

INVESTIGATION OF BIOTIC UPTAKE AND RELEASE OF PHOSPHORUS BY A WETLAND SEDIMENT

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Abstract

There is currently considerable controversy about the role of bacteria in the direct uptake and release of phosphorus by sediments. This paper reports work aimed at testing the hypothesis that microbial uptake and release of phosphorus by sediments is an important process in wetlands and that this process is influenced by the type of organic matter in the sediment. Experiments were undertaken with a wetland sediment to which was added both a carbon source (glucose or acetate) and orthophosphate (P). The carbon sources were chosen to represent a simple available carbon source (glucose) and a fermentation product (acetate). Control experiments were also undertaken using sterilised sediment with P added and an unsterilised sediment without added P. Under aerobic conditions, 100% of the added orthophosphate was taken up biologically by both the organic carbon-amended sediments, with 34-45% of this estimated to be microbial uptake. A smaller amount (ca. 9%) of the added phosphorus was taken up biotically by sediments that had no added carbon. When these aerobic uptake experiments were made anaerobic (and additional organic carbon added), only the acetate-amended sediment released an appreciable amount (ca. 30%) of the initially added phosphorus. We postulate that the acetate-amended sediment took up P for cell growth and also stored some P as polyphosphate (poly-P). This poly-P was then released under anaerobic conditions. The glucose-amended sediment used the added P for cell growth only, and this phosphorus was not released when the sediment was made anaerobic for a period of 10 days.

These preliminary results suggest an important additional role for sediment bacteria in the cycling of phosphorus. In the presence of bioavailable organic carbon and orthophosphate, sediment bacteria can grow well under aerobic conditions but do not seem to release the phosphorus under anaerobic conditions (at least not within the 10 days of the experiments reported here). However, in the presence of acetate, it appears the “luxury uptake” of added phosphorus can occur under aerobic conditions, and that this poly-P is released when the sediments are made anaerobic. More work is needed to determine the relative importance of these microbial uptake and release mechanisms in different sediment environments.

Keywords: Abiotic, acetate, biotic, uptake, release, glucose, phosphorus, poly-P bacteria, redox conditions, sediment, wetlands.

INTRODUCTION

Eutrophication of waterbodies in Australia and elsewhere is a major environmental issue [1]. Of the key factors causing eutrophication, phosphorus has been identified as the one that can potentially be managed to effect the greatest impact for the least cost [2]. For this reason there has been considerable focus in recent years on reducing phosphorus inputs to waterbodies from point sources and more recently also from diffuse sources. However, reducing the external loading alone may not guarantee a rapid recovery of the water body, since phosphorus may be released from the sediments [2 – 5].

The exchange of phosphorus between water and sediment is complex, involving interrelated chemical, biological and physical processes [4, 6]. Until quite recently, phosphorus transfer at the sediment-water interface has been considered to be largely abiotic process [7], with sediment bacteria assumed to play an indirect role only by modifying the redox conditions controlling the abiotic processes [8 -10]. However, there is now considerable evidence, indicating that both abiotic and biotic processes are important in the release of P from sediments, although the relative contribution from each process has yet to be fully quantified [9, 11, 12]. The physical and chemical processes are quite well known, but the role of bacteria in the biogeochemistry of sediments is still poorly understood [9, 12].

There are now many studies that support the view that microbial activity has a significant influence on the uptake and release of phosphorus by sediments [13 - 15]. Bacteria can affect phosphorus release both directly and indirectly. The direct action can be by: (i) decomposition of organic phosphorus compounds, (ii) degradation of stored polyphosphate under anaerobic conditions and (iii) mediating anaerobic dissolution of iron-P complexes [16]. However, this release of P can be moderated by subsequent uptake during microbial growth, which can occur under anaerobic conditions. Bacteria can also indirectly affect the release of phosphorus by: (i) lowering redox potential by consuming oxygen in the pore water, thus enhancing the release of phosphorus from iron-P complexes, (ii) reducing Fe (III) directly to Fe (II) when iron-reducing bacteria are present, and concurrently releasing phosphorus previously adsorbed to the iron oxide surface and (iii) reducing sulfate to sulfide (when sulfate-reducing bacteria are present) which then acts as a strong reducing agent facilitating the reduction of Fe (III) to Fe (II) with concurrent phosphorus release [16, 17].

