Development of a novel process integrating the treatment of sludge reject water and the production of polyhydroxyalkanoates (PHAs)

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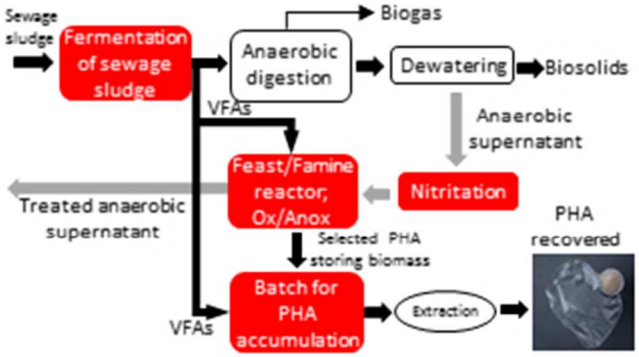

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Title: Development of a novel process integrating the treatment of sludge reject water and the production of polyhydroxyalkanoates (PHAs)

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ABSTRACT. Polyhydroxyalkanoates (PHAs) from activated sludge and renewable organic material could become an alternative product to traditional plastics since they are biodegradable and biocompatible. In this work, the selection of PHA storing bacteria was integrated to the side stream treatment for nitrogen removal via nitrite from sludge reject

water. A novel process was developed and applied where the alternation of a*erobic-feast and anoxic-famine* conditions accomplished selection of PHA storing biomass and nitrogen removal via nitrite. Two configurations were examined: in configuration 1 the ammonium conversion to nitrite occurred in the same reactor where the PHA selection process occurred, while in configuration 2 two separate reactors were used. The results showed the selection of PHA storing biomass was successful in both configurations, while the nitrogen removal efficiency was much higher (almost 90%) in configuration 2. The PHA selection degree was evaluated by the volatile fatty acid (VFA) uptake rate (-qVFAs) and the PHA production rate which were 182±14 mgCOD/gX h and 89±7mgCOD/gX h respectively. The characterization of biopolymers after the accumulation step, showed that it was composed of 3-hydroxybutyrate (3HB) (60%) and 3-hydroxyvalerate (3HV) (40%). The properties associated with the produced PHA suggest that they are suitable for thermoplastic processing.

Keywords: polyhydroxyalkanoate (PHA); feast and famine regime; nitrogen removal via nitrite; reject water

INTRODUCTION

Polyhydroxyalkanoates (PHA) are biodegradable polymers that can be produced by many different types of bacteria. Compared to conventional synthetic polymers, PHAs possess obvious ecological advantages since they are completely biodegradable and nontoxic[1,2] and can be produced from a renewable source. The family of PHA polymers, including polyhydroxybutyrate (PHB) and PHB-related copolymers, is versatile and thus, presents significant opportunities for marketability. PHA production from mixed cultures and renewable organic wastes as a carbon source [3,4,5] has become very attractive over the last years due to the decrease in the production cost of the process[6,7]. Activated sludge from

wastewater treatment plants (WWTPs) is a well-known source of PHA-storing organisms that store these polymers as carbon and energy reserve for biomass growth. PHA production from mixed cultures are accomplished by a sequence of operations which are the following[8,9]: i) acidogenic fermentation to produce volatile fatty acids (VFAs) from biodegradable organics; ii) selection of PHA storing biomass in a sequencing batch reactor (SBR); iii) batch step to maximise PHA accumulation in the bacteria.

The carbon limitation strategy under feast and famine conditions has been found to be favourable for the enrichment and long-term cultivation of PHA producing communities, while nitrogen limitation is a successful strategy that can be employed to accomplish high PHA contents during the PHA production step [10]. However, these processes are aerobic and thus are energy intensive; it is estimated that approximately 39 MJ are needed to produce 1 kg of PHA when the aerobic accumulation step is employed[11,12].

The integration of nutrient removal in wastewater treatment systems with the PHA production cycle is currently a challenge. Jiang et al. (2009)[13], Morgan-Sagastume et al. (2010)[14] and Pittmann et al. (2014)[15] evaluated the potential for the enrichment of PHA-storing organisms and the PHA-storage capacity of fermented sludge under different operational conditions. It was found that PHA producing organisms could be successfully enriched using the fermented sludge as feedstock, although very high nutrient loads did limit the maximum level of PHA accumulation[14]. Morgan-Sagastume et al. also examined a new concept of WWTP operation, where the selection of PHA storing biomass was accomplished based on the conversion of the readily biodegradable chemical oxygen demand (COD) from municipal wastewater without its pre-conversion to VFAs[16]. However, post-treatment was required, in particular for enhanced nutrient removal of the treated effluent. Anterrieu et al (2014)[17] studied the integration of PHA production with conventional nitrification and denitrification for treating sugar beet factory waters, by operating with an anoxic feast and

aerobic famine phase. In the sludge treatment line of municipal wastewater treatment plants, however, the nutrient loads are typically much higher, and side-stream treatment is an attractive option to avoid recycling these nutrients to the main treatment line. Short-cut nitrogen removal processes via the nitrite pathway have been found to provide an attractive means of achieving nutrient removal from sludge reject waters at lower operating expenses compared to conventional nitrification-denitrification[18]. Frison et al. (2014)[19] compared the potential PHA storage of sludge under nitrifying and non-nitrifying conditions in batch reactors, and found PHA production yields of 0.6 Cmol PHA/Cmol VFA in both systems. Nevertheless, the feasibility of integrating PHA production with nutrient removal via nitrite has never been investigated.

In this work, a novel process for the potential integration of the side stream biological nitrogen removal via nitrite with the selection of the PHA storing biomass from mixed cultures treating anaerobic supernatant is examined. A modified pathway for nitrogen removal was developed and applied in order to induce a feast and famine regime under aerobic and anoxic conditions, respectively. The novel approach is based on (i) selection of a PHA storing biomass in a sequencing batch reactor (SBR) by the alternation of aerobic feast conditions for ammonia conversion to nitrite followed by anoxic famine (FF) conditions for denitritation driven by internally stored PHA as carbon source; (ii) establishment of the desired COD/N ratio for the selection of PHA storing biomass by controlling the dosing of the external carbon source and the nitrogen contained in the anaerobic supernatant. A side-stream treatment technology able to integrate the conversion of fermented sludge to PHAs with nutrient removal via nitrite would enable the simultaneous reduction of nutrient loads to the main line of the WWTPs, lowering operational costs, with the recovery of a valuable resource, PHA, providing further economic advantages to the WWTP.

