(39) EFFECT OF ANAEROBIC SRT ON COMPLETE PHOSPHATE REMOVAL IN A POST-DENITRIFICATION SYSTEM

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A continuous flow post-denitrification (Dephanox type) lab-scale system fed with raw wastewater was operated in order to investigate the effect of the anaerobic solids retention time (anaerobic SRT) on phosphate removal. The presence of an internal settler just after the anaerobic tank enhanced the anaerobic SRT. Additional enhancement was provided by accumulation of solids into the internal settler under extreme operational conditions that seemed to be optimal for nutrients removal. Batch experiments with Dephanox sludge showed that the particulate COD fraction contributed with an additional 50% of phosphate release, thus an additional VFAs production can be inferred. In the case of the Dephanox sludge, the enhanced anaerobic SRT assisted effectively to achieve complete phosphate removal. As reference, the same batch experiments with AO and A_2O sludge from a full scale plant fed with primary effluent were carried out. The results showed that particulate COD fraction did not contribute significantly on the VFAs production. The combination of long SRT and abundant particulate COD apparently promoted fermentative organisms and denitrifying phosphorus accumulating organisms (DN-PAOs) acting in a syntrophic association on phosphorus removal.

Key Words: *Dephanox configuration, volatile fatty acids (VFAs), anaerobic solids retention time (anaerobic SRT), fermentative organisms, denitrifying phosphorus accumulating organisms (DN-PAOs)*

1. INTRODUCTION

Anaerobic-anoxic (A_2) configurations have been proposed by many researchers, just to name a few of them, Wanner *et al.*[1], Kuba *et al.*[2] and Bortone *et al.*[3] A_2 configurations showed to accumulate a high population of denitrifiying organisms capable of accumulation of high amounts of polyphosphate (DN-PAOs).[4] Since aeration is not needed for denitrification and phosphorus uptake, application of DN-PAOs allows simultaneous phosphorus and nitrogen removal in an overall economic process. Therefore aeration is applied only for the nitrification process allowing separation of nitrifiers and DN-PAOs in a post-denitrification two sludge configuration (Fig. 1). Separation of nitrifiers and DN-PAOs permits optimal control of nitrification and denitrifying dephosphatation separately, i.e. application of different SRT.

Pre-denitrification systems as A_2O have the denitrification stage before the nitrification stage, thus large recycle of nitrate from the aerobic to anoxic stage is required to achieve low nitrate concentration in the effluent. Therefore a complete nitrogen (nitrate) removal is not possible.

Post-denitrification systems as the Dephanox (Fig. 1) are two sludge systems. A side stream biofilm tank supplies the nitrate needed as electron acceptor in the anoxic tank. Theoretically complete denitrification is possible since the denitrification stage is located after the nitrification stage. Thus no recycle of nitrate is needed.

One of most sensitive operational parameters in a two-sludge post-denitrification configuration is the internal settler underflow rate [b] (Fig. 1). Low underflow rates can extend the anaerobic SRT as well as reduce the untreated ammonium that is bypassed to the anoxic tank. Most of the ammonium in the effluent is the bypassed untreated ammonium. Experimenting on an anaerobic-aerobic (AO) system Matsuo[5] proved that the anaerobic SRT was a key element for stable phosphorus removal, suggesting an optimal anaerobic SRT range (4.6 to 6.3 days). In the case of the Dephanox configuration, only when the internal settler is taken into account for the anaerobic SRT calculation (anaerobic SRT = 4.7 d) the anaerobic SRT is extended 43% complying with Matsuo's findings. Additional extension of anaerobic SRT can be achieved by accumulation of solids into the internal settler. It is possible to accumulate the solids by reduction of the bypass ratio (BPR). The BPR is defined as the ratio of underflow rate of the internal settler to influent flow rate (Fig. 1),

$$BPR = \frac{Q_{bp}}{Q_{RS} + Q} \quad (1)$$

where Q_{bp} = bypassed sludge flow rate from the internal settler

Q_{RS} = return sludge flow rate. In the experiments $Q_{RS} = 0.2\ Q$.

