Start-up of a sequencing batch reactor for the selection of polyhydroxyalkanoates accumulating cultures by means of a carbon and nitrogen decoupling strategy

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Abstract

Polyhydroxyalkanoates (PHA) could be produced in biological reactors fed with VFA-rich streams from acidogenic fermentation of organic wastes using mixed microbial cultures (MMC). This process depends on different strategies to obtain the maximum PHA storage. In this study, a start-up of the biological process to produce PHA was performed based on the selection of PHA-accumulating organisms. To this end, a sequencing batch reactor (SBR) working at 30ºC under uncoupled carbon and nitrogen feeding strategy with a cycle length of 6 hours and an SRT of 4.8 days was arranged. Synthetic wastewater rich in VFA (53.1% acetic, 21.3% propionic, and 25.6% butyric acid in chemical oxygen demand (COD) basis) was added in the feast step, whereas the ammonium nitrogen source (NH_4Cl solution) was supplied in the famine step. The reactor was operated with two different organic loading rates (OLR), namely 2.0 and 2.8 g COD $\cdot$ $L^{-1}\cdot$ day^{-1}. The change of OLR was based on the obtention of a feast/cycle ratio below 20%. Accumulation tests with a maximum duration of 7 hours were conducted with biomass purged in the selection SBR under the two OLRs applied, yielding a similar final PHA content. In the accumulation test using biomass from the SBR working at 2 g COD L^{-1} day^{-1} (Stage II), the PHA content of biomass increased from 9% PHA to 44% PHA (in SS basis). Similar results were obtained from the selected biomass of Stage III, obtaining 46% PHA starting from the value of 9% PHA (in SS basis). The PHA values recorded in the accumulation tests reflect a good selection of PHA storing microorganisms in the biomass purged from the selection SBR under the applied OLRs.

Keywords: Polyhydroxyalkanoates (PHA); Mixed microbial culture (MMC); Uncoupled; Volatile fatty acids (VFA); Selection reactor; Accumulating reactor

1. Introduction

The current consumption of resources is generating a large amount of waste, which implies that the natural reservoirs from which humans extract products are being depleted. For this reason, the circular economy concept is gaining attention to produce value-added products from waste. Consequently, wastewater treatment plants (WWTPs) are being transformed into resource recovery facilities (RRF). These plants are also known as biorefineries being a network of facilities that integrate biomass into conversion processes to produce biofuels, energy, and chemicals [1].

One of the products that could be obtained from biorefineries is Polyhydroxyalkanoates (PHA). PHA is a family of biodegradable polymers that can be accumulated by several microbes as carbon and energy reserves under stress conditions [2]. These thermoplastic polymers feature mechanical properties very similar to petroleum-derived polyolefins but, in this case, this innovative material is biodegradable, bio-compostable, and chiefly, its carbon source is renewable. There are two major co-polymers within PHA, namely polyhydroxybutyrate (PHB) and polyhydroxyvalerate (PHV). PHB has very similar properties to propylene [3]. However, if the biopolymer has HV, forming with HB the copolymer Poly(3-hydroxybutyrate-co-3-hydroxyvalerate), commonly known as PHBV, it will be more elastic and flexible [4].

There is a wide range of strategies to produce PHA. Many industries use pure microbial cultures (PMC) [5], although their production cost is relatively high mainly due to sterilization [6]. To reduce this cost, mixed microbial cultures (MMC) could be used as inoculum to produce PHA, reducing the investment and operational costs since sterilization is not required [4]. PHA production using mixed microbial cultures (MMC) and organic wastes is usually performed in three stages (i.e. fermentation, selection, and accumulation) [7]. Acidogenesis is the anaerobic biological step during the waste fermentation process (hydrolysis, acidogenesis, and acetogenesis) where organic matter breaks down into easily assimilable compounds, e.g. volatile fatty acids (VFA) and alcohols [8]. These VFAs are the carbon source for the selection of PHA-accumulating microorganisms (second stage) [9]. Then, the next stage is the accumulation, a process in which the stored PHA is maximized.

Several techniques have been considered for a good selection of PHA bacteria. On the one hand, the feast/famine strategy used is focused on the alternation of satiety and famine conditions during the cycle. In the first step (feast), microorganisms consume the external carbon source (VFA) which is accumulated as intracellular PHA. In the second step (famine) intercellular PHA is consumed for biomass growth. On the other hand, the strategy based on uncoupling the carbon and nitrogen in the feeding could be also carried out to enhance the proliferation of PHA storing microorganisms. This strategy consists of providing feed carbon sources in the feast phase and external nitrogen sources in the famine step to favour the proliferation of biomass with the ability to store carbon to grow [10].

This paper is focused on the start-up of an SBR reactor to select PHA accumulating microorganisms and determine their PHA accumulation capacity in aerobic batch accumulation tests.

