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Removal of micropollutants during biological phosphorus removal: impact of redox conditions in MBBR

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Abstract

Further biological polishing of micropollutants in WWTP effluents is limited by the lack of available carbon for cometabolic degradation. Metabolism of polyhydroxyalkanoates (PHAs) stored intracellularly during enhanced biological phosphorus removal (EBPR) could serve as carbon source for post-denitrification and micropollutant cometabolism.

The removal of nine micropollutants (i.e., pharmaceuticals and corrosion inhibitors) was investigated by using Moving Bed Biofilm Reactors (MBBRs), selecting phosphorus (PAO) or glycogen (GAO) accumulating organisms under different redox conditions. Three laboratory-scale MBBRs were operated in sequencing-batch mode under cyclical anaerobic and aerobic/anoxic conditions for phosphorus removal. Batch experiments were performed to evaluate the biodegradation potential of micropollutants along with the utilization of internally stored PHA. Experiments showed that aerobic PAO were able to efficiently remove most of the targeted micropollutants. The removal of benzotriazole, 5-methyl-1H-benzotriazole, carbamazepine, ketoprofen and diclofenac occurred simultaneously to phosphorus uptake and terminated when phosphorus was no longer available. Denitrifying PAO and aerobic GAO exhibited lower removal of micropollutants than aerobic PAO. Degradation profiles of stored PHA suggested a diverse utilization of the different fractions of PHA for phosphorus and micropollutant removal, with PHV (poly 3-hydroxyvalerate) most likely used for the cometabolism of targeted micropollutants.

1. Introduction

In the last decades, micropollutants such as pharmaceuticals, personal care products, household and industrial chemicals have been frequently detected in the aquatic environment (Daughton and Ternes, 1999; Reemtsma et al., 2006). Although they are typically found in effluents of municipal wastewater treatment plants (WWTPs) in concentration of ng L^{-1} to $\mu\text{g L}^{-1}$ (Ternes, 1998), effects of several micropollutants on aquatic organisms have been observed (Garric et al., 1996; Hoeger et al., 2005). The widespread occurrence of micropollutants indicates that conventional WWTPs are unable to completely remove these substances (Reemtsma et al., 2006). Currently, upgrading of conventional WWTP to improve removal of micropollutants from effluent wastewater mostly consider physical-chemical treatment, such as activated carbon or ozonation (Knopp et al., 2016; Stoquart et al., 2016). However, high costs associated with ozone treatment (Knopp et al., 2016), activated carbon regeneration and the possibility of the formation of oxidation products with equal or greater toxicity than the parent chemical (Benner et al., 2013), make biological treatment a desired alternative for removal of micropollutants.

Recent studies have proposed biofilm systems, e.g., MBBR, as a promising alternative to conventional activated sludge systems (CAS) with respect to the attenuation of micropollutants via biological treatment (Escolà Casas et al., 2015; Falås et al., 2016, 2012; Hapeshi et al., 2013; Torresi et al., 2016, 2017a). In MBBR, biofilms grow on specifically designed plastic carriers, which are suspended and retained in the system (Ødegaard, 1999).

Recently, Tang et al., (2017) investigated MBBR as a polishing technology for effluent wastewater, where an intermittent feeding strategy with influent wastewater (recirculated from primary clarification) was able to sustain the biomass growth, “boosting” the activity of the MBBR towards the

removal of a number of micropollutants (e.g., diclofenac). Indeed, tertiary biological treatment of micropollutants is limited by different factors such (i) the low concentration of micropollutants in the effluent wastewater which does not allow biomass growth, and (ii) the lack of carbon and nutrients in effluent wastewater necessary to support primary metabolisms of the micropollutant degrader organisms, in case cometabolism drives micropollutants degradation. Therefore, new strategies are needed to optimize tertiary biological treatment, and to provide an available carbon source for the primary metabolism, which drives the biodegradation of micropollutants even at the last stages of WWTP.

Methanol and ethanol are commonly used carbon sources that are added for post-denitrification in WWTP (Santos et al., 2001). However, in recent years, other solid substrates such as polyhydroxyalkanoates, PHAs (intracellularly stored), have been proposed for such purpose, as an alternative to soluble added substrates (Coats et al., 2011; Krasnits et al., 2013).

PHA are biopolymers that can be accumulated into the biomass during transient operation regarding carbon supply and redox conditions, as happens for example, during enhanced biological phosphorus removal (EBPR) (Dias et al., 2006). During an anaerobic phase, organic acids present in the wastewater, such as volatile fatty acids (VFA) are taken up and stored intracellularly as PHA by phosphate accumulating organisms, PAOs, by using the energy obtained from cleavage of previously stored polyphosphate and glycogen. In the subsequent aerobic and carbon poor conditions, PHA is used as carbon and energy source by the aerobic PAO for cell growth and to fuel the uptake and storage of polyphosphate and glycogen. When anaerobic conditions are followed by anoxic conditions, PHA can also support as carbon and energy source by the denitrifying PAOs (DPAOs) (Coats et al., 2011). During EBPR operation conditions, another group of bacteria named GAOs (glycogen- accumulating organisms) can also store PHA and compete with the PAO for the uptake of VFA, but without

removing any phosphate from wastewater, as their main energy source used for substrate uptake is glycogen (Lopez-Vazquez et al., 2009). As intracellular stored polymers (by both PAOs and GAOs), PHAs are easily decomposed and could be used as carbon source for phosphorus removal (Coats et al., 2011).

Only few studies have investigated the removal of micropollutants in systems performing EBPR. Ogunlaja and Parker (2018) studied the potential of PAO for removal of trimethoprim in a pilot-scale during biological nitrogen removal (BNR). They suggested a contribution of PAO to the removal of trimethoprim, although with lower biotransformation kinetics than other microbial groups (i.e., nitrifying and ordinary heterotrophic bacteria). Muz et al. (2014) studied the removal of six selected endocrine disrupting compounds in a lab-scale anaerobic-aerobic sequencing batch reactor (SBR) that exhibited more than 60% removal of most of the compounds, with exception of carbamazepine.

However, while these few studies gave an indication of PAO activity towards the removal of few micropollutants, no current evidences exist on the PAO and GAO activity for a wide range of micropollutants under different redox conditions.

Specific objectives of this research were to: (i) assess and compare the potential of aerobic PAO, anoxic DPAO and GAO enrichments for the removal of nine frequently detected micropollutants (two corrosion inhibitors and seven pharmaceuticals); and (ii) to investigate how PHA (3-hydroxybutyrate (3-PHB) and 3-hydroxyvalerate (3-PHV)) depletion profiles aligned with the micropollutants degradation profiles. Overall, this study was meant as “proof-of-concept” of a new operation of MBBR for tertiary treatment of micropollutants (based on a Swedish patent and previously discussed in Tang et al. (2017)). The new concept is currently tested in a pilot MBBR treating effluent municipal wastewater in a Danish WWTP.

