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Research article

# Polyhydroxyalkanoate production from fermentation of domestic sewage sludge monitoring greenhouse gas emissions: A pilot plant case study at the WRRF of Palermo University (Italy)

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ABSTRACT

This paper presents a comprehensive study on polyhydroxyalkanoate (PHA) production from sewage sludge. Greenhouse gas (GHG) emissions were monitored for the first time to assess the impact of climate change and environmental sustainability. The pilot plant was composed of a fermenter with a membrane and two biological reactors (namely, selection and accumulation). Results showed that despite a low organic loading rate (namely, 0.06 kg BOD kg $SS^{-1}$ $day^{-1}$), a good PHA yield was obtained (namely, 0.37 g PHA/g volatile fatty acids), confirming that sewage sludge can be a suitable feedstock. GHG emissions were 3.85E-04 g $CO_2eq$/g and 32.40 g $CO_2eq$/g, direct and indirect, respectively. Results provided valuable insights in view of finding a trade-off between PHA production and GHG emissions to prove the PHA production process as an effective solution for biosolids disposal at a low carbon footprint.

## 1. Introduction

Recently, the employment of the circular economy approach in the wastewater treatment plant (WWTP) operation led to the spread of new biotechnologies focused on resource recovery from wastewater (Guleria et al., 2022). Due to the high disposal costs and the high amount produced, sewage sludge has become the primary feedstock used to recover resources such as biogas, fertilisers and high-value chemicals (Andreoli et al., 2007; Kumar et al., 2022). Among them, polyhydroxyalkanoates (PHA) gained much attention in the last decade. They are biodegradable polyesters with properties similar to conventional petroleum-based plastics (Bugnicourt et al., 2014). Moreover, PHA can be produced by mixed microbial cultures (MMC) when fed with organic fermentation products such as volatile fatty acids (VFA) (Candry Pieter et al., 2022; Rodriguez-Perez et al., 2018). The possibility of producing PHA from MMC allowed the spread of a new process aimed at making the PHA inside the WWTP by using the sewage sludge as a new promising waste to be reused (Kourmentza et al., 2017; Mannina et al., 2020). Despite the increasing number of studies regarding sewage sludge-based PHA production, there are still several challenges regarding this feedstock (Varghese et al., 2022). The sewage sludge is usually poor in organic content compared to other feedstocks (Khatami et al., 2021). Therefore, acidogenic fermentation needed to produce volatile fatty acids (VFA), which are used to synthesise PHA intracellularly, has a generally low production rate (Perez-Esteban et al., 2022). To overcome these bottlenecks, most of the literature studies on sewage sludge-based PHA production focused on the co-fermentation with other feedstock or the pre-treatment of the sludge to increase the VFA production rate (Battista et al., 2022; Lorini et al., 2022; Moretto et al., 2020; Ramos-Suarez et al., 2021a; Rodriguez-Perez et al., 2018). More specifically, Lorini et al. (2022) used thermal pre-treated sludge to perform fermentation tests at a pilot scale for seven days. The sludge was kept at 78 °C for 48 h, as pre-treatment, and the fermentation tests were performed by maintaining temperature at 55 °C for five days. The results showed that the thermal pre-treatment and the thermophilic conditions enhanced the organic matter solubilisation and increased the amount of VFA produced up to 9.1 g $COD_{VFA}$/L. Zhang et al. (2019) obtained similar results by employing sludge pre-treated at even higher temperatures, 110 °C for 1 h, and then maintained at 55 °C during the fermentation tests. Such conditions favoured the COD solubilisation and the consecutive VFA production up to 8.4 ± 0.1 g $COD_{VFA}$/L. When referring to PHA production from sewage sludge, VFA production is still considered the pivotal step of the entire process: a higher concentration of VFA produced means an increase in the organic loading rate (OLR), which is

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going to positively affect the amount of the produced PHA (Crognale et al., 2022). Previous studies for PHA production from sewage sludge employ technologies aimed at maximising PHA yield neglecting the impact of carbon footprint and thus greenhouse gas (GHG) emissions.

WWTPs can contribute to anthropogenic greenhouse gas (GHG) emissions, both direct and indirect (Mannina et al., 2016b). Direct emissions (i.e., carbon dioxide ($CO_2$), methane ($CH_4$) and nitrous oxide ($N_2O$)) are the main gases that can be produced during the combustion of organic matter, anaerobic organic matter degradation and through the nitrification-denitrification process (Kampschreur et al., 2009; Yoshida et al., 2014). GHG indirect emissions are associated with energy consumption because of WWTP management by employing blowers, stirrers, heaters, etc. (Parravicini et al., 2016).

