



A comprehensive comparison between two strategies to produce polyhydroxyalkanoates from domestic sewage sludge

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ABSTRACT

Biopolymer materials, such as polyhydroxyalkanoates (PHA), are essential to restrain the use of petroleum-based plastic material fostered by European Union regulation. Despite the recent development regarding the production of PHA, the scale-up of sewage sludge-based technology is still in its infancy since the literature lacks studies on the environmental impact of the process. This work aims to stand as a pioneer study reporting the direct greenhouse gas (GHG) emissions and carbon footprint (CF) of two sewage sludge-based PHA production strategies. The two strategies, aerobic dynamic feeding (ADF) and aerobic/anoxic enrichment (AE/AN), were monitored and compared based on the system's efficiency in removing carbon and nutrients, PHA's production and productivity, nitrous oxide direct emissions and the CF. The produced PHA accounted for 38.21 g PHA g⁻¹ volatile suspended solids (VSS) % and 14.54 g PHA g⁻¹ VSS %, for ADF and AE/AN, respectively. The N₂O emissions were lower for ADF than the AE/AN enrichment, 0.39 N₂O–N L⁻¹ and 0.98 N₂O–N L⁻¹, respectively, as was the CF, which accounted for 3.56 kg CO₂ day⁻¹ and 6.91 kg CO₂ day⁻¹ for ADF and AE/AN respectively. This innovative study provides valuable insights into comparing the two above-mentioned strategies and a pilot for structuring and designing future studies comprehensively considering the environmental consequences of the process in future life cycle assessments.

1. Introduction

The escalating environmental concerns from the widespread use of petroleum-based plastics have catalysed an urgent quest for sustainable alternatives (Costa et al., 2023; Manali Shah et al., 2021; Rosenboom et al., 2022). Recently, European Union legislations have moved towards banning single-use plastic products, fostering the adoption of new bioplastic materials (European Commission, 2018; European Parliament/European Parliament C. of the E.U., 2019). In this context, polyhydroxyalkanoates (PHA) have emerged as promising new biopolymers due to their properties (Mannina et al., 2020; Puyol et al., 2017). The most common polymer, polyhydroxybutyrate (PHB), possesses a high melting point and good tensile strength, similar to polypropylene (Laycock et al., 2013). When mixed with the polyhydroxyvalerate (PHV), it forms copolymers having a higher elongation at break and elasticity compared to the PHB (Abate et al., 2024; Volova et al., 2023). The increasing need to implement a circular economy view in the water sector has fostered PHA production research as a resource recovered from wastes such as sewage sludge (Gherghel

et al., 2019; Yadav et al., 2020). The conventional sludge disposal is based on a linear economy model, implying an economic and environmental cost to dispose of the waste. However, by following the zero-waste concept, almost 50% of the nutrients in the wasted sewage sludge can be recovered while reducing the sludge's disposal cost (Das et al., 2020; Ding et al., 2021). Moreover, resources recovered, such as PHA, also imply an environmental benefit by producing valid biodegradable alternatives to conventional petroleum-based plastic (Capodaglio, 2023; Crutchik et al., 2020; Smol et al., 2020).

However, despite considerable advancements in PHA production methodologies, the technology still needs to be in its formative stages, underscoring an essential gap in comprehensive literature (Kourmentza et al., 2017; Rodriguez-Perez et al., 2018). PHA production by mixed microbial cultures (MMC) is usually based on a three-step process: i) carbon source production, usually volatile fatty acids (VFA) or glycerol, used as feedstock for PHA producer microorganisms; ii) enrichment of the PHA producers available in the microbial cultures of the sewage sludge; iii) PHA accumulation inside the microorganisms' cells (Saba-pathy et al., 2020; Yakesh Kannah et al., 2022). Despite not being the

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best feedstock to produce platform chemicals for PHA production by MMC (Mannina et al., 2020), sewage sludge is usually preferred for facilitating the sludge management and disposal in wastewater treatment plants (WWTP) while lowering the production cost (Li and Wilkins, 2020; Pakalapati et al., 2018). Several selection strategies have been developed to propose different ways to implement PHA production in wastewater treatment while producing high-quality polymers (De Donno Novelli et al., 2021). In aerobic dynamic feeding (ADF), the carbon source limiting conditions are the PHA production promoters since they are based on cycles of substrate availability (feast) and unavailability (famine) under aerobic conditions. The microorganisms that can produce PHA benefit from their consumption during the famine phase, surviving the carbon deficiency phase (Amulya et al., 2015; Ren et al., 2009; Valentino et al., 2017). Despite achieving the higher PHA produced (average 0.5 g PHA/g VSS with fermented sewage sludge), energy consumption due to the aerobic conditions contains its implementation to an industrial level (Bengtsson et al., 2017; Coats et al., 2007; Mengmeng et al., 2009). In view of implementing PHA production in the wastewater treatment process, the aerobic/anoxic (AE/AN) enrichment coupled with nitrification/denitrification was developed by Frison et al. (2015) and N. Basset et al. (2016). The process is based on aerobic feast and anoxic famine, where nitrate and nitrite produced during the feast are used as electron acceptors, taking advantage of the PHA produced as a carbon source to perform the denitrification (Conca et al., 2020; Estévez-Alonso et al., 2021). Despite being one of the most valuable strategies to adopt in wastewater treatment, a low PHA production rate (average 0.3 g PHA/g VSS) hampered its application.

