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Effects of overlying water aeration on phosphorus fractions and alkaline phosphatase activity in surface sediment

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Abstract

Microbial activity may influence phosphorus (P) deposit and release at the water sediment interface. The properties of DO (dissolved oxygen), pH, P fractions (TP, Ca-P, Fe-P, OP, IP), and APA (alkaline phosphatase activity) at the water sediment interface were measured to investigate microbial activity variations in surface sediment under conditions of two-month intermittent aeration in overlying water. Results showed that DO and TP of overlying water increased rapidly in the first week and then decreased gradually after 15 day of intermittent aeration. Microorganism metabolism in surface sediment increased pH and decreased DO and TP in the overlying water. After two-month intermittent aeration, APA and OP from surface sediment (0–2 cm) were both significantly higher than those from bottom sediment (6–8 cm) ($p < 0.05$), and surface sediment Fe-P was transferred to OP during the course of microorganism reproduction on the surface sediment. These results suggest that microbial activity and microorganism biomass from the surface sediment were higher than those from bottom sediment after two-month intermittent aeration in the overlying water.

Key words: aeration; disturbance; sediment; phosphorus fractions; alkaline phosphatase

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Introduction

Phosphorus (P) is often accepted as the most critical limiting nutrient for primary lake productivity (Dorich et al., 1985), and is usually introduced to lake ecosystems by wastewater and accumulated in sediment. P can be released into the overlying water under certain environmental conditions (Boström and Pettersson, 1982; Wentzel et al., 1989), which may lead to continuing water eutrophication.

Extensive studies have been conducted on environmental factors affecting P release from lake sediment, including temperature, pH, DO (dissolved oxygen), hydrological condition, and wind disturbance (Emil, 2000; Kim et al., 2003). Many studies have also investigated the mechanism of how DO affects surface sediment P release and transformation. To date, however, most previous research has focused on physical and chemical mechanisms under aerobic and anoxic conditions (Gomez et al., 1999; Kim et al., 2004).

Abundant benthic algae and microorganisms grow on surface sediment of shallow lakes (Mur and Schreurs, 1995), and microbial activity may influence nutrient deposit and release at the water sediment interface (Lau and Lane, 2002; Xie et al., 2003). Phosphatase is produced by plankton and microorganisms, which can hydrolyse OP (organic P) and release IP (inorganic phosphate). Traditional phosphatase activity research has mainly focused on marine systems, with only a few studies conducted on lake water and sediment APA (alkaline phosphatase activity) (Zhou and Fu, 1999; Zhou et al., 2002). Lake APA plays a key role in organic matter mineralization (Chrost et al., 1984), and is correlated with exotic lake nutrient pollutants such as organic P and organic nitrogen (Xue et al., 1995a). Other factors affecting APA in sediment have also been investigated including total sediment bacteria number, P release rate, P fraction, and P bioavailability (Sayler et al., 1986; Zhou et al., 2002; Zhang et al., 2007). Aeration is an effective method for improving water quality, and can accelerate oxidation and decomposition of pollutants by providing sufficient DO in water (Raul, 2007). However, the effects of long-term (two-month) aeration on P fraction transformation and APA activity at the surface sediment have not been considered.

We conducted a controlled experiment to investigate: (1) the variations of P release from surface sediment with intermittent aeration in the overlying water for two months;

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and (2) the transformation of P fractions (TP, Ca-P, Fe-P, OP, IP) and variation of APA in surface sediment (0–2 cm) during the study period.

1 Materials and methods

1.1 Sampling and pretreatment

Water and sediment samples were collected from a shallow landscape pond with an average water depth of 2.0 m near the Chinese Research Academy of Environmental Sciences, Beijing, in August 2009. Sediment samples (0–10 cm) were collected using a Petersen grab sample from different spots, and were then mechanically homogenized in a tank. The overlying water samples were collected at a depth of 0.5 m below the water surface and filtered by gauze immediately.

