Microbially Mediated Processes in Environmental Chemistry

(Lake Sediments as Model Systems)

Kurt W. Hanselmann*

Microbes alter environmental conditions through their metabolic activities which leads to changes in chemical equilibria. Thus microbes affect indirectly chemical processes like precipitation and dissolution, adsorption and desorption, oxidation and reduction, protonation and dissociation. Chemical compounds which can serve the microbes' needs for nutrients and energy are the primary prerequisite for microbial action per se and many geochemical events are influenced by concentration changes of metabolic substrates and products. – Sediments of eutrophic hardwater lakes offer ecosystems well-suited for microbial investigations. The short supply of oxidants and the almost unlimited organic «reducing power» cause a wealth of microbially mediated reactions and corresponding catalytic capabilities in numerous genera of bacteria. Understanding their interactions with each other and with a seasonally changing environment is a prerequisite to evaluate their role as mediators of geochemical reactions. Results from investigations of this kind have direct practical and theoretical implications for todays lake restoration projects. – In order to assess microbially mediated catalysis within sediments as a driving mechanism for the cycling of elements through their many specialized metabolic pathways, we need to better understand: The microbial habitats within sediments; the kind of microbes present and their interaction with each other and their surroundings; the influence of thermodynamic constraints on microbial existence; the microbial behavior towards transient environmental conditions; and the efficiency of microbes as biological diffusion barriers or diffusion promotors at the sediment-water interface. – In this presentation I will summarize our views on a few basic questions of environmental microbiology: Where do microbes live in sediments? How do they live where they are? What do they do to the environment? It should soon become clear to the reader that we feel the best approach to understanding biochemical processes in lake sediments must be through interdisciplinary studies involving microbiologists, chemists, and geologists.

1. The Role Microbes Play in the Environment

Microbes participate in the cycling of elements in the biosphere. Through their enzymes and the great variety of metabolic abilities they catalyze bio-chemical reactions which lead to the assimilation into biomass, to immobilization, and also to the release of molecules. In sedimentary, aquatic environments microbial substrates and products are either mobile within the interstitial water body and can thus be exchanged between microbial populations, or they get trapped in or on various solid sediment phases by adsorption, chelation, or precipitation processes. On a macroscale the dissolved molecules might be transported by diffusional fluxes or by biologically introduced turbations from the sediment into other lake compartments. Under certain conditions, however, they might be scavenged by other microbial populations at the sediment-water boundary zone and thus be cycled within sediment compartments only. Microbial activities are important for the recycling of bioelements and essential in initiating many of the chemical processes mentioned above as well as in controlling their kinetics in nature.

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Kurt Hanselmann: Born 1943 in Thal, SG. Educated as a secondary school teacher for mathematics and natural sciences, he taught in a Swiss mountain village for 3 years during which time he himself learned how to make science understandable and exciting to students and laymen (he still considers the communication of scientific discoveries to laymen to be an important part of his work today). In 1967 he began studying plant biology at the Universität Zürich and applied microbiology at the Eidgenössische Materialprüfungs- und Versuchsanstalt (EMPA). In 1970 he moved to the Worcester Foundation for Experimental Biology in Massachussetts (USA) where he studied cellular biochemistry and microbial energetics for 3 years. He holds a Ph.D. in microbiology from the Universität Zürich. After his graduation he was involved in training secondary school teachers and medical students and was an instructor in pre- and postgraduate courses in microbiology, plant biology, and biochemical energetics at the Universität and the Eidgenössische Technische Hochschule (ETH) Zürich. He was introduced to microbial ecology and biogeochemistry in Woods Hole in 1977 by Holger Jannasch. This experience and contacts with Helge Larsen, Jane Gibson, Eduard Leadbetter, and Robert Hungate greatly influenced his later activities. In 1980 he began to develop a research program on microbial ecology and environmental microbiology with Prof. Reinhard Bachofen at the Universität Zürich. With diploma- and Ph.D. students he presently works on microbial processes in redox transients in aquatic ecosystems and on the degradation of aromatic chemicals by microbial communities under anoxic conditions. In 1982, as a participant on a deep sea mission in the North Atlantic with the Dutch Institute for Sea Research he began an investigation on the suitability of deep sea sediments as sites for the deposition of radioactive wastes. While serving in the army in the region of alpine meromictic lakes he became aware of the usefulness of these lake ecosystems as models for the study of microbial processes in redox transition zones. In collaboration with Prof. Jacques Piccard he and his students are also investigating microbial habitats in sediments of eutrophic lakes with the aid of a small research submarine. He teaches Microbial Ecology at the Universität Zürich and is a lecturer for environmental biotechnology at the Department of Technology and Natural Sciences of the Hochschule für Wirtschafts- und Sozialwissenschaften in St. Gallen. – In his article for CHIMIA he emphazises a few research approaches in environmental microbiology with which he hopes to initiate an interest in collaborative work among those scientists from different fields in academia, industry, and government who are convinced of the need to investigate basic questions in the environmental sciences today.

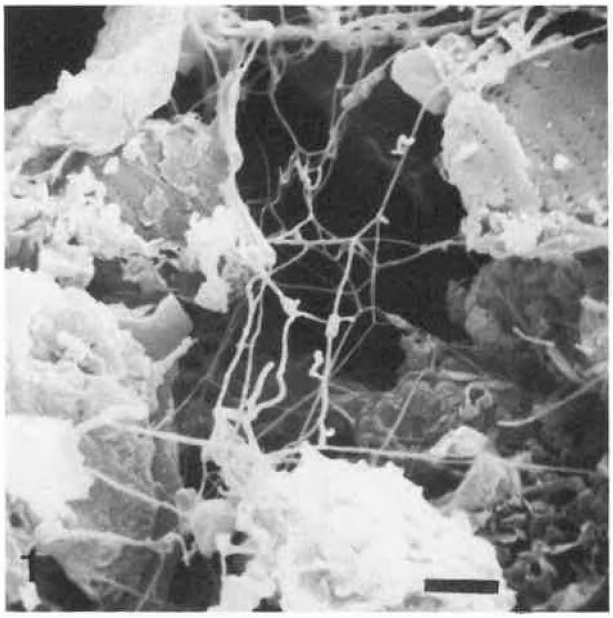

Fig. 1. Allochthonous and autochthonous particles «glued» together by bacterial slimes and adhesion threads in aggregates from Lake Zürich sediments (136 m). Bar = 50 µm.

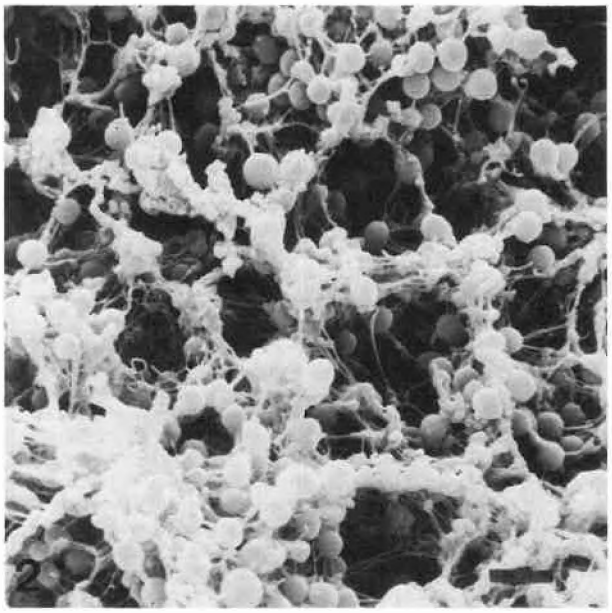

Fig. 2. Enriched growth of bacteria with adhesion threads within sediment aggregates after short perfusion with carbon- and energy-source (glucose) and incubation at 30°C. Bar = 1 µm.

2. Sediment Aggregates as Microbial Habitats

In sediment samples only a fraction of the indigenous bacteria can be obtained from the interstitial water; most of them appear to live firmly attached to particles within sediment aggregates. Even during sedimentation, organic and inorganic debris form aggregates which stabilize and probably remain intact in sedimentary deposits for a number of years. Aggregates seem to be most stable in sediment layers up to 10 cm below the surface[4]. They are mechanically stable and can only be disintegrated by sonication and by oxidation of the organic matrix with oxidants like H_2O_2. Reaggregation of sonicated aggregates does not occur spontaneously, indicating that the «glue» is a product of microbial activity. Aggregates are an irregular conglomerate of diatome skeletons, calcite crystals, clay platelets, and organic detritus held together by bacterial adhesion threads (Fig. 1). Bacteria are present in patches attached to certain surfaces only. They cannot be removed easily. Perfusion of these aggregates with nutrients leads to rapid enrichment of attached bacteria (Fig. 2).

Bacteria also attach to artificial surfaces which are incubated in sediments in situ (Fig. 3). Since these surfaces can be defined more easily, their use may contribute to the understanding of the chemical basis of microbial attachment mechanisms in the sediment environment. Many surfaces tested are first coated with patches of organic detritus, of metal oxides, oxide-hydroxides, and carbonates (Fig. 4) in the aerobic zones or with metal sulfides or carbonates, and other precipitates in the anaerobic regions before bacteria attach. Under favourable conditions the bacteria multiply and colonize the surfaces irregularly. We must envision the great variety of microbes found in sediment aggregates as a consequence of the multitude of surfaces and surface coatings with different reactivities within each microhabitat.

Observations indicate that sedimenting particles scavenge and transport numerous organic and inorganic molecules. These molecules form surface coatings which can serve as concentrated nutrient sources for bacterial growth. The bacteria produce stalks, slimes, and adhesion threads which tend to «glue» together even more particles leading to aggregate formation. These aggregates become microenvironments in which specialized chemical reactions will proceed. We also envision these aggregates as the sites within sediments where the more hydrophobic environmentally harmful chemicals adsorb, accumulate, and eventually get degraded by microbial enzymes. Microbial activity in sediments, therefore, is highest in surface films and flocs within the aggregates. For the catalysis of those processes which depend on syntrophic interactions, close spatial contact between the bacteria within these microstructures might be advantageous since it allows otherwise endergonic partial reactions to become exergonic overall-reactions through the rapid consumption of intermediate products. An example is *Syntrophobacter wolinii* which degrades propionate only if accompanied by a hydrogen scavenging methanogen or sulfate-reducer[1].

The aggregates probably disintegrate if certain nutrients, the oxidant, or the energy source become limiting below a certain sediment depth. In these layers bacteria might still be present in dormant stages and eventually will be buried along with those organic substances that were undegradable. Active microbial catalysis in the sediments is limited to the top layers which can support metabolism and growth, and aggregates are temporary habitats in these zones of high microbial activity. They are structurally and functionally comparable to similar structures present in soils. Detailed knowledge about the structures of bacterial habitats teaches us to consider in our experiments and interpretations the heterogeneous distribution of microbes within their microenvironment.

