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# Comparing two advanced selection strategies for polyhydroxyalkanoate production from domestic waste activated sludge

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## ABSTRACT

This study compares two membrane bioreactor-based enrichment strategies to produce polyhydroxyalkanoates (PHAs) from domestic waste-activated sludge. The aerobic dynamic feeding was implemented in layout 1 while layout 2 employs an aerobic/anoxic enrichment adopting an additional nitrification reactor. Both systems achieved around 38 % w/w of PHA with storage yields of 0.28–0.42 and 0.35–0.53 gCOD<sub>PHA</sub>/gCOD<sub>VFA</sub> for layouts 1 and 2, respectively. Layout 2 demonstrated an average N removal efficiency of 88.8 ± 3.9 %, slightly higher than layout 1 (82.7 ± 9.9 %). However, layout 2 showed greater nitrous oxide (N<sub>2</sub>O) emissions, averaging 0.7 ± 0.2 mg N<sub>2</sub>O-N/L almost doubling layout 1 (0.4 ± 0.1 mg N<sub>2</sub>O-N/L). Additionally, layout 2 exhibited a 42 % increase in carbon footprint compared to layout 1, reaching 10.2 kg CO<sub>2</sub>/day. This research highlights the high potential and drawbacks of the AE/AN enrichment strategy for integrating PHA production into wastewater treatment plant operations.

## 1. Introduction

Polyhydroxyalkanoates (PHAs) are a family of biodegradable biopolymers that received attention because of their huge potential to become an environment-friendly substitute for petroleum-based plastics [1]. Their versatility and biodegradability fostered their adoption for various applications in packaging, agriculture and medical devices [2,3]. However, one of the major drawbacks of PHA production is the high production cost from pure cultures, which is currently an obstacle to its large-scale commercialization. The use of waste streams as feedstock to produce PHA can open the road to sustainable and low-cost production methods [4].

One of the promising feedstocks to produce PHAs at reduced costs is waste-activated sludge (WAS), a by-product from wastewater treatment plants (WWTPs). WAS is rich in organic carbon sources, and the mixed microbial culture within this matrix contains several PHA producers' microorganisms that can be enriched and exploited to accumulate PHA [5,6]. This approach capitalizes on an existing waste stream and serves the aims of a circular bioeconomy in converting wastes into value-added bioproducts [7,8]. Different enrichment strategies have been used to enhance the PHA accumulation potential of microorganisms in WAS, with aerobic dynamic feeding (ADF) and aerobic/anoxic enrichment

(AE/AN) as the most promising approaches for further optimization and integration within the WWTP operation [9]. Besides potential contribution to PHA production, the WWTPs also stand at the forefront in the paradigm transition towards water resource recovery facilities (WRRFs). This transition is driven by the evolving understanding that wastewater is not simply a waste to be managed but a valuable source of water, energy and materials.

The forthcoming urban wastewater treatment directive (UWWTD) from the European Union accelerates this transition by enforcing more stringent limits on nitrogen (N) and phosphorus (P) discharges to protect aquatic ecosystems, fostering the water reuse practice whenever possible [10]. New requirements stimulate the need for innovative treatment processes and resource recovery strategies inside the WWTP. Recovery of PHAs from WAS could potentially fit this context since it includes compliance with regulatory discharge limits while retrieving added-value biopolymers [11–13]. The potential integration of PHA production with the WWTPs outlines an environmentally friendly solution by leveraging the existing infrastructure and employing advanced processes such as membrane bioreactors (MBRs). High-quality effluents could be produced aside from enriching microbial populations for PHA accumulation. Implementing PHA production within WWTPs may enhance the sustainability of the wastewater treatment processes by

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creating new resource recovery pathways, thus potentially reducing the environmental impact of both wastewater and the production of plastics.

Previous studies have already focused on comparing the ADF and the AE/AN enrichment strategy but have never taken into account the adoption of the MBR to improve the effluent quality and compare it with the legislation limits [14]. This study compared two MBR-based enrichment strategies, the ADF and the AE/AN enrichment, Layout 1 and 2, respectively [15]. The two strategies were tested on a pilot scale, and the same amount of WAS was treated. Two experimental periods with similar influent characteristics were chosen to compare the two strategies comprehensively. In view of assessing the potential implementation of one of these strategies in a WWTP, the criteria to compare the two systems considered the nutrient removal efficiency (N and P), direct GHG emission (as nitrous oxide – N<sub>2</sub>O), carbon footprint and amount of PHA produced.

## 2. Materials and methods

### 2.1. System description

Pilot-scale experiments were performed in the pilot plant hall of the WRRF at the University of Palermo (Mannina et al., 2021). This facility contains a wastewater and a sludge treatment line devoted to PHA production. The PHA production process begins with a 200 L fermenter with an ultrafiltration (UF) unit. The resulting fermented sludge liquid feeds a series of reactors.

Depending on the chosen enrichment strategy, the layout of the pilot plant varies (Fig. 1). A nitrification sequencing batch reactor (N-SBR) with a 30 L working volume produces a nitrite-rich effluent. The enrichment sequencing batch reactor (S-SBR) applies the feast-famine cycle to enrich microbial culture via the ADF or AE/AN strategy. At the start of the aerobic feast phase, the S-SBR is fed with fermented sludge liquid; if the ADF approach is used, the subsequent famine phase is aerobic, whereas if the AE/AN strategy is employed, the famine phase is anoxic, utilizing the effluent from the N-SBR. The S-SBR is paired with an MBR (total working volume 50 L) to yield a high-quality effluent.