In recent years, many studies have focused on improving substrate production, biomass enrichment, PHA accumulation and PHA extraction without considering the environmental sustainability of the technologies adopted regarding GHG emissions. Despite some efforts in applying new environmental-friendly solutions in PHA extraction (Pesante and Frison, 2023), the literature still lacks information regarding the environmental impact of the entire process. In particular, regarding the sewage sludge fermentation to produce VFA, which is always optimised by methods which increase the indirect GHG emissions (H. H. Liu et al., 2020; Y. M. Wu et al., 2023; Zhen et al., 2017).

Despite the importance of achieving a high amount of VFA and PHA in the process, more attention should be given to finding a trade-off to minimise GHG emissions. The paper presents the first comprehensive study regarding PHA production by MMC from wasted sewage sludge monitoring GHG emissions at a pilot scale. The sewage sludge was fermented without any pre-treatment and temperature control to keep low economic costs and indirect GHG emissions. The aerobic dynamic feeding (ADF) was the strategy applied during the biomass selection, followed by the PHA accumulation. Indirect GHG emissions were calculated for the entire process while direct GHG emissions, in terms of nitrous oxide ($N_2O$) emissions, were monitored during the biomass selection process.

## 2. Materials and methods

### 2.1. UNIPA pilot plant layout

The experiments were carried out at the Water Resource Recovery Facility (WRRF) of Palermo University (Mannina et al., 2021). Specifically, PHA was produced from a pilot plant composed of a fermenter equipped with an ultra-filtration unit and two sequencing batch reactors (SBR) devoted to PHA producers' selection (S-SBR) and PHA accumulation (A-SBR). The fermenter was a continuous stirred tank reactor (225 L) connected to a hollow fibre membrane with 0.03 μm porosity and 1.4 $m^2$ surface area installed inside the UF unit (43 L) and equipped with a gas recirculation pump to maintain the anaerobic conditions. VFA rich liquid was obtained after the filtration in the permeate collector, which fed S-SBR (75 L) and A-SBR (75 L) (Fig. 1).

### 2.2. Sewage sludge fermentation

The substrate was the wasted sewage sludge withdrawn from an aerobic reactor of the wastewater treatment pilot plant within the WRRF at Palermo University (Mannina et al., 2021). The sludge was not pre-treated and the fermentation was carried out without additives or temperature control for 6 ± 1 days. Soluble and total chemical oxygen demand (sCOD, TCOD), extracellular polymeric substances (EPS), soluble microbial products (SMP), total and volatile suspended solids (TSS, VSS) were analysed at the start and the end of the fermentation process while sCOD, VFA, ammonium ($NH_4^+$-N) and phosphate ($PO_4^{3-}$-P) were analysed each day. pH and temperature were also monitored during the fermentation. At the end of the fermentation, the mixed liquor was filtered by the UF unit with a net initial flow rate of 13.2 L/h (9 min filtration at 18 L/h and 1 min backwash at 30 L/h). In Tables 1 and 2, the sludge features are shown.

### 2.3. Selection and accumulation SBR

The S-SBR working volume was 30 L and the inoculum was the same as the substrate used in the fermentation. The selection was carried out with a hydraulic retention time (HRT) of 2 days and a cycle of 12 h divided into feeding, biological reaction, settling and effluent withdrawn. The ADF strategy was applied to select the PHA producers by using a ceramic diffuser at the bottom of the reactor to supply air. Temperature and dissolved oxygen (DO) were continuously monitored using a probe (WTW FDO® 925-P). Once a steady state was achieved, i.e., when the feast/famine (F/F) ratio remained constant (<5% deviation) for at least 14 days, the reactor performances were calculated. The operational parameters of the S-SBR are reported in Table 3. Finally, the biomass was withdrawn and stressed to accumulate PHA in the A-SBR with the same equipment as the S-SBR and a working volume between 40 and 50 L. The pilot plant operation was controlled by a programmable logic controller (Millennium 3, Crouzet) coupled with home-made software to automate the accumulation process (Mineo et al., 2023). The accumulation step is based on an automatic feed-on-demand strategy by using the DO concentration as a trigger to feed the substrate in the reactor, thus leading to the PHA accumulation.

### 2.4. Analytical methods

The S-SBR system was monitored by sampling the influent, mixed liquor inside the reactor and the effluent to evaluate the COD, $NH_4^+$-N, $PO_4^{3-}$-P, TSS and VSS twice a week while EPS, SMP and PHA once a week. During the accumulation TSS, VSS, PHA, influent COD and influent VFA were measured daily.

