Phosphorus release mechanisms during digestion of EBPR sludge under anaerobic, anoxic and aerobic conditions
Dongsu Bi, Xiaopin Guo and Donghui Chen

ABSTRACT
Three laboratory-scale digesters were operated in parallel under anaerobic, anoxic and aerobic conditions to reveal the release mechanisms of phosphorus when digesting enhanced biological phosphorus removal (EBPR) sludge. The variation rates of the parameters associated with phosphorus release were calculated and compared with that of a typical EBPR anaerobic process. The results show that both phosphorus-accumulating organisms (PAOs) and denitrifying phosphorus-accumulating organisms (DPAOs) played important roles in the phosphorus release during the digestion processes. Under anaerobic conditions, the PAOs hydrolyzed internal polyphosphorus (poly-P) into PO$_4^{3-}$/C$_0$-P concurrent with synthesis of polyhydroxyalkanoates (PHA). Under anoxic or aerobic conditions, PAOs and/or DPAOs assimilated part of the PO$_4^{3-}$/C$_0$-P from the digestive liquid using nitrate or oxygen as terminal electron acceptors. Nevertheless, the biological activities of PAOs under anaerobic conditions and DPAOs under anoxic conditions were limited. Moreover, it was the biomass hydrolysis degree that determined the phosphorus release capacity of the sludge, regardless of whether anaerobic, anoxic or aerobic conditions were adopted. Assuming that nitrate was the sole electron acceptor during anoxic digestion of EBPR biomass, the relationship between the consumption of nitrate and uptake of PO$_4^{3-}$/C$_0$-P associated with the denitrifying phosphorus removal (DPR) can be expressed as $\Delta P = 0.11 \times \Delta N$.

Key words | digestion, EBPR sludge, phosphorus, release mechanism

INTRODUCTION
Enhanced biological phosphorus removal (EBPR) is widely accepted as the most economical and sustainable process for the removal of phosphorus from wastewater (Oehmen et al. 2007). The phosphorus-accumulating organisms (PAOs) proliferated under cyclic anaerobic and aerobic conditions in EBPR accumulate excess P in the form of polyphosphorus (poly-P). Net phosphorus removal can therefore be achieved by wasting activated sludge after the aerobic period (Liu et al. 1996; Lin et al. 2005). Generally, the wasted EBPR sludge contains a high level of P content, i.e. about 3–6% of dry weight of the sludge.

Anaerobic digestion is one of the most common biological sludge treatment processes in use today due to its specific advantages including high treatment efficiency, low operation cost and methane-producing ability (Ucisik & Henze 2008). During the anaerobic digestion of wasted sludge, complex organic materials are hydrolyzed and then fermented into short chain volatile fatty acids (VFAs) (Wang et al. 1999). On the other hand, significant amounts of nitrogen and phosphorus can also be released into the supernatant during the digestion of wasted activated sludge (WAS), especially for the WAS from an EBPR process (Chen et al. 2007). This presents problems associated with treating rejected water for wastewater treatment plants (WWTPs). For the digestion supernatant, it generally contains high levels of VFAs, nitrogen and phosphorus, although the flow rate is not high (Yuan et al. 2006; Zupancic & Ros 2008). For example, the concentrations of chemical oxygen demand (COD), NH$_4^+$-N and PO$_4^{3-}$/C$_0$-P in the digestion supernatant generally vary over the range 1,000–10,000 mg L$^{-1}$, 200–500 mg L$^{-1}$, and 100–300 mg L$^{-1}$, respectively (Tong & Chen 2007). In general, the VFAs act as a carbon source that contributes to the removal of nutrients from the wastewater, while the NH$_4^+$-N and PO$_4^{3-}$/C$_0$-P

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increases the nutrient loading of wastewater and adversely affects the wastewater treatment performance (Tong & Chen 2009). It is therefore important to understand the release mechanism of nutrients during the anaerobic digestion process of WAS, especially for EBPR sludge.

Many studies have been carried out on the phosphorus release from activated sludge in WWTPs, and concluded that the type of substrate determines whether a net phosphorus uptake and release would occur in the presence of nitrate (Zou et al. 2006). Other reports stated that nitrate could be used as a terminal electron acceptor for phosphorus removal instead of oxygen under given conditions, and be reduced as nitrogen gas (Tsuneda et al. 2006).

