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THE METABOLIC VERSATILITY OF PAOs AS AN OPORTUNITY TO OBTAIN A HIGHLY P-ENRICHED STREAM FOR FURTHER P-RECOVERY

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ABSTRACT

The effects of two sequencing batch reactor operation strategies for phosphorus stream enrichment over the biological phosphorus removal performance have been studied. The objective of both strategies is of performing an extraction cycle in order to obtain a new stream highly enriched with phosphorus. In the 1st strategy the amount of influent volatile fatty acids (VFAs) is the same in each cycle; while in the 2nd strategy the influent VFAs concentration is increased during phosphorus extraction experiments. Despite the strong decrease of the stored poly-P inside the cells in both strategies after the recovery cycles, the ability of the systems to remove phosphorus was not affected. The $P_{release}/HAc_{uptake}$ ratio (changing from 0.73 to 0.21 mmol P $mmolC^{-1}$) together with FISH analyses (around 85% of *Accumulibacter* through the experimental period) confirmed that a shift from PAM to GAM occurred after phosphorus enrichment in the 2^{nd} strategy experiments. These results suggest that energy required for VFA uptake by polyphosphate-accumulating organisms (PAOs) was not only derived from polyphosphates degradation, but also from glycogen degradation. FISH also revealed that Type II *Accumulibacter* species are responsible of the metabolic shift. The strategy based on increasing influent VFAs concentration during phosphorus extraction experiments showed a higher extraction efficiency (from 46 to 76%), as higher phosphorus concentration within supernatant can be achieved (from 113.9 to 198.7 mg P l^{-1}). Following this strategy, it is possible to concentrate up to 81% of the incoming phosphorus in a single enriched stream. This suggests that, despite the extra addition of carbon source needed (9%), this strategy is more appropriate if phosphorus recovery for reuse purposes is required.

KEYWORDS

35 Phosphorus recovery, polyphosphate (poly-P), polyphosphate accumulating metabolism (PAM),
36 glycogen accumulating metabolism (GAM), enhanced biological phosphorus removal (EBPR),
37 sequencing batch reactor (SBR).
38
39 **1. INTRODUCTION**
40 Phosphorus (P) is essential for all life and is a key element in fertilizers to increase crop yields not
41 existing any other component that can substitute P in food production. Yet the world's main source
42 of P (phosphate rock) is non-renewable and is becoming increasingly scarce and expensive.
43 Phosphorus peak was estimated to occur by 2035, after which its demand would outstrip supply
44 (Cordell *et al*., 2009).
45
46 Approximately 17% of the total P in phosphate rock mined specifically for food production is lost
47 in human excreta via wastewater (approximately 3 million tonnes of elemental phosphorus per
48 year), which should be removed before its final disposal in inland and coastal waters in order to
49 prevent eutrophication (Cordell 2010). The classical biological P removal technology (Mino *et al*.,
50 1998) currently is presented as an opportunity not only for P removal but also for P recovery and
51 therefore provides a possible solution for the phosphate rock scarcity in a near future. PAOs are the
52 group of microorganisms primarily responsible for the P removal process. PAOs are equipped with
53 a polyphosphate accumulating metabolism under alternating anaerobic-aerobic/anoxic conditions.
54 Under these operational conditions PAOs are able to internally store the soluble P present in the raw
55 wastewater as polyphosphate (poly-P). All the P-recovery technologies from PAOs pass through a
56 previous P extraction before its final recovery mainly as struvite.
57
58 According to Yuan *et al*. (2012) there are a number of ways to recover P from sludge including: (a)
59 direct application of dewatered biosolids to the soil; (b) release of P from Enhanced Biological
60 Phosphorus Removal (EBPR) sludge by biological methods followed by recovery through chemical

methods; and (c) release of P from EBPR sludge by thermal-chemical methods, followed by either utilization of residue, or further processing for recovery. However, these methods are focused on recovering P in the sludge line of the wastewater treatment plants (WWTP) after the anaerobic digestion. This operation mode entails some disadvantages such as not being able to prevent an uncontrolled precipitation inside the digester and the downstream sludge management devices due to an important P release and pH increase during digestion (Doyle and Parsons, 2002). Other processes based on P recovery in the water line are the Phostrip process (Levin and Salla, 1987) and the BCFS process (van Loosdrecht *et al*., 1998). The Phostrip and BCFS process are technologies that besides achieving the phosphate effluent standards also recovers P from wastewater in the water line. However, this technology requires a phosphate stripping and further separation in a specific settler. One of the bottlenecks of this technology is that the phosphate concentration achieved in the stripper stream is not high enough to assure a high P recovery efficiency in a later crystallization process (around 25 mgP l^{-1} in the anaerobic phase, see Barat and van Loosdrecht, 2006). Other studies, recently published, studied the feasibility of the P recovery as P enriched stream in the water line using different configurations (Kodera et al., 2013; Shi et al., 2012; Wong et al., 2013; Xia et al., 2014).

On the other hand, different studies (Acevedo et al., 2012; Zhou et al., 2008) showed that the stripping of P in a Sequencing Batch Reactor (SBR) operated for EBPR could provide a highly enriched stream with soluble P after a decanting period and withdrawal at the end of the anaerobic stage. As the key point of these operation mode consists in achieving a very high P extraction during the SBR operation cycle, polyphosphate (poly-P) was expected to reach low level concentrations and therefore to reduce considerably the main PAOs energy source. Under these conditions, traditionally deterioration of P removal process was expected to occur due to the upgrowth of GAOs which use glycogen as energy source instead of poly-P (Oehmen et al., 2007). While GAO metabolism is based on the use of glycolysis to produce ATP for HAc consumption

under anaerobic conditions, PAOs use the hydrolysis of intracellular poly-P and the consequent release of phosphate for the same purpose. However, the same studies (Acevedo et al., 2012; Zhou et al., 2008) demonstrated the versatility of PAOs metabolism when these bacteria are starved for poly-P due to the P extraction. These authors observed that PAOs are able to resist under extreme conditions without poly-P during short periods when this component is removed from the bacteria.

Therefore, a new SBR-EBPR operation performance, consisting on the stripping of P in a SBR operated for EBPR, would provide a P recovery stream with low cost and high potential P recovery.