Q = influent flow rate (raw wastewater)

A previous research showed that particulate COD takes precedence when weak wastewater i.e. domestic wastewater is treated in a post-denitrification configuration[6], thus apparently the particulate COD effectively contributed in the production of VFAs that contributed for a complete and stable phosphate removal.

The objective of the present research is to elucidate the importance of particulate COD in conjunction with the extension of anaerobic SRT by operation of BPR.

2. MATERIALS AND METHODS

(1) Continuous flow post-denitrification system

A lab-scale two-sludge post-denitrification system also known as Dephanox (Fig. 1) was operated for 252 days under different operational parameters using municipal raw wastewater as influent with total COD=344 mg-COD·L^{-1} and dissolved COD=108 mg-COD·L^{-1} with a COD:N:P average ratio of 59:8:1 (east municipal wastewater treatment plant of Fukuoka city, Japan).. For the start up of the system, AO sludge from the same municipal plant was seeded into the system with a sludge volume index (SVI) of 120 mL·g^{-1}. Design and operational parameters of the Dephanox configuration are shown in Table 1.

For the anaerobic SRT calculation, the internal settler was taken into account. The content of solids in the internal settler was measured accurately by a momentaneous resuspension of the sludge in the internal settler and posterior TSS measurement of the homogeneous resuspended sludge. Previous to the momentaneous resuspension of the sludge contained in the internal settler, some preventions were taken to avoid overflow into the nitrification tank. The anaerobic SRT was calculated by the following equation:

$$Anaerobic\ SRT = \frac{\sum_{i=1}^{n} Va_i \cdot Xa_i}{Q_w \cdot X_w + (Q - Q_w) \cdot X_e} \quad (2)$$

where SRT = solids retention time

Va_i = volume of anaerobic units

Xa_i = concentration of solids in anaerobic conditions

X_w = concentration of solids in the tank from solids were removed (in this case from the anoxic tank)

Xe = concentration of solids in effluent

Q = influent flow rate

Qw = flow rate of liquid containing the solids to be removed (in this case from the anoxic tank)

n = Number of anoxic stages (reactors)

From Fig. 1, influent is introduced into the anaerobic tank (1) where phosphate is released from PAOs and most of the organic substrate is stored into the activated sludge flocs and into the bacterial cells. The internal settler (2) separates the activated sludge with intracellular and particulate organic substrate from ammonium-rich supernatant. The supernatant introduced in a biofilm tank (3) is nitrified. The substrate-rich sludge is bypassed to the anoxic tank

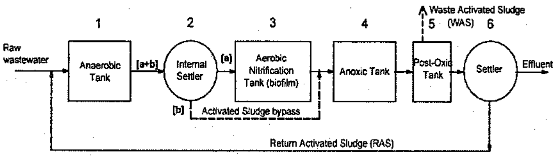

Fig.1 Dephanox type system characteristic: an internal settler bypasses ammonium from anaerobic tank directly to the anoxic tank, appearing in the effluent.

Table 1 Operational parameters of the lab-scale system.

Parameter	Unit	Per. a	Per. b	Per. c	Per. d	Per. e	Per. f
Volume of anaerobic reactor	L	12.1					
Volume of internal settler	L	11.7					
Volume of nitrification reactor (biofilm)	L	14.0					
Volume of anoxic reactor	L	19.6					
Volume of post-aeration reactor	L	5.0					
Influent flow rate	L·d^{-1}	110.0					
Solids wastage flow rate[1)]	L·d^{-1}	3.67					
System SRT (internal settler included)	d	10	10	10	10	18	33
BPR	–	0.33	0.25	0.17	0.13	0.08	0.04
Total HRT (internal settler included)	h	12					

[1)] Wastage from anoxic tank

(4), and is resuspended with the nitrified effluent from the biofilm tank (3). In the anoxic tank (4) nitrates are utilized as electron acceptor for enhanced phosphorus uptake by DN-PAOs. The post-oxic tank (5) polishes up and strips the N_2 gas from the sludge before the final settling (6).