The enriched biomass from the S-SBR is then used in the accumulation sequencing batch reactor (A-SBR) to produce PHA under the control of in-house software [16]. The two experimental periods compared in this work are selected from two long-term plant operations in view of similar influent characteristics, as shown below.

### 2.2. WAS acidogenic fermentation

The WAS collected from the aerobic reactor of the wastewater treatment line was fermented without any pretreatments or control of temperature or pH. The hydraulic and sludge retention times (HRT and SRT) were five days. Fermentation occurred in a 225 L stirred tank reactor, coupled to the UF unit with a hollow fiber polyvinylidene fluoride membrane (porosity: 0.03 μm; surface area: 1.4 m<sup>2</sup>). At the end of the five-day fermentation, the sludge was pumped into the UF unit and filtered to obtain the VFA-rich liquid. The starting pH of the fermentation was 7.2 ± 0.3, while the ending pH was 6.4 ± 0.2. The average temperature during the process was 19.6 ± 2.5 °C. Table 1 provides the average characteristics of the fermented sludge liquid.

Soluble chemical oxygen demand (SCOD); ammonium (NH<sub>4</sub><sup>+</sup>-N); orthophosphate (PO<sub>4</sub><sup>3-</sup>-P).

### 2.3. Enrichment strategies

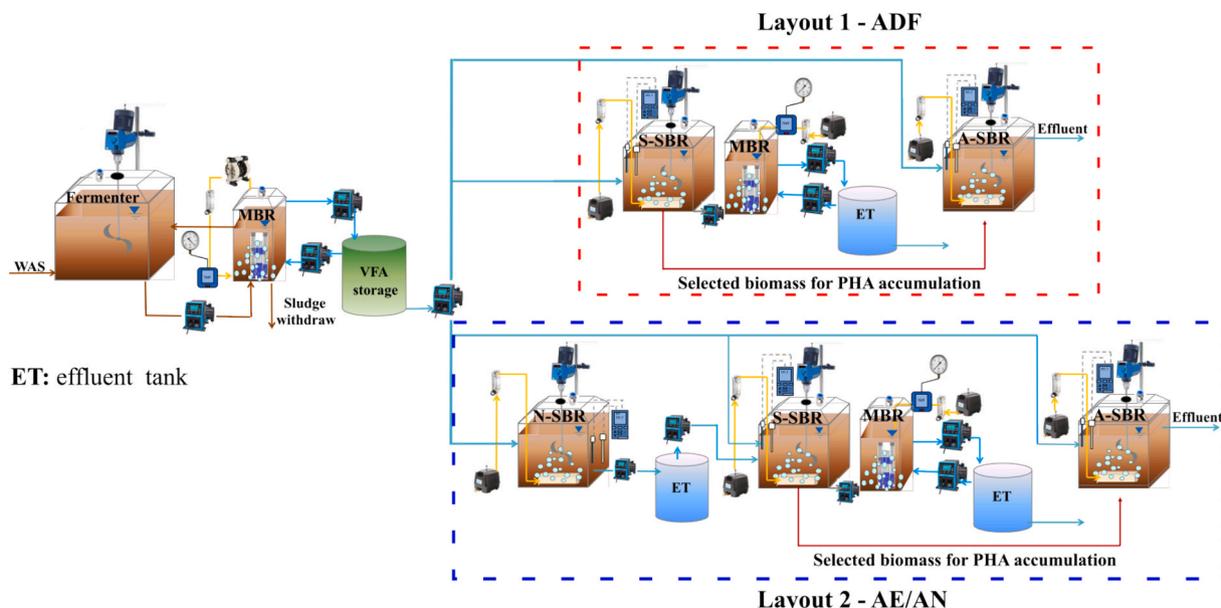
When using the AE/AN enrichment strategy, the N-SBR operates so that its effluent contains NO<sub>x</sub>, which serves as an electron acceptor instead of oxygen during the anoxic famine phase. Its operational cycle, comprising feeding, aerobic reaction, settling and effluent withdrawal, lasts 3.5 to 4 h, depending on the conditions [17]. The volume exchange ratio is set at 0.15. The NO<sub>x</sub>-rich effluent is then stored for use in the S-SBR.

Under AE/AN enrichment, the S-SBR cycle includes VFA feeding, aerobic feast, feeding from the N-SBR effluent, anoxic reaction and

**Table 1**

Average fermented liquid characteristics adopted as feeding for layout 1 (ADF) and layout 2 (AE/AN).

Parameter	U.M	Layout 1	Layout 2
SCOD	mg L <sup>-1</sup>	406.2 ± 125.2	378.6 ± 97.7
VFA	mg COD L <sup>-1</sup>	128.6 ± 45.6	146.4 ± 57.1
Acetic acid	mg COD L <sup>-1</sup>	95.0 ± 36.5	135.1 ± 53.1
Propionic acid	mg COD L <sup>-1</sup>	19.9 ± 12.9	7.1 ± 6.0
Butyric acid	mg COD L <sup>-1</sup>	15.5 ± 5.8	3.7 ± 2.8
Valeric acid	mg COD L <sup>-1</sup>	1.4 ± 1.0	–
VFA/SCOD	mg COD <sub>VFA</sub> mg COD <sup>-1</sup>	0.32 ± 0.04	0.4 ± 0.1
NH <sub>4</sub> <sup>+</sup> -N	mg N L <sup>-1</sup>	104.1 ± 30.0	87.1 ± 27.9
PO <sub>4</sub> <sup>3-</sup> -P	mg P L <sup>-1</sup>	14.3 ± 5.8	9.2 ± 4.8



