Microbial effects on phosphorus release in aquatic sediments
Ting-Lin Huang, Xiao-Chun Ma, Hai-bing Cong and Bei-Bei Chai

ABSTRACT
Microbial effects on phosphorus release were studied for the sediments of Tianjin source water by controlling DO and pH. The results show that: (1) In sterilised water, phosphorus began to release when pH = 9.1 and the stable release rate was 9.51 mg/(d·m²). It indicates that microorganisms may utilise anaerobic iron respiration to release Fe-P. (2) With unsterilised water, phosphorus release rate is 2.14 mg/(d·m²) when pH = 6.5, 8.60 mg/(d·m²) when pH is uncontrolled, and gets to 8.51 mg/(d·m²) when pH = 9.1. This indicates that microorganisms can dissolve insoluble phosphates to accelerate the ion exchange of OH⁻ and PO₄³⁻, which are derived from iron-bound ortho-P and aluminium-bound ortho-P.

Key words | APA, labile OP, phosphorus, release, sediment

INTRODUCTION
Nowadays, the adsorption–desorption of phosphorus and ortho-P co-release with reduced metal are considered as the key mechanisms that can explain phosphorus release from profound anaerobic lake sediments (Zhou et al. 2001). Recently, it has been discovered that microorganisms play an important role in the release of iron-bound phosphate. However, little is known about the mechanisms by which microorganisms affect the phosphorus release. Generally speaking, microorganisms can dissolve, mineralise and fix the phosphorus. But the phosphatase excreted from microorganisms can dramatically influence the process of organic phosphorus mineralisation. The experiments on Venice Lagoon and Xihu Lake show that the phosphorus release rate is proportional to the phosphatase activity (Chen et al. 1997; Zhou et al. 2001). Moreover, in the studies of Soyang Lake, Erken Lake and Donghu Lake, the phenomenon of eutrophication is found to be relative to the activity of alkaline phosphatase and the meaning of dramatic parameters (Zhou & Fu 1999; Zhou et al. 2000). Therefore, our research is focused on the sediment P release mechanisms under various environmental parameters especially microorganisms, which control the phosphorus release. Furthermore, the relationship of phosphatase activity and organic phosphorus (OP) is studied as well.

MATERIALS AND METHODS
Sampling
The sampling site was in Haihe River. Sediments were collected at the depth of 30 cm by a core sampler. They were preserved in polyethylene bags. The sediments were sieved to remove large pieces of stones and sand particles through Standard Testing Sieve. Then it was mixed thoroughly and 1,500 mL sediment–water mixture was measured and put into each tester for the release experiment. Residual sediments were air dried, sieved and finally preserved at 4°C for further analysis. The water was obtained through the microporous filtering film (0.45 μm pore size) and then slowly flowed into the testers.

Chemical analysis
A suitable volume of solution was collected from each sample every two days and the amount of soluble phosphorus was analysed by colorimetry after reaction
with molybdate and L-ascorbic acid (APHA et al. 1992). The phosphatase was tested by the P-NPP method (Berman 1970) and the labile OP was measured by fractionation of organic method (Bowman & Cole 1978). pH was obtained by a pHS-25 pH meter and dissolved oxygen (DO) was measured by a HI 9141 DO meter every day.

Release experiment design

Sediments were put into quadrate glass chambers with outer PVC housing to protect the sediments and water from light. A cap was mated with the bottom of each chamber and air holes and sampling ports were kept on the cap. Uviol lamps were fixed in the chamber 1#–3#. Overlying water was adjusted to different pH with NaOH and HCl. The temperature inside was controlled at 25°C by recycling hot water. Additionally, by adding oxygen and nitrogen, DO was controlled at 7–8 mg/L under aerobic conditions and 0–1 mg/L under anaerobic conditions.

The release rates of phosphorus release from the sediment were calculated according to the following formula (Wang et al. 2002):

\[
g = V(C_n - C_0) + \sum_{j=1}^{n} V_n C_j - 1, \quad R = \gamma tA
\]

(1)

where \( V \) (L) is the volume of overlying water. The analysed time is expressed as “\( n \)”. \( C_n \) (mg/L) is the concentration of orthophosphate measured every time. \( C_0 \) (mg/L) is the original concentration of orthophosphate in overlying water. \( V_n \) (L) represents the sample volume every time. \( \gamma \) (mg) is the release quantity measured every time. \( R \) (mg/(d·m²)) is the release rate. The release time is described as \( t \) (d). The touch area of sediment and water \( A \) (m²) in this experiment is 0.29 m².