From past studies [6, 12], two themes consistently emerge; first, the importance of organic matter in the uptake and release processes, and second, the role of bacteria that have the ability to store phosphorus as polyphosphates (poly-P bacteria). It has been suggested that under anaerobic conditions, poly-P bacteria can hydrolyse these polyphosphates to orthophosphate which is then released to the water column [8, 9, 12]. This metabolic process is stimulated in poly-P bacteria (e.g. *Acinetobacter* species), by low molecular weight organic acids, such as acetate [18].

This paper reports a preliminary investigation of the influence of two organic carbon source (glucose and acetate) and redox conditions on the uptake and release of phosphorus by a wetland sediment. The carbon sources were chosen to represent a simple available carbon source (glucose) and a fermentation product (acetate). This study is part of a larger investigation into the role of sediment bacteria in the biogeochemical cycling of phosphorus in sediments [19].

MATERIALS AND METHODS

Site description:

Sediment samples were obtained from the Monash University Research Wetland located in the Lance Creek catchment, South Gippsland. This wetland system has been constructed to investigate the role of wetlands in improving the quality of run off from agricultural catchments by reducing the levels of suspended solids and nutrients. The wetland system consisting of a series of four cells or ponds with a total volume of 1700 m³.

Sediment characterisation:

The top 5 cm layer of sediment was obtained (using an Eckman grab) from wetland 1 on 1 May 1996. Large vegetation was separated from the sediment sample gently by hand, so that only tiny roots remained. The sediment was kept at 4 °C in polyethylene bags until characterisation. The analytical methods used to characterise the sediment are summarised in Table 1. Full details are given in Standard Methods for the Examination of Waters and Wastewaters [20]. Three replicates were done for each analysis. Cells were counted using the 4'6-diamidino-2-phenylindole (DAPI) direct count method reported by Porter and Fieg [21].

Table 1. Methods for characterisation of Monash Wetland sediment

Parameter	Method
Dry weight	oven drying at 105 °C for 24 hr
Organic matter	loss on ignition at 450 °C
Total Fe	nitric acid digestion, AAS*
Total Extractable Fe	NH ₂ OH-HCl Extraction, AAS
Total Al	concentrated nitric acid digestion, AAS
Total Mg	concentrated nitric acid digestion, AAS
Total Ca	concentrated nitric acid digestion, AAS
Total Mn	concentrated nitric acid digestion, AAS
Total P	nitric acid digestion/ammonium molybdate method
Total carbon (TC)	by solid sample module schimadzu SSM-5000A
Total Organic Carbon (TOC)	by Total organic carbon analyser, TOC- 5000
Total Inorganic Carbon (TIC)	TIC=TC-TOC
Cell count after sampling	DAPI staining
Mean diameter of sediment partic	Malvern Master Sizer/E

* AAS=Atomic Absorption Spectrometer (Perkin Elmer model-1100)

Experimental set up

Six 2.5 L brown glass containers were used, the brown colour being selected to prevent algal growth by reducing light penetration during the experiment. Wet sediment (35 g) was added to 1 L of filtered (0.45 µm Millipore) water taken from the wetland. Five of the six containers were augmented with orthophosphate (1 mg P/L as KH₂PO₄) and different carbon sources as indicated in Table 2. The selection of organic carbon sources was influenced by finding from the area of enhanced biological phosphorus removal from sewage effluent where it has been well documented that the existence of a reduced carbon compound such as acetate enhances the phosphorus release in the anaerobic stage of the process [18]. We therefore selected acetate as a typical end product resulting from the fermentation of more complex organic compounds, and likely to support phosphorus release under anaerobic conditions, and glucose as a simple organic carbon source. The other container was kept as

a control. Formaldehyde was added to experiment 6 (4%v/v) to prevent the growth of bacteria during the experiment. Unfortunately, because of the preliminary nature of these experiments and the number of experiments run, it was not possible to run replicates.