(2) Batch experiments

In order to study the contribution of the particulate COD in the VFAs production, many anaerobic batch experiments were carried out. For reference purposes, AO sludge and A_2O sludge from the municipal east wastewater treatment plant of the Fukuoka city were studied. The full-scale AO system was fed with primary effluent, the full-scale A_2O system was fed with a mix of primary effluent-raw wastewater, and the lab-scale Dephanox sludge was fed with raw wastewater. Therefore three kinds of sludge fed with three kinds of influent were studied. Although direct comparison of Dephanox, AO and A_2O sludge is not possible because of different feeding and operational parameters, it is possible to get some reference.

When the system achieved complete phosphate removal, activated sludge was taken from the system's anoxic tank, aerated to ensure maximum phosphorus uptake, and washed with a mineral solution ($0.6 g \cdot L^{-1}$ $MgSO_4 \cdot 7H_2O$, $0.07 g \cdot L^{-1}$ $CaCl_2 \cdot 2H_2O$, $0.1 g \cdot L^{-1}$ NH_4Cl, $0.1\ g \cdot L^{-1}$ KCl, and 2 $ml \cdot L^{-1}$ trace mineral solution).[2] Finally the clean activated sludge was resuspended with the mineral solution to 1/5 of its original volume. The experiments were carried out in 500 ml (working volume) fermentors, pH was controlled to 7.0±0.1 and anaerobic conditions were kept by N_2 gassing for 5 hours in order to study the effect of long anaerobic conditions in particulate COD.

At the beginning of the experiment wastewater was added to complete the 4/5 of the original sludge's volume. The experiments carried in two fermenters had different wastewater feeding. One of the fermenters was fed with raw wastewater (RWW), therefore high particulate COD content, whereas the second fermenter was fed with filtered raw wastewater (FWW), therefore no presence of particulate COD. Previous to filtration, the raw wastewater was centrifuged and posteriorly filtrated by glassfiber filters (Whatman GF/C) with a nominal pore size of about 1.2 μm. The AO sludge and A_2O were experimented identically.

(3) Analytical methods

NH_4^+, PO_4^{3-}, NO_2^-, NO_3^-, were measured by automated analytical equipment (Autoanalyzer 3 from Bran-Luebbe). The COD measurement was made with dichromate, by a HACH DR/2400. MLSS and MLVSS were measured using glassfiber filters (Whatman GF/C).

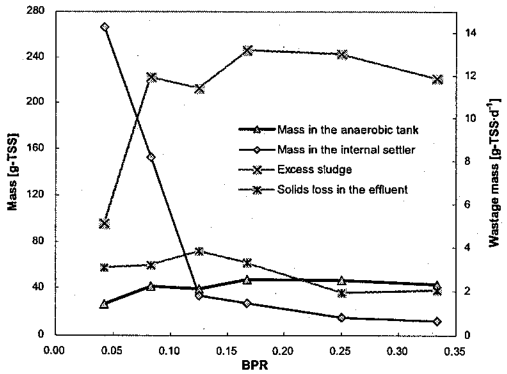

Fig.2 Components of the anaerobic SRT: Mass in the anaerobic tank [g-TSS], mass in the internal settler [g-TSS], excess sludge [$g\text{-}TSS \cdot d^{-1}$] and solids loss in the effluent [$g\text{-}TSS \cdot d^{-1}$].

3. RESULTS AND DISCUSSION

(1) Anaerobic SRT on enhanced biological phosphorus removal

The variation of the internal BPR had two simultaneous objectives: 1. enlargement of anaerobic SRT and 2. reduction of bypassed ammonium that effectively reduces the presence of ammonium in the effluent.

Since the internal settler is in anaerobic conditions and located just after the anaerobic tank, the internal settler was taken into account for the evaluation of the anaerobic SRT. Only the accumulation of solids into the internal settler (Fig. 2) can additionally enhance the anaerobic SRT while at the same time reduce the bypassed ammonium.

During the lab-scale post-denitrification system operation, different BPRs were applied in order to produce accumulation of solids in the internal settler, thus extend the anaerobic SRT. An effective and beneficial anaerobic SRT extension was observed for a narrow BPR interval ranging from 0.13 to 0.08 (Fig. 3).

The system developed a very low SVI during the experiments. From an initial 120 $mL \cdot g^{-1}$, the SVI improved to a lowest value of 55 $mL \cdot g^{-1}$ at 220 days of operation. A slight increase was produced at 250 days of operation when a very low BPR= 0.04 was applied. Under this low BPR a serious unbalance of MLSS took place with a failure in nutrients removal

(Fig. 4).