However, it is necessary to experimentally evaluate this potential P recovery and the effect of this new operation mode over the biological P removal process over time. Therefore, the aim of this paper is to carry out P extraction cycles following two different strategies (keeping influent VFAs concentrations constant or increasing them) with a SBR operated for EBPR, in order to: know the potential P recovery of the proposed new SBR operation mode and to study its effect over the biological P removal performance: process efficiency and microbial population dynamics.

2. MATERIALS AND METHODS

2.1. Experimental device

A laboratory scale SBR (total volume (V_T) of 7l) was operated under anaerobic-aerobic conditions for biological phosphorus removal. The SBR was operated with four 6-h cycle per day: filling period 4 min; anaerobic phase 1.5 h; aerobic phase 3.5 h; settling phase 52 min and withdrawing period 4 min. The phase length during some extraction cycles was modified as will be shown later.

The SBR was equipped with conductivity, ORP, pH, temperature and dissolved oxygen electrodes. The temperature was maintained at 20 °C. Dissolved oxygen (DO) concentration in the aerobic phase was controlled between 1.5 and 2.5 mg O_2 l^{-1}. Initial pH of the cycle was kept around 7.5 and

it was not controlled but did vary from 7 to 8.5 during the different phases of the cycle. Synthetic wastewater was used during the experimental period with a COD/P ratio of 13.3 COD mg P mg^{-1} (100 mg COD l^{-1} and 7.5 mg P l^{-1}). Synthetic wastewater used consisted of two solutions: the first one contained mineral compounds including K_2HPO_4 whilst the other one contained acetate and NH_4Cl (for detailed description of the SBR configuration and wastewater see Barat *et al*., 2008). Allyl-thiourea was added in a concentration of 2 mg l^{-1} in order to inhibit nitrification. The Solid Retention Time (SRT) and Hydraulic Retention Time (HRT) were kept constant around 10 d and 12 h, respectively.

In both strategies, the reactor was seeded with sludge from a real WWTP with biological phosphorus removal by means of an A/O scheme located in Valencia (Spain). The WWTP treated 33785 m^3 per day, and it was operated at a SRT of 10 days. The SBR was operated for approximately two months for each strategy of the study to obtain a sludge enriched in PAO bacteria. Operational conditions during the first strategy were maintained with no change. During some experiments of the second strategy, however, increases in length of anaerobic and aerobic phases were implemented aiming to achieve either complete VFAs uptake and glycogen regeneration.

2.2. Experimental design

During the experiments, an extraction cycle (hereafter known as recovery cycle) was made in order to obtain a new stream highly enriched with P. The different stages of this cycle are shown in Figure 1. The SBR was filled with synthetic wastewater and operated under anaerobic conditions. The sludge was settled in order to obtain an effluent highly enriched in phosphate after achieving complete VFAs uptake and consequently an increase in the amount of soluble phosphate. Then, the

maximum volume of the effluent was extracted and replaced with synthetic wastewater without acetate, following with aerobic conditions. Finally, the sludge was settled and the effluent was discharged.

The study was performed in two periods following different operation strategies:

- Slight and frequently P extraction (around 2 extractions per week): The first one involved adding the same amount of influent VFAs in each cycle (initial concentration in the reactor was equivalent to 100 mg HAc l^{-1} in each experiment). The goal of the experiments was to accomplish simple P extractions so as to obtain supernatant with high P concentration as frequently as possible.
- Strong and less frequently P extraction (1 extraction per week): The second one involved increasing influent VFAs concentration during P extraction experiment (initial concentration in the reactor was equivalent to 150, 250, 350, 350, 350 mg HAc l^{-1} in each experiment). The purpose of the experiments was to accomplish single P extraction in order to maximize P concentration within supernatant produced by the rise in VFAs concentration.

The experimental procedure of the study is presented in Figure 2. During the first strategy, four experiments were accomplished during 15 days. A recovery time of 6 days was necessary after completion of the first experiment due to a failure in temperature control. For the remaining experiments the recovery time was roughly 2 days. In contrast, during the second strategy 5 experiments were completed with a recovery time of approximately 7 days.

2.3. Analytical methods and microbial techniques

Experimental analysis was structured in 3 parts where phosphorus recovery cycle and cycles before and after recovery cycle were studied. A monitoring of the concentrations of VFAs and phosphate was made at least at the start of the cycle, end of anaerobic and aerobic phases. Samples were filtered through a 0.45 μm filter for these analyses. Moreover total phosphorous (P_T), total suspended solids (TSS) and volatile suspended solids (VSS) were also measured at the end of the anaerobic and aerobic phase. Samples for fluorescence in situ hybridization (FISH) were collected during aerobic phases.

VFAs were measured as proposed by Moosbrugger *et al*. (1992) using a Metrhom 716 DMS tritino. Phosphorus analyses were carried out according to Standard Methods (APHA, 2005) using a Lachat QuikChem800 flow injection analyzer. PT, TSS and VSS were performed in accordance with Standard Methods (APHA, 2005).

FISH technique was applied in order to study the population dynamics of PAOs as: PAOmix, Type I PAO and Type II PAO; and GAOs in the reactors. Cell hybridization was performed as described by Amann *et al*. (1990). The rRNA oligonucleotide probes used for FISH are listed in Table 1. Some probe associations were made for covering the adequate ranges: PAOmix (PAO462, PAO651, PAO846), DEFmix (TFO_DF218, TFO_DF618), DEF2mix (DF1020, DF988, H966, H1038) and EUBmix (EUB338, EUB338 II, and EUB338 III). EUBmix probes were labelled with FAM while the rest of the probes were labelled with TAMRA. All probes were used at a 35% formamide concentration. Hybridized cells were enumerated by means of capturing images with an epifluorescence microscope Leica DM2500 and a Leica DFC420c digital camera, using a software for image analysis (Borrás, L, 2008). A minimum of 20 randomly chosen microscopic fields were quantified from each sample. Each of the images was examined to determine the optimum threshold

184 values for each fluorochrome. The countable pixel area of the specific probe-fluorochrome signal
185 (Type I and Type II PAO, PAOmix, GB, DEFmix or DEF2mix probes) was then expressed as a
186 mean percentage of the pixel area count from the EUBmix probe signal. Error of the quantification
187 was calculated by dividing the standard deviation by the square root of "n", where "n" is the
188 number of fields examined.

