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Research note

## Luxury uptake of phosphorus by sediment bacteria

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### Abstract

This note reports the results of experiments aimed at confirming the luxury uptake of phosphorus (P) by sediment bacteria as polyphosphate (Poly-P). Aerobic suspensions of sediments from two different sites were spiked with 1 mg P/L as orthophosphate and augmented with acetate (a fermentation product) or glucose. The orthophosphate was rapidly taken up over a period of a few hours. When these aerobic uptake experiments were made anaerobic and additional organic carbon added, only the acetate-amended sediment released a significant amount of the added phosphorus. It was hypothesised that during the aerobic stage, and with the addition of acetate, some of the phosphorus was accumulated as Poly-P by sediment microorganisms, which was released during the subsequent anaerobic stage (provided acetate was still present). Two lines of evidence—transmission electron microscope analysis of sediment bacteria and <sup>31</sup>P-NMR analysis of sediment extracts—are presented to support the hypothesis that a portion of the phosphorus taken up during the aerobic experiments was stored as Poly-P. © 2002 Elsevier Science Ltd. All rights reserved.

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### 1. Introduction

The excessive growth of algae and bacteria in freshwater systems has been linked to the concentration of bioavailable phosphorus (P) present in the system. It has long been recognised that a significant release of phosphorus can occur from sediments under anoxic conditions. Phosphorus release by sediments consists of a series of interacting biotic and abiotic processes. The effects of these processes on phosphorus release are observed at a macroscale but are a reflection of processes occurring at microscales. This suggests that an understanding of microscale processes is needed to interpret the results observed on larger scales. A number of studies have speculated that microbial processes are important in the release of phosphorus from sediments [1–4]. Although these studies have proposed that P release from bacterial cells may be an important process

for anaerobic P release, most of the research on P release has ignored the direct role of sediment bacteria.

Under anaerobic conditions, carbohydrates (e.g. glucose) and some amino acids are fermented to provide a variety of products, predominantly acetate and other volatile fatty acids [2]. Acetate can then be used as a carbon source and/or electron donor in a number of anaerobic bacterial processes that can potentially play a role in the release of P from sediments. These processes are mainly sulphate reduction, iron and manganese reduction and polyphosphate (Poly-P) hydrolysis [3].

There has been considerable study of the ability of certain organisms to accumulate polyphosphate under aerobic conditions, and to release this as orthophosphate under anaerobic conditions [1,5]. Most of the advancement in understanding these organisms has come from workers studying the enhanced biological removal of phosphorus in sewage treatment processes [5–7]. Poly-P accumulating organisms are thought to be dominant under oscillating aerobic–anaerobic conditions because when anaerobic conditions occur, they are able to take up carbon sources and store them in the

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form of polyhydroxyalkanoates (PHA) accompanied by the hydrolyse of stored Poly-P (accumulated during aerobic conditions) and the release of orthophosphate. Then, when aerobic conditions occur, these organisms use the stored PHA as a carbon and energy source to take up orthophosphate and store it as Poly-P.

It has been speculated that Poly-P accumulating organisms could occur in sediments [1,2]. However, there is lack of evidence to show under what conditions this process could be important in P release from sediment bacteria.

The presence and location of intracellular inclusions of inorganic polyphosphates inside the cells of Poly-P accumulating bacteria can be determined by transmission electron microscopy (TEM) and  $^{31}\text{P}$ -NMR spectroscopy [8,9].  $^{31}\text{P}$ -NMR spectroscopy is a powerful tool for characterising the various P species present in environmental samples. It has been successfully applied to identify P compounds in bacteria, activated sludge obtained from wastewater treatment plants, algae, soils and marine sediments [8]. A number of researchers have demonstrated the usefulness of  $^{31}\text{P}$ -NMR for the analysis of Poly-P in activated sludge and also in NaOH extracts of freshwater sediments [8,10].

In a previous paper, we reported the effect of different organic carbon sources on P uptake and release by wetland and river sediment [4]. This study showed that provided acetate was present in both the aerobic and anaerobic stages, P taken up by the sediments during the aerobic conditions was subsequently released under anaerobic conditions. It was hypothesised that some of this phosphorus release was due to direct release from Poly-P accumulating bacteria present in the sediment. This paper reports further work undertaken to support this hypothesis, where we experimentally show, using  $^{31}\text{P}$ -NMR and TEM, that Poly-P does exist in these sediments.

