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REVIEW

Polyphosphate-accumulating microorganisms in aquatic sediments

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ABSTRACT: The direct contribution of microorganisms to the mobilisation and immobilisation of phosphorus (P) in aquatic sediments has been controversially discussed for more than a decade. Some authors have speculated that the microbial P pool is highly variable in the uppermost sediment layer, especially when excessive P accumulation in the form of polyphosphate (Poly-P) occurs. Poly-P storage is a widespread ability of many different organisms in nature. The phenomenon of Poly-P storage has been technically optimised in wastewater treatment plants (WWTP) providing conditions for enhanced biological phosphorus removal. New insights into the functioning of P elimination in WWTP were strongly linked to the development of novel methods, like ^{31}P nuclear magnetic resonance, for the detection of Poly-P and molecular biological methods for the identification of the specific microorganisms responsible for biological P elimination. Our review summarises current literature on Poly-P in aquatic systems and discusses different potential habitats and mechanisms for Poly-P storage in sediments that are more diverse than in WWTP. Poly-P in sediments may originate from benthic or pelagic hetero- and autotrophic organisms. Poly-P-accumulating organisms in sediments may be of high ecological importance, since they insert phosphorus into the benthic food chain and affect the permanent P mineral deposition in sediments by physiologically inducing rapid P release. Although several studies indicate that Poly-P substantially contributes to total P in the uppermost sediment layer (up to 10%), its origin and the microorganisms and mechanisms involved in Poly-P storage and cycling are largely unknown. Therefore, we also aim to stimulate future studies focusing on these important areas of sediment research.

KEY WORDS: Polyphosphate · Polyphosphate-accumulating organisms · Microbial diversity · Sediments · Phosphorus cycle · Diagenesis · Waste water treatment plant

INTRODUCTION

Recent literature shows that microbial activity in surface sediments affects the phosphorus (P) release from sediments into water by various mechanisms (e.g. Boström et al. 1988, Roden & Edmonds 1997, Gächter & Müller 2003). The uppermost millimetres of the sediment surface are 'hot spots' for microbial transformation processes in aquatic ecosystems, often harbouring extremely high numbers of bacteria. Therefore, it has been speculated that bacteria are directly involved in P exchange across the sediment–water interface, especially under fluctuating environmental conditions, such as the presence and absence of oxygen (e.g. Boström et al. 1988, Davelaar 1993, Gächter & Meyer 1993, Ingall & Jahnke 1997). These ideas are supported by several studies in wastewater treatment plants (WWTP), which have shown that several types of microorganisms are able to take up P in excess and store it inside the cell in the form of polyphosphate (Poly-P; e.g. Hesselmann et al. 1999). Oscillating redox conditions in WWTP favour the growth of Poly-P-accumulating organisms (PAOs), which are responsible for the high biological P elimination. Poly-P storage accounted for up to 10% of total dry mass in a pure culture of *Acinetobacter* 210A (Deinema et al. 1985).

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P in the biomass of sediment microorganisms as a regulator for the dynamics of benthic P fluxes has been controversially discussed throughout the last 2 decades. Experimental data (Gächter et al. 1988, Waara et al. 1993, Brunberg 1995), theoretical considerations (Davelaar 1993, Gächter & Meyer 1993) and detection of Poly-P in limnetic (Hupfer et al. 2004, Reitzel et al. 2006a) as well as marine sediments (Carman et al. 2000, Sannigrahi & Ingall 2005) provide strong indication that sediment microorganisms are indeed directly involved in P fixation. Other authors, however, question the relevance of microbial biomass as an important link for P cycling in sediments. For example, Golterman (2004) wrote: 'a rough estimate based on bacterial numbers in sediments shows that bacterial phosphate (including Poly-P) is not likely to be present in quantities for an important release'.

In general, the relative importance of microbial processes for P exchange across the sediment–water interface is difficult to determine because chemical and bacterial processes are tightly coupled and because sediment characteristics of different lakes show high variability. Our present knowledge of the origin and possible uptake and release mechanisms associated with Poly-P mediated by sediment microorganisms is very scarce, in particular for marine systems. The present review is focussed on summarising the main literature on microbial Poly-P storage in aquatic sediments and stimulating future studies on its ecological function.

INORGANIC POLYPHOSPHATE: A MOLECULE WITH MANY FUNCTIONS

This catchline coined by Kornberg et al. (1999) points to the multiple functions of Poly-P for living organisms. Inorganic Poly-P was already identified in yeast cells >100 yr ago (Liebermann 1890). It is a linear polymer comprised of a few up to several hundred residues of orthophosphate linked by high-energy phosphoanhydride bonds. This cell compound was considered a 'fossil molecule' for a long time because the importance of Poly-P as an energy storage component decreased during evolution from prokaryotes to eukaryotes (Kulaev & Kulakovskaya 2000). A number of enzymes of the Poly-P metabolism can still be found in prokaryotes, but not in eukaryotes. Poly-P most likely functioned in primitive organisms as ATP does in modern ones. Recent studies show that Poly-P is not only a nutrient storage component or convertible energy source, but fulfils several additional functions in various types of organisms, e.g. in cellular homeostasis and osmotic regulation (regulation of membrane transport), regulation of gene or even enzyme activities, stress response (e.g. Pick et al. 1990, Seviour et al. 2003, Kulaev et al. 2004), and detoxification of heavy metals by chelation (Kornberg 1995). Poly-P is localised in the cytoplasm and vacuoles either as granules, freely dissolved in the cytoplasm, or bound to cytoplasmic compounds such as RNA. Highly polymerised Poly-P, however, is located in the cytoplasm membrane, in the cell wall, or other organelle membranes (Kulaev et al. 2004).