2. Materials and Methods

2.1. Start-up and operation of Sequencing batch reactor (SBR)

2.1.1. SBR reactor

PHA accumulating microorganisms were selected using a 3L jacketed glass reactor connected to a water bath (JP Selecta Termotronic-100) to keep the temperature at 30ºC. This reactor was equipped with a mechanical stirrer and two porous stones that were connected to air compressors (Moure air pump 5, Epsilon). Peristaltic pumps (PERCOM-I) connected to timers (Smartwares 10.047.65 with programable mechanical temporizer) were used to temporize the sequence batch reactor (SBR). Moreover, four pumps were used to perform the feeding (VFA-rich wastewater feeding, NH_4^+-N source feeding) and withdrawal operations (sludge purge; treated wastewater discharge). The reactor was equipped with a pH probe (Mettler Toledo, HA405-DPA-SC-S8/225) connected to a pH meter (pH Crison28) and a dissolved oxygen (DO) probe (CellOx 325, WTW) connected to a DO portable meter (Oxi 3310, WTW) throughout the entire process. The DO profile was monitored continuously by connecting the probe to the computer to register the dissolved oxygen (DO).

The reactor followed cycles of 6 hours length with the following time distribution: (i) carbon source feeding of VFA in anaerobic conditions (15 min, 0.6 L), (ii) aerobic reaction where microorganisms consume the biodegradable carbon source and store internal PHA into the cells (147 min), (iii) biomass purge to obtain biomass with high PHA accumulation capacity (3 min, 0.156 L), (iv) aerobic feeding of ammonia nitrogen (4 min, 0.088 L), (v) aerobic reaction consuming the supplied N and PHA reserves for biomass growth (146 min), (vi) Biomass settling after the reactor (30 min) and (vii) treated effluent discharge (15 min, 0.6 L). The hydraulic retention time (HRT) and the sludge retention time (SRT) was set at 1.25 days and 4.8 days, respectively. Table 1 summarizes the operating conditions of the SBR divided into three stages depending on the recorded feast/total cycle time ratio, ammonium loading rate, and organic loading rate (OLR). As stated in Table 1, Stage II was characterized by the lower feast/cycle ratio and the increase of the ammonium nitrogen load, while Stage III was characterized by an increase of the organic loading rate (OLR).

Table 1. Operating parameters of the selection SBR reactor working with decoupled feeding of VFA and NH_4^+-N at 30ºC.

Parameter	Units	Stage I	Stage II	Stage II
Days of operation	-	1-117	118-169	170-209
HRT	days	1.25	1.25	1.25
SRT	days	4.8	4.8	4.8
Feast/cycle	% time	>20%	<17%	<17%
Organic loading rate	g COD · L^{-1} · day^{-1}	2.0	2.0	2.8
VFA feed	g COD · L^{-1}	2.5	2.5	3.5
Influent acetic acid	% COD	53.1	53.1	53.1
Influent propionic acid	% COD	21.3	21.3	21.3
Influent butyric acid	% COD	25.6	25.6	25.6
NH_4^+-N loading rate	mg NH_4^+-N · L^{-1} · day^{-1}	72	96	96

2.1.2. Inoculum and feed

The selection SBR was started up with 200 mL of waste activated sludge (WAS), collected from WWTP located in the Barcelona Metropolitan Area with 12.3 g TSS ·L^{-1} and 9.1 g VSS ·L^{-1}.

The synthetic feed used was a mixture of acetic (53.1% of COD), propionic (21.3% of COD), and butyric (25.6% of COD) for the two OLR used in this reactor (namely, 2.0 g COD· L^{-1}·day^{-1} for stages I and II, and 2.8 COD· L^{-1}·day^{-1} for stage III) which was based on previous results obtained from the acidogenic fermentation of organic fraction of municipal solid waste [11]. The pH of the synthetic feed was adjusted to 6.5 by adding 1.5 g $NaHCO_3$ · L^{-1} to the feed. Additionally, this synthetic feed contained macronutrients and micronutrients which were detailed in Table 2 based on Dapena-Mora et al. (2004) [12]. A synthetic solution containing NH_4^+-N was also prepared separately to decouple the carbon source from the nitrogen feeding. This N rich solution was prepared using different NH_4Cl concentrations to obtain an NH_4^+-N Loading Rate of 74 g N · L^{-1} · day^{-1} for Stage I and 96 g N · L^{-1} · day^{-1} for Stages II and III.

Table 2. Macronutrients and micronutrients concentration in the VFA-rich feed stream based on Dapena et. Al. [12].