1.2 Experimental apparatus and treatment

Experiments were conducted in six transparent glass tubes (650 mm diameter × 800 mm length) with gauze stoppers inserted at the top (Fig. 1, T1–T6). Two of six tubes (T3, T4) were connected to air pumps with airflow of 1.2 L/min, and two (T5, T6) were connected to rotary propellers with speed 60 r/min. Homogeneous sediment samples were injected into experiment tubes (T1, T3, T5) to a height of 10 cm from the bottom, with filtered pond water then slowly added to a height of 500 mm. Pond water was also added to the other tubes (T2, T4, T6) to a height of 500 mm without sediment in the bottom. All tubes were wrapped with black paper 15 cm from the bottom and left to stand in the dark without treatment for 12 hr. The tubes were then irradiated under a light intensity of 2500–2800 lx with a light/dark cycle of 12:12 hr. Four pumps connected to the tubes were operated from 10:00 to 16:00 every day. The experiments lasted for 60 days. Distilled water was added every 5 days to maintain the water column height of 500 mm. The surrounding temperature was maintained at (25 ± 3)°C by air conditioner.

1.3 Analytical methods

Levels of DO and pH were measured by sension portable meters (sension^{TM}156, HACH Company, USA). Concentrations of TP in overlying water were determined using standard methods (SEPA, 2002) on scheduled days during the experiment. The SMT method (Ruban et al., 1999) was used to analyze P fractions in the sediment at the beginning and end of the experiment. The method comprised five steps, namely measurements of Fe-P (P bound to Al, Fe and Mn oxides and hydroxides), Ca-P (P associated with Ca), OP (organic P), IP (inorganic P), and TP (total P). According to this method, Fe-P in 0.2 g of sediment was extracted by 1 mol/L of NaOH and Ca-P was extracted from the residual material by 1 mol/L of HCl. The IP was extracted by 1 mol/L of HCl from 0.2 g of sediment and the residual material was treated at 450°C and then extracted by 1 mol/L of HCl to analyze OP. The TP was determined by treating 0.2 g of sediment at 450°C, and then extracted by 1 mol/L of HCl.

The APA was determined by an ultraviolet-visible spectrophotometer as the release of *p*-nitrophenol from the model substrate *p*-nitrophenyl phosphate (PNPP). The reaction mixture contained 0.5 g of sediment, 10 mL of 0.05 mol/L Tris-buffer, pH 8.5, 5.0 mL of 0.2 mol/L NaN_3, and 5.0 mL of 0.12 mmol/L PNPP. Samples were incubated at 37°C for 2 hr with shaking. Reaction was terminated by the addition of 2.5 mL of 0.4 mol/L NaOH and all samples were then analyzed by spectrophotometer at 410 nm (Hadas and Pinkas, 1997). The results of specific APA were expressed as *p*-nitrophenol (mg/(kg·hr)). All samples were run in four replicates and data were analyzed by SPSS 16.0.

2 Results

2.1 Characteristics of overlying water and sediment

The DO and pH of overlying water samples measured in the field were 6.9 and 8.0 mg/L, respectively. For water samples, COD (chemical oxygen demand) was 7.97 mg/L, TP was 0.19 mg/L, and TN (total nitrogen concentration)

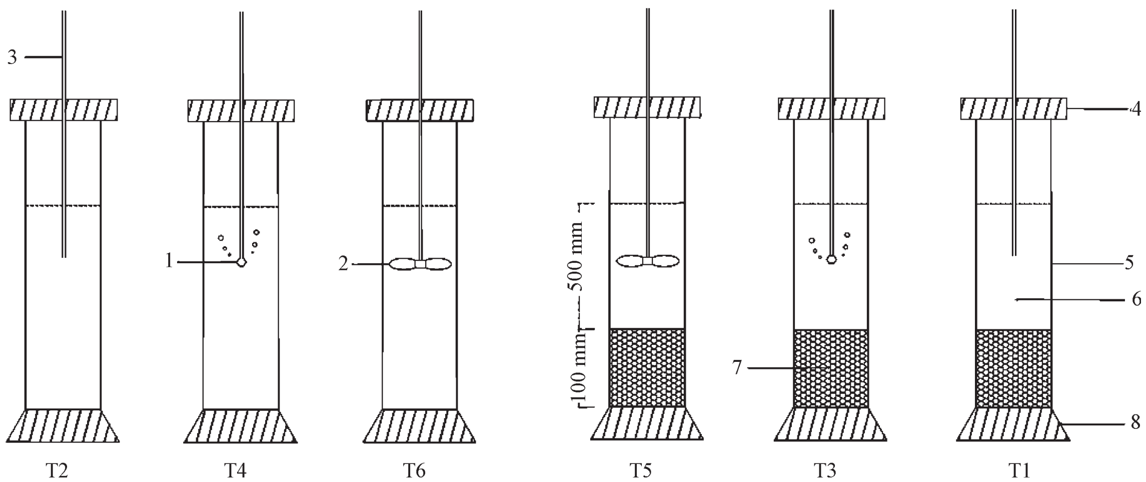

Fig. 1 Experimental apparatus. (1) air pump; (2) rotary propeller; (3) connection tube; (4) gauze stopper; (5) reactive tubes; (6) overlying water; (7) sediment; (8) rubber stopper.

