

## ORIGINAL ARTICLE

# Sulfide induces phosphate release from polyphosphate in cultures of a marine *Beggiatoa* strain

Jörg Brock<sup>1,2,3</sup> and Heide N Schulz-Vogt<sup>1,2</sup>

<sup>1</sup>Department of Microbiology, Max Planck Institute for Marine Microbiology, Bremen, Germany; <sup>2</sup>MARUM Center for Marine Environmental Sciences, University of Bremen, Bremen, Germany and <sup>3</sup>Institute for Microbiology, Leibniz University Hannover, Hannover, Germany

**Sulfur bacteria such as *Beggiatoa* or *Thiomargarita* have a particularly high capacity for storage because of their large size. In addition to sulfur and nitrate, these bacteria also store phosphorus in the form of polyphosphate. *Thiomargarita namibiensis* has been shown to release phosphate from internally stored polyphosphate in pulses creating steep peaks of phosphate in the sediment and thereby inducing the precipitation of phosphorus-rich minerals. Large sulfur bacteria populate sediments at the sites of recent phosphorite formation and are found as fossils in ancient phosphorite deposits. Therefore, it can be assumed that this physiology contributes to the removal of bioavailable phosphorus from the marine system and thus is important for the global phosphorus cycle. We investigated under defined laboratory conditions which parameters stimulate the decomposition of polyphosphate and the release of phosphate in a marine *Beggiatoa* strain. Initially, we tested phosphate release in response to anoxia and high concentrations of acetate, because acetate is described as the relevant stimulus for phosphate release in activated sludge. To our surprise, the *Beggiatoa* strain did not release phosphate in response to this treatment. Instead, we could clearly show that increasing sulfide concentrations and anoxia resulted in a decomposition of polyphosphate. This physiological reaction is a yet unknown mode of bacterial polyphosphate usage and provides a new explanation for high phosphate concentrations in sulfidic marine sediments.**

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**Keywords:** sulfur bacteria; marine sediment; polyphosphate; phosphorus cycle

## Introduction

Phosphorus is considered as the ultimate limiting nutrient, because over geological time scales the amount of biologically available phosphorus determines how much carbon is incorporated into the living biomass. In contrast to this, nitrogen often limits growth over shorter time scales, but is ultimately available in large amounts from the atmosphere through the activity of nitrogen-fixing microorganisms (Tyrrell, 1999). Therefore, it is crucial to know the sources and sinks of phosphorus in order to understand the global carbon cycle and thus the climate on the Earth. Phosphorus enters the biosphere in the form of phosphate by the weathering of phosphorus-rich rocks on land and is removed by phosphogenesis, which refers to the

formation of phosphorus-rich minerals on the seafloor (Föllmi, 1996). While weathering is mostly a physicochemical process, phosphogenesis is initiated by the spontaneous precipitation of phosphorus-rich minerals due to oversaturation in the pore water with respect to apatite, which can be induced by the activity of microorganisms. Depending on the initial degree of oversaturation, different mechanisms of precipitation reactions may prevail, resulting in a slow formation of dispersed apatite or a fast formation of phosphatic bodies (Krajewski *et al.*, 1994). In addition, calcium-associated polyphosphate of biological origin can reduce the kinetic nucleation barrier to the precipitation of calcium phosphate minerals, and diagenetic transformation into fine-grained, geologically stable authigenic apatite particles can thereby occur (Diaz *et al.*, 2008). In today's oceans, active phosphogenesis is mainly found beneath the nutrient-rich upwelling areas off the coasts of Peru, Chile and Namibia (Föllmi, 1996).

Large sulfur bacteria of the genera *Beggiatoa*, *Thioploca* and *Thiomargarita* are suspected to be involved in phosphogenesis, because they occur in

Correspondence: J Brock, Department of Microbiology, Max Planck Institute for Marine Microbiology, University of Bremen, Celsiusstrasse 1, D-28359 Bremen, Germany.

E-mail: jbrock@mpi-bremen.de

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remarkably high biomasses precisely in the areas of the most active modern phosphorite formation (Fossing *et al.*, 1995; Schulz *et al.*, 1999) and they are found as fossils in phosphorite rocks (Williams and Reimers, 1983; Reimers *et al.*, 1990; Bailey *et al.*, 2007). Recently, *Thiomargarita namibiensis* was found to accumulate polyphosphate, which can be released as phosphate under certain conditions producing steep peaks of phosphate in the pore water. The generated oversaturation then results in the formation of phosphorus-rich minerals in sediments off the coast of Namibia (Schulz and Schulz, 2005). Like other large sulfur bacteria, *T. namibiensis* gains energy by the oxidation of sulfide with oxygen or internally stored nitrate (Schulz and Jorgensen, 2001). Polyphosphate is a storage compound, which is accumulated at the expense of energy during favorable growth conditions and then can again be used to gain energy (Kornberg, 1995). As the accumulation and decomposition of polyphosphate is not part of the main energy-gaining metabolism, recurrent phosphate release must be initiated by an environmental stimulus. The identification of this stimulus is important for both the general understanding of polyphosphate in the microbial metabolism and the identification of the environmental conditions inducing phosphate release and phosphogenesis.

Most studies on bacterial polyphosphate accumulation and release were conducted on microbial communities from wastewater treatment plants, in which polyphosphate-accumulating bacteria are used for biological phosphorus removal. More than three decades ago, a correlation between the phosphate-removal capacity of activated sludge and the acetate concentration during the anaerobic treatment was observed (Fuhs and Chen, 1975). In later studies, Comeau *et al.* (1986) proved that addition of acetate to anoxic activated sludge triggered phosphate release, and based on this observation the authors postulated a biochemical model for enhanced biological phosphorus removal. According to this model, polyphosphate-accumulating bacteria store acetate in the form of poly- $\beta$ -hydroxybutyrate during the anaerobic treatment phase using the energy provided by the decomposition of polyphosphate. In the following oxic treatment phase, the same bacteria exhibit a 'luxury uptake' of phosphate along with accumulation of polyphosphate. This concept is generally accepted, although the identification and cultivation of the relevant polyphosphate-accumulating organisms has proven to be problematic (Seviour and McIlroy, 2008). Moreover, Comeau *et al.* (1986) report that sulfide addition stimulated phosphate release, but this result was not included into the concept of polyphosphate usage and, to our knowledge, has not further been investigated. However, sulfate reduction accompanied by the growth of sulfur-oxidizing bacteria is known to occur in anaerobic activated sludge (Okabe *et al.*, 1999) and a higher phosphate

release was detected simultaneously with an increase in sulfate reduction rates (Baetens, 2000).

