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Ferrous iron oxidation by denitrifying bacteria in profundal sediments of a deep lake (Lake Constance)

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Abstract

Profundal sediments of Lake Constance were sampled at 60–130 m water depth and studied with respect to the population sizes and depth distribution of mixotrophic and lithotrophic iron oxidizing, denitrifying bacteria. The sediment cores showed a distinct stratification with oxygen and nitrate being consumed within the first centimeter of the sediment. The sediment contained large amounts of acid extractable iron (0.8–1.4% of the dry mass). The proportion of ferrous iron in the solid phase increased dramatically from 16–35% in the oxidized surface layer to 79–97% below 4 cm depth, whereas the total iron content increased only slightly with depth. The concentration of dissolved ferrous iron in the pore water ranged between 10 and 80 μM, which was about three orders of magnitude lower than the total iron content of the solid phase. The redox profile of the pore water iron followed that of the solid phase. Most probable numbers (MPNs) of acetate oxidizing, denitrifying bacteria in the different cores ranged from 1.0×10^6 to 2.1×10^8 cells (ml sediment)$^{-1}$; highest numbers were found in the upper sediment layer. In most cores, bacteria capable of iron oxidation made up a significant part (1–58%) of the total cultivable denitrifying population. Between 1.0×10^4 and 5.8×10^5 cells ml^{-1} oxidized iron mixotrophically; the numbers of lithotrophic iron oxidizing denitrifiers were about one order of magnitude lower. MPNs of mixotrophic iron oxidizers and aerobic iron oxidizers among the denitrifying populations in the upper sediment layers were in the same range. Generally, the MPNs of the different iron oxidizing populations did not change significantly with depth.

Keywords: Ferrous iron; Iron oxidation; Iron cycle; Freshwater sediment; Denitrification

1. Introduction

Iron is the fourth-most abundant element in the Earth's crust and the most prevalent redox-active metal. Due to the rapid chemical oxidation of ferrous iron with oxygen and the low solubility of ferric iron minerals in water, iron accumulates in upper sediment layers as ferric oxides and ferric hydroxides [1]. Below the surface layer, ferric iron is reduced to ferrous iron by ferric iron-reducing bacteria [2,3] or through chemical reduction, e.g. with sulfide [4]. At the oxic–anoxic interface, ferrous iron is chemically or biologically reoxidized with oxygen; in the anoxic zone of the sediment, it can be oxidized biologically with nitrate. Since ferric iron will act again as electron acceptor for the oxidation of organic matter, a single iron molecule may be turned over up to 300 times before it is finally buried as ferrous iron in deeper zones of the sediment [5].

The stalked bacterium *Gallionella ferruginea* and sheathed bacteria belonging to the genus *Leptothrix* are the classical examples of bacteria catalyzing the aerobic oxidation of ferrous iron at neutral pH [6–8], although the biochemical basis of the iron-oxidizing activity has been substantiated only in a few cases [9]. A more recent study of an iron-oxidizing microbial mat showed that the majority of the microbial community consisted of non-appendaged, unicellular bacteria intimately associated with the iron oxides [10]. The recent isolation from groundwater of novel, rod-shaped, aerobic, iron-oxidizing strains of neutrophilic bacteria which are only distantly related to the classical iron oxidizers indicates that there might actually be a wide variety of microorganisms capable of this metabolism [11].

Ferrous iron can also be oxidized anaerobically, either

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Abbreviations: MPN, most probable number

by phototrophic bacteria [12 15] or by chemotrophic, denitrifying bacteria [16,17]. Straub and Buchholz-Cleven [18] have detected, enriched, and enumerated iron-oxidizing, nitrate-reducing bacteria in freshwater sediments of various streams, ponds, and ditches. However, nothing is known about the presence of such bacteria in deep lakes, and also their depth distribution within the sediment remains to be determined. Since an anaerobic oxidation of ferrous iron by denitrifying bacteria would allow the closing of the iron cycle within the anoxic zone of the sediment, more information is needed to assess both the function and the importance of this process in natural environments.