was 1.18 mg/L. The initial content of P fractions in the sediment measured as Fe-P, Ca-P, IP, OP, and TP were: (52.2 ± 1.07), (356 ± 19.6), (456 ± 8.75), (45.0 ± 3.75), (539 ± 15.8) mg/kg, respectively. Accounting for 66% of TP, Ca-P was the major fraction in the original sediment, while Fe-P and OP accounted for 9.7% and 8.3% of TP, respectively.

2.2 DO, pH and TP variations in overlying water

During the experimental period, DO in overlying water of different tubes showed different regulations (Fig. 2). In tubes without aeration (T1, T2), DO gradually increased to 6.0 mg/L during day 14 to day 30 and then fell to 3.0 mg/L at day 30 to day 45. In addition, a gradually decline of DO from 3.0 to 0.83 mg/L during day 2 to day 14 occurred in T1, and the average DO was significantly lower than that in T2 (T-test, $p < 0.05$). Thus, sediment can accelerate consumption of DO in overlying water. Aeration improved DO in the overlying water from 3.0 to 7.5 mg/L from day 2 to day 7. However, DO fell to the beginning concentration during day 30 to day 50. There was no difference in DO between T3 and T4 (T-test, $p > 0.05$), but a significant difference existed between T1, T3, and T5 (ANOVA, $p < 0.05$). As a result, sediment promoted the expenditure of DO in the overlying water. Aeration greatly improved DO in two to three days, and was sustained at 6–8 mg/L within two weeks. Water DO in all tubes began to decline by day 30, however, and dropped to 3–4 mg/L by day 50.

In tubes without aeration, pH in overlying water declined a little during the first two weeks and gradually increased to a peak at day 30 (Fig. 3). It then declined and reached a stable value during day 45–60 (T1, T2). The average value and variation rate of pH in T1 were obviously lower than those of T2 (Fig. 3), which suggests that sediment helped sustain pH stability in overlying water. Furthermore, the average value of pH in T3 and T5 were both significantly higher than those in T1 (ANOVA, $p < 0.05$). Thus, two-month intermittent aeration could increase the overlying water pH of the water sediment interface.

The overlying water TP in tubes (T1–T4) showed similar changes during the first two weeks and then began to decline after day 30, while the regulation of intermittent disturbance (T5 and T6) was not obvious (Fig. 4). The rate of TP variation in overlying water of T1 was significantly higher than of T2 (T-test, $p < 0.05$), which suggests that sediment greatly affected P concentration in overlying water. In addition, the rate of TP variation in T5 was higher than in T3, followed by T1 (Fig. 4, T1–T6). Thus intermittent aeration and intermittent disturbance could both accelerate the release of TP from sediment during the first two weeks, while the release of P was more rapidly under conditions of disturbance than aeration.

2.3 Variations of P fractions and APA in sediment

Sediment P fractions (TP, Ca-P, Fe-P, OP, and IP) in tubes T1, T3, and T5 were measured in surface (0–2 cm) and bottom sediment (6–8 cm) at the end of the experiment. In each tube, Ca-P, Fe-P, and OP from surface sediment (0–2 cm) were all higher than those from bottom sediment (6–8 cm) (Table 1). Among different tubes, surface sediment TP and Ca-P did not show significant variations from each other, while Fe-P and OP did (ANOVA, $p < 0.05$). Surface Fe-P in T3 was significantly lower than in T1, while surface OP in T3 was significantly higher than in T1 (T-test, $p < 0.05$). Surface OP was higher than in bottom sediment in each tube (ANOVA, $p < 0.05$). In addition, a large amount of bio-film was observed on

