Calcium phosphate precipitation in a SBR operated for EBPR: interactions with the biological process

R. Barat, T. Montoya, L. Borras, J. Ferrer and A. Seco

ABSTRACT

The aim of this paper is to study the precipitation process in a sequencing batch reactor (SBR) operated for EBPR (enhanced biological phosphorus removal) and the possible effects of this phosphorus precipitation in the biological process. Four experiments were carried out under different influent calcium concentration. The experimental results and the equilibrium study, based on the Saturation index calculation, confirm that the process controlling the calcium behaviour in a SBR operated for EBPR is the calcium phosphate precipitation. This precipitation takes place at two stages initially precipitation of the ACP and later crystallization of HAP. Also the accumulation of phosphorus precipitated was observed when the influent Ca concentration was increased. In all the experiments the influent wastewater ratio P/COD was kept constant. It has been observed that at high Ca concentration the amount of poly-P granules decrease, decreasing the ratio between phosphate release and acetate uptake (P_{ uptake}/Acuptake). Changes on PAO and GAO populations during the experimental period were ruled out by means of methylene blue stains for poly-P detection. These results confirmed the phosphate precipitation as a process that can affect to the PAO metabolism and the EBPR performance.

Key words | calcium, EBPR, glycogen accumulating metabolism (GAM), polyphosphate accumulating metabolism (PAM), precipitation

INTRODUCTION

Since early days of the use of enhanced biological phosphorus removal (EBPR), it was observed that the biologically induced precipitation processes could contribute significantly to the amount of phosphorus removed in the system (Arvin 1985).

These precipitation processes could have an important significance in a Sequencing Batch Reactor (SBR) operated for EBPR. In these reactors, in contrast to the continuous reactors, the concentration of different parameters, i.e. PO_{4}^{3-}, change drastically throughout the operation cycle. Therefore, if the phosphate concentration rises above a certain level, depending on the concentration of calcium and pH, calcium phosphate precipitation will occur.

Previous studies (Barat et al. 2006) observed a clear influence of influent Ca concentration on the EBPR process. These authors observed that at high Ca concentration the amount of poly-P granules available as an energy source decrease, decreasing the amount of phosphate released per unit of acetic acid taken. According to an extensive literature review, two different mechanisms of calcium fixation in activated sludge have been observed which could explain its effect on the biological process: fixation with phosphorus by biologically induced precipitation process and fixation as a counterion in the internal poly-P granules which are not available for the EBPR process (Scho¨nborn et al. 2001).

The aim of this paper is to study the precipitation process in SBR operated for EBPR and the possible effects of this phosphorus precipitation in the biological process.
MATERIALS AND METHODS

Experimental device

A laboratory scale SBR has been operated under anaerobic–aerobic conditions for biological phosphorus removal. Figure 1 shows a schematic diagram of the SBR operation cycle. The SBR was operated with four 6-h cycles per day. The SBR description and operation parameters are listed in Table 1. The SBR was controlled using a timer automaton. The reactor was equipped with conductivity, redox, pH, temperature and oxygen electrodes. The dissolved oxygen concentration in the aerobic phase was controlled at 3 mg l\(^{-1}\). The experiments were performed at controlled temperature (20 ± 1°C). The pH was not controlled and it ranged from 7.0 to 8.8. A data acquisition program was used to continuously store process information. The synthetic wastewater used consisted of two separated solutions prepared with decalcified tap water. One contained mineral compounds, including a significant amount of \(\text{K}_2\text{HPO}_4\), and the other one contained acetate (pH adjusted to 7.3 with NaOH). The composition of the synthetic wastewater is presented in Table 2.

![Figure 1 SBR process cycle.](image-url)
determined by a method proposed by Moosbrugger et al. (1992). Precipitated phosphorus was determined according to the method proposed by De Haas et al. (2000). Poly-P determination was performed with the Methylene Blue method (Seviour & Blackall 1999). Because of the strong floc formed in the SBR, the samples were disaggregated and diluted with a sequence of two blenders (Ziglio et al. 2002). After disaggregation, samples were dried on smears, and stained with Methylene Blue for poly-P visualization. Samples were examined under bright field at 1000× magnification. A minimum of 20 randomly chosen microscopic fields from each sample were quantified for Poly-P content.

### RESULTS AND DISCUSSION

**Calcium precipitation**

Figure 2 shows the measured values of acetic acid, phosphate, calcium and pH evolution in a cycle for each experiment.