In the present study, we determined the abundance and depth distribution of iron-oxidizing, nitrate-reducing bacteria among the denitrifying microbial community in the sediments of a deep, mesotrophic lake by most probable number (MPN) analysis. Fully aware that a cultivation-dependent enumeration will detect only a fraction of the total microbiota present [19,20], we deemed this approach justified because there is currently no reliable method available to quantify defined metabolic groups such as iron-oxidizing bacteria by other means. Even using specific rRNA-targeted probes and whole-cell hybridization, iron-oxidizing bacteria would be extremely difficult to enumerate by direct microscopic counts since they are closely attached to sediment particles or are encrusted with iron. Since both microbiological and chemical parameters are necessary to assess the potential for iron oxidation and to evaluate its significance in natural sediments, we also measured the concentration and the redox status of iron in the pore water and in the solid phase of the sediment.

2. Materials and methods

2.1. Sampling sites and field procedures

Samples were taken from profundal sediments of Lake Constance, Germany, at sites that represent undisturbed sediments outside the influence of the Rhine river. Sampling sites were located in the northwestern branch of the lake nearby Wallhausen (sampling site for core 9601 at 47°45.226′N and 009°08.075′E, water depth: 130 m) or close to the island of Mainau (sampling site for core 9602 at 47°42.121′N and 009°11.468′E, water depth: 60 m) and in the southern part of the lake near Güttingen (sampling site for cores 9706 and 9901 at 47°37.675′N and 009°18.352′E, water depth: 98 m). Sediment cores 9601 and 9602 were sampled in July 1996, sediment core 9706 in August 1997, and sediment core 9901 in December 1999. Samples were taken with a four-tube multicorer (core size: 8 cm × 37 cm) from the research vessel 'Robert Lauterborn'. Cores were cooled after sampling and processed within a few hours.

2.2. MPN analysis

MPN analyses were performed in three replicate series of tenfold dilutions of a sediment sample in anoxic bicarbonate-buffered mineral medium [21] with 1 mM sulfate as sulfur source. The medium was autoclaved and cooled under N_2/CO_2 (80:20, v/v); then trace element solution SL9 [22] and 7-vitamin solution [23] were added (1 ml l^{-1}), and the pH was adjusted to 7.2. In dilution series for aerobic ferrous iron oxidation in Fe(II)/oxygen gradient tubes, the medium was buffered with 20 mM 3-morpholinopropane sulfonic acid. Sodium nitrate, ammonium acetate, and yeast extract were added to the medium from sterile stock solutions. After addition of iron(II)sulfate from a 1 M anoxic stock solution kept under N_2 atmosphere, a grayish-green, fluffy precipitate formed, which consisted most likely of ferrous iron carbonate and phosphate.

For inoculation of dilution series, sediment sampled at defined depths of the core was immediately transferred to anoxic medium (tenfold dilution) and further processed under a N_2/CO_2 atmosphere. In the first dilution step, sterile glass beads were added, and the suspension was shaken vigorously on a Vortex mixer to homogenize the sediment. Further dilution steps were performed with 1-ml syringes (sediment from sampling sites 9601, 9602 and 9706) or glass pipettes (9901). Tubes were incubated at 28°C in the dark for 6 10 weeks and checked routinely for growth.

To enumerate nitrate-reducing bacteria, 5 mM nitrate, 3 mM acetate, and, when indicated, yeast extract (0.5 g l^{-1}) were provided as substrates. Tubes were considered positive when growth was accompanied by loss of acetate and nitrate; ammonium was determined to differentiate between denitrification and nitrate ammonification. The microorganisms in positive tubes were tested for the capability of anaerobic iron oxidation with nitrate by transfer to medium with 5 mM nitrate and 4 mM ferrous iron, with or without 3 mM acetate as organic cosubstrate. Tubes scored positive for anaerobic, nitrate-dependent iron oxidation only when ferric iron hydroxides were formed. Cultures were also tested for aerobic iron oxidation by transfer to Fe(II)/oxygen gradient tubes, as described previously [17]. Tubes scored positive for aerobic iron oxidation when oxygen penetrated less deeply and ferric iron accumulated in a sharper band in the upper part of the gradient tube than in non-inoculated controls.

Mixotrophic and lithotrophic iron-oxidizing, nitrate-reducing bacteria were enumerated by MPN analysis using anoxic medium containing 2 mM nitrate as electron acceptor and 10 mM ferrous iron with or without 0.5 mM acetate as electron donor. In both cases, tubes scored positive when ferric iron was formed and nitrate was consumed.