Macronutrient	Value	Unit	Micronutrient	Value	Unit
K_2HPO_4	0.58	$g \cdot L^{-1}$	$FeCl_3 \cdot 6H_2O$	1.5	$mg \cdot L^{-1}$
KH_2PO_4	0.23	$g \cdot L^{-1}$	H_3BO_3	0.15	$mg \cdot L^{-1}$
$MgSO_4 \cdot 7H_2O$	0.09	$g \cdot L^{-1}$	$CuSO_4 \cdot 5H_2O$	0.03	$mg \cdot L^{-1}$
$CaCl_2 \cdot 2H_2O$	0.07	$g \cdot L^{-1}$	KI	0.03	$mg \cdot L^{-1}$
EDTA	0.02	$g \cdot L^{-1}$	$MnCl_2 \cdot 4H_2O$	0.12	$mg \cdot L^{-1}$
			$Na_2MoO \cdot 2H_2O$	0.06	$mg \cdot L^{-1}$
			$ZnSO_4 \cdot 7H_2O$	0.12	$mg \cdot L^{-1}$
			$CaCl_2 \cdot 2H_2O$	0.12	$mg \cdot L^{-1}$

2.2. Accumulation Tests

The reactor used for the accumulation tests was a jacketed glass reactor of 1.5 L at 30ºC. It was equipped with a mechanical stirrer and one porous stone connected to a continuous DO supply system. Hence, an oxygen probe was used to control DO and to prove when the microorganisms completely consumed the carbon source. The reactor was operated in batch mode (feed on demand strategy) to promote the PHA storage in the microorganisms previously purged in the selection SBR. In each batch assay, 800mL of biomass obtained in the selection reactor was added. These tests were carried out using the selected biomass obtained on Stage II and Stage III of this study. The carbon source (VFAs) was added in the same proportion used for the selection of SBR. In this case, the synthetic feed used had double COD than the fed in the selection reactor, resulting in a 5 and 7 g $COD \cdot L^{-1}$ concentration for stage II and III, respectively. In addition, ammonium was not supplied during these tests. The feeding mode was performed with the addition of 100 mL of feed when DO was higher than 6 mg $O_2 \cdot L^{-1}$, indicating that the microorganisms finished the carbon source. This procedure was done about 4-5 times for every batch taking approximately 7 hours of the batch test. Ammonium was not supplied during the accumulation test.

2.3. Analytic methods

The analysis of Total Suspended Solids (TSS), Volatile Suspended Solids (VSS), and total ammonium nitrogen (NH_4^+) were performed following the 2450D, 2540E, and 4500-D methods, respectively, of the *Standard Methods for the Examination of Water and Wastewater* [13]. The ammonia was determined with an ammonia selective electrode method ammonia electrode Orion 9512HPBNWP connected to pH/ISE Benchtop Thermo Scientific Orion Dual Star. To analyse VFA concentration, the sample was filtered with a 0.45 μm pore size regenerated cellulose syringe filters. With a proper dilution, the sample was acidified with phosphoric acid (85% purity) and analysed by gas chromatography (Shimadzu GC 2010 plus) using a capillary column (Nukol™, 15 m x 0.53 mm x 0.5 μm) and flame ionization detector (FID).

The PHA content was analysed using the method described in Lahmna et. al. [14]. Briefly, the samples were frozen for 24h to -80ºC, and then, samples are freeze-dried (-52ºC) in a lyophilizer (Telstar, LyoQuest) for 24h. Methanol (20% sulphuric acid v/v) was used to break the bacteria membrane, while Chloroform stabilized with ethanol (Panreac, 119.38 M) to dissolve PHA. Benzoic acid (Sigma-Aldrich ≥ 99.5%) was used as an intern pattern to ensure the result validity. Moreover, the PHA pattern (Sigma Aldrich, 12% molar PHV) was used to assess the abundance of HB and HV. The samples were analysed by gas chromatography (Shimadzu GC 2010), with a capillary column Sapiens-X5MS (FTR-450262), 60mm x 0.25 mm x 0.25μm.

3. Results and discussion

3.1. Selection SBR operation

Table 3 shows the parameters monitored throughout the operation of the selection reactor in the 3 operating periods. The TSS and VSS were higher in the first period, which was attributed to the high quantity of inoculum initially used to carry out the selection of PHA accumulating organisms, which contained 12.3g $TSS \cdot L^{-1}$ and 9.1 $VSS \cdot L^{-1}$ of inoculum. Once the selection reactor started to operate correctly (VFA concentration in the effluent is nearly 0 mg $VFA \cdot L^{-1}$), the concentration of TSS and VSS decreased to an average concentration of 2.88g $TSS \cdot L^{-1}$ and 2.57g $VSS \cdot L^{-1}$ in Stage I,

1.92g TSS ·L^{-1} and 1.73 g VSS ·L^{-1} in Stage II and, 2.00 g TSS ·L^{-1} and 1.97 g VSS ·L^{-1} in Stage III (see Fig 1.). These values are similar to those reported by Albuquerque et.al. [15], who obtained a VSS concentration around 2.08 to 5.1 g VSS · L^{-1} when working with an SRT of 10 days and HRT of 1 day. These authors worked with three different carbon source concentrations, 30, 60, and 45 Cmmol·L^{-1}, with a cycle of 12 h in uncoupled conditions. Moreover, it is important to note that the biomass developed in this study had a constant ratio between the VSS/TSS at nearly 90% during the three operating stages. This indicates that the majority of TSS are microorganisms with the stored carbon source.