As pointed out by different authors, the Ca profiles show that this cation is not clearly involved in the biological phosphate dynamic as potassium and magnesium (Barat et al. 2005). Therefore, the Ca variations throughout the SBR operation cycle can be explained by calcium precipitation as will be discussed later. As can be seen, Ca remains quite constant during the anaerobic phase in all the experiments despite the phosphate increase probably due to the pH decrease during the anaerobic phase. Nevertheless, except in the experiment 1 with low Ca concentration, during the aerobic phase Ca concentrations change significantly. As can be seen in Figure 2 (markedly in experiments 3 and 4), Ca concentration drops quickly once the aerobic conditions were established. This fact could be

<table>
<thead>
<tr>
<th>Operational conditions in the experiments</th>
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<tr>
<td>Experiment</td>
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<tr>
<td>SRT (days)</td>
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<tr>
<td>Feed P/COD ratio (gP gCOD⁻¹)</td>
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<tr>
<td>Inf. Ca conc. (gCa m⁻³)</td>
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**Figure 2** Experimental results obtained through the experiments: VFA, phosphate, calcium and pH.
explained as a calcium phosphate precipitation caused by the high phosphate concentration and the CO₂ stripping increasing the pH value. Once the concentration of phosphate decreases to a certain level due to the biological phosphate accumulation, the concentration of calcium starts to increase until achieve values a little bit lower than the starting cycle concentrations. This calcium increase could be due to calcium phosphate dissolution caused by the phosphate uptake by PAO. Calcium phosphate precipitation is a very complex process involving various parameters, such as calcium and phosphate ions concentrations, ionic strength, temperature, ion types, pH and time (Boskey & Posner 1973; Van Kemenade & de Bruyn 1987). The calcium phosphate precipitation follows the Ostwald rule, which foresees that the least thermodynamically stable phase formed is the first one and this phase works as a precursor of the most stable phase, which is hydroxyapatite (HAP) in this case. The most common precursors for the HAP formation are brushite (DCPD, CaHPO₄·2H₂O), octacalcium phosphate (OCP, Ca₄H(PO₄)₃·2.5H₂O) and amorphous calcium phosphate (ACP, Ca₅(PO₄)₂·xH₂O) (Montastruc et al. 2003). The Ca/P molar ratios in these calcium phosphates all fall in between 1.0 and 1.7, which makes it difficult to distinguish the solid precipitated in our experiments by simple mass balances. Besides the calcium phosphate precipitation another solid that can affect to the calcium concentration through the SBR cycle is the calcium carbonate.

Due to the complexity of the precipitation process, to estimate the calcium phosphate formed and calcium carbonate precipitation in the experiments, the Saturation Index (SI) was calculated. The SI is used to describe the saturation state from a thermodynamic point of view of the aqueous phase composition versus different solids (Equation 1).

\[
\text{SI} = \log \frac{\text{IAP}}{K_{\text{SP}}} 
\]  

(1)

where IAP represents the ion activity product and K_{SP} the thermodynamic solubility product.

When SI is equal to zero the solution is in equilibrium; when SI is negative the solution is undersaturated and no precipitation occurs; when SI is positive the solution is supersaturated and precipitation could occur. Therefore, the SI values can be used to evaluate the effect of the solution conditions on the tendency and extent of the precipitation. The SI values were calculated for all the experiments using the equilibrium speciation model MINTEQA2 (Allison et al. 1991). MITEQA2 was used to calculate the chemical speciation of the aqueous phase during the cycle from the experimental values in each experiment. Once obtained the aqueous phase speciation the SI was calculated for each solid. Figure 3 shows the SI variation obtained in the experiment 3.

As can be seen in Figure 3, the SI profile of calcium carbonate predicts that it was possible its precipitation throughout the whole aerobic phase. Nonetheless, the experimental results showed clear calcium dissolution at the middle of the aerobic phase, suggesting an improbable calcite formation. This fact could be due to the inhibition of calcite crystallization by phosphate reported by Plant & House (2002). Regarding the calcium phosphate, OCP and DCPD do not precipitate because their SI predicts precipitation in the anaerobic phase while Ca concentration does not change under anaerobic conditions. The best fit resulted with ACP which was able to predict thermodynamically: non solid formation during the anaerobic phase, precipitation in the first half of the aerobic phase and dissolution at the end of the aerobic phase.

Therefore, the experimental results and the equilibrium study confirm that the process controlling the Ca behaviour in a SBR operated for EBPR is the calcium phosphate precipitation. This precipitation takes place at two stages initially precipitation of the ACP and later crystallization of HAP.

The conversion of ACP to the thermodynamically stable HAP is not straightforward. A study developed by Boskey &
Posner (1973) about the transformation of ACP in HAP showed that the required time for total conversion may vary with pH. In this work the transformation of ACP in HAP could be observed by the accumulation of phosphorus precipitated at the end of the aerobic phase (see Table 4). This phosphorus accumulation could not be possible only by the formation of ACP, which precipitates and is dissolved completely during the operation cycle.

Interactions with the biological process

As it is well known, PAO bacteria (responsible for EBPR) use alternating anaerobic-aerobic metabolism in which they anaerobically take up volatile fatty acids, especially acetate. Adenosine triphosphate (ATP) requirements for acetate transport (Smolders et al. 1994) and its conversion to acetyl coenzyme A are though to be provided by hydrolysis of glycogen and intracellular poly-P, which leads to soluble phosphorus release. Under anaerobic conditions two kinds of metabolism which compete for the acetic acid could coexist: polyphosphate accumulating metabolism (PAM) and glycogen accumulating metabolism (GAM) (Schuler & Jenkins 2003). The main difference is that in GAM activity glycogen is used as an energy source for anaerobic acetic acid uptake instead of internal poly-P (Liu et al. 1997).