VFA concentration was analysed by applying standard methods according to the literature (Rice et al., 2012). Filtered samples (0.45 μm) were mixed with 1 mL dimethyl carbonate (DMC-OEI) and 0.1 mL of potassium bisulphate ($KHSO_4$) solution and centrifuged at 4000 rpm for 10 min to evaluate the VFAs concentration. The upper layer of the treated samples was analysed by using an Agilent Technologies 7820A gas chromatograph (GC) equipped with a flame ionisation detector (FID) and a DB FFAA column (30 m × 0.25 × mm × 0.25 μm). Formic, acetic, propionic, isobutyric, butyric, isovaleric, valeric, isocaproic, hexanoic and n-heptanoic acids were analysed (Montiel-Jarillo et al., 2021) and their concentration was converted into COD by using the conversion factors proposed in the literature (Yuan et al., 2011).

PHA concentration was analysed by collecting mixed liquor samples during the selection and the accumulation, subsequently mixed with 10 mL of formaldehyde solution (37%) to inhibit the biological activity. Subsequently, samples were centrifuged at 8000 rpm for 30 min, the supernatant was removed and samples were lyophilised. Butanol and hydrochloric acid were added to weighted lyophilised samples, which were incubated at 100 °C for 8 h. An extraction with hexane and milliQ grade water was performed and the organic phase was collected and filtered (0.22 μm). A GC-FID equipped with a Restek Stabilwax column (30 m × 0.53 mm x 1.00 μm film thickness) was used to determine the polyhydroxybutyrate (PHB) and polyhydroxyvalerate (PHV) monomer

**Table 1**
Average sewage sludge features.

| Parameter | Units | Average | St. Dev. |
|---|---|---|---|
| sCOD | mg $L^{-1}$ | 133.59 | 93.91 |
| TCOD | mg $L^{-1}$ | 6320.39 | 2217.93 |
| $NH_4^+$-N | gN $L^{-1}$ | 36.65 | 25.02 |
| $PO_4^{3-}$-P | gP $L^{-1}$ | 24.03 | 21.42 |
| TSS | g $L^{-1}$ | 6.15 | 1.22 |
| VSS | g $L^{-1}$ | 4.35 | 1.11 |

**Table 2**
Sewage sludge features for each fermentation test.

| Fermentation week | F/M | SRT | Specific EPS | | Specific SMP | | pH start - end |
|---|---|---|---|---|---|---|---|
| | kg BOD kg $SS^{-1}$ $day^{-1}$ | days | Proteins | Carb. | Proteins | Carb. | |
| | | | mg $g^{-1}$ VSS | | mg $g^{-1}$ VSS | | |
| 1 | 0.03 | 19.1 | 21.49 | 0 | 10.12 | 0 | 7.65 - 6.87 |
| 2 | 0.21 | 17.7 | 23.14 | 8.13 | 4.3 | 0 | 7.71–7.05 |
| 3 | 0.36 | 5.4 | 14.14 | 26.86 | 54.84 | 0 | 7.41 - 6.91 |
| 4 | 0.26 | 9.1 | 48.9 | 0 | 32.7 | 0 | 7.23 - 6.60 |
| 5 | 0.14 | 12.1 | 58.84 | 0 | 7.18 | 0 | 7.41- 6.68 |
| 6 | 0.24 | 19.6 | 149.39 | 34.71 | 22.65 | 1.16 | 6.99 - 6.54 |

**Table 3**
Main operational features of the selection SBR.

| Weeks | F/M | vOLR | F/F |
|---|---|---|---|
| | kg BOD kg $SS^{-1}$ $d^{-1}$ | kg COD $m^{-3}$ $d^{-1}$ | min/min |
| 1 | 0.08 | 0.20 | 0.20 |
| 2 | 0.06 | 0.17 | 0.21 |
| 3 | 0.02 | 0.11 | 0.22 |
| 4 | 0.03 | 0.10 | 0.21 |
| 5 | 0.05 | 0.13 | 0.22 |
| 6 | 0.08 | 0.16 | 0.22 |
| 7 | 0.08 | 0.15 | 0.22 |

concentration. The protocol proposed by Mannina et al. (2019) was adopted both for VFA and PHA quantification.

Dissolved and gaseous $N_2O$ concentrations have been measured twice a week, according to the procedure described by Mannina et al. (2018). Briefly, mixed liquor sample collected during the selection were centrifuged at 8000 rpm for 5 min and 70 mL supernatant were stored in glass bottles with a total volume of 125 mL. 1 mL of a 2 N sulphuric acid solution was added to inhibit the biological activity and the bottles are subsequently sealed with a rubber septum. Bottles are mixed for 24 h, after which a sample is collected from the bottles' headspace. Gaseous $N_2O$ samples are collected directly from the reactor's headspace, and a GC equipped with an Electron Capture Detector (ECD) was used to quantify the $N_2O$ concentration.

