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Chapter 18

Microbial Metabolism of Iron Species in Freshwater Lake Sediments

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18.1 Introduction

Sediments develop by sedimentation of organic and inorganic residues of primary and secondary production as well as by inorganic precipitates, e.g., metal hydroxides, carbonates, silicates, and phosphates. The accumulation of this material at the bottom of freshwater lakes leads to an intensification of mainly microbial degradative activities which oxidise and transform the organic freight with concomitant reduction of oxygen and other electron acceptors. It is the activity of micro-organisms, especially of bacteria, which leads to the reduction of available electron acceptors, to an accumulation of reduced derivatives, and with that to changes of the redox potential in such sediments.

The basic processes involved in the degradation of organic matter by such microbial communities are known for a long time. As long as molecular oxygen is available it acts as the preferred electron acceptor, followed by nitrate, manganese(IV) oxide, iron(III) hydroxides, sulfate, and finally CO_2 with the release of nitrite, ammonia, dinitrogen, manganese(II) and iron(II) carbonates, sulfides, and finally methane as products of microbial reductive activities (STUMM & MORGAN,

1981). These preferences for the various acceptor systems are mainly determined by the redox potential and the availability of the redox systems under consideration, with the most positive ones at the beginning and the lower ones to the end, according to the scheme depicted in Table 18.1.

Tab. 18.1: Preferred redox potential ranges for the dominant microbial redox transformations in a freshwater lake sediment. After ZEHNDER & STUMM (1988) and numerous other sources.

Redox process	*Redox potential* [V]
Nitrate reduction	0.5 to 0.2
Manganese(IV) reduction	0.4 to 0.2
Iron(III) reduction	0.2 to 0.0
Sulfate reduction	0 to -0.15
CO_2 reduction	-0.15 to -0.22

Reduction of these electron acceptors with electrons from organic matter (average redox potential of glucose $\rightarrow$ 6 CO_2: -0.434 V; calculated using data from THAUER et al., 1977) provides metabolic energy in the mentioned sequence, and this sequence of preference is also translated via diffusive transport of the dissolved electron carrier systems into a spatial order in the sediment from the top to the bottom, with oxygen respiration at the sediment surface and methanogenesis in the deepest layers. The organic matter is to some extent oxidised to CO_2 but is also transformed into polymeric derivatives, similar to humic material in terrestrial ecosystems. These humic compounds (fulvic acids, "Gelbstoffe") are the dominant fraction of organic matter in lake water and persist in the sediment as the most important organic fraction. They contain aromatic and aliphatic residues from all chemical constituents of biomass, e.g. phenolic compounds, proteins, carbohydrates, fats, and even macromolecular cell structures as huge as entire murein sacculi. The longer this digestion process proceeds the less amenable this material will be to biochemical oxidation. Recent work in our lab has shown that proteinaceous humic constituents are degraded faster than others, shifting the total composition of the remnant material to a more aromatic character (KAPPLER et al., in prep.). Finally, hydroquinone as a model component for humic material releases electrons at an average redox potential of -0.328 V during its oxidation to CO_2 (glucose at -0.434 V, see above), indicating that conversion of such material to, e.g., methane and CO_2 ($E^{\circ\prime}$= -0.24 V), approaches not only kinetic but also energetic limitations.

The spatial sequence of redox processes in sediments on the basis of these considerations is preserved only in low-lying profundal sediments at water depths below 20 m; littoral sediments or sediments of shallow lakes are less stable and are subject to irregular mixing by wave action or bioturbation.

The redox potential measurable, e.g. by a platinum electrode, is determined mainly by reactive sediment constituents such as O_2, Fe^{2+} or Mn^{2+} ions, or hydrogen sulfide. Other carriers of low reactivity such as NH_4^+, iron hydroxides or carbonates, MnO_2, sulfate or methane do not directly influence the measurement of the redox potential. However, they do so indirectly in the presence of suitable catalysts, e.g. micro-organisms that are metabolically active.

The microbial activities involved in these redox processes are well-known as far as dissolved electron carrier systems are concerned, because organisms dealing with such carriers are easy to cultivate in the laboratory. Far less is known about the transformation of substantially insoluble electron acceptors such as manganese or iron compounds, due to their low solubility and difficulties in handling in the laboratory. In the present communication, we want to concentrate on new information on microbial activities involved in the transformation of iron compounds in lake sediments, and their possible impact on measuring the redox potential.