As the terminal electron acceptor for phosphorus uptake, oxygen and nitrate both play important roles in the EBPR process (Kuba & Loosdrecht 1996) although the roles they play in the digestion system of EBPR sludge remain unclear. In this study, three laboratory-scale digesters were therefore constructed to investigate the stoichiometric relationships of selected parameters and the role of nitrate associated with the release of phosphorus in order to improve the understanding of the phosphorus release mechanism in EBPR sludge digestion systems.

**MATERIALS AND METHODS**

**Source of sludge samples**

The sludge used for the digestion experiment was collected from a sludge-thickening tank of an EBPR WWTP in Shanghai, China. The plant treats about 45,000 m$^3$ d$^{-1}$ of wastewater (almost 100% domestic sewage) using an anaerobic-aerobic process. The phosphorus removal rate is about 80% when the influent concentration varies over the range 3.0–5.0 mg L$^{-1}$. After being transported to the laboratory, the collected sludge was rapidly concentrated by settling at 4°C for 60 min. The main characteristics of the EBPR sludge after settling are listed in Table 1.

**Digestion experiments**

Three laboratory-scale digesters (A, B and C) with a working volume of 2.0 L each were used in this study. A total of 6.0 L of sludge was evenly divided into three parts and rapidly transferred to three digesters, after which they were mixed by magnetic stirrers at 100 rpm. The operational approach of each digester is summarized in Table 2. The three digesters were sparged with nitrogen gas (N$_2$) for 2.0 min before being sealed. The digestion experiment was run for 20 days without feeding at 25°C, during which liquid and solid samples were taken every 2 days. The pH was not controlled, but was monitored during the experiment.

**Analytical techniques**

Sludge samples from the digesters were immediately filtered through a microfiber filter (0.45 μm). The filtrate was immediately analyzed for soluble COD (SCOD), NO$_3$–N, and PO$_4^{3–}$–P. The filter was assayed for volatile suspended solids (VSS), total phosphorus (TP) in VSS, polyhydroxyalkanoates (PHA) and glycogen. The SCOD analyses were conducted using a Hach DR/2000. The other sludge parameters, including VSS, TP, NO$_3$–N and PO$_4^{3–}$–P, were analyzed according to the standard methods (APHA 1998). PHA, glycogen and VFAs were measured according to the methods reported in the literature (Liu et al. 1997; Wang et al. 2002a; Jiang et al. 2007). All the analyses were carried out in triplicate, and all chemicals used in the analyses were of analytical grade.

**Data processing**

The change rates of liquid sample indexes during the experimental period, including PO$_4^{3–}$–P, NO$_3$–N, VSS and SCOD, were calculated via:

$$v = \frac{\rho_2 - \rho_1}{\Delta t}$$

(1)

**Table 1 | Characteristics of EBPR sludge (standard deviations are shown in parentheses; number of samples: 3)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Content</th>
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<th>Parameter</th>
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</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.7 (0.4)</td>
<td>TCOD (mg L$^{-1}$)</td>
<td>15050 (821)</td>
<td>SCOD (mg L$^{-1}$)</td>
<td>40 (7)</td>
</tr>
<tr>
<td>TSS (mg L$^{-1}$)</td>
<td>14820 (732)</td>
<td>TP (mg L$^{-1}$)</td>
<td>321.0 (74)</td>
<td>NH$_4^+$–N (mg L$^{-1}$)</td>
<td>12.5 (1.5)</td>
</tr>
<tr>
<td>VSS (mg L$^{-1}$)</td>
<td>10350 (567)</td>
<td>TN (mg L$^{-1}$)</td>
<td>553.0 (114)</td>
<td>PO$_4^{3–}$–P (mg L$^{-1}$)</td>
<td>4.36 (0.8)</td>
</tr>
</tbody>
</table>

TSS: total suspended solids; TCOD: total COD.
Table 2 | Experimental conditions of the digesters