2. Material and methods

2.1 Description of the sequencing batch MBBR system (long term-operation)

Three laboratory-scale MBBRs (2 L of operating volume in glass reactor) were operated in sequencing-batch mode for enhanced biological phosphorus removal. $R1_{PAO}$ and $R2_{APAO}$ were operated under cyclical anaerobic-aerobic conditions, while $R3_{DPAO}$ under cyclical anaerobic-anoxic conditions.

Each cycle consisted of two phases: (i) phase 1 was under anaerobic conditions that lasted 1 hour (reaction time) and it was similar in all three reactors; (ii) phase 2 was under aerobic conditions for $R1_{PAO}$ and $R2_{APAO}$, anoxic conditions in $R3_{DPAO}$ and it lasted 6 hours (reaction time) in all three reactors (Fig 1). Overall, the SBR operation for the three reactors consisted of the following phases: (i) fill (6 min), (ii) react under anaerobic conditions with VFA availability (1 hour), (iii) drain (100%, 12 min), (iv) fill (6 min), (v) react under aerobic ($R1_{PAO}$ and $R2_{APAO}$) or anoxic ($R3_{DPAO}$) conditions (no VFA present) (6 hours), (vi) drain (100%, 12 min). Thus, a cycle lasted in total 7.6 hours, including reaction time (7 h) and draining/filling time (0.6 h). A detailed explanation of the operation conditions was reported in Table S1 of the Supplementary information.

The duration of the anaerobic phase was set to be within the recommended 1-2 h contact period for biological phosphorus removal (Wang et al., 2011), while the aerobic (for $R1_{PAO}$ and $R2_{APAO}$) and anoxic ($R3_{DPAO}$) phases were kept long enough to provide sufficient contact time for the dissolved micropollutants and the biomass. The phases were automatically controlled by using a programmable power strip (EnerGenie power manager). A 100% drain between phases minimized the presence of dissolved organic carbon during phase 2 and the proliferation of other heterotrophic bacteria rather than PAO. For the three reactors and during both phases, a synthetic medium was used and consisted of: a

mixture of K_2HPO_4 and KH_2PO_4 (56% and 44%, respectively) to a final concentration of 30 mg L^{-1} of $PO_4\text{-P}$ and 20 mg L^{-1} of potassium, NH_4Cl to a final concentration of 5 mg L^{-1} of $NH_4\text{-N}$, $MgSO_4 \cdot 7H_2O$ to a final concentration of 15 mg L^{-1} of Mg and $CaCl_2 \cdot 2H_2O$ to a final concentration of 40 mg L^{-1} of Ca^{+} , and additional trace elements (section 1 in SI). High concentration of $PO_4\text{-P}$ was chosen for optimal conditions for PAO growth.

At the beginning of phase 1, an additional solution of synthetic VFA was added to an initial concentration of 250 mg L^{-1} of COD to ensure sufficient availability of carbon source for the anaerobic metabolism of the EBPR system. The VFA solution consisted of a mixture (mgCOD based) of sodium acetate (84% of, 0.31 g L^{-1}) and propionic acid (15%, 0.065 g L^{-1}), peptone and yeast (1%, 0.05 g L^{-1} respectively).

Sparging of nitrogen gas was used during phase 1 to provide mixing and anaerobic conditions ($DO < 0.2 \pm 0.05 \text{ mg L}^{-1}$) for all three reactors and mixing of the carriers for $R2_{APAO}$ under phase 2. Aeration with air pump provided a $DO > 2 \text{ mg L}^{-1}$ during phase 2 for $R1_{PAO}$ and $R2_{APAO}$. In the phase 2 of $R3_{DPAO}$, a solution of potassium nitrate (KNO_3) was additionally spiked to reach an initial concentration of 100 mg L^{-1} of $NO_3\text{-N}$. High concentration of nitrate was chosen to ensure a stoichiometric excess of electron acceptor during the anoxic phase.

Temperature was set at 20°C using a thermostat bath for $R1_{PAO}$ while $R2_{APAO}$ and $R3_{DPAO}$ were operated at ambient temperature ($20 \pm 1.5^\circ\text{C}$). pH was kept at 7.5 ± 0.5 by periodic addition of sodium hydroxide (16 mg L^{-1}) and HCL (10 mg L^{-1}). The number of carriers used in each reactor was 180, 150 and 160 pieces for $R1_{PAO}$, $R2_{APAO}$ and $R3_{DPAO}$ respectively, corresponding to an estimated exposed biofilm area of $0.1 \text{ m}^2 \text{ L}^{-1}$ (Piculell, 2016).

The inoculum biofilm carriers for the three MBBR consisted of AnoxK™ Z-400 (Veolia Water Technologies) from two different existing pilot plants. The AnoxK™ Z-400 carriers allow for the

development of biofilm with a maximum thickness of 400 μm . Inoculum 1 used for R1_{PAO} derives from a pilot plant operated with municipal wastewater for EBPR. Inoculum 2 used for both R2_{APAO} and R3_{DPAO} was taken from a pilot plant operated with municipal influent wastewater for COD and nitrogen removal. The difference between the two aerobic MBBR R1_{PAO} and R2_{APAO} was therefore only the biomass inoculum. R1_{PAO} was operated for approximately 150 days, while R2_{APAO} and R3_{DPAO} for 80 days.

To monitor the biomass activity during the long term SBR operation of the three reactors, samples for analysis of COD and $\text{PO}_4\text{-P}$ were taken during phase 1 (5 samples over 1 h of operation at 0, 15, 30, 45, 60 min) and only for $\text{PO}_4\text{-P}$ during phase 2 (7 samples over 6 hours of operation at 0, 0.5, 1, 1.5, 2, 4, 6 h).

Figure 1. Schematic representation of the three SBMBBRs. Phase 1 was anaerobic for all three reactors. During phase 2, R1_{PAO} and R2_{APAO} were kept under aerobic conditions, while R3_{DPAO} under anoxic conditions. The difference between the two aerobic MBBR R1_{PAO} and R2_{APAO} was the biomass inoculum.