## 2. Methods

Site description, sediment characteristics, sampling technique and the details of the experimental methods on aerobic and anaerobic experiments are given by Khoshmanesh et al. [4]. The P uptake/release experiments were undertaken under aerobic conditions for 3 days and then for a further 5 days under anaerobic conditions. The sediment suspensions were augmented with acetate or glucose, and orthophosphate. Two control experiments were run, one with the addition of formaldehyde and the other was the sediment only.

### 2.1. $^{31}\text{P}$ -NMR analysis

At the end of the aerobic stage, a sample of sediment suspension was extracted (using NaOH-EDTA) accord-

ing to the modified method of Hupfer et al. [8]. The extraction procedure is fully described by Khoshmanesh et al. [4]. After extraction, the sediment suspension was centrifuged at 27,000 g and the supernatant concentrated 15 times using a freeze drier and stored at  $-20^{\circ}\text{C}$  before measuring the  $^{31}\text{P}$ -NMR spectrum in a Bruker DRX-400 NMR spectrophotometer. An 85% solution of phosphoric acid was used as an external reference for zero adjustment. The Poly-P signal was expected at a chemical shift of  $-20$  ppm [11].

### 2.2. Transmission electron microscopy (TEM)

TEM was used to observe the inner structure of the sediment bacteria and agglomerates of polyphosphates. The sediment suspensions were taken at the end of the aerobic stage of the P uptake experiments. Sediment bacteria were dislodged by sonication, washed off, concentrated by centrifugation, resuspended and then filtered through  $0.2\ \mu\text{m}$  GS (Millipore) filters. The bacteria retained on filters were fixed with glutaraldehyde (5%). They were then treated with osmium tetroxide (1%) for 1 h, dehydrated with ethanol (in a series of 10–90% each for 10 min then  $3 \times 10$  min in 100%) and embedded in Epon 812/Araldite resin as follows: 75% ethanol/25% plastic—30 min; 50% ethanol/50% plastic—1 h; 25% ethanol/75% plastic—30 min; 100% plastic 3 times—30 min; 100% plastic overnight. The embedded filters were sectioned with a Reichert Ultracut E ultramicrotome. Sections of 90–120 nm were put on copper grids stained with saturated uranyl acetate for 5–10 min and then further stained with Reynolds lead citrate for 10 min. The contrasted sections were observed through the electron microscope (TEM Joel 200CX).

## 3. Results and discussion

### 3.1. $^{31}\text{P}$ -NMR analysis

$^{31}\text{P}$ -NMR analysis of the NaOH-EDTA extracts of sediment samples taken at the end of the aerobic stage indicated that only the acetate-amended sample contained Poly-P (peak at chemical shift  $-20$  ppm) [11]. Fig. 1 shows the  $^{31}\text{P}$ -NMR spectra of (a) the acetate-amended sample and (b) the control sample (no added carbon). These data suggest that Poly-P accumulating organisms only accumulated P under aerobic conditions when acetate was available. Hupfer et al. [8] used  $^{31}\text{P}$ -NMR to show that 31–50% of the non-reactive P in the NaOH-EDTA extracts of sediment from a eutrophic lake was present as Poly-P. This part of the study has confirmed the speculation by many workers that sediment bacteria may store Poly-P [1,3,4], particularly when acetate is available to these bacteria.