### 2.5. Calculations

The PHA concentration in microbial cells was calculated as the ratio between PHA and VSS concentrations and reported as g PHA/g VSS. PHA productivity was defined as the ratio of the mass of PHA produced daily (g PHA/day).

The emission factor was calculated in relation to the Total Kjeldahl Nitrogen (TKN), as reported by the literature (Mannina et al., 2016a; Tsuneda et al., 2005). To evaluate the indirect emissions, the total electrical consumption in kWh was converted to grams of equivalent $CO_2$ (g $CO_2$eq) based on 2022 emissions in Italy (Scarlat et al., 2022) and subsequently divided into the amount of treated sewage sludge to obtain the grams of equivalent $CO_2$ per grams of treated sludge (g $CO_2$eq/g treated sludge) .

## 3. Results and discussion

### 3.1. Production of volatile fatty acids

Overall, domestic sewage sludge's fermentation was carried out for 6 weeks to produce the ultrafiltrated effluent used as a carbon source to select the PHA producers and accumulate PHA. Fig. 2 summarises the sCOD measured at the first and peak day of the fermentation coupled with the VFA/sCOD ratio (a) and the VFA distribution (b). Measured sCOD was always less than 500 mg/L showing a low fermentation yield. Indeed, sewage sludge acidogenic fermentation has low VFA production mainly due to the hydrolysis step (W. W. Liu et al., 2020). Due to this reason, substrate pre-treatment is usually performed to enhance the VFA production yield and rate. However, such a practice leads to an increase in indirect GHG emissions (e.g., thermophilic fermentation and thermal pre-treatment) and economic costs (e.g., alkali addition to control pH) (Guleria et al., 2022), which was far as a strategy from the performed study aimed at limiting the plant carbon footprint. The first week of fermentation achieved the highest sCOD concentration (namely, 450 mg/L) and the lowest sCOD production, comparing the first day to the