2.2 Chemicals

Nine compounds were studied, that can be divided in two groups: (i) two corrosion inhibitors, i.e., benzotriazole and 5-methyl-1H-benzotriazole and (ii) seven pharmaceuticals, i.e., a) analgesic (diclofenac and carbamazepine); b) anti-inflammatory (ibuprofen and ketoprofen); c) fibrate (gemfibrozil and bezafibrate) and d) herbicide (clofibric acid). Compounds were obtained from Sigma-

Aldrich (Munich, Germany) and they were chosen according to the Swiss strategy for micropollutants as well as the most detected pharmaceuticals in effluents of WWTP (Margot et al., 2015). Formic acid and HPLC-gradient grade methanol were purchased from Sigma-Aldrich (Schnelldorf, Germany). The chemical properties of the nine compounds are shown in the Table S2 in SI. Chemical properties for each compound were retrieved using ACD/Labs predictions and the database Molinisticts, PubChem.

2.3 Batch experiments for micropollutants

Batch experiments for the three reactors were performed to assess the removal of the nine micropollutants in the three MBBRs during phosphorus removal. The batch experiments were performed when a stable performance in COD and phosphorus removal was observed.

2.3.1 Batch experiment for $R1_{PAO}$ reactor for biotic removal

For reactor $R1_{PAO}$, two batch experiments at two different times of operation were performed: after approximately 30 (Batch 1) and 150 days (Batch 2) of operation. A change in performance in phosphorus release and uptake was observed after 100 days of operation that suggested a shift of microbial community from PAO to GAO (discussed in section 3.1).

For both batch experiments, phase 1 was performed to achieve PHA accumulation under anaerobic conditions at same initial feeding conditions (VFA availability) as of the long term operation. In this phase (lasting 1 hour), only COD and phosphorus but not micropollutants were monitored, by collection of 5 samples at 0, 15, 30, 45, 60 min. After 1 hours of operation, the feed water was drained and 60 carriers (11% filling ration) were transferred into new glass reactors of 1 L. Micropollutants

biodegradation was instead monitored during phase 2 under aerobic conditions over 24 hours (11 samples at 0, 0.3, 0.5, 1, 2, 3, 4, 6, 20, 22, 24 h for R1_{PAO}). Simultaneously to the micropollutants sampling, samples for analysis of phosphorus were taken. In Batch 1, during phase 2, two different conditions were tested: (i) aerobic conditions with initial targeted PO₄-P concentration of 8 mg L⁻¹ (R1_{PAO} (lowP)) and (ii) aerobic condition with initial concentration of 30 mg L⁻¹ (R1_{PAO} (highP)). This was done to investigate if there was a notable enhancement or inhibition of the primary substrate (phosphorus) on the cometabolic activity of the PAO community, by using low and high strength wastewater. In Batch 2, when GAO community most likely dominated PAO in the community, only high concentration of phosphorus (30 mg L⁻¹) as used during long term operation was tested (R1_{GAO}). For both batch experiments, reference substances (dissolved in methanol in a stock solution of 100 mg L⁻¹) were first spiked into a glass beaker and the methanol was let evaporate. Subsequently, the substances were re-dissolved in the synthetic wastewater (two different solutions for high and low P as described previously) and used for the batch experiments. Reference substances were spiked to an initial concentration of 50 µg L⁻¹.

2.3.2 Batch experiment for R2_{APAO} and R3_{DPAO} reactors for biotic removal

Batch experiments for R2_{APAO} and R3_{DPAO} were performed similarly as described for R1_{PAO}. After 1 hour under anaerobic conditions and with VFA availability, the reactors were drained and 70 carriers (14% filling) for each reactor were transferred into a new glass reactor (1 L). As for R1_{PAO}, micropollutants were first spiked in a glass beaker and, once the methanol was evaporated, the mix was re-suspended in the new synthetic feed. In the batch for R2_{APAO} and R3_{DPAO}, only the synthetic feed with a phosphorus concentration of 30 mgP L⁻¹ was used, as the main objective of this test was to investigate the different metabolism of aerobic and denitrifying PAO, rather than the effect of primary substrate on the cometabolism of micropollutants. Therefore, similar concentration of PO₄-P as used

during “long term operation” of the MBBR was chosen. Additionally, for R3_{DPAO} a spiking of KNO₃ to an initial concentration of NO₃-N of 100 mg L⁻¹ was used to maintain anoxic conditions. The second phase (aerobic R2_{APAO} and anoxic R3_{DPAO}) was set to 24 hours and samples for analysis of micropollutants were taken at regular intervals (12 samples at 0, 0.3, 0.5, 1, 2, 3, 4, 6, 9, 12, 21, 24 h for R2_{APAO} and R3_{DPAO}). Simultaneously to the micropollutants sampling, samples for analysis of phosphorus were taken. Bubble aeration was used in R2_{APAO} for mixing the carriers and to reach a DO concentration of 7 ± 0.5 mg L⁻¹. Nitrogen sparging was used for mixing of carriers in R3_{DPAO} to reach a DO concentration lower than 0.5 mg L⁻¹.

2.3.3 Batch experiment for sorption of micropollutants to biomass

Batch experiments for sorption were also performed with biomass from R2_{APAO} and R3_{DPAO} to estimate single values of sorption coefficient K_{d,eq} (L g⁻¹). Batch experiments were performed in 0.5 L glass beaker with the synthetic wastewater (as described previously for the biotic batch experiment) spiked with reference substances (to a concentration of 50 µg L⁻¹ for each compound). Carriers (30 for each reactor) were used to reach a biomass concentration of 0.6 and 0.7 gVSS L⁻¹ for R2_{APAO} and R3_{DPAO} respectively and the carriers were mixed by sparging air. Biomass was inhibited using sodium azide (initial concentration of 0.2 mg L⁻¹). Sorption coefficient K_{d,eq} (L g⁻¹), were calculated according to the formula:

$$K_{d,eq} = \frac{C_{L,0} - C_{L,eq}}{C_{L,eq} X_{biomass}} \quad (\text{Eq.1})$$

Where C_{L,0} is the initial measured dissolved concentration of micropollutant, C_{L,eq} is ‘asymptotic’ concentration at equilibrium estimated by fitting measured concentration profiles in batch sorption experiments with a first-order decay equation, as previously used in Torresi et al. (2017b). X_{biomass} is

the biomass concentration in the reactor. Estimated $K_{d,eq}$ does not account for biofilm porosity and mass transfer into pores.

2.3.4 Batch experiment for other abiotic removal of micropollutants (control)

The experiment for abiotic removal was divided into two parts as applied previously (Torresi et al., 2016): (i) without plastic carriers and using only synthetic wastewater spiked with the references substances to assess abiotic degradation and sorption onto glass walls, and (ii) with new carriers added to synthetic wastewater to investigate sorption onto plastic carriers. The abiotic experiment was performed at each batch experiment performed for biotic removal of micropollutants.

