**Comparison of anaerobic phosphorus release from activated sludge with three carbon sources**

Xiaohu Dai, Zhanbei Gu, Lingling Dai, Changming Shen, Wei Zhou, Jihui Huang, Wei Wang and Zhigang Liu

**ABSTRACT**

Three carbon sources, namely sodium acetate, sewage and effluent, were used to simulate the process of phosphorus release in an actual sewage treatment plant, in order to explore the phosphorus release performance of the sludge, the relationship between phosphorus release and chemical oxygen demand (COD) in sewage, and the stability of phosphorus-rich sludge. The results showed that the type and concentration of carbon sources had significant effects on the rate of phosphorus release, reaction equilibrium time and phosphorus release amount. When sodium acetate was used as the carbon source, the phosphorus release rate reached 12.54 mg P (g VSS·h)$^{-1}$, and tended to be stable at 4.0 hours. The phosphorus release amount in the first 2.5 hours accounted for 36.88% of the total phosphorus in the sludge. When sewage was used as the carbon source, the phosphorus release rate did not reach equilibrium even at 5.0 hours, and the phosphorus release amount increased by 1.0 mg L$^{-1}$ for every 10.5 mg L$^{-1}$ increase sewage COD within a certain range. When effluent was used as the carbon source, the visual phosphorus release amount was only 0.83 mg L$^{-1}$ after standing for 24 hours and the actual phosphorus release amount was 17.98 mg L$^{-1}$. These conclusions can provide technical support for the optimization of phosphorus removal in sewage treatment plants.

**Key words** | activated sludge, anaerobic phosphorus release, carbon sources

**HIGHLIGHTS**

- The effect of anaerobic phosphorus release from activated sludge with three carbon sources was compared.
- A formula was obtained by linear regression for the correlation between COD concentration and phosphorus release amount.
- A phosphorus load contribution of 8.38% to the influent was due to the sludge treatment process.
- Parameters obtained in the experiments can be used to optimize sewage treatment plant performance.
INTRODUCTION

Biological phosphorus removal (BPR) is a commonly used for phosphorus removal in sewage treatment plants. Phosphorus is released under anaerobic conditions, then excess phosphorus is absorbed under aerobic conditions by phosphorus accumulating organisms (PAOs) in the activated sludge, and phosphorus is removed from sewage by discharging phosphorus-rich sludge (Barnard et al. 2017). The essential step in BPR is anaerobic phosphorus release, as it directly affects the efficiency of phosphorus removal in sewage treatment plants. Generally, the more polyhydroxyalkanoates (PHAs) stored in PAOs in the anaerobic section, the more phosphorus can be absorbed in the following aerobic section (Petersen et al. 1998; Feng et al. 2019).

Four main factors affect anaerobic phosphorus release from activated sludge. (1) The characteristics of the activated sludge, such as the microorganisms present and the amount of glycogen and polyphosphate in the PAOs, influence the BPR's efficiency directly (Romanski et al. 1997; Liu et al. 2000; Dai et al. 2018). (2) Many studies have shown that the type and concentration of carbon sources directly affect the release of phosphorus from activated sludge; in particular, biodegradable organic compounds, such as acetate and propionate, can significantly promote the release of phosphorus from activated sludge (Marais et al. 1983; Chai et al. 2005; Hao et al. 2008; Li et al. 2018). (3) Environmental factors can promote or inhibit the phosphorus release process; for example, too much nitrate will inhibit anaerobic phosphorus release (Niel et al. 1998; Wang et al. 2004), while an appropriate increase in the initial phosphorus concentration can promote phosphorus release (Panswad et al. 2007). In addition, suitable pH, temperature, oxidation-reduction potential (ORP), dissolved oxygen (DO), and other conditions can ensure the activity of microorganisms (Kumar et al. 1998; Schuler & Jenkins 2002; Filipe et al. 2010; Bond et al. 2015), and therefore favor phosphorus release. (4) The operation parameters and mode of the process, mainly the ratio of sludge reflux and the hydraulic retention time (HRT) in the anaerobic section, also have a major impact on phosphorus release (Coats et al. 2011).