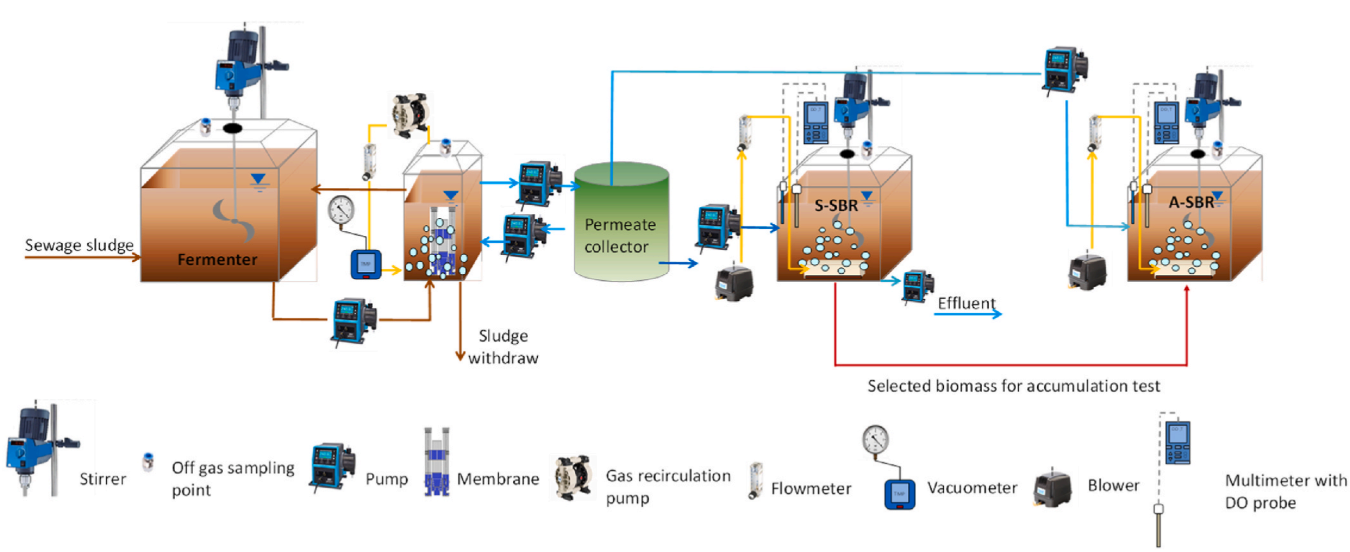


**Fig. 1.** Schematic representation of the PHA production line.

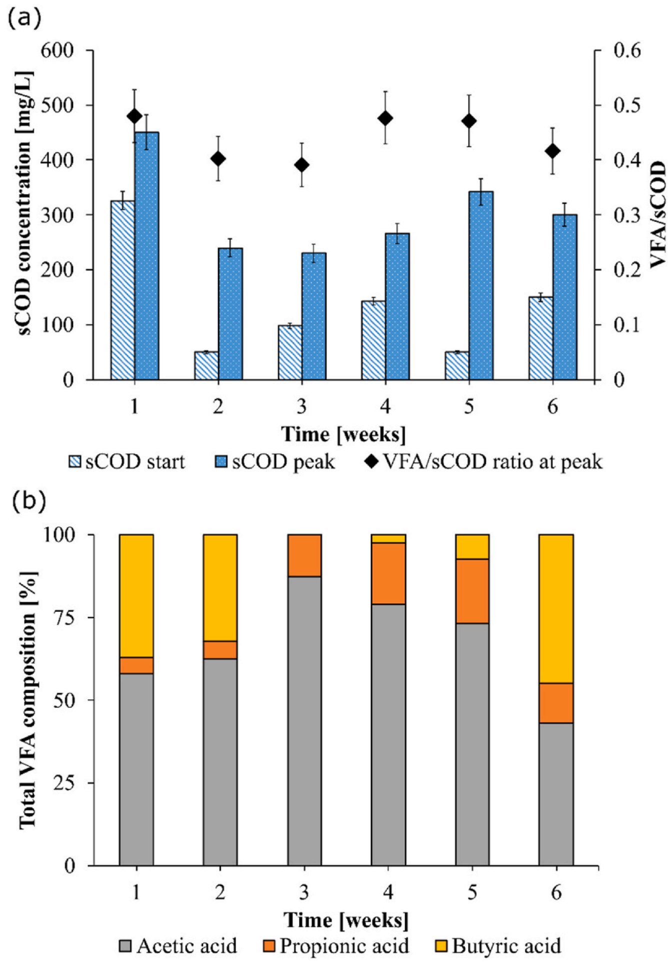


**Fig. 2.** sCOD concentration at the start and peak day and VFA/sCOD ratio (a), VFA distribution at the peak day (b).

sCOD peak. During week 5, on the other hand, it was achieved 341 mg/L of sCOD but started with 50 mg/L sCOD concentration, thus showing the highest production rate. Such a fact was related to the low food-to-microorganism ratio (F/M) and high sludge retention time (SRT) (Table 2) which led to a worsening of VFA production (Ucisik and Henze, 2008). EPS concentration did not affect the overall process performance, probably due to the abundance of inert materials. Only acetic, propionic and butyric acid had a share of the total VFAs higher than 0.01 %, the reason why they are the only fatty acids reported in Fig. 2b. Acetic acid accounted, on average, for 67.24 ± 3.66 % of the total VFA, being the main produced fatty acid. This result is probably related to the low VSS during the fermentation (Bouzas et al., 2002). Propionic acid accounted for 12.06 ± 6.19 % of the total VFA share, reaching a peak on weeks 4 and 5 of 18.85 ± 0.57 %. The propionic acid production may be related to the F/M and SRT applied in the plant where the sludge was withdrawn, indicating that high SRT and low F/M (Table 2) may be favourable to its synthesis (Chen et al., 2021; M. Y. Wu et al., 2023). Butyric acid was the longest chain fatty acids measured during the experimental period, with an average share of 20.70 ± 19.60 %. The highest concentrations were measured during weeks 1, 2 and 6 where the butyric acid accounted, on average, for 38.08 ± 6.31 %. The results indicate that extremely high SRT (17.7–19.6 days) combined with low F/M (0.03–0.24 kg BOD kg $SS^{-1}$ $day^{-1}$) are favourable for butyric acid synthesis, the reason why butyric acid was not detected on week 3 and only 2.52 and 7.45 % was measured during weeks 4 and 5.

### 3.2. Nutrient removal and PHA production

Pseudo-steady state conditions were reached after two weeks having an F/F ratio almost constant (namely, a deviation of 5%). The limited time for achieving pseudo-steady state conditions was related to the low vOLR connected to the VFA concentration obtained during the fermentation (Table 3). As reported in Fig. 3, sCOD, ammonia and phosphate removal efficiencies were always higher than 84, 68 and 57 %, respectively. sCOD removal efficiencies were consistent, while the ammonia removal efficiency kept increasing until reaching a plateau from week six, showing that a high-efficiency nitrification process was established even at relatively high influent ammonia concentration (>100 mg N/L).

The accumulation was performed by adopting a feed-on-demand strategy controlled by a home-made software (Mineo et al., 2023).