18.2 Iron Compounds in Lake Constance Sediments

Since iron(III) hydroxides and -oxihydroxides displaying extremely low solubility the free water column of oxygenated lake water contains iron mainly as constituents of living organisms or in complexes of biological origin, and after degradation of the organic residues iron precipitates as iron(III)hydroxides, -oxihydroxides, carbonates, silicates, or phosphate. These compounds accumulate in the sediments to high concentrations, averaging at around 1 to 5 % of the sediment dry matter (20 to 100 mM), depending on the type of lake and the chemistry of its catchment area. With these high concentrations, iron(III) is a very important electron acceptor in the sediment, compared to oxygen (around 0.3 mM), nitrate (<0.1 mM), or sulfate (around 0.2 mM in many freshwater lakes). Reduction of ferric iron hydroxides etc. leads to release of Fe^{2+} ions and the formation of ferrous carbonate (siderite), depending on the activity of bicarbonate and carbonate. The Fe^{2+} ion can diffuse through the sediment and may transfer electrons to other oxidised ferric iron or manganese minerals; they are also a redox-active species that contributes to the redox potential measured by the platinum electrode. However, their concentration is usually low and limited by the solubility of siderite. Besides the nano-particle movement, the substantially insoluble iron minerals are immobile and do not interfere with diffuse transport processes.

The distribution of Fe^{2+} in the pore water, and HCl-soluble (1 M) Fe(II) and Fe(III) in a profundal sediment core from Lake Constance is shown in Figure 18.1a, in comparison to an oxygen profile in Figure 18.1b. Oxygen profiles were measured with micro-electrodes; iron was quantified with the ferrozine method in extracts of slices of the sediment core obtained under a dinitrogen gas atmosphere before and after HCl (1 M) extraction and reduction with hydroxylamine. Oxygen is depleted by microbial reduction in the upper 5 cm of the sediment, with maximal depletion activity (highest slope) at 4 to 5 cm depth. Dissolved Fe^{2+} ions reach a concentration maximum (0.04 mM) at 4 to 7 cm depth, decreasing upwards as a result of chemical or microbial reoxidation, and down-

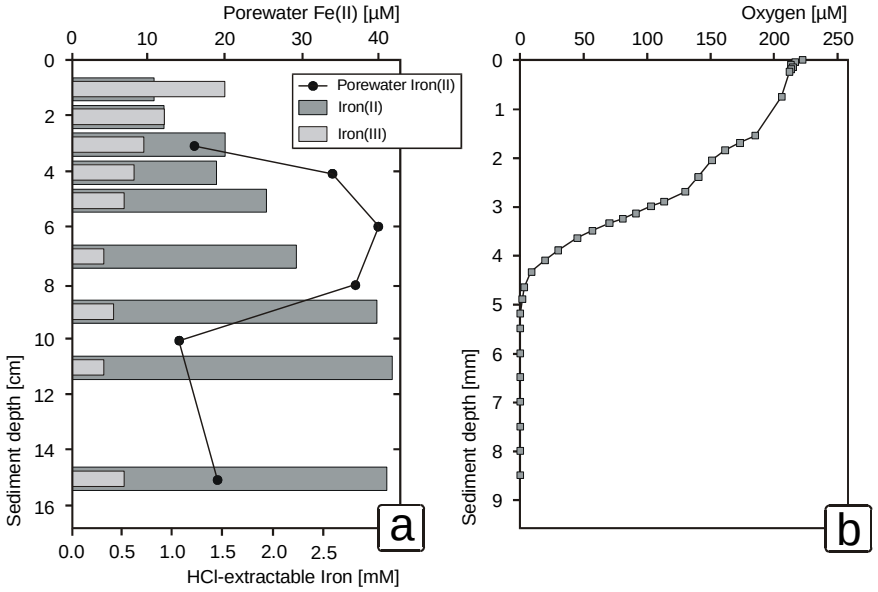

Fig. 18.1: Profiles of distribution patterns of iron species (a) and oxygen (b) in a sediment core taken July 17, 1996 in Lake Constance, Überlinger See, off the shore of Wallhausen, at 130 m water depth.

wards probably due to precipitation. In the non-water-soluble, HCl-extractable fractions, iron(III) dominated in the upper sediment layers whereas the reduced forms became dominant at depths lower than 4 cm. Nonetheless, there was always a small fraction of oxidised iron present even in lower sediment layers, indicating that this iron(III) was not accessible to microbial reduction. Comparison with similar profiles from more acidic fresh water lake sediments could provide information on whether this limited efficiency of iron reduction is due to competition against siderite formation. The increase of the total HCl-extractable iron content with depth from 2.0 to 3.5 mg/g may be a consequence of calcite dissolution and decomposition of the organic matter.

18.3
Microbial Oxidation of Iron Compounds

Aerobic bacterial oxidation of iron(II) is a well-known phenomenon and is catalysed in acidic environments by *Thiobacillus*-like bacteria, at neutral pH by morphologically conspicuous, filamentous bacterial forms such as *Gallionella* or *Leptothrix* species (SCHLEGEL, 1994). The latter form fluffy or slimy structures in springs and surface waters with high input of Fe^{2+}, and these structures are sup-

posed to prevent convective water mixing and, consequently, prevent chemical oxidation of iron(II) by oxygen-saturated water.