In this study we tested whether acetate or sulfide is responsible for the release of phosphate by a marine *Beggiatoa* strain through the decomposition of polyphosphates. Members of the genus *Beggiatoa* are filamentous, highly motile sulfur bacteria, which occur abundantly in sulfidic sediments all over the world (Teske and Nelson, 2006) and are also encountered as fossils in phosphorite deposits (Williams and Reimers, 1983; Reimers *et al.*, 1990). Typically, the long filaments populate organic matter-rich sediments, in which they form a mat in the narrow zone where oxygen and sulfide overlap, close to the sediment surface.

## Materials and methods

### *Strain and cultivation*

The investigated marine *Beggiatoa* strain grows in the presence of a single accompanying bacterium. This *Pseudovibrio* strain occurs in low cell numbers but could not be removed during purification and therefore seems to be required for the growth of the culture. The *Beggiatoa* filaments have an average diameter of 6  $\mu\text{m}$ ; the cells possess sulfur inclusions, a central vacuole, probably for nitrate storage, and polyphosphate inclusions of different sizes.

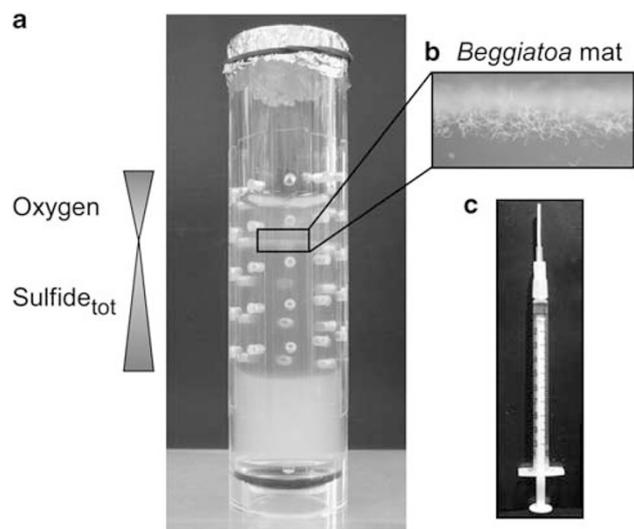
For cultivation, a mineral medium with opposing gradients of oxygen and sulfide modified after Nelson and Jannasch (1983) was strongly buffered with 1,4-Piperazinediethanesulfonic acid disodium salt (20  $\text{mmol l}^{-1}$ ) to maintain a pH of 7 and thus avoid phosphate precipitation. The initial phosphate concentration of the medium was lowered to 20  $\mu\text{mol l}^{-1}$ . The gradient medium was prepared in a Plexiglas tube of 20 mm inner diameter and 12 cm length, closed at the bottom and filled with 7.5 ml sulfidic bottom agar and 15 ml sulfide-free top agar. On the wall of the tube 2-mm-wide holes were drilled in 1-mm-depth intervals and closed with an autoclavable tape (Figure 1).

### *Distribution of Beggiatoa filaments*

Due to the highly refractive internal sulfur globules, filaments of *Beggiatoa* appear white and can easily be seen in a transparent agar medium. The distribution of the filaments in the top part of the gradient medium was recorded by a sensicam CCD camera (PCO, Kelheim, Germany) while the tubes were exposed to an amber Luxeon LED (Philips, Amsterdam, The Netherlands).

### *Experiments*

In order to test the influence of different sulfide fluxes on the release of phosphate, gradient media with 8, 16, 24 and 36  $\text{mmol l}^{-1}$   $\text{Na}_2\text{S}$  in the bottom agar were prepared.  $\text{Na}_2\text{S}$  was added to the bottom agar after autoclaving, and afterward the pH of the bottom agar was adjusted to about 7 by addition of



**Figure 1** Gradient sampling tube with an oxygen/sulfide gradient medium. (a) The tube consists of polymethyl methacrylate (Plexiglas) and features spherical drilled holes of 2 mm diameter separated by a vertical distance of 1 mm for high-resolution sampling. The holes are sealed with an autoclavable adhesive tape. (b) A *Beggiatoa* mat forms at the interface of oxygen and sulfide. (c) A microrhizon attached to a 1-ml syringe is used for sampling of liquid from the agar.

1 mol l<sup>-1</sup> HCl. After solidification of the bottom agar at 4 °C the top agar was added. If necessary the pH was adjusted to 7 after autoclaving by addition of 1 mol l<sup>-1</sup> HCl. The tubes were closed by aluminum foil, allowing exchange of headspace gas with the atmosphere. After aging for 1 day, a sulfide gradient had established in the top agar and the experiment was started by inoculation with 80 µl of a *Beggiatoa* culture, which contained 8 mmol l<sup>-1</sup> Na<sub>2</sub>S in the bottom agar and the same concentration of phosphate and buffer as noted above. Sampling of three parallel cultures was carried out after 7 days of oxic growth or after 7 days of oxic growth followed by 24 h of anoxic incubation. Anoxic conditions were obtained by closing the tubes with a rubber stopper and flushing with nitrogen for 5 min.