MATERIAL AND METHODS

2.1 Experimental set-up

Figure 1 shows two alternative configurations applied for nitrogen removal via nitrite and the selection of PHA storing biomass downstream from the anaerobic digester. In configuration 1, the nitrogen removal through nitritation/denitritation and the PHA selection process occurred in a single SBR by applying a feast and famine regime. Specifically, during aerobic conditions, ammonium was oxidized to nitrite, while during the anoxic famine conditions, nitrite was reduced to nitrogen gas using the internally stored PHA as carbon source (Figure 1a). In configuration 2, two separate SBRs were used to accomplish the ammonium conversion to nitrite and the selection of the PHA storing biomass (Figure 1b). Nitritation was carried out in a dedicated SBR and then the liquid was fed to the selection reactor while operated under famine conditions, accomplishing the denitritation using the stored PHA. In the selection reactor, the COD/N ratio was adjusted by feeding the anaerobic supernatant as main source of nutrients (nitrogen and phosphorus).

The PHA accumulation step of the examined process was performed in a fed-batch reactor to maximize the cellular PHA content of the biomass harvested from the selection stage. The volatile fatty acids (VFAs) that are required for the feast conditions in the selection reactor and the PHA accumulation in the batch reactor were recovered from the acidogenic fermentation of sewage sludge. Fermented liquid from sewage sludge was used as VFA source. The process is described in detail in the work of Longo et al. (2015)[20]. The technical details of the applied configurations are given below.

2.1.1 Configuration 1 (Figure 1a). An SBR having a working volume of 26 L was used for the selection of PHA storing bacteria. Full details of the SBR are reported in the supporting

information (SI). The cycles of the SBR consisted of 5 min of feeding, 300 min of reaction phase (aerobic and anoxic), 15 min of settling and 5 min of discharge. Within the reaction period, the aerobic phase was varied from 50 to 100 min during period 1, and from 100 to 150 min during period 2 (Configuration 1). This variation was introduced in order to compare the effect of the availability of nitrite on the process. Two experimental periods were performed using configuration 1 and adopting different COD/N ratios (Table 1). The target COD/N was achieved by maintaining the volumetric organic loading rate (vOLR) stable at 674±72 gCOD/m^3d and altering the volumetric nitrogen loading rate (vNLR) by adjusting the feeding of the anaerobic supernatant. Table 1 reports the operating conditions adopted during the examined periods. In the first period, the feast conditions were established at the beginning of the aerobic phase by applying a COD/N ratio of 5.6±0.1 gCOD/gN, while in the second period the COD/N was around 2.0±0.02 gCOD/gN. The solids retention time (SRT) was kept within the range of 12-15 days throughout each experimental period.

(Table 1)

(Figure 1a and 1b)

2.1.2 Configuration 2 (Figure 1b). In a first step, a nitritation SBR (N-SBR) was applied as a pre-treatment stage in order to enhance the conversion of ammonium to nitrite (nitritation). A detailed description of the operating conditions of the N-SBR are given in the SI. The anaerobic supernatant exiting from the N-SBR reactor contained ammonium in the range of 25 to 67 mgN/L, while the average level of nitrite was approximately 350 mgN/L. After settling, the clarified effluent from the N-SBR was temporarily collected in a storage tank (80 L of volume) and then was fed to the selection SBR during the first 10-12 minutes of the anoxic phase based on a volumetric nitrogen loading rate of 530±11 gN/m^3d, which corresponded to a volumetric nitrite loading rate of 423±95 gNO_2-N/m^3d. The loading rate of

147 the carbon source during the feast was balanced with the nitrite loading rate applied during
148 the famine phase based on the ratio 2.2±0.1gCOD/gNO_2-N. The operation cycle of the
149 selection SBR consisted of 50 minutes of aerobic phase, followed by 250 minutes of anoxic
150 conditions. The length for the feeding, settling and discharging were respectively 15, 30 and
151 15 minutes. The SRT was kept in the same range as for Configuration 1, 12-15 days.
152

153 **2.2 PHA accumulation**

154 The biomass was collected under famine conditions (end of the anoxic phase from the
155 selection SBR) and was concentrated gravimetrically by applying 30 min of settling. The aim
156 was to reduce the level of nutrients contained in the biomass obtained from the selection SBR.
157 Then, the biomass was placed in triplicate fed-batch glass reactors with working volumes of
158 1 L that were equipped with blowers, diffusers and probes for the measurement of the
159 dissolved oxygen (DO, type WTW, CellOx® 325), the pH (Polyplast Pro) and the
160 temperature (PT100). The on line signals were automatically recorded. The DO level was
161 always maintained above 2 mg/L. The oxygen uptake rate (OUR) was determined using the
162 respirometer MARTINA (SPESS, Italy). The substrate was divided in fixed- volume aliquots
163 in order to dose manually each time approximately 1 gCOD/L of VFAs. New aliquots were
164 dosed when consecutive OUR values were ~ 50% less than the previously recorded values. In
165 order to test different COD/N/P ratios during the PHA accumulation, three different carbon
166 sources were applied; a synthetic mixture of VFA (COD/N/P 100:0:0), sewage sludge
167 fermentation liquid (SFL) (COD/N/P 100:9.7:2.1) and sewage sludge fermentation liquid
168 with wollastonite (WSFL) (COD/N/P 100:7.8:0.06). In the latter, the use of wollastonite
169 during fermentation improves the COD_{VFA}/NH_4-N/PO_4-N ratio, since it limits the net release
170 of nutrients (ammonia and phosphates). The duration of the accumulation test was 6-8 h.
171

2.3 Calculations

In this study the VFAs concentration, expressed as mgCOD/L, was calculated as follows:

$$VFAs = \sum (HAc + HPr + HBt + iso\text{-}HBt + HPt + isoHPt + HHe + HHp) \quad (1)$$

where: HAc is acetic, HPr is propionic, HBt is butyric, iso-HBt is iso-butyric, HPt is pentanoic, isoHPt is iso-pentanoic, HHe is hexanoic and HHp is heptaoic acid.

The PHA monomeric concentrations were converted into COD units by following the oxidation stoichiometry: 1.38 mgCOD/mg(3-hydroxybutyrate, HB), 1.63 mgCOD/mg(3-hydroxyvalerate, HV) and 1.82 mgCOD/mg(3-hydroxyhexanoate, HH). The total PHA concentration was calculated as follows:

$$PHA\ (mgCOD/L) = \sum (PHB + PHV + PHH) \quad (2)$$

where: PHB is polyhydroxybutyrate; PHV is polyhydroxyvalerate; PHH is polyhydroxyhexanoate.

The amount of PHA (g/L) was subtracted from the VSS (g/L) to calculate the concentration of the active biomass (X, g/L). The latter was transformed as COD concentration by the stoichiometric ratio of 1.42 gCOD/gVSS.