As for VFA, samples were taken at the input port of the reactor (feed, nitrogen-rich solution) and at the output ports (sludge purge, effluent discharge). The VFA concentration in the sludge purge was always lower than the VFA concentration in the feed, which means that microorganisms stored this carbon source in aerobic conditions as PHA inside the cellular matter itself before the end of the feast stage, as expected. VFA was always completely removed in the treated effluent discharge (at the end of the SBR cycle).

Table 3. Average (minimum-maximum) values of the operating parameters in the selection SBR

	Stage I	**Stage II**	**Stage III**
ORL (g COD·L^{-1}·day^{-1})	2.0	2.0	2.8
TSS (g · L^{-1})	2.88 (1.08 – 5.48)	1.92 (1.07 – 3.55)	2.00 (1.66 – 2.31)
VSS (g · L^{-1})	2.57 (0.98 – 4.86)	1.73 (0.98 – 3.55)	1.97 (1.58 – 2.27)
% VSS/TSS	89 (52 – 99)	89 (79 – 97)	98 (98-100)
VFAs sludge purge (mg VFA· L^{-1})	28 (0 – 170)	11 (7-32)	42 (42-110)

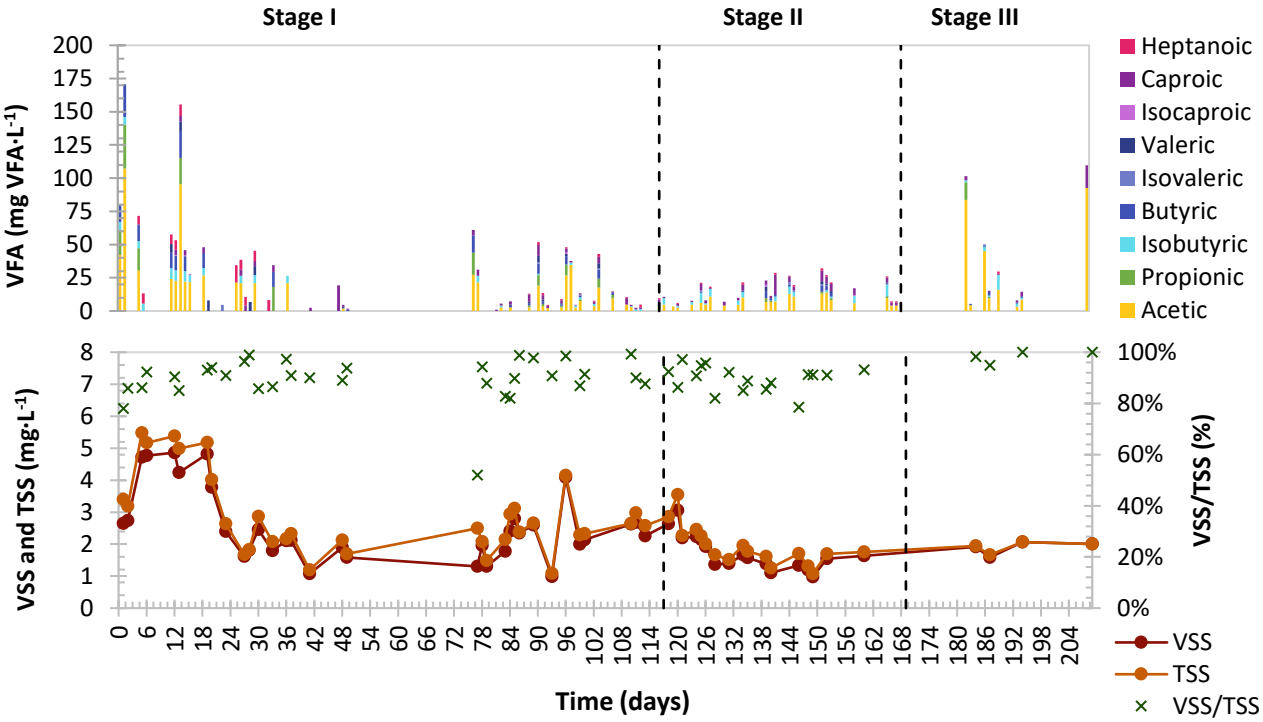

Fig 1. VFA, VSS, TSS, and VSS/TSS ratio in the sludge purge stream of the selection SBR. Note that VFA were always completely removed in the treated effluent discharge (at the end of the SBR cycle)

3.2. SBR cycle analysis

In this section, a representative SBR cycle monitored (Fig. 2) which consists of DO, pH, VFA, NH_4^+-N, and PHA content obtained during the Stage III in which concentrations of 2.49 g TSS · L^{-1} and 2.39 g VSS · L^{-1} were obtained is described in detail.

