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Monitoring of microbial storage products and the efficiency of an activated sludge plant performing anoxic phosphorus removal under different operational conditions

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ABSTRACT

The present work focuses on the operation of a continuous flow laboratory scale plant for enhanced biological phosphorus removal (EBPR) from synthetic wastewater. After the acclimatization period, basic operational conditions, namely the influent COD concentration and the anoxic to anaerobic tank volumes ratio, varied, in order to evaluate their impact to the system performance. The progressive increase of the influent COD concentration from 200 mg COD/l to 300 and 400 with constant influent phosphate concentration of 15 mg PO_4^{-3}–P/l, led to proportional increase of the intracellular PHAs used during the anoxic phosphorus uptake process and to the respective amount of nitrate denitrified. However, the highest phosphorus uptake rate and the maximum net phosphorus removal were observed for the influent COD/P concentrations ratio of 20 (300 mg COD/l). Interestingly, this was also the ratio where the maximum carbohydrates synthesis and biomass P content during the anoxic phase were observed, indicating this, as the optimum ratio for the system efficiency. In a second operational period and for this particular COD/P ratio, three different anaerobic hydraulic residence times (HRT) were imposed, by increasing the volume of the anaerobic tank, in order to achieve anoxic to anaerobic HRT ratios of 2.33, 1.75 and 1.4. The experimental results indicated that decreased ratio values led to increased PHAs utilization for phosphorus removal. Furthermore, despite the considerable increase of the specific P uptake rate, the specific anaerobic P release rate remained almost constant, irrespective to the anoxic to anaerobic HRT ratio. The maximum net phosphorus removal was observed for the minimum ratio, indicating that increased anaerobic contact time enhances the activity of polyphosphate accumulating organisms (PAOs) in the EBPR plant. The biomass growth yield was not influenced by the anoxic to anaerobic HRT ratio and remained constant at 0.27 gVSS/gCOD. Phosphorus removal under alternative anaerobic/anoxic conditions proved its practical feasibility and satisfactory efficiency in a continuous flow activated sludge system.

Keywords: Acetate; Continuous flow plant; Denitrifying phosphorus removal; DPAOs; Polyhydroxyalkanoates

1. Introduction

Application of biological nutrient removal (BNR) processes in wastewater treatment is necessitated for the protection of water bodies from eutrophication. Phosphorus removal from wastewater streams can be achieved by using either chemical or biological phenomena; the latter are proved to be more suitable from environmental as well as economical point of view. Enhanced biological phosphorus removal (EBPR) in

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wastewater treatment is conventionally achieved by biomass recirculation between anaerobic and aerobic conditions. This configuration enhances the selection of organisms, widely known as polyphosphate accumulating organisms (PAOs) that accumulate phosphate in much higher levels than conventional heterotrophic bacteria. Phosphorus is finally removed from the system via rejection of surplus sludge. However, biomass recirculation between anaerobic and anoxic conditions, where phosphorus removal and denitrification occur simultaneously is also an interesting alternative, combining advantages such as optimized utilization of organic substrate and lower aeration demands [1]. Various BNR plants designs favor the growth of denitrifying phosphorus accumulating organisms (DPAOs), thus achieving concomitant phosphorus and nitrogen removal from the wastewater [2].

In EBPR several biochemical processes are involved. During the anaerobic phase the available organic substrate, mainly in the form of short chain fatty acids, is uptaken by the bacteria and stored in the form of polyhydroxyalkanoates (PHAs). At the same time, the biomass stored polyphosphate is hydrolyzed and orthophosphate is released in the mixed liquor. During this phase, bacterial glycogen is also consumed producing reduced coenzymes essential for PHAs synthesis [3–7]. In the subsequent aerobic or anoxic phase, the stored PHAs are consumed for both cellular growth and excess phosphorus uptake. Glycogen is synthesized and phosphate is stored in the form of polyphosphate. Several bacterial species have been proposed as candidate PAOs and specifically DPAOs. However, no isolation attempt has been proved successful to date [2,8,9]. Although several studies on biological anoxic phosphorus removal has been conducted, reports concerning process stoichiometry and the characteristics of DPAOs are relatively limited [10,11].