The fraction of the PHA in the biomass was calculated considering the following equation:

$$PHA(\%) = \frac{gPHA}{gVSS} \times 100 \quad (3)$$

where the gPHA and the VSS were respectively the PHA and the volatile suspended solids of the biomass. The specific VFA uptake rate (-qVFA, mgCOD/gCOD$_X$h) and the PHA storage rate (qPHA, mgCOD/gCOD$_X$h), were determined by linear regression analysis by plotting the concentarion of VFAs and PHA as a function of time. The results were normalized for the active biomass concentration. The rates were standardized at 20°C of temperature by the Arrenhius equation[21]. The PHA storage yield ($Y_{PHA/VFA}$, $gCOD_{PHA}/gCOD_{VFA}$) and the growth

196 yield ($Y_{X/VFA}$, $gCOD_X/gCOD_{VFA}$) were calculated as the ratio between each maximum
197 specific rate (qPHA and qX, respectively) and the -qVFA.
198
199 **2.4 Analytical methods**
200 The concentrations of the mixed liquor suspended solids (MLSS), mixed liquor volatile
201 suspended solids (MLVSS), chemical oxygen demand (COD), soluble chemical oxygen
202 demand (SCOD), total Kjeldahl nitrogen (TKN), ammonium (NH_4-N), phosphorous (TP)
203 were determined according to standard methods[22]. Nitrite (NO_2-N), nitrate (NO_3-N) and
204 phosphate (PO_4-P) concentration were determined by the ion chromatograph Dionex ICS-900
205 with AS14 as column, while the concentration of HAc, HPr, HBt, iso-HBt, HPt, isoHPt and
206 HHe and heptaoic acid was determined by liquid chromatography through a Dionex ICS-
207 1100 with IonPac ICE-AS1 as column. Samples of biomass were collected from the selection
208 and accumulation reactors and the liquor was removed through centrifugation. Then, the
209 thickened biomass was freeze-dried with a lyophilisation unit (Lio 5P, 5Pascal, Milano, Italy)
210 and analysed for the content of PHA using the method developed by Lanham et al., (2013)[23].
211 The specific ammonium uptake rate (sAUR) and specific nitrogen uptake rate (sNUR) were
212 determined in situ following the procedures that are given in SI. The nitrogen mass balances
213 were calculated for each experimental period (see SI).
214 The final biopolymers produced during the accumulation stage were also extracted from the
215 freeze dried biomass by applying the chloroform method (50 mL/g of freeze dried biomass)
216 followed by the addition of methanol as precipitation agent[1]. Then, the biopolymers were
217 analysed through size exclusion chromatography (SEC, Polymer Lab) in order to determine
218 the molecular number (M_n), average molecular weights (M_w) and the polydispersity indices
219 (PDI). The glass transition temperature (Tg), melting temperature (T_m) and melting enthalpy

(DHm) were determined by differential scanning calorimetry (DSC, TA Instruments). More information about the procedures are available on SI.

RESULTS AND DISCUSSION

3.1 Efficiency of single stage reactor configuration

The impact of combining the ammonium oxidation to nitrite, denitritation and selection of the PHA storing biomass in a single reactor was evaluated by altering the percentage of the aerobic versus the total cycle duration. The aerobic reaction duration varied from 17 to 30% (Period 1, days 0 -50) and from 30 to 50% (Period 2, days 51 -108) of the total cycle duration. In configuration 1, the main electron acceptor available during the feast conditions was oxygen, while nitrite was the only electron acceptor present during the famine period. If no additional external carbon source is present, an efficient famine condition under the anoxic environment may occur when enough nitrite is available for the complete utilization of PHA. Thus, the stoichiometry of the process imposes that the ratio of PHA stored (as COD) to the nitrite denitrified should not exceed the stoichiometric value of $[1.72/(1-Y_{HD})]$ ($gCOD_{PHA}/gNO_2$-N), where Y_{HD} is the growth yield of the denitrifying bacteria using storage compounds.

3.1.1 Period 1. At the beginning of period 1, the biomass showed a typical feast and famine response (the duration of the feast phase was approximately 16% of the total cycle duration), although the ammonium conversion to nitrite was 36±13%, producing only 6.5 $mgNO_2$-N/L at the completion of the aerobic phase (Figure 2 a and b). During feast conditions the -qVFA was 96±33 mgCOD/gX h (Figure 5 and Table 2). Despite the relatively high DO concentration during the aerobic phase, within the first 15 days (0-15 days) the nitrification activity of the biomass seems to be negatively affected by the presence of VFA during the

feast phase, since the sAUR decreased from the initial value of 15-18 mgN/gX h (measured in the inoculum) to 4.18±1.93 mgN/gX h. This is likely due to the faster biomass growth rate of VFA consuming heterotrophic organisms as compared to nitrifying autotrophs (Henze et al. 2000). As a consequence, the ratio between the PHA stored (COD based) and the nitrite concentration at the beginning of the anoxic phase was 15.5 $gCOD/gNO_2$-N, which was too high to allow for complete PHA degradation under famine conditions. The lack of nitrite available under anoxic conditions resulted in poor nitrogen removal efficiency for this period (Table 2). Under denitrifying famine conditions, the efficiency of PHA consumption was not more than 41±1% due to the shortage of nitrite as electron acceptor (Figure 5) and the PHA accumulated in the selection reactor up to 0.21 $gCOD_{PHA}/gCOD_X$ (Figure 2b). Maintaining efficient famine conditions where the stored PHA is consumed has been found to be of high importance in achieving a selected culture with a high PHA storage capacity[24].

During the first period, the –qVFA decreased down to 4 mgCOD/gX h (day 28, Figure 5) and the $Y_{PHA/VFA}$ from 0.31 to 0.07$gCOD/gCOD_{VFA}$ (Figure 5). To promote the growth of the PHA storing bacteria during the anoxic/famine conditions, the ammonium conversion to nitrite was enhanced by doubling the duration of the aerobic phase, while maintaining constant the applied vNLR and the vOLR (days 29 to 50). The amount of nitrite increased up to 26 $mgNO_2$-N/L and the nitrogen removal efficiency was 70.1%. The higher duration of the aerobic phase favoured the degradation of the PHA up to 66% (Figure 5, day 39) under famine conditions, due to the more abundant presence of electron acceptors. However, it was found that ~30% of the previously stored PHA was consumed under aerobic/famine conditions before the anoxic-famine period initiated. The increase in the aerobic period resulted in an aeration time that exceeded the feast phase duration for the applied vOLR, leading to some aerobic PHA degradation. The PHA content at the end of the anoxic famine conditions was constantly below 0.01 gCOD/gCOD. Furthermore, at the end of period 1

(days 29-50), the –qVFA increased together with the $Y_{PHA/VFA}$ up to 108±18 mgCOD/gX h and 230±150 $mgCOD_{PHA}/gCOD_{VFA}$ (Figure 5), respectively.