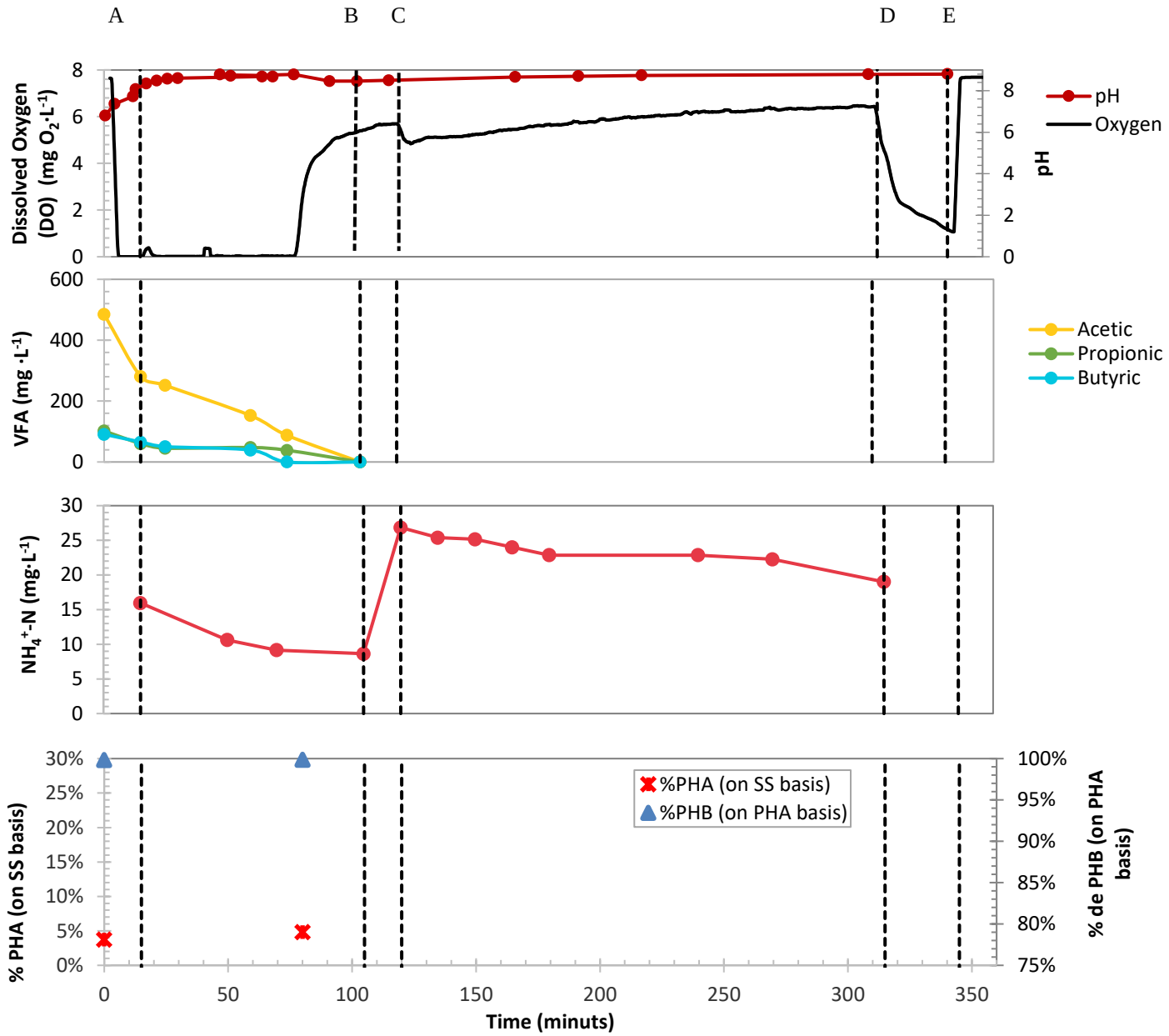

Fig 2. DO, pH, VFA, NH_4^+-N, and PHA profiles of a representative SBR cycle of period III (A = feed addition; B = purge; C = ammonia addition; D = sedimentation; E = effluent discharge)

As observed in Fig 2., the DO in the feast phase (A-B) was nearly 0 mg ·L^{-1}, although it was constantly supplied. This fact does not concern the selection of microorganisms where they did not lose the PHA storing capacity. [16]. When VFA were completely depleted, a rise in DO concentration was observed. A minor decrease was experienced when NH_4^+-N was added since microorganisms consumed this nutrient for their growth. Fig 2. shows that the feast phase is much shorter than the famine (ratio of 0.17), which is below the value of 0.2 and allows an internal selection pressure [9,14].

Fig 2. reveals that microorganisms consume acetic, propionic, and butyric acids in aerobic conditions before the sludge purge as was expected. The acetic acid consumption rate was higher than those observed for propionic and butyric acids, probably related to the higher complexity of those carbon compounds. For this reason, acetic acid exhaustion suggests that it was the primary substrate for PH, which agrees with Wijeyekoon, et al. [18]. During the second aerobic reaction phase with ammonium (C-D), microorganisms consumed stored PHA, ammonia, and other nutrients to grow. The PHA concentration was measured at the beginning of the cycle and in the purge when the PHA storage was maximum (7% in SS basis). As expected, this PHA was mainly composed of PHB due to the composition of the synthetic feed, since it could be attributed to the proportion of the even and odd number of carbons of VFA. HB is obtained when it comes from acids with a pair number of carbons. By contrast, odd carbons give rise to HV [19, 20]. Similar values (around 9 % PHA n a VSS basis) were obtained by different authors but with different conditions. Firstly, Ahmadi et al. [21] obtained this value (9% in a TSS basis) using a feed composed of sugar and VFA with a different distribution of the cycles which