### RESULTS AND DISCUSSION

Changes of pH, DO and microorganisms

The release characteristics of phosphorus under different conditions (Table 1) are shown in Figures 1–3. Throughout the experiment, phosphorus began to release when DO < 0.5 mg/L in 4# chamber (pH = 6.5; Original water-Original sediment), and the average release rate of 10 days was 0.41 mg/(d·m²). Under anaerobic conditions, the release rate was gradually increased and finally reached 2.14 mg/(d·m²). In contrast, little PO_4^{3-}-P was released during the whole procedure in 1# chamber (pH = 6.5, Enzyme deactivated sediment–Sterilised water).

From Figure 2, we can see that large amounts of phosphorus are released in 5# chamber (uncontrolled pH; Original water–Original sediment) with the rate of 8.60 mg/(d·m²), while in 2# chamber (uncontrolled pH; Enzyme deactivated sediment–Sterilised water) only small quantities are transported to the overlying water under anaerobic conditions. The concentration of PO_3^{4-}-P in 2# chamber (uncontrolled pH; Enzyme deactivated sediment–Sterilised water) gets to the highest value of 0.11 mg/L on the twelfth day under anaerobic conditions, and soon drops to 0.02 mg/L.

Under alkaline conditions, pH, the dominating environmental parameter, is more helpful to the phosphorus release than DO. It was found that phosphorus was released as quickly as 8.51 mg/(d·m²) when pH = 9.1 and DO = 7–8 mg/L in 6# chamber (pH = 9.1; Original water–Original sediment). In 3# chamber (pH = 9.1; Enzyme deactivated sediment–Sterilised water), the phosphorus began to release under aerobic conditions after 20 days, and the stable release rate was 9.51 mg/(d·m²).

It is well known that under anaerobic acidic conditions, Fe^{3+} is easily reduced to co-release with PO_4^{3-}. Micro-

### Table 1 | Controlled experimental conditions for phosphorus release

<table>
<thead>
<tr>
<th>Number of release chambers</th>
<th>1#</th>
<th>2#</th>
<th>3#</th>
<th>4#</th>
<th>5#</th>
<th>6#</th>
</tr>
</thead>
<tbody>
<tr>
<td>State</td>
<td>Enzyme deactivated sediment–Sterilised water</td>
<td>Enzyme deactivated sediment–Sterilised water</td>
<td>Enzyme deactivated sediment–Sterilised water</td>
<td>Original water–Original sediment</td>
<td>Original water–Original sediment</td>
<td>Original water–Original sediment</td>
</tr>
<tr>
<td>Water pH</td>
<td>6.5</td>
<td>Uncontrolled</td>
<td>9.1</td>
<td>6.5</td>
<td>Uncontrolled</td>
<td>9.1</td>
</tr>
</tbody>
</table>
organisms are able to use Fe$^{3+}$ as electron acceptors to metabolise polyhydroxybutyrate. The resulting release of iron-bound ortho-P would supply abundant ortho-P for uptake and synthesis of intercellular poly P.

Under high pH values, the exchange of OH$^-$ and PO$_4^{3-}$ will take place and the phosphorus will be released from sediments. When DO < 1mg/L, the cooperation of the displacement and reduction occurs, and phosphorus release rate is greatly increased. The process of PO$_4^{3-}$ release from Fe-P can be described through following equations:

$$\text{FePO}_4 + 2\text{OH}^- - e^- \rightarrow \text{PO}_4^{3-} + \text{Fe(OH)}_2$$  \hspace{1cm} (2)

$$\text{Fe(OH)}_2\text{H}_2\text{PO}_4 - e^- \rightarrow \text{H}_2\text{PO}_4^- + \text{Fe(OH)}_2$$  \hspace{1cm} (3)

Under anaerobic conditions, PO$_4^{3-}$ and H$_2$PO$_4^-$ will gradually get to the equilibrium concentrations in overlying water. So the release rate decreased from 8.51mg/(d·m$^2$) to 5.13mg/(d·m$^2$) in 6# chamber (pH = 6.5; Original water—Original sediment). Plentiful studies on phosphorus decomposing microorganisms have shown that the microorganisms like mildew are able to decompose the indissoluble phosphate as Fe-P and Al-P, then PO$_4^{3-}$ releases from Fe-P, Al-P and is finally transferred to overlying water. Moreover, Fe$^{3+}$ and Al$^{3+}$ quickly react with OH$^-$ as the following equations describe:

$$\text{Fe}^{3+} + \text{OH}^- \rightarrow \text{Fe(OH)}_3$$  \hspace{1cm} (4)

$$\text{Al}^{3+} + \text{OH}^- \rightarrow \text{Al(OH)}_3$$  \hspace{1cm} (5)