Poly-P occurs ubiquitously in bacteria, fungi, protozoa, insects, algae, higher plants and even in mammals. Its accumulation is common in natural freshwater and marine phytoplankton (e.g. Miyata & Hattori 1986, Feuillade et al. 1995). The highest biomass-specific amounts of Poly-P, however, have been found in microorganisms, which store it as a nutrient and energy reserve, especially under fluctuating environmental conditions (Kulaev & Kulakovskaya 2000). Many genera of microorganisms in aquatic ecosystems are able to rapidly take up P beyond their physiological need ('luxury uptake'). Synthesis and storage of Poly-P are common among microorganisms (especially for cyanobacteria; McDignum et al. 2005), when P is sufficiently available after phases of acute P starvation ('overplus uptake'). The increased stress due to limited P availability implies the synthesis of a high-affinity uptake system for orthophosphate and additional enzymes, which transform orthophosphate into insoluble Poly-P inside the cell. These adaptations may provide competitive advantages when inorganic nutrients become limiting (e.g. McDignum et al. 2005, Eixler et al. 2006). Additionally, laboratory experiments have demonstrated that Poly-P storage in autotrophic microorganisms can also be influenced by various other environmental conditions, e.g. light (Sianoudis et al. 1986), sulphur (Lawerence et al. 1998) and nitrogen availability (Küsel et al. 1989). For heterotrophic microorganisms (both eukaryotes and prokaryotes), it has been further shown that Poly-P synthesis is linked to responses to physical and chemical stress in the environment (Brown & Kornberg 2004, Thomas & O'Shea 2005).

Much of the evidence that Poly-P represents an important energy source for microorganisms has been mainly derived from investigations of activated sludge. Biochemical models have been developed based on these studies to describe Poly-P cycling under alternating redox conditions (Comeau et al. 1986, Wentzel et al. 1991). According to these models the energy of Poly-P hydrolysis is used to take up labile organic carbon sources (e.g. low molecular fatty acids, alcohols and sugars) under anaerobic conditions and to store them in the form of polyhydroxyalkanoates (PHAs), in particular Poly-β-hydroxybutyrate (PHB). By doing so, some aerobic bacteria implement an 'emergency supply' for the maintenance of the major cellular functions. In the subsequent aerobic phase, bacteria use

the stored PHA as a source of carbon and energy for growth and other cell functions, including Poly-P resynthesis. The temporal separation of uptake and consumption of organic resources for growth is a highly efficient eco-physiological adaptation to alternating redox conditions. It offers a competitive advantage over other obligate aerobic bacteria that exclusively use external carbon sources.

The following section discusses mechanisms of microbial Poly-P accumulation, and conditions under which they may occur in natural aquatic sediments.

MICROBIAL POLYPHOSPHATE STORAGE IN AQUATIC SEDIMENTS

Potential ecological niches for PAO

Speculations that Poly-P bacteria are directly involved in the P exchange across the sediment–water interface are based on several studies in wastewater treatment plants/enhanced biological phosphorus removal (WWTP/EBPR). The phenomenon of excess P storage in WWTP and laboratory-scale reactors, without any addition of chemicals was discovered in the 1960s and has been intensively studied ever since (see review of Seviour et al. 2003). Alternating anaerobic and aerobic conditions favours growth of Poly-P-storing bacteria in WWTP; thus, a high efficiency of P removal from the wastewater is achieved by withdrawing the sludge containing the P-enriched bacteria. In this regard, the optimisation of the EBPR technology led to an increase in the P content in activated sludge from 1–2% up to 3–8% of the total dry weight (Röske & Uhlmann 2005).

The role of bacteria in the observed elevated P enrichment in the activated sludge has been controversially discussed in the literature for a long time. The following facts, however, are now widely accepted

- PAOs are the main actors of P elimination in WWTP/EBPR (Seviour et al. 2003)
- Poly-P degradation is coupled with the capacity for carbon storage (Santos et al. 1999)
- PAOs need labile organic matter, since it stimulates the cellular storage of the PHB/PHA used for Poly-P synthesis (Potgieter & Evans 1983)
- Nitrate prevents the release of Poly-P in the absence of oxygen because denitrifying bacteria substantially decrease the supply of organic substrates (Kortstee et al. 1994), and even store Poly-P themselves (Barak & van Rijn 2000)
- Sulphate reduction hinders Poly-P-storing bacteria by competition for organic acids (Yamamoto-Ikemoto et al. 1994), and by the toxic effects of the generated sulphide
- The low molecular mass fraction of Poly-P is exclusively utilised as an energy source under anaerobic conditions (Mino et al. 1985).

Toerien et al. (1990) stated that the optimised biological P elimination in activated sludge systems must depend on microorganisms that are favoured by oscillating redox conditions in nature. It is therefore assumed that the fluctuating redox conditions prevailing at the sediment–water interface may favour Poly-P-storing heterotrophic bacteria (Davelaar 1993, Gächter & Meyer 1993). Lake sediments usually comprise strong vertical gradients of electron donors and acceptors. The resulting redox gradient leads to an ecologically well-defined distribution of microbial populations (Hanselmann 1986). Possible ecological niches of Poly-P-storing bacteria in sediments are microzones in which oxygen and/or nitrate are available from the overlying water together with labile organic matter such as short-chain organic acids from deeper horizons. Variations in redox gradients frequently occur, and are controlled by hydrodynamic conditions in the overlying water, such as stratification of the water body, bioturbation and sedimentation events of material rich in organic matter. Redox fluctuations may show sporadic or even regular temporal patterns, and in this regard microbial Poly-P storage may be induced by alternating redox conditions, particularly in the following microhabitats