included two settles (after the first and the second reaction respectively). Secondly, Albuquerque et al. [15] obtained 9% PHA in a VSS basis, with a feeding mixture of sugar molasses with 30 Cmmol·L^{-1} under uncoupled feeding of carbon and nitrogen source and a cycle length of 12 hours. Finally, Chen et al., [22] showed values of PHA around 9.55-18.90 % PHA in VSS basis under SRT of 12 days using a coupled nitrogen and carbon source (fermented sugar cane wastewater with 1.41 g COD · L^{-1} · d^{-1}). Chen et.al. [22] who used a similar organic loading rate (2 g COD · L^{-1} · d^{-1} of sodium acetate), SRT of 10 days, and a coupled feeding of carbon source and nitrogen, achieved a PHA percentage of 23% (in VSS basis) in the selection reactor. Another case would be Villano et.al. [24], who used a higher substrate organic loading rate (8.5 g DQO · L^{-1} · d^{-1} of a synthetic mixture of acetic acid (85% in COD) and propionic acid (15% in COD)) using a coupled C and N regime with no settling phase, yielding a maximum PHA of 19 ± 0.8% w/w.

3.3. PHA accumulation tests

During the run of the selection reactor, different accumulation assays were performed with the purged selected biomass. In this case, two representative accumulation tests are displayed in Figures 3 and 4, according to the OLR used in the selection SBR.

Regarding the first accumulation test, the purged biomass obtained in Stage II had a TSS and VSS concentration of 1.59 g TSS· L^{-1} and 1.45 g VSS· L^{-1} respectively, with a 0.96 VSS/TSS ratio. The COD used to feed the accumulation reactor had a 5 g COD · L^{-1} with the proportional percentage of acetic, propionic, and butyric acids (see Section 2.1.2). The performance of the accumulation assay is illustrated in Fig 3. where DO, VFA, and PHA evolution during the test are presented. This assay was performed with 5 spikes of VFA rich wastewater performed at 0.2 hours, 1.9 hours, 2.3 hours, 3.4 hours, and 4.8 hours, when VFA were depleted. When the spikes of synthetic wastewater were performed, the DO profile showed a slight decrease related to VFA consumption and a subsequent DO increase when the organic substrate was depleted. DO abruptly decreased before the third and fifth spike since the oxygen supply was turned off and biomass was settled before separating a liquid fraction of the reactor's content. Although the VFA dosage was the same in each spike, over time, microorganisms become more satiated and took longer to consume the feeding acids (see Table 4).

Following this strategy, microorganisms stored carbon in the form of intracellular PHA with an initial concentration of 308.89 ± 133.86 mg PHA · L^{-1} (9.76 ± 0.04% PHA in SS basis) reaching values as high as 1363.38 ± 86.72 mg PHA · L^{-1} (44.09 ± 0.05% PHA in SS basis) in the last sample of the assay. As occurred in the selection reactor, 90% corresponded to PHB. Applying similar conditions (4.25 g COD · L^{-1}) but different VFA feed proportion (85% acetic and 15% propionic, in COD basis) and cycle length of 12 hours with uncoupled C and N regime, Lorini, et.al. [25] obtained 63-70% gPHA/gVSS at the final of the accumulation assay. These results agree with Valentino et al. [26] who reported a final value of 38±4 % COD_{PHA} · COD_{VSS}^{-1} in an accumulation reactor operated 6.5 hours with a synthetic feed of acetic and propionic acids (2 g COD_{VFA} · COD_{VSS}^{-1})

Table 4. The average speed of VFA degradation of each pulse in the accumulation test performed on purged biomass from the selection SBR working under 2 g COD · L^{-1} · day^{-1}

Pulse	Initial PHA (% on SS)	Initial VFA (mg · L^{-1})	Final PHA (% on SS)	Average VFA degradation speed (mg VFA · min^{-1} · L^{1})	Time spent (min)
1	7	198.85	9	5.41	46
2	9	198.85	-	3.81	48
3	-	198.85	26	3.54	63
4	26	198.85	-	2.32	89
5	-	198.85	41	1.94	101

