Effect of organic loading rate on the production of Polyhydroxyalkanoates from sewage sludge

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ABSTRACT

The aim of this work was to study the effect of organic loading rate on the production of Polyhydroxyalkanoates (PHA) from sewage sludge. Synthesis of PHA using sewage sludge as platform was achieved in this work. Three pilot-scale selection-sequencing batch reactors (S-SBR) were used for obtaining a culture able to accumulate PHA following a strategy of aerobic dynamic feeding (ADF) at different volumetric organic-loading-rate (vOLR): 1.3, 1.8 and 0.8 g COD L^{-1} d^{-1} for S-SBR 1, S-SBR 2 and S-SBR 3, respectively. Decreasing the vOLR enhanced the general performance of the process as for organic matter removal (from 99.2% ± 0.3 % in S-SBR-3 to 92 ± 2 in S-SBR-2) while the opposite trend was recorded for PHA production (6.0 PHA % w/w in S-SBR-3 vs 13.7 PHA % w/w in S-SBR-2 at the end of the feast phase). Furthermore, indirect and direct emissions, as N2O, were evaluated during the process for the first time. Finally, three accumulation tests were performed achieving 24 % w/w.

Keywords: Aerobic dynamic feeding, Polyhydroxyalkanoates, Resource recovery from wastewater, Selection - sequencing batch reactor, Sewage sludge.

1 Introduction

In the last decade, a paradigm change in wastewater treatment plants (WWTPs) has become necessary for ensuring environmentally sustainable development. Sewage sludge from WWTPs has been considered a source of volatile fatty acids (VFAs), biofuels and biopolymers with high market value (Mannina et al., 2020). Biopolymers like polyhydroxyalkanoates (PHA) have become the most interesting alternative to conventional plastics (Sabapathy et al., 2020). Up to decades ago, PHA was mainly produced by pure cultures while up to now researchers have focused on different alternatives such as the mixed microbial consortia (MMC). VFAs produced from sludge anaerobic digestion are the main building blocks used by MMC to produce PHA as an energy storage product (Pakalapati et al., 2018). This process allowed taking benefit from natural selection and competition principles between the microorganisms to favour the ones with the ability to store PHA (selection step) (Kourmentza et al., 2017). Once the PHA producer microorganisms in the MMC have been selected, they are subjected to a continuous feed-on-demand process (accumulation step), to produce the maximum amount of PHA possible (Mannina et al., 2020).

The use of microbial consortia allows the use of different waste streams including, for example, municipal wastewater and sewage sludge (Ahmadi et al., 2020).

Moreover, the possibility of using municipal WWTPs as platform for PHA production has gained attention, since microbial consortia from the secondary reactor would be more favourable than pure cultures in several aspects, such as the economic cost of pure cultures and the required carbon source (Conca et al., 2020). Less investment and operating costs are needed as wastewater and fermented sewage sludge can be used as a cheap substrates and microbial consortia operation and maintenance are easier than pure cultures (Albuquerque et al., 2011; Serafim et al., 2008).

In this sense, the integration of PHA production by microbial consortia within existing wastewater treatment infrastructures has been introduced by Anterrieu et al. (2014), but it is still a challenge to keep the organic carbon and nutrients removal within the legislative limits while creating, at the same time, the best conditions for PHA production (Yukesh Kannah et al., 2022). In the way of understanding the main operating parameters that affect PHA production by microbial consortia, limited studies exist regarding the effect of the volumetric organic-loading-rate (vOLR) on the feast/famine strategies applied for culture selection (Simona et al., 2022; Morgan-Sagastume, 2016). On the one hand, an increase in vOLR generally leads to a biomass growth thus enhancing the overall PHA productivity (Dionisi et al., 2006). On the other hand, low vOLR (<1.2 g COD L^{-1} d^{-1}) allows achieving PHA-storage rates over 15% (Valentino et al., 2014; Dionisi et al., 2006). Pardelha et al. (2012) reported that low vOLRs (< 0.2 g COD L^{-1} d^{-1}) in combination with low Feast-to-Famine (F/F) ratio are favourable growth conditions for microorganisms that synthetize PHA with low hydroxyvalerate (HV) fractions. Despite the above studies highlighted the important role played by vOLR in obtaining high PHA-storage microbial consortia, knowledge is still in its infancy.

Therefore, the aim of this work was to evaluate the efficiency of different feeding schemes in three selection-sequencing batch reactors (S-SBR) with the final goal of increasing the PHA content of the biomass of each reactor. In this sense, each S-SBR was operated with a different vOLR using an aerobic dynamic feeding (ADF) strategy. Additionally, in the light of the possible integration of this process in WWTP, direct and indirect emissions were calculated with N_2O being the greenhouse gas (GHG) considered. As far as the authors' knowledge goes, this is the first time a work reports direct GHG emission analysis and indirect GHG emission evaluation related to the PHA production process. Finally, the accumulation was carried out using the biomass selected with different vOLR.