189

190 Table 1. Oligonucleotide probes used in this study.

191

192 **3. RESULTS AND DISCUSSION**

193 An example of the resulting profiles for conductivity, pH, VFAs and orthophosphates ($P\text{-}PO_4$)
194 obtained for the recovery cycle and the cycles before and after this one during both strategies of the
195 study are shown in Figure 3. As can be seen from these figures, there is a reduction in P after the
196 extraction of supernatant with high P concentration during the recovery cycle. Moreover, extraction
197 itself apparently did not affect the EBPR processes due to the fact that profile's trend of cycles
198 before and after the recovery cycle are similar. When comparing results obtained for both strategies,
199 it was noted that the higher the initial VFAs concentration (100 mgCOD l^{-1} and 350 mgCOD l^{-1} in
200 the 1^{st} and 2^{nd} strategy respectively), the higher P concentration could be obtained and therefore
201 potentially recovered (70 mgP-PO4 l^{-1} and 170 mgP-PO4 l^{-1} in the 1^{st} and 2^{nd} strategy respectively).

202

203 **3.1. Biological process performance**

204 As previously mentioned, the effect of the new SBR-EBPR operation mode over biological process
205 performance has been studied.

206

207 Commonly the $P_{release}/HAc_{uptake}$ ratio is used in order to indicate the presence of poly-P or glycogen
208 accumulating metabolism. In this study, $P_{release}/HAc_{uptake}$ ratio and the evolution of phosphorus

during the experimental phase have been determined to evaluate the biological process performance. Moreover, it is highly interesting to identify and quantify PAOs and GAOs by means of FISH analysis to verify the effect of the P extraction over the microbial population dynamics. These results are shown in the following sections.

3.1.1. Phosphorus analysis

Results for phosphate ($P\text{-}PO_4$), total phosphorus (P_T) and VSS were used to make the phosphorus balance. Organic phosphorus (P_{org}) was estimated as 2% of the VSS (Metcalf and Eddy Inc., 2003). Poly-P in the system was calculated given the value for P_T and then subtracting $P\text{-}PO_4$ and P_{org}. Figure 4 shows trends in P_T, poly-P and $P\text{-}PO_4$ at the end of either anaerobic or aerobic phases during both strategies of the study. As can be seen in Figure 4, P_T concentrations remarkably decreased due to extraction of phosphorus in enriched supernatant during the recovery cycles. In the first strategy, reductions of P_T are constant, while in the second strategy P_T reductions increased along the experiments, as expected by increasing the concentration of HAc. The concentrations of P_T before each extraction remained largely constant during the second strategy but not for the first one due to operational problems (mechanical failures with the electromagnetic valve of the waste sludge). Poly-P concentrations remarkably decreased when the recovery experiments were held (see Table 2). As can be seen, phosphate at the end of aerobic phase remained under 0.5 mg P l^{-1} during both strategies. Hence, the ability of the systems to remove P did not changed with the P extraction. Moreover, variations in poly-P due to extraction in the second strategy of the study were greater than those in the first one. Thus, P extraction was more aggressive during the second strategy. Poly-P concentration values after the extraction during last three experiments of this strategy were found to be less than 10 mg P l^{-1} (almost 0 mg P VSS^{-1}). Furthermore as can be seen in Figure 4b, seven days were enough in the second operation strategy to recover PAOs with poly-P between two consecutive extractions.

3.1.2. Yield for phosphorus release per HAc consumption

Trends in $P_{release}/HAc_{uptake}$ ratio and P-PO_4 concentration at the end of anaerobic phase for both strategies of the study are shown in Figure 5. $P_{release}/HAc_{uptake}$ ratio and P-PO_4 did not change during the experimental phase of the first strategy despite the P extractions. However, in the second strategy, the trend of these parameters was to decrease after each extraction cycle. Then, progressive recovery of these values was achieved during successive days. Acevedo et al., (2012), used the $P_{release}/HAc_{uptake}$ ratio to indicate the variations between PAM and GAM activities. Values between 0.48 and 0.80 mmol P mmol C^{-1} correspond to PAO enriched cultures (Smolders *et al.*, 1995; Kisoglu *et al*., 2000). In contrast, values below 0.02 mmol P mmol C^{-1} are related to GAO enriched cultures. In the first strategy, the ratio was found to be above 0.52 mmol P mmol C^{-1}, thus, PAM was predominant. It is believed that a significant metabolic shift in PAOs did not take place during this strategy due to the fact that poly-P reduction was not high enough after P extraction. Nevertheless, as can be seen in Figure 5b corresponding to the second strategy, values for $P_{release}/HAc_{uptake}$ in the last three experiments decreased from approximately 0.64 mmol P mmol C^{-1} to 0.21 mmol P mmol C^{-1} confirming a clear and quick variation between PAM and GAM. These low ratio values obtained are not due to the presence of GAOs as FISH results showed (discussed later), but to the fact that almost all the poly-P storage was depleted during the extraction.

These observations are in accordance with other studies which showed that PAOs are able to behave like GAOs under different conditions. Zhou et al. (2008) and Acevedo et al. (2012) observed that PAOs could shift from PAM to GAM when poly-P depletion was imposed on the culture. Therefore, findings in the present work suggest that a shift from PAM to GAM occurred after P recovery experiments.

3.1.3. Microbial population dynamics

In order to verify that the P recovery experiments did not favor the growth of GAOs, the microbial dynamics population was studied using FISH. As can be seen in Figure 6, biomass was highly enriched in *Accumulibacter* during experimental phase with approximately 70% of total bacteria in the first strategy and 85% in the second. In the first strategy of this study (Figure 6a), *Competibacter* probes were used to determine percentage of GAO bacteria, whose value fell below 2% of total bacteria. FISH results for the second one (Figure 6b) showed that *Competibacter*, *Defluviicoccus*-cluster 1 and *Defluviicoccus*-cluster 2 were not present.