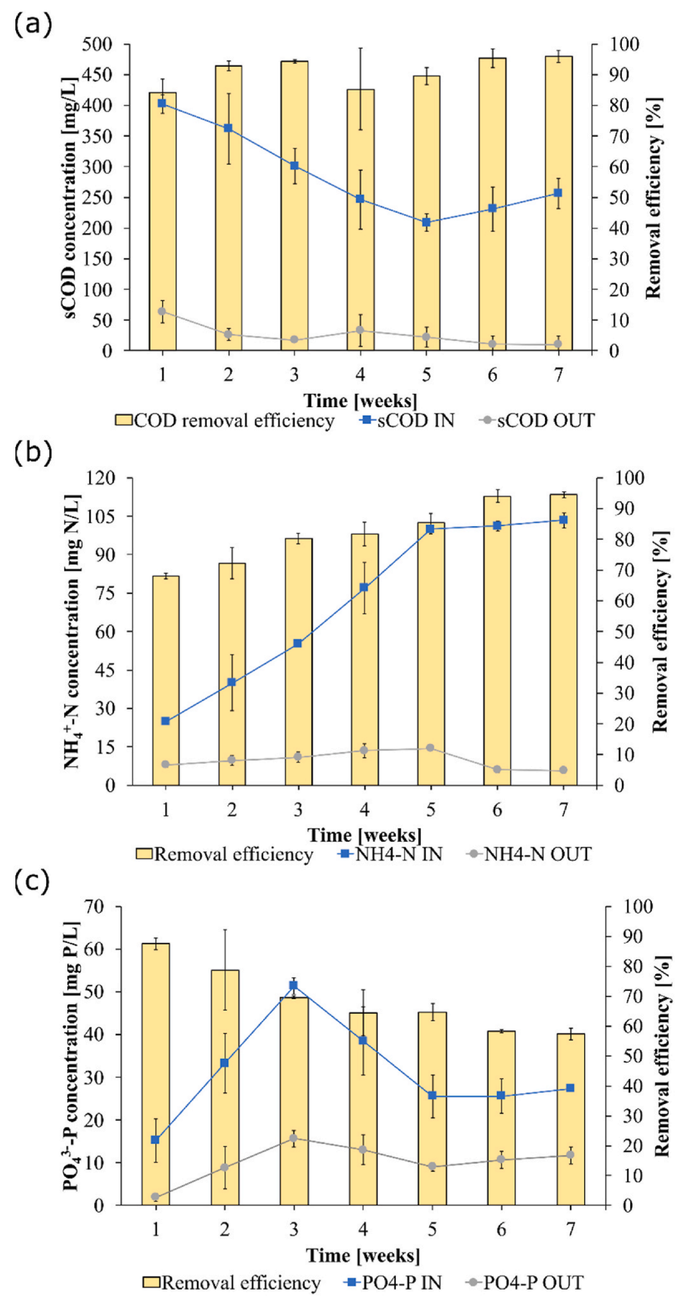


**Fig. 3.** sCOD (a), ammonia (b) and phosphate (c) removal efficiencies monitored during the selection SBR operation.

The carbon source used was the VFA rich liquid obtained from the fermentation. As shown in Fig. 4, the accumulation was carried out for four days with the final PHA concentration of 0.37 g PHA/g VSS. The PHB accounted for 97.7 %, while the remaining was PHV. This ratio is in accordance with the literature since the acetic and butyric acid was the main VFA produced and the biomass was selected with low OLR and F/F ratio (Conca et al., 2020; Pardelha et al., 2012; Szacherska et al., 2021). The obtained PHA was higher than the literature: 0.21 and 0.28 g PHA/g VSS, Valentino et al. (2015) and Morgan-Sagastume et al. (2015), respectively. Both literature studies, as in the present study, employed sewage sludge as feedstock. The higher obtained PHA yield was most likely related to a more extended accumulation period compared to literature where the accumulation was carried out for less than 24 h (Morgan-Sagastume et al., 2015; Valentino et al., 2015). Despite the application of strategies less favourable for PHA accumulation, the treatment of sludge during the acidogenic fermentation process is going to enhance PHA production. Conca et al. (2020) applied a mesophilic temperature (37 °C) during the acidogenic fermentation coupled with the dynamic thickening of sewage sludge, which resulted in a fermentation reject liquid rich in VFA (547 ± 149 mg COD/L). The aerobic/anaerobic enrichment was used as a selection strategy, despite being less performant in PHA production than the ADF (Frison et al., 2015; Lorini et al., 2022). When the real fermentation liquid was used to perform the accumulation, the PHA amount accounted for 0.42 g PHA/g VSS in 6.5 h, thus producing a higher amount of PHA in less time compared to this study. Lorini et al. (2022) applied a sludge thermal pretreatment (70 °C for 48 h) before performing the fermentation at 55 °C for 5 days. This pretreatment allowed us to achieve a fermentation liquid containing 8.4 ± 0.3 g COD/L of VFA, a considerably higher amount than those reported in the literature (Ramos-Suarez et al., 2021). Six PHA accumulation tests were performed, reaching an average value of 0.53 ± 0.03 g PHA/g VSS, the highest value achieved at the pilot scale from fermented sewage sludge. Still, despite the noticeable positive effect of sludge pre-treatment, there is no information in the literature regarding the environmental impact of those treatments.