2.4 Quantification of micropollutants

During batch experiment, at each sampling time, 3 mL of sample was filtered with a syringe filter and transferred to a glass vial. 0.8 mL acetonitrile was immediately added into the filtered sample to stop further bioactivity. All samples were preserved at -20 °C in a freezer before analysis by HPLC-MS/MS. For analysis, the samples were taken from the freezer and left to reach room temperature. 900 µL of each sample was transferred into a HPLC vial and 100 µL of internal standard solution consisting of mecoprop and atrazine was afterwards added into the HPLC vial. Lastly, 100 µL of sample from the HPLC vial was injected and analysed by HPLC-MS/MS. Further information regarding HPLC-MS/MS are reported in section 2 in SI. The range of limit of quantification (LOQ) for targeted micropollutants was from 0.05 to 10 µg/L and overall 98% of the theoretical concentration was recovered.

2.5 Analytical methods

Samples taken for analysis of conventional pollutants (soluble COD, PO₄-P, NO₃-N, NO₂-N) were filtered through 0.45 µm glass fiber filters (Sartorius, Göttingen, Germany). Hach Lange kits (LCK 339, LCK 341, LCK 349 and LCK 214) were used and samples were analyzed using a spectrophotometer (Hach Lange DR2800).

Biomass PHA content was determined by Gas Chromatography (GC) with Flame-Ionization Detection (GC-FID) according to the method described in Wang et al., 2017. Firstly, biomass was detached by the use of sterile brush and DI water, collected in Eppendorf tube (2 ml) and samples were kept at -20 °C in a freezer until further analysis. Prior to the analyses, collected biomass from carriers was freeze-dried for 24 hours. Briefly, dried PHA-rich biomass samples were digested at 100 °C in 2 mL each of acidified methanol (20% sulfuric acid v/v) and 1 mL of chloroform containing an exact amount of heptadecane (HD) (approximately 1 g L⁻¹) as internal standard. Two µL of sample were injected in a gas chromatographer equipped with a FID detector and a column (60m, 0.53mm internal diameter, 1 µm film thickness) coupled with a guard-column (0.32 mm internal diameter). Samples were analysed using helium as a carrier gas, at constant pressure (14.5 psi), and initial temperature of injection of 280°C. The main two measured compositions of PHA were 3-hydroxybutyrate (3-PHB) polymer and 3-hydroxyvalerate (3-PHV) copolymer. The compounds were confirmed by retention time and mass spectral matching with known PHA standards (a commercial co-polymer of PHB-PHV (88:12 molar)) and quantified based on the internal standard.

The biomass concentrations as TAS (total attached solid) was measured as difference in weight of a clean aluminium plate containing the biomass of 3 carriers (detached from carriers by using DI water

and a sterile brush) and set in at 105 °C for >24 h. VAS (volatile attached solids) were subsequently measured by setting the aluminium plate at 510 °C for 15 min and measuring the difference in weight.

2.6 Removal efficiency, rates and kinetics estimation

COD removal rates, PO₄-P release and removal rates were calculated by linear regression of soluble COD and soluble PO₄-P, respectively, under non limiting condition (COD > 100 mg L⁻¹, PO₄-P > 2-20 mg L⁻¹ for R_{1PAO} (low) and (high), respectively). Removal efficiencies (%) of micropollutants during batch experiment were calculated by measuring initial and final dissolved concentration of micropollutants in the bulk solution. Removal efficiencies were calculated over 6 hours, considering the duration of phase 2 of the three systems during long-term operation.

The pseudo first-order biotransformation rate constants k_{bio} (L g_{VAS} d⁻¹) (accounting for sorption processes) were identified using the activated sludge model framework for xenobiotics (ASM-X) (Polesel et al., 2016) performed in Graph pad Prism 7.0. Statistical analyses and PHA degradation rate (K, h⁻¹) was retrieved by using first order degradation rate in Graph pad Prism 7.0.

3. Results and discussion

3.1 Long-term operation of SBMBBR operation

Figure 2. Data of long-term operation of the SBMBBR reactor of COD removal and phosphorus release rates under phase 1 (anaerobic phase) and phosphorus removal under phase 2. (a) R1_{PAO} reactor; (b) R2_{APAO}, full symbols and R3_{DPAO}, empty symbols.

R1_{PAO} was operated for approximately 150 days. Phosphorus release and COD removal rates (expressed as carrier surface normalized, $g d^{-1} m^{-2}$) were calculated during the anaerobic phase (phase 1). For R1_{PAO}, a gradual increase of PO_4 -P release and COD removal was observed during the first month of operation up to 6 $g_P d^{-1} m^{-2}$ and 13 $g_{COD} d^{-1} m^{-2}$, while phosphorus removal under aerobic phase reached values of 3.5 $g_P d^{-1} m^{-2}$ (Fig. 2a). At the end of phase 1, the COD and PO_4 -P

concentration was approximately of 150 and 50 mg L⁻¹, respectively, while at the end of phase 2 the PO₄-P was approximately of 5 mg L⁻¹. The phosphorus release and COD uptake ratio was equal to 0.45, that was slightly lower than what reported in another SBMBBR for phosphorus and nitrogen removal (0.52, Pastorelli et al., 1999). The ratio between phosphorus uptake and release was around 0.7.

After batch 1 (after 30 days of operation) the reactor experienced a decrease in phosphorus release and uptake (Fig. 2), but exhibiting similar COD removal, suggesting a drastic change of the microbial community composition. This could be associated to possible proliferation of GAO bacteria or PAO bacteria presenting a similar metabolism than GAO (i.e., removing VFA under anaerobic conditions and store it as PHA using glycogen as the primary energy source) (Erdal et al., 2008; Zhou et al., 2008). According to Lopez-Vazquez et al. (2009), based on a modelling investigation, the composition of VFA in this study (84% acetate, 15% propionate), temperature of 20 °C and pH between 6.5 and 7 should have allowed for the coexistence of PAO and GAO.

Activity of the biofilm in R2_{APAO} and R3_{DPAO} sharply increased, reaching after 70 days of operation values of COD removal of 34 and 32 g_{COD} d⁻¹ m⁻² and phosphorus release of 8 g_P d⁻¹ m⁻² for R2_{APAO} and R3_{DPAO}, respectively. At the end of phase 1, the COD and PO₄-P concentration in both reactors was approximately of 25 and 50 mg L⁻¹, respectively, while at the end of phase 2 the PO₄-P was approximately of 10 mg L⁻¹. Final concentration of NO₃-N in R3_{DPAO} at the end of phase 2 was approximately of 65 mg L⁻¹. Values of anaerobic COD removal were higher than what was reported in two SBMBBRs for phosphorus removal, i.e. 27.1 g_P d⁻¹ m⁻² (Helness and Ødegaard, 2001; Pastorelli et al., 1999) as well as compared to R1_{PAO}. For both reactors, phosphorus removal during aerobic and anoxic phase reached values of 1.3 g_P d⁻¹ m⁻², which was lower than the value measured by Pastorelli et al. (1999).