The main objectives of the current work are the enrichment of an activated sludge consortium with DPAOs and the study of the effects of different operational parameters on the intracellular storage products transformations and the performance of anoxic EBPR.

The present study was conducted in two operational periods lasting for more than six months each. During the first operational period three different influent COD/P ratios were examined by varying the influent COD concentration. During the second operational period three anoxic/anaerobic tank volumes ratios and hence three anoxic to anaerobic HRT ratios were imposed by altering the anaerobic tank volume. Each operational period started by seeding the laboratory plant with activated sludge with no phosphorus removal ability, withdrawn from the municipal wastewater treatment plant (WWTP) of the city of Xanthi. Almost 60 d of continuous plant operation after each inoculation at a mean hydraulic residence time of 15 h, biomass enrichment with DPAOs was achieved as verified by FISH analysis. From this point on and for the rest of each operational period biomass PHAs, glycogen, and phosphorus content, as well as concentration of acetate, phosphate and nitrate were monitored regularly since they are all considered to participate in enhanced biological phosphorus removal [3].

2. Materials and methods

2.1. Experimental setup

A continuous flow lab scale activated sludge plant was built according to Fig. 1 and operated for a time period of more than one year in order to conduct this study. The system consists of two stirred tank reactors and a sedimentation tank. Synthetic wastewater, with acetate as carbon source, is introduced to the anaerobic reactor for intracellular PHA synthesis and breakdown of polyphosphate by DPAOs. The mixed liquor is then pumped to the anoxic reactor, where nitrate (NO_3^-) is continuously dosed. Here, DPAOs accomplish denitrification on the anaerobically stored PHA, using the energy produced, for replenishment of their polyphosphate content, so as phosphorus uptake from the mixed liquor is achieved. Anoxic tank mixed liquor is transferred to the sedimentation tank where sludge is being separated from the supernatant and pumped back to the anaerobic reactor. Effective volumes of the system tanks were 1.2/1.6/2 l for the anaerobic, 2.8 l for the anoxic and 1.3 l for the sedimentation tank. Influent flow rate was fixed at 0.3 l/h, resulting to a mean hydraulic residence time of approximately 15 h. Sludge recirculation rate was equal to the influent flow (100% recirculation), in order to avoid sludge accumulation in the sedimentation tank bottom. Mixed liquor temperature was constantly

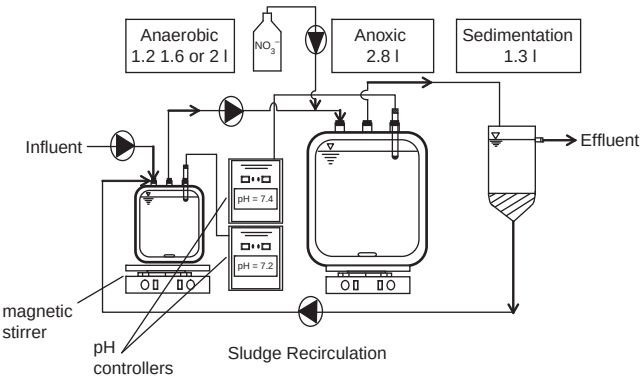

Fig. 1. Laboratory scale continuous flow activated sludge plant.

Table 1
Synthetic medium composition

Synthetic medium	Influent (mg/l)
Carbon source	
CH_3COOH	200, 300, 400 mg COD/l
Phosphorus source	
K_2HPO_4 0.049 g/l	15 mg PO_4^{-3}–P/l
KH_2PO_4 0.028 g/l	
Basic medium	
$MgSO_4 \cdot 7H_2O$	0.6 g/l
$CaCl_2 \cdot 2H_2O$	0.07 g/l
NH_4Cl	0.227 g/l
Trace Mineral	2.0 ml/l
$FeCl_3 \cdot 6H_2O$	1.5 g/l
H_3BO_3	0.15 g/l
$CuSO_4 \cdot 5H_2O$	0.03 g/l
KI	0.03 g/l
$MnCl_2 \cdot 4H_2O$	0.12 g/l
$Na_2MoO_4 \cdot 2H_2O$	0.06 g/l
$ZnSO_4 \cdot 7H_2O$	0.12 g/l
$CoCl_2 \cdot 6H_2O$	0.15 g/l