Table 2. Experimental conditions

Experiment No	P added (1 mg/L)	Organic C added (100 mg C/L)	Formaldehyde added (4% v/v)
1	×	×	×
2	√	×	×
3	√	Acetate	×
4	√	Glucose	×
5	√	Acetate + Glucose	×
6	√	×	√

x-no, √-yes

Aerobic experiments

Humidified air was bubbled with similar flow rates through all samples. After 30 minutes of bubbling, phosphorus and a carbon source were added (Table 2). A 30 mL sample was taken immediately from each container using a 40 mL syringe as the zero time sample. Samples were then taken after 1 h, and subsequently after each 24 h up to 162 h. Before sampling, the container was shaken by hand. At the time of sampling, pH (Activon-A211) and dissolved oxygen (DO - YSI Model 57) were also measured. The samples were centrifuged at 3450 rpm (Beckman Avanti™) for 15 minutes and the supernatant was filtered through 0.45 µm membrane filters. Filtrates were frozen and analysed for filterable reactive phosphorus (FRP-FIAstar 5010 System, ammonium molybdate method, APHA, 1985) within two weeks. Replicate samples were analysed for each container. DO was kept >5 mg/L in all containers during the aerobic experiment.

Anaerobic experiments

After 162 hours, air was replaced with N₂ gas which was bubbled through the containers for 45 minutes until the DO had dropped to less than 1 mg/L. Samples were then taken,

centrifuged and filtered. Additional carbon (100 mg C/L) was added to test containers 3, 4 and 5 as indicated in Table 2. The containers were then filled with N₂ and the tops sealed with parafilm^M. pH and DO were measured daily and a sample taken for FRP determination at regular intervals up to 244 hours. N₂ gas was bubbled through each container during sampling and the pH and DO were measured. At the end of experiment, around 80% of the initial sediment suspension volume remained. DO values were kept at < 0.5 mg/L, during these experiments.

Cell counts

Bacterial numbers were determined by staining the cells using DAPI (4', 6-diamidino-2-phenylindole, Sigma Chemical Co., St Louis, Mo, USA) and counting the stained cells under epifluorescence microscopy. Five mL of sediment suspension was taken at 164, 234 and 330 hours during the anaerobic experiment, preserved using formaldehyde (4% v/v) and kept at 4°C before staining and cell counting. Sediment suspensions were diluted and sonicated in a water bath for 3 minutes three times with 30 second stops in between. Cell counting was done by filtering 5-10 µL of the DAPI stained sediment suspension on prestained (Irgalin black treated) 0.2 µm polycarbonate filters.

RESULTS & DISCUSSION

Sediment characteristics

The sediment tested had characteristics typical of a wetland sediment (see Table 3). The organic carbon content was 5.4 % (dry wt), total iron content 31,300 ug/g (dry wt), with almost 40% of this in NH₂OH-HCl extractable forms, and the total phosphorus concentration was 1025 ug/g (dry wt).

pH

The results for pH are presented in Figure 1. The pH remained relatively constant (7.5 to 8.0) during the six aerobic experiments, but varied from 7.9 to 6.8 during the anaerobic experiments for all containers except that with glucose added. In this latter case the pH ranged from 8.0 to 6.3 (Figure 1). The reason for the variation in pH during the anaerobic experiments was possibly due to different rates of bacterial organic matter degradation, producing different amounts of CO₂ in the solution, with a consequent influence of this

solution CO₂ on the pH. This hypothesis is supported by the fact that the glucose-amended system had the highest bacterial growth (see Figure 3) and also achieved the lowest pH.

Table 3. Characteristics of Monash wetland sediment.

Parameter	Result
Dry weight	43.0 ± 1.0 %
Organic matter	12.0 ± 0.9 %
Total Fe	31.3 ± 0.4 mg/g dw
Total Extractable Fe	12.0 ± 1.4 mg/g dw
Total Al	29.0 ± 0.1 mg/g dw
Total Mg	3.9 ± 0.1 mg/g dw
Total Ca	3.50 ± 0.05 mg/g dw
Total Mn	0.55 ± 0.01 mg/g dw
Total P	1025 ± 7 µg/g dw
Total carbon (TC)	5.38 ± 0.02 %
Total Organic Carbon (TOC)	5.40 ± 0.10 %
Total Inorganic Carbon (TIC)	0.00 ± 0.01 %
Cell count after sampling	0.8 ± 0.2 E+09 Cells/mg dw
Mean diameter of sediment particles	31.0±0.2 µm