As mentioned above, it is essential to release sufficient phosphorus from activated sludge during sewage treatment and as little as possible during sludge treatment in order
for sewage treatment plants to optimize phosphorus removal. A sewage treatment plant in Jiangsu Province in China was selected for the optimization of its phosphorus removal, and the flowchart is shown in Figure 1. The HRT of the biological treatment of sewage in the plant is 18.0 hours (including 2.4 hours in the anaerobic section, 6.0 hours in the anoxic section and 9.6 hours in the aerobic section), and the sludge retention time (SRT) is 15 days. Sludge will also spend 16.5 hours (including sludge thickening for 10.9 hours and sludge storage for 5.6 hours) in the process of sludge treatment.

Based on these, samples taken from the inlet and outlet of the anaerobic tank and the key sludge treatment sites were analyzed to establish the status of the sewage treatment plant. Then three carbon sources, namely sodium acetate (degradable carbon source), sewage (general carbon source) and effluent (lower carbon source) were used in an activated sludge anaerobic phosphorus release experiment to explore the phosphorus release performance of activated sludge, the relationship between phosphorus release and chemical oxygen demand (COD) in sewage, and the stability of phosphorus-rich sludge under lower carbon source conditions. The aim was to provide technical support for the optimization of phosphorus removal in the sewage treatment plant.

MATERIALS AND METHODS

Experimental materials

The experimental activated sludge, sewage and effluent were obtained from the sewage treatment plant. Activated sludge was the mixture taken from the end of the aerobic tank, sewage was the influent of the anaerobic tank, and effluent was the supernatant of mixture. The characteristics of activated sludge, sewage and effluent are shown in Table 1.

Methods

The experiment using sodium acetate as the degradable carbon source was as follows: 5.0 L activated sludge was placed directly into a reaction vessel with plugged glass (effective volume = 5.0 L), and sodium acetate (analytical grade) as COD = 300 mg L\(^{-1}\) was added immediately before sealing the reaction vessel. A magnetic stirrer was used to create a suspension. The changes in pH, DO and temperature were measured so as to ensure that pH = 7.0 ± 0.5, DO < 0.1 mg L\(^{-1}\) and T = 25 ± 1°C during the experiment. Samples were taken at 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0 and 5.0 hours and centrifuged (894 g) for 3.0 min, then the concentration of orthophosphate in the supernatant was measured. This process was carried out three times.

5.0 L activated sludge was centrifuged (894 g) for 3.0 min, then the residue at the bottom and some sewage were transferred to the reaction vessel, so that the total volume of the mixture was 5.0 L. Other operating conditions were the same as for the experiment with sodium acetate. The process was carried out six times.

5.0 L mixture taken from the end of the aerobic tank, of which the supernatant was the effluent, and placed directly into a reaction vessel without being stirred or centrifuged. At 0, 1.5, 3.0, 5.0, 8.0, 12.0, 16.0, 20.0, and 24.0 hours, 0.1 L of the supernatant was taken to measure its orthophosphate concentration. After the last sampling, the mixture was stirred to mix well and centrifuged (894 g) for 3.0 min, then the orthophosphate concentration in the supernatant was measured. The same process was conducted three times.

Determination methods

PO\(_4^{3-}\)-P, total phosphorus (TP), NO\(_3\)-N and COD were determined by ‘Examination methods for municipal sewage; Determination of soluble phosphate; Stannous
chloride spectrophotometric method’ (CJ/T 51-2018(29.1)), ‘Water quality; Determination of the total phosphorous; Ammonium molybdate spectrophotometric method’ (GB/T 1183-1989), ‘Examination methods for municipal sewage; Determination of nitrate; ultraviolet spectrophotometric method’ (CJ/T 51-2018(25.1)) and ‘Water quality; Determination of the chemical oxygen demand; Fast digestion; Spectrophotometric method’ (HJ/T 399-2007), respectively. The mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) were measured according to the Standard Methods for the Examination of Water and Wastewater (20th edition). The phosphorus content in activated sludge was determined by ‘Determination method for municipal sludge in wastewater treatment plant; Determination of total phosphorus; molybdenum-antimony anti-spectrophotometric method after sodium hydroxide melting’ (CJ/T 221-2005(50)). JPSJ-605F (Leici, China) and PHB-3 (Sanxin, China) were used for the determination of DO, temperature and pH, respectively.