In recent years, the literature has focused on achieving a higher PHA production and polymer quality rather than comprehensively assessing which technology can be the best solution to produce PHA (Kourmentza et al., 2017). This assessment must also concern the environmental impact of the adopted solutions (Khatami et al., 2021; Vicente et al., 2023). Greenhouse gas (GHG) emissions and carbon footprint (CF) are indeed partially considered, leading to a knowledge gap regarding the environmental sustainability of the adopted solutions (Parravicini et al., 2016; Rodríguez-Caballero et al., 2015). Moreover, to maximize VFA production, several high-energy demand pretreatments are performed on sewage sludge (i.e. ultrasound, thermal, alkaline, enzymatic pretreatment), which will inevitably increase the environmental impact of the applied technology (Fang et al., 2020; Liao et al., 2018; Liu et al., 2020). This relates not only to the direct and indirect GHG emissions, but also to the number and scale of reactors used to produce PHA, which is tied to the PHA's productivity rate (Nguyenhuynh et al., 2021).

The findings of this study hold major significance towards sustainable PHA production aligned with the circular economy paradigm. By comparing MMC's based PHA production strategies and their associated environmental implications, this research aims to provide insights crucial for filling the knowledge gap under the imperative integration of environmentally conscious practices into the PHA production process. The experimental activities were carried out in pilot-scale vessels, enhancing the findings' significance within the context of future commercialization. A comprehensive comparison between the ADF and AE/AN enrichment has been carried out to assess the effectiveness and sustainability of the different solutions. The two strategies have been compared based on: i) the system performance in carbon and nutrient (nitrogen and phosphorous) removal efficiency; ii) the amount of PHA produced and the productivity rate; iii) the direct nitrous oxide emission both in gaseous and liquid form; iv) the environmental impact of each strategy, assessed by calculating the CF.

2. Materials and methods

The experiments were carried out at the Water Resource Recovery Facility (WRRF) of Palermo University (Mannina et al., 2021). The PHA production line is composed of a fermenter (working volume: 200 L) equipped with an ultra-filtration unit and two sequencing batch reactors

(SBR) devoted to PHA producers' selection (S-SBR, (working volume: 30 L) and nitrification (N-SBR, working volume: 30 L). At the end of the enrichment process, the selected biomass was used to perform batch-test accumulation automatically controlled by a tailored home-made software (Isern-Cazorla et al., 2023; Mineo et al., 2023). The software applies a feed-on-demand strategy based on the dissolved oxygen (DO) concentration measured in the accumulation reactor. The inoculum of the fermenter, S-SBR and N-SBR was wasted sewage sludge from the water treatment line of the WRRF (Cosenza et al., 2023).

2.1. Pilot plant layouts

When the ADF strategy was adopted (Fig. 1a), the fermented sludge liquid containing VFA was used as a carbon source to feed the S-SBR. The selection cycle lasted 12 h and was composed of: i) feeding (10 min), ii) feast/famine (F/F) regime (11 h and 10 min), iii) settling (30 min) and iv) supernatant removal (10 min). When the AE/AN enrichment strategy was adopted (Fig. 1b), the fermented sludge liquid was used to feed both the S-SBR and the N-SBR. The N-SBR cycle lasted 3 h and 30 min and was composed of: i) feeding (10 min), ii) aerobic biological reaction (2 h and 40 min), iii) settling (30 min) and iv) supernatant removal (10 min). The S-SBR cycle was composed of an aerobic feast phase (1–3 h) and an anoxic famine phase (8–10 h), at the start of which the NO_x rich supernatant recovered from the N-SBR effluent was fed to the S-SBR to be able to perform the denitrification. The average parameters for the two enrichment strategies are reported in Table 1.

Once a steady state was achieved in the S-SBR, i.e., when the F/F remained constant (<5% deviation) for at least 14 days, the reactor performances were calculated and monitored for four weeks. Finally, the biomass was withdrawn and stressed to accumulate PHA in the batch accumulation reactor (Mannina et al., 2019). Briefly, 3 L biomass was withdrawn from the S-SBR, and 2 L of supernatant were removed after settling. Biomass was washed twice with tap water, and the supernatant was removed each time to ensure no carbon source was available. After, the biomass was left aerated overnight to achieve endogenous conditions. Subsequently, the accumulation was performed using the fermented sludge liquid as a carbon source and the DO as a parameter to run the accumulation automatically, as Mineo et al. (2023) reported. DO was set at 2 mg/L in all the reactors. A programmable logic controller controls the pilot plant operation (Millennium 3, Crouzet). The aerobic conditions were established in all the reactors by using an air blower connected to a ceramic diffuser at the bottom of the reactors. In anoxic conditions, a mixer stirring at 125 rpm was used. T and DO were continuously monitored in all the reactors using a probe (WTW FDO® 925-P).

2.2. Analytical methods

S-SBR and N-SBR were monitored twice weekly by sampling the influent, mixed liquor inside the reactor and effluent. Biological Oxygen Demand (BOD), Soluble Chemical Oxygen Demand (sCOD), Nitrogen as Ammonia (NH₄⁺-N), Nitrites (NO₂⁻-N) and Nitrates (NO₃⁻-N), Phosphorus as Phosphate (PO₄³⁻-P), Total and Volatile suspended solids (TSS and VSS), EPS and SMP concentration were analysed according to the standard methods (Rice and Bridgewater, 2012).