Aerobic experiments

The changes in the concentration of filterable reactive phosphorus during the aerobic and anaerobic experiments are shown in Fig. 2. The same general trend was apparent in all experiments, with an initial rapid uptake of P followed by a further slower uptake. The rapid uptake is possibly an abiotic process controlled by the physico-chemical sorption of orthophosphate onto the sediment particles [22], while the slower uptake step is likely to be due to microbial uptake [23]. House et al. [23], in studying the kinetics of phosphorus uptake on river bed sediments, concluded that the net microbial uptake of P may contribute significantly during the period of slow kinetics. We hypothesis that during the slow uptake stage, the bacteria transfer phosphorus into cells where it is either incorporated into the cell biomass or is accumulated as intracellular products such as polyphosphate (Poly-P) [24].

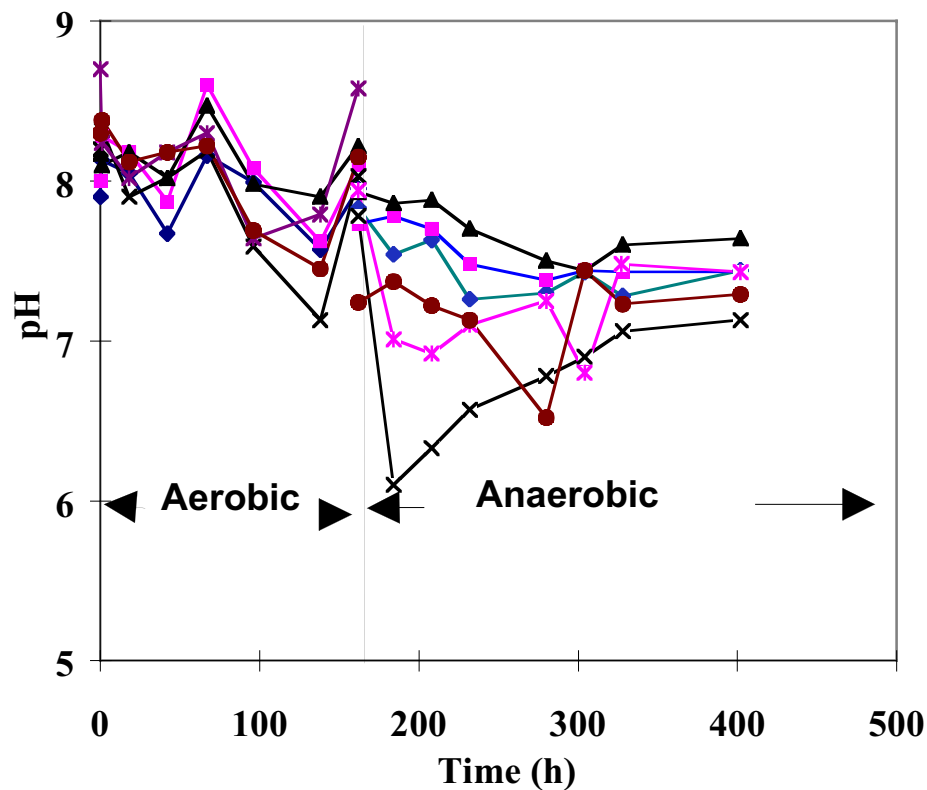


Figure 1. pH (in aerobic and anaerobic) of solutions during the experiments. Key to the experiments: ◆ Control (1); ■ P only added (2); ▲ P and acetate added (3); × P and glucose added (4); * P + acetate and glucose added (5); ● P and formaldehyde added (6).

Table 4 contains a summary of the percent of the added phosphorus that was taken up during the aerobic experiments, and the proportion of total P uptake due to bacterial uptake. The latter was obtained by subtracting the total P uptake in the sterilized container (No 6) from the total P uptake in each container. The amount of P released during the anaerobic stage was obtained by subtracting the final P concentration in the solution (at the end of these experiments) from the concentration at the beginning.