- Littoral sediments: Diurnal changes of oxygen concentrations in the overlying water are caused by oxygen depletion due to respiration at night and oxygen over-saturation by photosynthesis during day time (Dodds 2003). Furthermore, diurnal and seasonal changes in the depth of oxygen penetration into littoral sediments have been observed
- Surficial sediments: The oxygen penetration depth into sediments is controlled by periodic internal seiches in stratified lakes (Lorke et al. 2003), and by the circulation frequency of the water body in polymictic lakes. However, oxygen penetration depth and the consequent shift of redox zones in the sediment also depend on variations in the vertical flux of organic carbon to the sediment. In addition, organic P can account for a high portion (up to 50%) of total P in detritus-rich sediments (e.g. Penn et al. 1995)
- Resuspended sediments: Repeated resuspension of sediment particles (and their attached microorganisms) is often accompanied by alternating changes of aerobic and anaerobic conditions (Gerhardt & Schink 2005)
- Microzones in the rhizosphere of macrophytes and tubes of macrozoobenthos: The aerenchyma of macrophyte roots and bioirrigation of tubes by the inhabiting zoobenthos transport oxygen to deeper horizons and supply the surrounding pore water with

organic acids that support the growth of aerobic bacteria (Aller 1994, Bodelier 2003). The supply by oxygen follows diurnal (macrophytes) or pulsative patterns (macrozoobenthos).

Storage of Poly-P is not solely limited to heterotrophic organisms, and autotrophs may also substantially contribute to the Poly-P pool in sediments. It has been well documented that different genera of phototrophic organisms are able to store P in excess in the form of Poly-P (e.g. Pick et al. 1990, Sudo et al. 1997, Eixler et al. 2006). Photoautotrophic organisms potentially contribute to P binding in aquatic sediments by Poly-P accumulation. The following scenarios are likely to occur in sediments

- Benthic microalgae and photoautotrophic bacteria (periphyton) store Poly-P in euphotic zones of aquatic environments (e.g. littoral sediments, riverbed sediments, wetland sediments)
- Pelagic algae are also able to store large quantities of Poly-P (Reynolds 1984). Studies on Poly-P metabolism, however, have been mostly carried out with cyanobacteria (e.g. Sicko-Goad & Jensen 1976, Jacobson & Halman 1982), and it has been shown that Poly-P storage in cells of *Microcystis aeruginosa* preferentially occurs in settling colonies (Reynolds et al. 1981). Settling of aggregated living microalgae, residues of zooplankton, and microalgae, which are intensively colonised by bacteria, fungi and micrograzers, might introduce substantial amounts of Poly-P into the sediments
- Several cyanobacteria are able to store Poly-P as a nutrient source in the sediment during their life cycle, which consists of benthic and pelagic stages. For example, *Microcystis* sp. colonies overwinter and survive for extended periods when buried in sediments (Preston et al. 1980, Brunberg 1995). The biomass of *Microcystis* sp. in the benthos of Lake Vallentunasjön temporarily encompassed >90% of the total living benthic biomass (Brunberg 1995). In the eutrophic Green Lake, colonies of a benthic population of *Gleotrichia echinulata* contained large Poly-P bodies and ascended from the sediments into the pelagic zone (Barbiero & Welch 1992). Their epilimnetic growth in Lake Erken has been shown to largely depend on these internal P reserves, which have been acquired during the benthic phase of their life cycle (Istvanovics et al. 1990).

Evidence and quantification of polyphosphate in aquatic sediments

The contribution of Poly-P on P fixation and release in sediments has been estimated by observations at a laboratory scale. Fleischer (1986) demonstrated that facultative anaerobic bacteria (*Pseudomonas fluorescens*) are able to rapidly assimilate P from solubilisation of Fe(III), with a rapid release under subsequent anaerobic conditions. This author speculated that Poly-P stored in bacteria is the source of most of the released P. Likewise, Gächter et al. (1988) observed that bacteria isolated from the sediments of eutrophic Lake Sempach bind and release P similarly to sewage sludge bacteria when exposed to alternating aerobic–anaerobic conditions. Furthermore, *in situ* experiments in Lake Sempach indicate that P release from the sediment cannot conclusively be explained by iron dissolution alone. Waara et al. (1993) have carried out laboratory experiments with the bacterial isolate *Pseudomonas* sp., which was exposed in sediments of Lake Vallentunasjön. In contrast to the findings by Fleischer (1986), they did not find the expected rapid release of P from the cells under anaerobic conditions. Stimulation of bacterial biomass by glucose addition, however, clearly led to a ca. 30% increase in the non-reactive NaOH-extractable P (NaOH-NRP), which in activated sludge mainly includes Poly-P (Uhlmann et al. 1990). In laboratory experiments with and without addition of *Microcystis* sp., Brunberg (1995) found that variations in bacterial biomass and cellular P content cause measurable changes of total P in the sediment (especially of the NaOH-NRP fraction). This notion is supported by the findings of Törnblom & Rydin (1998), who used sediments from Lake Erken in laboratory experiments. Microbial activity and bacterial biomass production immediately increased in response to the deposition of seston and resulted in increasing P uptake rates from the overlying water and an increase in the NaOH-NRP pool at the sediment surface. These results are further supported by Goedkoop & Pettersson (2000), who found a significant relationship between seasonal changes in bacterial biomass and in the NaOH-NRP fraction of the same sediment. Since the amount of NaOH-NRP also correlates well with the chlorophyll *a* content of settling seston, discrimination between potential P uptake by bacteria and the supply with photoautotrophic organisms via sedimentation is impossible. All the above-mentioned conclusions are based on the interpretation of indirect results, and not on direct evidence and quantification of Poly-P. More recently, it has been shown that the NaOH-NRP fraction does not serve as a 'conservative measure of Poly-P' (Goedkoop & Pettersson 2000), because it contains a wide spectrum of different P-binding forms.