2 Materials and methods

2.1 Description of the pilot plant

The pilot plant of the Wider Uptake Project EU is located at the Water Resource Recovery Facility of Palermo University campus (Mannina et al., 2021). The plant layout is composed of a wastewater treatment pilot-plant with an oxic-settling-anaerobic (OSA) configuration, followed by a sludge deviation line based on PHA production. Figure 1 reports the experimental set-up of this work. A synthetic mixture of VFAs act as a carbon source in the selection-SBR (S-SBR), aimed to favour the growth of the PHA-storing organisms. Finally, the selected biomass is used in the accumulation batch reactor to increase the amount of PHA produced (Mannina et al., 2021). The sewage sludge for the

PHA production was obtained from the aerobic tank of the wastewater treatment pilot-plant (see further details in Mannina et al., 2021).

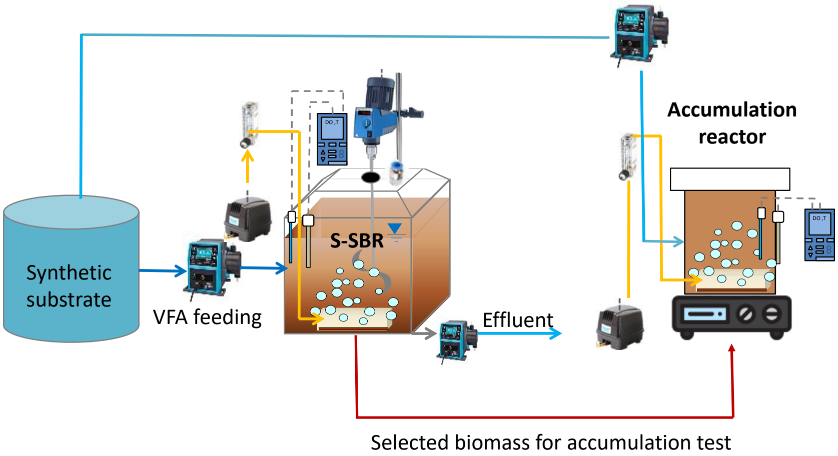

Figure 1. Process scheme developed at pilot scale for PHA production.

2.2 Experimental set-up

The selection SBRs (S-SBR) consisted of a 30 L working volume vessel with a volumetric exchange ratio of 25%. Each cycle was composed of four phases: feeding (variable depending on the applied vOLR), biological reaction (660 min), sludge sedimentation (30 min) and effluent withdrawal (5 min) for a total cycle time of 12 hours (Conca et al., 2020) and a hydraulic retention time (HRT) of 2 days. In the reaction phase, the feast and famine strategy was carried out by feeding the concentrated substrate for 20, 18 and 10 seconds in S-SBR 1, S-SBR 2, S-SBR 3, respectively. Water was also supplied for 3 min to achieve the desired initial concentration in each S-SBR. During the feeding phase, a synthetic substrate, mimicking a real VFA-rich stream, was used to evaluate the influence of three different vOLR. The concentrated synthetic substrate contains a solution of 150 g L^{-1} of organic matter measured as chemical oxygen demand (COD), 7.5 g N L^{-1} and 1.5 g P L^{-1} for a C:N:P ratio of 100:5:1. Sodium acetate (CH_3COONa) and propionic acid (CH_3CH_2COOH) were used as the COD components with a 70:30 ratio (Frison et al., 2021). The final solution composition was: 135.0 g L^{-1} sodium acetate, 29.8 g L^{-1} propionic acid, 28.6 g L^{-1} ammonium chloride and 8.4 g L^{-1} potassium phosphate bibasic. Ceramic diffusers in the bottom of the reactor were used to supply air for maintaining a dissolved oxygen (DO) concentration of 6.5-8.0 mg O_2 L^{-1} to achieve an acceptable ammonia removal efficiency for the highest nitrogen loading rate reactor (S-SBR 2). A DO probe (WTW FDO® 925-P, Weilheim, Germany) was installed inside each reactor for the DO measurement. The feeding and discharging pumps (Watson-Marlow qdos30, Falmouth, United Kingdom) were installed to maintain a feed flowrate of 390 mL min^{-1} for S-SBR 1, 600 mL min^{-1} S-SBR 2 and 480 mL min^{-1} for S-SBR 3. Finally, 15 L d^{-1} of effluent was discharged and collected in an effluent storage tank. The operational details for the three S-SBRs are represented in Table 1. The performance during feast and famine phases and the parameters that characterised the SBR performance were calculated once steady state was achieved, i.e., when the F/F ratio remained constant (< 2% deviation) for at least ten days, approximately.