The ratio between phosphate release and acetate uptake ($P_{rel}/Ac_{uptake}$) is a typical parameter of the biological phosphorus removal process, used as a good indicator of the metabolism involved in the acetic acid uptake under anaerobic conditions (Schuler & Jenkins 2003). There is a strong variability in the $P_{rel}/Ac_{uptake}$ ratio found in literature, changing from 0.03 to 0.8 gP gCOD$^{-1}$. A good review of these values could be observed in Schuler & Jenkins (2003). According to literature review, this variability could be due to pH variations (Comeau et al. 1986; Smolders et al. 1994; Filipe et al. 2001), the ratio between phosphorus and chemical oxygen demand (P/COD) in raw wastewater (Randall et al. 1992; Schuler & Jenkins 2003), and the ratio between PAO and GAO bacteria present in the sludge (Satoh et al. 1994; Manga et al. 2001). When GAM activity increases relative to PAM activity, $P_{rel}/Ac_{uptake}$ should decrease.

During the whole experience, anaerobic and aerobic samples were taken for poly-P examination. Poly-P granules stained bright pink-violet, and GAO forming tetrads stained same colour but only on cell wall (Figure 4). From these analyses not appreciable changes in PAO and GAO populations were observed. The percentage of PAO (as poly-P granules) obtained for the aerobic phase was 60–70%, and the percentage of GAO was less than 11% for all the experiments. It is suggested that PAO population was stable and highly enriched during the experimental period. However, further research will be necessary to obtain reliable results. In situ identification by FISH could give consistent values of both populations (Serafim et al. 2002).

Likewise the results obtained by Barat et al. (2006), in the four experiments carried out in this study, different values for the $P_{rel}/Ac_{uptake}$ ratio were obtained. However, these variations are not due to influent P/COD ratio (see Table 1), pH values (similar in all the experiments, see Figure 2) and variations in the ratio between PAO and GAO bacteria. These results confirmed that the $P_{rel}/Ac_{uptake}$ variations were directly related to the influent Ca concentration (Figure 5) in which can be observed that the higher influent Ca concentration the lower phosphorus released per acetic acid consumed. Therefore, these results could indicate a change in the bacterial metabolic pathway, prevailing PAM activity at low influent Ca concentration and GAM activity at high concentration, as was suggested by Barat et al. (2006).

| Table 4 | Phosphorus precipitated at the end of the anaerobic phase of each experiment |
|---------|------------------|------------------|------------------|------------------|
| Experiment | 1 | 2 | 3 | 4 |
| TSS (g m$^{-3}$) | 1,257 | 1,902 | 1,877 | 2,134 |
| VSS (%) | 61.3 | 58.9 | 61.0 | 62.5 |
| Precipitated P (gP m$^{-3}$) | 2.8 | 6.0 | 27.3 | 20.9 |

Figure 4 | Methylene blue stain for the detection of Poly-P granules. Micrograms took at 1000x magnification.
As pointed out in the introduction, two different mechanisms of calcium fixation in activated sludge have been reported which could explain this metabolic change: fixation with phosphorus by biologically induced precipitation process, studied in this paper, and fixation as a counterion in the internal poly-P granules stored as “inert” poly-P (Bonting et al. 1993; Schönborn et al. 2001). Both mechanisms affect to the amount of poly-P granules available as an energy source to store the acetic acid as PHA (PAM activity): the precipitation stores phosphate as a solid phase and the fixation stores phosphate as “inert” poly-P granules, decreasing in both cases the energy source from poly-P and displacing the energy source to glycogen metabolism. The precipitation study carried out in this work confirmed the calcium phosphate precipitation in the experiments and its accumulation as HAP throughout the experimental period when the influent Ca concentration was increased. These results confirmed the phosphate precipitation as a process that can affect to the PAO metabolism.

However, further research is needed in order to confirm if the phosphate precipitation is the only mechanism affecting this metabolic change or the coexistence with the accumulation of phosphate as “inert” poly-P, reported by Schönborn et al. (2001).

CONCLUSIONS

The Ca profiles in all the experiments show that Ca is not clearly involved in the biological phosphate dynamic as potassium and magnesium. The experimental results and the equilibrium study confirm that the process controlling the Ca behaviour in a SBR operated for EBPR is the calcium phosphate precipitation. This precipitation takes place at two stages initially precipitation of the ACP and later crystallization of HAP.

Different values for the ratio $P_{rel}/Acuptake$ were obtained for different influent Ca concentrations. These variations were not due to influent P/COD ratio and pH. Microbiological observations confirm that appreciable changes in PAO and GAO populations were not observed in Methylene Blue stains. The ratio $Prel/Acuptake$ has been found to be highly dependent on the Ca concentration, increasing as Ca concentration decreases. These results suggest a change in the bacterial metabolic pathway, prevailing PAM activity at low influent Ca concentration and GAM activity at high concentration. The accumulation of phosphorus precipitated as calcium phosphate at high influent Ca concentration throughout the experimental period confirmed the phosphate precipitation as a process that can affect to the PAO metabolism.

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