The sterilised sample (No 6) showed the lowest P uptake. We assumed that this experiment measures the abiotic-only uptake because addition of formaldehyde should prevent the growth of microorganisms. A total of 68% of the added P was taken up in experiment 2, which when compared with the sterilised sample suggests that around 10% of the added P was taken up by bacteria. Introduction of a bioavailable carbon source (acetate or glucose)

to containers 3, 4 and 5 resulted in a significantly higher uptake of phosphorus compared with those test containers that contained no added carbon source (Table 4).

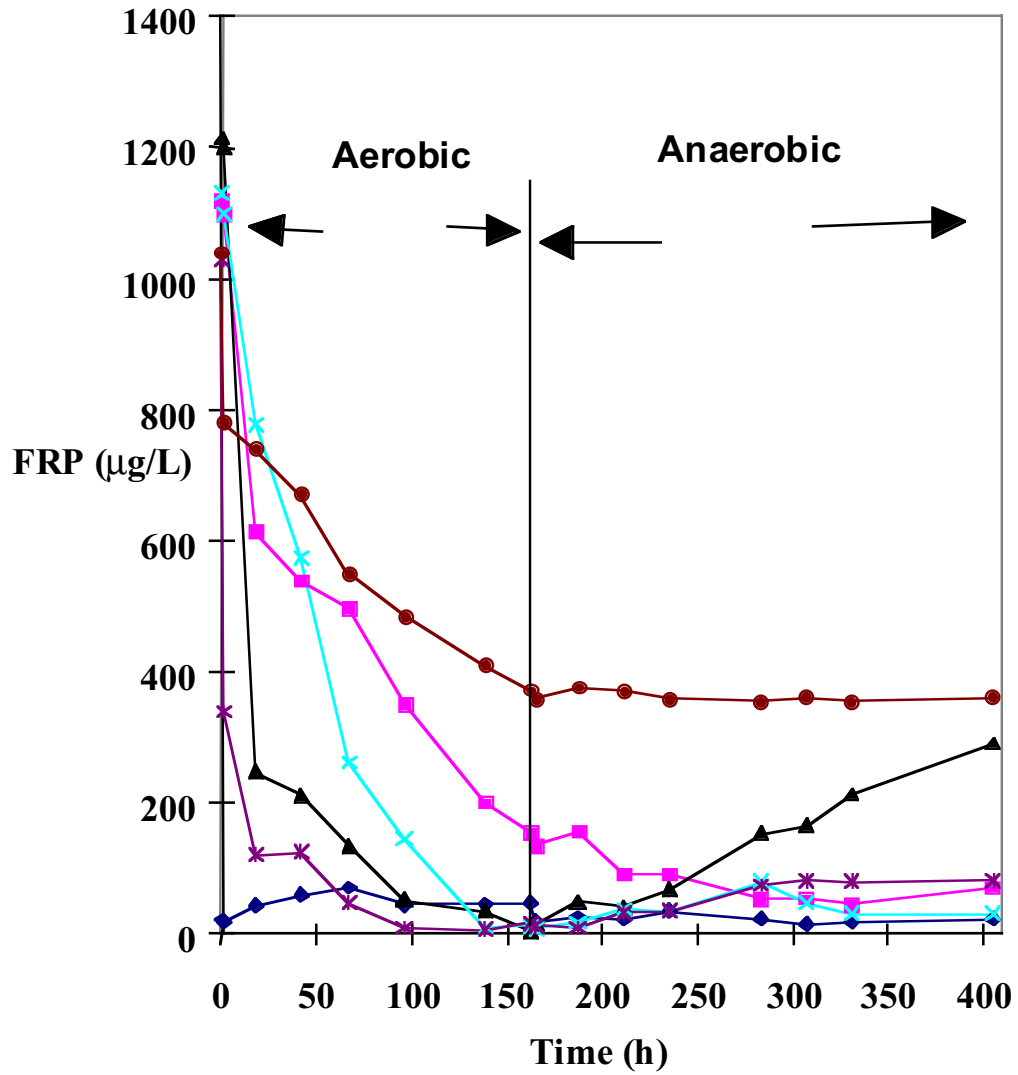


Figure 2. Residual filterable reactive phosphorus (FRP) concentration in the test containers during aerobic and anaerobic experiments. Key to the experiments: ◆ Control (1); ■ P only added (2); ▲ P and acetate added (3); × P and glucose added (4); * P + acetate and glucose added (5); ● P and formaldehyde added (6).