This configuration allowed to apply stressing conditions to microbial consortia with intermittent feeding at different vOLR. The ADF alternates an excess of carbon source (feast) followed by carbon deficiency (famine), under aerobic conditions, making possible to favour PHA accumulating bacteria. The key is to provide a sufficient length of feast phase to complete substrate consumption and a much long enough famine phase to allow the consumption of previously accumulated PHA (Hao et al., 2018). From the literature (Guleria et al., 2022; Almeida et al., 2021), nowadays, the most studied and efficient strategy employed is ADF. In the feast phase, the carbon source is stored as PHA granules. There is the existence of a competitive advantage for PHA storing microorganisms over the rest. In the famine phase, PHA storing microorganisms grow from the accumulated PHA.

The biomass selected with three different vOLRs was used to perform three accumulation tests run in fed-batch mode. Two 2 L glass reactors, magnetically stirred and aerated, were used. The reactors were equipped with probe ports to monitor DO while the temperature was controlled by using a thermostat (Corio CD-CB6) maintained at 20 °C. The biomass (3 L) was collected from the selection reactor, washed with tap water and let to settle down to be able to discard 1 L of supernatant. Then it was mixed with 1 L mineral medium without ammonium chloride. Finally, the biomass was left in aeration and stirred overnight before starting the experiments. The carbon source used was the same as the one used during the selection step (Table 1).

Table 1. Operational characteristics for the three selection-SBR and the relative accumulation tests.

	OLR	**F/M (accumulation)**
	g COD L^{-1} d^{-1}	g COD g^{-1} VSS
s-SBR 1	1.3	3.1
s-SBR 2	1.8	3.7
s-SBR 3	0.8	2.5

2.3 S-SBR monitoring and analytical techniques applied

The S-SBR system was followed by sampling the influent, mixed liquor inside the reactor and the effluent during the cycles to evaluate the COD, ammonium (NH_4-N), phosphate (PO_4^{3-}-P), total and volatile suspended solids (TSS and VSS), extracellular polymeric substances (EPS), sludge volumetric index (SVI) and the PHA concentration.

For measuring SVI, TSS and VSS standard methods stated in APHA/AWWA/WEF (2012) were applied. For the COD, NH_4-N and PO_4^{3-}-P analysis, Sigma Aldrich (Merck KGaA, Darmstadt, Germany) kits were used in filtered samples (0.45 µm). The absorbance for all the samples prepared for COD, ammonium and phosphate was measured at 600 nm wavelength in UV-VIS spectrophotometer (Spectroquant® NOVA 60 photometer, Billerica, USA). The EPS extraction and analysis method used is based on Le-Clech et al. (2006) to characterise the relative concentration of proteins (eEPSp) and carbohydrates (eEPSc). EPS extracted samples are measured at 700 nm wavelength for proteins and 625 nm for carbohydrates in a UV-VIS spectrophotometer (UVmini-1240, Shimadzu, Japan). The calibration curve for proteins was done with a standard solution of albumin bovine (BSA) and for carbohydrates a glucose standard solution was used. This parameter was measured because the literature points out that the increase in EPS content allows biomass to settle down better contributing to maintain the stable operation of the enrichment system (Wen et al., 2022) (see supplementary materials). Dissolved and gaseous N_2O concentration has been measured according to the procedure described by Mannina et al. (2018) by using a gas chromatograph (GC) equipped with an Electron Capture Detector (ECD). The emission factor was calculated by considering the total nitrogen entering in the system, as reported by Tsuneda et al. (2005). The indirect emissions were calculated by considering the two pumps, one blower and one electric valve used in the pilot plant. The electrical consumption in kWh was then converted to grams of equivalent CO_2 (g CO_2eq) based on 2022 emissions in Italy reported by Scarlat et al. (2022) and subsequently divided into the amount of produced PHA to obtain the grams of equivalent CO_2 per grams of produced PHA (g CO_2eq/g PHA).