3. Appearance of the Macroenvironment

Having introduced the microsites which are inhabited by microbes I now turn to the question of how their activities express themselves macroscopically. The visual ap-

pearance of sediment surfaces is often a clear indication of processes going on in the underlying deposits as well as in the water above. This is a consequence of biochemical processes which are mainly controlled by the oxygen supply and the seasonal productivity in the water above and by the prevailing biologically catalyzed processes in the sediments underneath. A few examples might serve to demonstrate this contention. Under conditions of unlimited oxygen supply, reduced sulfur compounds will be oxidized along with the oxidation of the biomass. The sediment surface will appear brown to grey, indicating oxic sediments poor in organic matter. White surface veils which can be found sometimes in gigantic mat developments are an enrichment of predominantly *Beggiatoa spp.* (Fig. 5). *Beggiatoa spp.* belong to the colorless sulfur bacteria which oxidize sulfide to elemental sulfur intracellularly under microaerophilic conditions[16]. These bacteria develop under conditions where the H_2S produced in a metal-limited environment is allowed to diffuse to a sediment surface existing in a low oxygen environment.

Black sediment surfaces indicate strong reducing conditions at the interface. Patchiness at the sediment surface is an indication of local heterogeneities, e.g. white Beggiatoa-mats can be seen within seemingly homogeneous metal oxide and hydroxide covers. It also indicates that lake deposits are not as uniformly laid down by the regular rain of sedimenting particles as has traditionally been envisioned. Conversely Beggiatoa-veils are irregularly broken up by patches of «naked», black sediment areas (Fig. 5).

The pillow-like formations which we have been able to observe with the aid of a small submarine in Lake Geneva are a morphological surface structure that extends over many square kilometers in the deeper basin of this lake (Fig. 6). It is interesting to note that microbiologically mediated oxidation reactions differ between areas on the crest of the pillows and areas in the trenches between them[2].

Appreciating sediment heterogeneities permits one to make more sensible conclusions on the kinds and the importance of microbially mediated chemical reactions at different sediment-water interfaces and helps one to avoid making generalizations too early.

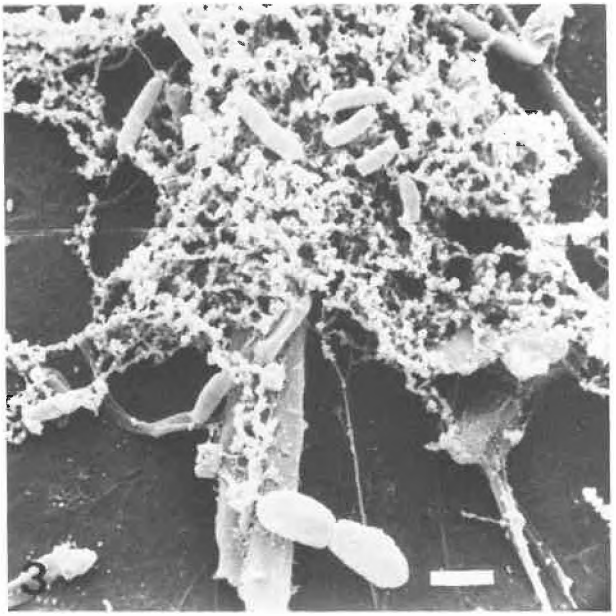

Fig. 3. Bacteria attached to teflon surface immersed for 20 days at the sediment-water interface in Lake Zürich (136 m, December 1983). Bar = 2 μm.

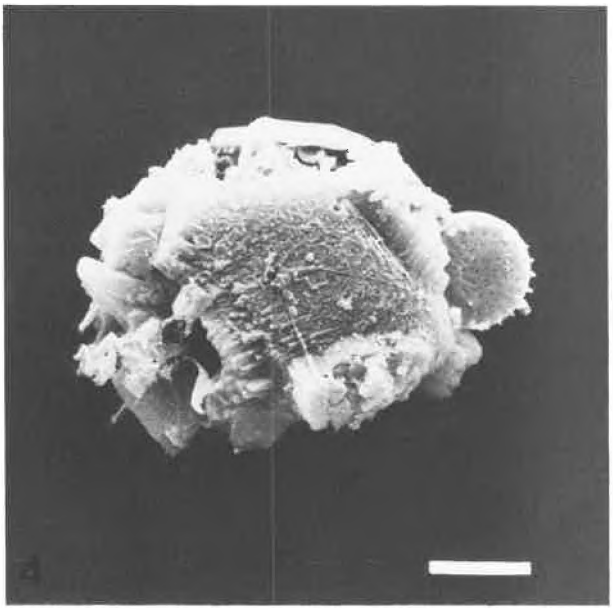

Fig. 4. Calcite crystal formed around diatome as a result of biogenic calcite precipitation in the photic zone of the epilimnion. Bar = 5 μm.

4. Interactions in the Macroenvironment

Microbial activity in sediments is governed by the supply of reductants, oxidants, and nutrients in an environment favourable to life (Fig. 7). Reductants and nutrients are supplied in the form of dead biomass in which most of the chemical elements are in a reduced state (Fig. 7:1). In the course of transformation some of the nutrients are re-assimilated into new bacterial cells, others are released into the water and into deeper sediment strata. More nutrients in the water lead to new biomass. The microbes extract energy for maintenance and growth predominantly from the oxidation of carbon compounds. Those molecules that are not oxidized completely and those for whose mineralization microbial enzymes are lacking get deposited and will later be conserved in geological formations (Fig. 7:3). Oxidants are replenished in gaseous, dissolved, or solid form through the water column (Fig. 7:4). They originate from erosion in the catchment area (Fig. 7:5) and may be cycled several times through endogenic biological and chemical processes (Fig. 7:6). In sediments with carbon excess all available oxidants are used by various microbial populations (Fig. 7:7). Mineralization always leads to the formation of «inorganic carbon» (Fig. 7:8) which exchanges with the overlying water and the deeper sediment strata where it may lead to the precipitation of carbonates (Fig. 7:2). Iron and manganese sulfides and metal carbonates form an electron sink within the sediment ecosystem. Other reduced end products (Fig. 7:9) diffuse into oxygen-rich waters where they are reoxidized (Fig. 7:10) so that the electrons once captured from water by photosynthesis will now return to the oxygen of the water molecule after having been involved in several microbial metabolic reactions.

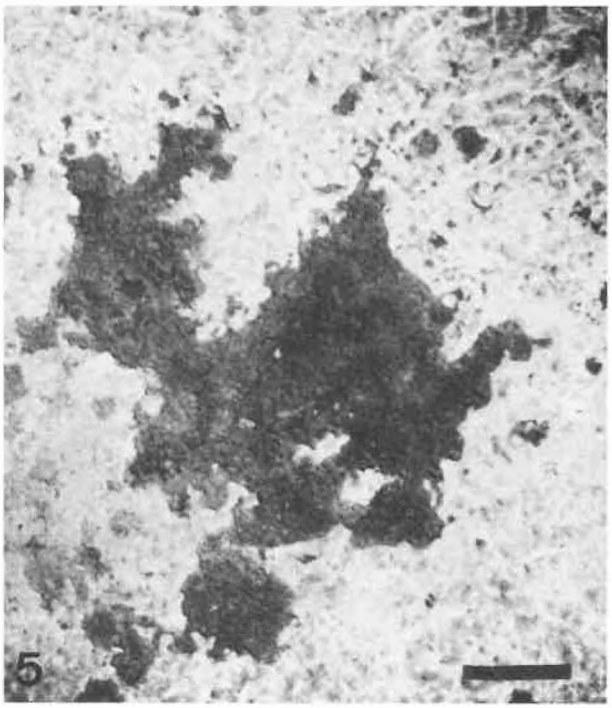

Fig. 5. Heterogeneity within Beggiatoa-mat. Continuous white veils are irregularly broken up by patches of sediment rich in metal sulfides (Zugersee, 35 m, December 1983). Bar = 10 cm.

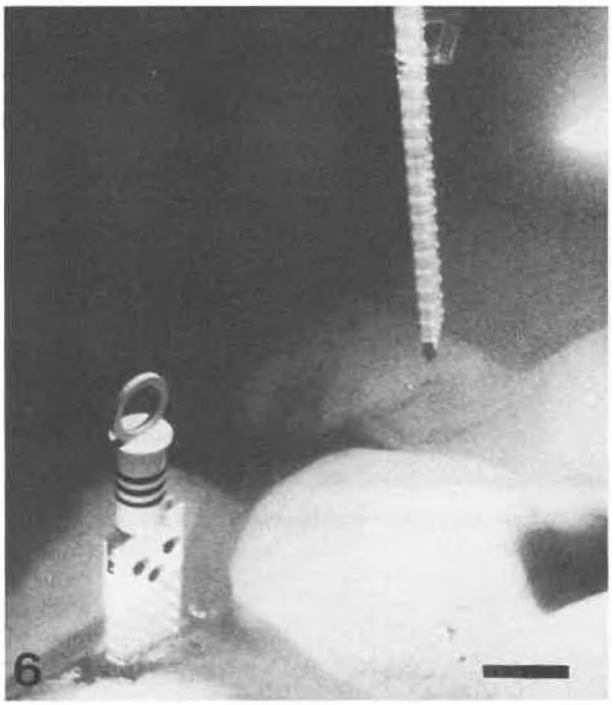

Fig. 6. Pillow-like structures in the deeper regions of Lake Geneva. Equilibrium diffusion plates are positioned in the trenches and on top of the pillows by the hydroelectric manipulator of the submarine F. A. Forel (240 m, at Lausanne-Ouchy). Bar = 10 cm.

5. Spacial and Temporal Transients due to Oxidant Limitation

Through metabolic activities microbes alter both, the oxidation-reduction and the acid-base conditions of their environment. This is most clearly presented, if one formulates a general mineralization reaction as

$$\langle HCHO \rangle + H_2O \rightarrow CO_2 + 4\,H^{\oplus} + 4\,e^{\ominus}$$

which intends to stoichiometrically link proton release during electron cycling to the oxidation of organic matter. In the following section the consequences of this observation are discussed.

Microbial activity in sediments of eutrophic lakes at depths that cannot be reached by light may be limited by two factors: a multitude of substrates are inaccessible to enzymatic conversion; and available oxidants necessary to dispose of the electrons that could be set free during the oxidation of organic matter are limited. In the ocean oxygen and sulfate are the dominating oxidants. In most eutrophic lakes, however, the large amount of organic material to be oxidized requires that several biologically accessible oxidants be employed. Some of the more common oxidants employed by microbes and the reduction products are listed in Table 1. The electron accepting processes are written as electrochemical half-cell reactions. They can thus be combined with any electron donating half-cell reaction listed in Tables 2 and 3.