PAO Type I and Type II clades were also studied during both strategies. It was observed that initial predominant PAO species was Type II in both cases. In the first strategy, variations between Type I and Type II populations were not detected (data not shown), suggesting that slight and frequently P extraction does not affect PAO type population dynamic. Results for the second strategy (Figure 6b) show that there was a change in the population dynamics of *Accumulibacter* species. Percentage for Type I remained constant whereas Type II decreased after the third experiment, which was the first of the series of high-efficiency extractions. Besides, sum of both types of PAOs was found to be lower than the total amount of *Accumulibacter* detected with PAOmix probe.

Previous studies showed an increase in PAO Type II as the poly-P content decreased (Acevedo *et al*., 2012), suggesting that PAO Type II were the main responsible for the metabolic change. In Acevedo *et al*. (2012) the initial biomass at the beginning of the experiments was: a high content of PAO Type I and a low content of PAO Type II (66% and 8% respectively). Under strong P extraction conditions PAO Type II increased up to 36-48% while Type I decreased up to 23%. However, in the present study it was not observed a similar PAO behavior between Type I and Type II, probably because the PAO population distribution at the beginning of the experiments was clearly different between both studies. In the present study the starting point consisted on a biomass with a low content of PAO Type I and a high content of PAO Type II (4% and 75% respectively).

286 Regarding the Type I population, it remains quite stable at low levels during the whole experimental
287 period (below 15%) which agrees with the results obtained by Acevedo et al. (2012), which
288 suggested that the PAO Type I were not involved in the metabolic change at low Poly-P levels. On
289 the other hand, PAO Type II decreased as poly-P content decreased due to the P extraction in the
290 recovery cycles. However despite the PAO Type II reduction over the experiments with strong P
291 extraction, its proportion was quite high (43% in the strongest P extraction) which is in the range of
292 the highest abundances found in Acevedo *et al*. (36-49%). This suggests again, as in Acevedo et al.
293 (2012), that PAO Type II were responsible of the observed metabolic change. But its reduction
294 along the experiments also suggested that a subgroup of PAO within PAO Type II group could be
295 the responsible of the metabolic change while other PAO Type II disappeared as the poly-P content
296 decreased.
297
298 Another aspect to be highlighted is that the sum of both types of PAOs was lower than the total
299 amount of Accumulibacter detected with PAOmix probe specially during the last three experiments
300 with complete poly-P extraction. These results are in accordance with Acevedo et al. (2012) which
301 suggested a growth of other PAO clades not included in the probes used in this study since
302 percentage for PAOmix remained almost constant.
303
304 In conclusion, not only have both extraction strategies of this study proved not to affect P removal,
305 but they also have evidenced not to promote GAO bacteria growth in EBPR system.
306
307 **3.2. Potential P recovery**
308 In order to evaluate the potential P extraction of the proposed new SBR operation mode, process
309 efficiency for both strategies were studied and then compared to each other. Table 2 shows the
310 percentages of P extraction and poly-P reduction followed in this study during both strategies. The
311 average efficiency of extraction for the first strategy was approximately 35% of total P at the start

312 of cycle. During the last three extractions in the second strategy, roughly 75% P was extracted from
313 initial total P. Moreover, poly-P reduction was close to 99% of initial poly-P in this experiments
314 which evidence that phosphorus recovery was maximized (see Table 2).
315
316 Table 2. Efficiency of phosphorus extraction and poly-P reduction during experiments.
317
318 As can be seen in Figure 7, an estimation of the overall result would be that recovery of 59% of
319 incoming P could be achieved when accomplishing two experiments per week following the first
320 strategy. In contrast, when implementing the second strategy, 81% recovery of incoming P in only
321 one experiment could be attained weekly. However, this strategy entails an increment of the carbon
322 source requirements of around 9 % per week. Despite the extra cost of carbon source, the second
323 strategy showed having higher extraction efficiency due to the fact that a higher P concentration
324 within supernatant can be achieved. Consequently, results obtained suggested that this strategy is
325 more appropriate if phosphorus recovery for reuse purposes is required. Nevertheless, further
326 research is needed in order to assess the long-term effect of the P extraction step and the economic
327 feasibility of the second strategy. On the other hand, other authors obtained lower P recovery
328 efficiencies, around 79% (Xia et al., 2014), 60% (Barat and van Loosdrecht, 2006) and 70.2% (Zou
329 et al., 2014) using a side stream system in the water line.
330
331 The next step for the application of this new operation mode in a SBR-WWTP aiming the final P-
332 recovery will imply the treatment of the P enriched stream in a crystallization reactor for P recovery
333 as struvite (Pastor el al. 2004). The struvite precipitation requires the presence of N, P and Mg in a
334 molar ratio of 1:1:1. Despite the high P concentration in the supernatant obtained from the SBR, it
335 will be necessary to increase the N and Mg concentration in order to achieve the optimum molar

ratio for its precipitation. The nitrogen source can be found from other streams present in the WWTP such as the supernatant obtained from the anaerobic sludge process, which is highly enriched in NH_4 and PO_4 also (Pastor et al., 2008). On the other hand, the Mg could be added as an external Mg source such as $MgCl_2$ or seawater (Martí et al., 2010; Rubio-Rincón et al., 2014).

4. CONCLUSIONS

Taking into account the results from this study, it can be concluded that none of the strategies used for P recovery had a negative impact on the biological process performance. Although a deterioration of the P removal process was expected to occur due to the poly-P reduction as well as an upgrowth of GAOs, the ability of the systems to remove P did not changed with the extraction strategies even during the second strategy where P extraction was more aggressive. Microbiological observations confirmed that the low $P_{release}/HAc_{uptake}$ ratios obtained in the second strategy were due to the effect of low amount of poly-P available. This suggests that a shift from PAM to GAM occurred after P recovery experiments as the PAO energy required for the uptake of the VFA was necessarily not only derived from poly-P degradation, but also from glycogen degradation. FISH results for the second strategy also show that there was a change in the population dynamics of Accumulibacter species.

Comparing both strategies for P enrichment, the second one showed to have higher extraction efficiency. Following this strategy it is possible to recover up to 81% of the incoming P per week. Consequently, results obtained suggested that this strategy is more appropriate if P recovery for reuse purposes is required despite the extra cost of carbon source. However, further research is needed in order to assess the long-term effect of the P extraction step and the economic feasibility of the second strategy.

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471 Figure 1. Stages implemented during the phosphorus recovery cycle.