The effect of volatile fatty acids in combination with anoxia on phosphate release was tested by injecting acetate or propionate (32 µl of a 1 mol l<sup>-1</sup> solution) directly into the bacterial mat in three parallel cultures (7 days old, 8 mmol Na<sub>2</sub>S mol l<sup>-1</sup> in the bottom agar) followed by 24 h of anoxic incubation. As a control, three parallel cultures (6 days old) were left oxic for 24 h after the injection.

Additionally, it was tested whether phosphate release is an active process of living cells or occurs as a consequence of cell death. Thick mats of *Beggiatoa* were harvested and immediately inactivated by exposure to 60 °C for 15 min. A 1-mm-thick layer of dead filaments was placed on top of a sterile and phosphate-free gradient medium that contained 36 mmol l<sup>-1</sup> sulfide in the bottom agar. Phosphate profiles were measured in three parallels after 3 days of oxic conditions or after 2 days of oxic conditions followed by 24 h of anoxic incubation.

### Phosphate profiles

For phosphate determination, samples of 80 µl were taken from subsequent depth layers through the holes in the wall of the tubes using microrhizons (Rhizosphere Research Products, Wageningen, The Netherlands). After addition of 420 µl H<sub>2</sub>O phosphate concentrations were determined colorimetrically by the ascorbic acid method modified after Hansen and Koroleff (1999) using a Beckman DU 640 Spectrophotometer (Beckman Coulter, Fullerton, CA, USA) or a SpectroDirect Spectrophotometer (Aqualytic, Dortmund, Germany).

### Sulfide profiles

Sulfide profiles were determined with commercially available microelectrodes (Unisense, Aarhus, Denmark). Total dissolved sulfide concentrations were calculated using a simultaneously measured pH profile according to Kühl *et al.* (1998). The flux of total sulfide was calculated as the sum of the H<sub>2</sub>S and the HS<sup>-</sup> fluxes according to Fick's first law of diffusion:

$$J = -D^0 \times \partial C / \partial x$$

The used diffusion coefficients ( $D^0$ ) were 1.4910<sup>-9</sup> for HS<sup>-</sup> and 1.7510<sup>-9</sup> m<sup>2</sup> s<sup>-1</sup> for H<sub>2</sub>S (Schulz, 2006).

### Microscopy and staining of polyphosphate inclusions

Filaments of *Beggiatoa* were imaged by differential interference contrast (DIC) microscopy to visualize internal cell structures such as sulfur globules, which appear as dark refractive spots. 4',6-Diamidino-2-phenylindole dihydrochloride (DAPI) is a common stain for DNA with a blue signal and a maximum emission wavelength of 460 nm when bound to DNA. DAPI as well binds to polyphosphate, but then the dye's maximum emission wavelength is shifted to 525 nm, resulting in a yellow signal (Tijssen *et al.*, 1982). For polyphosphate staining 5 µl of a DAPI solution (1 g l<sup>-1</sup> in H<sub>2</sub>O) was added to 50 µl of a fresh *Beggiatoa* sample and incubated overnight at room temperature. Polyphosphate inclusions were observed by fluorescence microscopy using an Axioplan universal microscope (Zeiss, Oberkochen, Germany) with a HBO 50 mercury lamp (Osram, München, Germany) for UV light and a UV-G 365 filter set (G 365 exciter filter, FT 395 chromatic beam splitter and an LP 420 barrier filter, Zeiss, Oberkochen, Germany).

## Results

### Culture growth

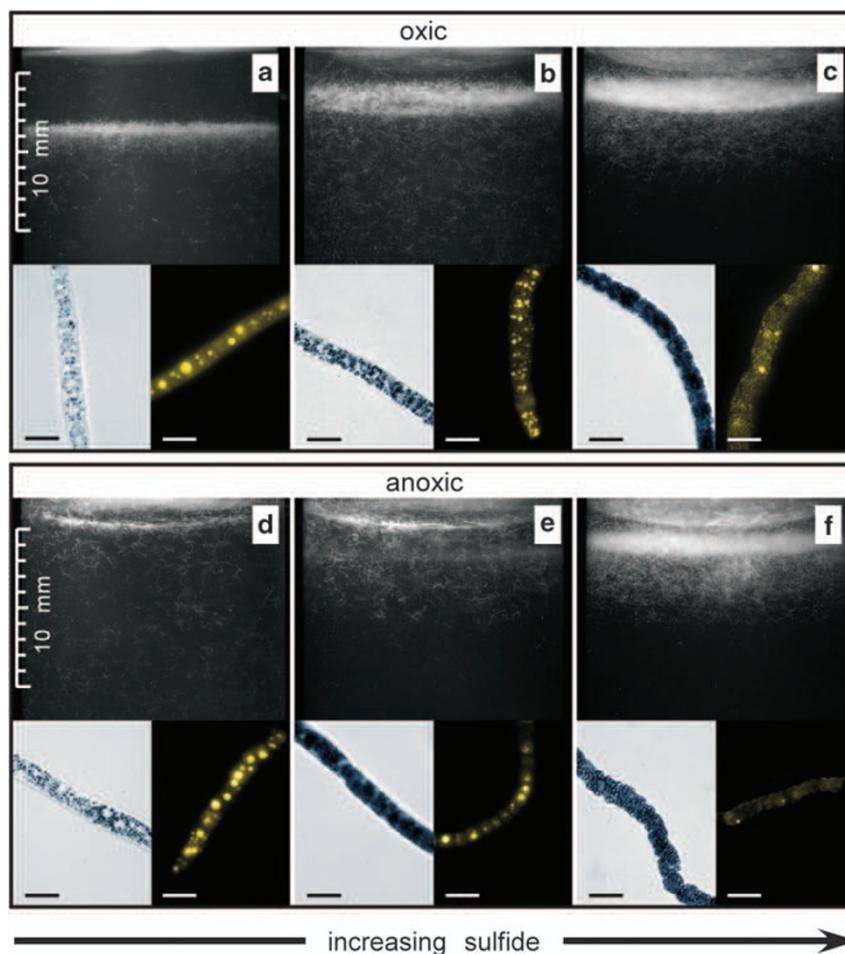
All cultures showed the formation of a distinct *Beggiatoa* mat 1–2 days after inoculation. Higher sulfide concentrations in the bottom agar resulted in higher growth rates and higher positions of the *Beggiatoa* mats in the gradient medium (Figure 2).