As a consequence of this veritable menu of oxidants in limnic sediments we normally find microbes belonging to a variety of taxonomic groups and having many different catalytic abilities. Lake sediments therefore present an excellent teaching tool and a model ecosystem well-suited for microbial research. Electrons from H_2O can be extracted by oxygenic phototrophic organisms exclusively (Table 3, reaction 26). Nitrifying bacteria like *Nitrosomonas spp.* and *Nitrobacter spp.* will couple reactions 27–29 to oxygen reduction. Manganese oxidizing bacteria (reactions 30–32) are represented by several aerobic genera – *Caulococcus, Hyphomicrobium, Metallogenium, Pedomicrobium, Leptothrix, Flavobacterium, Siderocapsa, Aeromonas, Nocardia, Micrococcus, Pseudomonas* – and probably some denitrifying anaerobes. Many of these are indigenous to lake sediments. Iron oxidation (reaction 33) can be mediated by members of the genera *Siderocapsa, Leptothrix, Gallionella, Planktomyces, Metallogenium, Hyphomicrobium, Siderococcus*, and others among the aerobes. It is to be expected that anaerobic acetogens, sulfur and sulfate reducers, methanogens, fumarate reducers, denitrifyers, and manganese reducers also possess the potential for ferrous iron oxidation. Sulfide and several other compounds containing reduced sulfur are oxidized aerobically by species of the *Thiobacilli, Thiomicrospira, Beggiatoa, Thioploca*, and others, and anaerobically mostly by members of the phototrophic families *Chromatiaceae* and *Chlorobiaceae* in the light and by *Thiobacillus denitrificans* in the dark (reactions 34–37). Methane oxidation (reaction 38) is carried out aerobically by members of the methylotrophic genera *Methylococcus, Methylomonas*, and *Methylosinus* among others. It is thermodynamically possible that sulfate, fumarate, manganese, and nitrate could also serve as oxidants for methane. Little is known however about the microbes involved in these oxidations. Hydrogen oxidizing capacity (reaction 39) is found in numerous genera of aerobic and anaerobic bacteria since it can be oxidized by most of the oxidants listed in Table 1.

The sequence in which each oxidant is used follows the rule of metabolic energy efficiency. Less efficient reactions are carried out as soon as the better oxidants are

depleted. Of those oxidants which are fed to sediments from the hypolimnetic water by diffusion, the most efficient ones are always used at the sediment surface. The oxidant gradients created in this way indicate the approximate location of particular oxidation processes. In the example given in Fig. 8 for the top of the pillow-like structure in Lake Geneva, denitrification stops directly below the sediment surface; below 5 cm sulfate reduction ceased, allowing the methanogens to be most active in deeper layers[2]. In the trenches the sulfate diffuses as deep as 25 cm indicating that oxygen and nitrate – the preferentially used oxidants – do not become limiting as rapidly as in the sediments on top of the pillows. Although both locations are equally supplied with sulfate from the water column the amount of reductant seems to be lower in the trenches. The shapes of the methane profiles can be interpreted in two ways. Either sulfate reducing organisms inhibit methanogenesis, or the methane which is produced in deeper layers diffuses into the sulfate reduction zone where it is oxidized by the sulfate reducers.

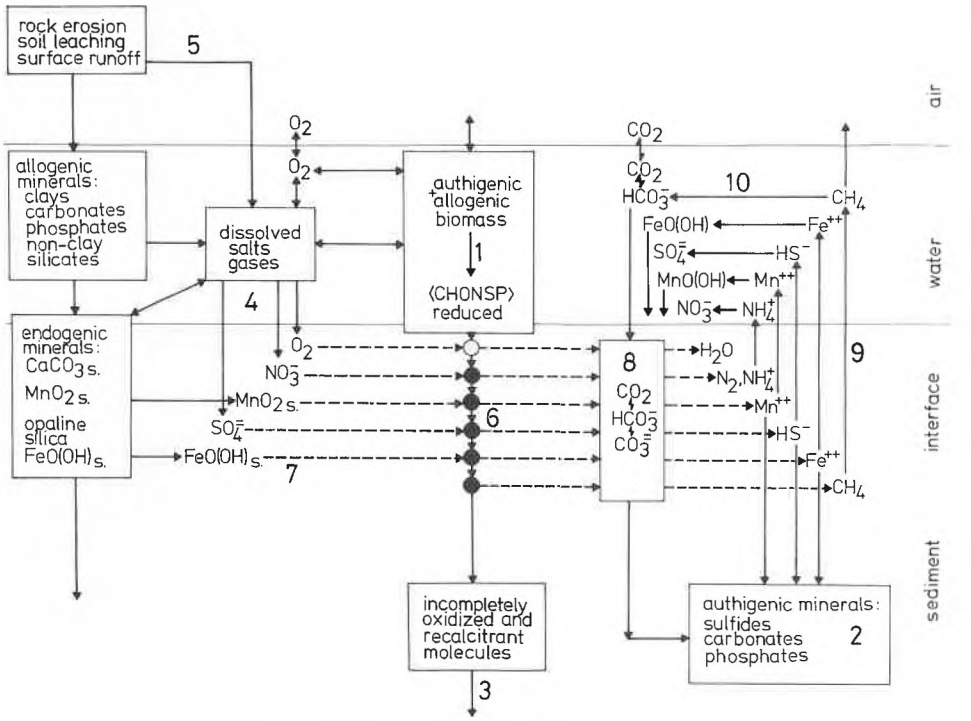

Fig. 7. Coupling of biological and geochemical processes at the sediment-water interface. The numbered reactions are explained in the text.

Table 1. Microbially mediated electron-acceptor reactions.

No.	Electron-acceptor	Oxidation state of electron-acceptor	Reduction reaction	Δ n [a]	Δ H⊕ [b]	Δ C_T [c]	Δ ANC [d]	Δ G^0 [kJ·mol^{-1}] [e]	Δ $G^{0'}$ [kJ·mol^{-1}] [f]	$E^{0'}$ [V] [g]
1	O	0	$4e^\ominus + O_{2(g)} + 4H^\oplus \rightarrow 2H_2O$	−4	−4	0	+4	−474.36	−314.6	−0.815
2	N	+V	$5e^\ominus + NO_3^\ominus + 6H^\oplus \rightarrow \frac{1}{2}N_{2(g)} + 3H_2O$	−5	−6	0	+6	−600.20	−360.56	−0.747
3		+III	$3e^\ominus + NO_2^\ominus + 4H^\oplus \rightarrow \frac{1}{2}N_{2(g)} + 2H_2O$	−3	−4	0	+3	−442.14	−282.38	−0.976
4		+V	$2e^\ominus + NO_3^\ominus + 2H^\oplus \rightarrow NO_2^\ominus + H_2O$	−2	−2	0	+3	−158.06	−78.18	−0.405
5		+V	$8e^\ominus + NO_3^\ominus + 10H^\oplus \rightarrow NH_4^\oplus + 3H_2O$	−8	−10	0	+10	−679.57	−280.17	−0.363
6	Mn	+III	$1e^\ominus + MnO(OH)_{(s)} + 3H^\oplus \rightarrow Mn^{2\oplus} + 2H_2O$	−1	−3	0	+3	−144.69	−24.87	−0.258
7		+IV	$2e^\ominus + MnO_{2(s)} + 4H^\oplus \rightarrow Mn^{2\oplus} + 2H_2O$	−2	−4	0	+4	−237.21	−77.45	−0.401
8	Fe	+III	$1e^\ominus + FeO(OH)_{(s)} + 3H^\oplus \rightarrow Fe^{2\oplus} + 2H_2O$	−1	−3	0	+3	−91.23	+28.59	+0.296
9		+III	$1e^\ominus + Fe(OH)_{3(s)} + 2H^\oplus \rightarrow FeOH^\oplus + 2H_2O$	−1	−2	0	+2	−55.12	+24.76	+0.257
10	S	+VI	$8e^\ominus + SO_4^{2\ominus} + 10H^\oplus \rightarrow H_2S_{(g)} + 4H_2O$	−8	−10	0	+10	−237.65	+161.75	+0.210
11		0	$2e^\ominus + S_{(s)} + 2H^\oplus \rightarrow H_2S_{(g)}$	−2	−2	0	+2	−33.56	+46.32	+0.240
12	C	0	$2e^\ominus + \langle HCHO\rangle + 2H^\oplus \rightarrow \langle HCH_2OH\rangle$	−2	−2	0	+2	−44.85	+35.03	+0.182
13		+IV	$8e^\ominus + HCO_3^\ominus + 9H^\oplus \rightarrow CH_{4(g)} + 3H_2O$	−8	−9	−1	+8	−175.44	+184.02	+0.238
14		+IV	$8e^\ominus + 2HCO_3^\ominus + 9H^\oplus \rightarrow CH_3COO^\ominus + 4H_2O$	−8	−9	−2	+8	−144.43	+215.03	+0.279
15		+IV	$4e^\ominus + HCO_3^\ominus + 5H^\oplus \rightarrow \langle HCHO\rangle + 2H_2O$	−4	−5	−1	+4	−18.05	+181.65	+0.471
16	H	+I	$1e^\ominus + H^\oplus \rightarrow \frac{1}{2}H_{2(g)}$	−1	−1	0	+1	0	+39.94	+0.414

[a] Number of electrons exchanged; [b] number of protons exchanged; [c] changes in the inorganic carbon pool; [d] changes in the acid-neutralizing capacity; [e] standard Gibbs free enthalpy change (pH 0); [f] standard Gibbs free enthalpy change (pH 7); [g] electrochemical potential (pH 7)[18].

Table 2. Microbially mediated electron-donor reactions: Oxidation of organic carbon compounds.