The system started with a high BPR = 0.33 and an anaerobic SRT=4.0 [d]. During this condition no accumulation of sludge into the internal settler was observed. Significant amount of ammonium was bypassed to the anoxic rector avoiding nitrification, therefore lack of electron acceptor (nitrate) hampered the phosphorus uptake. Ammonium removal was as low as 70%, since most of it was bypassed untreated ammonium. When complete nitrification is achieved in the nitrification tank, all the ammonium detected in the effluent is untreated ammonium bypassed by the internal settler. Therefore to achieve high removal of ammonium, low BPRs should be applied. Complete ammonium removal is possible only when the bypassed untreated ammonium is balanced with the DN-PAOs consumption of ammonium for growth purposes.

From BPRs 0.25 to 0.13 a high and stable phosphate removal was observed. From 98 to 100% of phosphate removal efficiency was achieved (Fig. 4). Experimental data from previous researches showed that metabolic selection of A_2 conditions improve considerably sludge settleability.[1),4),6)] Identically, in spite that the lab-scale post-denitrification system was seeded with an anaerobic–anoxic (AO) sludge that contained filamentous microorganisms (as any other AO sludge does), the settleability kept improving steadily due to the selective properties mentioned above. Although solids accumulation in the internal settler was increased in more than 100% during this range, increment of solids concentration in the effluent and distribution of sludge in the whole system could offset the anaerobic SRT extension (Fig. 2 and Eq. 2). In spite of more than 100% of solids accumulation increase in the internal settler could be measured, the anaerobic SRT was extended slightly for BRPs of 0.25 to 0.13 (Fig. 3). Fig. 3 shows a moderate increase of phosphate release in the internal settler that may be explained by an additional production of VFAs from the particulate COD accumulated in this unit.

In regards to ammonium removal efficiency, in this range (BPRs from 0.25 to 0.13), it was increased as much as 21% by only reducing the bypassed ammonium flow.

From BPRs of 0.13 to 0.08 significant accumulation of sludge was observed. The anaerobic SRT could be effectively extended by BRP reduction (Fig. 3). Sludge settleability kept its constant improvement during this range till a minimum of 55 $mL \cdot g^{-1}$ at 220 days. The anaerobic SRT started to enhance after BPR $\leq$ 0.13 and the anaerobic SRT was extended from 4.8 [d] to 13 [d] (Fig. 3) observing

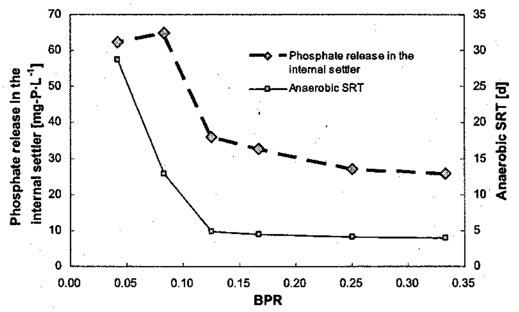

Fig.3 Phosphate release in the internal settler and anaerobic SRT extension.

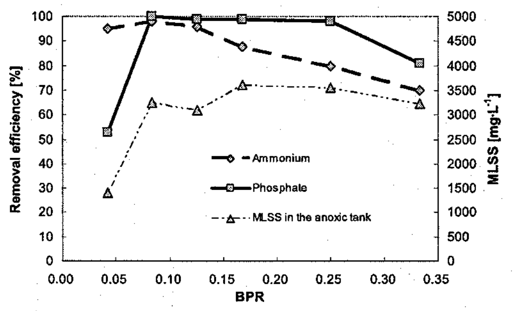

Fig.4 Concentration of MLSS in the anoxic reactor, phosphate removal and ammonium removal efficiency.

stable phosphate removal (96%) with a considerable reduction of bypassed ammonium (Fig. 4). The high denitrifying dephosphatation activity of the A_2 sludge has been reported by many previous researches,[3),4),6)] and one of them suggested that particulate COD plays an important role in microorganisms selection.[6)] Fig. 3 shows a notably high increase of phosphate release into the internal settler that may be explained by an additional production of VFAs from the particulate COD accumulated in the internal settler under the extended anaerobic SRT (for BPRs of 0.13 to 0.08) assisting in the denitrifying dephosphatation process in the anoxic reactor.