After 1 week the mat positions varied from 5.0 mm depth in the cultures with the lowest sulfide flux to 0.5 mm depth in the cultures with the highest flux (Figures 3a and 4, hatched lines). The addition of acetate or propionate followed by oxic incubation induced an upward movement of the mat from 3 to 1 and 5 to 2 mm depth, respectively, within 24 h (Figure 3, hatched lines). Under anoxic incubation the *Beggiatoa* mat dispersed in all cultures and the *Beggiatoa* filaments moved to the top of the gradient medium within 24 h (Figures 2d–f, 3 and 4, gray lines).

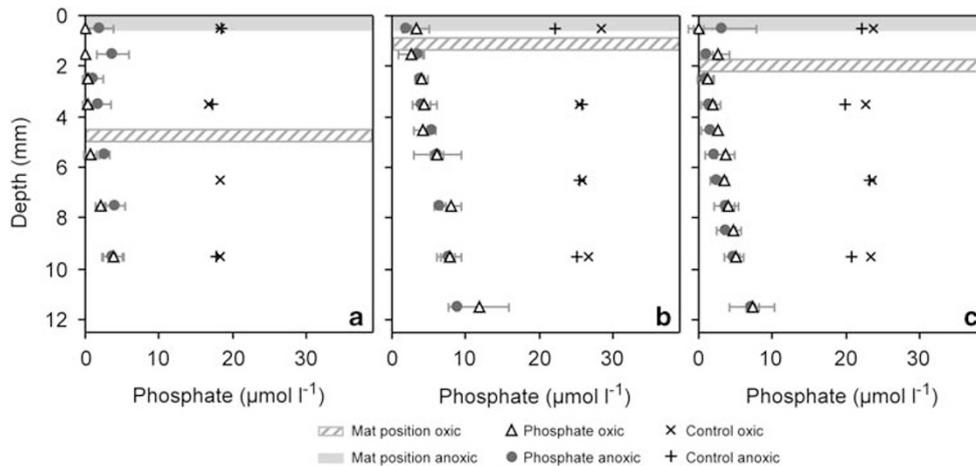
#### Phosphate uptake and release in response to addition of fatty acids

Under oxic conditions the profiles of dissolved phosphate (Figure 3, triangles) showed a decrease in concentration from 20 to nearly  $0 \mu\text{mol l}^{-1}$  at the top of the gradient media where the *Beggiatoa* mats

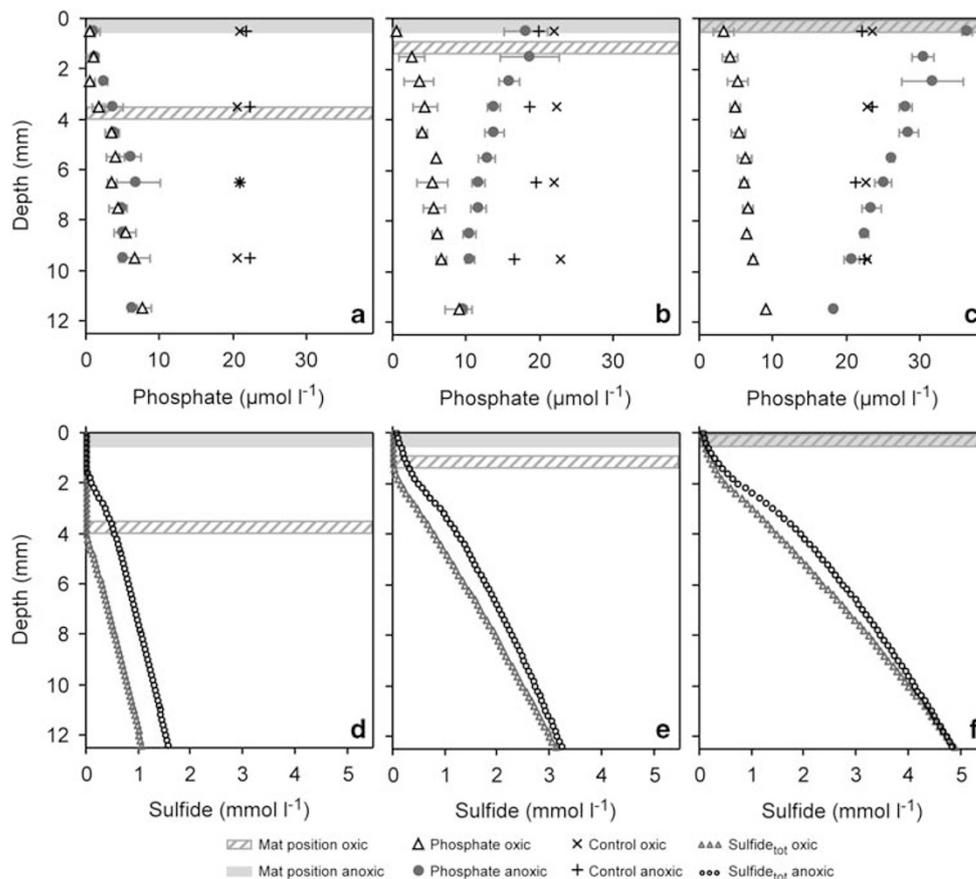
were situated, owing to bacterial growth and accumulation of polyphosphate (Figure 3, triangles). Below the *Beggiatoa* mats, the phosphate concentration increased with ongoing depth. As *Beggiatoa* filaments were hardly found in these deeper layers, the phosphate concentration was regulated by diffusion. In sterile controls the initial phosphate concentration of about  $20 \mu\text{mol l}^{-1}$  was found throughout the depth of the medium (Figure 3, crosses). No significant difference in phosphate profiles was evident when acetate or propionate (Figures 3b and c) was added to cultures without volatile fatty acids (Figure 3a). After 24 h of anoxic incubation no change in the phosphate profiles could be observed (Figure 3, filled circles). However, under oxic conditions the *Beggiatoa* mat responded to addition of acetate or propionate with an upward movement from 3 to 1 mm or 5 to 2 mm depth, respectively, within 24 h (Figure 3, hatched lines).