For PHA analysis the protocol described by Mannina et al. (2019) was followed. Briefly, sludge samples taken at the end of the feast phase and during the accumulation tests were mixed with formaldehyde solution to inhibit the biological activity. Then, they were centrifuged at 8000 rpm for 40 minutes and the obtained pellet was stored at -80ºC overnight and finally lyophilized. The lyophilized samples were weighed and transferred to test tubes in which butanol and chlorhydric acid were added; then the test tubes were incubated at 100ºC for 8h. Finally, hexane and MilliQ grade water were added and tubes were vortex mixed. The organic phase was collected, filtered (0.22 µm) and introduced into gas chromatography (GC) vials for further analysis. The PHA concentration in the vials was measured following the protocol stated by Montiel-Jarillo et al. (2017), in which an Agilent Technologies 7820A GC equipped with a flame ionization detector and a Restek Stabilwax column (30 m x 0.53 mm x 1.00 µm film thickness) was used. The concentration of PHB and PHV was determined through standard curves obtained using PHB and PHB-co-HV (8% PHV) Sigma Aldrich standards (see supplementary materials).

2.4 Calculations

The F/F ratio was determined by considering the length of the feast and the famine phase as determined by the DO profile. The following equation was considered (Conca et al., 2020):

$$\frac{Feast}{Famine}\left(\frac{min}{min}\right) = \frac{T\ feast}{T\ famine} \tag{1}$$

The PHA amount estimation in microbial cells and the storage yield were calculated following the protocol explained by Mannina et al. (2019) as follows:

$$PHA\ amount\ in\ microbial\ cells = \frac{Mass\ PHA}{Mass\ lyophilised\ biomass} \tag{2}$$

The PHA amount inside microorganisms would be the fraction of PHA contained in the lyophilised biomass and it was calculated based on GC results. PHA concentration was calculated as COD equivalents by using the following stoichiometry: 1.67 g COD/g HB and 1.92 g COD/g HV.

3 Results and Discussion

3.1 Influence of different vOLR in reactor performance

Three vOLR were applied in the three different S-SBR to analyse which works better under ADF conditions with the aim of balancing the removal efficiencies and the enrichment of the biomass in PHA-storing microorganisms. The S-SBR performance of each reactor is dependent on the applied vOLR, which in this case in related to the applied F/F ratio. Table 2 contains the mean values of the monitored variables of the three S-SBR once steady state was achieved, while Figure 2 reports sCOD, NH_4^+ and PO_4^{3-} removal efficiencies during the whole operation.

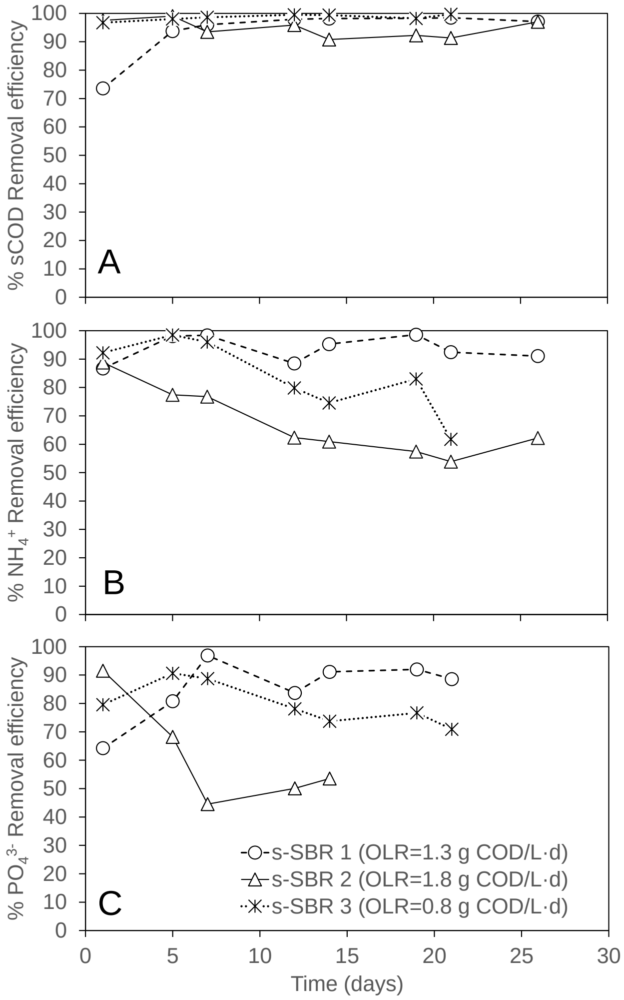

Figure 2. Performance of A) sCOD, B) NH_4^+ and C) PO_4^{3-} removal efficiencies (%) for the three s-SBR along the whole experimentation time corresponding to three different OLR.

Table 2. Average experimental data for the three S-SBRs processes at steady state.