472

473 Figure 2. Experimental design for a) first strategy and b) second strategy of the study.

474

475 Figure 3. VFAs, P-PO_4, pH, and conductivity variations during P recovery cycle and the cycles
476 before and after it during a) the first strategy, b) the second strategy of the study.

477

478 Figure 4. Evolution of phosphorus compounds during a) first strategy and b) second strategy of the
479 study. The arrow indicates the reduction of total phosphorus (-ΔPT) for each recovery cycle.

480

481 Figure 5. Evolution of $P_{release}/HAc_{uptake}$ ratio and phosphate at the end of anaerobic phase for a) the
482 first strategy, b) the second strategy of the study.

483

484 Figure 6. Population dynamics for a) PAOmix and GAOmix during first strategy, b) PAOmix,
485 PAO Type I and PAO Type II during second strategy of the study.

486

487 Figure 7. The weekly phosphate input and output during both strategies.

Table 1

Table 1. Oligonucleotide probes used in this study.

Probe	**Sequence (5' → 3')**	**Specificity**	**Reference**
EUB 338	GCTGCCTCCCGTAGGAGT	*Eubacteria*	Amann *et al.* 1990
EUB 338 II	GCAGCCACCCGTAGGTGT	*Planctomycetes*	Daims *et al.* 1999
EUB 338 III	GCTGCCACCCGTAGGTGT	*Verrucomicrobiales*	Daims *et al.* 1999
PAO 462	CCGTCATCTACWCAGGGTATTAAC	*Rhodocyclus tenuis subgrup*	Crocetti *et al.* 2000
PAO 651	CCCTCTGCCAAACTCCAG	*Candidatus Accumulibacter phosphatis*	Crocetti *et al.* 2000
PAO 846	GTTAGCTACGGCACTAAAAGG	*Rhodocyclus tenuis subgrup*	Crocetti *et al.* 2000
Acc-I-444	CCCAAGCAATTTCTTCCCC	Clade IA and other Type I clades	Flowers *et al*., 2009
Acc-II-444	CCCGTGCAATTTCTTCCCC	Clade IIA, IIC and IID as Type II clades	Flowers *et al*., 2009
GB	CGATCCTCTAGCCCACT	*Gammaproteobacterial group*	Kong *et al*. 2002
TFO_DF218	GAAGCCTTTGCCCCTCAG	*Defluvicoccus*-related (cluster 1)	Wong *et al.* 2004
TFO_DF618	GCCTCACTTGTCTAACCG	*Defluvicoccus*-related (cluster 1)	Wong *et al.* 2004
DF1020	CCGGCCGAACCGACTCCC	*Defluvicoccus*-related (cluster 2)	Meyer *et al.* 2006
DF988	GATACGACGCCCATGTCAAGGG	*Defluvicoccus*-related (cluster 2)	Meyer *et al.* 2006
H966	CTGGTAAGGTTCTGCGCGTTGC	(DF988 helper)	Meyer *et al.* 2006
H1038	AGCAGCCATGCAGCACCTGTGTGGCGT	(DF988 helper)	Meyer *et al.* 2006

Table 2

Table 2. Efficiency of phosphorus extraction and poly-P reduction during experiments.

STRAT.	EXP.	START	END ANAEROBIC			EXTRACTION EFFICIENCY[1]	
		Poly-P (mgP/l)	P-PO4 (mgP/l)	Porg (mgP/l)	Poly-P (mgP/l)	%P EXTRACTED[2]	%poly-P REDUCT.[3]
1	1	145.5	72.4	15.4	81.1	38	37
	2	183.7	67.7	13.7	124.6	29	21
	3	111.5	60.5	14.1	58.6	39	36
	4	132.0	60.3	12.4	71.6	35	31
2	1	187.0	113.9	15.6	80.65	46	57
	2	208.5	162.5	18.2	53.69	59	74
	3	192.0	198.7	23.9	3.74	75	98
	4	185.6	184.8	19.1	8.17	75	96
	5	150.8	156.8	20.4	1.39	76	99

[1]These values are referred to a normalized extraction volume of 85% of the total volume.

[2] $\%\, P_{extracted} = \frac{P\_PO_{4\ end\ anaerobic} \cdot 0.85 \cdot V_T}{P_{T\ end\ anaerobic} \cdot V_T} \cdot 100$, where $P_{T\ end\ anaerobic} = \left(P\_PO_4 + P_{org} + poly\_P\right)_{end\ anaerobic}$

[3] $\%\, poly\_P_{reduction} = \left(1 - \frac{poly\_P_{end\ anaerobic}}{poly\_P_{start}}\right) \cdot 100$

Figure 1

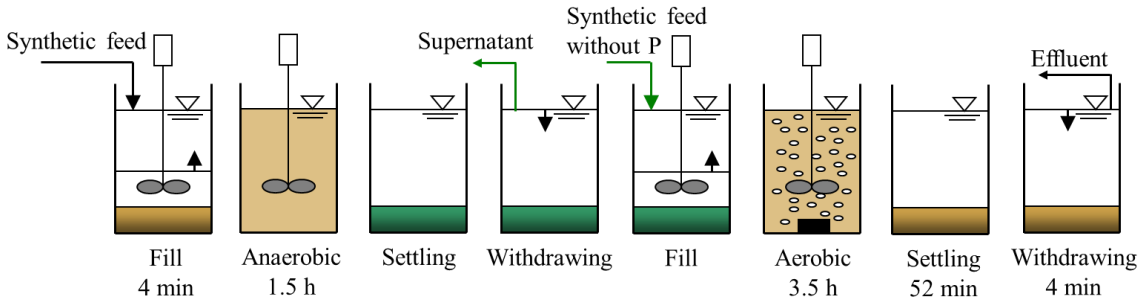

Figure 1. Stages implemented during phosphorus recovery cycle.