3.2 Batch experiment 24 hours

3.2.1 Conventional pollutants and PHA accumulation

Table 1 shows the calculated rates of COD removal, phosphorus release and uptake rates measured during the 24 hours batch experiments performed for the three reactors, normalized for the amount of biomass (expressed as g_{VAS}). R_{1PAO} (lowP) and (highP) indicate the results from batch 1 experiment performed for R_{1PAO} (section 2.3.1) that tested the influence of low and high initial phosphorus concentration in two separate batch experiment. As mentioned in 3.1, in batch 2 for R_{1PAO} , a GAO microbial population most likely was enriched in the system, and thus R_{1GAO} rates correspond to the results of batch 2. Profiles of measured dissolved concentration of COD, phosphorus and associated linear regressions during anaerobic phase are reported in Fig. S1 in SI.

A faster phosphorus removal rate was observed at higher initial phosphorus concentration (R_{1PAO} (highP), $0.12 \text{ gP d}^{-1} g_{VAS}^{-1}$) than for R_{1PAO} (lowP) ($0.04 \text{ gP d}^{-1} g_{VAS}^{-1}$, Table 1). Additionally, phosphorus removal occurred only in the first 4 hours of the experiment for R_{1PAO} (lowP) and 6 hours for R_{1PAO} (highP), although phosphorus was still available in R_{1PAO} (high P) (Fig. S2 in the SI).

In the second batch, R_{1GAO} showed nor release neither uptake of phosphorus, as expected, but high COD removal under anaerobic phase (Table 1). Batch experiment for R_{2APAO} and R_{3DPAO} resulted in higher (~3 fold) COD removal and P release rate compared to R_{1APAO} . On the other hand, phosphorus removal rates were comparable in all three reactors. Denitrification in R_{3DPAO} exhibited two different rates, a faster rate up to 9 hours ($0.1 \text{ gN d}^{-1} g_{VAS}^{-1}$) and a slower rate ($0.04 \text{ gN d}^{-1} g_{VAS}^{-1}$) for the rest of the experiment. At the end of the batch for R_{3DPAO} , the remaining nitrate concentration was around 50 mg L^{-1} of $\text{NO}_3\text{-N}$, maintaining anoxic condition for the duration of the experiment.

PHA compositions in the three reactors at the end of the anaerobic phase (i.e., initial to the aerobic/anoxic phase) are presented in Table 1. The fraction of PHAs in the biomass of R1_{APAO} consisted mostly of PHV. Considering the feed composition (15% propionate, 84% acetate), this finding was in contrast to what is usually reported in literature, as the use of propionate was shown to induce mainly PHV production whereas PHB by acetate (Jiang et al., 2011). R1_{GAO} exhibited similar composition and concentration of PHA as in R1_{APAO}, although GAOs have been suggested to store anaerobically higher PHA concentration than PAOs (Oehmen et al., 2007).

R2_{APAO} and R3_{DPAO} reached significantly higher PHV accumulation compared to R1_{PAO} (4 and 3-fold, respectively), with higher values in the aerobic reactor R2_{APAO}. PHB was accumulated only in R2_{APAO} and R3_{DPAO}, and not in R1_{PAO}. Considering the similar conditions in which the three reactors were operated (e.g., VFA availability, main feed composition, temperature, pH) and the obtained different PHB and PHV biomass composition, it is likely that the biomass inoculum used for the experiment (R1_{PAO} different from R2_{APAO}/R3_{DPAO}) might have differently influenced the enrichment of PAO and GAO community for removal of phosphorus.

Table 1. Rates measured during batch experiments for the three reactors. VAS: volatile attached solids.

	R1_{PAO}			R2_{APAO}	R3_{DPAO}
	R1_{PAO} (lowP) (Batch 1)	R1_{PAO} (highP) (Batch 1)	R1_{GAO} (Batch 2)		
COD removal rate (g d ⁻¹ g _{VAS} ⁻¹)	0.7		1.7	1.9	2.0
PO₄-P release rate (g d ⁻¹ g _{VAS} ⁻¹)	0.2		0	0.5	0.5
PO₄-P removal rate (g d ⁻¹ g _{VAS} ⁻¹)	0.04	0.12	0	0.1	0.08
Denitrification rate (g d ⁻¹ g _{VAS} ⁻¹)	/	/	/	/	0.04
VAS (g L⁻¹)	1.5	1.5	0.5	1.6	1.8
%PHV initial (gPHV/g _{VAS})	1.2		0.9	4.7	3.5
%PHB initial (gPHB/g _{VAS})	<LOQ		<LOQ	1.5	1.7

3.2.2 Biotransformation of micropollutants in R1_{PAO} (aerobic conditions)

For R1_{PAO}, the nine targeted micropollutants were investigated in a batch experiment at low (8 mg L⁻¹) and high (30 mg L⁻¹) initial phosphorus concentration (blue symbols and line in Fig. 3). This was done to investigate if there was a notable enhancement or inhibition of the primary substrate (phosphorus) on the cometabolic activity of the PAO community. With exception of phosphorus concentration, feed composition, pH, T and biomass concentration were kept equal in the two reactors (R1_{PAO} (lowP) and R1_{PAO} (highP)).

Biotransformation kinetics of most of the investigated chemicals could be described with first-order equation ($R^2 > 0.9$, Table S2 in SI), thereby allowing for the estimation of pseudo-first order biotransformation rate constants k_{Bio} (L g_{VAS}⁻¹ d⁻¹) (Table 2).

We observed a substantial difference (20% up to 60%) in removal between R1_{PAO} (lowP) and R1_{PAO} (highP) for benzotriazole, 5-methyl-1H-Benzotriazole, carbamazepine, ketoprofen and diclofenac (Fig.3 and Table 2), with R1_{PAO} (high P) exhibiting the highest removal. Additionally, the removal of those compounds occurred only in the first 4 hours of the experiment for R1_{PAO} (lowP) and 6 hours for R1_{PAO} (highP), when the phosphorus was still available in the two reactors (Fig. S2 in the SI).