**Figure 2** Response of a marine *Beggiatoa* strain to increasing sulfide fluxes and anoxia. (a–c) After 7 days of oxic conditions *Beggiatoa* filaments have established a distinct mat at the interface of oxygen and sulfide. With increasing sulfide fluxes the mats are situated at higher positions. (d–f) The same cultures after 24 h of anoxic incubation. The mats have dispersed and the filaments have migrated to the top. Insets show micrographs of filaments from the respective cultures. In the left images sulfur globules are visualized as dark spots by DIC microscopy. In the right images polyphosphate inclusions stained with DAPI show a yellow fluorescence. Filaments contain more sulfur granules with increasing sulfide fluxes. At higher sulfide concentrations, anoxic conditions cause the decomposition of polyphosphate inclusions. Scale bars are 10  $\mu\text{m}$ . (Pictures a–f by courtesy of Anne Bachmann.)



**Figure 3** Influence of volatile fatty acids on phosphate release. Mean phosphate concentrations and standard deviations of three parallels after 6 days of oxic growth followed by the injection of a volatile fatty acid into the mat and 24 h of oxic incubation ( $\Delta$ ), or 24 h of anoxic incubation ( $\bullet$ ). The hatched horizontal lines indicate mat positions after oxic condition and the gray lines after anoxic incubation. (a) Profiles without addition of volatile fatty acids, (b) after addition of acetate and (c) after addition of propionate to cultures with  $8 \text{ mmol l}^{-1}$  sulfide in the bottom agar. Under oxic conditions phosphate is rapidly taken up, but after switching to anoxic conditions phosphate is not released in the absence or in the presence of acetate or propionate.



**Figure 4** Influence of different sulfide fluxes on phosphate release. Cultures with (a, d)  $16 \text{ mmol l}^{-1}$ , (b, e)  $24 \text{ mmol l}^{-1}$  and (c, f)  $36 \text{ mmol l}^{-1}$  sulfide in the bottom agar are shown. (a–c) Mean phosphate concentrations and standard deviation of three parallels after 7 days of oxic growth ( $\Delta$ ) and after 7 days of oxic growth followed by 24 h of anoxic incubation ( $\bullet$ ). (d–f) Concentrations of total sulfide under oxic ( $\Delta$ ) and after switching to anoxic conditions ( $\circ$ ). The hatched horizontal lines indicate mat positions after oxic condition and the gray lines after anoxic incubation. Under oxic conditions phosphate is rapidly taken up. With increasing sulfide more biovolume is produced and a mat is established at a higher position. During the following anoxic incubation steeper sulfide gradients stimulate an increasing release of phosphate.

**Table 1** Concentration of phosphate and total dissolved sulfide at the top of the culture as well as total sulfide fluxes in the *Beggiatoa* mat at different sulfide concentrations in the bottom agar

$c(\text{Na}_2\text{S})$ ( $\text{mmol l}^{-1}$ ) <sup>a</sup>	$c(\text{PO}_4^{3-})$ ( $\mu\text{mol l}^{-1}$ ) <sup>b</sup>		$c(\text{Sulfide}_{\text{tot}})$ ( $\mu\text{mol l}^{-1}$ ) <sup>b</sup>		$\text{Sulfide}_{\text{tot}}$ flux ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) <sup>c</sup>	
	Oxic	Anoxic	Oxic	Anoxic	Oxic	Anoxic
8	0	0–4	0	0	0.14	0.11
16	0–1	0–2	0	0	0.19	0.19
24	0–1	16–23	0	70	0.43	0.36
36	2–5	30–32	45	63	0.61	0.53

The values were determined after 7 days of oxic growth or after 7 days of growth followed by 24 h of anoxic incubation. A release of phosphate after anoxic incubation coincides with the presence of sulfide at the top of the culture when *Beggiatoa* filaments are exposed to sulfide.

<sup>a</sup>Initial concentration in bottom agar.

<sup>b</sup>Concentration at the top.

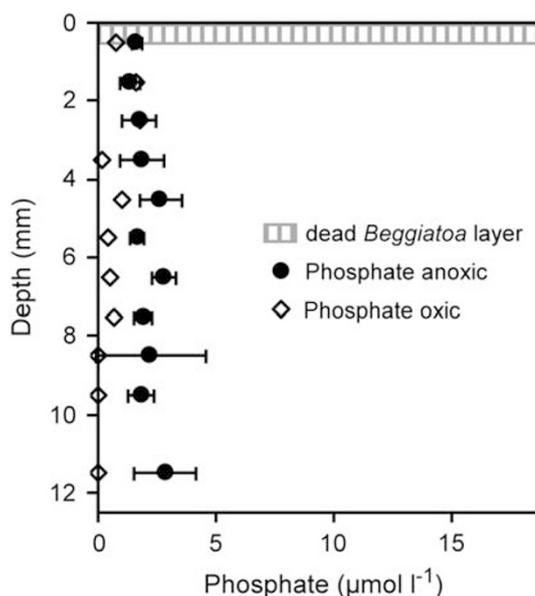
<sup>c</sup>Sum of  $\text{HS}^-$  and  $\text{H}_2\text{S}$  flux.