The PHA produced was analysed following the literature protocol (Mannina et al., 2019). Briefly, mixed liquor samples (20 mL) were collected during the selection, and the accumulation was mixed with 1 mL of formaldehyde solution (37%) to inhibit the biological activity. The samples were centrifuged at 8000 rpm for 30 min, the supernatant was removed and the samples were lyophilised. Weighted lyophilised samples were mixed with butanol and hydrochloric acid and incubated at 100 °C for 8 h. The extraction was performed by adding one hexane aliquot and two milliQ grade water aliquots. The recovered organic phase was then filtered (0.22 µm) and stored in 2 mL vials. Samples were analysed by an Agilent gas chromatographer (GC) (8860) equipped with

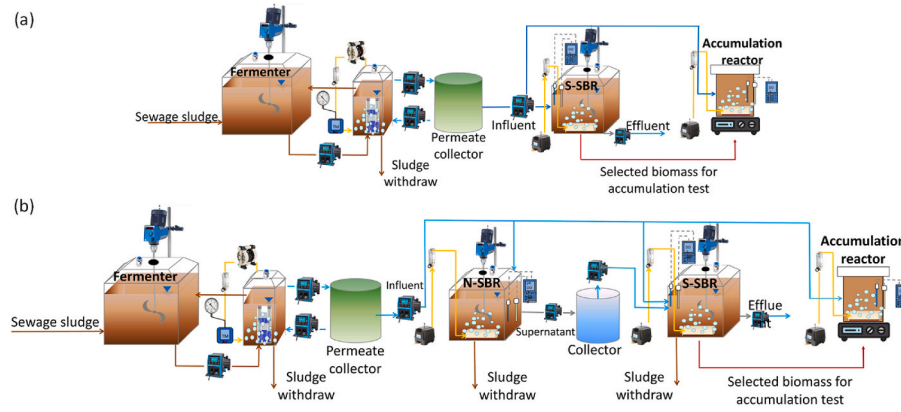


Fig. 1. Schematic representation of the ADF layout (a) and AE/AN enrichment layout (b).

Table 1
ADF and AE/AN enrichment parameters.

Parameter	Acronym	U.M.	ADF		AE/AN			
			S-SBR		N-SBR		S-SBR	
			Average	St.dev.	Average	St.dev.	Average	St.dev.
Food to Microorganism ratio	F/M	kg BOD kg SS ⁻¹ d ⁻¹	0.18	0.09	0.15	0.06	0.17	0.05
Volumetric Organic Loading Rate	vOLR	kg COD m ⁻³ d ⁻¹	0.13	0.02	0.14	0.02	0.16	0.09
Temperature	T	°C	16.03	2.51	14.30	2.10	14.51	1.97
Extra Polymeric Substances	EPS	mg/g VSS	221.72	46.74	300.92	63.49	179.16	59.08
Soluble Microbial Products	SMP	mg/g VSS	52.71	13.38	32.46	17.91	19.24	5.48

a flame ionisation detector and a Restek Stabilwax column (30 m × 0.53 mm x 1.00 µm film thickness), which allowed to determine the PHB and PHV monomer concentration. Helium was used as carrier gas at 29 mL min⁻¹. The injector and detector temperature were 220 °C and 250 °C, respectively. The oven temperature programme was set to 70 °C for 2 min, increased to 160 °C at 10.

°C/min and held for 2 min. The run was set to 5 min while the split ratio was 40:1 (Montiel-Jarillo et al., 2017; Werker et al., 2008). PHB and PHV calibration was performed by using Poly [(R)-3-hydroxybutyric acid] (363,502-10G) and Poly (3-hydroxybutyric acid-co-3-hydroxyvaleric acid) (403,105-10G) standards from Merck - Sigma Aldrich, Germany.

Dissolved and gaseous nitrous oxide (N₂O) concentrations have been measured twice a week, according to the procedure described by Mannina et al. (2018). Dissolved N₂O was analysed by collecting mixed liquor samples from the S-SBR and N-SBR, which were centrifuged at 8000 rpm for 5 min 70 mL of supernatant were stored in glass bottles (total volume 125 mL) and mixed with 1 mL of a 2 N sulphuric acid solution to inhibit the biological activity. Rubber septum sealed bottles were mixed for 24 h, after which a sample was collected from the bottles' headspace. Gaseous N₂O samples are collected directly from the reactor's headspace. N₂O concentration was measured by a GC equipped with an electron capture detector and a Porapak-Q 80/100 mesh column (6 ft x 1/8 in x 2.1 mm).

2.3. Calculations

The PHA concentration was calculated as the sum of the PHB and PHV monomer concentration (Conca et al., 2020). Overall PHA concentration was calculated as a weight percentage based on the gram of PHA per gram of VSS as reported by Equation (1):

$$PHA \% = \frac{w}{w} = \frac{g\ PHA}{g\ VSS} * 100 \quad (\text{Equation 1})$$

PHA productivity was defined as the gram of PHA produced hourly

(g PHA/hour). Nitrification and denitrification efficiency were calculated as reported by Battistoni et al. (2008), while the Nitrite Accumulation Rate (NAR) has been calculated according to Kowal et al. (2022).

The CF assessment was performed by quantifying and summing the direct, indirect and derived emissions. Direct emissions (DE) have been calculated considering the equivalent CO₂ emission due to the organic carbon oxidation (CO₂,OrgOx), endogenous respiration (CO₂,Endog.) and N₂O emission (CO₂eq,N₂O). CO₂, OrgOx (Equation (2)) and CO₂, Endog (Equation (4)) have been quantified according to Boiocchi et al. (2023).

$$CO_2, \text{OrgOx} = FCS * rO_2 (\text{kg CO}_2 / \text{day}) \quad (\text{Equation 2})$$

Where FCS is the conversion factor indicating the amount of CO₂ emitted per kg of consumed O₂ (equal to 1.1 kg CO₂/kg O₂ (Boiocchi et al., 2023)) and rO₂ is the amount of oxygen consumed per day [kg O₂/d] (Equation (3)).

$$rO_2 = Vrs * \left(\frac{1}{f} - 1.42 * Y \right) \quad (\text{Equation 3})$$

Where Vrs is the BOD5 removed per day [kgBOD5/d] based on the measured data, f equals 0.68 and Y is the calculated cell growth rate [kgVSS/kgBOD5].

$$CO_2, \text{Endog} = FC\text{Endog} * mVSS (\text{kg CO}_2 / \text{day}) \quad (\text{Equation 4})$$

Where FCEndog is the conversion factor describing the amount of CO₂ emitted per kg of produced VSS (equal to 1.947 kg CO₂/kgVSS (Boiocchi et al., 2023)) and mVSS is the mass of VSS based on the mass balance.