kept at 20 ± 1°C via indoor temperature control and pH was fixed in each tank by using pH controllers, with automated addition of NaOH or HCl, when necessitated. The pH was set at 7.2 and 7.4 for the anaerobic and anoxic reactor, respectively, in order to minimize chemical phosphorus precipitation. Composition of the synthetic wastewater is shown in Table 1 [10]. Acetic acid at various concentrations was used as the sole carbon source. Addition of nitrate in the anoxic reactor was such that residual concentration of 1–2 mg NO_3^-–N/l, was constantly detected in the effluent.

2.2. *Analytical methods*

MLSS, MLVSS, COD, and orthophosphate were determined according to Standard Methods [12]. Nitrate was determined by a modified second derivative UV spectroscopy method [13]. Biomass total phosphorus content was determined after persulfate digestion [14] by the stannous chloride method [12]. PHAs were determined by gas chromatography after acidic propanolysis [15]. Biomass total carbohydrates content was determined after sludge digestion with 0.6 M HCl [16] by the anthrone method [17,18]. Sludge samples were periodically fixed in 4% formaldehyde solution and FISH was performed according to [19]. The Cy-3 labeled oligonucleotide probes employed are listed at Table 2. PAO462, PAO651 and PAO846 are all specified for most *Accumulibacter* [20] and were applied as a mixture containing equal amounts of each probe (PAOmix) [11].

Table 2
Oligonucleotide FISH probes employed in this study

Probe	Sequence 5′-3′
PAO462	5′-CCG TCA TCT ACW CAG GGT ATT AAC-3′
PAO651	5′-CCC TCT GCC AAA CTC CAG-3′
PAO846	5′-GTT AGC TAC GGC ACT AAA AGG-3′

3. Results and discussion

A series of analyses were conducted on regular basis during the first experimental period, which lasted for several months after acclimatization in order to determine the effect of three acetate concentrations to the biomass nitrogen and phosphorus removal ability. The anaerobic tank volume was fixed at 1.6 l for this series of experiments. Once the optimum COD concentration was determined, the impact of the anoxic to anaerobic tank volume ratio on the system performance was studied. In parallel during the same time period, biomass samples were subjected to FISH analysis for the validation of the presence of DPAOs in the activated sludge. The determined parameters were the specific denitrification rate, the specific phosphorus release and uptake rates, the biomass polyhydroxyalkanoates and carbohydrates contents and the biomass total phosphorus content.

In Fig. 2, the effect of the COD concentration on the specific phosphorus release rate and the specific nitrogen and phosphorus removal rates is depicted. For both acetate doses of 200 and 300 mg COD/l, the acetate uptake in the anaerobic tank was complete, whereas for the maximum influent acetate concentration of 400 mg COD/l, there was a residual acetate concentration of 60 mg COD/l detected in the anaerobic tank effluent. As depicted in this figure, the optimum COD concentration regarding phosphorus uptake rate was found to be 300 mg/l. The maximum denitrification rate was observed for the COD concentration of 400 mg/l but this could be partly attributed to nitrate reduction by ordinary denitrifying heterotrophic organisms which utilize the excess acetate residues passing to the anoxic tank, for nitrate reduction. The specific denitrification rates observed were between 2.9 to 4.3 mgN–NO_3^-/g VSS·h, similar to those already reported in previous studies [21,22].