**Fig. 1.** Schematic representation of the layouts adopted: layout 1 – aerobic dynamic feeding (ADF) and layout 2 – aerobic/anoxic enrichment (AE/AN).

effluent removal via the MBR. The entire cycle length was approximately 10 h, while the feast phase would range from 1 to 3 h, depending on how the process was developing. In contrast, when the ADF strategy is employed, the famine step proceeds aerobically without additional feeding. The aerobic feast phase ends once the dissolved oxygen (DO) concentration stabilizes, marking the start of either the anoxic or aerobic famine phase. The feast-to-famine ratio was kept around  $0.20 \pm 0.05$ . The HRT and volume exchange ratio for the S-SBR are maintained at 2 days and 0.25, respectively. A WTW FDO 925 probe was used to monitor DO and temperature in the reactors. Table 2 outlines the main operating parameters for both the N-SBR and S-SBR.

Food to microorganisms ratio (F/M); organic loading rate (OLR); nitrogen loading rate (NLR); total suspended solids (TSS); volatile suspended solids (VSS).

#### 2.4. Accumulation reactor

The PHA accumulation was performed in the fed-batch mode in an SBR reactor (working volume of 10 L) following the feed-on-demand strategy. The mixed liquor withdrawn from the S-SBR was left to settle for 20 min to remove the supernatant. Before starting the accumulation phase, the sludge was maintained under aeration overnight to establish endogenous conditions. The accumulation is carried out by monitoring the DO as the only parameter with which the accumulation can be run automatically by software installed in a s Pi4 Model B [16]. The Raspberry Pi4 Model B was connected to a multimeter (WTW Multi 3630 IDS) for measuring the DO concentration inside the reactor and to a programmable logic controller (Crouzet M3 XD26) to control the air blower and the feeding pump. A volume of 1 L of fermented WAS liquid was supplied per pulse. Once the maximum level inside the reactor was reached after 6–7 feedings, the sludge was left to settle for 20 min, after which the supernatant was discarded. After removing the supernatant, the cycle would restart with a substrate-feeding pulse.

#### 2.5. Analytical methods

SCOD, VFA,  $\text{NH}_4^+\text{-N}$ , and  $\text{PO}_4^{3-}\text{-P}$  were analyzed in fermentation by sampling the mixed liquor twice weekly. The N-SBR and S-SBR were monitored twice a week by collecting samples from the influent, the mixed liquor within the reactor and the effluent. SCOD,  $\text{NH}_4^+\text{-N}$ ,  $\text{PO}_4^{3-}\text{-P}$ , nitrate ( $\text{NO}_3^-\text{-N}$ ) and nitrite ( $\text{NO}_2^-\text{-N}$ ) were monitored in the influent and effluent. TSS, VSS and dissolved  $\text{N}_2\text{O}$  were monitored in the mixed liquor and the effluent. Gaseous  $\text{N}_2\text{O}$  samples were collected by directly sampling the headspace volume of the reactors [18]. Following previous studies,  $\text{N}_2\text{O}$  sampling was carried out after the feast phase in the S-SBR and at the end of the feeding phase in the N-SBR [19]. In the A-SBR, mixed liquor samples were taken to measure TSS, VSS and PHA concentration, which was calculated as the combined concentration of polyhydroxy butyrate (PHB) and polyhydroxy valerate (PHV) [20].

SCOD,  $\text{NH}_4^+\text{-N}$ ,  $\text{PO}_4^{3-}\text{-P}$ ,  $\text{NO}_3^-\text{-N}$ ,  $\text{NO}_2^-\text{-N}$ , TSS, and VSS were analyzed following the standard method [21,22]. VFAs were quantified using a gas chromatograph (GC) (Agilent 8860) equipped with a flame ionization detector (FID) and a DB FFA column ( $30 \text{ m} \times 0.25 \text{ mm} \times 0.25 \mu\text{m}$ ),

**Table 2**  
Operational conditions of the adopted reactors.

Parameter	U.M	Layout 1 S-SBR	Layout 2 S-SBR	Layout 2 N-SBR
Influent C/N	g COD g $\text{NH}_4^+$	$4.3 \pm 0.2$	$4.5 \pm 1.1$	$4.9 \pm 1.0$
F/M	kg BOD kg $\text{SS}^{-1} \text{d}^{-1}$	$0.2 \pm 0.1$	$0.2 \pm 0.1$	$0.2 \pm 0.1$
SRT	days	$36.7 \pm 12.9$	$10.0 \pm 7.0$	$5.6 \pm 2.3$
OLR	kg COD $\text{m}^{-3} \text{d}^{-1}$	$0.3 \pm 0.1$	$0.3 \pm 0.2$	$0.3 \pm 0.1$
NLR	kg N $\text{m}^{-3} \text{d}^{-1}$	$0.06 \pm 0.02$	$0.16 \pm 0.02$	$0.12 \pm 0.02$
TSS	g $\text{L}^{-1}$	$1.1 \pm 0.2$	$1.4 \pm 0.4$	$1.2 \pm 0.2$
VSS	g $\text{L}^{-1}$	$0.9 \pm 0.2$	$1.0 \pm 0.3$	$0.9 \pm 0.1$
Temperature	$^{\circ}\text{C}$	$21 \pm 4$	$23 \pm 5$	$20 \pm 5$

as described in the literature [23]. The same GC-FID setup, featuring a Restek Stabilwax column ( $30 \text{ m} \times 0.53 \text{ mm} \times 1.00 \mu\text{m}$ ), was employed to determine PHB and PHV concentrations from lyophilized sludge samples [24]. According to the literature,  $\text{N}_2\text{O}$  concentrations were measured using a GC equipped with an electron capture detector and a Porapak-Q 80/100 mesh ( $6 \text{ ft} \times 1/8 \text{ in} \times 2.1 \text{ mm}$ ) column [25].