No.	Electron-donor	Oxidation state of carbon	Oxidation reaction (e.g.)	Δ n [a]	Δ H⊕ [b]	Δ C_T [c]	Δ ANC [d]	Δ G^0 [kJ·mol^{-1}] [e]	Δ $G^{0'}$ [kJ·mol^{-1}] [f]	$E^{0'}$ [V] [g]
17	C	+II	$\langle HCOOH\rangle + H_2O \rightarrow HCO_3^\ominus + 3H^\oplus + 2e^\ominus$	+2	+3	+1	−2	+22.77	−97.05	−0.503
18		0	$\langle HCHO\rangle + 2H_2O \rightarrow HCO_3^\ominus + 5H^\oplus + 4e^\ominus$	+4	+5	+1	−4	+18.05	−181.65	−0.471
19		−II	$\langle HCH_2OH\rangle + 2H_2O \rightarrow HCO_3^\ominus + 7H^\oplus + 6e^\ominus$	+6	+7	+1	−6	+62.9	−216.68	−0.374
20		−IV	$\langle HCH_3\rangle_{(g)} + 3H_2O \rightarrow HCO_3^\ominus + 9H^\oplus + 8e^\ominus$	+8	+9	+1	−8	+175.44	−184.02	−0.238
21		−II	$\langle HCH_2OH\rangle \rightarrow \langle HCHO\rangle + 2H^\oplus + 2e^\ominus$	+2	+2	0	−2	+44.85	−35.03	−0.182
22		−IV	$\langle HCH_3\rangle_{(g)} + H_2O \rightarrow \langle HCHO\rangle + 4H^\oplus + 4e^\ominus$	+4	+4	0	−4	+157.39	−2.37	−0.006
23		−III / +III	$CH_3COO^\ominus + 4H_2O \rightarrow 2HCO_3^\ominus + 9H^\oplus + 8e^\ominus$	+8	+9	+2	−8	+144.43	−215.03	−0.279
24		−III/−II/+III	$CH_3CH_2COO^\ominus + 7H_2O \rightarrow 3HCO_3^\ominus + 16H^\oplus + 14e^\ominus$	+14	+16	+3	−14	+260.79	−378.25	−0.280
25		−III/−II/+III	$CH_3CH_2COO^\ominus + 3H_2O \rightarrow HCO_3^\ominus + 7H^\oplus + 6e^\ominus + CH_3COO^\ominus$	+6	+7	+1	−6	+116.36	−163.22	−0.282

[a]–[g] See legend to Table 1 and ref.[18].

Table 3. Microbially mediated electron-donor reactions: Further oxidation of mineralization products.

No.	Electron-donor	Oxidation state of electron-donor	Oxidation reaction	Δn	$\Delta H^{\oplus}$	ΔC_T	Δ ANC	ΔG^0 [kJ·mol^{-1}]	$\Delta G^{0'}$ [kJ·mol^{-1}]	$E^{0'}$ [V]
				[a]	[b]	[c]	[d]	[e]	[f]	[g]
26	O	− II	$H_2O \rightarrow \frac{1}{2}O_{2(g)} + 2H^{\oplus} + 2e^{\ominus}$	+ 2	+ 2	0	− 2	+ 237.18	+ 157.3	+ 0.815
27	N	− III	$NH_4^{\oplus} + 2H_2O \rightarrow NO_2^{\ominus} + 8H^{\oplus} + 6e^{\ominus}$	+ 6	+ 8	0	− 7	+ 521.51	+ 201.99	+ 0.349
28		+ III	$NO_2^{\ominus} + H_2O \rightarrow NO_3^{\ominus} + 2H^{\oplus} + 2e^{\ominus}$	+ 2	+ 2	0	− 3	+ 158.06	+ 78.18	+ 0.405
29		− III	$NH_4^{\oplus} + 3H_2O \rightarrow NO_3^{\ominus} + 10H^{\oplus} + 8e^{\ominus}$	+ 8	+ 10	0	− 10	+ 679.57	+ 280.17	+ 0.363
30	Mn	+ II	$Mn^{2\oplus} + 2H_2O \rightarrow MnO(OH)_{(s)} + 3H^{\oplus} + 1e^{\ominus}$	+ 1	+ 3	0	− 3	+ 144.69	+ 24.87	+ 0.258
31		+ III	$MnO(OH)_{(s)} \rightarrow MnO_{2(s)} + 1H^{\oplus} + 1e^{\ominus}$	+ 1	+ 1	0	− 1	+ 92.52	+ 52.58	+ 0.545
32		+ II	$Mn^{2\oplus} + 2H_2O \rightarrow MnO_{2(s)} + 4H^{\oplus} + 2e^{\ominus}$	+ 2	+ 4	0	− 4	+ 237.21	+ 77.45	+ 0.401
33	Fe	+ II	$Fe^{2\oplus} + 2H_2O \rightarrow FeO(OH)_{(s)} + 3H^{\oplus} + 1e^{\ominus}$	+ 1	+ 3	0	− 3	+ 91.23	− 28.59	− 0.296
34	S	− II	$H_2S_{(g)} \rightarrow S_{(s)} + 2H^{\oplus} + 2e^{\ominus}$	+ 2	+ 2	0	− 2	+ 33.56	− 46.32	− 0.240
35		0	$S_{(s)} + 4H_2O \rightarrow SO_4^{2\ominus} + 8H^{\oplus} + 6e^{\ominus}$	+ 6	+ 8	0	− 8	+ 204.09	− 115.43	− 0.199
36		− II	$H_2S_{(g)} + 4H_2O \rightarrow SO_4^{2\ominus} + 10H^{\oplus} + 8e^{\ominus}$	+ 8	+ 10	0	− 10	+ 237.65	− 161.75	− 0.210
37		− II/ + VI	$S_2O_3^{2\ominus} + 5H_2O \rightarrow 2SO_4^{2\ominus} + 10H^{\oplus} + 8e^{\ominus}$	+ 8	+ 10	0	− 10	+ 210.04	− 189.36	− 0.245
38	C	− IV	$CH_{4(g)} + 3H_2O \rightarrow HCO_3^{\ominus} + 9H^{\oplus} + 8e^{\ominus}$	+ 8	+ 9	+ 1	− 8	+ 175.44	− 184.02	− 0.238
39	H	0	$H_{2(g)} \rightarrow 2H^{\oplus} + 2e^{\ominus}$	+ 2	+ 2	0	− 2	0	− 79.88	− 0.414

[a]–[g] See legend to Table 1 and ref.[18].

Oxygen gradients in eutrophic sediments are steep and, in the absence of surface turbation, limited to the top few millimeters. In spite of the solubility increase through elevated pressure and low temperature in the hypolimnion, there is seldom enough oxygen available at the sediment-water interface to completely oxidize the sedimenting biomass. Many organisms isolated from this uppermost layer are facultative anaerobes with the ability to switch their metabolism to fermentation or denitrification as soon as oxygen ceases to be available[10]. Therefore, the chemical gradients in sediments are a consequence of microbial activity which is governed by thermodynamic constraints. In other words, the oxidation processes and the population stratification are regulated by physical diffusion, since it is the imbalance between rates of supply and utilization of oxidants which leads to the «gradient ecosystem» at the sediment-water interface. From the shapes of concentration profiles one may calculate physical fluxes into and out of sediments from which one may derive information about microbial activities in situ.

In addition to the spacial gradients in different depths, sediments of our holomictic, eutrophic lakes are also influenced by seasonal transients. Presently little is known and one may only speculate about the metabolic behavior of microbes during the transition phases in winter when oxygen-depleted hypolimnetic water is exchanged for oxygen-rich water, and during summer and fall when anoxic conditions develop in the hypolimnion. How does the microbial community react? What kind of organisms could get established under these transient conditions? Again, lake sediments offer beautiful model ecosystems to study problems of microbial metabolism in temporal transients. The results could be directly applied to a better understanding of the reactions occuring in sediments during lake restoration measures.

Vertical gradients of oxidants and organic matter in lake sediments indicate that the common oxidants are depleted before the organic matter has been mineralized completely. The only oxidant still available in abundance is CO_2, which upon reduction itself becomes part of an organic molecule. Thus, microbes capable of this organotrophic CO_2-fixation increase the organic content by reducing some of the CO_2 while oxidizing further some of the organic matter from biomass. There are three known groups of microorganisms involved in these processes. Hydrogenotrophic methanogens reduce CO_2 with H_2 released by fermenting organisms, to CH_4:

$$4\,H_2 + HCO_3^{\ominus} + H^{\oplus} \rightarrow CH_4 + 3\,H_2O$$

while hydrogenotrophic acetogens (e.g. *Acetobacterium spp.*) yield acetate according to:

$$4\,H_2 + 2\,HCO_3^{\ominus} + H^{\oplus} \rightarrow CH_3COO^{\ominus} + 4\,H_2O$$

Another group of acetogens, which is not well characterized yet, is capable of oxidizing a few fermentation products coupled to organotrophic CO_2-reduction, e.g.

$$CH_3OH + \tfrac{1}{2}\,HCO_3^{\ominus} \rightarrow \tfrac{3}{4}\,CH_3COO^{\ominus} + H_2O + \tfrac{1}{4}\,H^{\oplus}$$

$$CH_3CH_2OH + HCO_3^{\ominus} \rightarrow \tfrac{3}{2}\,CH_3COO^{\ominus} + H_2O + \tfrac{1}{2}\,H^{\oplus}$$

Thus, in oxidant-limited sediments the acetogens may convert degradation products (methanol, ethanol etc.) which are not accessible to the methanogens present into the substrate for acetotrophic methanogenesis. Only a few methanogens are capable of existing on acetate alone[6,17]; however, some of these can regularly be enriched from lake sediment samples and it may be assumed that they constitute the main portion of the methanogenic populations in sediments of eutrophic lakes. Microbial synthrophism, a third process of importance in habitats with restricted oxidation, will be discussed in section 7.

Some molecules are not further degraded in these restricted environments.

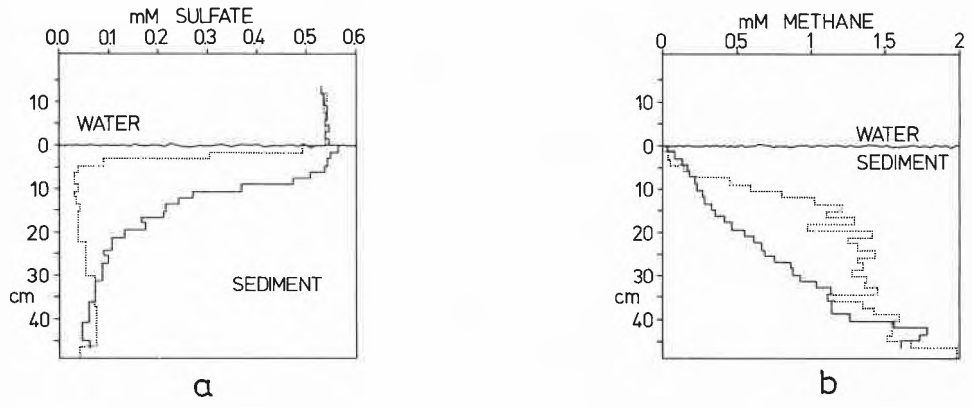

Fig. 8. Concentrations of sulfate (a) and methane (b) in the top 45 cm of sediments of Lake Geneva (250 m depth, May 1983). Dotted lines: measured on the crest of the pillow-like structures; full lines: measured in the trenches between the pillows.