3.1.2 Period 2. Once the feast and famine cycle and the nitrogen removal efficiency showed a steady profile, the vNLR was increased to 0.38±0.03 kgN/m^3d in order to enhance the treatment capacity of the system, while maintaining constant the vOLR. The duration of the aerobic reaction phase was slightly altered during this period in order to ensure sufficient conversion of ammonium to nitrite and to control the COD_{PHA}/NO_2-N ratio between 2.0 and 2.2 $gCOD/gNO_2$-N when the anoxic/famine condition took place. More specifically, aerobic conditions were maintained for 45±12% of the total cycle duration. The sAUR slightly increased up to 5.5±0.5 mgN/gX h and within 100-150 min of aerobic conditions, the nitrite concentration was 32 mgN/L. As result, a stable feast-famine regime was established during days 51-108 (Figure 3 a and b). Under aerobic conditions, the average –qVFA was 136±29 mgCOD/gX h (Figure 5), resulting in the decrease of the ratio of the feast to the total cycle length from 18-19% (period 1) to 14-15% (period 2). The higher capacity of the biomass to store PHA was confirmed by the $Y_{PHA/VFA}$, which increased from 230±15 (period 1) to 371±15 (period 2) $mgCOD_{PHA}/gCOD_{VFA}$ (Figure 5). Furthermore, 72±16% of the PHA was degraded during the aerobic and anoxic famine conditions (Figure 5). Although the denitritation efficiency of the denitrificable nitrogen was always higher than 94%, the average nitrogen removal in the same period was low (48.8±3.4%, average value, see SI) since a significant residual ammonium concentration remained. Thus, an alternate process configuration (Configuration 2) was studied in order to increase nitrogen removal and augment PHA production further.

(Figure 2a and 2b)

(Figure 3a and 3b)

(Figure 4a and 4b)

(Figure 5)

3.2 Efficiency of the configuration with two separate reactors

To cope with the residual ammonium concentration, configuration 2 applied a two stage process for nitritation and selection of PHA storing biomass. Ammonium was first oxidized in the N-SBR (Figure 1b) and then the liquid was fed in the selection reactor when the anoxic reaction phase started. Figure 4 (a and b) shows the typical profiles of nitrogen, PHA and VFA concentration that were obtained in the selection SBR after adopting configuration 2 (day 108 to 150). The VFA were completely depleted within the first 30 min of the aerobic phase, resulting in a –qVFA of 182±14mgCOD/gX h (Figure 5). Under feast conditions, the PHA of the biomass rapidly increased at a qPHA of 89±7 mgCOD/gX h, which is higher compared to the respective value of configuration 1. The $Y_{PHA/VFA}$ increased as well, from 371±15 to 433±10 $mgCOD_{PHA}/gCOD_{VFA}$ (Figure 5). After 50 min of aerobic phase, the anoxic-famine phase started by switching off the blower and feeding the effluent of the N-SBR. In configuration 2, nitrite was the only electron acceptor for the PHA degradation for denitritation during the famine conditions. Furthermore, this strategy allowed a better control of the applied COD/NO_2-N, avoiding nitrite limitation in the famine phase and enabling higher nitrogen removal efficiency (Table 2). Figure 4(a) shows the increase of the nitrite content during the anoxic phase (up to 75 mgNO_2-N/L). During the famine conditions, the PHA was consumed, reaching a minimum concentration of 0.006 $gCOD_{PHA}$/gCOD at the end of the anoxic cycle. This fact indicates that almost all the stored PHAs were degraded. The PHA consumption efficiency was indeed higher when compared with that of configuration 1 (83±4%, Figure 5). Additionally, during the anoxic phase, the ammonium nitrogen decreased from 28 to 21 mgN/L; this reduction was correlated with the growth of the PHA storing

bacteria. The rate of PHA degradation during the famine phase is independent of the type of electron acceptor present[16]. The famine (anoxic) duration and the carbon stored were enough to achieve almost 90% of denitritation. The nitrite at the end of the cycle decreased to 15 mgNO_2-N/L. However, the nitrite concentration in the effluent was 7-10 mgNO_2-N/L, indicating that denitritation occurred during the sedimentation but without any rising of sludge. This was confirmed by the low content of solids in the effluent (<15 mg/L).

Configuration 2 is advantageous compared to configuration 1, since it enhances the overall nitrogen removal efficiency up to 79±4.4%, when applying a vNLR of 0.53±0.11 kgN/m^3d. Furthermore, the PHA production rate and yield per VFA were enhanced. Coupling side-stream nitritation/denitritation processes with PHA production through mixed microbial cultures has the potential to be economically advantageous, since recovery of a valuable resource can be incorporated into the wastewater treatment plant, while reducing nutrient loads to the head of the plant, reducing operational costs in the mainstream. Furthermore, the implementation of anoxic conditions during the famine phase of PHA production systems by mixed cultures is a useful means of saving aeration energy.

(Table 2)

3.3. PHA accumulation

The PHA storage capacity gradually increased during the overall experimental period, as a confirmation of a good acclimation response of the selected biomass. In this work, the maximal capacity of biomass to store PHA was examined under the operation of configuration 2, when better PHA productivity was obtained. After 8 hours, the biomass was able to accumulate up to 19±2%, 21±5% and 41±4% (gPHA/gVSS x100) when sewage sludge fermentation liquid (COD:N:P = 100:9.7:2.1, no N or P limitation), primary sludge fermentation liquid with wollastonite (COD:N:P = 100:7.8:0.06, P- limited), and a synthetic

mixture of VFA (COD:N:P = 100:0:0, N and P-limited) were fed, respectively (Table 3). The results were in the same range with other authors which used fermentation liquid from sewage sludge[14]. The carbon source was also used as substrate for biomass growth. Despite the fact that the synthetic mixture of VFAs presented a favourable ratio of COD:N:P to limit the growth of new bacteria, the yield of active biomass per substrate consumed varied between 0.20 and 0.28 gCOD/gCOD. This was due to the nitrogen and phosphorus present in the liquor + biomass withdrawn from the selection reactor for the accumulation tests, lowering the COD driven for PHA production. Limiting further the presence of nutrients during the accumulation step would be of interest for process optimisation purposes in future research in order to maximise PHA productivity.

The biopolymers that were produced with the WSFL and SFL had similar characteristics in terms of 3HV and 3HB percentage (Table 3). With the use of the synthetic mixture of VFAs as carbon source, the biopolymer was composed of 35% HB and 65% of HV and HH. This correlates well with the VFA profile added during each test, where the synthetic VFA mixture contained a higher fraction of HV precursors (Table 3).