### RESULTS AND DISCUSSION

#### The status of the sewage treatment plant

Samples were taken from the inlet and outlet of anaerobic tank twice a week for 4 weeks, and the PO₄³⁻P concentration of each sample was measured. The results showed that the PO₄³⁻P concentrations of the influent were between 0.56 and 6.06 mg L⁻¹, with an average of 2.10 mg L⁻¹, and the PO₄³⁻P concentrations of the effluent were between 5.27 and 17.4 mg L⁻¹, with an average of 10.62 mg L⁻¹ (Figure 2). So the concentrations of PO₄³⁻P increased significantly after the anaerobic phosphorus release process.

The concentrations of PO₄³⁻P and TP in the sewage, the effluent of the secondary setting tank, the supernatant of the sludge thickening tank and the filtrate of the centrifugal dehydrator were measured twice a week for 4 weeks (Figure 3). The average concentrations of PO₄³⁻P and TP in the supernatant of the sludge thickening tank were 1.17 and 2.72 mg L⁻¹, respectively, which were higher than in the effluent of the secondary setting tank (where the average concentrations were 0.11 and 0.51 mg L⁻¹), but still lower than in the sewage (where the average concentrations were 2.09 and 3.73 mg L⁻¹). However, the average concentrations in the filtrate of the centrifugal dehydrator were the highest: 30.54 mg L⁻¹ and 47.49 mg L⁻¹, respectively.

According to the plant’s own data, about 4,000 m³ sludge with a moisture content of 99.2% is produced every day. The sludge moisture content is about 97.0% after gravity thickening and about 78.5% after centrifugation, with 2,933 m³ supernatant and 918 m³ filtrate produced. The phosphorus load contribution of 8.38% to the influent was due to the sludge treatment process according to the data in Figure 3, which was less than the result of a previous report (van Loosdrecht & Salem 2006). The main reason is assumed to be that the excess sludge was mixed with chemical sludge, which contained more Fe element due to the use of iron salt precipitating and removing phosphorus in the sewage treatment plant.

### Table 1 | Characteristics of activated sludge, sewage and effluent

<table>
<thead>
<tr>
<th>Experimental materials</th>
<th>Index</th>
<th>Range</th>
<th>Mean ± std</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activated sludge</td>
<td>pH</td>
<td>6.8–7.0</td>
<td>6.9 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>MLSS (g L⁻¹)</td>
<td>3.48–4.72</td>
<td>3.99 ± 0.39</td>
</tr>
<tr>
<td></td>
<td>MLVSS (g L⁻¹)</td>
<td>2.04–2.88</td>
<td>2.39 ± 0.28</td>
</tr>
<tr>
<td></td>
<td>Phosphorus content (g TP (g MLSS)⁻¹)</td>
<td>1.84–2.73%</td>
<td>2.39% ± 0.34%</td>
</tr>
<tr>
<td>Sewage</td>
<td>pH</td>
<td>6.8–7.3</td>
<td>7.0 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>COD (mg L⁻¹)</td>
<td>84.3–262.0</td>
<td>193.2 ± 59.0</td>
</tr>
<tr>
<td></td>
<td>SCOD (mg L⁻¹)</td>
<td>30.4–72.4</td>
<td>52.9 ± 14.7</td>
</tr>
<tr>
<td></td>
<td>PO₄³⁻P (mg L⁻¹)</td>
<td>0.82–3.19</td>
<td>2.14 ± 0.89</td>
</tr>
<tr>
<td></td>
<td>TP (mg L⁻¹)</td>
<td>1.92–6.32</td>
<td>3.84 ± 1.60</td>
</tr>
<tr>
<td></td>
<td>NO₃⁻-N (mg L⁻¹)</td>
<td>0.28–1.04</td>
<td>0.70 ± 0.29</td>
</tr>
<tr>
<td>Effluent</td>
<td>pH</td>
<td>6.8–7.0</td>
<td>6.9 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>COD (mg L⁻¹)</td>
<td>19.9–39.8</td>
<td>28.1 ± 6.2</td>
</tr>
<tr>
<td></td>
<td>NO₃⁻-N (mg L⁻¹)</td>
<td>3.41–8.51</td>
<td>6.54 ± 1.74</td>
</tr>
<tr>
<td></td>
<td>PO₄³⁻-P (mg L⁻¹)</td>
<td>0.024–0.13</td>
<td>0.050 ± 0.038</td>
</tr>
<tr>
<td></td>
<td>TP (mg L⁻¹)</td>
<td>0.12–2.1</td>
<td>0.40 ± 0.45</td>
</tr>
</tbody>
</table>