They are preserved, altered by purely geochemical reactions, and buried in the sediment. For this reason oxidant-limited sediments might also serve as model systems to study initial processes in oil formation.

The methanogens, the fermenting, and the sulfate-reducing bacteria cannot efficiently exploit the energy present in the substrates for growth and metabolism. Through their way of life they produce volatile, energy-rich products like CH_4, NH_3, H_2S (among others) which diffuse into upper strata where appropriate oxidants (O_2, $NO_3^{\ominus}$) are present and they are oxidized by denitrifying and aerobic bacteria (Fig. 7:9 and 10). They are the last link in the electron transfer chain since the electrons have now been returned to the oxygen atom of the water molecule or to the nitrogen in N_2.

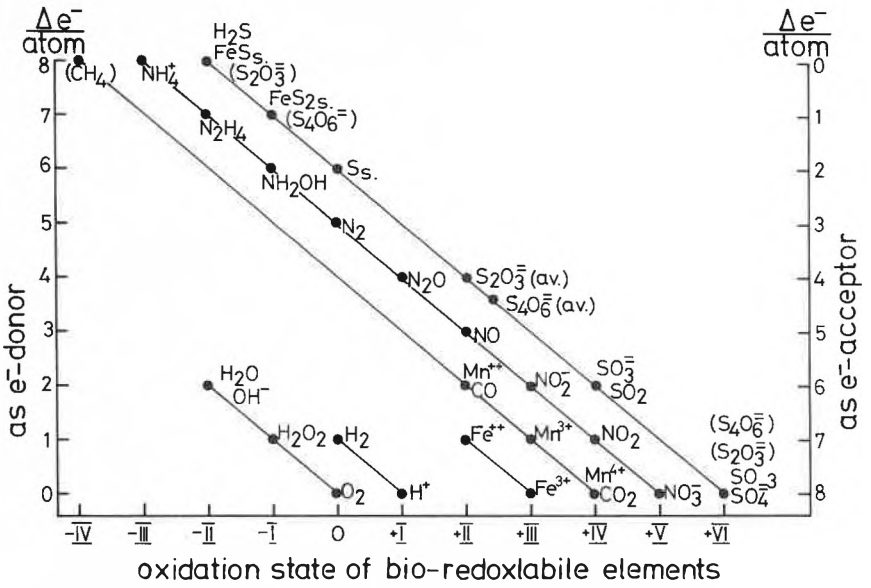

Fig. 9. Capacity of inorganic compounds as electron acceptors and electron donors in microbially mediated processes.

6. Equilibrium Thermodynamics: a Conceptual Framework

Simple considerations taken from the theory of equilibrium thermodynamics enable one to explain and to predict microbial action and behavior in natural ecosystems. Microbes employ only a small number of elements in their oxidation reactions: S, N, C, H, O, Mn, and Fe. The electron-scavengeing and electron-donating capacities of some of the compounds used in microbial redox metabolism are listed in Fig. 9. In order to conceptually combine the oxidation of organic molecules with inorganic oxidants, one assigns formal oxidation states to the carbon in organic compounds. Mean oxidation states and the corresponding electron donating capacities are related for several microbial substrates and products as depicted in Fig. 10. The carbon atom which is tetravalently bound to other carbons (R_4C) is assigned the oxidation state 0 (Fig. 11). The carbon is reduced if R is replaced by hydrogen, it is oxidized through bonds with oxygen, ammonia-nitrogen, or other electronegative elements like chlorine. The concept of these «artificial» oxidation states allows one to treat reduction and oxidation of organic molecules in terms of electron flow via microbial electron transport enzymes. In Fig. 11 the concept is applied to microbially mediated oxidation reactions. The stoichiometric coupling between carbon oxidation and the utilization of electron acceptors can be predicted from the figure. The dashed line illustrates an example. Microbial hydrocarbon degradation requires as a first step an activation of the methyl-carbon. The six electrons released during the oxidation to the carboxy-carbon can be taken up by oxidants with various electron accepting capacities. Due to the extremely reduced state of hydrocarbons their complete oxidation would require large amounts of oxidants. The conservation in geologic formations of fossil hydrocarbons may partially be due to the rapid depletion of oxidants during the

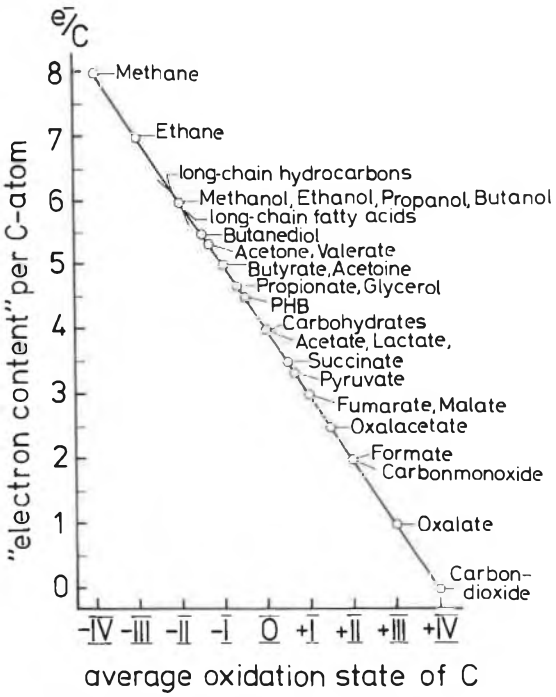

Fig. 10. Mean oxidation states of carbon in several microbial substrates and products.

original deposition of the organic matter in a manner similar to the situation in sediments of the eutrophic lakes of today.

Stoichiometries between inorganic reductants and microbially employed electron acceptors may be depicted from the lower part of Fig. 11.

From comparisons of the Gibbs free enthalpy changes of redox reactions one may distinguish between thermodynamically feasible and impossible reactions. The Gibbs free enthalpy changes of electron donor and acceptor reactions (Tables 1 to 3) have been correlated as shown in Fig. 12. With the aid of the graphs one may rapidly find those reactions which are exergonic under standard conditions and pH 7. Electrons originating from the complete oxidation of acetate for example can be accepted by ferric iron, elemental sulfur, hydrogencarbonate, sulfate, formaldehyde, fumarate, manganese, nitrate, and oxygen. Therefore microbial mediation of these electron transfer reactions can be predicted. Sulfate reducing bacteria, on the other hand, are unable to oxidize ammonia, nitrite, or manganese. The graph stipulates, however, the existence of bacteria capable of anaerobically oxidizing ammonia to nitrite employing denitrification to N_2 or $NO_2^{\ominus}$ as electron accepting mechanisms. These purely thermodynamic considerations for standard conditions do not prove that a biological process will actually

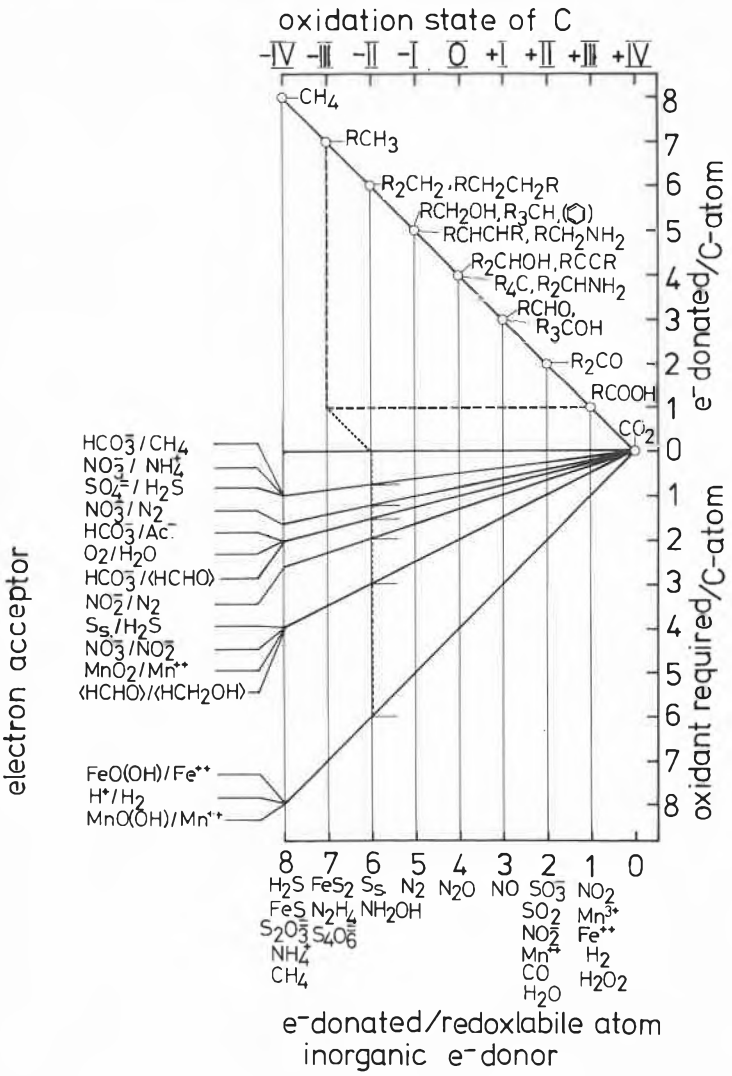

Fig. 11. Stoichiometric coupling between oxidation of carbon atoms of different oxidation states and microbially employed oxidants with various electron-accepting capacities. Bottom part of figure: Stoichiometry of inorganic reductants with microbially employed electron acceptors. The figure allows for any combination of oxidation and reduction reactions, one can, however, not distinguish between thermodynamically possible and impossible combinations of reactions.

exist or take place as predicted under the various and often changing natural conditions; they merely exclude highly improbable reactions.

7. Microbial Coupling of Electron Flow through Sulfur and Carbon Cycling

The final steps in the anaerobic degradation of biomass-carbon are sulfate-reduction which prevails in anoxic marine ecosystems and methanogenesis which is (with exceptions) dominant in lake sediments. There are two principal means by which sulfate reducing bacteria oxidize organic substrates (Table 4): through complete oxidation to CO_2 or by producing mainly acetate and propionate as products of long-chain fatty acid degradation[15]. Although both processes are $H^{\oplus}$-producing, the excess of base ($HCO_3^{\ominus}$ and $HS^{\ominus}$) produced is large enough to neutralize them and still lead to an increase in acid-neutralizing capacity (ANC). Electrons flowing from carbon through the sulfur cycle participate in an elaborate array of redox processes in which bacteria from various families and genera are involved (Fig. 13). The complexity might reflect the large span of oxidation states, i.e. electron-accepting and electron-donating capacities of sulfate and hydrogensulfide, respectively (Fig. 9).