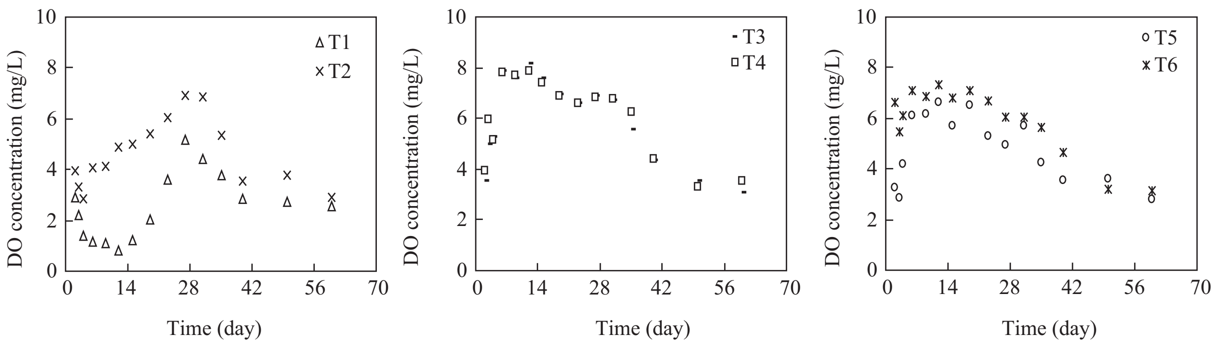

Fig. 2 Variations of DO in overlying water.

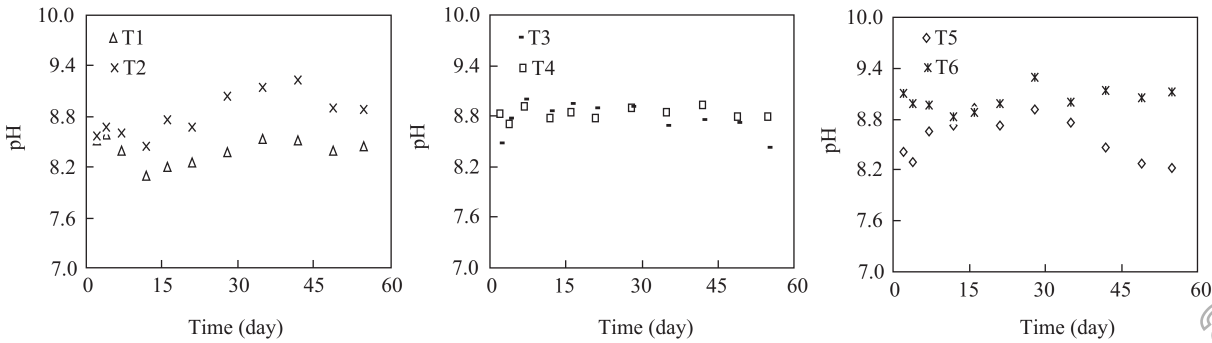

Fig. 3 Variations of pH in overlying water.

Table 1 P fractions concentration of surface and bottom sediment

Tube	TP (mg/kg)		Fe-P (mg/kg)		Ca-P (mg/kg)		IP (mg/kg)		OP (mg/kg)	
	0–2 cm	6–8 cm	0–2 cm	6–8 cm	0–2 cm	6–8 cm	0–2 cm	6–8 cm	0–2 cm	6–8 cm
T1	560 ± 15.8	528 ± 20.7	58.8 ± 2.04	51.9 ± 1.66	363 ± 10.7	352 ± 13.1	429 ± 26.9	450 ± 8.61	88.9 ± 3.22	73.9 ± 0.805
T3	513 ± 35.5	535 ± 16.6	52.2 ± 1.23	50.1 ± 2.62	354 ± 4.81	348 ± 5.69	431 ± 12.9	431 ± 17.3	96.7 ± 1.57	82.4 ± 3.76
T5	560 ± 20.8	534 ± 7.05	58.2 ± 2.58	55.3 ± 1.31	368 ± 10.9	369 ± 2.15	470 ± 14.5	465 ± 10.6	49.0 ± 6.46	33.0 ± 0.793

Data are present as mean ± SD (n = 3).

the surface sediment and inner wall of the tubes after intermittent aeration in overlying water for 30 days. From these results, we concluded that surface sediment Fe-P was transferred to OP during the massive multiplication of microorganisms on the surface sediment. The APA in surface (0–2 cm) and bottom (6–8 cm) sediment of three tubes was measured on day 55 (Fig. 5). Surface sediment APA showed obvious regulations of T1 < T3 < T5 and significant differences (ANOVA, $p < 0.05$). Although surface sediment APA was much higher than that from bottom sediment in each tube (T-test, $p < 0.05$), there was no significant difference between bottom sediment APA from the different tubes (T-test, $p > 0.05$).