<table>
<thead>
<tr>
<th>Digester no.</th>
<th>Conditions</th>
<th>Controlled measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Anaerobic</td>
<td>Sealed and stirred at 100 rpm using magnetic stirrers.</td>
</tr>
<tr>
<td>B</td>
<td>Anoxic</td>
<td>2250.0 g NaNO₃ powder was added at the beginning; other experimental conditions as for digester A.</td>
</tr>
<tr>
<td>C</td>
<td>Aerobic</td>
<td>Aerated with air pumps to ensure that the dissolved oxygen (DO) was c. 2.0 mg L⁻¹; some freshwater was periodically injected into the digester to maintain water balance.</td>
</tr>
</tbody>
</table>

where \( \nu \) is the change rate of a parameter (mg L⁻¹ d⁻¹); \( \rho_1 \) and \( \rho_2 \) are the concentration values of a parameter in the sludge sample at the beginning and at the end, respectively (mg L⁻¹); and \( \Delta t \) is the value of the time interval (d).

The change rate of the parameters in VSS during the experimental period, including TP, PHA and glycogen, are calculated via:

\[
\nu = \frac{(c_2\rho_{VSS2} - c_1\rho_{VSS1})M}{1,000 \Delta t}
\]

where \( \nu \) is the change rate of a parameter (mg L⁻¹ d⁻¹); \( c_1 \) and \( c_2 \) are the concentration values of a parameter in VSS at the beginning and at the end, respectively (mmol g⁻¹); \( \rho_{VSS1} \) and \( \rho_{VSS2} \) are the concentration values of VSS in the sludge sample at the beginning and at the end, respectively (mg L⁻¹); \( M \) is the atomic weight of C or P (mg mmol⁻¹); and \( \Delta t \) is the value of the time interval (d).

**RESULTS**

**Decay of sludge and gas production**

Decay of sludge, which can be expressed as the release of SCOD and the decrease of VSS, is the main objective of anaerobic digestion (Saiki et al. 1999). As shown in Figure 1, the release of SCOD and the decrease of VSS occurred concurrently over time, indicating that a part of the biomass was converted to the soluble substrates. Specifically, the VSS decreased from 10,350 to 4120, 10,350 to 3920 and 10,350 to 2,500 mg L⁻¹ in digesters A, B and C, respectively (Figure 1(b)), and the reduction degree in the three digesters followed the order: A < B < C. This indicates that the hydrolysis rate of EBPR biomass was accelerated under both anoxic and aerobic conditions. The SCOD variations increased from 40 to 930,1,012 and 790 mg L⁻¹ in digesters A, B and C, respectively (Figure 1(a)). The VFA production in A and B on the 10th day was also assayed and shown in Table 3. Generally, SCOD can further convert to CH₄ gas during anaerobic digestion. However, a higher SCOD accumulation occurred in B than in A, suggesting that the gas generation pathways were blocked under anoxic conditions. Overall, 107.0 and 85.0 mL of gas were produced in digesters A and B, respectively, every day, with the CH₄ percentage varying over the range 15–25%. The gas productions trend agreed well with the SCOD accumulation.

**Release of phosphorus**

The phosphorus variations in digesters A, B and C are shown in Figure 2. The biomass TP, i.e. the total phosphorus in VSS, gradually decreased with time in both digesters A and B from 1.2 to 0.43 and 1.2 to 0.78 mmol g⁻¹ VSS, respectively (Figure 2(a)). The biomass TP in digester A was lower than B, which indicates that more phosphorus had been released under anaerobic conditions while this phosphorus release was suppressed under anoxic conditions. As for digester C, the biomass TP increased slowly with time (Figure 2(a)) from 1.2 to 1.42 mmol g⁻¹ VSS. These findings indicate that the total phosphorus per VSS increased under aerobic conditions, although the VSS decreased with time. Nevertheless, the concentration of PO₄³⁻-P in digesters A, B and C obviously increased throughout the experiment (Figure 2(b)), i.e. from 4.32 to 102.9, 83.7 and 43.1 mg L⁻¹, respectively. These changes in the liquid PO₄³⁻-P in A and B were concordant with the biomass TP changes. However, in digester C, the PO₄³⁻-P increased along with the increasing biomass TP, possibly due to the decay of VSS.