(Table 3)

Characterisation of the recovered PHA supports their applicability for thermoplastic processing. The analyses revealed that the biopolymers were composed of long molecular chains, with a similar molecular weight varying between $6x10^5$ and $8x10^5$ g/mol and also a similar chain length distribution (PDI 1.22-1.35). In general, the low crystallinity in combination with a low Tg (between -1.1 to -0.5°C) indicate biopolymers with amorphous characteristics[14]. Overall, the biopolymer characteristics observed were in the same range as observed in other studies[14,24].

(Table 4)

370 **3.4 Aeration demand of the novel process and future perspectives**

371 The experimental results acquired in this study were used to estimate the potential aeration
372 savings (thus, decrease in energy costs) when the process for PHA production is integrated in
373 the side stream nitrogen removal via nitrite from the anaerobic supernatant. The calculations
374 are reported in detail in the SI (Table S5). The production of 1 kg of PHA with 60% HB and
375 40% HV, required the equivalent of 3.7 kgCOD of VFA, which are partially oxidized (1.3
376 kgCOD) and partially used to grow new bacterial cells (0.9 kgCOD, $Y_{X/VFA}$=0.25
377 kgCOD/kgCOD) during the accumulation stage. The amount of oxygen needed during the
378 accumulation can be estimated to be 1.7 kgO_2 considering that 1.3 kgO_2 is required for every
379 kg of COD that is oxidized[25]. The percentage of PHA in the biomass could achieve 35%
380 (gPHA/gVSS), thus 2.6 kg of active biomass should be produced during the feast/famine
381 stage by providing 10.2 kg of COD (VFA). The oxygen required for the selection stage is the
382 oxygen provided only during the aerobic feast phase, which represents 20% of the reaction
383 phase duration of the SBR. The oxygen estimated during the aerobic feast phase is 4.6 kg of
384 O_2, meaning that the overall process required 6.4 kgO_2 for 1 kg of PHA. Compared with the
385 conventional aerobic PHA production (selection and accumulation phase), the aerobic and
386 anoxic feast/famine selection plus aerobic accumulation process could save approximately
387 58% of the oxygen requirement.
388 In the current work, the biological nitrogen removal via nitrite was integrated with the
389 selection of PHA storing biomass in the sludge treatment line. The integration of PHA
390 production within a WWTP at full scale was the driving force for the development of our
391 novel treatment scheme. A twofold objective is achieved in the novel process that is
392 proposed; enhanced selection of PHA stored biomass and wastewater and reject water
393 treatment for nitrogen removal. Thus, the examined process provides true added value
394 towards the effective treatment of nitrogen in highly contaminated effluents within WWTPs,

aiming at the same time to maximize resource recovery through the polymer production, which could enhance the sustainability of the WWTP.

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4 References

1. Madkour, M. H.; Heinrich, D.; Alghamdi, M. A.; Shabbaj, I. I.; Steinbüchel, A., PHA Recovery from Biomass. *Biomacromolecules* **2013,** 14, 2963−2972.

2. Yu, J.; Chen, L. X. L., The Greenhouse Gas Emissions and Fossil Energy Requirement of Bioplastics from Cradle to Gate of a Biomass Refinery. *Environ. Sci. Technol.* **2008,** 42, 6961–6966.

3. Bengtsson, S.; Pisco, A. R.; Johansson, P.; Lemos, P. C.; Reis, M. A. M., Molecular weight and thermal properties of polyhydroxyalkanoates produced from fermented sugar molasses by open mixed cultures. *J. Biotechnol.* **2010,** 147 (3), 172-179.

4. Dionisi, D.; Caruccia, G.; Petrangeli Papinia, M.; Riccardi, C.; Majone, M.; Carrasco, F., Olive oil mill effluents as a feedstock for production of biodegradable polymers. *Water Res.* **2005,** 39, 2076 –2084.

5. Yuan, H.; Chen, Y.; Zhang, H.; Jiang, S.; Zhou, Qi.; Gu, G. Improved Bioproduction of Short-Chain Fatty Acids (SCFAs) from Excess Sludge under Alkaline Conditions. Environ. Sci. Technol., 2006, 40 (6), pp 2025–2029.

6. Braunegg, G.; Bona, R.; Koller, M., Sustainable polymer production. *Polymer-Plastics Technol. Eng.* **2004,** 43 (6), 1779–1793.

7. Research Watch. *Environ. Sci. Tecnol.* **1996**, 30 (9), 1996.

8. Tamis, J.; Marang, L.; Jiang, Y.; van Loosdrecht, M. C. M.; Kleerebezem, R., Modeling PHA-producing microbial enrichment cultures—towards a generalized model with predictive power. *New Biotechnol*. 2014, 31 (4).

9. Dionisi, D.; Majone, M.; Tandoi, V.; Beccari, M., Sequencing batch reactor: influence of periodic operation on performance of activated sludge in biological a astewater treatment. *Ind. Eng. Chem. Res.* **2001,** 40, 5110– 5119.

10. Johnson, K.; Kleerebezem, R.; van Loosdrecht, M. C. M., Influence of the C/N ratio on the performance of polyhydroxybutyrate (PHB) producing sequencing batch reactors at short SRTs. *Water Res.* **2010,** 44, 2141–2152.

11. Meesters, K. H. P. *Production of poly (3 hydroxyalkanoates) from waste streams*; Technical University of Delft: Delft, **1998**.

12. Ju, J.; Si, Y. A Dynamic Study and Modeling of the Formation of Polyhydroxyalkanoates Combined with Treatment of High Strength Wastewater. *Environ. Sci. Technol.* **2001**, 35, 3584-3588.

13. Jiang, Y.; Chen, Y.; Zheng, X., Efficient polyhydroxyalkanoates production from a waste-activated sludge alkaline fermentation liquid by activated sludge submitted to the aerobic feeding and discharge process. Environ. Sci. Technol. 2009, 43, 7734-7741.

14. Morgan-Sagastume, F.; Karlsson, A.; Johansson, P.; Pratt, S.; Boon, N.; Lant, P.; Werker, A., Production of polyhydroxyalkanoates in open, mixed cultures from a waste sludge stream containing high levels of soluble organics, nitrogen and phosphorus. *Water Res.* **2010**, 5196-5211.