Direct evidence for microbial Poly-P storage in aquatic sediments has been obtained using a variety of chemical methods, summarised in Table 1. Uhlmann & Bauer (1988) presented the first direct evidence of Poly-P storing bacteria in the sediment of a eutrophic reservoir by direct observation with transmission elec-

Table 1. Poly-P in sediments. NMR/LS: liquid-state nuclear magnetic resonance; NMR/SS: solid-state NMR; EM: electron microscopy

Location	Main results	Method	Source
Lake sediments			
Reservoir Saidenbach (Germany)	Detection of P-rich inclusions in sediment bacteria (0–1 cm)	EM and X-ray	Uhlmann & Bauer (1988)
Lake Lucerne, Lake Baldegg (Switzerland)	Poly-P: max. 50% of non-reactive P in NaOH extracts (0–0.5 cm)	EM and X-ray, NMR/LS	Hupfer et al. (1995)
Lake Gömmaren, Lake Långsjön (Sweden)	Poly-P: Distinct signals in 0–7 and 8–16 cm (L. Gömmaren) and in 0–5 cm (L. Långsjön)	NMR/LS	Carman et al. (2002)
Twenty-two European lakes (Germany, Austria, Switzerland)	Poly-P: max. 11% of total P (0–1 cm); no Poly-P was evident in deeper layers (>2 cm); detection of Poly-P in trap material	NMR/LS	Hupfer et al. (2004)
Lake Sønderby (Denmark)	Poly-P: 3.2% of total P (0–1 cm); evidence also in anoxic layers up to 23–24 cm depth	NMR/LS	Reitzel et al. (2006a)
Lake Erken (sweden)	Poly-P: 8.3% of total P (0–1 cm); Poly-P traces up to 5–6 cm depth; detection of Poly-P in trap material	NMR/LS	Reitzel et al. (2007)
Marine sediments			
Effingham Inlet/west coast of Vancouver Island	Poly-P: 8% of the total P (1–2 cm) from an oxic site; no Poly-P in deeper samples and in anoxic site	NMR/SS	Sannigrathi & Ingall (2005)
Nambian Shelf	Poly-P-storing sulphur bacteria (*Thiomargarita namibiensis*) are responsible for phosphorite deposition	Toluidine Blue staining, EM	Schulz & Schulz (2005)
Marine sinking particles from several oceans	Poly-P: max. 2% of total P	NMR/LS	Paytan et al. (2003)

tron microscopy (TEM). Poly-P granules are visible as electron dense structures that are darker than the surrounding cell material. The combination of TEM with an energy dispersive X-ray microanalysis allows the direct quantification of the elemental composition of these inclusions (Fig. 1). In addition to P, which has the highest signal intensity, Ca, Mg and K are often other main elements of Poly-P granules (Goldberg et al. 2001). The visible inclusions and their elemental composition in lake sediments are similar to those of bacteria from activated sludge. However, a reliable quantification of the microbial Poly-P content in a whole sediment sample by electron microscopy is impossible. By combining X-ray microanalysis with Poly-P quan-

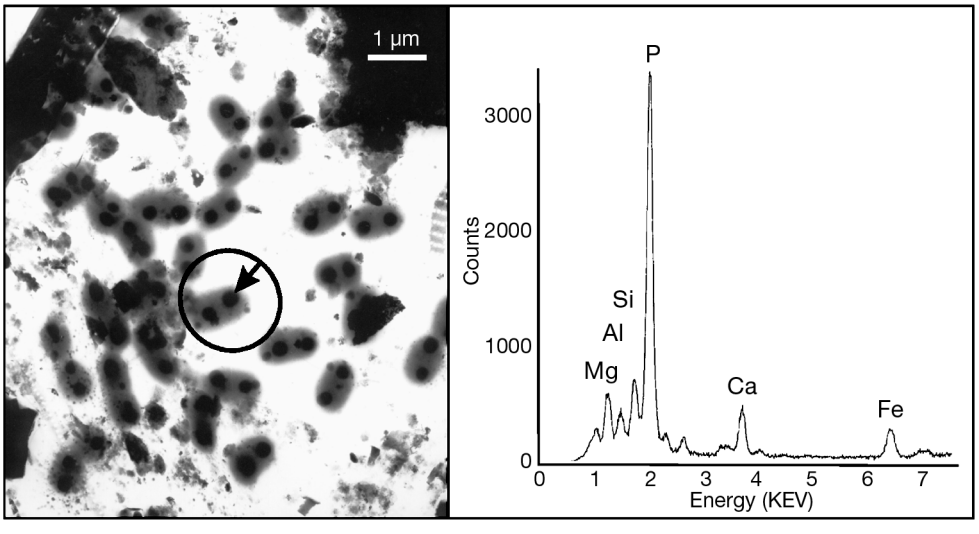

Fig. 1. Left panel: Transmission electron microscopy micrograph of bacteria with Poly-P granules (arrow) from sediment surfaces (0 to 0.5 cm) of Lake Baldegg (Switzerland). Right panel: X-ray microanalysis of elemental composition of an electron dense inclusion (modified from Hupfer et al. 1995)

tification by means of ^{31}P NMR (nuclear magnetic resonance) spectroscopy, Hupfer et al. (1995) found that Poly-P granules identified by electron microscopy corresponded to distinct signals of Poly-P in NMR spectra in the surface sediments of 2 Swiss Lakes. ^{31}P NMR spectroscopy is a powerful tool to distinguish several P compounds, such as phosphonates, phosphate, phosphate monoesters, phosphate diesters, pyrophosphate and Poly-P (Cade-Menun 2005).

