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Maintenance of phosphorus removal in an EBPR system under permanent aerobic conditions using propionate

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ABSTRACT

Enhanced biological phosphorus removal (EBPR) is an efficient and sustainable technology to remove phosphorus from wastewater preventing eutrophication in natural waters. It is widely accepted that EBPR requires an optimal anaerobic hydraulic retention time to obtain stable P-removal from wastewater. Thus, it is suggested that deterioration of the EBPR efficiency regularly observed in full-scale wastewater treatment plants (WWTPs) is normally caused by an excessive aeration of activated sludge that increments the amount of oxygen recycled to the anaerobic reactor and consequently, the anaerobic conditions are not totally preserved. Furthermore, it has been reported a progressive decrease in P-removal capacity in an EBPR lab-scale system enriched with acetate as the sole carbon source under permanent aerobic conditions. Hence, to evaluate the stability of P-removal with a different carbon source, an EBPR-SBR was operated with propionate under permanent aerobic conditions. As a result, net P-removal was successfully accomplished in the SBR without any anaerobic phase during 46 days of aerobic operation. Moreover, the system was shifted after this period to the standard anaerobic–aerobic conditions and reliable P-removal was maintained. FISH (fluorescence in situ hybridisation) analysis showed a significant presence of *Accumulibacter* (70, 50 and 72%, in different periods) and the absence of *Competibacter*. The results indicate that using propionate as carbon source it is possible to maintain in a long term an enriched culture of phosphorus accumulating organisms (PAO) able to remove phosphorus under permanent aerobic conditions.

1. Introduction

Enhanced biological phosphorus removal (EBPR) is an efficient and sustainable biological technology to remove phosphorus from wastewater. It is based on the enrichment of activated sludge with phosphorus accumulating organisms (PAO) by introducing alternating anaerobic–aerobic conditions, which favour PAO growth over ordinary heterotrophic organisms (OHO). Under anaerobic conditions, PAO store volatile fatty acids (VFA) as polyhydroxyalkanoates (PHA) with concomitant P-release into solution derived from the hydrolysis of polyphosphate (poly-P) reserves. The required reducing equivalents are provided by the catabolism of stored glycogen [1]. In a subsequent aerobic (or anoxic) stage, PHA is used as carbon and energy sources for growth and replenishment of the internal glycogen and poly-P pools. An amount of phosphate higher than that released is taken up by PAO and the poly-P pools are restored. As a result, net P-removal is accomplished by periodic sludge waste after this phase.

On the other hand, even though the two most common VFA present in municipal wastewater are acetate and propionate, most of works on EBPR consider acetate as sole carbon source. However, the metabolism of propionate by PAO has attracted recently considerable attention [2–4] and it is suggested that it could be a more favourable substrate for EBPR [5–9].

Although many works are focused on this complex process, the complete metabolic behaviour of the EBPR sludge is still unclear and it is prone to unpredictable failures, primarily because the process design is highly empirical due to an incomplete understanding of sludge microbial ecology [10]. For example, Brdjanovic et al. [11] hypothesised that one cause of EBPR efficiency deterioration could be excessive aeration, which would result in a gradual depletion of PHB and/or saturation of the biomass by poly-P. The two most common causes of excessive aeration in continuous wastewater treatment plants (WWTPs) are heavy rainfall periods and failures in the aeration control, which increment the amount of oxygen recycled to the anaerobic reactor. In both scenarios, the anaerobic conditions are reduced and the aerobic phase is extended leading to a detriment in P-removal.

To the best of our knowledge, successful EBPR operation without a physical separation between the electron donor (organic matter)

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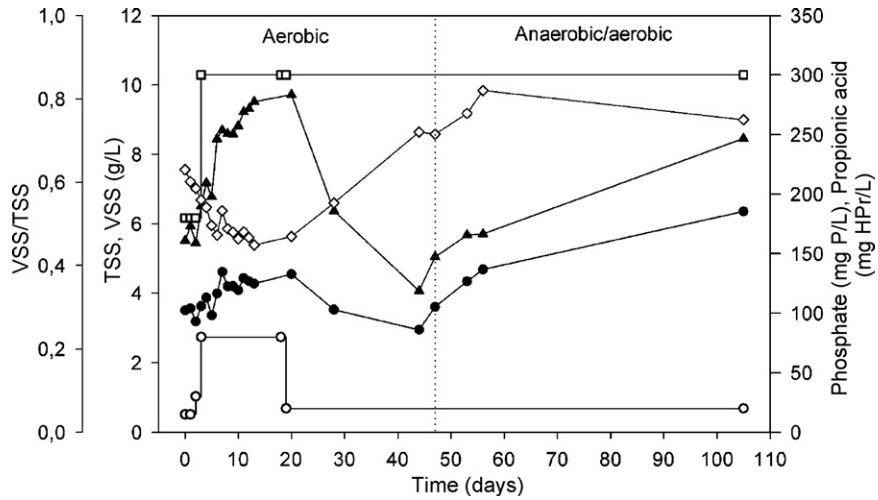