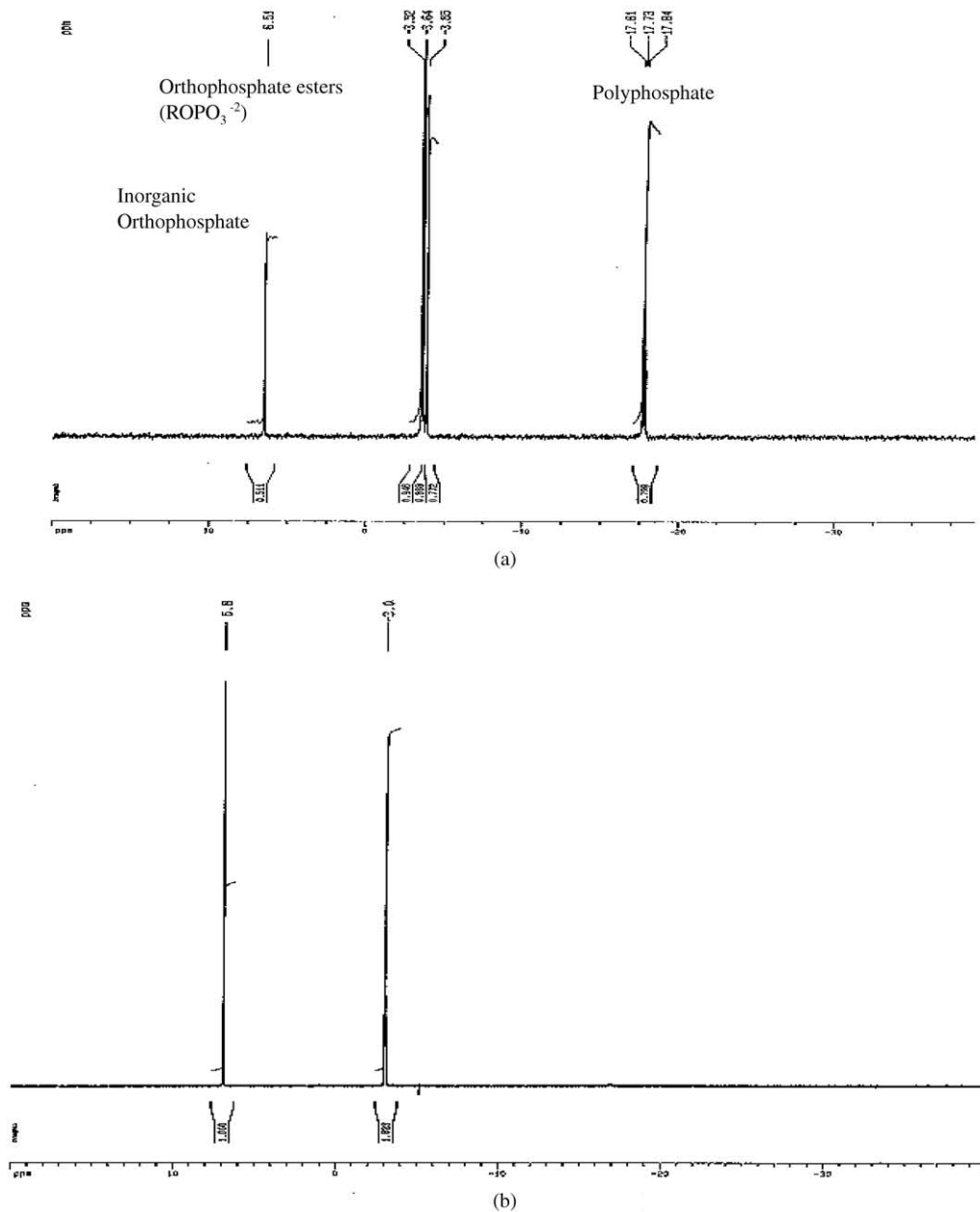


Fig. 1.  $^{31}\text{P}$ -NMR results for the NaOH-EDTA extract: (a) acetate-amended experiment, (b) control.

### 3.2. TEM analysis

Sediment samples taken at the end of the aerobic P uptake experiments were used for TEM examination. TEM electron micrograph (Fig. 2a) indicated electron-dense bodies (considered as Poly-P) inside the cells, only in the acetate-amended samples. No electron dense bodies were observed in the control samples (Fig. 2b) and other samples.

In the acetate-amended samples, cells were observed on the sectioned filters together with abiotic particles.

There were enough cells per section to be counted. The sectioned bacteria from these samples contained both electron dense cells as well as normal cells. We observed a wide range of cell sizes in these samples, and noted that the electron dense bodies mostly occurred in the larger cells and tended to occupy the whole cell.

The control samples contained less cells, and smaller cells with denser cell walls, than the acetate-amended samples. This suggests that the addition of acetate promoted larger cells with thinner cell walls, or perhaps different microbial populations.