**Production of PHA and consumption of glycogen**

According to the metabolic model of the EBPR, PHA is used as an energy source for phosphate uptake in the aerobic or anoxic phase, while glycogen is the main source of reducing power for PHA synthesis (Wang et al. 2002b). To better understand the role of PAOs in phosphorus release during anaerobic, anoxic and aerobic digestion, the change profiles of PHA and glycogen were determined for digesters A, B and C in Figure 3. After digestion for 20 days, the PHA in VSS increased from 0.67 to 2.91 and 0.67 to 1.42 mmol C g⁻¹ VSS in digesters A and B, respectively. The glycogen in VSS decreased from 4.5 to 2.85 and 3.56 mmol C g⁻¹ VSS in digesters A and B, respectively. These findings indicated...
that part of the degraded glycogen might have been converted to PHA, and this transfer was enhanced more significantly under anaerobic conditions than under anoxic conditions. However, for digester C where aerobic conditions were adopted, both the PHA and glycogen increased slightly.

Table 3
| The VFA production in different digesters on day 10 (mg L⁻¹); standard deviations are shown in parentheses and the number of samples is 3. Note that HAc = acetic, HPr = propionic, i-HBu = iso-butyrice, HBu = n-butyric, i-HVa = iso-valeric |
|-----------------|-------|-------|-------|-------|-------|-------|-------|-------|
| Parameter       | HAc   | HPr   | i-HBu | HBu   | i-HVa | HVa   | In total |
| A               | 121.5 (24.5) | 65.3 (12.3) | 32.1 (8.7) | 8.9 (2.1) | 43.2 (7.5) | 5.2 (1.2) | 276.2 |
| B               | 113.5 (14.3) | 53.2 (11.7) | 38.7 (12.3) | 15.2 (4.4) | 49.2 (6.9) | 17.6 (5.5) | 287.4 |

Figure 1 | Changes in SCOD and VSS over time in three digesters: (a) SCOD and (b) VSS. Error bars represent the standard deviation of triplicate samples.

Figure 2 | Changes of TP and PO₄³⁻-P with time in different digesters: (a) TP and (b) PO₄³⁻-P. Error bars represent the standard deviation of triplicate samples.

Figure 3 | Changes of PHA and glycogen with time in three digesters: (a) PHA and (b) glycogen. Error bars represent the standard deviation of triplicate samples.
Consumption of nitrate under anoxic conditions

The NO$_3$-N change in digester B was also investigated (data not shown) to reveal the anoxic P release or uptake mechanism. The NO$_3$-N concentration decreased from 189.70 mg L$^{-1}$ to 11.70 mg L$^{-1}$ after anoxic digestion for 20 days, with a variation rate of $-8.9$ mg L$^{-1}$. These findings suggest that a large amount of nitrate was depleted during the experiment in digester B.

DISCUSSION

Phosphorus release process under anaerobic, anoxic and aerobic conditions

In digester A (anaerobic processes), phosphorus was released slowly after the EBPR biomass containing high amounts of poly-P was added. Such low phosphorus release is possibly due to the low SCOD availability during the first 4 days of the digestion (Figure 2). According to Lu et al. (2007), poly-P and glycogen were utilized simultaneously under anaerobic and anoxic starvation conditions for maintenance energy production, with glycogen being the primary energy source until the glycogen content reached very low levels. During the initial 4 days of the digestion when insufficient external carbon sources were available, the PO$_4^{3-}$-P release amount was therefore higher in A and B than in C (Figure 2(b)), accompanied by the higher glycogen hydrolysis in A and B than in C (Figure 3(b)). It should be noted that more glycogen was degraded in A than in B and C (Figure 3(b)), primarily because glycogen provided a significant fraction of the maintenance energy. Thereafter, as more SCOD was released, the VFAs increased through the hydrolysis and acidification of the biomass (Yuan et al. 2006; Jiang et al. 2007). Accordingly, the phosphorus release was prompted due to sufficient VFA availability for PAOs to synthesize PHA. For this reason, the released P amount in A was highest among the three digesters (Figure 2). Further, more glycogen was degraded in A than in B and C during the latter part of the digestion as they were hydrolyzed to generate enough reducing power for the PHA synthesis in digester A.