Parameter	s-SBR 1 Average ± st. dev	s-SBR 2 Average ± st. dev	s-SBR 3 Average ± st. dev
sCOD OUT (mg COD L^{-1})	55 ± 9	183 ± 74	6.3 ± 0.6
NH_4-N OUT (mg N L^{-1})	6 ± 4	59 ± 2	13 ± 2
PO_4-P OUT (mg P L^{-1})	1.2 ± 0.2	20 ± 2	4 ± 1
sCOD Removal efficiency (%)	98.0 ± 0.3	92 ± 2	99.2 ± 0.3
NH_4-N Removal efficiency (%)	94 ± 4	59 ± 4	75 ± 3
PO_4-P Removal efficiency (%)	90 ± 2	50 ± 4	75 ± 2
TSS concentration (g TSS L^{-1})	4.5 ± 0.1	4.7 ± 1.5	4.8 ± 0.9
VSS concentration (g VSS L^{-1})	3.8 ± 0.2	3.6 ± 1.1	3.2 ± 0.4
TSS concentration in the effluent (g TSS L^{-1})	0.08 ± 0.01	0.3 ± 0.2	0.20 ± 0.05

Regarding sCOD, the three S-SBR showed a removal efficiency higher than 90% from the second week after steady state was achieved. The ammonium removal showed the same trend for all the reactors, starting to decrease after 2-3 weeks. In this case, the S-SBR 1 (NH_4^+ removal efficiency between 87-100%) performed better than the S-SBR 3 (NH_4^+ removal efficiency between 65-85%) while the S-SBR 2 presented the lowest values (NH_4^+ removal efficiency between 85-50%). The S-SBR 1 was the best in removing phosphate with a medium value of 90 ± 2 % (Table 2), while the S-SBR 3 performed slightly worse than the S-SBR 1 and much better than the S-SBR 2.

In the framework of urban WWTP, few works report removal efficiencies of the enrichment reactor, mainly because they only focus on the PHA production, but not in the integration of this process within the WWTP. In this sense and to the best of authors' knowledge, very few works reported performance of the enrichment reactor (Table 3): i) Morgan-Sagastume et al. (2015) reported a vOLR of 3 g COD L^{-1} d^{-1} and removal efficiencies of 24, 46 and 60 for NH_4^+, PO_4^{3-} and COD, respectively; ii) Bengtsson et al. (2017) applied a vOLR of 1.8 g COD L^{-1} d^{-1} and they obtained removal efficiencies of 95%, 62% and 76% for NH_4^+, PO_4^{3-} and COD, respectively; finally iii) Valentino et al. (2019) reported removal efficiencies between 85-97% for NH_4^+, 69-82% for PO_4^{3-} and 86-92% for COD for a vORL of 4 g COD L^{-1} d^{-1}. Unfortunately, none of them have studied different vOLRs with the aim of trying to balance the removal efficiencies and the enrichment of the biomass in PHA-storing microorganisms.

From our results, it can be concluded that reactor's performance was worsened when the highest vOLR (1.8 g COD L^{-1} d^{-1}) was applied, most likely because the treatment capacity of the system was exceeded which is in line with previous studies (Morgan-Sagastume et al., 2015). However, despite obtaining higher removal efficiency for ammonia (75%) and phosphate (75%) at the lower vOLR (0.8 g COD L^{-1} d^{-1}), the effluent values (NH_4^+-N OUT and PO_4^{3-}-P OUT) reported in Table 2, are still high compared to legislation limits (Directive (EU) 91/271/EEC). Therefore, this still represents a challenge in the integration of this process within WWTPs.

Table 3. Summary of studies showing the operational parameters of the enrichment reactor.

Feedstock characteristics	vORL (g COD L^{-1} d^{-1})	F/M ratio (kg BOD kg^{-1} VSS·d^{-1})	Type of reactor	PHA amount in microbial cells during enrichment (% gPHA g^{-1} VSS))	HV share (% w/w)	F/F (min min^{-1})	HRT (d)	SRT (d)	COD removal (%)	N-NH_4^+ removal (%)	P-PO_4^{3-} removal (%)	SVI (mL g^{-1} TSS)	TSS concentration in the effluent (g TSS L^{-1})	Within legal discharge limits to water receiving bodies? (Yes/No)	Reference
Synthetic VFA	0.8 1.3 1.8	0.5 0.3 0.4	SBR	6.0 6.6 13.7	1.0 1.3 5.5	0.16 0.19 0.28	2	3	99 98 92	75 94 59	75 90 50	81 89 116	0.20 0.08 0.30	No	This study
Fermented Citrus processing wastewater	1.0 2.0 3.0	0.22 0.43 0.63	SBR	N.A.	N.A.	0.14 0.10 0.16	4.4 2.2 1.5	22 8.8 5.3	98 98 93	N.A.	N.A.	50 40 150	N.A.	No	Corsino et at., 2022
Synthetic VFA	1.3	0.67	SBR	9.1	N.A.	0.20		7-10	99	N.A.	N.A.	100	1.68	No	Frison et al. 2021
Fermented waste activated sludge	2.0-2.2	N.A.	SBR	6.6	9.5	0.07	2.0	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	-	Lorini et al. 2022
Synthetic substrate and fermented agro-industrial residual feedstock	1.8	N.A.	SBR	19-36	N.A.	N.A.	3.3-5.9	6	76	95	62	N.A.	N.A.	-	Bengtsson et al. 2017
Fermented waste activated sludge and organic fraction of municipal solid waste	4.0	N.A.	SBR	12*	14	0.1	1	1	86-92	85-97	69-82	N.A.	1.93	No	Valentino et al. 2019
Fermented waste activated sludge and mixture of organic fraction of municipal solid waste	4.0	1.57	SBR	14	N.A.	0.07**	1	2	N.A.	N.A.	N.A.	N.A.	N.A.	-	Moretto et al. (2020)
Cellulosic primary sludge fermentation liquid	1.3	0.57	SBR	10	N.A.	0.09	1.7-2.3	6-7	100*	89*	N.A.	N.A.	N.A.	-	Conca et al. (2020)