Note: SCOD – COD determination of samples after filtration by medium-speed qualitative filter paper.
Phosphorus release with sodium acetate as a degradable carbon source

The phosphorus release trends were almost similar when activated sludge taken at a different time was added to the same amount of sodium acetate (Figure 4), meaning that the characteristics of activated sludge from the end of aerobic tank were stable. The rate of phosphorus release was highest at the beginning, 12.54 mg P (g VSS·h)$^{-1}$, then decreased gradually, and approached 0 at 4.0 hours. The PO$_4^{3-}$-P concentration of the supernatant was finally about 44.40 mg L$^{-1}$, and the phosphorus release amount was $17.07 \pm 1.46$ mg P (g VSS)$^{-1}$, which accounted for 39.5% of the total phosphorus in the sludge. In these experiments, the trends of phosphorus release from activated sludge were similar to the results from Hou et al. (2007) and Li et al. (2018), but both the phosphorus release amount and maximum phosphorus release rate were slightly lower, which might be related to the fact that nitrate in the mixture was not eliminated to simulate the actual situation and the sludge was different.

By comparing the phosphorus release effects of activated sludge with different concentrations (Figure 4(a)), the PO$_4^{3-}$-P concentration increased by 41.96, 40.75 and 41.58 mg L$^{-1}$ at 2.5 hours, much higher than the average PO$_4^{3-}$-P concentration of 10.62 mg L$^{-1}$ at the end of the actual anaerobic tank, also with an HRT of 2.5 hours. The amount of phosphorus released accounted for 36.88% of the TP in the sludge at 2.5 hours, which was lower than the results in the literature, probably because they changed the type and concentration of carbon sources and treatment methods to further increase the release ratio of phosphorus.
in sludge (Mavinic et al. 1998; Hao et al. 2008). All these results indicated that the limiting factor of the anaerobic phosphorus release process in the selected plant was not the characteristics of the activated sludge, but the type and quality of carbon sources.

**Phosphorus release with sewage as a general carbon source**

COD in sewage can be directly used as a carbon source for anaerobic phosphorus release in sewage treatment plants. The variation in phosphorus release was almost similar, although sewage with different COD concentrations was used as a general carbon source (Figure 5). The rate of phosphorus release was highest at first, then decreased gradually, but phosphorus was being released even after 5.0 hours compared to being close to 0 after 4.0 hours when sodium acetate was used as the degradable carbon source, owing to the slowly biodegradable COD in sewage. After the anaerobic phosphorus release of activated sludge for 2.5 hours, the concentration of PO$_4^{3-}$-P in the supernatant increased by 3.11–21.45 mg L$^{-1}$, and the phosphorus release amount accounted for 67.7% to 86.4% of that at 5.0 hours. The phosphorus release amount at 5.0 hours accounted for 3.12%–30.36% of the TP in sludge, which were lower than that when sodium acetate was added as a degradable carbon source. These experimental results verified that the presence of enough high-quality carbon could further improve the amount of phosphorus release.