The PHA productivity decreased sharply after the first day, where a productivity of 9.2 g PHA/d was recorded (Fig. 4). This is related to the low OLR applied in the selection step and the low F/M ratio (0.4 g COD/g VSS) in the accumulation. The result is in accordance with the literature since biomass can produce PHA rapidly but cannot increase the storage capacity (Heo and Liu, 2021; Pinto-Ibieta et al., 2021).

Despite carrying out the fermentation without pre-treatment, a decent amount of PHA was achieved (0.37 g PHA/g VSS), which is very close to the cut-off value reported in the literature (Bengtsson et al., 2017). The results prove that achieving a sustainable amount of PHA to perform an economically feasible process at a pilot scale with low VFA production (lower than 250 mgCOD/L) while minimising the indirect GHG emissions is possible.

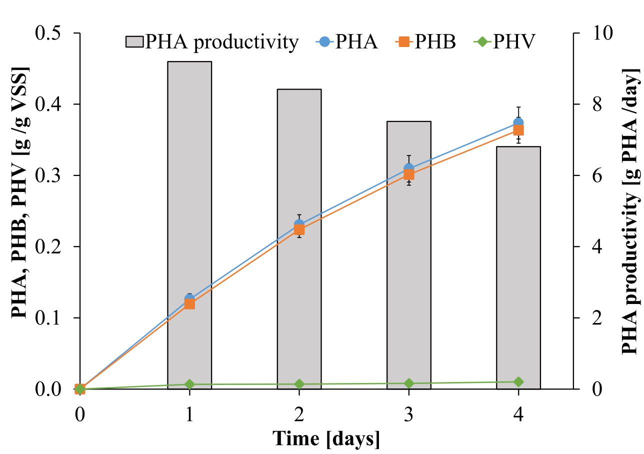


**Fig. 4.** PHA concentration and productivity during the accumulation test.

### 3.3. GHG emissions

Fig. 5 reports $N_2O$ and nitrogen balance for the monitored period. The highest and lowest $N_2O$ gaseous concentrations were 0.47 and 0.1 mg $N_2O$–N $L^{-1}$s measured during weeks 5 and 7, respectively. Dissolved $N_2O$ concentration revealed a similar pattern to the gaseous one, reaching the highest concentration during week 5 (0.25 mg $N_2O$–N $L^{-1}$) and the lowest during week 7 (0.04 mg $N_2O$–N $L^{-1}$). Since the selection strategy adopted was aerobic dynamic feeding, the $N_2O$ gaseous concentration in the selection SBR was much higher than the dissolved form (Duan et al., 2021). Gaseous concentrations were comparable to those measured by Rodriguez-Caballero et al. (2015) in an SBR system while the dissolved $N_2O$ was similar to both pilot and batch SBR treating domestic wastewater (Mannina et al., 2018b; Park et al., 2001).

The emission factor showed a decreasing trend during the reactor operation, with the exception of week 5, probably because of the biological activity improvement over time, which was more effective in removing ammonia, as previously shown in 3.2. The influent ammonia in the fifth week was 100 mg N/L, reaching a peak after a continuous increase from the second week. This increase could have been a stress factor to the biomass, thus increasing the $N_2O$ production. Nonetheless, the biomass was able to adapt to this new condition easily, as shown by the ammonia removal efficiencies and $N_2O$ emissions during weeks 6 and 7. Emission factors are comparable to those calculated by other studies regarding the aerobic reactors with similar C:N ratio (5.8 ± 5.2 g