From the original BPR=0.33 to BPR=0.13, the total ammonium removal efficiency improved as much as 39%. In many individual observations complete ammonium removal was observed. It could be stated that complete simultaneous ammonium and phosphate removal was achieved for $0.08 \leq BPR \leq 0.13$ showing a notably high denitrifying dephosphatation activity of the A_2 sludge.

For the last range, BPRs of 0.08 to 0.04 produced a high accumulation of sludge into the internal settler. The volume of accumulated sludge in the internal settler was increased from 40% to as much as 90% of the internal settler's working volume, accumulating an extreme amount of solids (Fig. 2). This extreme accumulation although extended considerably the anaerobic SRT to 29 [d] affected the sludge distribution in the system (Fig. 4). The MLSS concentration in the anoxic tank was decreased from a steady average of 3400 mg/L to a very low 1400 mg/L. Such a low MLSS concentration strongly hampered the phosphate removal process.

(2) Batch Experiments - Particulate COD on VFAs Production

In order to elucidate the high denitrification phosphorus accumulating activity of the post-denitrification system, anaerobic batch experiments were carried out (Fig 5). Sludge from an AO and A_2O configurations was also experimented for comparison purposes (Fig. 6 and 7). The Dephanox, AO and A_2O systems were cultivated with raw wastewater, a mix of raw wastewater and primary effluent, and primary effluent respectively. Therefore sludge was "acclimated" to different kinds of influent with different particulate COD content. Evidently the sludge characteristics were affected by the influent type not only in their microorganism population but also in the suspended solids concentration, thus although a direct comparison is not possible, the results can be used as reference.

The batch experiments were carried out using raw wastewater (RWW) and filtered wastewater (FWW), therefore the contribution of particulate COD in VFAs production and consequent phosphorus release could be measured.

In anaerobic conditions DN-PAOs release orthophosphate as source of energy for acetic acid (HAc) consumption and storage as intracellular carbon source in the form of poly-β-hydroxybutyrate (PHB). Therefore increased phosphate release implies an increased PHB storage as intracellular carbon source by the DN-PAOs. Consequently an increased storage of PHB provides the supplementary carbon sources for an enhanced P removal in anoxic conditions.

In the batch experiment residual VFAs could not be measured after the initial 20 minutes because of the rate of VFAs consumption was higher than the rate of VFAs production. An indirect estimation of VFAs production can be inferred by the phosphate released since a fixed ratio of 1 to 1.5 g-P per every g-C of consumed HAc was reported by a previous research.[7] In the case of Dephanox sludge, the presence of particulate COD increased the phosphate release from 26 to 39 mg-P·g-VSS^{-1}. Using the previous equivalence, 13 mg-C·g-VSS^{-1} were estimated as VFAs additional production from the particulate fraction of COD. It represents an increment of 50% in VFAs production.

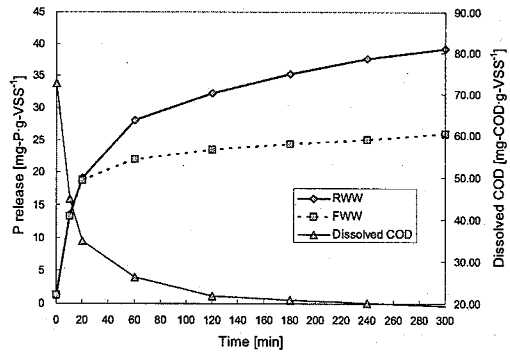

Fig.5 Phosphorus release in an anaerobic batch experiment using wastewater with and with out particulate COD for the post-denitrification sludge.

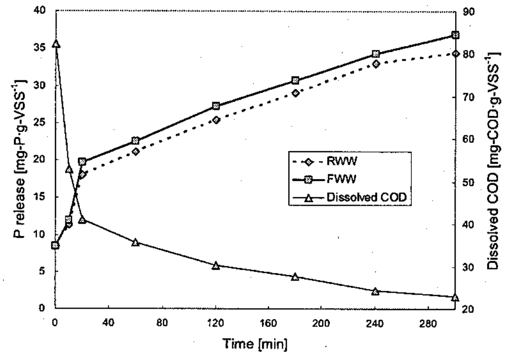

Fig.6 Phosphorus release in an anaerobic batch experiment using wastewater with and with out particulate COD for the AO sludge.