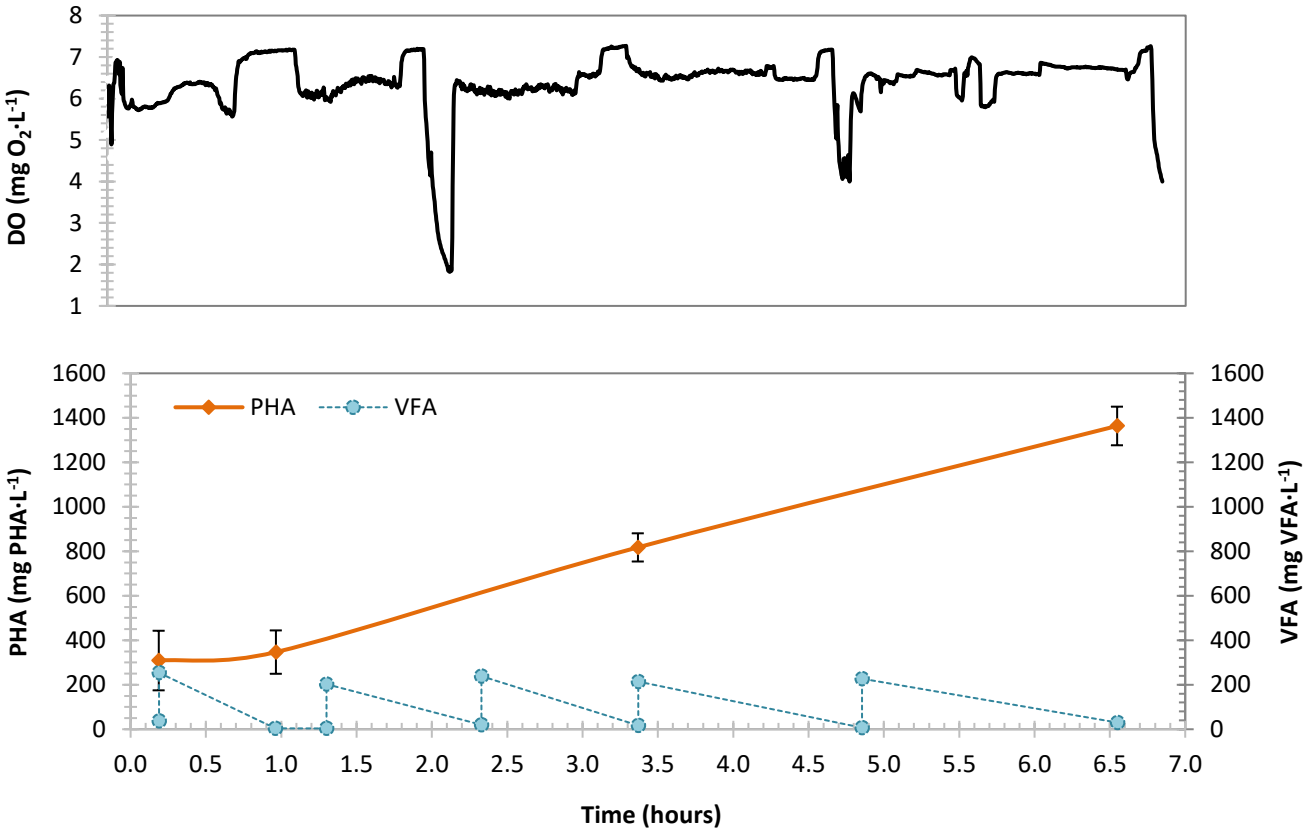

Fig 3. Accumulation of Stage II of the selective reactor with COD = 5 g COD · L^{-1}
VFA concentration (sum of acetic, propionic, and butyric acids)

The next accumulation test was performed with biomass extracted from the selection reactor of Stage III. The purged biomass used consists of 1.81 g TSS· L^{-1} and 1.84 g VSS· L^{-1}, being a VSS/TSS ratio of 98%. The COD used in the feed was 7 g COD · L^{-1} of acetic, propionic, and butyric acids. Fig 4. represents the evolution of PHA content, VFA concentration, and DO concentration during the test (note that there were no sedimentation steps as in the previous test). The spikes were added at 0.1 hours, 1.85 hours, 3.1 hours, 4.4 hours, and 6.8 hours. Due to the fast consumption of VFA, the second and the third spikes were not performed immediately after the depletion of VFAs.

With the same feed-on-demand strategy, the microorganisms seemed to be satiated with the last pulse as is shown in Fig 4. Table 5 shows the average VFA consumption rate which decreased dramatically during the last spike. The maximum recorded PHA content of the biomass was 1620.14 ± 122.33 mg PHA · L^{-1} (46.12 ± 0.00% PHA in SS basis) which started with 246.18 ± 217.41 mg PHA · L^{-1} (9.16 ± 0,08% PHA in SS basis). Lorini et.al [25] started from biomass that had a 10.119 g COD · L^{-1} synthetic feed to select the biomass in SBR. Then, after the accumulation test, values of 70% PHA in a VSS basis were reported with nitrogen limitation and a feed of 2 g COD_{VFA}·$gVSS^{-1}$. Similarly, Villano et.al. [24] obtained 46±2 % w/w on average with a 6 h batch test through a feeding compound by acetic acid and propionic acid (85% and 15% in a COD basis) under nitrogen limitation. Therefore, the present study succeeded in the start-up of a selection reactor to generate a sludge purge enriched in PHA-storing microorganisms, that could increase its biomass PHA content above 40% with a feed on demand PHA accumulation strategy, a value that has been reported as a rough threshold towards commercially viable PHA recovery [27].