This suggests a first indication of cometabolic activity of PAO towards the removal of those compounds that ceased when the primary substrate (phosphorus) and/or the internal stored PHA was no longer available. When comparing R1_{PAO} (lowP) and R1_{PAO} (highP) in the first 4 and 6 hours respectively, a significant ($p < 0.05$) faster removal was observed at initial lower phosphorus concentration for carbamazepine, ketoprofen and diclofenac. As no evidence exists on the removal of those targeted micropollutants under biological enhanced phosphorus removal, biodegradation kinetics and efficiencies are compared to previous studies performed on MBBR and on activated sludge.

Benzotriazole and 5-H-methyl-benzotriazole removal was previously investigated in a two-stage aerobic MBBR and activated sludge (Mazioti et al., 2015), showing k_{Bio} (normalized on suspended solids) 10 times lower than the ones reported in this study. The predicted removal of benzotriazole ranged within 40-70% in the high and low loaded MBBR, respectively, which is comparable to this study (during the first 6 hours of the experiment). On the other hand, significant higher removal of 5-methy-1H-benzotriazole (29-76%) was observed in this study compared to Mazioti et al. (2015) (<20%), suggesting the efficiency of a PAO enriched community towards the removal of this compound. Carbamazepine is known to be a recalcitrant compound under most of the conditions in biological treatment (Falås et al., 2016). In this study, we observed a removal of 47-66% in the first 4 and 6 hours under aerobic phosphorus removal in $R1_{\text{PAO}}$ (lowP) and $R1_{\text{PAO}}$ (highP), respectively, suggesting an efficient cometabolic activity of PAO enriched community. Although this is a promising result for carbamazepine, further investigation was carried out in the following experiment ($R2_{\text{APAO}}$) to support this finding (section 3.2.2). Similarly, diclofenac presented a significantly faster removal k_{Bio} (in the first 4 and 6 hours in both reactors) compared to other studies with nitrifying MBBR (Torresi et al., 2016) and other biological process configurations (Vieno and Sillanpää, 2014), indicating the need for further investigation.

No major removal of clofibric acid (the main metabolite of clofibrate) was observed, while Falås et al. (2012) showed a removal in aerobic MBBR, although 3-8 times lower than what was observed in activated sludge (Joss et al., 2006). Bezafibrate was equally removed (~10%, Table 2) in both $R1_{\text{PAO}}$ (lowP) and $R1_{\text{PAO}}$ (highP), with k_{Bio} in the range of the one presented in Falås et al. (2016). This supports the hypothesis that bezafibrate is generally removed in mainly aerobic environment, also during phosphorus removal. Ibuprofen was removed faster in $R1_{\text{PAO}}$ (lowP) compared to $R1_{\text{PAO}}$ (highP), however with k_{Bio} similar or lower than what previously was measured in aerobic MBBR (Falås et al.,

2012; Torresi et al., 2016) and in general lower than what was observed for nitrifying-aerobic activated sludge (Joss et al., 2006; Suarez et al., 2010). This suggests that PAO activity for ibuprofen was lower than other heterotrophic and nitrifying bacteria. K_{Bio} for gemfibrozil was double at initial higher phosphorus concentration and in general higher than what was measured in a nitrifying MBBR (Falås et al., 2012).

In the batch experiment 2, in $R1_{GAO}$, most of the micropollutants were not removed (symbols and continuous red lines in Fig. 3) and only a removal of approximately 20% was measured for benzotriazole, clofibric acid, gemfibrozil and ibuprofen. Only ibuprofen presented k_{Bio} comparable to batch 1, however with kinetics significantly lower than what usually measured for activated sludge (Joss et al., 2006) or nitrifying MBBR (Torresi et al., 2016). Overall, results from batch 2 suggest that a community mostly populated by GAO, although able to store PHA at concentration similar to $R1_{APAO}$ (Table 1), may not exhibit a significant biotransformation activity for the targeted micropollutants.

Figure 3. Measured and simulated concentration profiles of micropollutants in reactor R1_{PAO} (lowP), R1_{PAO} (highP) in blue and R1_{GAO} in red. Dotted lines at 4 and 6 h indicate the time when phosphorus is no longer available for R1_{PAO} (lowP) and (highP), respectively. Black symbols and lines show results of the control experiment (abiotic removal).

Table 2. Biodegradation rate constants and removal efficiency calculated for the first 6 hours of experiment for R_{1PAO} during batch 1 (R_{1PAO} (lowP) and (highP)) and batch 2 (R_{1GAO}).

	R_{1PAO} (low P)		R_{1PAO} (high P)		R_{1GAO}	
	k_{Bio}	Removal	k_{Bio}	Removal	k_{Bio}	Removal
	($L\ g_{VAS}^{-1}\ d^{-1}$)	(%)	($L\ g_{VAS}^{-1}\ d^{-1}$)	(%)	($L\ g_{VAS}^{-1}\ d^{-1}$)	(%)
Benzotriazole	12.5 ± 3.9	32	12.4 ± 0.7	69	1.5 ± 0.9	18
5-methyl-1H-Benzotriazole	5.6 ± 1.6	29	25.9 ± 6.7	76	n.d	/
Carbamazepine	45.4 ± 16.1	47	12.1 ± 1.7	66	n.d	/
Ketoprofen	49.6 ± 11.2	44	6.4 ± 0.2	54	n.d	/
Diclofenac	47.2 ± 11.6	52	11.9 ± 2.0	72	n.d	/
Clofibric acid	n.d	/	n.d	/	5.0 ± 1.8	27
Bezafibrate	1.1 ± 0.3	16	0.3 ± 0.2	10	n.d	/
Ibuprofen	2.4 ± 0.6	48	0.4 ± 0.4	25	0.9 ± 0.6	21
Gemfibrozil	4.6 ± 0.8	26	2.3 ± 0.6	36	25.9 ± 15.3	16

3.2.2 Abiotic removal of micropollutants

As described previously (Torresi et al., 2017b), sorption of spiked micropollutants was considered significant when a relative concentration drop of measured initial and final concentration was higher than 10%, thus accounting for analytical uncertainty. Profiles of aqueous concentration of the sorptive micropollutants measured during batch experiments for R_{2APAO} and R_{3DAO} are shown in Figure in S3.