MPNs were calculated as described by Cochran [24]. Since the standard deviation for the $\log_{10}$ of MPN values

derived from a tenfold serial dilution with three parallels is 0.33, the difference between the $\log_{10}$ of two MPN values has to be >0.93 to be statistically significant ($P=0.95$) [25].

2.3. Analytical procedures

Sediment cores were processed anoxically in a modified glove box, which allowed introduction of the cores vertically through the bottom of the chamber. Samples were taken from different layers of the cores using syringes that were cut off at the tip.

For iron analysis in the pore water, sediment samples (about 20 ml) were transferred to stainless steel centrifuge tubes, which were then sealed inside the glove box and centrifuged at $12\,000\times g$ for 30 min at 4°C. The supernatant was separated from the pellet inside the glove box and iron was determined as described below.

For determination of HCl-extractable iron in the solid phase, 0.1-ml sediment samples were taken inside the glove box and transferred to tubes containing 10 ml 1 M HCl, sealed with butyl rubber stoppers, and incubated on a rotary shaker at 30°C for 24 h. Afterwards, subsamples were centrifuged and iron was analyzed in the supernatant. To account for heterogeneity of the sediment, averages were calculated for three replicate samples taken at each depth of the sediment core.

For iron analysis in gradient tubes, the agarose medium was cut with a razor blade into 2-mm slices, which were each transferred to 1 ml 5 M HCl. The slices were dissolved during 15-min incubation in a water bath at 40°C; iron was then measured as described below.

Ferrous iron was quantified photometrically with ferrozine; total iron was measured after reduction of all iron to the ferrous state with hydroxylamine hydrochloride [28]. Ferric iron was calculated as the difference between total iron and ferrous iron. 100-µl aliquots of each sample were added to 900 µl HCl (1 M) or to 900 µl hydroxylamine hydrochloride (10% (w/v) in 1 M HCl), respectively, and 1 ml ferrozine solution (50% ammonium acetate, 0.1% ferrozine (w/w) in distilled water) was added. The absorption at 562 nm was measured 2 min after ferrozine addition. All iron determinations were performed in triplicate.

Porosity of the sediments was determined by drying a quantity (25 g) of fresh sediment at room temperature to constant weight; density was determined from the volume of sediment slurries prepared from a quantity (25 g) of fresh sediment and known quantities of water.

Turbidity indicating growth in MPN tubes was measured photometrically at 578 nm. Acetate was quantified by gas chromatography as described previously [26]. Ammonium was quantified by flow injection analysis as described previously [27]. Nitrate and nitrite were quantified by high-performance liquid chromatography using an anion-exchange column (GromSil Anion GSSA 20508R1205, 125 mm×4.6 mm ID) and UV detection at 220 nm. A mobile phase of acetonitrile:methanol:30 mM KCl (65:15:20, v/v/v) was used at a flow rate of 1 ml min ^{1}. To prevent a chemical reaction of nitrite and iron, and to protect the column, iron-containing samples were treated with one volume of 0.5 M phosphate buffer (pH 7.0), incubated at room temperature for 15 min and centrifuged at $14\,000\times g$ for 5 min. The supernatant was used for nitrate and nitrite determination. The detection limit of this method was 10 µM for both nitrate and nitrite. Nitrite formation in growth experiments was also monitored semiquantitatively with test sticks (Merck, Darmstadt, Germany).

3. Results

3.1. Sediment characteristics

At all stations, the sediment consisted of fine-grained material without any major macroscopic heterogeneity. The top layer of all cores had a brown color, which extended less than 1 cm below the sediment surface. Microelectrode profiles of oxygen and nitrate indicated that this layer coincided with the region of oxygen and nitrate reduction (A. Kappler, M. Benz, B. Schink, A. Brune, unpublished results).

The content of acid-extractable iron in the sediments was in the range of 0.8 1.4% for dry sediment, which corresponds to 140 250 µmol (g dry mass) ^{1} and increased slightly with depth at all stations (data not shown). The ferrous iron content of the acid-extractable iron increased strongly over the upper 6 8 cm from 16 35% in the oxidized surface layer to 79 97% below 4-cm depth (Fig. 1). This was most pronounced in the case of core 9602, where the ferrous iron content increased from 30% of the acid-extractable iron in the top sediment layer to almost 100% at 6-cm depth. Also the fraction of dissolved ferrous iron ions in the pore water, determined for core 9706, increased with depth. The reduction status of iron in the pore water was slightly lower than in the solid phase in the upper layers, but surpassed it below 10-cm depth (Fig. 1). There was no clear correlation between the total iron content of the pore water and depth (not shown); the absolute concentration of dissolved iron species in the pore water ranged between 10 and 80 µM in the cores.