The variations of the PHAs, carbohydrates and biomass total phosphorus content in correlation to the influent COD concentration are depicted in Fig. 3. In this Figure the percentage of PHAs utilization, carbohydrates synthesis and phosphorus storage during the anoxic phase are shown. For the acetate concentration of 300 mg/l COD, phosphorus storage in the anoxic tank

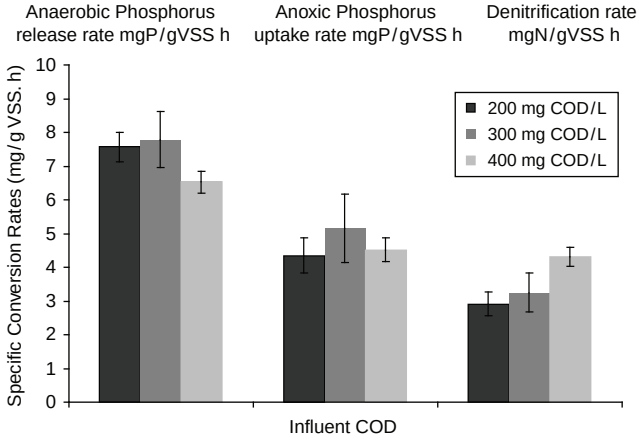

Fig. 2. Effect of influent acetate concentration on the specific phosphorus release rate and the specific phosphorus uptake and denitrification rates (Anoxic to Anaerobic tank volumes ratio: 1.75).

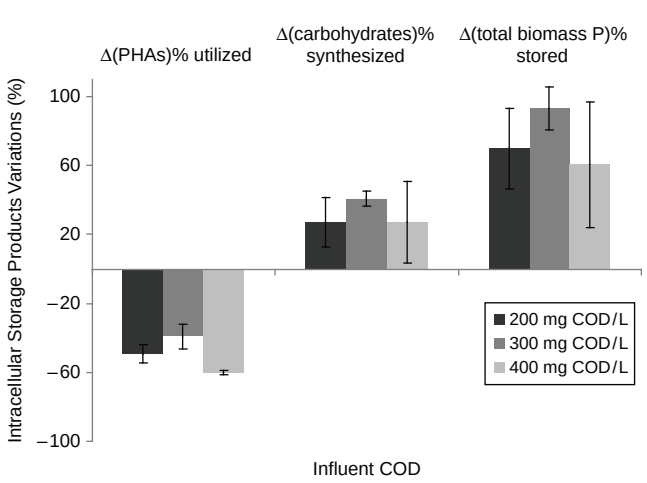

Fig. 3. Effect of influent acetate concentration on the biomass polyhydroxyalkanoates, carbohydrates and total phosphorus content variations in the anoxic reactor. (Anoxic to Anaerobic tank volumes ratio: 1.75; values represent % difference from original parameter value).

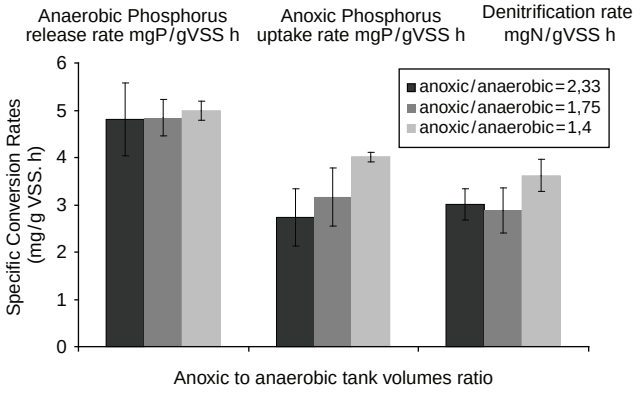

Fig. 4. Effect of the anoxic to anaerobic tank volumes ratio on the specific denitrification and phosphorus uptake rates and on the specific phosphorus release rate for 300 mg/l influent COD concentration.

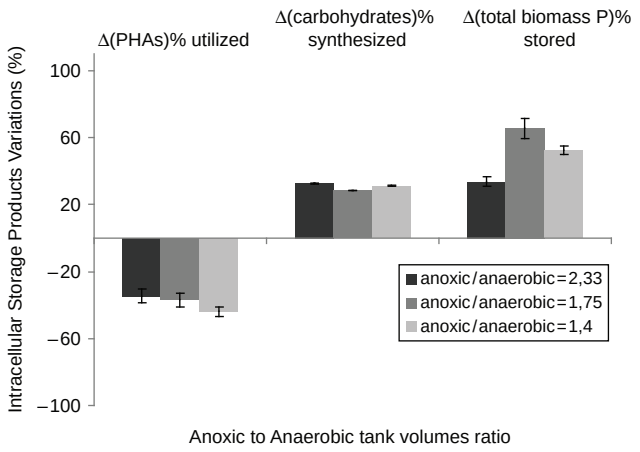

Fig. 5. Effect the anoxic to anaerobic HRT ratio on the biomass polyhydroxyalkanoates, carbohydrates and total phosphorus content variations for 300 mg/l influent COD concentration (Values represent percentage difference from original parameter value).