An example of Poly-P quantification by means of ^{31}P NMR in a surficial sediment is shown in Fig. 2. An extensive ^{31}P NMR survey of sediments from 22 European lakes with different trophic status and morphometry has shown that Poly-P storage is not a rare or episodic phenomenon. In the top 0.5 cm of the sediment, the percentage of Poly-P ranged between 1.5 and 11.4% of total P (Hupfer et al. 2004). In this study, Poly-P was not detected in deeper layers of most of the selected sediment cores. Contrary to this, the ^{31}P NMR analysis of a sediment core of Lake Sønderby (Reitzel et al. 2006a) demonstrated that Poly-P might not only be detectable in substantial amounts at the sediment surface, but also in anoxic layers up to 24 cm depth. Traces of Poly-P were also evident in the anoxic layers (5 to 6 cm depth) in Lake Erken (Reitzel et al. 2007). The relative contribution of Poly-P in settling seston traps increased between 8 and 15 m, whereas no Poly-P could be detected in the phytoplankton at the same time. The authors explain the accumulation of Poly-P in traps by the colonisation of settling seston by Poly-P-storing bacteria. Khoshmanesh et al. (2002) concluded from laboratory investigations that microorganisms from a wetland sediment in Australia were able to form Poly-P under aerobic conditions whenever low molecular dissolved organic carbon was available. Their conclusion was derived from ^{31}P NMR and TEM investigations that showed the presence of Poly-P only in acetate-amended experiments, but not in the controls. Moreover, Carman et al. (2000) have investigated various marine and lacustrine sediments by ^{31}P NMR spectroscopy and found distinct signals of Poly-P middle groups in 2 of 3 investigated lake sediments, even in thick layers between 0 and 5 (7) cm depth. It is remarkable that no Poly-P was detected in Baltic Sea sediment samples from sites with permanent anoxic conditions in the overlying water. Similar findings have been reported by Sannigrahi & Ingall (2005), who used solid-state ^{31}P NMR for comparison of 2 sites in a fjord in British Columbia. In these studies, Poly-P was only evident at oxic sediment surfaces, but not at anoxic ones. The authors calculated that Poly-P release might explain a substantial portion of the observed higher P release from sediments with overlying anoxic waters.

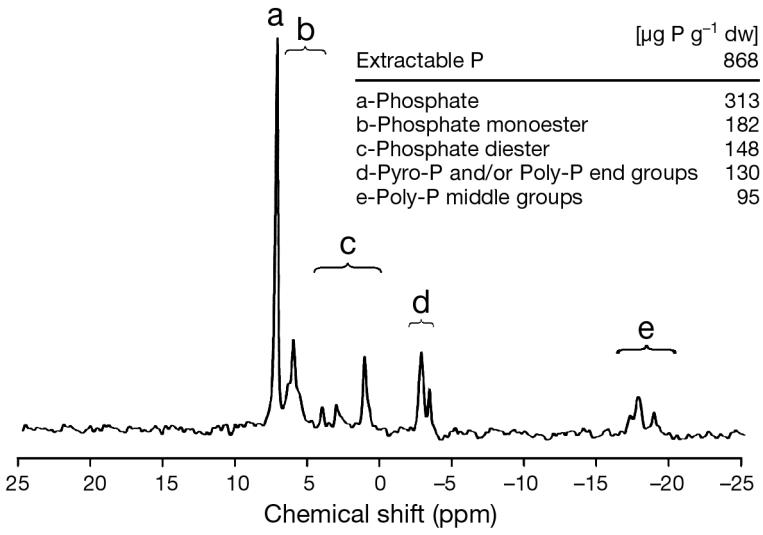

Fig. 2. ^{31}P nuclear magnetic resonance spectra of NaOH extracts from surface sediments (0 to 1 cm) of Lake Erken (21 m water depth) taken in April 2004 (redrawn from Reitzel et al. 2006b). The table shows the quantification of the detected P species. The total P content of the sediment sample was 3100 µg P g^{-1} dry wt

In a marine study by Schulz & Schulz (2005), further evidence is provided for benthic P fluxes resulting from Poly-P utilisation by the sulphide-oxidising bacterium *Thiomargarita nambiensis* in shelf sediments of the Namibian coast. High numbers of intracellular Poly-P inclusions were found by histological staining and electron microscopy under oxic conditions. Very high pore water P concentrations (ca. 8 mg l^{-1}) occurred in a narrow layer densely populated by this bacterium. The observed rates of P release by separated cells under anoxic conditions in the laboratory as well as thermodynamic considerations suggest an episodic P release by bacteria. This mechanism may potentially be responsible for the observed high P peak in the pore water and the extensive hydroxyapatite deposits in sediments of the Namibian coast.

Detection, quantification and characterisation of PAO

Although little is known about the phylogeny, ecology and physiology of PAOs in sediments, several methods have been used for their detection, quantification and characterisation in activated sludge. These approaches are also suitable for similar studies in lake sediments. The combination of molecular, e.g. fluores-

cence *in situ* hybridisation (FISH) and Poly-P staining techniques (DAPI or Neisser staining; see Serafim et al. 2002) in activated sludge showed that members of the *Alphaproteobacteria* and *Actinobacteria* contain intracellular Poly-P granules (Kawaharasaki et al. 1999). The design of new, more targeted oligonucleotide probes and the combination of different molecular methods such as denaturing gradient gel electrophoresis (DGGE) and clone libraries have highlighted the importance of hitherto uncultured PAO. In particular, bacteria closely related to the genus *Rhodocyclus* (*Betaproteobacteria*) and some *Actinobacteria* seem to play an important ecological role in WWTP. By using FISH, the percentages of specific bacterial groups, including PAO, have been determined in laboratory-scale and full-scale plants (Table 2). The subsequent application of post-FISH Neisser, methylene blue, or DAPI staining indicated that bacteria closely related to *Rhodocyclus* indeed store Poly-P inside their cells (Crocetti et al. 2000, Onda et al. 2002, Onuki et al. 2002). In addition, clone libraries from activated sludge revealed many clones related to the genus *Rhodocyclus* (Bond et al. 1995, Hesselmann et al. 1999, Liu et al. 2001, McMahon et al. 2002), indicating their important role for Poly-P accumulation in WWTP. This finding is also supported by DGGE and subsequent sequencing of excised bands, since many sequences from WWTP belong to the genus *Rhodocyclus* (Ahn et al. 2002, Onda et al. 2002, Onuki et al. 2002).