These data, together with pore water profiles of sulfate and sulfide concentrations and apparent redox potential (E_h) of the sediment cores (A. Kappler, M. Benz, B. Schink, A. Brune, unpublished results), allowed the identification of the zonation of the major redox processes and the approximate limits of the iron reduction and sulfate reduction zone (see Fig. 1a). Below the brown top layer (A) harboring oxygen and nitrate reduction followed a layer (B) of green-brown color, which extended to a depth of about 5 6 cm, where the E_h of the pore water decreased

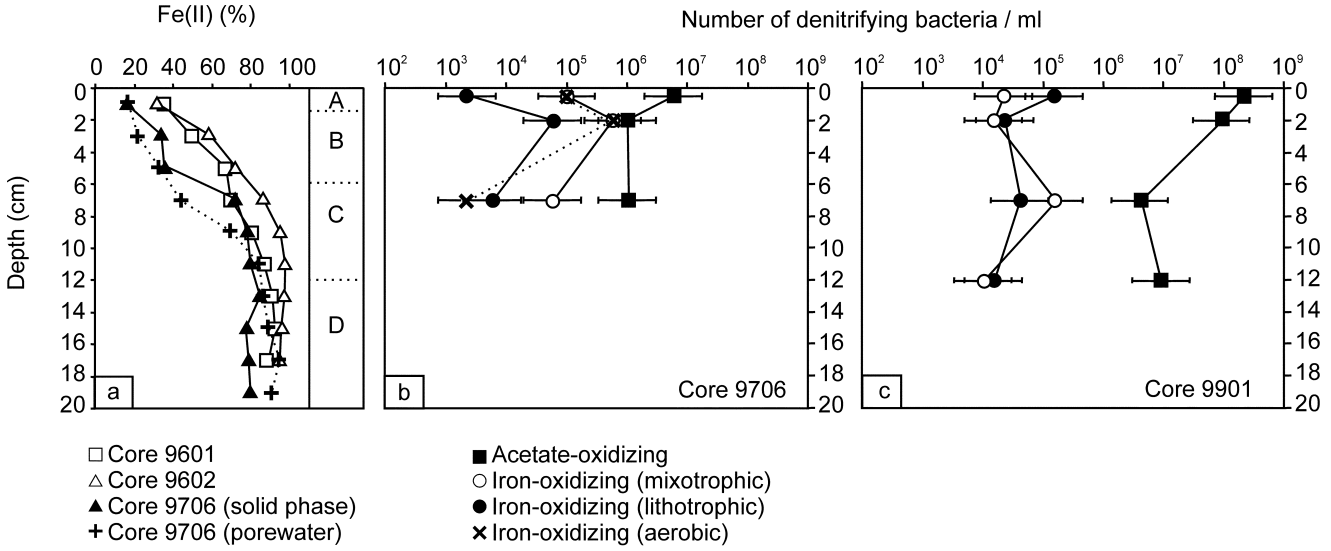

Fig. 1. Depth profile of ferrous iron content and depth distribution of denitrifying bacteria in profundal sediments of Lake Constance. Panel a shows the relative proportion of ferrous iron among the acid-extractable iron in the solid phase of sediment cores 9601 (□), 9602 (△) and 9706 (▲) and in the pore water of core 9706 (+). The depth interval sampled was 2 cm for the solid phase and the pore water. Absolute iron content increased slightly with depth and was in the range of 8 11 mg (g dry mass) ^{1} for cores 9601 and 9602, and 6 14 mg (g dry mass) ^{1} for core 9706. Panels b and c show the depth distribution of denitrifying and aerobic, ferrous iron-oxidizing bacteria among the total population of acetate-oxidizing, denitrifying bacteria in core 9706 and 9901, respectively. Cell densities were determined by MPN analysis; the error bars indicate the 95% confidence limits for the respective data points. The predominant redox processes in the different sediment layers were aerobic respiration/denitrification (A), iron reduction (B), sulfate reduction (C), and methanogenesis (D) (see text).

