670621

Sabapathy PC, Devaraj S, Meixner K, Anburajan P, Kathirvel P, Ravikumar Y, Zabed HM, Qi X (2020) Recent developments in Polyhydroxyalkanoates (PHAs) production – a review. Bioresour Technol 306:123132. https://doi.org/10.1016/j.biortech.2020.123132

Salamanca-Cardona L, Ashe CS, Stipanovic AJ, Nomura CT (2014) Enhanced production of polyhydroxyalkanoates (PHAs) from beechwood xylan by recombinant *Escherichia coli*. Appl Microbiol Biotechnol 98:831–842. https://doi.org/10.1007/s00253-013-5398-4

Samrot AV, Samanvitha SK, Shobana N, Renitta ER, Senthilkumar P, Kumar SS, Abirami S, Dhiva S, Bavanilatha M, Prakash P, Saigeetha S, Shree KS, Thirumurugan R (2021) The synthesis, characterization and applications of polyhydroxyalkanoates (PHAs) and PHA-based nanoparticles. Polymers (Basel). https://doi.org/10.3390/polym13193302

Saunders AM, Albertsen M, Vollertsen J, Nielsen PH (2016) The activated sludge ecosystem contains a core community of abundant organisms. ISME J 10(1):11–20. https://doi.org/10.1038/ismej.2015.117

Scaccia N, Vaz-Moreira I, Manaia CM (2020) Persistence of wastewater antibiotic resistant bacteria and their genes in human fecal material. FEMS Microbiol Ecol 96(6):fiaa058. https://doi.org/10.1093/femsec/fiaa058

Shahid S, Razzaq S, Farooq R (2021) Polyhydroxyalkanoates: next generation natural biomolecules and a solution for the world's future economy. Int J Biol Macromol 166:297–321. https://doi.org/10.1016/j.ijbiomac.2020.10.187

Sruamsiri D, Thayanukul P, Suwannasilp BB (2020) In situ identification of polyhydroxyalkanoate (PHA)-accumulating microorganisms in mixed microbial cultures under feast/famine conditions. Sci Rep 10(1):3752. https://doi.org/10.1038/s41598-020-60727-7

Szacherska K, Moraczewski K, Rytlewski P, Czaplicki S, Ciesielski S, Oleskowicz-Popiel P, Mozejko-Ciesielska J (2022) Polyhydroxyalkanoates production from short and medium chain carboxylic acids by *Paracoccus homiensis*. Sci Rep 12:7263. https://doi.org/10.1038/s41598-022-11114-x

Vogel FA, Schlundt C, Stote RE, Ratto JA, Amaral-Zettler LA (2021) Comparative genomics of marine bacteria from a historically defined plastic biodegradation consortium with the capacity to biodegrade polyhydroxyalkanoates. Microorganisms 9(1):186. https://doi.org/10.3390/microorganisms9010186

Wang Y, Huang J, Zhu F, Zhou S (2021) Airborne microplastics: a review on the occurrence, migration and risks to humans. Bull Environ Contam Toxicol 107(4):657–664. https://doi.org/10.1007/s00128-021-03180-0

Werker A, Bengtsson S, Korving L, Hjort M, Anterrieu S, Alexandersson T, Johansson P, Karlsson A, Karabegovic L, Magnusson P, Morgan-Sagastume F, Sijstermans L, Tietema M, Visser C, Wypkema E, van der Kooij Y, Deeke A, Uijterlinde C (2018) Consistent production of high quality PHA using activated sludge harvested from full scale municipal wastewater treatment. Water Sci Technol 78(11):2256–2269. https://doi.org/10.2166/wst.2018.502

Werker A, Lind P, Bengtsson S, Nordström F (2008) Chlorinated-solvent-free gas chromatographic analysis of biomass containing polyhydroxyalkanoates. Water Res 42:2517–2526

Xia S, Duan L, Song Y, Li J, Piceno YM, Andersen GL, Hermanowicz SW (2010) Bacterial community structure in geographically distributed biological wastewater treatment reactors. Environ Sci Technol 44(19):7391–7396. https://doi.org/10.1021/es101554m

Yu K, Zhang T (2012) Metagenomic and metatranscriptomic analysis of microbial community structure and gene expression of activated sludge. PLoS ONE 7(5):e38183. https://doi.org/10.1371/journal.pone.0038183

Zeng T, Wang L, Zhang X, Song X, Li J, Yang J, Chen S, Zhang J (2022) Characterization of microbial communities in wastewater treatment plants containing heavy metals located in chemical industrial zones. Int J Environ Res Public Health 19(11):6529. https://doi.org/10.3390/ijerph19116529

Zhang T, Shao MF, Ye L (2012) 454 pyrosequencing reveals bacterial diversity of activated sludge from 14 sewage treatment plants. ISME J 6(6):1137–1147. https://doi.org/10.1038/ismej.2011.188