#### 2.6. Calculations

PHA production was expressed as the percentage of PHA relative to VSS (% g PHA/g VSS). PHA productivity was calculated by quantifying the mass of PHA produced each day (g PHA/day). PHA storage yield was also given in the weight ratio of PHA produced over VFA fed into the reactor as  $\text{g COD}_{\text{PHA}}/\text{g COD}_{\text{VFA}}$ . Oxidation stoichiometries were considered  $1.67 \text{ g COD/g}$  for PHB conversion and  $1.92 \text{ g COD/g}$  for PHV conversion. Stoichiometries of VFA oxidation proposed by Yuan et al. (2011) were used [26]. The  $\text{N}_2\text{O}$  emission factor was calculated considering the gaseous and dissolved  $\text{N}_2\text{O}$  concentration, the reactors' HRT, the reactors' headspace and the total nitrogen concentration in the influent as reported by Tsuneda et al. (2005) [27]:

$$EF_{\text{N}_2\text{O}} = \frac{\frac{\text{N}_2\text{O}-\text{N}_g}{\text{HRT}_{\text{hs}}} + \frac{\text{N}_2\text{O}-\text{N}_d}{\text{HRT}}}{\text{TN}_{\text{influent}}} \quad (1)$$

$\text{N}_2\text{O}-\text{N}_g$  indicates the gaseous  $\text{N}_2\text{O}$  concentration,  $\text{N}_2\text{O}-\text{N}_d$  represents the dissolved  $\text{N}_2\text{O}$  concentration,  $\text{HRT}_{\text{hs}}$  denotes the retention time in the reactor headspace, HRT is the reactor's retention time and  $\text{TN}_{\text{influent}}$  is the total nitrogen concentration entering the system. To assess the environmental impact of these solutions, the carbon footprint (CF) was calculated by summing up the direct, indirect and derived emissions contribution as reported by Boiocchi et al. (2023) [28]. Direct emissions (DE) took into account the equivalent  $\text{CO}_2$  emission from the organic carbon oxidation ( $\text{CO}_2\text{,OrgOx}$ ), endogenous respiration ( $\text{CO}_2\text{,Endog.}$ ) and  $\text{N}_2\text{O}$  emission ( $\text{CO}_2\text{eq,N}_2\text{O}$ ). Indirect emissions (IE) are calculated from the equivalent  $\text{CO}_2$  emission due to energy consumption ( $\text{CO}_2\text{eq, En}$ ) and wasted sludge treatment, transportation and landfill disposal ( $\text{CO}_2\text{eq,Sludge}$ ). The derived emissions (DerE) are calculated from the pollutants discharged into the receiving water bodies, considering the contribution for BOD ( $\text{CO}_2\text{eq,effBOD}$ ) and dissolved  $\text{N}_2\text{O}$  concentrations ( $\text{CO}_2\text{eq,effN}_2\text{O}$ ).

### 3. Results and discussion

#### 3.1. Efficiency of nutrient removal and GHG emissions

The efficiency in carbon, nitrogen and phosphorus removal for both studied processes is reported in Table 3. Overall, both layouts ensured high carbon and nitrogen removal, with removal efficiencies of around 95 % for SCOD removal and around 85 % for TN removal. A slight decrease in TN removal was registered in layout 1 S-SBR. There, TN removal is related to the nitrification occurring during the feast phase, producing nitrites and nitrates not consumed during the famine because of the aerobic conditions. On the contrary, the AE/AN enrichment in layout 2 provided a complete nitrogen removal pathway through nitrification-denitrification despite the non-optimal operating conditions of the N-SBR (Table 2). Orto phosphate removal efficiency was around 50–55 %, indicating a slightly lower performance during layout 1 compared to layout 2. Considering the differences in the influent  $\text{PO}_4^{3-}\text{-P}$  concentration, it is more likely that this was the cause of the different removal efficiencies rather than an improved phosphorus removal process in the AE/AN enrichment. Coupling an MBR to the S-SBR proved to be a successful approach to improve the system performance and achieve a higher-quality effluent.

Despite the systems showing similar efficiencies in contaminants removal, one of the major differences was the direct GHG emissions, as reported in Fig. 2. The S-SBRs'  $\text{N}_2\text{O}$  emission (Fig. 2a) showed a 33 %

**Table 3**

Influent and effluent concentrations and average contaminants removal efficiency for layout 1 (ADF) and layout 2 (AE/AN).