Figure 2

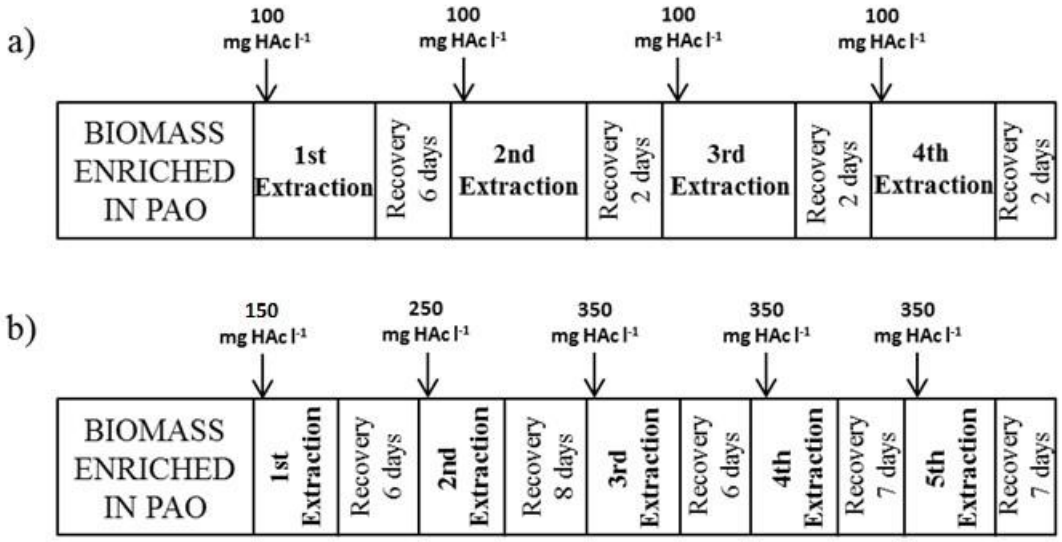

Figure 2. Experimental design for a) first strategy and b) second strategy of the study.

Figure 3

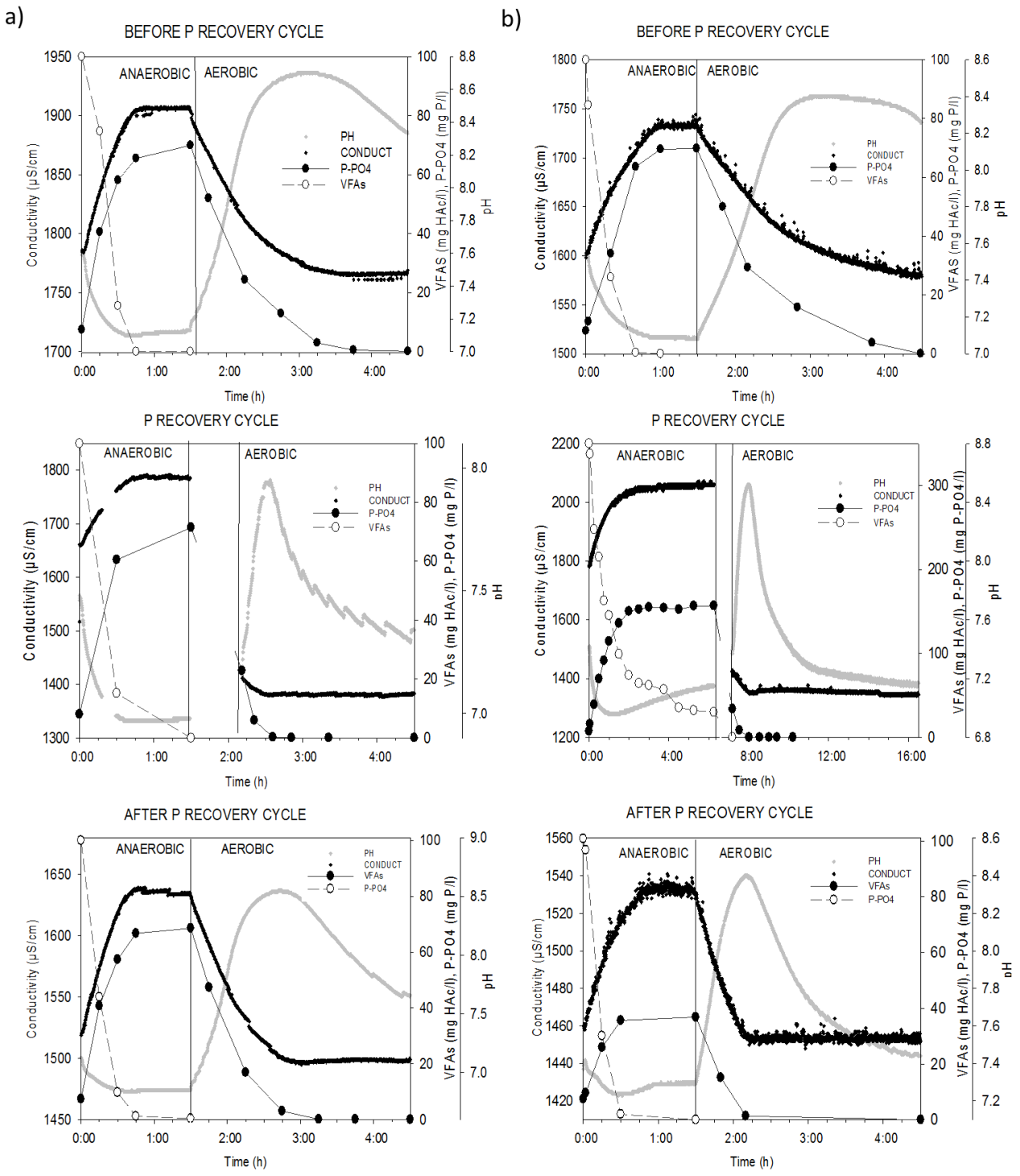

Figure 3. VFAs, P-PO_4, pH, and conductivity variations during P recovery cycle and the cycles before and after this one for a) the first strategy, b) the second strategy of the study.