3 Discussion

Benthic algae and microorganisms at the water sediment interface can stimulate P release (Sundbäck et al., 1991) via their metabolism or by altering aspects of the surrounding environment. The mechanism regarding the effect of overlying water aeration on surface sediment P transformation by microbial activity has rarely been considered, however. In this study, we provide evidence showing that: (1) significant variations of APA and P fractions occurred in surface sediment with two-month intermittent aeration in overlying water; and (2) APA and OP in surface sediment (0–2 cm) were both significantly higher than those from bottom sediment (6–8 cm).

Aeration rapidly increased overlying water DO in the first week. The DO began to decline after intermittent aeration for 30 days and was maintained at 2.5–3.5 mg/L after day 50, even though aeration continued. Previous research has shown that abundant microorganisms grow at the sediment water interface, where microbial reactions create an anaerobic and reductive environment (Haglund et al., 2003). Thus, we concluded that the decline in overlying water DO was a result of microorganism consumption at the water sediment interface. Abundant bio-film was also observed growing on inner wall of the experimental test tubes (T3, T5), and a significant amount of DO in overlying water could be consumed due to massive multiplication of microorganisms on the surface sediment.

The pH in the overlying water is affected by many factors, especially microbial metabolism at the water sediment interface (Jiang et al., 2006). In this study, overlying water pH of T1 was significantly lower than that of T2 (T-test, $p < 0.05$) and intermittent aeration increased the overlying water pH (T-test, $p < 0.05$) of the water sediment system. Many carbonate and organic acids produced by abundant microorganisms growing on the surface sediment, which caused the decline of pH in overlying water. Previous studies have reported that algae photosynthesis

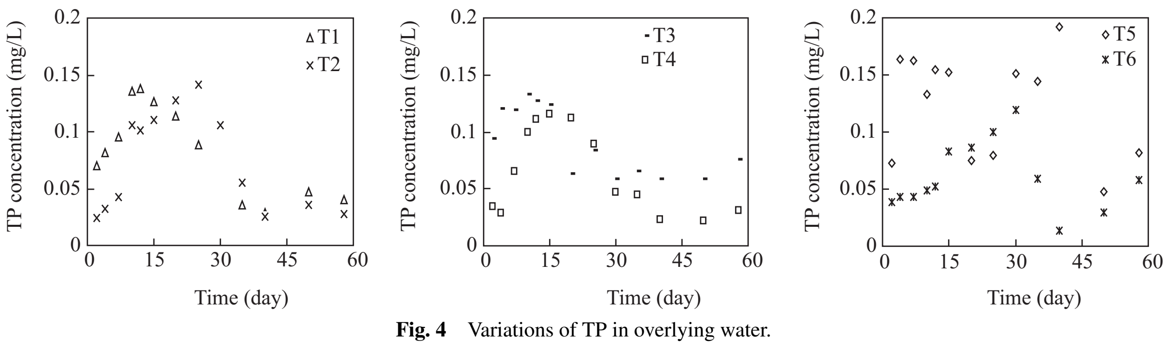

Fig. 4 Variations of TP in overlying water.

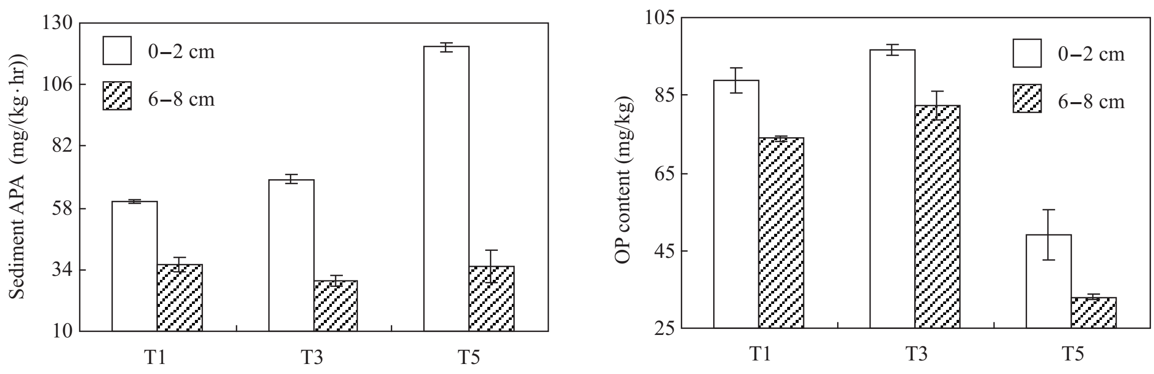

Fig. 5 Variations of OP content and APA in sediment.

may result in an increase in water pH (Kennedy and Hutchins, 1992). In this study, abundant filamentous green algae were observed on the sediment surface or bottom surface of each tube after 30 days. The photosynthesis of such filamentous green algae in tubes of intermittent aeration were likely responsible for the increase in the pH in overlying water.