from oxidized values in layer A to negative values between 150 and 200 mV. Layer B, which was identified as the zone of iron reduction on the basis of the redox profiles of iron in the solid phase, was delimited by layer A and layer C. The black color of layer (C), which extended down to 9 12 cm depth, indicates the presence of iron sulfides. Layer C was clearly identified as the zone of sulfate reduction by the sulfate concentration profile in the pore water. Below 9 12 cm depth was the zone of methane production (layer D), where all sulfate was depleted and where the sediment assumed a grayish color, which extended beyond the lower end of the sediment cores (about 20 cm).

The depth profiles were very similar among the cores taken from the same sampling site. Also cores from different stations did not differ much with regard to the penetration depth and concentration of the discussed parameters (data not shown).

3.2. *Vertical distribution of denitrifying bacteria*

The numbers of acetate-oxidizing, denitrifying bacteria in the sediment cores 9601 and 9602 (Table 1) and in core 9706 (Fig. 1) did not differ significantly with depth within each core. Among the three cores, the average population densities of denitrifying bacteria within a core ranged from 2.6×10^{6} to 1.1×10^{7} cells (ml sediment) ^{1}, which corresponded to 1.1 4.8×10^{7} cells (g dry mass) ^{1}, when density (1.01 1.16 g cm ^{3}) and porosity (76 84%) of the respective sediment layers were taken into account (details not shown). In contrast, the numbers of denitrifying bacteria in the upper layers (A and B) of core 9901 (Fig. 1) were significantly higher than the numbers of bacteria in the lower layers (C and D) of this core. The average population density was 1.5×10^{8} cells (ml sediment) ^{1} in layers A and B and 6.5×10^{6} cells (ml sediment) ^{1} in layers C and D. Ammonium was not found to be an end product of

Table 1
Depth distribution of acetate-oxidizing, denitrifying bacteria and mixotrophic, ferrous iron-oxidizing, denitrifying bacteria in profundal sediments of Lake Constance

Depth (cm)	Layer[a]	MPN (10^{5} cells ml ^{1})			
		Acetate-oxidizing, denitrifying bacteria		Mixotrophic, ferrous iron-oxidizing, denitrifying bacteria	
		9601	9602	9601	9602
0.5	A	220	37	2.2	5.8
1.5	B_1	58	58	1.0	5.8
4.5	B_2	58	58	5.8	1.0

[a]Layer A represents the zone of oxygen and nitrate reduction, layers B_1 and B_2 represent the upper, more oxidized, and the lower, more reduced sublayers of the iron reduction zone (see Fig. 1 and text).

nitrate reduction in any of the dilution series, indicating the absence of nitrate ammonification.

3.3. Ferrous iron oxidation by denitrifying bacteria

The denitrifying enrichment cultures obtained from the serial dilutions of sediment from the different layers of cores 9601, 9602, and 9706 were tested for their ability to catalyze iron oxidation. Varying proportions (1 58%) of the denitrifying enrichment cultures were able to oxidize ferrous iron in the presence of acetate (Fig. 1, Table 1). The average population density of iron-oxidizing mixotrophs within a core ranged from 3.0×10^5 to 4.2×10^5 cells (ml sediment) ^{1}; the differences in the MPNs obtained for the different sediment layers are generally not statistically significant. However, in core 9706, the number of denitrifying bacteria capable of oxidizing iron lithotrophically were more than one order of magnitude lower than that of the mixotrophs and represented 2 10% of the iron-oxidizing, denitrifying populations in the respective layers (Fig. 1b).

In the case of core 9901, the MPNs of mixotrophic and lithotrophic, iron-oxidizing, denitrifying bacteria did not differ significantly and did not change with depth (Fig. 1c). The average population density of iron-oxidizing denitrifiers within the core was 5.3×10^4 cells (ml sediment) ^{1}, which is slightly lower than the values obtained for the other cores (see above). However, due to the high population density of organotrophic denitrifiers, especially in the upper layers of this core, the proportion of iron oxidizers among the denitrifying bacteria was much lower than in the other cores.