Figure 4

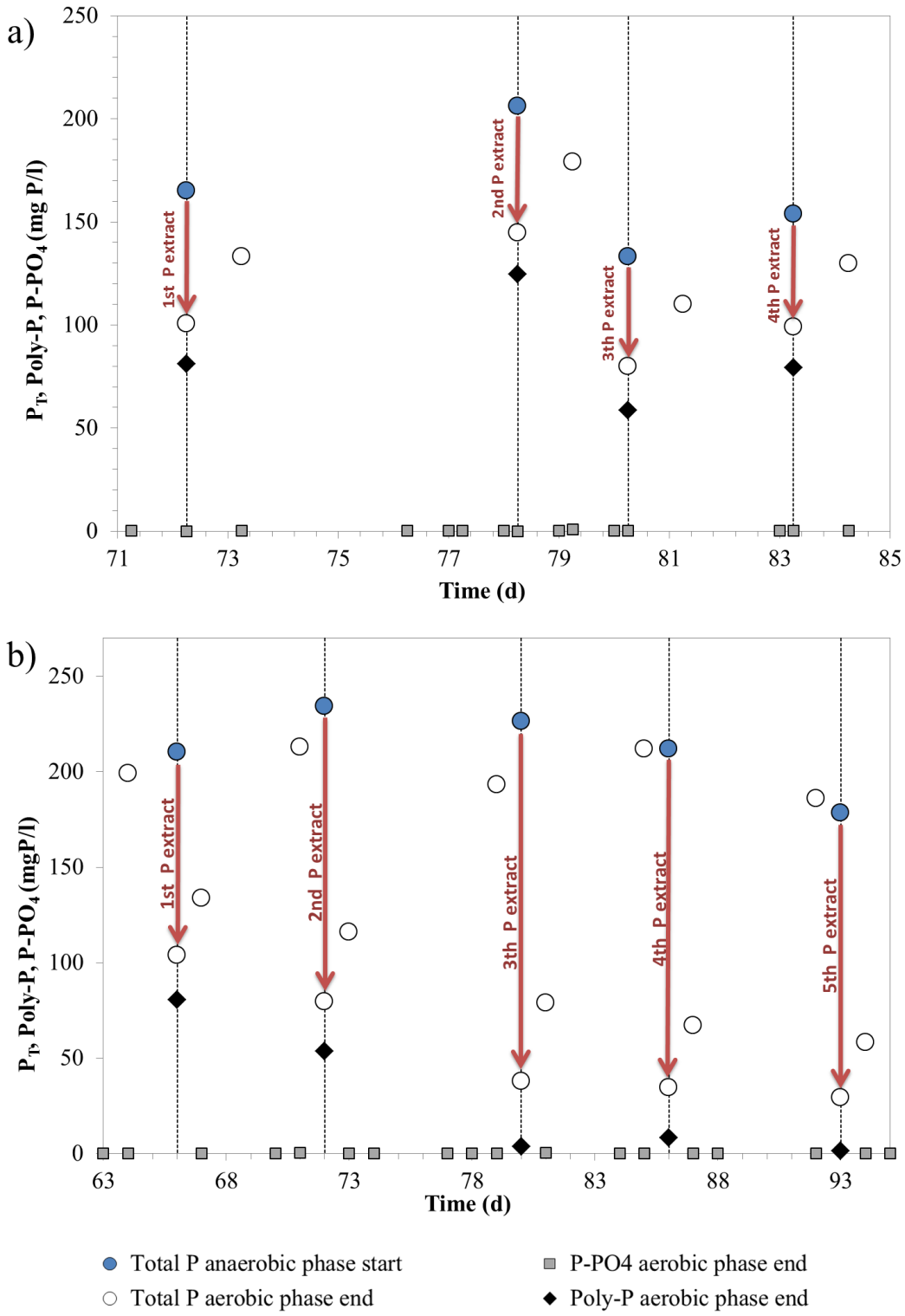

Figure 4. Evolution of phosphorus compounds during a) first strategy and b) second strategy of the study. The arrow indicates the phosphorus recovery (-ΔPT) for each extraction cycle.