The CO₂eq, N₂O has been quantified based on the measured N₂O concentration according to Equation (5).

$$CO_{2eq}, N_2O = Qg * Cg, N_2O * GWP_{N_2O} (\text{kg CO}_2 / \text{day}) \quad (\text{Equation 5})$$

Where Qg [m³/day] is the average gas flow, Cg, N₂O [kgN₂O/m³] is the average measured gaseous N₂O concentration and GWP_{N₂O} [kgCO₂eq/kgN₂O] is the N₂O global warming potential (equal to 298 according to

IPCC, 2022).

Indirect emissions (IE) account for the equivalent CO₂ due to energy consumption (CO₂eq,En) (Equation (6)) and to the wasted sludge treatment, transportation and landfill disposal (CO₂eq,Sludge) (Equation (7)).

$$CO_2eq, En = En * FCEn (kg CO_2eq / day) \quad (\text{Equation 6})$$

Where En (kWh/day) is the total energy consumption based on the measured data and FCEn [kgCO₂eq/kWh] is the conversion factor of the energy (equal to 0.252 kgCO₂eq/kWh according to Scarlat et al., 2022).

$$CO_2eq, Sludge = mSludge * FCSludge (kg CO_2eq / day) \quad (\text{Equation 7})$$

Where mSludge (ton/day) is the mass of wasted sludge per day based on the measured data and FCSludge (kgCO₂eq/ton) is the emission factor due to the sludge treatment, transportation and landfill disposal (equal to 714.74 kgCO₂eq/ton according to Zhao et al., 2023).

The derived emissions (DerE) are related to the pollutants discharged into the receiving water bodies (Equation (8)).

$$DerE = CO_2eq, effBOD + CO_2eq, effN_2O (kg CO_2eq / day) \quad (\text{Equation 8})$$

Specifically, the contribution of BOD and dissolved N₂O has been calculated according to Equations (9) and (10), respectively.

$$CO_2eq, effBOD = mBOD * FCBOD (kg CO_2eq / day) \quad (\text{Equation 9})$$

Where mBOD (kgBOD/day) is the mass of discharged BOD per day based on the measured data and FCBOD (kgCO₂eq/kgBOD) is the conversion factor due to the BOD discharge (equal to 0.96 kgCO₂eq/kgBOD according to Boiocchi et al., 2023).

$$CO_2eq, effN_2O = Qw * Cl_{N_2O} * GWPN_2O (kg CO_2eq / day) \quad (\text{Equation 10})$$

Qw (m³/day) is the average treated flow rate, Cl_{N₂O} (kgN₂O/m³) is the average measured liquid N₂O concentration discharged.

3. Results and discussion

The two enrichment strategies were compared by considering the system's performance in terms of carbon, nitrogen, and phosphorous removal efficiencies, PHA accumulation, and the carbon footprint of the process.

3.1. System performance

Carbon, nitrogen and phosphorous removal efficiencies are reported in Fig. 2. Overall, sCOD removal efficiency (Fig. 2a) was always higher than 80% overall. S-SBR removal efficiency remained constant through the different configurations (90.80 ± 4.73% for ADF and 90.33 ± 1.76% for AE/AN), thus proving in both cases the high system performance (Morgan-Sagastume et al., 2015; Valentino et al., 2019). N-SBR removal efficiency was slightly lower than S-SBR, achieving an average removal of 87.16 ± 5.42%. This result may be related to the shorter cycle time of N-SBR, compared to S-SBR, to perform the Ammonia Oxidizing Bacteria (AOB) enrichment and favour the Nitrite Oxidizing Bacteria (NOB) washout (Conca et al., 2020; Musa and Idrus, 2020).

Ammonium removal efficiency (Fig. 2b) reached the highest value with the AE/AN strategy. S-SBR ADF achieved an average removal of 75.57 ± 6.51%, comparable to other studies regarding the ADF strategy (Moretto et al., 2020a). The AE/AN strategy reached higher removal efficiencies, namely 86.20 ± 6.33% for S-SBR and 89.55 ± 1.20% for N-SBR, thus proving the system efficiency. Still, despite the high ammonium removal efficiency, the calculated NAR in the N-SBR was 20.45 ± 1.84%. This result may be related to the low temperature measured inside the reactor (Table 1), which hampered the nitrite production pathway, as already reported in the literature (Conca et al.,