Since the solubilities of Fe(OH)$_3$ and Al(OH)$_3$ are extremely low (2.64 × 10$^{-39}$ and 1.1 × 10$^{-33}$), more phosphorus is released to the overlying water. In contrast, under sterilisation conditions, indissoluble phosphate in
Sediments is released very slowly and the quantities are very small during initial 20 days.

**pH changes in 2# and 5# chambers**

The pH of original overlying water was 7.38. Under aerobic conditions, in both 2# and 5# chambers pH values fluctuated around 7.5 (see Figure 4) However, pH in chamber 5# was gradually increased from 7.52 to 8.35 as DO declined below 1mg/L. In chamber 2#, pH fell a little and the average value was 7.2.

The pH of Tianjin source water is 7.38. Under aerobic conditions, pH fluctuated between 7.3 and 7.6 at the beginning. Then it rapidly got to 8.35. Anoxic chamber experiments were bubbled with nitrogen gas, thereby scrubbing out chamber water CO2 and presumably maintaining or increasing pH. The displacement of OH\(^{-}\) and PO\(_{4}^{3-}\) enhances the phosphorus transportation from sediment to water (Marc Watson Beutel 2002). The pH of 2# decreases and is maintained at 7.2. The results show that microorganisms are the dominant parameter that controls the phosphorus release.

**Changes of OP and phosphatase in sediments**

Based on the different mineralisation rates, OP fractions can be divided into four kinds, including Labile OP, Moderately Labile OP, Moderately Resistant OP and Highly Resistant OP. Moderately Labile OP describes the most easily mineralised OP. Through Bowman-Cole method it has been found that Labile OP in soil could increase as phosphatides and nucleic acids grow (r\(^2\) = 0.863; r\(^2\) = 0.868) (He & Li 1987). Since alkaline phosphatase is mainly to supply nutrition to the cells of microorganisms from circumambience, anabolism of alkaline phosphatase is closely related to the concentration of phosphorus.

In comparison experiments (1# and 2#), the activity of alkaline phosphatase fluctuated within a narrow range. But the activity in 4# and 5# chambers increased quickly after the anaerobic experiments, with 74.2% rise in 4# and 33.8% rise in 5#. It is clear that there was great mineralization of Labile OP in 4# under aerobic condition with the efficiency of 47%. After anaerobic experiment, the Moderately Labile OP declined to the minimum level which was 0.52 mg/kg in 4# chamber and 0.57 mg/kg in 5# chamber. The results are shown in Table 2.

The results show that little difference of Labile OP was found when pH \(>\) 7. The phosphatase in the sediment of Tianjin source water is mostly alkaline. As a nonspecific enzyme, the alkaline phosphatase mostly exists in sediments and is able to catalyze the hydrolysis of OP. As a result, the Labile OP can be hydrolyzed into simple inorganic phosphate and released to the overlying water. Although the relationship between Labile OP and alkaline phosphatase can't be described as linearity, significant uniformity appears in this experiment. The mineralizing efficiency of Moderately Labile OP is much higher than other kinds of OP based on Bowman-Cole method.

**CONCLUSIONS**

The microbial effects on phosphate release characteristics and the trend are studied by simulating the circumstance of sediment–water interface. The results show that microorganisms play an important role in the course of P release from sediment. Under neutral and acidic conditions, microorganisms are able to use Fe\(^{3+}\) as electron acceptors and induce the release of phosphate. Under alkaline conditions, microorganisms may dissolve the indissoluble phosphate to accelerate the exchange of OH\(^{-}\) and PO\(_{4}^{3-}\) that comes from Fe-P and Al-P. The activity of alkaline phosphatase is mainly to supply nutrition to the cells of microorganisms from circumambience, anabolism of alkaline phosphatase is closely related to the concentration of phosphorus.
phosphatase in sediments is coincident with the release trend of dissolved phosphate in overlying water. Due to microorganisms, the mineralising efficiency of Moderately Labile OP is much higher than other kinds of OP based on Bowman-Cole method, and the relationship between Labile OP and alkaline phosphate shows negative correlation.

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