The formula $y = 0.095x - 4.57$ ($y =$ phosphorus release amount, mg L$^{-1}$; $x =$ COD concentration, mg L$^{-1}$; $R^2 = 0.829$; $P = 0.0073$) could be obtained by linear regression according to the correlation of COD concentration and phosphorus release amount at 2.5 hours. A significant positive correlation between phosphorus release amount and COD concentration was shown in the formula. When sewage was used as carbon source, the phosphorus release amount could increase by 1.0 mg L$^{-1}$ for every 10.5 mg L$^{-1}$ increase in COD concentration within a certain concentration range. At the same time, it was also noted that the phosphorus release amount at the COD concentration of 172 mg L$^{-1}$ was higher than at 182 mg L$^{-1}$, and the phosphorus release amount at 206 mg L$^{-1}$ was slightly higher than at 253 mg L$^{-1}$, indicating that the release of phosphorus from activated sludge was also influenced by the quality of COD and the characteristics of activated sludge to some extent. Considering the concentration of COD, N and P in sewage and the ratio of return sludge in the selected plant, a carbon source should be added to strengthen phosphorus release in the anaerobic tank or agents should be added to enhance chemical phosphorus removal to ensure that the phosphorus in the effluent meets the discharge requirements when the COD in sewage is continuously lower than 125 mg L$^{-1}$.

**Phosphorus release with effluent as a lower carbon source**

It is common that phosphorus is released from sludge in the absence of a carbon source, as in the process of sludge thickening. When sludge was kept static for 24.0 hours without a supplementary carbon source, the concentration of PO$_4^{3-}$-P in the supernatant increased slowly at the beginning, but was still less than 0.10 mg L$^{-1}$ after 5.0 hours, and only reached 0.83 mg L$^{-1}$ at 24.0 hours although the rate of

![Figure 5](image-url)
phosphorus release had accelerated (Figure 6). However, the PO$_3^-$-P concentration rose to 17.98 mg L$^{-1}$ after the sample was thoroughly mixed, which was much greater than before, indicating that the sludge released phosphorus with the carbon source produced by sludge fermentation (Mavinic et al. 1998), but a lack of disturbance limited the speed of phosphorus entering the liquid phase. Finally, the phosphorus release amount reached 5.48 mg (g VSS)$^{-1}$, and accounted for about 14.78% of the TP in sludge.

The experimental result shown in Figure 6 was similar to the actual measured result. Most shown in the phosphorus released from the sludge did not enter the supernatant, but collected in the sludge at the bottom of the tank. However, it could enter the liquid phase by disturbance. Shortening the retention time and avoiding anaerobic conditions can reduce the release of phosphorus during sludge treatment.

CONCLUSIONS

The type and concentration of carbon sources had significant effects on the rate of phosphorus release, reaction equilibrium time and phosphorus release amount. When sodium acetate was used as a degradable carbon source, the maximum phosphorus release rate reached 12.54 mg P (g VSS·h)$^{-1}$, and the amount of phosphorus released in the first 2.5 hours accounted for 36.88% of the TP in the sludge, which tended to be stable at 4.0 hours. When sewage was used as a general carbon source, the phosphorus release rate did not reach equilibrium even at 5.0 hours. Within a certain concentration range (84.0–262.0 mg L$^{-1}$), sludge phosphorus release increased by 1.0 mg L$^{-1}$ for every 10.5 mg L$^{-1}$ increase in COD in sewage, and sludge phosphorus release was significantly positively correlated with COD in sewage. The actual phosphorus release amount reached 17.98 mg L$^{-1}$ and accounted for about 14.78% of the TP in sludge with effluent as a lower carbon source. The concentration of phosphorus in the influent increased by 8.38% due to the supernatant and filtrate produced in the process of sludge treatment.

ACKNOWLEDGEMENTS

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

REFERENCES


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