Recently, the combination of FISH with microautoradiography (MAR-FISH) has enabled researchers to simultaneously study both structure and *in situ* substrate uptake patterns of individual microbial cells within a given bacterial community. In accordance with the above-mentioned studies, different morphotypes of *Betaproteobacteria*, in particular *Rhodocyclus*-related bacteria, accumulate $^{33}P_i$ aerobically in activated sludge of full-scale WWTP (Kong et al. 2002, 2004). This finding is in good agreement with existing biochemical models of Poly-P storage (van Loosdrecht et al. 1997, Mino et al. 1998).

Moreover, bacteria other than those related to *Rhodocyclus*, mainly *Alpha-* and other *Betaproteobacteria*, also incorporate $^{33}P_i$, but constitute only a minor fraction of the total bacterial community in activated sludge (Kong et al. 2004). Members of the *Actinobacteria* also assimilate orthophosphate and store Poly-P granules. This has been shown in cultivation-dependent approaches for different isolates, e.g. *Microlunatus phosphovorus* (Santos et al. 1999) and *Tetrasphaera elongata* (Onda & Takii 2002), and more recently with the cultivation-independent MAR-FISH method for *Tetrasphaera*-related bacteria in activated sludge. These *Tetrasphaera*-related bacteria aerobically took up $^{33}P_i$ and stored Poly-P after anaerobic uptake of amino acids. In contrast to the existing biogeochemical models for typical PAO (see above), these bacteria do not assimilate acetate or store PHA (Kong et al. 2005). However, they often exhibit high percentages of the total microbial community in diverse full-scale WWTP, indicating an importance in the EBPR similar to that of *Rhodocyclus*-related bacteria. Differences in substrate availability in different types of waste stimulate the growth of several chemotaxonomic groups in different ways (Liu et al. 2001). Until now, it remains ambiguous whether other genera besides *Rhodocyclus* and *Tetrasphaera* are responsible for EBPR.

Despite 30 yr of research, many questions still remain unanswered regarding the phylogeny and ecological role of specific microbial communities in activated sludge from WWTP/EBPR. Almost all PAOs are extremely difficult to isolate, and to date no pure culture of a typical PAO exists. These methodological restrictions further complicate the studies of diversity and ecology of PAOs in natural sediments. A high bacterial diversity has been found in natural sediments when using culture-independent molecular methods (e.g. Wobus et al. 2003). Our own results, also obtained by culture-independent approaches, show that potential PAOs are always present in a variety of sediment samples of lakes and rivers in northeastern Germany (S. Gloess, M. Hupfer, H.-P. Grossart unpubl. results). However, information on the abundance, physiology and ecological role of PAOs in natural sediments is still scarce.

ECOLOGICAL RELEVANCE OF POLY-P ACCUMULATING ORGANISMS IN SEDIMENTS

Sediments act as sinks and sources for P. As a limiting factor, P controls the primary productivity in many aquatic ecosystems. The internal P cycle often delays the response of aquatic systems to changes in external P load by buffering due to simultaneous retention and mobilisation of P in sediments. For a long time, the exchange of P between sediment and water has mainly been explained by abiotic factors, e.g. its coupling to the redox-sensitive iron cycle in conjunction with oxygen availability in the overlying water (Einsele 1936, Mortimer 1941). More recently, however, benthic bacteria have been incorporated into these schemes, based on their potential influence on the ability of lake sediments to retain inorganic P, mainly by reducing iron and sulphate (Roden & Edmonds 1997, Gächter & Müller 2003). Today, it is well accepted that benthic microorganisms are directly involved in the transformation of P via both the transfer of inorganic P to organic P (biosynthesis) and of organic P to soluble compounds (mineralization).

Table 2. Relative fractions of different microbial groups in percent of total cell counts (DAPI) or of eubacteria (EUB). Studies have been performed using fluorescent *in situ* hybridisation (FISH) with various 16S and 23S rRNA oligonucleotide probes in laboratory-scale and full-scale plants (for details see section 'Detection, quantification and characterisation of PAO'). The following oligonucleotide probes (www.microbial-ecology/probebase) have been used: ALF1b (*Alphaproteobacteria*, some *Deltaproteobacteria*, *Spirochaetes*), BET42a (*Betaproteobacteria*), GAM42a (*Gammaproteobacteria*), HGC69a (*Actinobacteria*), CF319 (Cytophaga–Flavobacterium-group of the *Bacteroidetes*), Aca23a (*Acinetobacter*), PAO462/PAO651/PAO846 (members of the Candidatus '*Accumulibacter*' cluster), MP2 (*Microlunatus phosphovorus*), Actino1011 (*Tetrasphaera japonica*, EBPR clones Ebpr19, Ebpr20 [*Actinobacteria*]). SBR: sequence batch reactor; VFA: volatile fatty acid; A/O: anoxic/oxic; WWTP: wastewater treatment plants; EBPR: enhanced biological phosphorus removal