Parameter	U.M	Layout 1 S-SBR	Layout 2 S-SBR	Layout 2 N-SBR	
SCOD	Influent	mg/L	426.2 ± 125.2	403.2 ± 97.7	378.6 ± 97.7
	Effluent	mg/L	13.9 ± 5.3	16.3 ± 12.4	24.7 ± 14.9
	Removal efficiency	%	96.6 ± 0.9	95.5 ± 3.8	92.7 ± 7.1
NH <sub>4</sub> <sup>+</sup> -N	IN	mg N/L	101.0 ± 25.5	97.4 ± 18.0	87.9 ± 20.0
	OUT	mg N/L	16.3 ± 10.2	10.4 ± 5.6	12.2 ± 7.1
	Removal efficiency	%	82.7 ± 6.6	88.8 ± 4.2	83.1 ± 3.3
TN	Influent	mg N/L	107.9 ± 31.5	142.7 ± 28.6	95.9 ± 28.2
	Effluent	mg N/L	27.4 ± 8.8	25.3 ± 2.8	45.4 ± 2.7
	Removal efficiency	%	74.6 ± 9.9	82.3 ± 3.9	52.7 ± 11.6
PO <sub>4</sub> <sup>3-</sup> -P	Influent	mg P/L	14.3 ± 5.8	12.7 ± 6.2	9.2 ± 4.8
	Effluent	mg P/L	7.9 ± 4.6	5.5 ± 4.4	3.5 ± 2.3
	Removal efficiency	%	48.4 ± 15.8	57.4 ± 12.5	55.3 ± 23.0

emission reduction during layout 2 compared to layout 1. On average, N<sub>2</sub>O emitted concentration accounted for 0.21 ± 0.02 and 0.19 ± 0.01 mg N<sub>2</sub>O-N/L in gaseous and dissolved form, respectively, for layout 1 S-SBR. A slight reduction was registered for the S-SBR in layout 2, with N<sub>2</sub>O emission accounting for 0.16 ± 0.04 and 0.10 ± 0.03 mg N<sub>2</sub>O-N/L in gaseous and dissolved form, respectively. However, despite the slight decrease, the emission factor did not exhibit substantial differences between the two enrichment strategies, accounting on average for 0.16 ± 0.09 and 0.13 ± 0.03 % of the TN<sub>influent</sub> for layouts 1 and 2, respectively (Fig. 2b). The slight discrepancies registered may be related to the anoxic famine conditions, which favoured the denitrification in layout 2 S-SBR compared to layout 1, acting more as an N<sub>2</sub>O sink process rather than a production pathway [29]. This result suggests that the main pathway involved in N<sub>2</sub>O generation in the ADF operation is related to the incomplete ammonia oxidation pathway with hydroxylamine (NH<sub>2</sub>OH) as an intermediate. The heterotrophic denitrification during the AE/AN famine phase showed a slightly lower N<sub>2</sub>O emission than the ADF. It has to be underlined that N<sub>2</sub>O emission factors were always below 0.3 % except for the first two weeks of layout 1, where it can be assumed that the start-up conditions worsened the reactor's performance.

A higher N<sub>2</sub>O emission was measured for the N-SBR (Fig. 2a), which resulted in layout 2 emitting almost double the amount of layout 1, achieving 0.39 ± 0.07 and 0.74 ± 0.16 mg N<sub>2</sub>O-N/L as average overall emission for the layouts 1 and 2, respectively. Overall, N-SBR N<sub>2</sub>O emission accounted on average for 0.27 ± 0.05 and 0.21 ± 0.04 mg N<sub>2</sub>O-N/L for gaseous and dissolved form, respectively. The average emission factor accounted for 0.31 ± 0.08 %, with a peak of 0.42 %. Despite being considered "low" N<sub>2</sub>O emissions, it has to be highlighted that the AE/AN was introduced to treat three to ten times higher nitrogen loads than those adopted in this work, which will likely lead to an N<sub>2</sub>O emission increase [20,30]. The results show that the N-SBR is the main contributor to N<sub>2</sub>O emission in the AE/AN system because the ammonia-oxidizing bacteria activity regulates the reactor's efficiency. In the N-SBR, the N<sub>2</sub>O emission pathway could be related to the NH<sub>3</sub> oxidation and partially to the nitrifier denitrification [31–33]. If future studies confirm the low contribution of the nitrifier denitrification pathway, a mitigation strategy focused on nitrate could be tested. As reported in the literature, the electron acceptor adopted during the

famine phase does not affect the system efficiency in PHA degradation [34]. This implies that replacing nitrites with nitrates would not alter the performance of the S-SBR operation and could potentially reduce N<sub>2</sub>O emissions in the N-SBR without compromising system performance. Recent studies have highlighted that the coexistence of different electron acceptors during denitrification (e.g., NO<sub>3</sub><sup>-</sup> and N<sub>2</sub>O) may enhance N<sub>2</sub>O consumption rates, thereby reducing overall emissions [35–37]. Although this approach is still far from a full-scale application, it could be tailored for an AE/AN enrichment-based PHA production system treating high-ammonia influent and offering a mitigation strategy that does not interfere with N-SBR operation.

### 3.2. Carbon footprint assessment

In order to assess the environmental impact of the two strategies compared, the CF was evaluated. Fig. 3 shows the CF for S-SBR and N-SBR for the two enrichment strategies, while the contribution of the fermenter unit is reported below. Being a common unit for the two strategies, the fermentation's CF has been excluded from Fig. 3. Overall, it showed an IE of 1.99 kg CO<sub>2eq</sub>/day composed of 1.94 and 0.05 kg CO<sub>2eq</sub>/day for CO<sub>2</sub>, En and CO<sub>2</sub>sludge, respectively. Fig. 3a shows the overall CF assessment for layouts 1 and 2. DerE is not shown in the figures as they accounted for around 0.01 % of the total emission. The beneficial effect on the indirect emission due to the lack of aeration during the anoxic famine in layout 2 S-SBR is overcome by the N-SBR reactor operation, leading to a 25 % IE increase [38]. The DE shows the same trend, where the N-SBR operation led to a 100 % increased emission compared to the ADF. The contribution of the reactors towards DE and IE is shown in Fig. 3b. Regarding DE, the N-SBR and S-SBR show similar emissions, thus leading to a double emission in the AE/AN enrichment. It has to be underlined that the A-SBR contribution lacks the CO<sub>2</sub>OrgOx contribution since the effluent quality of the A-SBR was not monitored. Since the effluent is supposed to be discharged into the environment for the S-SBR, monitoring the effluent quality should be a mandatory requirement to assess the feasibility of process integration. Regarding IE, it is shown that the anoxic phase led to a 16 % reduction of the S-SBR IE due to a 23 % reduction in the energy consumed (CO<sub>2eq</sub>, En). However, the reduction is unbalanced by the N-SBR operation, whose emission is similar to that of the A-SBR since both reactors apply continuous aerobic conditions.