Table 4: Percent P uptake in aerobic experiment and the total amount of P released during the anaerobic stage

Experiment No.	P or release (%)					
	1*	2	3	4	5	6
	Control					Sterilised
Added carbon	Nil (control)	Nil	Acetate	Glucose	Acetate Glucose	Nil
Aerobic conditions						
Total P uptake (%)	2±1	68±8	100±2	99±1	98 ±2	59±5
Biotic P uptake (%)	0	9±2	45±2	40±5	34±4	0
Anaerobic conditions						
P release (%)	0.3±0.	-6.0±0.	30±2	2.0±0.	7±0.	0.2±0.

* No orthophosphate added

Anaerobic experiments

The colour of the sediment suspension in all containers (except No 6) changed to a dark grey by about 48 hours after commencing the anaerobic conditions. The sediment suspension in container 6 (the sterilised control) remained brown during the whole experiment.

The cell count results during the anaerobic experiment are given in Figure 3. As expected, the addition of a carbon source stimulated bacterial growth, with the highest cell numbers observed in experiment 4 (glucose added). Test container 3 (acetate added) showed the highest P uptake (Table 4), although there were lower cell number in comparison with experiments 4 and 5. This suggests that some of the P uptake in experiment 3 may have been accumulated by bacteria as poly-P. Under anaerobic condition, the amount of P released in experiment 3 was considerably higher than in the other carbon-amended experiments (4 and 5), suggesting that some of the accumulated P was released.

The direct release of P from poly-P bacteria via the uptake of acetate (or other low molecular weight organic acids) has been extensively studied in the enhanced biological removal of P in wastewater treatment plants [18]. Phosphorus release from sediments due to indirect bacterial processes (e.g. mineralisation, changed redox conditions) would not be expected to depend on the specific type of bioavailable carbon source.

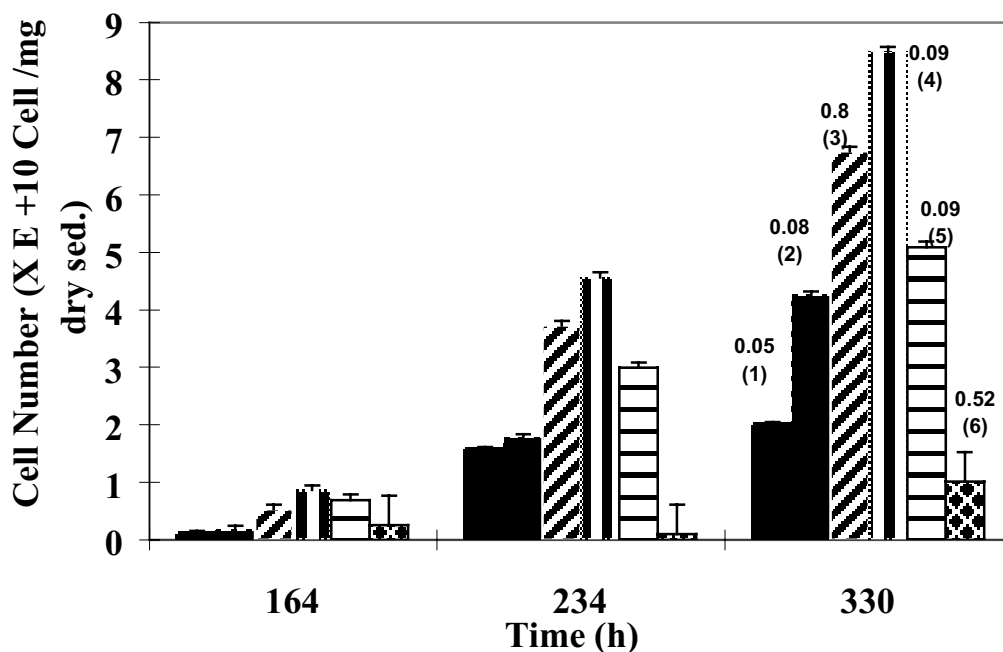


Figure 3. Cell numbers in the sediments from test containers 1 to 6 at 164, 234 and 360 h after the start of the experiment. Note that anaerobic conditions existed after 164 hours. The error bars shown are standard errors and numbers in parentheses indicate the test container number as follows: Control (1); P added (2); P and acetate added (3); P and glucose added (4); P +acetate and glucose added (5); P and formaldehyde added (6).