15. Pittmann, T., Steinmetz, H. Polyhydroxyalkanoate production as a side stream process on a municipal waste water treatment plant. *Biores. Technol.* **2014**, 167, 297–302

16. Morgan-Sagastume, F.; Valentino, F.; Hjort, M.; Cirne, D.; Karabegovic, L.; Gerardin, F.; Johansson, P.; Karlsson, A.; Magnusson, P.; Alexandersson, T.; Bengtsson, S.; Majone,

445 M.; Werker, A., Polyhydroxyalkanoate (PHA) production from sludge and municipal
446 wastewater treatment. Water Sci. Technol. 2014, 69, 177-184.
447 17. Anterrieu, S., Quadri, L., Geurkink, B., Dinkla, I., Bengtsson, S., Arcos-Hernandez,
448 M., Alexandersson, T., Morgan-Sagastume, F., Karlsson, A., Hjort, M., Karabegovic, L.,
449 Magnusson, P., Johansson, P., Christensson, M., Werker A. Integration of biopolymer
450 production with process water treatment at a sugar factory. *N Biotechnol.* **2014,** 31(4), 308-
451 323; DOI: 10.1016/j.nbt.2013.11.008.
452 18. Cervantes, F.J. *Environmental Technologies to Treat Nitrogen Pollution*. IWA
453 Publishing, London, 2009.
454 19. Frison, N.; Katsou, E.; Malamis, S.; Oehmen, A.; Fatone, F., Nutrient removal via
455 nitrite from reject water and polyhydroxyalkanoate (PHA) storage during nitrifying
456 conditions. *J. Chem. Technol. Biotechnol.* **2014;** DOI 10.1002/jctb.4487
457 20. Longo, S., Katsou, E., Malamis, S., Frison, N., Renzi, D., Fatone, F. Recovery of
458 volatile fatty acids from fermentation of sewage sludge in municipal wastewater treatment
459 plants. *Biores. Technol.* **2015,** 175, 436–444.
460 21. Carrera, J., Vicent, T., Lafuente, F.J. Influence of temperature on denitrification of an
461 industrial high-strength nitrogen wastewater in a two-sludge system. *Wat. SA*. **2003,** 29, 11–
462 16.
463 22. APHA, Standard Methods for the Examination of Water and Wastewater, 20th ed.;
464 American Public Health Association: Washington, DC, 1998.
465 23. Lanham, A.B., Ricardo, A.R., Albuquerque, M.G.E., Pardelha, F, Carvalheira, M.,
466 Coma, M., Fradinho, J., Carvalho, G., Oehmen, A., Reis, M.A.M. Determination of the
467 extraction kinetics for the quantification of polyhydroxyalkanoate monomers in mixed
468 microbial systems. *Proc. Bioch.* **2013**, 48, 1626–1634.

469 24. Albuquerque, M.G.E., Torres, C.A.V., Reis, M.A.M., Polyhydroxyalkanoate (PHA)
470 production by a mixed microbial culture using sugar molasses: Effect of the influent substrate
471 concentration on culture selection. *Wat. Res.* **2010**, 44, 3419-343318.
472 25. Gurieff, N,; Lant, P., Comparative life cycle assessment and financial analyses of
473 mixed culture polyhydroxyalkanoate production. *Bioresour. Technol.* **2007**, 3393-3403.
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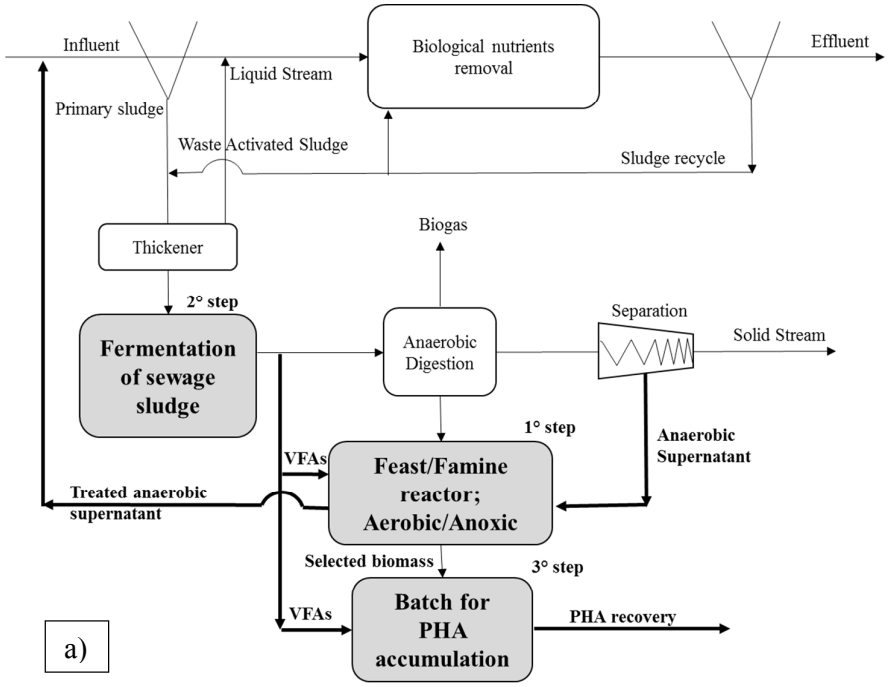
475



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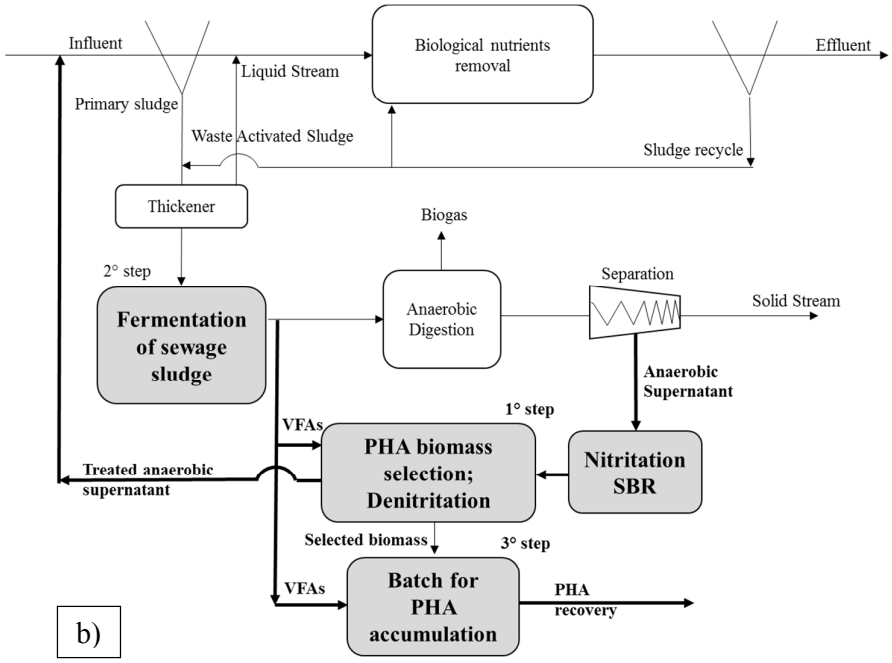

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478 **Figure 1.** Configurations for the combined selection of PHA storing biomass and nitrogen
479 removal from sludge reject water by applying (a) the aerobic/anoxic feast/famine regime and
480 nitrogen removal via nitrite in a single stage reactor and (b) a two-stage process of nitritation
481 (first reactor for the production of electron acceptor) followed by an aerobic/anoxic
482 feast/famine selection reactor.