Only diclofenac, gemfibrozil and ketoprofen presented significant removal (%) due to sorption to biomass in 24 hours of experiment. As observed in Torresi et al. (2017b), equilibrium in thick biofilm ($> 200\ \mu m$) may not be reached within the duration of the batch experiment (usually performed within

4 hours, and here in 24 hours). Therefore calculation of the distribution coefficient $K_{d,eq}$ was based on the approximation of the asymptotic equilibrium concentration, as suggested in Torresi et al. (2017b). Value of $K_{d,eq}$ were 1.3-1.6 for diclofenac, 0.9-0.7 for ketoprofen and 0.3-0.3 L g⁻¹ for gemfibrozil for R2_{APAO} and R3_{DPAO}, respectively. Interestingly, no significant difference was observed in the two $K_{d,eq}$, estimated for aerobic and anoxic carriers. In our previous work, sorption of diclofenac to biofilm (Z-carriers) were found insignificant, as well as in other studies (reviewed in Vieno and Sillanpää (2014)). This could be due to the ionization state of diclofenac that at pH typical of wastewater treatment plant of 7.5 (similarly to this study) has a negatively ionized carboxylic acid moiety, which repels the negatively charged biomass. Thus, the significant sorption coefficient measured in both R2_{APAO} and R3_{DPAO} should be further investigated. Mazioti et al. (2015) showed that sorption was of minor importance for both benzotriazole and 5-methyl-1H-Benzotriazole in an aerobic MBBR, similarly to this study. Values of sorption for ketoprofen are higher than $K_{d,eq}$ measured in primary activated sludge and MBR (0.22 L g⁻¹, Radjenović et al., 2009) while for gemfibrozil similar sorption coefficient was observed (0.3 L g⁻¹).

No major removal due to other abiotic processes (e.g., sorption to glass wall, plastic, volatilization and hydrolysis) was observed for the targeted micropollutants (black symbols in Fig. 3 and. Fig. 4).

3.2.3 Biotransformation of micropollutants in R2_{APAO} (aerobic conditions) and R3_{DPAO} (anoxic conditions)

Reactors R2_{APAO} and R3_{DPAO} were operated to compare the performance of PAO community under aerobic and anoxic conditions, respectively. Generally, the batch experiment for R2_{APAO} was performed under similar conditions of R1_{PAO} (highP), although the two reactors were inoculated with different

biomass, taken from two existing pilot plants. When comparing $R2_{APAO}$ and $R1_{PAO}$ under aerobic conditions, the five compounds that previously presented a slowdown of degradation when phosphorus was over (Fig. 3) were still degraded in $R2_{APAO}$ for the rest of the experiment but with lower k_{Bio} . Despite this, k_{Bio} for benzotriazole and 5-methyl-1H-Benzotriazole were in the range of the kinetics measured in a two stage aerobic MBBR treating full strength wastewater (0.58-2.46 for benzotriazoles and 0.7 for 5-methyl-1H-Benzotriazole, Mazioti et al., 2015). The removal of carbamazepine under aerobic conditions was poor, not supporting what previously found for $R1_{PAO}$. However, the difference in biomass composition may have influenced the measured activity of the two aerobic PAO communities. Due to analytical uncertainties, ibuprofen could not be measure in this experiment. Interesting, diclofenac and ketoprofen presented a lag-phase prior degradation until 4 hours of batch experiment for $R2_{APAO}$ (Fig. 4). Diclofenac exhibited k_{Bio} in the range of what observed by using MBBR carriers from a full-scale plant (Falås et al., 2012) and a lab-scale three-stage MBBR configuration (Escolà Casas et al., 2015), but lower of what observed in a polishing MBBR pilot fed with intermitting feeding (k_{Bio} of $5.5 \text{ L g}^{-1} \text{ d}^{-1}$) (Tang et al., 2017). Conversely to what observed in $R1_{PAO}$, clofibric acid was biodegraded to a certain extent, with k_{Bio} significantly higher to what was observed in a previous aerobic MBBR (Falås et al., 2012). Overall, obtained removal efficiency (%) calculated over the 6 hours of batch experiment for $R2_{APAO}$ was comparable to the efficiency $R1_{PAO}$ (with exception of bezafibrate), confirming the potential of the enriched PAO community in the biodegradation of these compounds.

When comparing aerobic to anoxic conditions ($R2_{APAO}$ and $R3_{DPAO}$), only benzotriazole and carbamazepine were removed to similar extent, while most of the compounds were not removed or presented lower removal under anoxic conditions. Removal of carbamazepine has previously been observed in anoxic post-denitrifying MBBRs (Torresi et al., 2017a), confirming its removal under those

conditions. Conversely, diclofenac under anoxic condition was confirmed to be non-degradable as previously observed in pre- and post-denitrifying MBBR (Polesel et al., 2017; Torresi et al., 2017a).

Figure 4. Measured and simulated concentration profiles of micropollutants in reactor R2_{APAO} and R3_{DPAO}.

Table 2. Biodegradation rates and removal efficiency calculated for the first 6 hours of the batch experiment for R2_{APAO} (aerobic PAO) and R3_{DPAO} (denitrifying PAO). Asterisks * indicate compounds that show significant sorption removal ($K_{d,eq}$ included in the estimation of the biotransformation kinetics).

	R2_{APAO}		R3_{DPAO}	
	k_{Bio} (L g _{VAS} ⁻¹ d ⁻¹)	Removal (%)	k_{Bio} (L g _{VAS} ⁻¹ d ⁻¹)	Removal (%)
Benzotriazole	1.5 ± 0.3	42	1.6 ± 0.2	55
5-methyl-1H-Benzotriazole	0.5 ± 0.1	29	0.1 ± 0.1	25
Carbamazepine	0.2 ± 0.0	12	0.3 ± 0.1	16
Ketoprofen*	1.2 ± 0.1	14	n.d	6
Diclofenac*	1.8 ± 0.1	8	n.d	0
Clofibric acid	1.5 ± 0.3	38	0.6 ± 0.2	20
Bezafibrate	n.d	/	n.d	/
Gemfibrozil*	0.8 ± 0.0	33	n.d	0

3.3 PHA utilization and micropollutants biodegradation in R2_{APAO} and R3_{DPAO}

PHB and PHV concentrations in the biomass were measured over 24 hours of batch experiment, performed for the three reactors. Unfortunately, for R1_{PAO}, only the initial concentration of PHA was quantifiable, while the subsequent 10 samples were under the level of quantification (LOQ). Therefore, for R1_{PAO}, the removal observed in Fig. 3 could indicate a possible cometabolic activity of the PAO community with the uptake of phosphorus, with the degradation of stored PHA, or a combination of the

two mechanisms. Therefore, only concentration profiles measured during the batch experiment for $R2_{APAO}$ and $R3_{DPAO}$ are presented (Fig. 5).