VFA from primary sludge	3.0	N.A.	SBR	< 4	34-26	0.13	0.13	1.8*	60	24	46	34-180	1.3	No	Morgan-Sagastume et al. (2015)

* (Calculated from reported values). **Feast to cycle length ratio

Concerning other performance parameters such as the SVI, the values were below 150 mL g^{-1} TSS owning the three enriched biomass good settling properties. The different SVI in the three S-SBR can be explained by the difference in the EPS concentration. The low protein amounts recorded in S-SBR 2 would influence the settling behaviour in this reactor by increasing biomass SVI (116 mL g^{-1} TSS). Also, this show how different vOLRs may influence the biomass response in front of higher vOLRs (Huang et al., 2022)). Despite the relatively high amount of PHA produced at the end of the feast in the S-SBR 2 (see next section), the reactor showed the worst COD and nutrients removal efficiency as well as the worst settling characteristics.

In the light of a possible integration of the enrichment reactor within WWTPs, lot of work have still to be done, mainly because the effluents of this type of reactors still need a post treatment, for nutrients, TSS or even for COD removal.

3.2 Achieved PHA yields for each vOLR

To evaluate the PHA storage yields during the selection process, sludge samples were drawn at the end of the feast phase after the establishment of steady state conditions in all the reactors. Similarly, for the S-SBR 1, samples were drawn through the first 6 hours of the cycle to evaluate the cycle performance.

3.2.1 F/F ratio and PHA storage yields

In Table 4 the F/F ratio of the different experimental periods is reported. The F/F ratio decreased from an average of 0.28 min/min for the higher vOLR, to 0.16 min/min for the lowest vOLR. This indicates that by decreasing the vOLR without changing the cycle length and other operational parameters, a lower F/F rate is achieved as pointed out by previous studies (among others, Valentino et al., 2020). For the first and second S-SBR the F/F ratio showed a variable value due to several changes in the DO profiles during the cycles (see supplementary materials).

The S-SBR 2 reactor, with the highest vOLR, showed the highest amount of accumulated PHA, but also the HV share was the highest one (5.5 % w/w of PHV, Table 4) compared to S-SBR 1 and S-SBR 3, despite of having the highest F/F ratio of the three S-SBR. The PHA accumulated in S-SBR 1 and in S-SBR 3 were almost half of that recorded for S-SBR 2, with no appreciable difference between them even in terms of the HV share (1.3 % w/w PHV and 1.0 % w/w PHV for S-SBR 1 and S-SBR 3, respectively). These results are in accordance with the literature, indicating that at high vOLR the biomass tends to accumulate more PHA, although increasing the HV fractions synthesized (Pardelha et al., 2012; Conca et al, 2020; Estévez-Alonso et al., 2022) whilst the opposite happen at lower vOLR and low F/F ratio (< 0.2 min/min) which is the connection between S-SBR 1 and 2.

Table 4. Summary of the food to microorganism (F/M) ratio, F/F ratio and PHA accumulated among the different OLR.

Parameter	F/M ratio (kg BOD kg^{-1} VSS·d^{-1})	F/F ratio (min/min)	PHA amount in microbial cells (% w/w)	HV share (% w/w)
s-SBR 1	0.3 ± 0.1	0.19 ± 0.08	6.6	1.3
s-SBR 2	0.4 ± 0.2	0.28 ± 0.01	13.7	5.5
s-SBR 3	0.5 ± 0.2	0.16 ± 0.08	6.0	1.0

3.2.2 Cycle profile performance during biomass selection

Concentration profiles of organic matter (as COD), DO and PHA during the 55th cycle of S-SBR 1 are shown in Figure 3. t0 represents the start point of a new cycle, corresponding to the end of the effluent withdraw of the previous cycle thus explaining the value of 0 mg L^{-1} in the DO concentration. After 120 min (end of feast phase), DO sharply increased to 7.5 mg L^{-1} during the famine phase.