Electrons released during oxidation of biomass-carbon enter the cycle at the steps of sulfate- and sulfur-reduction (Fig. 13A, B: a and b). They exit the cycle under aerobic conditions via oxygen (dissimilation) or get partially incorporated (assimilation) into the carbon of the cell mass and into sulfur reserves of aerobically sulfide oxidizing bacteria (c) or they leave the cycle with the metal sulfides (precipitation). Metal sulfide precipitates ($MeS_{(s)}$) are a depository electron sink. In the anaerobic

Table 4. Oxidation of fatty acids by sulfate reducing bacteria.

	Δ n	Δ ANC	$\frac{\Delta H^{\oplus}}{\lvert\Delta n\rvert}$	$\frac{\Delta C_T}{\lvert\Delta n\rvert}$	$\frac{\Delta \text{ ANC}}{\lvert\Delta n\rvert}$
A) Complete oxidation to CO_2					
1. *even* numbered fatty acids ($h \geq 0$)					
$CH_3(CH_2)_{2h}COO^{\ominus} + (^3/_2h+1)\ SO_4^{2\ominus} \rightarrow 2(h+1)\ HCO_3^{\ominus} + (^3/_2h+1)\ HS^{\ominus} + {}^1/_2h\ H^{\oplus}$	12h+8	+ (3h+2)	$+\frac{1}{8}\cdot\frac{h}{3h+2}$	$+\frac{1}{2}\cdot\frac{h+1}{h+2}$	$+\frac{1}{4}$
2. *odd* numbered fatty acids ($h \geq 0$)					
$CH_3(CH_2)_{2h+1}COO^{\ominus} + {}^1/_4(6h+7)\ SO_4^{2\ominus} \rightarrow (2h+3)\ HCO_3^{\ominus} + {}^1/_4(6h+7)\ HS^{\ominus} + {}^1/_4(2h+1)\ H^{\oplus}$	12h+14	+ (3h+3^1/$_2$)	$+\frac{1}{8}\cdot\frac{2h+1}{6h+7}$	$+\frac{1}{2}\cdot\frac{2h+3}{6h+7}$	$+\frac{1}{4}$
Desulfobacter postgatei (acetate only), *Desulfococcus multivorans*, *Desulfonema limicola*, *Desulfonema magnum*, *Desulfosarcina variabilis*, *Desulfovibrio barsii*, *Desulfotomaculum acetoxidans* (acetate, but not propionate to CO_2, produces propionate from valerate).					
B) Incomplete oxidation to acetate and propionate					
1. *even* numbered fatty acids ($h \geq 0$)					
$CH_3(CH_2)_{2h}COO^{\ominus} + {}^1/_2h\ SO_4^{2\ominus} \rightarrow (h+1)\ CH_3COO^{\ominus} + {}^1/_2h\ HS^{\ominus} + {}^1/_2h\ H^{\oplus}$	4h	+ h	$+\frac{1}{8}$	0	$+\frac{1}{4}$
2. *odd* numbered fatty acids ($h \geq 0$)					
$CH_3(CH_2)_{2h+1}COO^{\ominus} + {}^1/_2h\ SO_4^{2\ominus} \rightarrow h\ CH_3COO^{\ominus} + CH_3CH_2COO^{\ominus} + {}^1/_2h\ HS^{\ominus} + {}^1/_2h\ H^{\oplus}$	4h	+ h	$+\frac{1}{8}$	0	$+\frac{1}{4}$
Desulfobulbus propionicus (propionate only), *Desulfotomaculum nigrificans*, *Dm. orientis*, *Dm. ruminis*, *Desulfomonas pigra*, *Desulfovibrio desulfuricans*, *Dv. vulgaris*, *Dv. salexigens*, *Dv. africanus*, *Dv. baculatus*, *Dv. gigas*, *Dv. thermophilus*, *Dv. sapovorans*.					

process in the light electrons appear in cell mass, storage materials (glycogen, polyhydroxybutyrate = PHB), and sulfur reserves (d). They are therefore assimilated completely.

Sulfate as the primary oxidant stems from the dissolution of gypsum and anhydrite (Fig. 13:1); it diffuses into anoxic sediment strata (Fig. 13:2) where it is employed as the oxidant in dissimilatory sulfate reduction (Fig. 13:3). In the presence of metals, sulfides precipitate (Fig. 13:4). Free hydrogensulfide diffuses towards the redox-transition zone as well (Fig. 13:5) where it is reoxidized by aerobic chemotrophic or anaerobic phototrophic processes. A few large sulfur bacteria (e.g. *Beggiatoa, Thiotrix*) store elemental sulfur intracellularly before oxidizing it back to sulfate (Fig. 13:6). Other thiobacteria (e.g. *Thiobacilli, Thiomicrospira*) and several facultatively phototrophic *Chromatiaceae* oxidize hydrogensulfide aerobically in the dark to sulfate (Fig. 13:7). Thiosulfate and tetrathionate may be intermediates of the bacterial – or of a purely chemical – oxidation process (Fig. 13:8). *Thiobacillus denitrificans* employs nitrate as oxidant; it is capable therefore of anaerobically oxidizing hydrogensulfide to sulfate in the absence of light (Fig. 13:9). Some of the sulfur droplets produced as intermediates in the sulfur cycles never get oxidized further; they get buried together with the dead cells harboring them (Fig. 13:10). Elemental sulfur serves as oxidant for *Desulfuromonas spp.*, several *Desulfovibrio spp.*, and *Campylobacter spp.* (Fig. 13:11). *Chromatiaceae* and *Chlorobiaceae* as well employ elemental sulfur as electron acceptor in the dark. *Rhodopseudomonas sulphidophila*, a member of the phototrophic non-sulfur bacteria, is capable of oxidizing hydrogensulfide to sulfate (Fig. 13:12). *Chromatiaceae* convert hydrogensulfide via elemental sulfur which is stored temporarily inside the cell to sulfate (Fig. 13:13), while *Chlorobiaceae, Ectothiorhodospira spp.*, a few *Cyanobacteria*, and a few *Rhodospirillaceae* form sulfur droplets extracellularly (Fig. 13:14). Thiosulfate can be produced by some *Chlorobiaceae* and it can serve as electron donor to several *Chromatiaceae, Chlorobiaceae*, and *Rhodospirillaceae* in the light (Fig. 13:15). If the bacterially mediated oxidation of hydrogensulfide is limited or inhibited one may expect a number of products from purely chemical oxidation reactions (Fig. 13:16).

In lake sediments where sulfate becomes limiting, methanogenesis is the final process in anaerobic mineralization. Methanogenic bacteria[7] have been isolated from a variety of ecosystems (Table 5). All have very reduced substrate preferences, i.e. are stenotrophic. Most of them can use H_2 and CO_2, fewer isolates use methanol, carbon monoxide, formate, methylamines, or acetate (Table 5). Stenotrophic organisms are obligately dependent on companion populations which produce the substrates for them. Acetate is the most abundant

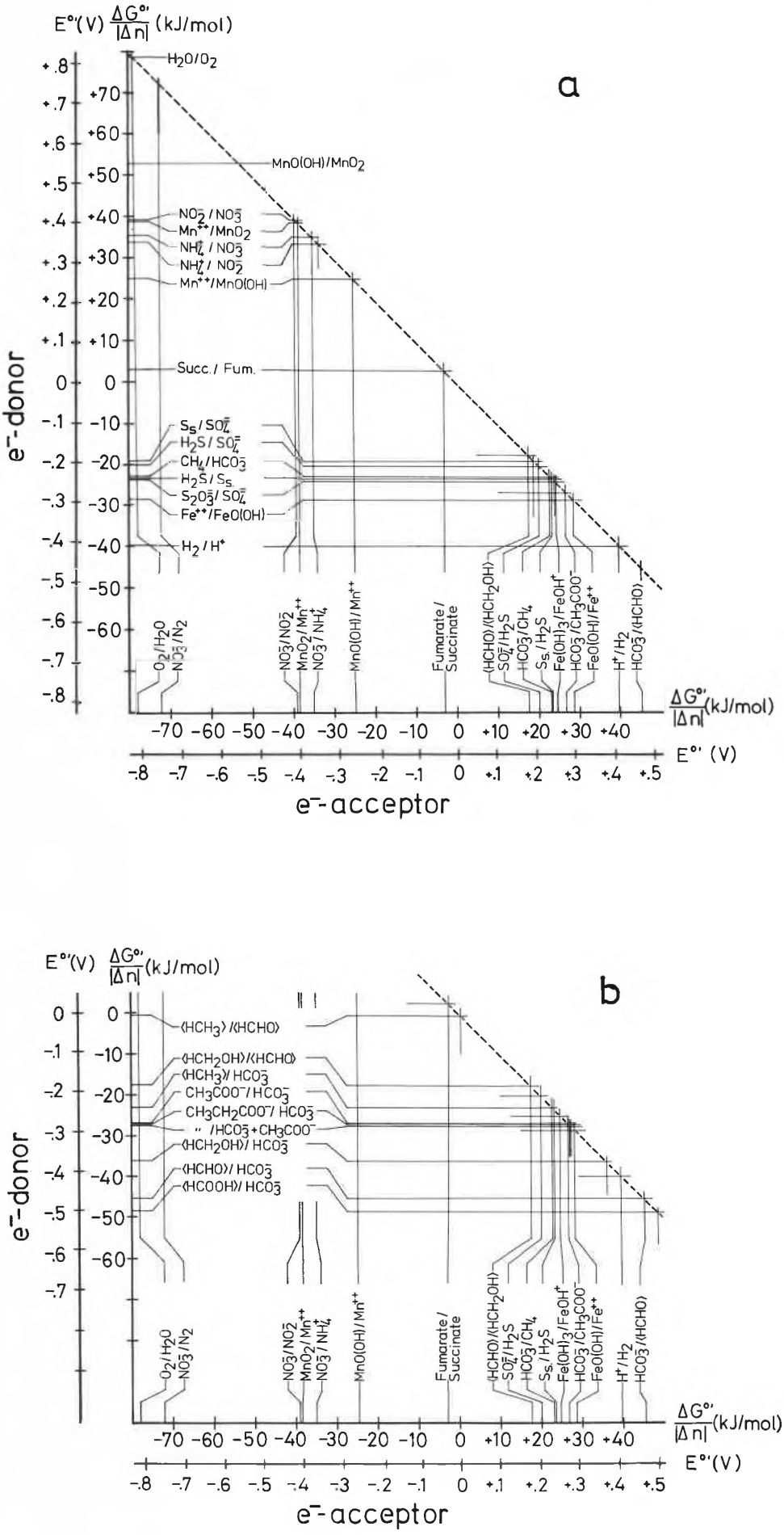

Fig. 12. (a) Thermodynamic correlation under standard conditions and pH 7 between electron donating substrates and electron acceptors commonly cycled in microbially mediated reactions. For a given electron donor one may deduce those electron acceptors which would lead to an exergonic overall redox-process. (b) For a few organic electron donors only.