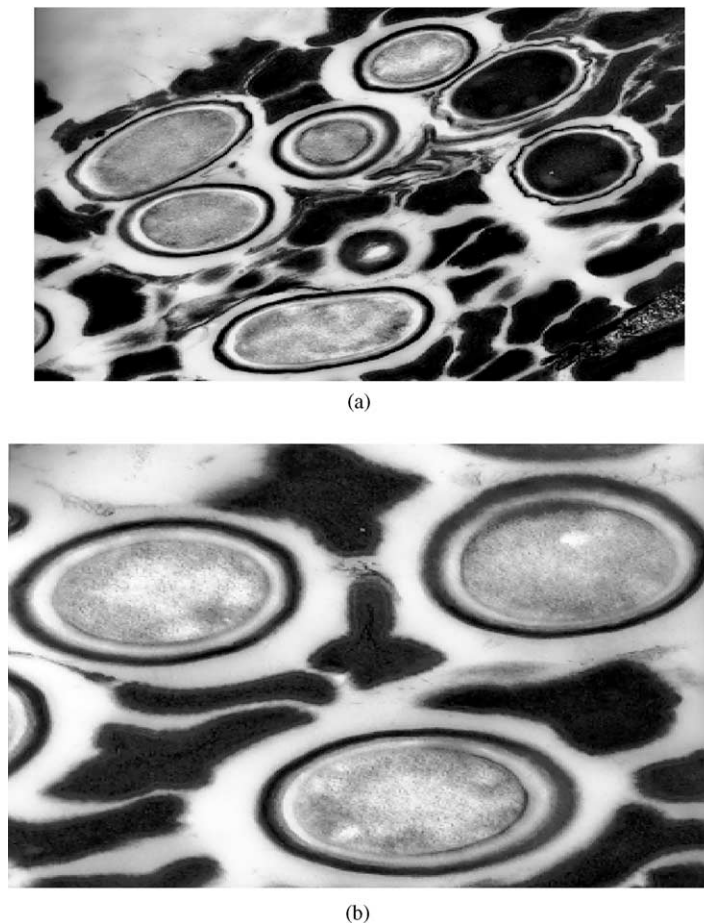


Fig. 2. TEM electron micrographs of cells: (a) acetate-amended sediment, (b) control. Magnification  $21 \times 1000$ .

The TEM examination showed that about 76–80% of the total cells (counted per section) in the acetate-amended sediments contained electron-dense polyphosphate granules. Many of the cells contained large central granules rather than small granules dispersed throughout the cells. Some cells contained no electron-dense bodies. Therefore, counting the cells in random sections of embedded sample could give an approximation of the community structure of the system in terms of percentage of Poly-P versus non-Poly-P accumulating bacteria.

#### 4. Conclusions

The results of the  $^{31}\text{P}$ -NMR and TEM analysis confirmed that, under aerobic conditions when only acetate was available, Poly-P accumulating organisms take up phosphorus as polyphosphate granules inside the cell. Unfortunately, these methods do not permit quantification of the amount of Poly-P accumulated.

This information is needed to estimate the relative contribution of the direct release of P from these bacteria in the overall P release. However, some sediment bacteria are clearly able to assimilate remarkable amounts of phosphorus. In fact, in some cells Poly-P granules (Fig. 2a) occupied almost the total cell volume. Florentz et al. [12] in their study on the phosphorus metabolism of microorganisms from wastewater reported similar findings. Poly-P cannot be synthesised abiotically at low temperatures and most likely would hydrolyse quickly in the presence of enzymes expected to be present in sediments [8].

Clearly, the finding that sediment microorganisms grown under experimental conditions in the presence of acetate, accumulate Poly-P supports the hypothesis that these organisms are an important transient sink for P in sediments, and that acetate may be an important source of organic carbon in such systems. The importance of low molecular weight fatty acids, such as acetate, in sediment P uptake and release is likely to be restricted to freshwater systems. Recent work by Boschker et al. [13]

has shown that in anaerobic brackish sediments, acetate and propionate were predominantly consumed by sulphate-reducing bacteria.

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