All enrichment cultures obtained from dilution series of denitrifying bacteria in sediment core 9706 were also tested for their ability to oxidize ferrous iron aerobically (Fig. 1b). MPNs of aerobic ferrous iron-oxidizing bacteria in sediment layers A and B, calculated from the patterns of positive tubes, were identical to those of mixotrophic iron-oxidizing denitrifying bacteria. Only in layer C was the number of aerobic iron oxidizers more than one order of magnitude lower than that of anaerobic iron-oxidizing mixotrophs.

Nitrite and oxygen react chemically with Fe(II), which might give rise to false-positive results [29]; therefore all anoxic dilution series were routinely checked for the formation of nitrite during the incubation. Nitrite concentrations were usually below the detection limit of the HPLC system (5 μM) and always below 100 μM. Controls with 100 μM sodium nitrite did not show chemical oxidation of ferrous iron within 4 months of incubation. Tubes were counted positive for nitrate-dependent iron oxidation only if iron oxidation was above the background observed in non-inoculated control tubes (<0.7 mM Fe(III) after 6 10 weeks of incubation), which is probably caused by a slight air penetration through the butyl rubber stoppers.

4. Discussion

The present study demonstrates considerable population sizes of iron-oxidizing, denitrifying bacteria and thus a significant potential for anaerobic, microbial iron oxidation in profundal lake sediments. It is also the first report on the depth distribution of iron-oxidizing denitrifiers in lake sediments.

4.1. Ferrous iron oxidation by denitrifying bacteria

The average population densities of denitrifying bacteria in the different cores were between 2.6×10^6 and 1.5×10^8 cells ml ^{1}; the values are in the same range as those reported for other sediments [30]. The significantly higher number of organotrophic denitrifiers in the upper sediment layers of core 9901 may be attributed to seasonal differences in the input of organic matter, e.g. at the end of the summer stratification period. Since these and other differences between the cores are probably related to the different sampling stations and sampling times, such differences will not be discussed further.

The present study was based on the assumption that the bacteria capable of anaerobic iron oxidation should represent a subset of the total denitrifying population that uses ferrous iron as an alternative electron donor. This assumption was based on the results obtained in previous studies [16,17], which demonstrated that all pure cultures of bacteria described to date that catalyze nitrate-dependent ferrous iron oxidation can also grow organotrophically with nitrate. It is further validated by the observation that direct enrichment for iron-oxidizing denitrifiers leads to MPNs similar (not shown) or even lower (core 9901) than those obtained from enrichments of organotrophic denitrifiers.

Various earlier studies on denitrification in sediments have shown that denitrifying bacteria represent a large fraction of all bacteria present [30 32]. The finding that ferrous iron-oxidizing, denitrifying bacteria make up about 58% of the total denitrifiers counted (this study) therefore indicates a substantial biological potential for microbial nitrate-dependent ferrous iron oxidation.

While the population densities of mixotrophic iron oxidizers in profundal sediments of Lake Constance are similar to those recently reported for various littoral sediments [18], the numbers of bacteria oxidizing iron lithotrophically, i.e. in the absence of acetate as cosubstrate (cores 9706 and 9901), were 1 2 orders of magnitude higher. These results are not necessarily in contradiction: littoral sediments are subject to irregular mechanical mixing, and especially the upper centimeters are disturbed by convection or bioturbation. This exposes the upper sediment layers to oxygen with variable intensity and duration, and ferrous iron should be oxidized aerobically to a far higher extent than anaerobically; part of the ferrous iron may even be oxidized chemically in the water column.

Since this would deprive bacteria that strictly depend on the availability of ferrous iron as their electron donor, the profundal sediments might represent a more favorable habitat for lithotrophic iron oxidizers.

Since nearly all denitrifying bacteria can use molecular oxygen as alternative electron acceptor [34,35], the same would be conceivable also for nitrate-reducing iron oxidizers; this has been documented already for a pure culture [17]. The MPNs obtained for aerobic bacteria capable of ferrous iron oxidation and mixotrophic, iron-oxidizing, denitrifying bacteria among the total denitrifying populations in the upper sediment layers (A and B) of core 9706 were identical (Fig. 1b). This suggests that the two groups may actually represent the same population of facultatively aerobic, iron-oxidizing denitrifiers; however, the presence of additional, strictly aerobic, iron-oxidizing bacteria cannot be excluded.