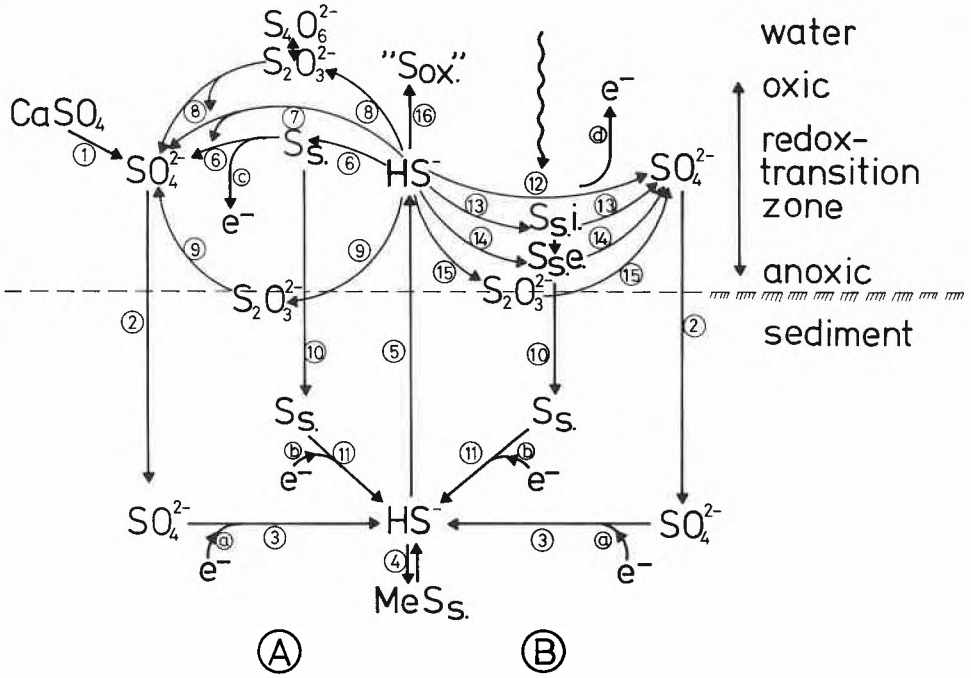

Fig. 13. Electron flow and sulfur cycling at sediment-water interfaces. A: in the absence of light; B: under conditions where light reaches the redox-transition zone. The numbered steps are explained in the text.

methanogenic substrate in lake sediments. It accumulates, together with propionate, temporarily in many sediments indicating an imbalance between its production and its utilization (Fig. 14)[2]. Probably most of the acetate stems from fermentation reactions and from the incomplete oxidation by sulfate reducing bacteria (Table 4). A third group of acetogens synthesize acetate from CO_2 employing H_2 or one of several fermentation products as reductants (see section 5). This last group of organisms has not yet been well characterized. Their quantitative contribution to the acetate pool is unknown.

There are three principal possibilities for the dissimilatory oxidation of acetate by anaerobes outside of the phototrophic world (Table 6):

1. by sulfate-reducing bacteria employing sulfate, sulfite, or thiosulfate as oxidants[14,15];
2. by sulfur-reducing bacteria[9];
3. by methanogens[6,17].

Acetotrophic methanogenesis is the dominant process in lake sediments. Bacteria utilizing sulfur and sulfate as electron acceptors have been isolated from lake sediments; their quantitative contribution to acetate degradation is unknown however.

Table 5. Substrates and habitats of some methanogenic bacteria.

	H_2/CO_2	HCOOH	CH_3COOH	CH_3OH	CO	CH_3NH_2, $(CH_3)_2NH$, $(CH_3)_3N$	$CH_3CH_2N(CH_3)_2$	Habitat
Methanobacterium formicicum	+	+			+			B
bryantii	+							A
thermoautotrophicum	+				+			A
Methanobrevibacter ruminantium	+		+					B
arboriphilus	+							A
arboriphilus DH1	+				+			A
smithii	+	+			+			A
Methanothermus fervidus	+							C
Methanococcus vannielii	+	+						A
voltae	+	+						A
mazei			+	+		+		A
deltae	+	+						A
maripaludis	+	+						A
thermolithotrophicus	+	+						A
jannaschii	+							C
Methanomicrobium mobile	+	+						B
paynteri	+							A
Methanogenium cariaci	+	+						A
marisnigri	+	+						A
tatii	+	+						C
olentangyi	+							A
thermophilicum	+	+						A
Methanospirillum hungatei	+	+						A
Methanotrix soehngenii			+					A
Methanococcoides methylutens				+		+		A
Methanosarcina barkeri	+		+	+	+	+	+	A
vacuolata	+		+	+	+	+		A
acetivorans			+	+		+		A
Methanoplanus limicola	+	+	+					A
Methanolobus tindarius				+		+		A
Methanoplasma elisabethii	+	+						A

Habitats: A = anaerobic sediments (mostly marine, seldom limnic), sludge of sewage plants, heartwood of decaying trees; B = rumen; C = geothermal springs.

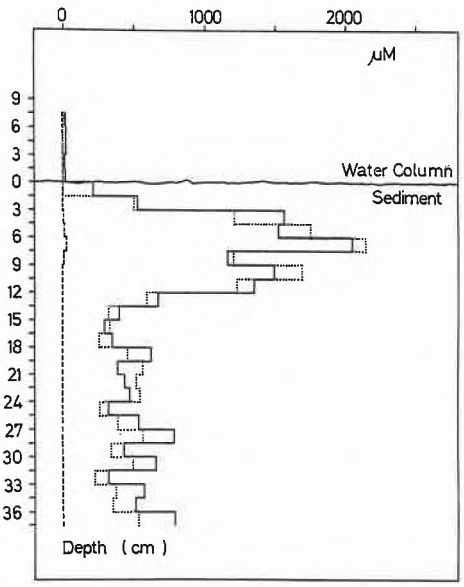

Fig. 14. Concentrations of acetate (full line), propionate (dotted line), and isobutyrate (dashed line) in sediments of Lake Zürich (4 m, December 1981). The higher concentrations of acetate and propionate indicate a production horizon 6 to 7 cm below the sediment surface and an imbalance between production and utilization of these microbial intermediates.

Among other possibilities propionate might be produced by the oxidation of straight-chain, odd-numbered fatty acids by sulfate-reducing bacteria with incomplete oxidation (Table 4); from valerate by *Desulfotomaculum acetoxidans*[14], and from succinate by *Propionigenium modestum* which also degrades several other substrates to propionate[11].

There are three principal possibilities for anaerobic propionate oxidation by non-phototrophic anaerobes:

1. by sulfate-reducing bacteria with sulfate as oxidant degrading the propionate completely to CO_2 (*Desulfococcus spp., Desulfonema spp., Desulfosarcina spp.*) or incompletely to acetate and CO_2 (*Desulfobulbus propionicus*)[15];

2. by *Desulfobulbus propionicus* under denitrifying conditions;
3. fermentatively to acetate, H_2, and CO_2 by *Syntrophobacter wolinii* when coupled with product-scavenging (mostly H_2-utilizing) organisms[1].

The reaction mediated by *Syntrophobacter wolinii* is endergonic; it can become exergonic only if the partial pressure of hydrogen is kept low. This is accomplished if *Syntrophobacter wolinii* coexists with hydrogen utilizing methanogens or sulfate-reducers. No methanogen is known to metabolize propionate directly. They are known, however, to synergistically interact with *Syntrophobacter wolinii*[1]. Direct reduction of CO_2 to CH_4 with electrons from propionate, alcohols, and other reduced organic substrates is thermodynamically feasible (Fig. 12b); H_2 remains the only known reductant for CO_2 by methanogens so far.

Biomolecules that have not been degraded to the level of methanogenic substrates by the time they reach sediment depths where oxidants become limiting cannot be altered further biologically. They are buried and subjected to geochemical alteration reactions. If anaerobic degradation in lake sediments is to be an efficient mineralization strategy, microbial synergism would have to lead to the production of a few methanogenic substrates from the immense variety of organic molecules produced by living organisms. Besides being limited by the lack of oxidants, mineralization, therefore, may also be restricted by metabolic inadequacy. The enzymes necessary to attack any feasible organic molecule that ends up in a lake sediment and to convert it into methanogenic substrates might simply not be available. This limitation is particularly grave for man-made chemicals of our times. Fermenting bacteria and sulfate-reducers have broad metabolic abilities. Coupling of fermentation, acetogenesis, and acetotrophic methanogenesis should, therefore, lead to complete mineralization even in the absence of common oxidants. Observations in eutrophic lakes demonstrate, however, that it does not. The limitation is probably the inability of anaerobes to break certain chemical bonds and structures that are products of the strongly oxidizing, aerobic world.

8. Geochemical Consequences of Microbial Activities

Microbes consume substrates and release different products. They thereby constantly change the conditions in the environment which consequently affects geochemical equilibria. Three parameters which reflect these changes are included in Tables 1 to 3. They are proton production and consumption ($\Delta H^{\oplus}$), changes in the content of inorganic carbon (ΔC_T), and in the capacity to neutralize acid (ΔANC). Although hydrogencarbonate is the most abundant acid regulating species in our hard-water lakes, other acid-base pairs will respond according to their abilities to accept or donate protons (Table 7). The consequences of electron-transfer coupled acid-base changes in microbial habitats are depicted in Fig. 15 for predominantly inorganic (Fig. 15a) and exclusively organic (Fig. 15b) electron donors. It can be predicted that complete oxidation of methanol-carbon by sulfate-reducers for example, makes the habitat conditions more basic. If methanogenesis is the methanol-utilizing process one would expect a slight acidification. Metal oxide-hydroxides as electron acceptors impair the most pronounced proton uptake with inorganic and with organic reductants.

Table 6. Acetate conversion by sulfate-, sulfite-, thiosulfate-, and sulfur-reducing bacteria and by methanogens.