In Figure 3, the COD uptake was very fast in the first 180 min, then during the famine phase it decreased slowly. For the PHA, PHB and PHV, the highest amount was obtained at the end of the feast phase, while during the famine phase, it was consumed for cell growth.

Results are in line with the literature (Valentino et al., 2019), the DO profile decreases also at the beginning of the cycle until 2 mg L^{-1} because of the increase in metabolic activity. sCOD is consumed by the biomass, DO concentration increased and stabilised at 6.0 mg L^{-1}, a lower value than the one we obtained at 7.5 mg L^{-1} during the famine phase. Regarding the PHA concentration, the maximum obtained in this work (500 mg COD_{PHA} L^{-1}) is doubling the one obtained by Valentino et al. (2019) (220 mg COD_{PHA} L^{-1}), this could be related to the different F/F ratio applied in both works. Finally, the P(HB-co-HV) obtained had a higher proportion of PHB than PHV which is in line of Albuquerque et al (2010) results.

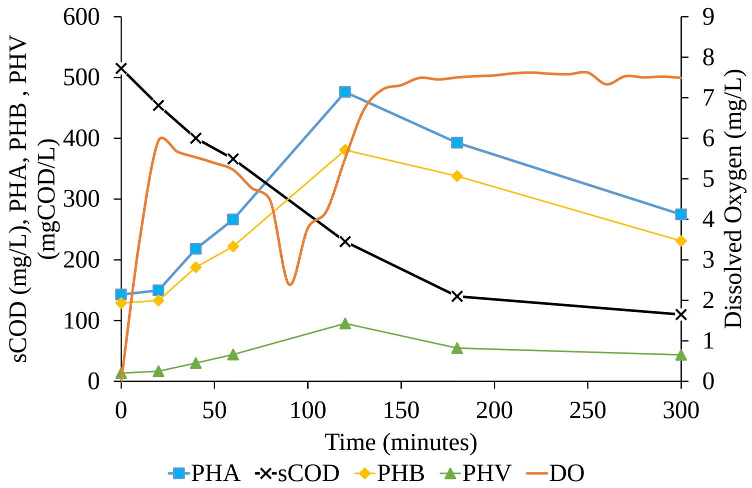

Figure 3. Cycle profile for S-SBR 1 (OLR 1.3).

3.2.3 Accumulation tests

Figure 4 shows PHA produced at the end of accumulation tests of 8 hours along with the storage yield. T1, T2 and T3 were performed by using the biomass selected during S-SBR 1,2 and 3, respectively. The carbon source used and TSS concentration was the same for each accumulation tests such that the F/M ratio differences (table 1) can be related to the different vOLR used in the selection step. The experiments were carried out to evaluate the influence of the vOLR in the accumulation step with any aim to achieve the highest amount of accumulated PHA.

As expected, T2 showed the highest amount of PHA produced (24.18 % w/w) followed by T1 (13.46 % w/w) and T3 (11.74 % w/w). Higher vOLR in the selection step is more effective in enhancing the microorganism' PHA production and storage ability. Indeed, the storage yields were 0.18, 0.24 and 0.14 for T1, T2 and T3, respectively. The enhancement of the PHA production ability is also reported by the HV:HB ratio (0.31, 0.45 and 0.3 for T1, T2 and T3, respectively).

Results show that higher vOLR applied in selection step also affects the accumulation by inducing a higher PHA production mainly increasing the share of HV polymer synthesised. This consideration should also be taken into account when considering the integration of PHA production process in a WWTP: to achieve higher production of PHA by adopting VFAs as substrate, a high vOLR is mandatory. Still, focusing only on the amount of PHA produced, the effluent quality will not be enough for the legislation limits underlying how it is important to find a trade-off between the reactor performance and recovered resources.