### 3.3. PHA accumulation

The PHA accumulation was carried out in the pilot-scale reactor A-SBR. The accumulation started on the 60th day of the system operation and lasted until day 114th, as shown in Fig. 4. The ADF-enriched biomass achieved a peak of 0.37 gPHA/gVSS with an HV:HB ratio of 0.08 and an average PHA productivity of 0.26 gPHA/day (26 gPHA/m<sup>3</sup>). Similar results were achieved by the AE/AN enriched sludge, which produced 0.38 gPHA/gVSS with an HV:HB ratio of 0.11. A slight decrease in PHA productivity was observed, accounting on average for 0.19 g PHA/day (19 gPHA/m<sup>3</sup>) for layout 2. The results show no substantial difference in the PHA produced between the two layouts. The main difference was related to the PHA productivity, which achieved a higher peak value (4th day of accumulation) in layout 1 compared to layout 2. Also, the two strategies achieved similar storage yields, 0.38 ± 0.10 gCOD<sub>PHA</sub>/gCOD<sub>VFA</sub> for the ADF and 0.43 ± 0.15 gCOD<sub>PHA</sub>/gCOD<sub>VFA</sub> for the AE/AN enrichment. The enrichment OLR was 0.3 kg COD/m<sup>3</sup> for both strategies. ADF showed slightly lower production compared to other WAS-based PHA production processes. Valentino et al. (2019b, 2019a) [39,40] and Moretto et al. (2020) [41] applied WAS co-fermentation with the organic fraction of municipal solid waste at the pilot scale, achieving a maximum of 0.49, 0.46 and 0.52 gPHA/gVSS, respectively. The same enrichment strategy was used in their work with a higher enrichment OLR, respectively 2–3.5, 4 and 4.4 kg COD/m<sup>3</sup> day, which resulted in an increased amount of PHA produced. As