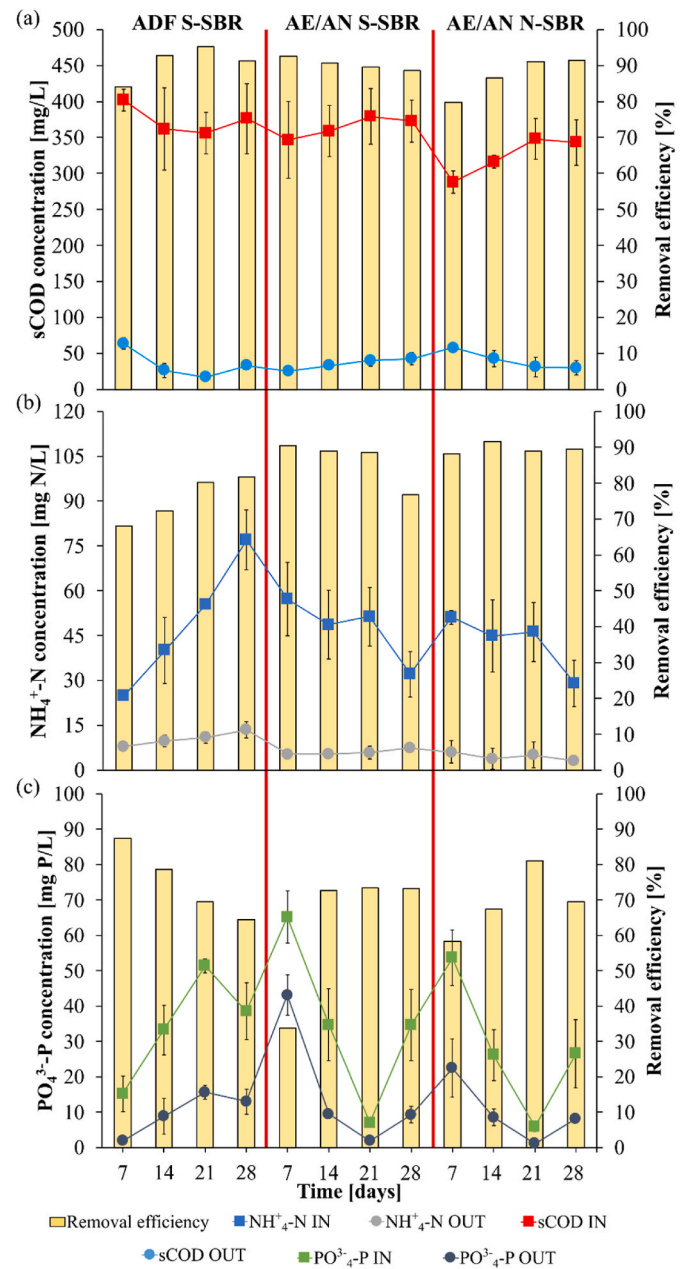


Fig. 2. ADF and AE/AN enrichment general performance.

2020; Frison et al., 2015). In fact, in view of maintaining a low carbon footprint, the temperature was not controlled inside the reactors, thus disturbing the AOB enrichment and NOB washout, causing the NAR deficiency. Despite the low nitrite production, the average nitrification efficiency for N-SBR was 75.39 ± 15.15%, while the S-SBR denitrification efficiency accounted for 68.50 ± 20.02%. As reported in the literature (Salehizadeh and Van Loosdrecht, 2004), PHB degradation is independent of the electron acceptor used, meaning that nitrate production was still suitable for the anabolic metabolism during the famine. Despite achieving relatively good efficiencies, a higher nitrite production would have also increased the denitrification efficiency for S-SBR. As already pointed out by Conca et al. (2020), adopting an operational temperature of around 25 °C in the N-SBR will enhance nitrite productivity, while no direct correlation has been found with the sludge retention time (Jubany et al., 2009; Wu et al., 2016).

The ADF S-SBR reached the highest phosphate removal efficiency (Fig. 3c) of 75.04 ± 10.19%, while a slight decrease during the AE/AN

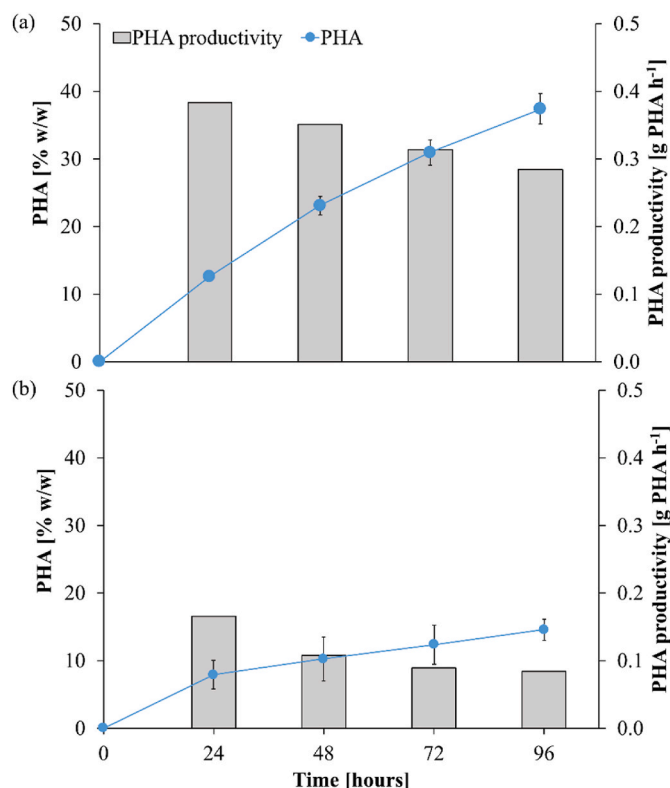


Fig. 3. PHA concentration and productivity for the biomass enriched by ADF (a) and AE/AN (b).

was registered, achieving $63.25 \pm 19.63\%$ and $69.05 \pm 9.40\%$ for S-SBR and N-SBR, respectively.