was maximum as well as carbohydrates production. PHAs utilization was maximum when the influent COD concentration was 400 mg/l, but this could be due to the higher PHAs synthesis in the anaerobic phase. Complete phosphate removal from the mixed liquor of the anoxic tank was observed for the experiments with influent COD concentration of 300 mg COD/l, which could have caused the lower anoxic PHAs consumption depicted in Fig. 3. By taking into account, the increase of the biomass total phosphorus content during the anoxic phase and the phosphorus uptake rate as the most important parameters for efficient and stable EBPR, the influent acetate concentration of 300 mg/l COD was considered as optimum and this concentration was used throughout the rest of the study.

After the completion of the first experimental set, the laboratory plant operation was stopped. A second acclimatization period of more than 60 d followed the inoculation of the laboratory plant with biomass which had no phosphorus removal ability. In the second experimental period and since the optimum influent COD concentration was determined, the effect of the anoxic to anaerobic tank volumes ratio (i.e., anoxic to anaerobic HRT) on the laboratory plant performance was examined.

In Fig. 4 the effect of the anoxic to anaerobic HRT ratio on the specific phosphorus release, the specific phosphorus uptake and the specific denitrification rate is depicted. The specific phosphorus release rates observed during the anaerobic phase remained practically constant regardless of the anoxic to anaerobic HRT ratio. The conclusion that the length of the anaerobic period has minor impact on the overall EBPR performance, assuming that sufficient time has been given for substrate uptake by the bacteria [23], has been reported in the literature although for different types of treatment plants [24–26].

On the other hand, the specific denitrification rate increased at the lower anoxic to anaerobic HRT ratio and was almost constant for the other two ratios. The specific phosphorus uptake rate showed strong correlation to the anoxic to anaerobic HRT ratio, since it increased from 2.73 to 3.16 and 4.01 mgP/gVSS·h as the anoxic to anaerobic HRT ratio decreased from 2.33 to 1.75 and 1.4, respectively. It is reported that longer anaerobic HRT favors the PAOs in terms of substrate uptake [27] and might result to higher specific phosphorus uptake rates in the subsequent anoxic phase. Recently, a survey of full scale WWTPs in Netherlands [28] concluded that the anoxic HRT rather than the anaerobic one has greater impact on the overall EBPR performance.

Fig. 5 demonstrates that the PHAs utilization degree and the biomass total phosphorus content increase were also influenced by the anoxic to anaerobic HRT ratio when biomass from the anaerobic tank is subjected to anoxic conditions. The carbohydrates synthesis degree, at the same time remained more or less constant. The discrepancies observed when corresponding values of the two experimental periods are compared could be attributed to differences in the biomass used to seed the laboratory plant for the start up of the two experimental periods.

The anoxic to anaerobic HRT ratio had minimal impact on the biomass growth yield, which remained substantially constant at about 0.27 g VSS/gCOD, similar to values already reported for denitrifying EBPR sludge [2,10,29]. Phosphorus uptake to nitrogen denitrified ratio increased along with the anaerobic volume, as can be seen in Table 3 and reached 1.11 mg PO_4^{-3}–P/ mg NO_3^- – N. Similar values are documented in several studies [22,30,31].

The validation of the presence of PAOs in the acclimatized biomass came from Fluorescence In Situ Hybridization (FISH) of a number of samples using the appropriate, PAO specific probes. A typical epifluorescence image of the biomass, after the acclimatization period is shown in Fig. 6.