On the other hand, in digester C (aerobic conditions) the PHA contents did not vary significantly even though the SCOD increased slightly during digestion (Figure 1). Also, the phosphorus release was lower compared to that of A and B, confirming that P uptake might occur by PAOs using PHA as the primary source of energy for P uptake and maintenance under such aerobic starvation conditions (Figure 3). Moreover, although more SCOD was released to the bulk after day 10, the PHA did not vary significantly but remained constant. This was primarily due to the presence of oxygen, i.e. the released SCOD was oxidized to CO$_2$ using oxygen as electron acceptor but not used for PHA synthesis. The released amount of phosphorus in digester B was between that for A and C (Figure 2(b)), possibly because in digester B a part of the released PO$_4^{3-}$-P was taken up again by PAOs/denitrifying phosphorus-accumulating organisms (DPAOs) using nitrate as an electron acceptor, which was classified as participating in denitrifying phosphorus-accumulating organisms (DPAOs) using nitrate as an electron acceptor.

Stoichiometric relationships associated with phosphorus release

The change rates of the parameters associated with phosphorus release in the three digesters were summarized and compared with the data from a typical EBPR anaerobic process (Table 4). Under anaerobic conditions, PAOs assimilate VFA to synthesize PHA using energy produced by hydrolysis of poly-P (Liu et al. 1996; Wang et al. 2002b; Pijuan et al. 2005) and the reducing power from glycogen hydrolysis. Since PHA production and consumption of glycogen were observed in digester A, poly-P hydrolysis in the PAO cell could have occurred actively to produce sufficient energy for PHA synthesis and maintenance. However, the differences between digester A and the typical anaerobic process in EBPR system were great with regards to both PO$_4^{3-}$-P release and PHA production (Table 4). This indicates that the biological activities of PAOs in digester A were not sufficient. On the other hand, the release rate of PO$_4^{3-}$-P was only a little less than one-third of the decrease rate of TP, suggesting that most of the TP in VSS was dispersed into the digestive liquid rather than translated into PO$_4^{3-}$-P directly. It was therefore the WAS hydrolysis degree that primarily determined the release of phosphorus. The production rate of PHA was negative for both digester B and C, which may indicate that PAOs/DPAOs uptake part of the PO$_4^{3-}$-P from the digestive liquid using nitrate or oxygen as an electron acceptor.

Nitrate consumption under anoxic conditions

According to physicochemical principles, addition of nitrate cannot induce the physicochemical incidents during the digestion of WAS. Significant precedence in the literature has shown that nitrate has an intensive effect on the
action of microorganisms in an EBPR system (Tsuneda et al. 2006; Zou et al. 2006). It has been shown that the addition of nitrate not only decreased the biological phosphorus release of PAOs by competing with substrate, but partially inhibited the activity of PAOs. Soejima et al. (2006) have reported that the DPR action in the presence of nitrate may also reduce the amount of phosphorus released. The phosphorus release processes were therefore inhibited in digester B.

It was assumed that nitrate was the sole electron acceptor and that the relationship between the consumption of nitrate and uptake of PO$_4^{3-}$-P associated with the DPAOs is defined (Zou et al. 2006):

$$\Delta P = 1.51 \times \Delta N$$

(3)

Using the amounts of PO$_4^{3-}$-P release in digester A as a reference value, the differences in PO$_4^{3-}$-P release between digester A and B can be seen in the uptake amounts associated with the DPAOs. The relationship between the consumption of nitrate and uptake of PO$_4^{3-}$-P in digester B are therefore:

$$\Delta P = 0.11 \times \Delta N$$

(4)

The difference between the coefficients of the two equations is rather distinct, which indicated that the DPR action of the microorganisms in digester B was also not sufficient.

**CONCLUSIONS**

Although the release of phosphorus resulted from the combined action of many factors, phosphorus release was primarily derived from the hydrolysis of WAS, regardless of whether it was under anaerobic, anoxic or aerobic conditions. The phosphorus release process is accelerated by the biological poly-P release of PAOs under anaerobic conditions, while the process will be inhibited by the actions of DPAOs when nitrate is present. The process can also be inhibited to some extent under aerobic conditions. In addition, most of the TP in VSS released into the digestive supernatant under anaerobic conditions remains as other forms of phosphorus rather than being translated into PO$_4^{3-}$-P.

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