The PHA accumulation was performed in duplicate at batch scale for four days. ADF and AE/AN enriched biomass were treated using the same sewage sludge fermented liquid as influent to neglect the carbon source effect on PHA production. As reported in Fig. 3, the ADF reached 38.21% w/w (0.9 g PHA/L) of accumulated PHA, while the AE/AN enrichment achieved only 14.54% w/w (0.26 g PHA/L). Also, the PHA productivity was significantly different, reaching a peak of 0.38 g PHA/hour for the ADF (0.38 g PHA/L hour) and 0.17 g PHA/hour for AE/AN (0.16 g PHA/L hour). Significant differences in the substrate, especially in COD and ammonium, were not registered during both selection strategies. This may suggest that the difference in PHA production is not related to the substrate used during the selection step. Compared to the ADF, the AE/AN enrichment may be disadvantaged towards PHA production due to the different microbial community composition (Lorini et al., 2022). In fact, due to the anoxic famine conditions, a disturbance of the PHA producers can be assumed, thus reducing the amount of PHA that can be produced, as reported in several studies (N. Basset et al., 2016; Frison et al., 2015; Morgan-Sagastume et al., 2010). However, future studies on comprehensively comparing different strategies to produce PHA from sewage sludge's MMC should report in depth metagenomic analysis to address the previous assumption. PHA production achieved with the ADF strategy is comparable to other sewage sludge ADF-based PHA production studies in the literature (Moretto et al., 2020b; Morgan-Sagastume et al., 2015; Valentino et al., 2019). Still, the accumulation reactor in this study operated for a period of 16 times longer than the above-cited studies. The reason is that no pretreatment was performed during the acidogenic fermentation step, leading to a considerably lower amount of COD and VFA produced, leading to a less efficient PHA accumulation (Isern-Cazorla et al., 2023). On the contrary, Moretto et al. (2020b) and Valentino et al. (2019) co-digested thickened sludge with the organic fraction of municipal solid wastes while controlling the temperature over the process while Morgan-Sagastume et al.

(2016) adopted thickened waste sludge with pH and T control. Despite the great potential of the AE/AN as a strategy to couple the denitrification process in the WWTP and the PHA production, there is still a lack of information regarding the PHA production for the AE/AN enrichment, especially compared to other more performing selection strategies. Results clearly show that, despite being better for carbon, nitrogen and phosphorous removal, the AE/AN strategy is still far from reaching the PHA production of the biomass enriched by the ADF.

3.2. GHG emissions

N₂O liquid and gaseous concentrations are reported in Fig. 4a and b, respectively. Overall, ADF showed the lowest N₂O emission (on average 0.26 ± 0.06 mg N₂O-N/L for the gaseous phase and 0.13 ± 0.04 mg N₂O-N/L for the liquid phase) with a decreasing trend throughout the experimental period. On the other hand, the AE/AN strategy revealed the highest N₂O emissions, comparable to those related to full-scale WWTPs (Campos et al., 2016). N-SBR emitted, on average, 0.41 ± 0.01 mg N₂O-N/L and 0.13 ± 0.02 mg N₂O-N/L for gaseous and liquid phase, respectively, the highest gaseous N₂O concentration recorded. The AE/AN S-SBR revealed a lower N₂O emission than the N-SBR, reaching 0.25 ± 0.03 mg N₂O-N/L for the gaseous phase and 0.20 ± 0.01 mg N₂O-N/L for dissolved N₂O. Furthermore, during the AE/AN, N₂O emission remained constant. The effluent nitrogen mass balance has been performed to understand better and relate the GHG emissions, as shown in Fig. 5. During ADF, in the first weeks almost a third of the influent was metabolised (37.69 and 25.10%), decreasing until reaching the minimum at week 4 (8.49%), indicating a decrease of metabolism

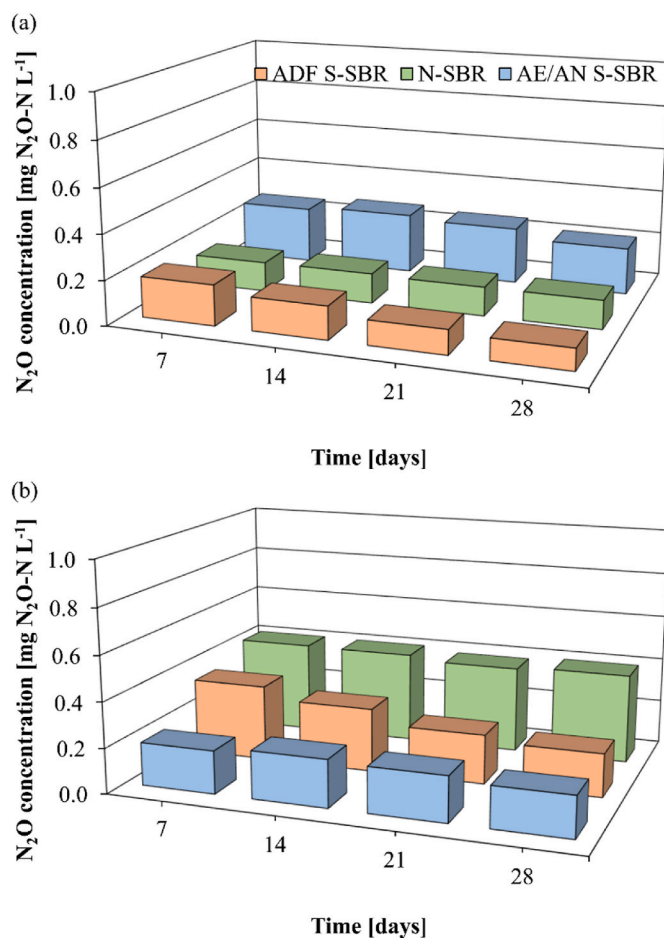


Fig. 4. Liquid (a) and gaseous (b) N₂O emission for ADF S-SBR, AE-AN S-SBR and N-SBR.