### Phosphate uptake and release in response to increasing sulfide concentrations

The shape of the phosphate profile under oxic conditions did not change significantly with increasing sulfide concentrations in the bottom agar (Figure 4, triangles). Only in the culture with the highest sulfide flux did the phosphate concentrations not reach  $0 \mu\text{mol l}^{-1}$  at the position of the mat but remained around  $3 \mu\text{mol l}^{-1}$ . Sulfide profiles measured with microelectrodes showed that under these conditions sulfide diffused up to the top of the medium. Accordingly, *Beggiatoa* filaments in these cultures were permanently exposed to higher sulfide concentrations compared with cultures in which a lower sulfide flux allows a complete oxidation of sulfide within the mat (Figure 4f; Table 1).

The *Beggiatoa* filaments migrated to the top of the gradient medium during anoxic incubation at all sulfide fluxes tested (Figures 2d–f; Figure 4, gray lines). Anoxic conditions for 24 h resulted in increasing phosphate release when the *Beggiatoa* strain was cultivated in the presence of steeper sulfide gradients (Figures 4b and c, filled circles; Table 1). Sulfide microprofiles showed that filaments in these cultures were exposed to elevated sulfide concentrations during anoxic conditions (Figures 4e and f; Table 1). At  $16 \text{ mmol l}^{-1} \text{ Na}_2\text{S}$  in the bottom agar the flux of total sulfide was  $0.19 \mu\text{mol m}^{-2} \text{ s}^{-1}$  under oxic and anoxic conditions. At a sulfide concentration of  $36 \mu\text{mol l}^{-1} \text{ Na}_2\text{S}$  in the bottom agar the flux of total sulfide decreased from  $0.61 \mu\text{mol m}^{-2} \text{ s}^{-1}$  under oxic conditions to  $0.53 \mu\text{mol m}^{-2} \text{ s}^{-1}$  after anoxic incubation, indicating a decrease in sulfide uptake.

In the control experiment in which the dead filaments were placed on top of a phosphate-free medium, no significant release of phosphate could be detected under oxic or anoxic conditions (Figure 5). The *Beggiatoa* filaments, which originally contained polyphosphate in similar amounts as in the experiments, were mostly fractured, and possessed after the treatment a lower amount of polyphosphate inclusions compared with untreated



**Figure 5** Control experiment with dead *Beggiatoa* filaments. A thick *Beggiatoa* mat was harvested and inactivated by exposure to  $60^\circ\text{C}$  for 15 min. A 1-mm-thick layer of dead filaments was placed on top of a sterile, phosphate-free medium with  $36 \text{ mmol l}^{-1}$  sulfide in the bottom agar. Phosphate profiles after 3 days of oxic conditions ( $\diamond$ ) and 2 days of oxic followed by 24 h of anoxic conditions ( $\bullet$ ) showed no significant phosphate release.

filaments. It is likely that the majority of polyphosphate was dispersed in the medium and was therefore not detectable by DAPI staining.

### Accumulation of polyphosphate

When incubated oxically, the *Beggiatoa* strain accumulated polyphosphate in high amounts within the cells as visualized by DAPI staining (Figures 2a–c). At low sulfide concentrations big round polyphosphate inclusions with a diameter up to  $3 \mu\text{m}$  were found in the center of the cells in addition to a few smaller inclusions. With increasing sulfide concentrations the size of the

polyphosphate inclusions decreased and at very high concentrations also their number.

At low sulfide concentrations no change in size and number of polyphosphate inclusions was visible after 24 h of anoxic incubation (Figures 2a and d), whereas the amount of the inclusions decreased at medium sulfide concentrations (Figures 2b and e). At high sulfide concentrations, inclusions were hardly found after anoxic incubation (Figure 2f). In addition to the decrease in size and number of polyphosphate inclusions the fluorescence signal weakened, which could be an effect of the increasing amount of sulfur globules diffracting the fluorescence signal.

### Sulfur storage

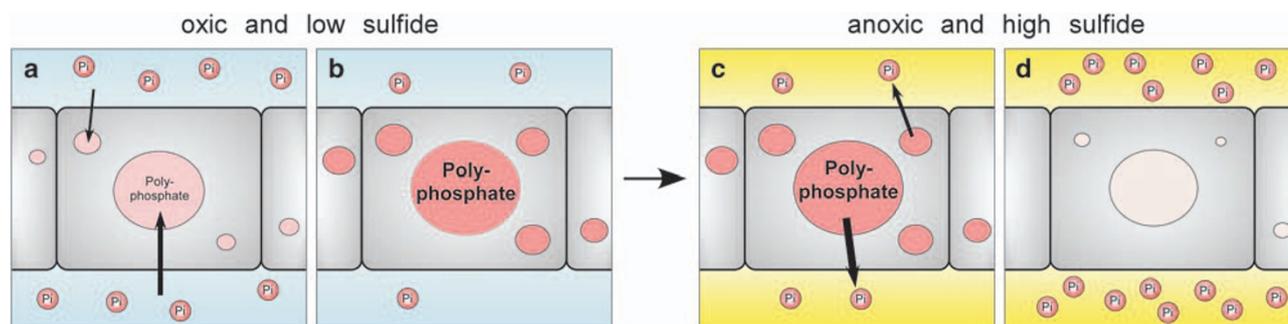
Under oxic conditions, the number of sulfur globules, which were visible as dark spots by differential interference contrast microscopy, increased along with sulfide concentrations in the bottom agar (Figures 2a–c). At low sulfide concentrations only some sulfur globules were found, whereas at high sulfide concentrations the cells were filled with sulfur globules and the filaments appeared completely dark. After 24 h of anoxic incubation the amount of sulfur globules had increased considerably for the respective sulfide concentrations (Figures 2d–f). The sulfur globules did not increase only in number but also in size (Figures 2c and f).