Laboratory-scale with EBPR	ALF 1b	BET 42a	GAM 42a	HGC 69a	CF 319	Aca 23a	PAO 462	PAO 651	PAO 846	PAO mix	MP2	Actino 1011	Counts relative to:	Source
SBR sludge fed with acetate (run P1)	14	77	1	1		0		20					EUB	Kong et al. (2002)
SBR sludge fed with a mixture of VFAs	25	54	12	ND			25			53			EUB	Levantesi et al. (2002)
(A/O)- and Phoredox-sludge	12–17	22	7.5–8.5	12–17	12–17	3–5			3–5		3–5	10	DAPI	Eschenhagen et al. (2003)
SBR sludge (named P)	4	45	<1	35									DAPI	Bond et al. (1999)
SBR sludge fed with acetate and peptone	7	64	<0.1	10	4						3		DAPI	Kawaharasaki et al. (1999)
SBR sludge fed with acetate	9.5	41.3	6.8	17.9	4.5				35.2			16.7	DAPI	Liu et al. (2001)
SBR sludge fed with glucose, high P loading	14	30	12	14		3								
SBR sludge fed with acetate, high P loading	15	34	12	16		4							EUB	Sudiana et al. (1999)
SBR sludge	12	80		28	14								EUB	Crocetti et al. (2000)
SBR fed with acetate	3	89	2	<1	5	<0.01								
SBR fed with a mixture	9	34	7	20	16	5							EUB	Hesselmann et al. (1999)

Full-scale		ALF 1b	BET 42a	GAM 42a	HGC 69a	CF	Aca 23a	PAO mix	Counts relative to:	Source
Municipal WWTP with EBPR	Aerobic zone	13	33	10	17	1	1		DAPI	Kämpfer et al. (1996)
	Anaerobic zone	13	25	12	27	1	1			
Process plant without N recycling with EBPR	End of aerobic stage	10	25	3	9			18	DAPI	Zilles et al. (2002)
Aerated-anoxic Orbal process plant with EBPR	End of aerobic stage	4	12	7	15	4		13		
Municipal WWTP	Aeration basin/high load	10	42	34					EUB	Wagner et al. (1994)
	Aeration basin/low load	37	37	7					EUB	
Municipal WWTP (1-state-system)	No information	12	59.5	24.1	8.8	22.8			DAPI	Manz et al. (1994)
SBR-treating dairy sewage	No information	2.1	6.0	6.5	22.9	85			DAPI	

By assembling the available information on the potential role of PAO in P regeneration in aquatic sediments, we propose the following simplified scenario (Fig. 3). The settling of seston-associated PAO influences P sedimentation rates and the seasonal patterns of Poly-P occurrence at the sediment surface (Fig. 3, transfer process a). For example, Gächter & Mares (1985) have observed that settling particles in lakes do not always lose P, but also act as sinks for soluble P. It has been hypothesised that even decaying algae and their decomposers with low P contents can take up orthophosphate during sedimentation. The stripping of P from the water by sedimentation of PAO could thus efficiently decrease the availability of P for epilimnetic primary production.

Upon sedimentation, microbial Poly-P likely becomes a highly variable P pool in sediments, which is controlled by several uptake and release mechanisms: at the oxic and detritus-rich sediment surface, settled organic P, including Poly-P, can be released from decaying algae and bacteria by means of (alkaline) phosphatase activities or by physiological responses of living cells to dramatic changes in environmental parameters following sedimentation. On the other hand, P uptake for bacterial growth could be due to decomposition of non-living organic matter, by enzymatically catalysed dissolution of iron-bound P (Fig. 3, transfer process b), or direct uptake from the sediment pore water (Fleischer 1986, Boström et al. 1988). Heterotrophic benthic Poly-P bacteria have a dual function in P turnover. On the one hand, they influence the organic P pool by hydrolysis and subsequent P uptake in their biomass during the decomposition of organic matter. On the other hand, they regulate the P availability through metabolism of inorganic Poly-P. Another general ecological function of bacteria is to assimilate and re-introduce organic matter into the food chain (Davelaar 1993). The consumption of detritus and associated microorganisms by meio- and macrozoobenthos increases the P regeneration due to mineralization of P-containing organic matter (Andersson et al. 1988). It is well documented that benthic animals excrete dissolved P in substantial quantities (e.g. Devine & Vanni 2002). Furthermore, benthivorous fishes, as top predators in the benthic food chain, translocate P from the sediment into the water by P excretion (Persson & Svensson 2006). These P regeneration pathways in the food chain will be reinforced when benthic animals graze on P-rich PAO instead of bacteria with much lower P content (Fig. 3, transfer process c).

Temporal fluctuations of the oxic/anoxic boundary layer within the sediment can lead to a strong induction of microbial P release (Fig. 3, transfer process d). This mechanism could result in rapid increases in P concentrations of the sediment pore water, which are