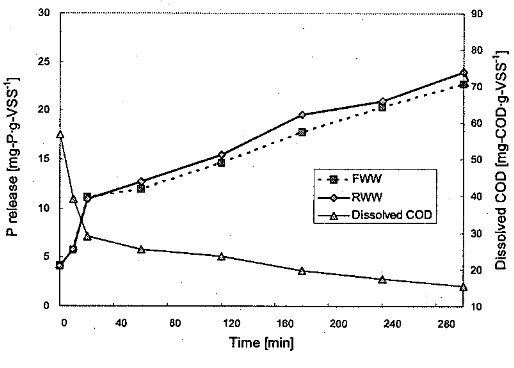

Fig.7 Phosphorus release in an anaerobic batch experiment using wastewater with and with out particulate COD for the A_2O sludge.

According to the American standard methods[8] and most of the standard methods, the glassfiber filter (Whatman GF/C) with a nominal pore size of about 1.2 μm is used for the separation of dissolved solid from suspended (or filterable) solids in wastewater. Although this definition has been used from the particle-size point of view, researches in the field of simulation point out that a considerable amount of particles passes thought the filter pore. From a fermentative point of view, particles with nominal size less than 1.2 μm are still particulate COD and not dissolved COD.[9)] Thus this fraction although considered dissolved can not be considered readily biodegradable substrate. Consequently in order to obtain an influent with only dissolved COD, not only filtration but also previous chemical flocculation should have been applied.

In the batch experiments carried out in this research, chemical flocculation was not applied, and therefore the results of the batch experiments (Fig. 5, 6 and 7) suggest that effectively a contribution of slow biodegradable substrate (fine particulate COD that passed through filtration) started taking place 20 minutes after filtered wastewater (FWW) was exposed to the activated sludge. At this point, the phosphate release rate had a sudden reduction showing a depletion of readily biodegradable substrate (dissolved) and sole contribution of fine particulate COD in all the batch experiments can be inferred.

The batch experiment with post-denitrification sludge showed a remarkable difference in phosphorus release (Fig. 5). An increment of about 50% of phosphorus release was observed at the end of the batch experiment. The experiment proved that particulate COD effectively contributed in the VFAs production process. Hydrolysis acted on the particulate or high molecular weight soluble substrates. Hydrolysis is catalyzed by hydrolytic enzymes excreted by bacteria present in the sludge. As a second phase acidogenesis or anaerobic oxidation of short-chain fatty acids produce the complementary acetate that assisted in the stable and complete phosphate removal. In the case of the post-denitrification sludge methanogenesis can not take place due to the high rate of VFAs consumption by DN-PAOs. In this sense the fermentation process is carried out partially in a process known as pre-fermentation.[10)] These results imply the presence of two fundamental kinds of organisms: fermentative organisms and DN-PAOs acting coupled in a syntrophic relationship. Therefore pre-fermenters and DN-PAOs have a biological relationship in which they are dependent.

The AO and A_2O sludge did not show significative activity of pre-fermentative organisms. The experiments (Fig. 6 and 7) do not show additional phosphate release and consequently the complementary production of VFAs. Thus no significative presence of pre-fermentative organisms in the AO and A_2O sludge can be inferred.

4. CONCLUSIONS

The low SVI (around 60 $mL \cdot g^{-1}$) developed in the post-denitrification system allowed significant extension of the anaerobic SRT for very low BPRs (BPRs<0.13). High and stable phosphate removal was observed for a wide range of BRP (0.25 to 0.08). Accumulation of sludge in the internal settler extended the phosphate release implying an extended production of VFAs by prefermentation. It stabilized the phosphorus removal and enhanced the nitrogen removal process. Batch tests showed that a metabolic selection of the post-denitrification configuration promoted a high presence of two fundamental kinds of organisms: fermentative organisms and DN-PAOs acting coupled in a syntrophic relationship.

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