It is possible that an increase in P release from an acetate-amended sediment under anaerobic condition, with no release from the control sediment, is due to direct release of P from the sediment poly-P bacteria [9, 12, 25]. Alternatively, if P is released from both the glucose-amended and acetate-amended sediments, this would be indicative of an anaerobic release process that does not depend on the type of bioavailable carbon source. Finally, if there is no difference in the P release in the control, carbon augmented and sterilised samples, an abiotic (physico-chemical) release processes is suggested.

Release of P under anaerobic conditions only occurred in experiment 3 (acetate-amended) (Figure 2). During the initial aerobic stage the growth of aerobes and facultative anaerobes would have been favoured relative to the growth of obligate anaerobes [4]. Release of phosphorus in the acetate-added experiment (No 3) compared with the sterilised experiment (No 6), indicates that a significant amount of P release occurred via biotic processes. It is

not possible to distinguish between the direct and indirect bacterial release under the conditions employed in our experiments. Randall et al. [25] found that the enhanced biological phosphorus removal in sewage treatment plants depended on the presence of fermentation products (acidogenesis), suggesting that the direct release of phosphorus from poly-P bacteria may be the dominant process leading to release of P from sediments under the special conditions employed in our experiments.

Some P was released in the glucose plus acetate experiment (No 5) compared to the control (No 1) and sterilised experiments (No 6), but the amount released after 244 hour was about one quarter of the released in the experiment with acetate added (No 3). This suggests that acetate and glucose together do not provide the conditions favourable for the growth of poly-P bacteria under aerobic conditions. Most probably bacteria other than poly-P bacteria dominated under these conditions [24].

Under aerobic conditions, the glucose-amended experiment (No 4) had the highest number of bacteria ($8.9 \pm 1.5 \times 10^9$ cells/mg dry wt), probably because the added carbon and P was all used for cell growth. However, the acetate-amended experiments which had an equivalent amount of carbon added, had less bacterial growth ($5.3 \pm 0.42 \times 10^9$ cells/mg dry wt), suggesting that only part of the added carbon and P was used for cell growth, with the rest used by the poly-P bacteria to produce polyphosphate [18]. This interpretation is supported by the results from the anaerobic experiments where larger amounts of P were released from experiment 3. There was little bacterial growth in the control experiment, and this growth was very slow after 70 h from becoming anaerobic. Slightly higher bacterial growth was observed in the P-only experiment (No 2) compared to the control experiment. The experiments reported in this paper point to the importance of both bacteria and the organic carbon substrate in the uptake and release of P by sediments.

CONCLUSIONS

Under aerobic conditions, when orthophosphate was added to a suspension of wetland sediment, with and without additional organic carbon source (acetate or glucose), an initial rapid uptake of P was followed by a slower uptake in all experiments. It is hypothesised that the initial uptake is abiotic sorption of P onto the sediment particles and the second slower uptake is mainly due to microbial uptake.

There was both a greater number of bacteria and a greater amount of phosphorus taken up in the carbon-amended experiments compared to the control and sterilised sediments. Under anaerobic conditions only the addition of acetate caused direct release of phosphorus from the sediment. The other carbon sources did not result in phosphorus release.

The results indicated that bacteria play a considerable role in phosphate uptake by sediments under aerobic and release under anaerobic conditions. Further and more detailed study on river sediments also supports the findings reported here. The ³¹P-NMR-analysis on river sediment extract amended with acetate indicated poly-P to be present. These results will be reported in future [19].

ACKNOWLEDGMENT

The authors wish to thank Sandra Sdraulig, Ashley Liang and Tom Portelli for analysing the sediment and for their support during the laboratory work. The financial support of an Australian Postgraduate Award (to AK) is also appreciated.

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