## Discussion

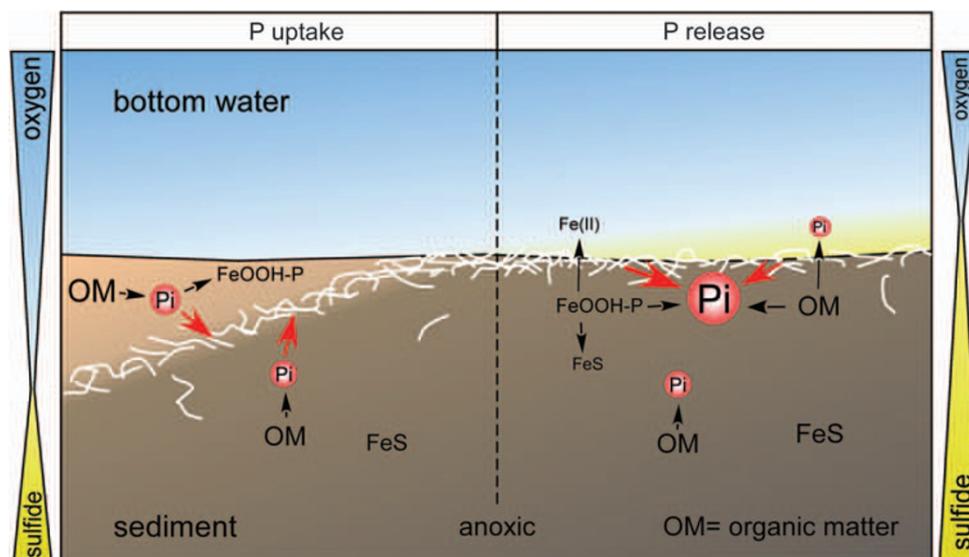
Our study demonstrates a very high phosphate accumulation capacity of the investigated marine *Beggiatoa* strain under oxic conditions. The excessive energy, which can be gained through sulfide oxidation and does not need to be invested in overall metabolism and cell growth, is spent for a 'luxury uptake' of phosphate, which is stored in the form of polyphosphate as demonstrated by DAPI staining (Figure 2). If sulfide exceeds concentrations at which the supply of oxygen is not sufficient for a

complete oxidation of the sulfide, the capacity to build up polyphosphate decreases (Figures 2c and 4c). In contrast to the results obtained in studies on activated sludge from wastewater treatment plants (Fuhs and Chen, 1975; Comeau *et al.*, 1986), we never observed phosphate release in direct response to the addition of fatty acids under anoxic conditions (Figure 3). However, the upward movement of the *Beggiatoa* filaments under oxic conditions indicates the usage of these compounds. Therefore, it can be concluded that polyphosphate is not decomposed to provide energy for the uptake of fatty acids and the synthesis of polyhydroxyalkanoates. Instead, our data reveal that exposure to high sulfide concentrations under anoxic conditions is the direct stimulus for polyphosphate decomposition (Figure 2) and phosphate release in the studied *Beggiatoa* strain (Figures 4 and 6). This release is due to the metabolism of living filaments, because it did not occur in controls with heat-inactivated filaments (Figure 5).

The most common source of high sulfide concentrations in nature is the anaerobic oxidation of organic carbon, including acetate (Widdel and Pfennig, 1981), by sulfate-reducing bacteria. Earlier studies on the giant sulfur bacterium *T. namibiensis* showed that phosphate was released under anoxic conditions in response to acetate addition ( $10\text{ mmol l}^{-1}$  final concentration) (Schulz and Schulz, 2005). In this experiment, *Thiomargarita* cells were taken directly from their original, sulfidic sediment, because no culture of this bacterium is available. In view of the new findings reported here, we suspect that acetate was used by sulfate-reducing bacteria, which were derived from the mucus sheaths of *Thiomargarita*, leading to sulfide formation in the medium, which in turn induced the decomposition of polyphosphate. This explanation is in agreement with the observation that phosphate release in anoxic sediments is enhanced in lakes with increased sulfate concentrations (Caraco *et al.*, 1993). As one possible mechanism, the authors suggest an increased phosphate release from microbial polyphosphate pools.



**Figure 6** Proposed phosphate uptake and release by *Beggiatoa*. (a, b) Under oxic conditions and exposure to low sulfide concentrations phosphate is taken up by *Beggiatoa* and accumulated as polyphosphate. The phosphate concentration in the medium decreases. (c, d) When the conditions change to anoxia and exposure to sulfide increases, the *Beggiatoa* decompose polyphosphate and release phosphate. This leads to an increase of phosphate in the medium.



**Figure 7** Postulated impact of sulfur bacteria on the phosphorus cycle in coastal marine sediments. Owing to bacterial degradation of organic matter, phosphate is released into the pore water. Under oxic conditions phosphate is then taken up by sulfur bacteria and accumulated internally as polyphosphate (red arrows, left side). Another part of the released phosphate is adsorbed to iron hydroxides. With increasing sulfide concentrations, the sediment becomes completely anoxic and phosphate is released due to dissolution of iron hydroxides. The sulfur bacteria decompose polyphosphate once they have no access to oxygen and are exposed to sulfide. The phosphate is released (red arrows, right side) and the concentration of phosphate in the pore water increases drastically. Part of the released phosphate diffuses into the bottom water.

In addition to the active microbial release of phosphate from polyphosphate, purely chemical processes, collectively referred to as reductive dissolution, could explain the increased phosphate concentrations recorded in some anoxic sediments. According to the classical model established by Einsele (1936), phosphate is retained in oxic lake sediments by coprecipitation on iron hydroxides. This phosphate is released from anoxic sediments when iron is reduced, thus dissolving iron hydroxides and releasing the associated phosphate, as shown in Figure 7. Einsele also showed experimentally that sulfide reduces iron hydroxides and thereby induces the release of phosphate. In spite of the plausibility of this model, Einsele and later authors (Boström *et al.*, 1988; Hupfer and Lewandowski, 2008) realized that it does not always sufficiently explain the observed phosphate fluxes in lake sediments. In marine environments, reactive iron concentrations are lower, as are iron-associated phosphate concentrations (Blomqvist *et al.*, 2004). Nevertheless, high sulfide concentrations in anoxic marine bottom water often co-occur with increased phosphate concentrations, which is interpreted as a concurrent release of sulfide and phosphate by the bacterial degradation of organic matter (Shen *et al.*, 2002). In sulfide incubation experiments with sediments from eutrophic lagoons, Heijs *et al.* (2000) detected a 10-fold higher phosphate release related to the initial iron-bound amount of phosphate. Interestingly, these sediments showed a high biological sulfide oxidation potential, which the authors attributed to the presence of colorless sulfur bacteria.