Fig. 1. Time course of TSS, VSS, VSS/TSS ratio and changes in the feed composition during both SBR periods studied. (●) VSS, (▲) TSS, (◊) VSS/TSS, (○) phosphate, (□) propionic acid.

and the electron acceptor (oxygen) has not been reported yet in full scale WWTP. In conventional continuous WWTP, the inclusion of a first anaerobic reactor allows the separation of organic matter and oxygen. These conditions favour PAO against OHO because PAO are more efficient to anaerobically uptake VFA due to their polyphosphate reserves. Nevertheless, some recent studies showed the feasibility to achieve net P-removal under strictly aerobic conditions in well-controlled lab-scale EBPR reactors [12–14]. In these studies, aerobic acetate uptake was linked to phosphate release, PHA storage and glycogen degradation. After substrate depletion, phosphate was taken up linked to PHA degradation, glycogen synthesis and PAO growth. The phases with and without available external substrate are known in the literature [15–16] as feast and famine, respectively. According to the pattern observed, these stages were analogous to the anaerobic–aerobic conditions of the typical EBPR process [13]. Moreover, Pijuan et al. [17] studied the P-removal deterioration in enriched PAO sludge subjected to permanent aerobic conditions. An aerobic SBR was operated using acetate as carbon source, observing the abovementioned feast/famine phases. However, net P-removal was only maintained during the first 4 days of operation, showing that the tested conditions did not provide a stable operation for aerobic EBPR.

Finally, Ahn et al. [18] proposed recently a new operational scheme for aerobic phosphate removal from wastewater consisting of an aerobic SBR where the feed stage (acetate addition) was temporally separated from phosphate addition, which begins the famine stage. The authors showed that this process was capable to remove phosphate from 10 to 12 mg P/L to less than 0.1 mg P/L over an extended period, using wastewater with low COD content.

This paper aims to propose an alternative for stable aerobic P-removal. It tests if EBPR can be maintained in a SBR under permanent aerobic conditions using propionate as the sole carbon source. Additionally, after this strict aerobic operation, the stability of net P-removal is examined when the SBR is changed to conventional EBPR anaerobic–aerobic operation.

2. Materials and methods

2.1. Equipments and experimental design

The 10 L SBR used for the experiments was seeded with sludge obtained from an EBPR anaerobic–aerobic SBR [19]. It was operated at four cycles per day with a hydraulic residence time (HRT) of 12 h. Each cycle consisted of 120 min of anaerobic phase (5 initial min for feeding), 180 min of aerobic phase, 55 min of settling and 5 min of extraction of 5 L of supernatant. The sludge residence time (SRT) was kept at 10 days by periodic sludge wastage during the end of the aerobic period but before stirring was stopped.

After 5 months of stable and efficient EBPR operation, the SBR was changed to permanent aerobic operation (from day 1 to 46) to investigate the short-term and long-term aerobic P-removal capability of PAO. Each cycle consisted of 300 min of aerobic phase (5 initial min for feeding), 55 min of settling and, in the last 5 min, withdrawing of 5 L of supernatant. SRT was kept also at 10 days.

After the 46 days of aerobic operation, the SBR was operated with standard anaerobic–aerobic cycles for 59 days (from day 47 to 105) to evaluate the behaviour of the system regarding P-removal stability.

Temperature in the SBR was kept at 25 °C and the pH was maintained below 7.5 with HCl 1 M. Dissolved oxygen (DO) was controlled during the aerobic phase between 3.5 and 4.5 mg DO/L with an on/off controller to avoid oxygen limitations. Oxygen Uptake Rate (OUR) was estimated on-line by measuring the DO decrease rate between 4.5 and 3.5 mg DO/L during the off position of the aeration valve. The average biomass concentration maintained in the reactor during the whole period of study was around 4 g VSS/L, although some variations of TSS and VSS were observed (Fig. 1).

2.2. Synthetic media

Two separate solutions called "concentrated feed" (constituting 0.25 L per 5 L synthetic wastewater) and "P-water" (constituting 4.75 L per 5 L synthetic wastewater) collectively formed the synthetic wastewater used in this study. The "concentrated feed" consisted of (g/L RO water): 12 (or 7.2) propionic acid (to achieve 300 mg/L or 180 mg/L in the SBR after feeding), 2.0 NH_4Cl, 0.88 $MgSO_4 \cdot 7H_2O$, 3.2 $MgCl_2 \cdot 6H_2O$, 0.84 $CaCl_2 \cdot 2H_2O$, 0.4 yeast extract, 0.10 allylthiourea (ATU) to inhibit nitrification and 6 mL of nutrient solution. The P-water consisted of 1:0.6 KH_2PO_4/K_2HPO_4 (molar ratio). The concentration of P and propionic acid changed along the study (see Fig. 1). Prior to the permanent aerobic cycles performed in the SBR, it was operated with initial concentrations of 15 mg/L of P and 180 mg/L of propionic acid. Afterwards, in the third day of permanent aerobic operation, they were incremented up to 80 and 300 mg/L, respectively, for a better monitoring of the variables measured in the study cycles. Later on (day 19), phosphate concen-