Figure 5

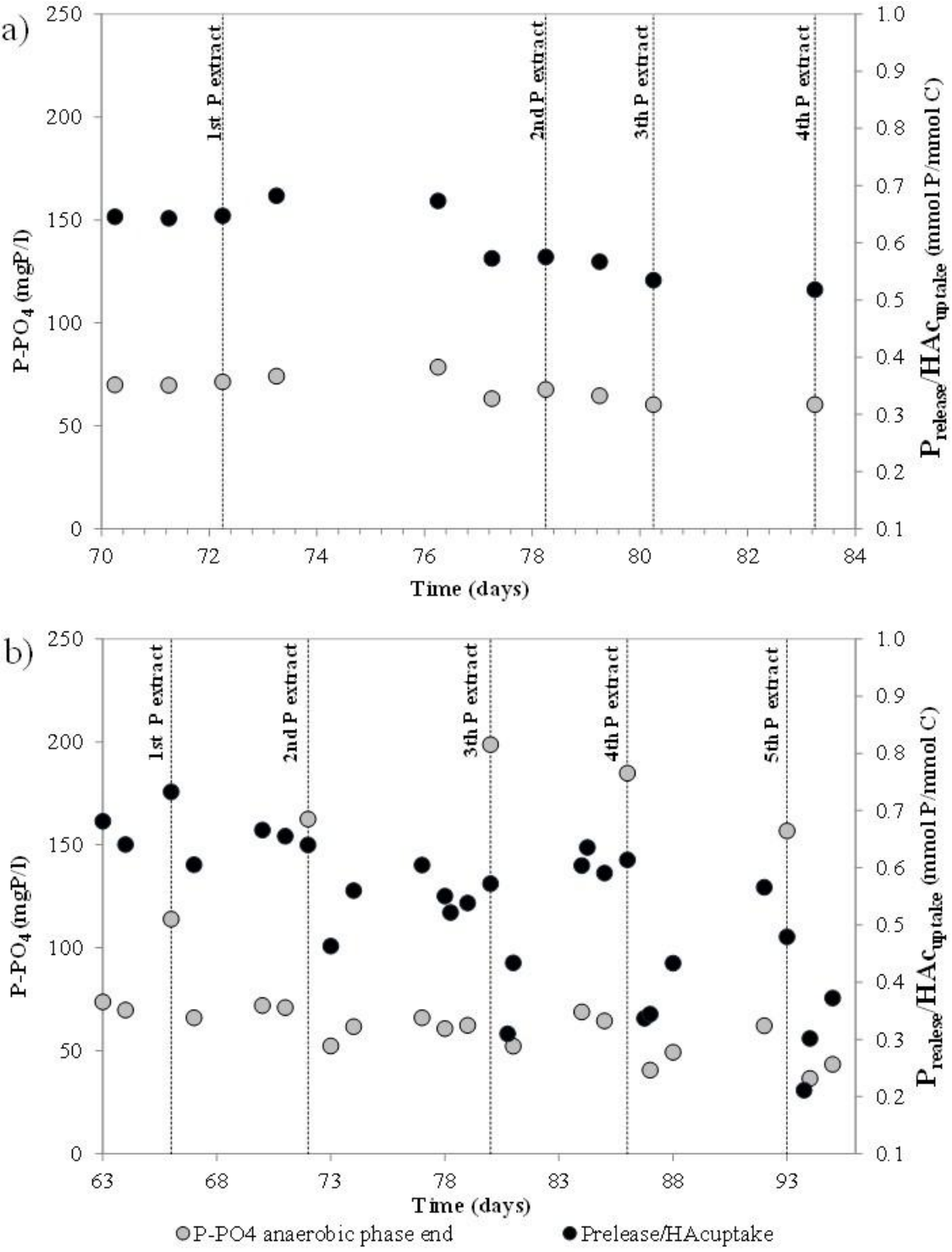

Figure 5. Evolution of $P_{release}/HAc_{uptake}$ ratio and phosphate at the end of anaerobic phase for a) the first strategy, b) the second strategy of the study.

Figure 6

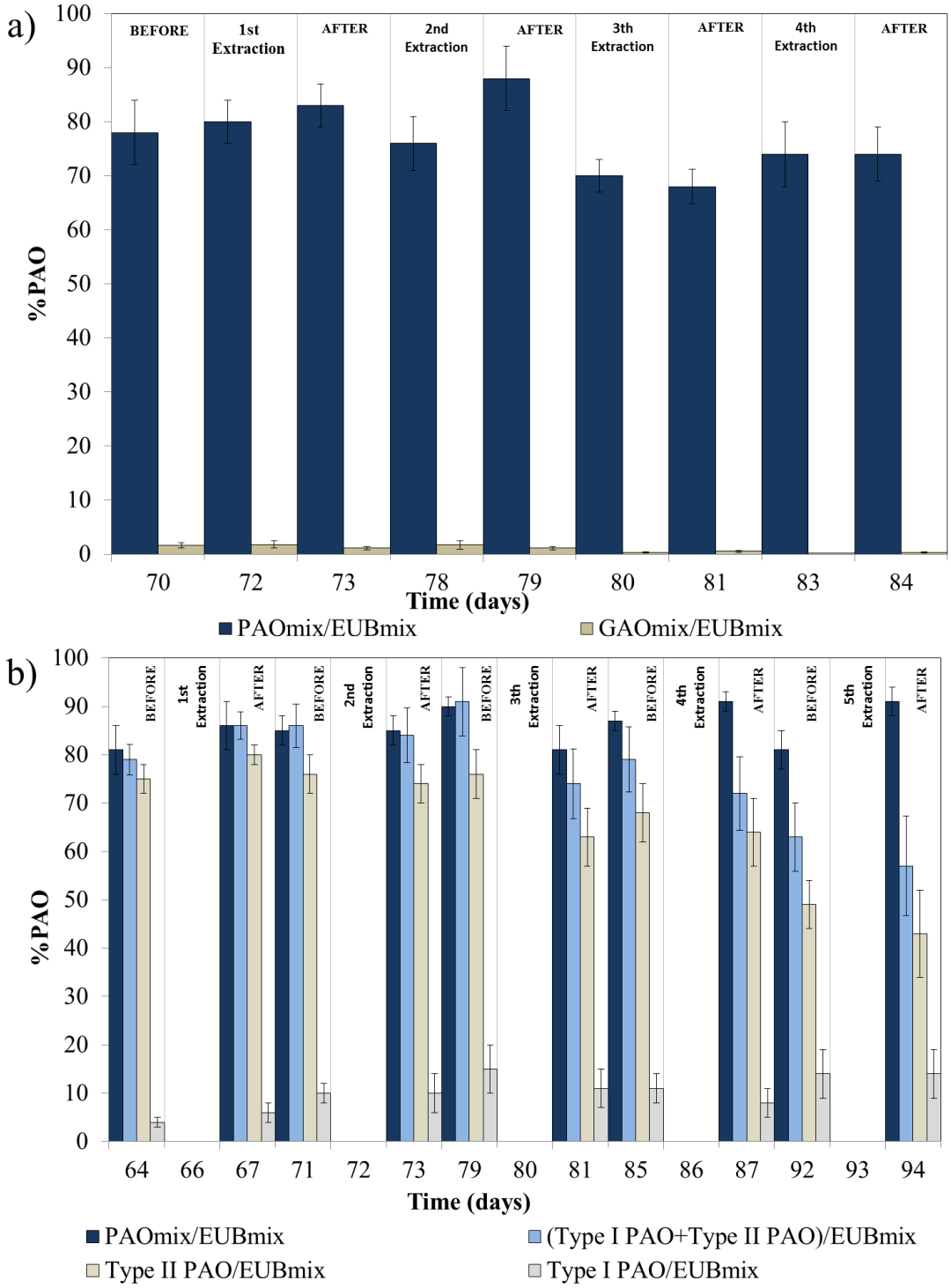

Figure 6. Population dynamics for a) PAOmix and GAOmix during first strategy, b) PAOmix, PAO Type I and PAO Type II during second strategy of the study.

Figure 7

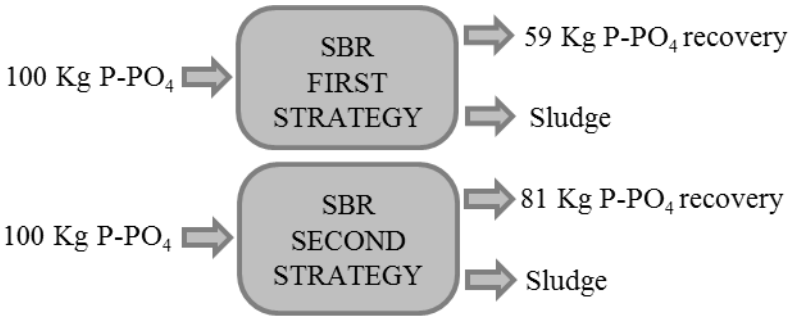

Figure 7. Weekly phosphate input and output from both strategies.