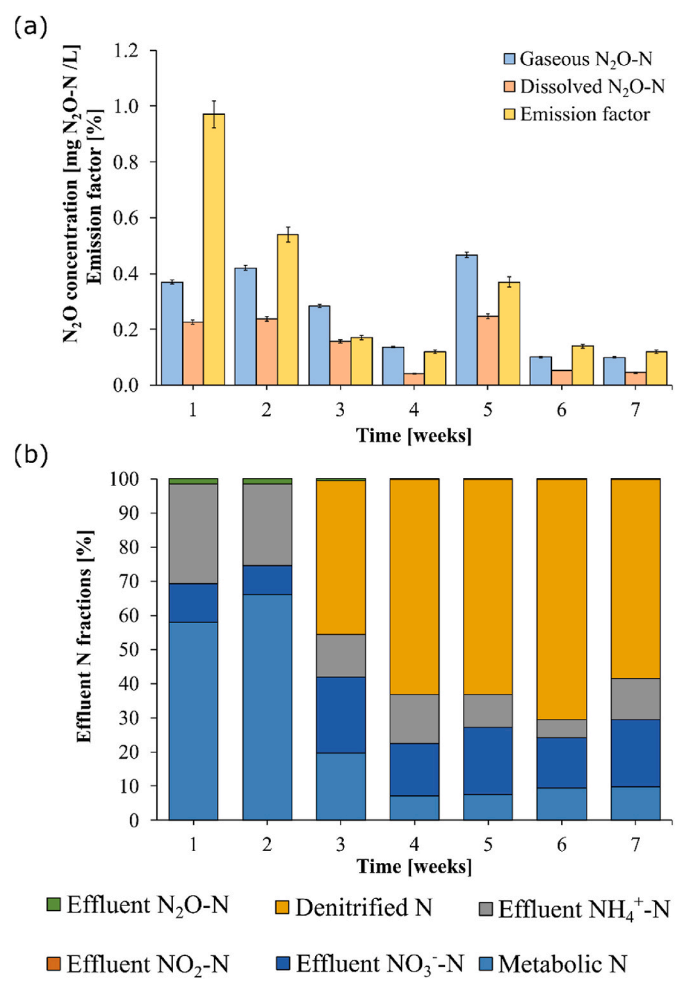


**Fig. 5.** $N_2O$ concentration and emission factor (a) and effluent nitrogen mass balances (b) for the selection SBR operational period.

COD/g N) (de Haas and Andrews, 2022; Gruber et al., 2021; Mannina et al., 2018a). Direct GHG emissions accounted for 3.85E-04 g $CO_2$eq/g treated sludge while the indirect emissions, calculated considering all the utilities adopted in the pilot plant and the conversion factor from Scarlat et al. (2022), accounted for 32.40 g $CO_2$eq/g treated sludge.

These results show how the direct and indirect GHG emissions in a PHA production pilot plant can be comparable to those monitored in WWTPs. The above findings highlight that GHG emissions have to be taken into account for having an environmentally sustainable PHA production process; therefore, more attention must be given to carbon footprint and non-entirely aerobic strategies for selecting PHA producer microorganisms (e.g., anaerobic - aerobic, aerobic - anoxic and anoxic - aerobic enrichment). Therefore, a trade-off between GHG emissions and PHA yield must be sought.

Fig. 5b shows the nitrogen mass balance calculated during the selection SBR operation. During the first two weeks a large amount of influent nitrogen was metabolised (62.05 %) with low ammonia removal (26.67 % of effluent ammonia) and the highest $N_2O$ production (1.39 %). Subsequently, in the following weeks, the biomass activity was reduced to 10.71 % of metabolised nitrogen while both autotrophic and heterotrophic activity raised since effluent ammonia decreased to 10.80 % and the denitrified nitrogen accounted for 59.95 %, as can be seen by the removal efficiency reported in 3.2. Finally, $N_2O$ accounted for around 0.24 % from week 3 to week 7, by the emission factor calculated.

As previously stated, the biomass rapidly adapted to the influent ammonia concentration increase registered during the second and the third week. The denitrification activity, appreciable from week 3, was the main biological response to the stress condition. As Conthe et al. (2019) reported, the denitrification acted as an $N_2O$ sink, scavenging the $N_2O$ produced by ammonia oxidation, thus reducing the direct emission. The increase in the denitrification activity is also related to the increased amount of carbon source (PHA) stored during the feast phase due to the selection process (Li et al., 2022).

## 4. Conclusions

Sewage sludge collected from a pilot scale WWTP was used to produce VFA, select the PHA producers' microorganisms and stress the PHA accumulation. Low carbon footprint acidogenic fermentation was aimed to produce VFA, successfully used as a carbon source to produce PHA. The results demonstrated that it is possible to achieve the amount of PHA to have an economically viable process (0.37 g PHA/g VSS) without applying sludge pre-treatment or chemicals addition, thus limiting the indirect GHG emissions. To the authors' knowledge, this is the first study where the direct GHG emissions were monitored during a PHA production process at a pilot scale. Results showed that direct and indirect emissions, which accounted for 3.85E-04 and 32.40 g $CO_2$eq/g treated sludge, respectively) should be considered to assess the environmental sustainability of the PHA production process, which is still far from scaling up.

### CRediT authorship contribution statement

**Giorgio Mannina:** Conceptualization, Methodology, Resources, Writing – original draft, Writing – review & editing, Visualization, Supervision. **Antonio Mineo:** Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization.

### 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.

### Data availability

The data that has been used is confidential.

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