Table 3
Effect of the anoxic to anaerobic tank volume ratio on the phosphorus to nitrogen removal ratio and the biomass growth yield

Anoxic to anaerobic HRT ratio	P to N removal ratio g/g	Standard deviation	Biomass growth yield g VSS/g COD	Standard deviation
2.33	1.06	±0.15	0.25	±0.01
1.75	1.09	±0.03	0.27	±0.01
1.4	1.11	±0.08	0.27	±0.00

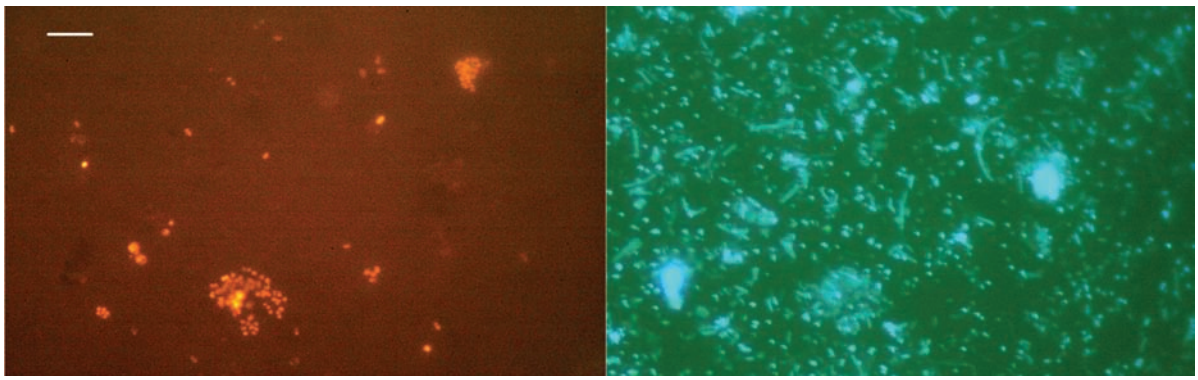

Fig. 6. Epifluoresce micrographs of activated sludge sample after the second acclimatization period, showing the same microscopic field stained with PAOmix (left) and DAPI (right) (Bar represents 10 μm).

4. Conclusions

The results of this study show that there is an optimum influent COD/P concentrations ratio of 20 (300 mg COD/l and 15 mg PO_4^{-3}–P/l) for the laboratory scale plant, when specific phosphorus uptake rate is of concern. Phosphorus storage and carbohydrates synthesis during the anoxic phase were also maximum for this COD/P concentrations ratio. PHAs utilization at the same time was minimum, implying efficient stored carbon utilization during phosphorus removal. When the effect of anoxic to anaerobic HRT ratio was examined, the results showed moderate variations among the three ratios studied. The specific phosphorus release rate remained constant and so did the specific denitrification rate with the exception of the ratio of 1.4 when it slightly increased. However, the specific phosphorus uptake rate increased proportional to the anaerobic tank volume and so did the PHAs utilization degree, while carbohydrates synthesis remained constant irrespective to theHRT ratio. The biomass growth yield was found equal to 0.27 g VSS/gCOD and showed minor differences among the three experimental sets of the second operational period.The phosphorus to nitrogen removal ratio increased as the anaerobic volume also increased. In order to verify that all the abovementioned results correlate with the presence of DPAOs in the activated sludge, FISH analysis was performed, which undoubtebly proved the existence of PAOs in the laboratory scale plant.

Conclusively influent COD/P concentrations were found to affect the system performance since for the intermediate value tested, the laboratory plant achieved the higher specific P release and specific P uptake rates. The anoxic to anaerobic tank volumes ratio and hence the respective HRT ratio had minor impact on the laboratory plant performance since only the specific anoxic phosphorus uptake rate slighty increased along with the anaerobic HRT. The continuous flow anaerobic/anoxic laboratory plant had satisfactory performance irrespective to the operational alterations imposed proving that anoxic EBPR is a promising alternative to the conventional aerobic EBPR systems. By integrating experimental results for the PAO metabolism and the relevant intracellular storage products with the process engineering, anoxic EBPR can be optimized regarding its performance and operation.

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