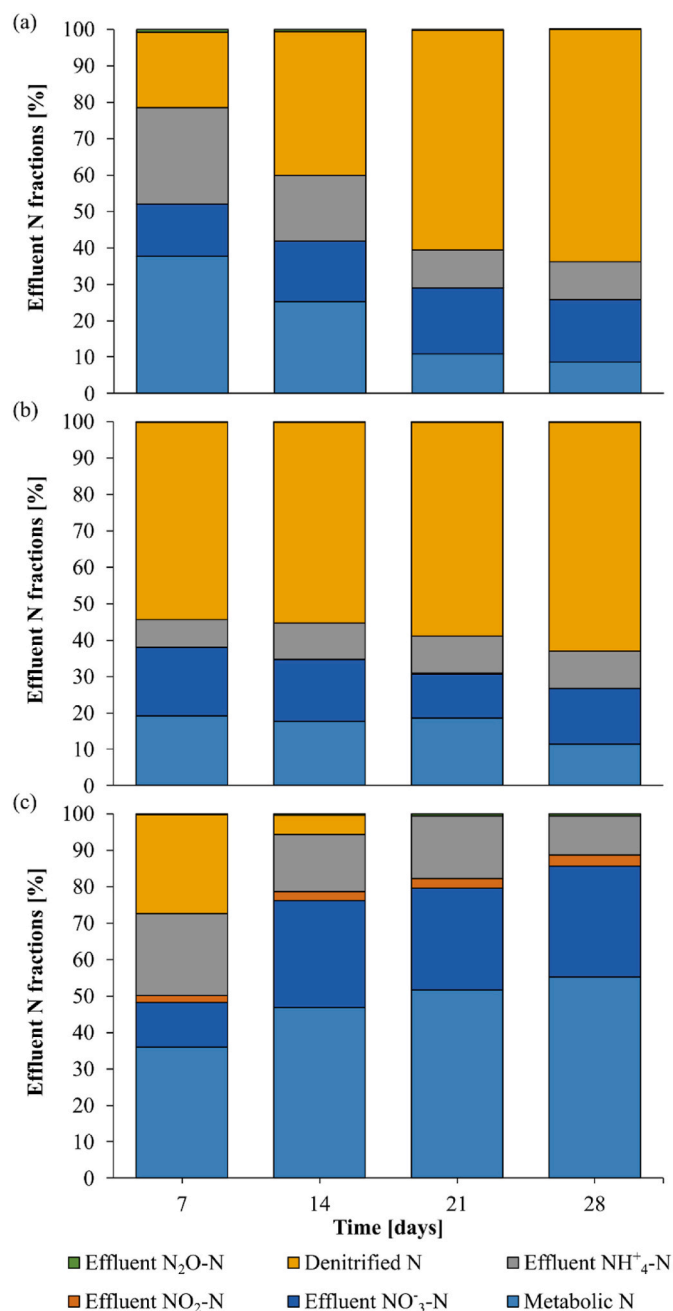


Fig. 5. Effluent nitrogen mass balance for the ADF S-SBR (a), the AE-AN S-SBR (b) and the N-SBR (c).

activity which led to a higher ammonium removal (Fig. 2), achieving 10.36% of effluent nitrogen as ammonium in week 4, and lower N₂O emission since, in week 4, 0.14% of effluent nitrogen was N₂O, as opposed to week 1 with 0.89%.

The AE/AN S-SBR showed a similar trend relative to the metabolic nitrogen, slowly decreasing from 19.09% in week 1–11.48% in week 4. As a result of the selection strategy adopted, it revealed the highest denitrified nitrogen share, on average $57.70 \pm 3.89\%$. It also revealed the lowest N₂O share ($0.27 \pm 0.03\%$), mainly due to the enhanced denitrification, which, as reported in the literature, acts as an N₂O sink by removing the N₂O produced during the ammonia oxidation within the aerobic feast step (Conthe et al., 2019; Oba et al., 2022; Zhou et al., 2022). In fact, for the AE/AN S-SBR, the N₂O emission was monitored both in aerobic and anoxic conditions, revealing that the emissions were 25–30% higher in the aerobic feast phase compared to the anoxic famine

conditions. The dissolved N₂O was mainly affected by the two different phases, decreasing from an average of 0.51 ± 0.12 mg N₂O-N/L in the feast phase to 0.25 ± 0.03 mg N₂O-N/L in the anoxic famine. On the other hand, the N-SBR revealed the highest gaseous N₂O concentration (Fig. 4b) and an effluent nitrogen share similar to the ADF S-SBR ($0.47 \pm 0.13\%$). The increased metabolic nitrogen share ($47.45 \pm 8.32\%$) indicates a low autotrophic activity (Zhang et al., 2022), as already revealed by the low NAR due to the disadvantageous temperature conditions.

Establishing anoxic famine conditions is a key element in reducing the N₂O emission by enhancing the denitrification step in the selection reactor. On the other hand, adopting the AE/AN as a selection strategy with a preliminary nitrification reactor will positively affect the system performance, particularly nitrogen removal, while increasing the N₂O emission, thus nullifying the beneficial effects of the anoxic famine conditions.

3.3. Carbon footprint

The CF obtained for the ADF was 7.93 kg CO₂/day, while for the AE/AN, it was equal to 12.49 kg CO₂/day, thus showing a noticeable increase in the AE/AN strategy. As Fig. 6a and b reported, all the emissions increased for the AE/AN. Direct and indirect emissions were the main factors leading to the increase in CF, depending on the number of reactors in the pilot plant scheme. To better understand the influence of the N-SBR, Fig. 6c shows the contribution of each reactor for DE, IE and DerE. The AE/AN S-SBR showed a slight reduction in the indirect emissions (3.46 kg CO₂/day) compared to the ADF S-SBR (4.07 kg CO₂/day) due to the anoxic conditions, while the DE and DerE only showed slight differences, 3.56 and 0.30 kg CO₂/day for the ADF S-SBR and 3.78 and 0.29 kg CO₂/day for the AE/AN S-SBR. The N-SBR significantly impacted the carbon footprint, especially in direct emissions (3.12 kg CO₂/day), revealing the highest CO₂eq, N₂O contribution of 0.0017 kg CO₂/day, as already pointed out by the N₂O emissions.