In any case, the aerobic iron-oxidizing bacteria in our enrichment cultures are clearly different from the well-known aerobic ferrous iron-oxidizing bacteria *Leptothrix ochracea* and *G. ferruginea*, to which aerobic iron oxidation is usually attributed. Apart from the fact that the latter are not known to reduce nitrate, the enrichment cultures obtained in this study did not contain morphotypes that formed conspicuous appendices or filamentous sheaths [6,7]; the cells were morphologically inconspicuous short rods. Also Emerson and Revsbech [10] have reported that in the mat-like aggregations of ferric hydroxides of an iron seep, the major populations of bacteria responsible for aerobic iron oxidation were not *Gallionella* or *Leptothrix* species, but unicellular, morphologically inconspicuous, unidentified bacteria. Emerson and Moyer [11] isolated and characterized several pure cultures of rod-shaped, aerobic, iron-oxidizing bacteria from groundwater samples that were only distantly related to the classical iron oxidizers.

4.2. Sediment characteristics and depth profiles of different iron species

Iron oxidation in profundal sediments depends exclusively on the presence of oxygen or nitrate [16]. Layer A, the region of oxygen and nitrate reduction, is therefore the only sediment layer where the metabolic potential of the denitrifying populations investigated in this study (viz., aerobic and anaerobic iron oxidation) would be of relevance. The penetration of oxygen and nitrate into a sediment is controlled mainly by bioturbation, molecular diffusion, and microbial activities [33,43 45]. In profundal sediments of Lake Constance, bioturbation is not as important as in the littoral zone, where macrozoobenthos has a strong influence on transport processes [44]. Diffusive transport and consumption by microbial activities and chemical oxidation reactions limit the penetration depth of oxygen into the sediment to a few millimeters [44]. The oxic anoxic transition zone of sediments represents a perfect habitat for aerobic microbial iron oxidation where convective mixing is impeded and microbial oxidation of chemically unstable ferrous iron can proceed under conditions of diffusion-limited oxygen supply [33]. Also the availability of nitrate depends on the diffusive supply from the water column and on the nitrate-producing and nitrate-consuming activities of the microbiota.

Therefore, the relatively uniform distribution of all metabolic groups, i.e. chemotrophic, mixotrophic, and lithotrophic denitrifiers, and denitrifiers capable of aerobic iron oxidation, throughout the upper sediment layers (A and B) is puzzling, especially if one considers that in undisturbed sediments, the bacteria in layer B should have been without an electron acceptor already for many years [38].

Due to the presence of acid-stable iron minerals, e.g. pyrite, the acid-extractable iron does not represent the total iron content of the sediment [1]. Nevertheless, the strong increase with depth in the reduction status of the acid-extractable iron in solid phase and pore water indicates the reduction of ferric iron by chemical and biological processes. The high ferrous iron content of the sediment, together with the ferrous iron constantly formed by iron-reducing bacteria, represents a large pool of substrates for iron-oxidizing processes.

Although iron is rather reactive, it is present in sediments nearly exclusively in a solid state and does not participate directly in diffusive transport processes. Translocations of redox equivalents and redox transitions are probably restricted to dissolved ferrous iron species as free iron ions and chelated iron forms, or to organic compounds such as fulvic or humic acids, which may act as mediators or as complexing agents [36,37]. Since dissolved ferrous iron species are much easier to access than insoluble iron minerals, they probably represent the major electron donor in microbially catalyzed ferrous iron oxidation and may also mediate electron fluxes from the solid phase. The iron concentrations in the pore water (10 80 μM) were slightly higher than the concentrations expected on the basis of the solubility of the iron ion in the presence of hydroxyl and carbonate ions (2 5 μM [39]). This may be explained by the chelating effect of soluble organic matter, especially of phenolic substances in the pore water, or by the presence of colloidal iron hydroxides.

4.3. Conclusions

In view of previous results, it becomes apparent that nitrate-dependent anaerobic ferrous iron oxidation is not a unique phenomenon, but seems to occur widely in different natural sediments [16 18]. Considering the importance of microbial iron reduction [3,5,40] and the recently described microbial reduction of humic acids by iron-reducing [41] and fermenting bacteria [42], which can act as mediators in the chemical reduction of ferric iron, the reoxidation of ferrous iron with oxygen and nitrate

emerges as an important link in the electron flow from organic matter to oxygen in natural sediments.

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