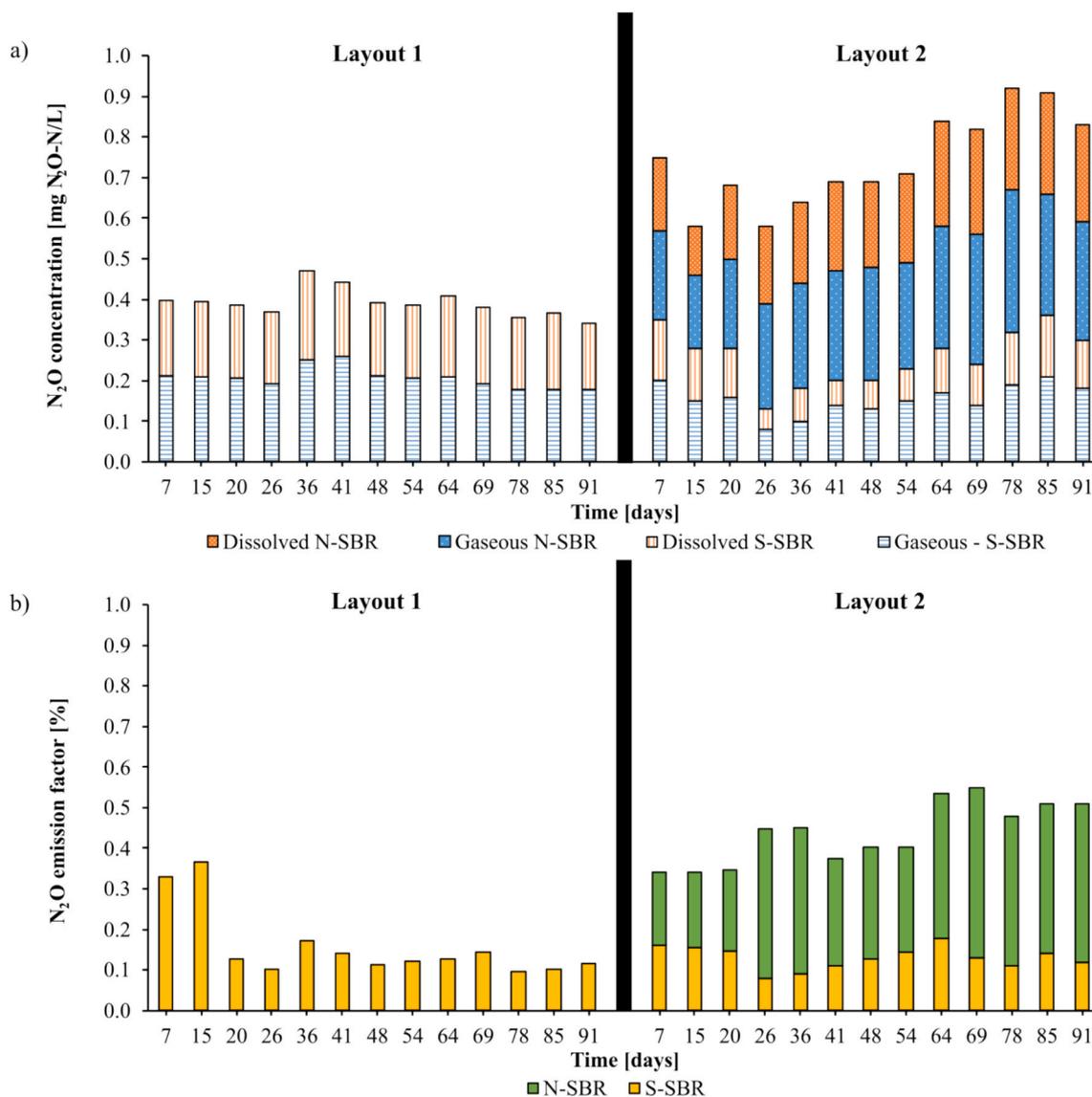


Fig. 2. Nitrous oxide concentration emitted (a) and relative emission factors (b) for layout 1 (ADF) and layout 2 (AE/AN).

reported by literature, increasing the enrichment of OLRs increases the amount of PHA produced in the accumulation up to a threshold [42,43]. Also, a slight difference in the storage yield was observed, achieving 0.33–0.44, 0.44–0.50 and 0.59 gCOD<sub>PHA</sub>/gCOD<sub>VFA</sub>, respectively.

On the other hand, the AE/AN enrichment showed a slightly higher PHA production compared to Frison et al. (2015) [30]. By adopting a primary sludge and WAS co-fermented substrate, the authors achieved 0.19–0.21 g PHA/g VSS, 45 % less than the one reported in this work despite the higher enrichment OLR (0.72–0.82 kg COD/m<sup>3</sup> day). However, it must be underlined that an increased amount was achieved in this work because of the longer accumulation time adopted. In another paper adopting the same substrate as Frison et al. (2015) [30], Conca et al. (2020) [20] achieved 0.44 g PHA/g VSS with a storage yield of 0.58–0.61gCOD<sub>PHA</sub>/gCOD<sub>VFA</sub>. This result is comparable to the values reported by the ADF's relative literature and much higher than that achieved in this work. However, the authors adopted a C/N of 50 g COD/g N during the accumulation, while in this work, the A-SBR fed the same as the S-SBR, the accumulation C/N was around 4.5 g COD/g N. Moreover, a much higher OLR was adopted in the work of Conca et al. (2020) [20], reaching 1.3–1.6 kg COD/m<sup>3</sup> day, thus indicating that the lower PHA produced was related to the unfavorable enrichment conditions adopted in this paper compared to literature.

### 3.4. Assessing the potential of the two strategies

If the PHA production has to be implemented in regular WWTP operation, the limits imposed by the upcoming UWWTD must be considered. Indeed, urban areas larger than 100,000 person equivalents have to implement tertiary treatment for N and P removal, achieving effluent concentrations < 6 mg/L for N and < 0.5 mg/L for P [10]. According to the results presented in this work, none of the MBR-based strategies can produce such a high-quality effluent. The AE/AN (layout 2) seems to be the more promising in view of completely removing N due to S-SBR denitrification. Improving the N-SBR operation may lead to S-SBR effluent respecting the N discharge limit, as partially shown by the literature (Conca et al., 2020) [20]. P concentrations do not meet the limits for both strategies since it can be assumed that biological growth is the primary removal mechanism involved. Tertiary treatment would still be necessary on PHA effluent, meaning that biochar adsorption or chemical precipitation has to be considered in the WAS-based process [44]. However, the major drawback at a larger scale, treating high N-rich influents, is the high emissions registered for AE/AN (layout 2). Despite improving the performance compared to the single reactor scheme [30], introducing the N-SBR led to high indirect and direct emissions, comparable if not higher than the S-SBR. Even assuming that