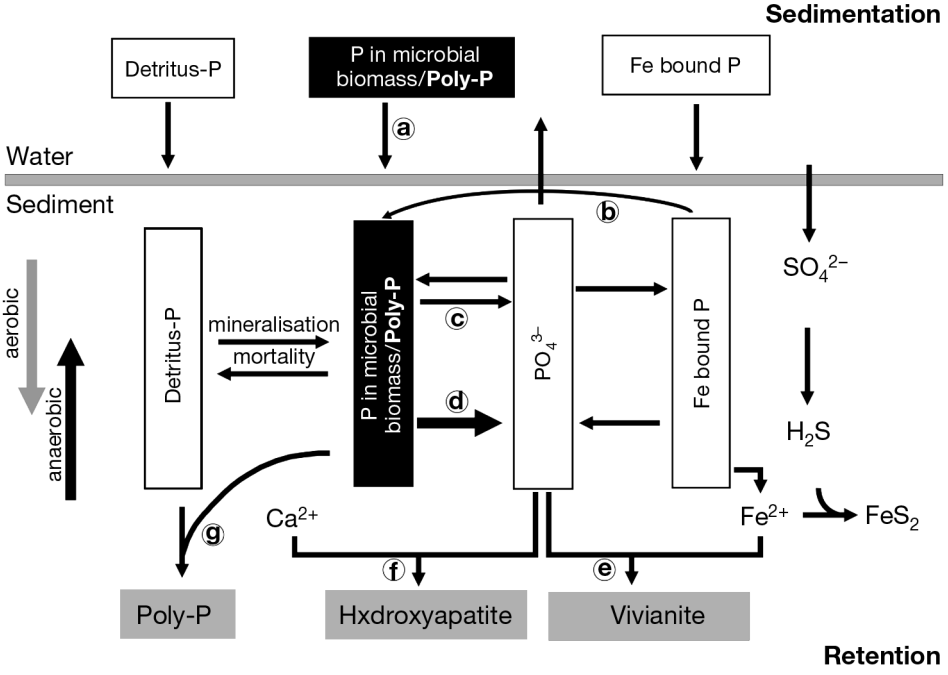

Fig. 3. Simplified scheme of the supposed functions of biomass P, including Poly-P in P diagenesis in aquatic sediments. Transfer processes between the most important P pools are shown (partially based on Boström et al. [1988] and Roden & Edmonds [1997]). a: increase of P sedimentation; b: enzymatic dissolution of iron-bound P; c: P transfer in the benthic food chain; d: rapid P release under changed redox conditions; e: formation of FE(II) minerals (e.g. vivianite); f: formation of Ca-P minerals (e.g. hydroxyapatite); g: permanent deposition of Poly-P (see section 'Ecological relevance of PAO in sediments')

at times higher than those mediated by iron-controlled P release alone. Such high P concentrations can even induce authigenic P mineral precipitation such as vivianite (Fig. 3, transfer process e) and hydroxyapatite (Fig. 3, transfer process f). The presence of high concentrations of Ca^{2+} or Fe^{2+} is a key condition to attain saturation for mineral formation. In anaerobic, iron-rich sediments microbial reduction of Fe(III) oxide produces Fe^{2+}. The concentration of Fe^{2+} in the pore water depends on the sulphate concentration in the system and the extent of sulphate reduction (Roden & Edmonds 1997). High sulphate concentrations in marine and in some lacustrine systems favour the formation of sulphide and the subsequent immobilisation of iron as FeS_2, which prevents the precipitation of Fe(II)-P minerals. In calcite-rich sediments the formation of apatite-like minerals can indeed be induced by bacterial P release (Schulz & Schulz 2005). In general, mineral formation increases the permanent burial of P and removes P from the biosphere for geological time scales.

P retention in sediments is also influenced by Poly-P enclosed in the cells of dormant or dead PAO and by the production and accumulation of non-metabolisable organic P (Fig. 3, transfer process g) (Gächter & Meyer 1993, Ingall & Jahnke 1997). It has been proposed that the metabolism by PAO could be an alternative way for the redox-sensitive P cycling in sediments (e.g. Davelaar 1993, Ingall et al. 2005). The pathways illustrated in Fig. 3 show that the ecological consequences of P regeneration in sediments differ from each other when P is initially stored in PAO and not bound to iron. In fact, Poly-P seems to be a highly variable P pool in sediments. Sediment core studies have shown that the contribution of Poly-P to the release of P during diagenesis should not be disregarded when compared with the dissolution of P bound to iron hydroxides (Hupfer et al. 2004, Reitzel et al. 2007). Some preliminary studies suggest that microbial Poly-P formation in natural sediments can be induced by alternating anoxic and oxic conditions (Khoshmanesh et al. 2002, Hupfer et al. 2004).

Although there is substantial information on the topic, the ecological role of PAOs in natural sediments remains highly speculative, since up to now most of the PAOs are neither phylogenetically, nor physiologically characterised.

CONCLUSIONS AND FUTURE RESEARCH NEEDED

Major statements of our review are

- Modern Poly-P detection methods revealed that Poly-P in aquatic sediments can frequently account for a substantial fraction of total P in surface sediments (maximum ca. 10% of total P). A relationship between the occurrence and content of Poly-P in sediments and the trophy or other lake and sediment characteristics has not yet been shown
- Poly-P in sediments may originate from various sources, such as benthic or pelagic hetero- and autotrophic organisms. Aggregates of settling seston potentially colonised by microorganisms could act as efficient carriers for Poly-P deposition to the sediment surface. Field experiments need to test whether Poly-P in sediments is of pelagic or benthic origin
- The reliable quantification of Poly-P by ^{31}P NMR methods enables the monitoring of Poly-P dynamics under laboratory and field conditions in specific habitats, especially when the detection sensitivity can be further increased. Further experiments are necessary to verify under which conditions storage and release of Poly-P occur at the sediment surface (e.g. light, oxygen, redox gradients, substrates)
- The combination of TEM with energy-dispersive X-ray microanalysis has shown that Poly-P in sediments often occurs inside microbial cells. Since benthic and activated sludge systems are characterised by periodic or episodic changes of redox conditions and the availability of various carbon sources, a certain parallelism between PAO in benthic and activated sludge systems has been proposed. Although such a parallelism seems likely, direct proof is still missing. In addition, potential mechanisms and niches of microbial Poly-P accumulation in aquatic sediments are more diverse than in activated sludge. These differences may also result in distinct differences in the physiology and ecology of PAO from both systems
- Poly-P bacteria have the potential to control a significant portion of the P fluxes between the sediment and the overlying water by redox-dependent changes of their physiology. The metabolism of PAOs and their rapid P release may even result in authigenic P mineral formation and, thus, in an increase of the permanent P deposition in sediments
- The combination of different methodological approaches allows studies on the dominant PAO in sediments; in particular, the detection and separation of PAOs from natural sediment samples allows their precise phylogenetic characterisation. This knowledge will contribute to our understanding of the ecological function of PAO in the internal P cycle of aquatic systems. Until now, however, it has been uncertain whether key organisms known to carry out Poly-P storage in activated sludge play a similar role in natural sediments
- Once the physiology of PAOs and their role in Poly-P cycling in sediments are sufficiently understood, it will be possible to combine these findings with our current knowledge on geochemical cycles. This will

be an important step to further improve the existing quantitative models for P exchange between sediments and the overlying water.

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