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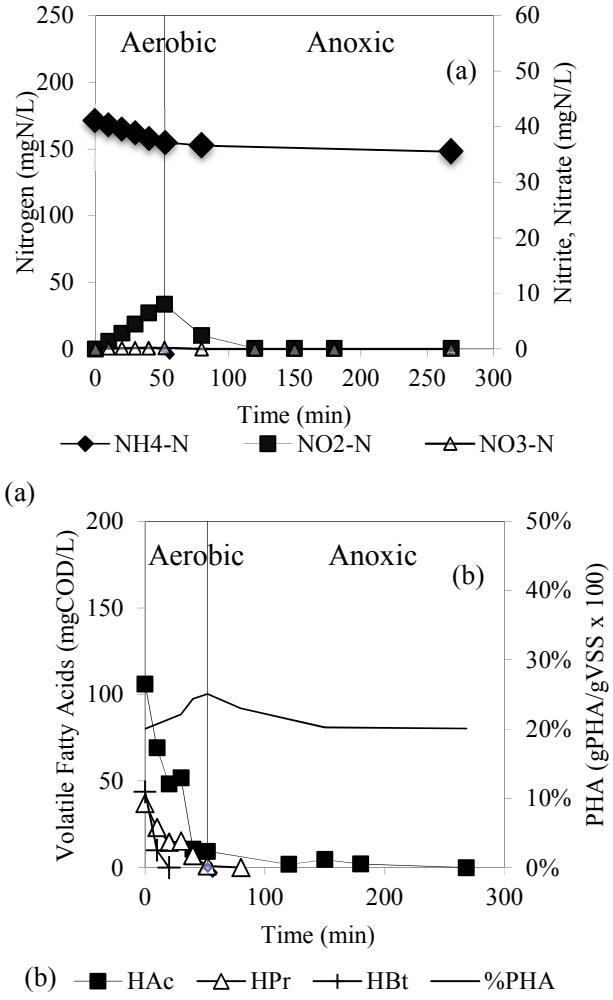

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486 **Figure 2.** Typical profiles of (a) nitrogen and (b) VFAs and PHA profile during the feast and

487 famine phase of the SBR for period 1 of configuration 1.

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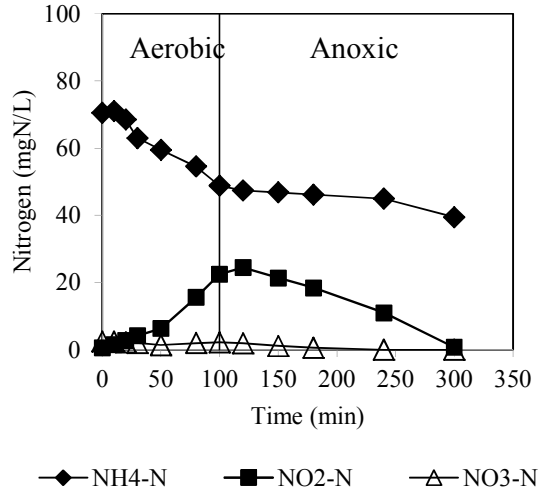
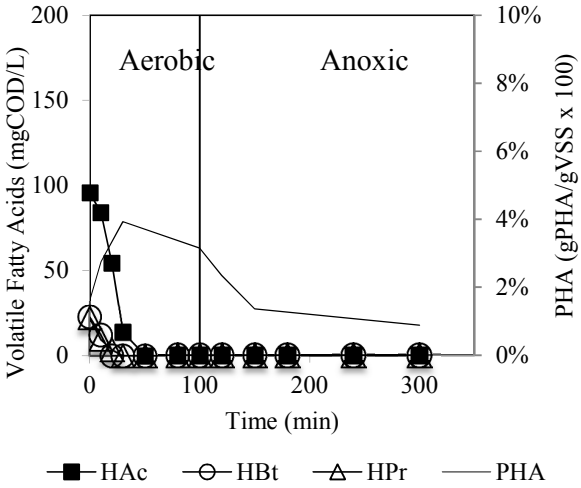
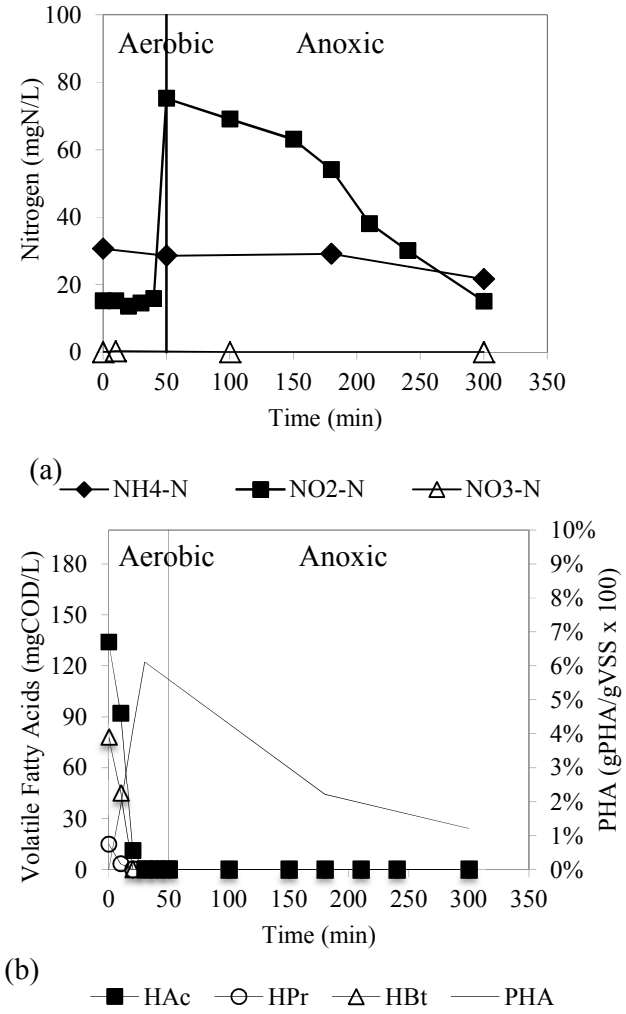


(a)



(b

Figure 3. Typical profiles of (a) nitrogen and (b) VFAs and PHA during the feast and famine phase in the selection reactor during period 2 of configuration 1.