PHA degradation rates in the biofilm reactors were previously observed to follow first order degradation rates (Krasnits et al., 2013). In $R2_{APAO}$, both PHB and PHV exhibited a degradation rate K of $0.35 \pm 0.16 \text{ h}^{-1}$. For $R3_{DPAO}$, a faster degradation rate K for PHB ($K=0.33 \pm 0.10 \text{ h}^{-1}$) than for PHV ($K=0.10 \pm 0.05 \text{ h}^{-1}$) was observed. Values of degradation rates of PHA are similar to those estimated for denitrifying biofilm reactors fed with synthetic wastewater (Krasnits et al., 2013) and in activated sludge (Beun et al., 2000). However, higher PHA degradation rates were measured in the same study when using real wastewater (Krasnits et al., 2013). Lower PHA degradation rates in biofilm compared to suspended sludge were associated with diffusion limitation exhibited in biofilms (Krasnits et al., 2013).

For $R2_{APAO}$ under aerobic conditions, the fraction of PHB was removed in the first 4 hours of experiment (Fig. 5), simultaneously to the removal of phosphorus, while the removal of PHV continued during the 24 hours. Noticeable, a lag-phase of 4 hours before biodegradation was observed for the compounds diclofenac and ketoprofen (Fig. 4), which may be related to a possible inhibition in their biodegradations by PHB and phosphorus removal. The lag-phase terminated when PHB was fully consumed. Due to the absence of PHB in reactor $R1_{PAO}$, the inhibition might not have been visible in the aerobic batch in $R1_{PAO}$. Under anoxic condition, in $R3_{DPAO}$, phosphorus and nitrate removal occurred simultaneously to the micropollutant biodegradation. As shown in Fig. 5, PHB was mainly degraded during time when phosphorus removal occurred (in this case approximately 5 hours), during simultaneous removal of PHV. Additionally, in $R3_{DPAO}$, PHV degradation occurs over the 24 hours of the experiment, due to the reducing energy needed for the denitrification. As no additional carbon source was present in the feed during phase 2 of the batch experiment, it is likely that PHV was related

with the biodegradation of micropollutants. As no evidences are present regarding PHA degradation and micropollutant removal, further research should be carried out to assess the specific contribution of the different PHA fractions on biodegradation of trace compounds.

Figure 5. Measured and simulated concentration profiles of phosphorus, PHB, PHV and nitrate in reactor R2_{APAO} and R3_{DPAO}.

3.4 Application and future considerations

One of the major limitations of using biological treatment as polishing technology in WWTPs is the difficulty in maintaining sufficient biofilm growth and activity at low available concentration of carbon, nitrogen and other nutrients in the effluent wastewater. Intermittent feeding of aerobic MBBR between pre-clarified wastewater (by-passed at the last stage of WWTP and recirculated to primary

settler) and effluent wastewater has been shown to be a valid solution to maintain substantial biomass concentration and activity at a tertiary biological treatment (Tang et al., 2017). This study was performed to investigate another possibility for operation and feed strategy of polishing MBBR: an intermittent feeding of the biofilm with pre-clarified wastewater under anaerobic conditions, which in return will favour the enrichment of PAO community and PHA storage. The results suggest that the PHA stored in under anaerobic conditions can subsequently be used in the effluent wastewater stream to maintain biofilm growth, to treat the remaining phosphorus and nitrogen (if anoxic conditions are applied) and in addition also micropollutants. Such operational strategy is currently under investigation in a polishing pilot-scale MBBR (funded by Innovationsfonden Denmark and based on the Swedish patent 1650321-1.a by Veolia Water Technologies), consisting of two wastewater lines (Fig. 6): a side stream (treating pre-clarified wastewater) under anaerobic conditions, where PHA are stored, and a second line (treating effluent wastewater) under consecutive anoxic and aerobic conditions. Intermittently, the flows between the two lines are shifted and the reactor previously exposed to anaerobic conditions (reactor C in Fig.6) is set on the polishing line (in the position of A reactor) to treat effluent wastewater, as previously described in Tang et al. 2017. Notably, the new concept relies on the side stream of pre-clarified wastewater being returned to the main wastewater treatment line (i.e., after pre-clarification), not affecting the quality of the effluent wastewater.

In addition to the testing of the new operational concept for tertiary treatment of micropollutants, the pilot will serve as validation of the results of this study that relied on synthetic wastewater and was found highly dependent on the inoculum media.

Figure 6. Schematic view of the polishing pilot that test the intermitting feeding concept developed by Veolia Water Technologies (Swedish patent 1650321-1.a) as additional polishing technology to treat effluent wastewater.

4 Conclusion

Three lab-scale MBBRs were operated under different redox conditions to investigate the potential of removing nine targeted micropollutants, by monitoring the biodegradation of stored PHA. Based on targeted batch experiments, we could draw the following conclusions:

- Although the three lab-scale MBBR were operated with similar conditions (e.g., feed composition, pH, temperature), the different inoculum biofilm strongly influenced the activity and the PHA composition of the microbial community.
- Under aerobic conditions ($R1_{PAO}$), higher removal (%) of most of the micropollutants was observed at higher initial $PO_4\text{-P}$ concentration (30 mg L^{-1}) compared to lower concentration (8 mg L^{-1}), suggesting a cometabolic activity of the PAO enriched community. Removal of benzotriazole, 5-methyl-1H-benzotriazole, carbamazepine, ketoprofen and diclofenac occurred only in the first 6 hours of experiment, simultaneously to phosphorus removal and terminated when phosphorus was no longer available.
- When the microbial community in $R1_{APAO}$ shifted to mainly GAO enriched community ($R1_{GAO}$), the removal of most of micropollutants was insignificant, with exception of clofibric acid and ibuprofen.
- Under aerobic conditions, $R2_{APAO}$ exhibited similar removal efficiency to $R1_{APAO}$ of most of the micropollutants (with exception of bezafibrate), although with lower k_{Bio} .
- The removal efficiency of most of targeted micropollutants was significantly reduced (from 20 to 80% reduction) under anoxic conditions ($R3_{DPAO}$).
- Results from measured PHA concentration showed that phosphorus removal likely was driven by simultaneous degradation of PHB and PHV fractions during the first 4-6 hours of batch

experiment. On the other hand, biodegradation of PHV occurred over the whole duration of the experiment, simultaneously to the removal of the targeted micropollutants and nitrate (in R_{3DPAO}).

Supplementary information

E-supplementary data of this work can be found in online version of the paper

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ACCEPTED MANUSCRIPT

Graphical abstract

Highlights

- MBBR operated for biological phosphorus removal under different redox conditions
- Aerobic PAO efficiently removed nine targeted micropollutants
- Removal occurred simultaneously to phosphorus uptake for several micropollutants
- Denitrifying PAO and GAO exhibited lower removal than aerobic PAO
- PHV and PHB contributed differently to removal of phosphorus and micropollutants