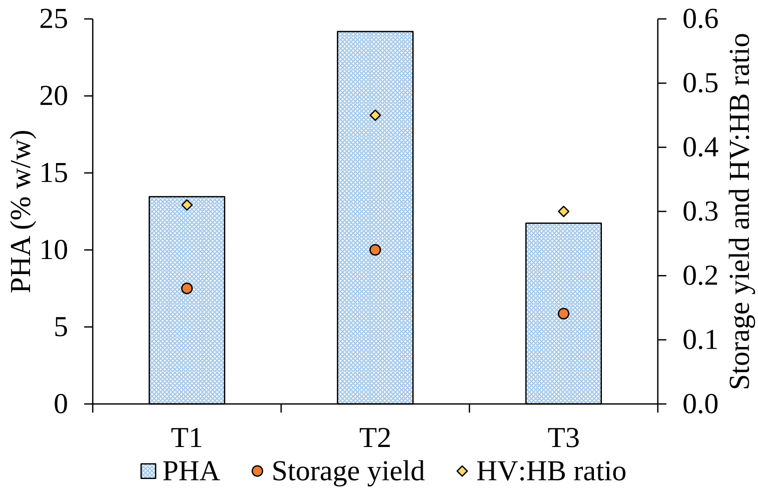

Figure 4. Final PHA content and storage yield.

3.3 Direct and indirect emissions

Figure 5 shows the N_2O emission factor (EF) for S-SBR 1, calculated considering the N_2O direct emissions measured during the feast. Due to the air supply in the reactor, the EF is in line with previous studies for conventional activated systems (Tkakur and Medhi, 2019). As can be seen, the EF decreased during the experimental period as the steady state condition was reached, starting from 2.08 % at day 2 to 0.48 % on day 26. Also, the direct and indirect emissions were related to the amount of PHA produced during the accumulation of S-SBR 1. The direct emissions, which considered only the N_2O, methane as expected was neglectable respect to N_2O, were 0.02 g CO_2eq/g PHA while the indirect emissions reached 489.06 g CO_2eq/g PHA. These results show that, despite the effort to enhance the PHA production process, more focus should be given to the environmental impact of the process to be able to assess its sustainability. Greenhouse gas emissions should be monitored in the entire PHA production process in order to find the trade-off between effluent quality, PHA produced and environmental impact both from direct and indirect emissions. Detailed mechanisms of N_2O production have to be analysed more in detail for such systems which was out of the scope of this study.

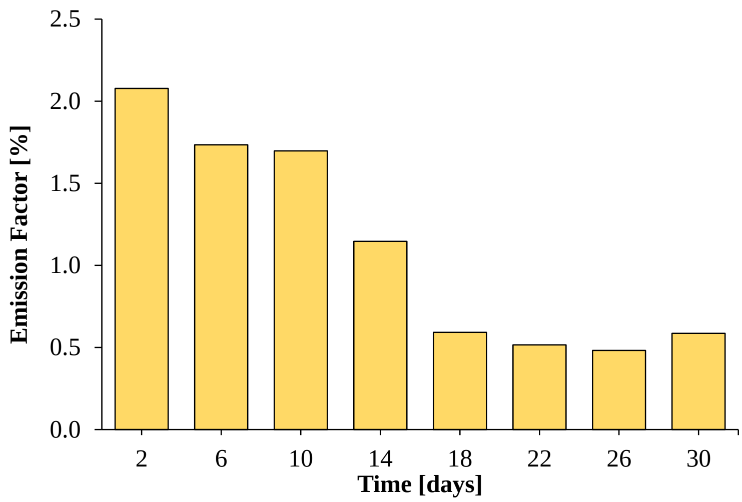

Figure 5. N_2O emission factor based on total nitrogen for S-SBR 1.

4 Conclusions

PHA producer microorganisms' selection is the main step involved in the PHA production process from MMC, which allows to integrate resource recovery in the WWTP management. In this work, three vOLR were applied to three SBRs (1.3, 1.8 and 0.8 g COD L^{-1} d^{-1} for S-SBR 1, 2 and 3, respectively) as different feeding schemes to select PHA producers using sewage sludge as a platform. High vOLR (1.8 g COD L^{-1} d^{-1}) allows to obtain a better enrichment in PHA-storage microorganisms (13.7 % w/w at the end of the feast phase) with the highest HV share measured (5%). This result is also confirmed by the accumulation test, where the biomass produced from S-SBR 2 achieved a PHA concentration of 24.2 % w/w after 8 hours with a HV share of 31 %. Despite the high PHA production, the reactors' performance was the worst among the three S-SBRs with a medium sCOD, ammonia and phosphate removal of 92, 59 and 50 % respectively. Results show that the vOLR plays a key role in the biopolymer production, copolymer produced and general performance of the process, thus underlying the importance of finding a cut-off value to valorise the amount of PHA produced while discharging an effluent within the legislative limits. Indirect GHG emissions (489.06 g CO_2eq/g PHA) monitored during the selection step were higher than direct ones (0.02 g CO_2eq/g PHA) pointing out that the PHA plant layout has to be designed taking into account such important data.

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Appendix. Supplementary data

E-supplementary data for this work can be found in e-version of this paper online.

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