(a)

(b)

Figure 4. Typical profiles of (a) nitrogen and (b) VFAs and PHA during a cycle of the selection SBR in configuration 2.

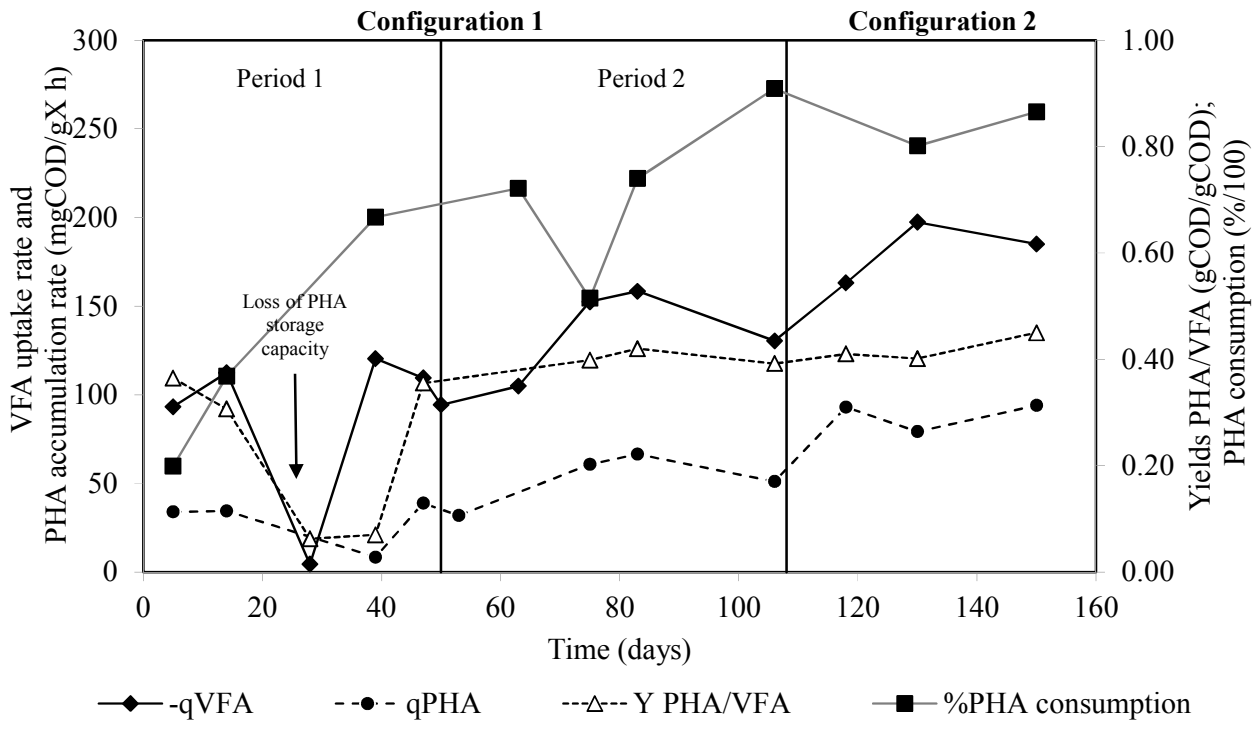


Figure 5. –qVFA, qPHA, $Y_{PHA/VFA}$ and efficiency of PHA consumption during the overall experimental periods of configurations 1 and 2

504 **Table 1**. Operating conditions for the 'selection' SBR that was applied for the examined
505 configurations.

Parameter	Configuration 1		Configuration 2
	Period 1 (days 0-50)	Period 2 (days 51-108)	Period 3(days 109-138)
vNLR (gN/m^3d)	110±27	380±29	530±11
OLR ($gCOD/m^3d$)	623±55	760±43	1166±106
COD/N ($gCOD_{VFA}$:gNH_4-N)	5.6±0.06	2.0±0.02	2.2±0.1
F/M (gCOD:gX)	0.33±0.08	0.22±0.05	0.20±0.07
Aerobic reaction time/Anoxic reaction time	0.30±0.14	0.73±0.37	0.20

506
507
508 **Table 2.** Performance of configuration 1 & 2 for PHA biomass enrichment; –qVFA: VFA
509 uptake rate, qPHA, PHA accumulation rate, $Y_{PHA/VFA}$, storage PHA yield based on the VFA.
510 (*E_{nn}: nitrifying efficiency upon the nitrificable incoming nitrogen;**E_{dd}: the denitrifying
511 nitrogen efficiency upon the denitrificable nitrogen).

Performance	Configuration 1		Configuration 2
	Period 1	Period 2	
Enn(%)*	39	49	87
Edd(%)**	94	97	89
-qVFA(mgCOD/gX h)	96±33	136±29	182±14
qPHA(mgCOD/gX h)	36±0.2	53±9	89±7
$Y_{PHA/VFA}$($mgCOD_{PHA}/gCOD_{VFA}$)	230±15	371±15	437±40

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514 **Table 3.** Performance of the batch PHA accumulation using different types of carbon source.

Parameter	Synthetic mixture of VFA	WSFL	SFL
Duration of accumulation	8.5	8.5	8.5
COD(VFA):NH_4-N:PO_4-P	100:0:0	100:7.8:0.06	100:9.7:2.1
Initial/Final* NH_4-N (mgN/L)	35.7/27.2	20.1/185.5	35.2/146.5
Initial/Final* PO_4-P (mgP/L)	12.5/8.4	11.6/8.1	25.4/45.3
%PHAs (gPHA/gVSS x 100)	44±5%	21±2%	19±2%
HAc/HPr (gCOD/gCOD)	1.4	1.1	1.1
HV (%)	65 (HV+HH)	41	42
$Y_{PHA/VFA}$(gCOD/gCOD)	0.46±0.06	0.40±0.04	0.40±0.04
$Y_{X/VFA}$ (gCOD/gCOD)	0.26±0.02	0.25±0.09	0.23±0.06

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516 **Table 4.** Main properties of the biopolymers obtained with the different carbon sources after

517 the accumulation tests.

Carbon source	Mw (g/mol)	PDI (Mw/Mn)	Tg (°C)	T_{m1} (°C)	T_{m2} (°C)	ΔHm (J/g)	$T_{d\text{-}trans}$ (°C)
Synthetic mixture of VFA	$6.2x10^5$	1.30	-1.1	138	147	21	267
SFL	$6.5x10^5$	1.29	-0.5	136	144	24	275
WSFL	$7.4x10^5$	1.25	-1.6	141	153	27	276

518 Mw: average molecular weight, PDI: polydispersity index, Mn: molar number, Td-trans:
519 decomposition temperature (DSC analyses), Tg: glass-transition temperature, Tm: melting
520 temperature, ΔHm: melting enthalpy

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