Only recently was it discovered that iron(II) could also be oxidised also by anaerobic processes, e.g. by the action of anoxygenic phototrophic bacteria (WIDDEL et al., 1993; EHRENREICH & WIDDEL, 1994; HEISING & SCHINK, 1998) or by nitrate-reducing bacteria (STRAUB et al., 1997; BENZ et al., 1998a). The former process appears not to be of major importance in lakewater sediments, not even in littoral zones, because only small numbers of iron-oxidising phototrophs were found in such sediments (HEISING & SCHINK, unpubl.). Nitrate-dependent iron oxidation, on the other hand, appears to play a major role in iron oxidation in oxygen-deficient sediments: iron-oxidising nitrate reducers were counted in high numbers in the surface sediments of Lake Constance (BENZ et al., 1998a) and at least in some cases we could show that there were clear indications of ferric iron formation in oxygen-free sediment layers where nitrate was the only available electron sink (BENZ & SCHINK, unpubl.). These nitrate-dependent ferrous iron oxidisers isolated from numerous dilution series were also able to oxidise ferrous iron in diffusion-controlled gradient cultures with oxygen as electron acceptor. Since the spatial resolution of chemical analysis of iron species in the sediment gradient systems is insufficient for the time being, we cannot definitively decide whether the dominant function of these bacteria in the sediment is oxygen- or nitrate-dependent iron oxidation. Nonetheless, the profiles given in Figure 18.1a and b clearly indicate that iron(III) dominates to a large extent in the upper sediment layers that are entirely depleted of oxygen.

These bacteria were inconspicuous in shape, simple, short and rod-shaped, not forming any kind of unusual hypercellular structures. Since the sediment provides a structured environment impeding convective mixing of water from oxygen-containing and from lower layers rich in reduced iron, such aerobically iron-oxidising bacteria do not need to structure their environment themselves if they inhabit the sediment at the transition zone between oxygen-supplied and reduced layers. Perhaps these recently discovered iron oxidisers are far more important for ferrous iron oxidation on a global scale than the well-known and well-described *Gallionella*, *Sphaerotilus*, and *Leptothrix* species.

18.4 Reduction of Ferric Iron Hydroxides

Reduction of ferric to ferrous iron is chemically easy; the difficulty in microbial iron reduction is the extremely low solubility of ferric iron hydroxides. Ferric iron cannot be taken up by bacteria as such; aerobic bacteria have developed very refined and specific complexing agents (siderophores) to secure their iron supply for assimilatory purposes (NEIDHARDT, 1989). Such extremely energy-consuming systems cannot be applied by bacteria which use ferric iron as an electron acceptor in their energy metabolism. Delivery of electrons to insoluble ferric iron minerals would require immediate attachment of the iron-reducing bacterial cell to the mineral surface, but electron transfer between two non-dissolved particles becomes difficult even over extremely short distances. It was suggested recently that

humic compounds could act as electron carriers between iron-reducing bacteria and insoluble iron minerals (LOVLEY et al., 1996). This concept is worth to be examined in more detail because humic compounds are actually present in sediments at comparably high concentrations (up to 1 % of total dry matter) and redox reactions between humic compounds and ferric iron minerals have been described repeatedly in the literature (SZILAGYI, 1970). Moreover, we could show recently that several fermenting bacteria can also use humic acids as electron acceptors (BENZ et al., 1998b), and electron transfer through humic compounds to iron(III)-oxides may therefore be a rather widespread type of respiratory activity not confined to typical iron-reducing bacteria.

We demonstrated recently that the iron-reducing bacterium *Geobacter sulfurreducens* excretes during growth significant amounts of a soluble c-type cytochrome into the growth medium. This cytochrome has a standard redox potential at -0.167 V and reduces iron(III)-hydroxide at very high rate. Thus, it acted as an extracellular iron(III)-hydroxide reductase and transferred electrons by diffusion over limited distances (SEELIGER et al., 1998). The same cytochrome could also mediate electron transfer to manganese(IV)oxide and to partner bacteria. Calculations of diffusion kinetics, actual cytochrome concentrations in growing cultures and growth rates gave reasonable evidence that this cytochrome contributed to a significant part to the iron reduction activity of growing cultures in the laboratory, and should do so *in situ*, in sediments and hydromorphic soils as well. Although this has not yet been tested, it appears reasonable to argue that such extracellular cytochromes would also react with platinum electrodes and would therefore help to characterise redox potentials of microbial communities in sediments to such monitoring devices.

18.5 Conclusions

This contribution concentrated on the transformation of iron compounds by new, so far unknown microbial activities. By mass, iron minerals represent the most important electron carrier system in most freshwater sediments. Due to their low solubility they do not interfere directly with the redox potential measured with the platinum electrode but do so only indirectly through Fe^{2+} which is present in the Fe(III)/Fe(II) redox transition zone at maximal albeit low concentrations (in the micromolar range). Humic compounds and extracellular cytochromes of certain iron-reducing bacteria may contribute further to the transfer of electrons between iron minerals, iron-metabolising bacteria, and platinum electrodes as monitoring devices. Oxidation of ferrous iron in freshwater sediments may be catalysed to a large extent in the oxygen-free layer of sediments with nitrate as oxidant by nitrate-reducing bacteria which have been described only recently and are able to use also oxygen as electron acceptor.

18.6 Acknowledgements

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