Table 5. The average speed of VFA degradation of each pulse in the accumulation test performed on purged biomass from the selection SBR working under 2.8 g COD · L^{-1} · day^{-1}

Pulse	Initial PHA (% on SS)	Initial VFA (mg · L^{-1})	Final PHA (% on SS)	Average VFA degradation speed (mg VFA · min^{-1} · L^{1})	Time spent (min)
1	8	555.13	20	8.25	54
2	20	555.13	29	8.41	51
3	29	555.13	38	5.54	78
4	38	555.13	39	2.68	144
5	39	555.13	46	0.33	96

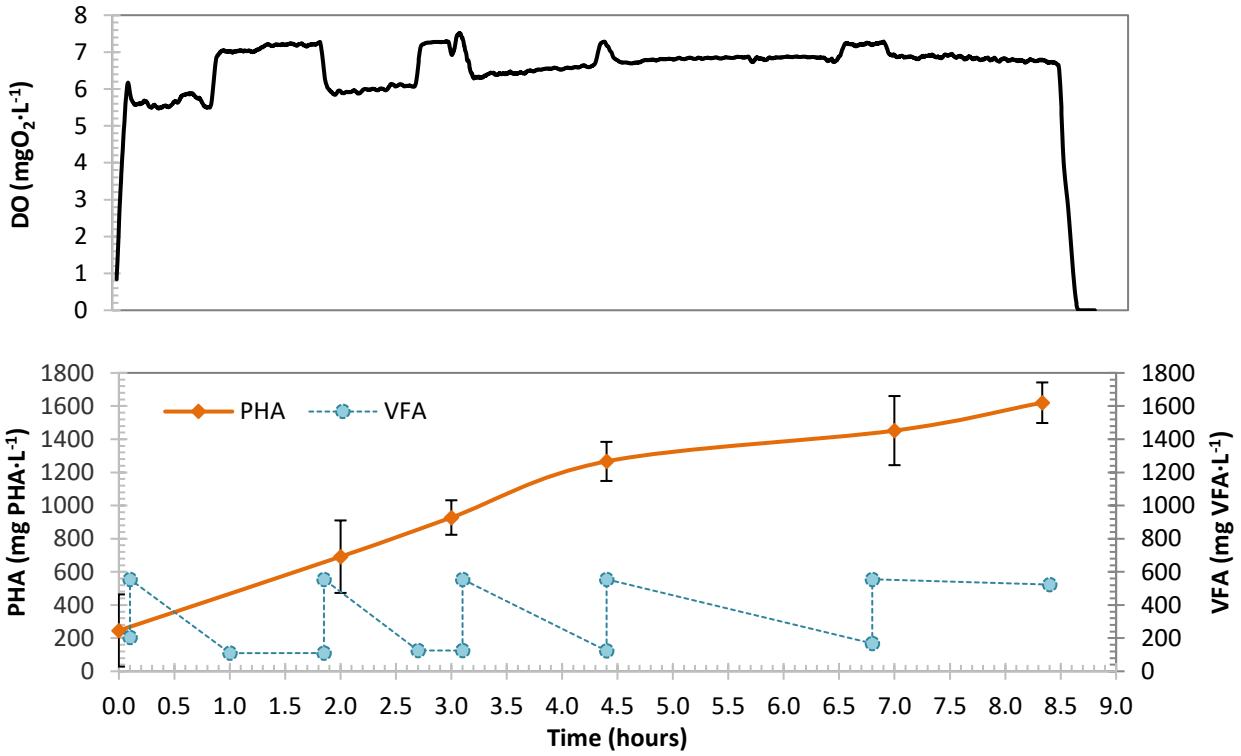

Fig 4. Accumulation of Stage III of the selective reactor with COD = 7 g COD · L^{-1}
VFA concentration (sum of acetic, propionic, and butyric acids)

4. Conclusions

This study allowed to establish a set of operational conditions for the selection of accumulating PHA microorganisms using uncoupled feeding of biodegradable carbon and nitrogen with MMC using VFA rich wastewater. The dissolved oxygen profile of the selection SBR allowed us to understand the process performance. The SBR working conditions, that were beneficial for a proper microorganism selection, were based on an HRT of 1.25 days and an SRT of 4.8 days under an OLR of 2-2.8 g COD·L^{-1}·day^{-1} with the proportions of 53.1, 21.3, and 25.6 % (in COD basis) of acetic, propionic, and butyric acids, respectively. The uncoupled ammonium loading rate was 96 mg N-NH_4^+·L^{-1}·day^{-1} and the feast/famine ratio was lower than 17%. Accumulation assays showed that PHA-storing microorganisms selection were successfully selected in the SBR. At the experimental conditions of 30ºC, using a feed of 5-7 g COD·L^{-1}, which was based on acetic, propionic, and butyric acid with the same proportion of SBR feed with an absence of nitrogen, the percentage of PHA was above 40 % in SS basis, a value that could lead to commercially viable PHA recovery.

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