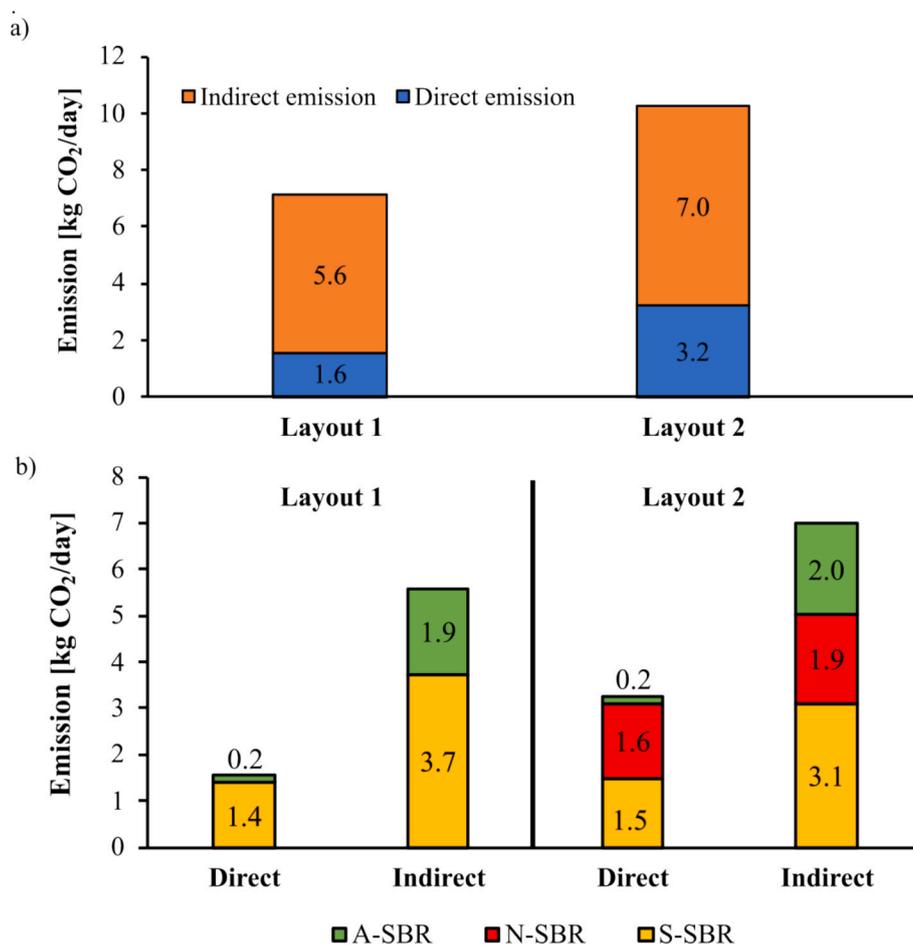


Fig. 3. Overall emission (a) and specific contribution of each reactor for direct and indirect emissions (b).

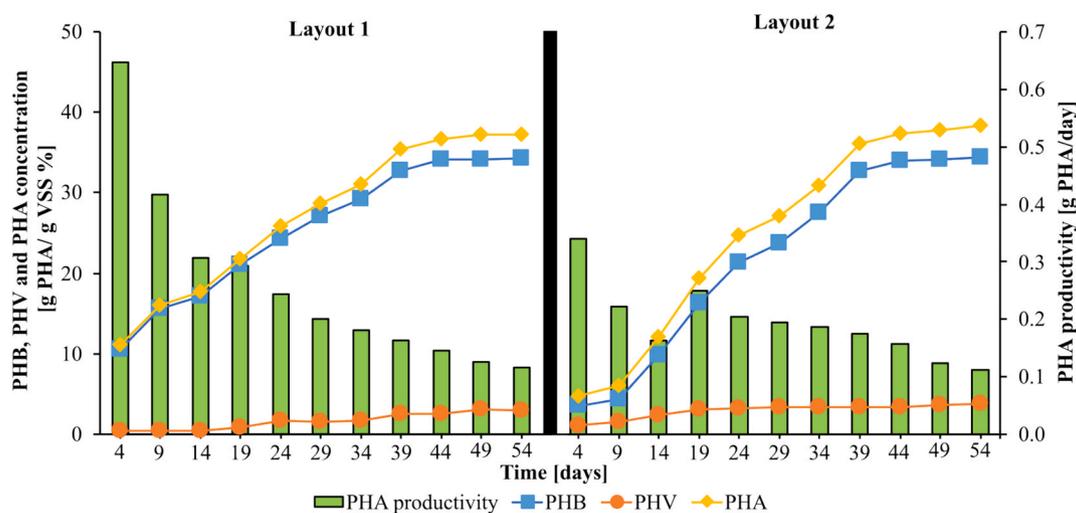


Fig. 4. Results of the PHA accumulation.

indirect emissions can be balanced by the amount of PHA produced by the system, indicating a net zero or low carbon emissions system [45], direct emissions still pose a potential high-risk towards N-SBR applications (layout 2). N<sub>2</sub>O mitigation strategies could be tested but should not interfere with the AOB activity. A possible solution could be the improvement of the single-reactor scheme, even if it has been poorly tested and N<sub>2</sub>O emissions data are lacking [34,38,46]. In view of solving the potential low NO<sub>x</sub> concentration in the famine feed when the single-

reactor scheme is used, the system could be applied to conventional activated sludge systems, which do not achieve complete denitrification. In this way, the main biological reactor will act as the N-SBR, while the PHA production line will be a sludge treatment and denitrification-based N removal line. In view of integrating PHA with urban wastewater treatment, AE/AN enrichment seems to be one of the most promising strategies. However, the literature poorly evaluated the strategy, which did not focus on its major drawback: GHG emissions. In view of the

environmentally sustainable application of the PHA process, nutrient removal and GHG emissions must be monitored and minimised while maximising the produced PHA.

#### 4. Conclusions

This study compared two MBR-based enrichment strategies, ADF (layout 1) and AE/AN (layout 2) – for producing PHA from domestic WAS. Both systems showed high COD removal (> 95 %) with nitrogen removal efficiencies of 83 % for layout 1 and 89 % for layout 2. Although layout 2 showed slightly better efficiency in N removal, it was associated with significant direct N<sub>2</sub>O emissions of 0.74 ± 0.16 mg N<sub>2</sub>O-N/L and a 42 % higher carbon footprint than layout 1, reaching 10.2 kg CO<sub>2</sub>/day. Both systems produced a considerable amount of PHA (around 38 % w/w). Despite the AE/AN enrichment strategy's potential for complete N removal while producing PHA, the direct and indirect GHG emissions could pose a significant challenge to its practical application. Therefore, the environmental sustainability of these processes should be considered when implementing PHA production strategies in urban WWTPs. Future studies should focus on monitoring and mitigating GHG emissions while maximising PHA production to ensure a genuinely sustainable approach.

#### CRedit authorship contribution statement

**Antonio Mineo:** Writing – original draft, Validation, Methodology, Formal analysis. **Mark M.C. van Loosdrecht:** Writing – review & editing, Visualization, Validation, Investigation. **Giorgio Mannina:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Investigation, Data curation, Conceptualization.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Data availability

The data that has been used is confidential.

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