During the experiment period, overlying water TP in tubes (T1–T4) increased for the first two weeks and then declined after 30 days, while the regulations in T5 and T6 did not. P can be released from sediment under both aeration and non-aeration conditions (Gomez et al., 1999), which was also observed in our experiment as the overlying TP of each tube increased during the first two weeks. Previous studies have reported that decreasing TP in overlying water can be attributed to absorption of surface sediment and utilization by microorganisms at the sediment water interface (Lau and Lane, 2002; Wu et al., 2008). After 30 days, abundant filamentous green algae were observed on the sediment surface and inner surface of each tube, which likely caused the decrease of TP in the overlying water. In addition, faster decline of overlying water TP occurred in T3 and T5 compared to T1 (Fig. 4). Accordingly, we observed much more filamentous green algae growing on sediment surface under aeration (T3, T5) conditions than under control conditions.

Fe-P was used to estimate available P in the sediment and is an indicator of available algal P (Zhou et al., 2001). In previous reports, an increase in Fe-P was considered a consequence of human activity, and Fe-P was easily released for phytoplankton growth under anoxic conditions at the sediment-water interface (Ting and Appan, 1996; Ruban et al., 1999, 2001). When soluble IP in water is low, plankton or microorganisms may produce phosphatase to hydrolyse organic P and release inorganic phosphate. Sediment Fe-P and OP are considered a potential resource for overlying water, while Ca-P is relatively stable and difficult to release in physical and chemical processes (Ruban et al., 1999). In this experiment, APA and OP in surface sediment were all significantly higher than in bottom sediment for T1, T3, and T5. Most extracellular enzymes are associated with bacteria (Chrost, 1990), and benthic microorganisms can synthesis OP to sustain their growth. Thus, our results suggest that microbial mass in surface sediment (0–2 cm) was much larger than in bottom sediment (6–8 cm).

Surface sediment OP and APA both showed significant regulation of T3 > T1 (Fig. 4, T-test, $p < 0.05$), while Fe-P showed significant regulation of T3 < T1. TP did not show significant variation (Table 1, T-test, $p < 0.05$) between T3 and T1. Sediment APA was associated with microorganisms and algae biomass (Chrost, 1990; Zhang et al., 2007). As APA and OP in the surface sediment both showed significant regulation of T3 > T1, much more algae were observed on the sediment surface of T3 than of T1. These results suggest that two-month intermittent aeration in overlying water was favorable to the growth of microorganisms and filamentous green algae, during which surface sediment Fe-P was utilized to synthesis OP for their multiplication, and APA in the surface sediment increased accordingly. The OP in surface sediment showed significant regulation of T5 < T3, while surface sediment APA showed significant regulation of T5 > T3 (Fig. 5, T-test, $p < 0.05$), and average TP in overlying water was T5 > T3. On condition of turbulence, suspended particulates reduced bacteria settling on the surface sediment, thus OP decreased and was released into the overlying water. However, the APA increased in spite of the decline in microorganism quantity, which suggests that microbial activity in surface sediment was greatly enhanced under conditions of two-month intermittent disturbance in overlying water.

4 Conclusions

The DO and TP in overlying water rapidly increased during the first week and declined after 15 days under intermittent aeration. Filamentous green algae grew in all test tubes and abundant bio-film grew in tubes under intermittent aeration after 30 days. The metabolism of microorganisms in the surface sediment increased pH while decreased DO and TP in overlying water.

Sediment APA was associated with microorganism and algae biomass. In the present study, APA and OP from surface sediment (0–2 cm) were both significantly higher than those from bottom sediment (6–8 cm) in each tube. As a result, microbial activity and microorganism biomass from surface sediment was higher than from bottom sediment after two-month intermittent aeration in overlying water. The Fe-P was utilized to synthesis OP for microorganism reproduction and APA was elevated due to the increase in microbial activity in the surface sediment.

Acknowledgments

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