These results, combined with the low PHA production and high GHG emissions, clearly discourage adopting the AE/AN enrichment as a selection strategy. Despite the great potential in coupling the PHA production within the WWTP denitrification, it clearly shows its limitations, especially when compared to the ADF. The two reactor configurations used to achieve good nitrification and denitrification performances, as reported by Frison et al. (2015), increase direct and indirect emissions without achieving a benefit in PHA production. Being the first results presented in literature regarding pilot scale studies, they cannot be directly compared with PHA production's life cycle assessments based on full-scale models (Morgan-Sagastume et al., 2016; Roibás-Rozas et al., 2020; Vogli et al., 2020). Future pilot and full-scale studies regarding PHA production should provide clear information regarding the CF and GHG emissions considering the entire process, from feedstock fermentation to PHA extraction, to achieve a big step towards understanding and enhancing the most efficient and sustainable solution.

4. Conclusions

This study is among the first in the literature to report pilot-scale experimental results regarding the environmental impact of two strategies used to produce PHA from sewage sludge. The presented results stand as valid insights given that future studies should be structured and designed comprehensively, considering the environmental implications of the PHA production process. The ADF strategy proved the best solution for selecting PHA producers' microorganisms without applying nitrogen dosage or temperature control to the pilot-scale reactors. ADF and AE/AN selection strategies were tested and compared in similar conditions to evaluate performance and sustainability better. ADF-enriched biomass achieved nearly 40% w/w of PHA accumulated in four days, while the AE/AN enriched biomass achieved almost 15% w/w. The N-SBR introduced in the AE/AN enrichment strategy increased

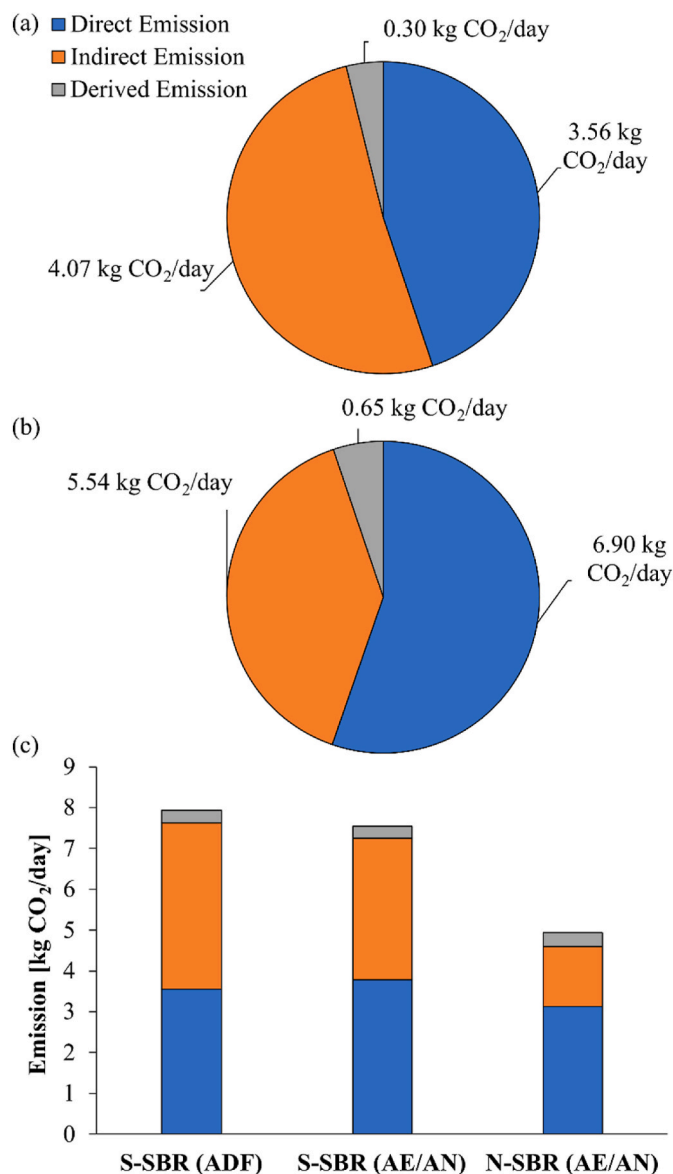


Fig. 6. Percentage of direct, indirect and derived emissions for ADF (a) and AE/AN enrichment (b) and for each reactor (c).

the amount of N₂O produced, up to 0.98 ± 0.12 mg N₂O-N L⁻¹, and the CF, by achieving a total emission of 12.49 kg CO₂/day. Despite being introduced as an efficient and sustainable way to couple the denitrification process with the PHA production, the two-reactor AE/AN enrichment scheme is not as efficient and sustainable as the ADF. The results regarding the N₂O emission and the CF assessment, presented and discussed for the first time in literature, prove that a trade-off between efficiency and sustainability is yet to be found. Future studies regarding the PHA should focus on providing information on the sustainability of the different technologies adopted, especially at the pilot scale, to move forward with the circular economy approach which initiated this trend.

CRedit authorship contribution statement

Giorgio Mannina: Resources, Project administration, Investigation, Conceptualization. **Antonio Mineo:** 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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