In addition to the two classical models, biological phosphate release in response to acetate and chemical release of iron-bound phosphate, we suggest a third alternative mechanism by which a switch to anoxia may induce increased phosphate concentrations in marine sediments: Phosphate is being trapped by sulfur bacteria through 'luxury uptake' under oxic conditions. In response to elevated sulfide concentrations and anoxia, enhanced decomposition of bacterial polyphosphate leads to strong phosphate release. We assume that this mode of phosphate release is the dominant mechanism in coastal sediments with a high input of phosphorus bound in organic matter and dense populations of sulfur bacteria (Figure 7).

Even though the effect of sulfide on phosphate release by the studied *Beggiatoa* strain is obvious, it still remains unclear why anoxic exposure to sulfide has this physiological effect. Among the many functions of polyphosphate, such as an ATP substitute, this compound is important in the physiological adjustment to stress, such as pH changes and nutrient limitation (Kornberg *et al.*, 1999). In the present case, an explanation could be that the energy provided by the decomposition of polyphosphate is needed to endure sulfide exposure in the absence of a suitable electron acceptor like oxygen or nitrate, which is known to be used by sulfur bacteria for sulfide oxidation (Teske and Nelson, 2006). As changes in the redox conditions are frequent in sulfidic sediments populated by *Beggiatoa*, *Thioploca* and *Thiomargarita*, this so far unknown usage of polyphosphate could act as a kind of 'safety system', which enables survival

under unfavorable conditions of low redox potential. On a stoichiometric level it is a good example of how key resources such as energy and mineral nutrients lead to a high variability in the elemental composition of autotrophs (Sterner and Elser, 2002). The C:P ratio of sulfide-oxidizing bacteria due to a change from oxic to anoxic and sulfidic condition is highly dynamic and is of general ecological importance. It remains to be shown whether other bacteria from habitats with comparably fluctuating conditions like sulfidic hot springs, hydrothermal vents or sulfidic cyanobacterial mats show similar responses.

#### *Impact on precipitation of phosphorus-rich minerals*

The active release of phosphate by polyphosphate-accumulating sulfur bacteria may lead directly to the precipitation of phosphorus-rich minerals as observed off the coast of Namibia (Schulz and Schulz, 2005), or it could mainly enhance the flux of phosphate from the sediment into the bottom water. The process that prevails depends on the rate of phosphate release and the sediment depth at which the release occurs. In general, it seems reasonable to assume that a frequent change from oxic to anoxic conditions in a sulfidic environment, inducing pulses of bacterial phosphate release, will enhance the chances for phosphorite formation on average.

One important difference between freshwater and seawater is that sulfate concentrations are considerably higher in seawater. Consequently, bacterial sulfate reduction is a much more important process in marine sediments compared with freshwater environments. Assuming that phosphogenesis is stimulated by phosphate release of polyphosphate accumulating bacteria and that the key stimulus for bacterial phosphate release is a change from oxic to anoxic conditions in the presence of high sulfide concentrations, we would expect that large accumulations of phosphorites occur more in marine than in freshwater environments, which is certainly the case (Föllmi, 1996). Furthermore, phosphogenesis should occur at locations with very high sulfate reduction rates and changing redox conditions. Indeed, pronounced phosphogenesis is today found in areas of local upwelling, where sulfate reduction rates are exceptionally high (Thamdrup and Canfield, 1996). Within these upwelling areas, phosphorites are preferentially formed in shelf sediments that are located at the boarder of oxygen-depleted water masses (Burnett *et al.*, 1983), where frequent changes between oxic and anoxic conditions in the bottom water prevail. In addition, active phosphate release in sediments populated by large sulfur bacteria is mostly found in the same depth as a peak of lipid biomarkers indicative of sulfate-reducing bacteria (Arning *et al.*, 2008). Recently, Arning *et al.* (2009) showed that Pleistocenic phosphorite crusts off Peru

(ca. 1 million years old) formed as a result of closely coupled bacterial sulfate reduction and sulfide oxidation.

Massive phosphorite deposits from the geological past usually formed in organic matter-rich sediments such as black shales (Piper and Codispoti, 1975) and are often associated with oceanic anoxic events (Handoh and Lenton, 2003). This supports our assumption that phosphorites formed preferentially in sediments with high sulfate reduction rates. On the other hand, phosphorites are typically enriched in uranium, which indicates a change from oxic conditions, wherein uranium is soluble, to anoxic conditions, wherein uranium precipitates (Baturin and Dubinchuk, 2005). All this is principally in agreement with our observations, which suggest an increased release of bacterial-bound phosphate, inducing the precipitation of phosphorus-rich minerals, in response to a switch from oxic to anoxic conditions under exceptionally high sulfide fluxes. Other factors that seem to have an important role in this process are pH, alkalinity and calcite within the sediment (Reimers *et al.*, 1990; Tribovillard *et al.*, 2010). Consequently, if the massive phosphorite deposits of the past were formed at the borders of expanding anoxic water bodies, the gradually increasing burial of phosphorus into the sediment would have counteracted further eutrophication. As a result, the rate of oxygen-consuming organic matter degradation would have been decreased. Concurrently with a flux of oxygen from the prior oxygen-enriched atmosphere, oceanic anoxia would have been reversed by this negative feedback mechanism (Handoh and Lenton, 2003).

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