	$\Delta G^{0'}$ [kJ·mol^{-1}]	ΔANC $\lvert\Delta n\rvert$	$E^{0'}$ [V]
$CH_3COO^{\ominus} + SO_4^{2\ominus} + H^{\oplus} \rightarrow 2\,HCO_3^{\ominus} + H_2S_{(g)}$	− 53.28	+ 2	− 0.069
$CH_3COO^{\ominus} + \frac{4}{3}HSO_3^{\ominus} + \frac{1}{3}H^{\oplus} \rightarrow 2\,HCO_3^{\ominus} + \frac{4}{3}H_2S_{(g)}$	− 131.99	+ $1\frac{1}{3}$	− 0.170
$CH_3COO^{\ominus} + S_2O_3^{2\ominus} + H^{\oplus} + H_2O \rightarrow 2\,HCO_3^{\ominus} + 2\,H_2S_{(g)}$	− 80.89	+ 2	− 0.105
$CH_3COO^{\ominus} + 4S_{(s)} + 4\,H_2O \rightarrow 2\,HCO_3^{\ominus} + 4\,H_2S_{(g)} + H^{\oplus}$	− 29.75	0	− 0.039
$CH_3COO^{\ominus} + H_2O \rightarrow HCO_3^{\ominus} + CH_{4(g)}$	− 31.01	0	− 0.040

For thermodynamic values see ref.[18].

The most pronounced geochemical consequences that result are:

1. Minerals are eroded by the acids produced (carbonates) or through redox processes (e.g. iron and manganese oxides and oxide-hydroxides, iron phosphates).
2. New minerals are formed due to changes in the ion concentration (e.g. metal sulfides, carbonates, etc. as listed in Table 8).

The effect of microbes on calcite dissolution and preservation can serve to demonstrate point 1. Calcite formation in eutrophic, calcium- and hydrogencarbonate-rich lakes takes place regularly during times of active photosynthetic CO_2-fixation in the photic zone:

$$2\,HCO_3^{\ominus} + Ca^{2\oplus} \rightarrow \langle HCHO\rangle + CaCO_{3\,(s)} + O_2$$

For each CO_2 reduced to the oxidation state of biomass, two $HCO_3^{\ominus}$ are required, one serves as the carbon source the other as the proton source. Deprotonation of hydrogencarbonate and consumption of protons by photosynthesis leads to the formation of carbonate and its precipitation as calcium carbonate at the elevated pH (Fig. 4). The crystals reach the sediment rapidly and are there subjected to environments characterized by bacterial mineralization events. Acid producing processes contribute to the dissolution of the crystals according to:

$$CaCO_{3\,(s)} + H^{\oplus} \rightarrow Ca^{2\oplus} + HCO_3^{\ominus}$$

From the generalized mineralization equations combined in Fig. 15 potentially calcite-eroding processes can be depicted. Eroded calcite crystals, however, are only found in microhabitats with active proton production. This is the case in horizons with high rates of acetic and propionic acid formation and under conditions of heterotrophic CO_2-fixation where acid production is coupled to an overall basicity decrease. These are conditions to be expected in the sediments of our eutrophic lakes. Generally the strong alkalinity in limnic sediments will neutralize metabolic acids effectively between pH 6.36 and pH 10.33, the pK_a-values of carbonic acid and hydrogencarbonate, respectively. Changes within this pH-range can often be observed; they allow us to draw conclusions about the prevailing microbial processes in a particular sediment. Addition of strong base or acid (Table 7) can upset environmental conditions to pH-values below or above the hydrogencarbonate buffering limits.

The release of reduced iron and manganese species are also a consequence of anaerobic mineralization (Fig. 16). The mobile ions may diffuse from the production horizon into zones with a free carbonate concentration high enough to lead to the formation of rhodochrosite and siderite.

9. Consequences for Nutrient Cycling in Lakes

Nutrient cycling between sediments and hypolimnetic water has been the subject of intensive investigation for decades[3,8]. A recent motivation for this kind of study stems from the interest in phosphate release from sediments and its effect on internal lake fertilization. Another motivating force derives from the effect of the sediment on rates of mobilization and immobilization of toxic compounds.

With regard to bacterial physiology one would have to distinguish between substances which are essential nutrients or energy sources for bacterial growth and those that are only non-essential alternatives. Many denitrifying bacteria for example can switch to ferric iron as electron acceptor as soon as nitrate is exhausted. Through this non-essential alternative some denitrifyers become indirectly involved in phosphate release, since reduction of $Fe^{3\oplus}$ is linked to phosphorus cycling[12].

Phosphate is an essential nutrient for cell growth. In the process of mineralization of

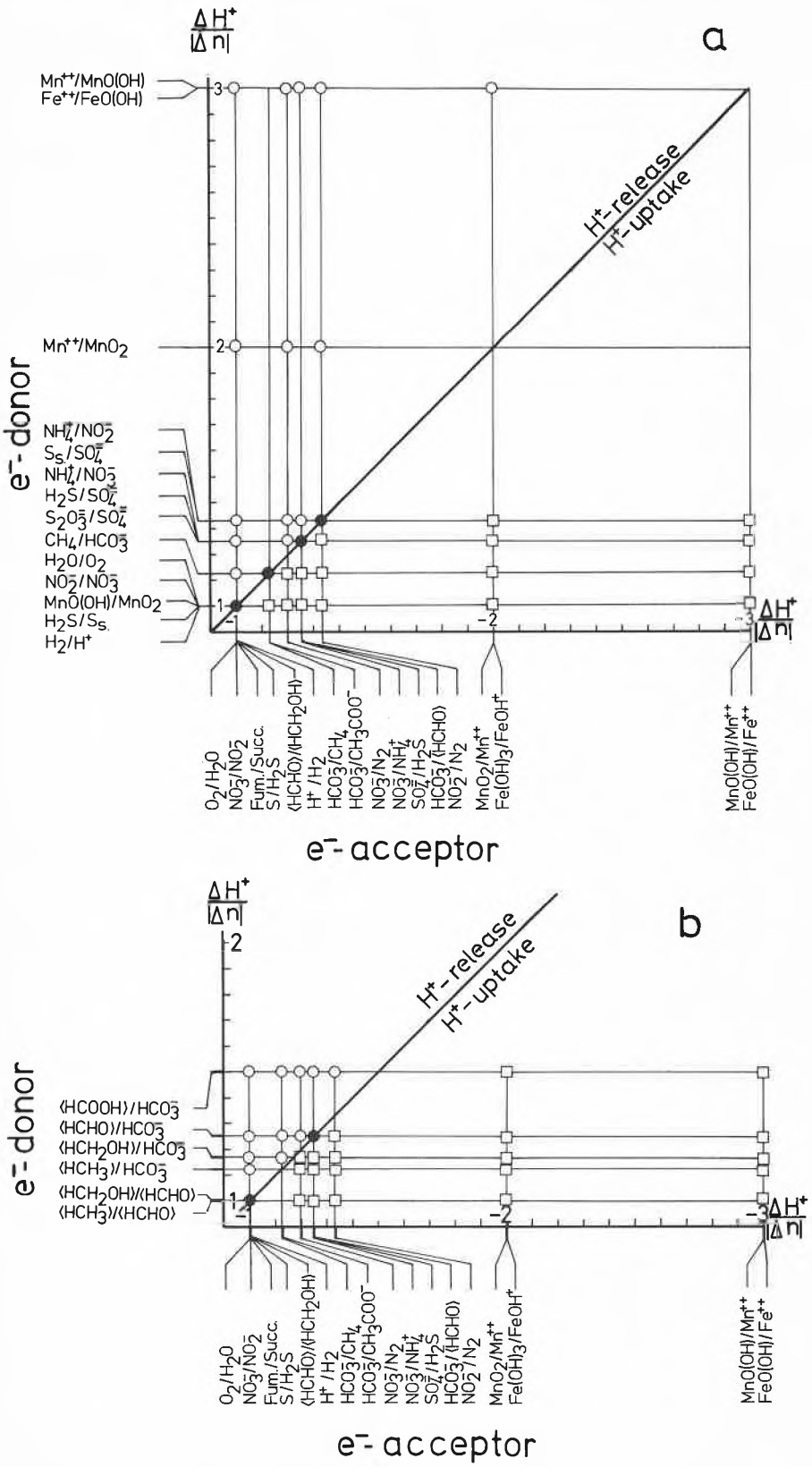

Fig. 15. Proton uptake and proton release as a consequence of electron transfer. The numbers of protons exchanged ($\Delta H^{\oplus}$) per electron transferred (Δn) are correlated for electron donor (a: mostly inorganic, b: exclusively organic) and electron acceptor reactions. For detailed numerical values see Tables 1–3.

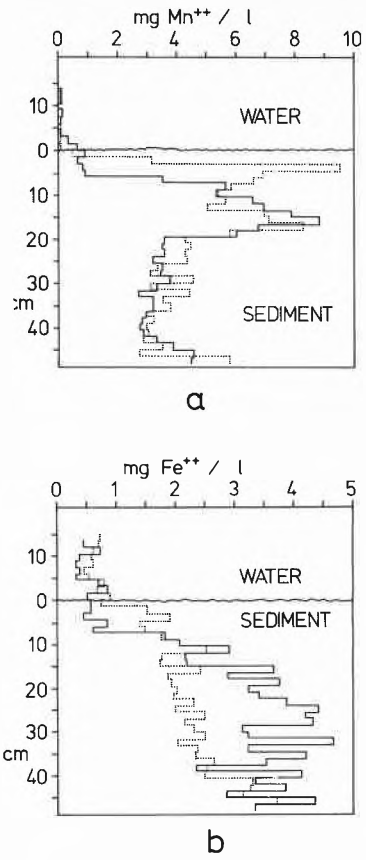

Fig. 16. Concentrations of mobile species of manganese (a) and iron (b) in the top 45 cm of sediments of Lake Geneva (250 m depth, May 1983). Dotted lines: on the crest of the pillow-like structures; full lines: in the trenches between the pillows.

dead biomass it is released under oxic and anoxic conditions. Due to its status as a growth limiting nutrient in lakes, a large portion will rapidly be incorporated into the new biomass of the mineralizing populations. This is true for aerobic conditions if cell growth is not energy- or oxidant-limited. In anaerobic environments where growth is confined by the efficiency of energy conversion, less new biomass is formed and more nutrients, e.g. phosphate, are released.

Oxic sediment surfaces containing iron and manganese oxides and oxide-hydroxides may function as nutrient traps through their adsorptive properties[13]. Bacteria might be involved in the formation of these oxides through ferrous iron oxidation. We observe the growth of *Siderocapsa*-webs on artificial surfaces exposed for only a few days at the sediment-water interface in Lake Zürich. These heterotrophic organisms catalyze the oxidation of $Fe^{2\oplus}$ without gaining energy for growth from the reaction. The ability to oxidize ferrous iron is widespread in the microbial world[5]. Whether all the bacteria described as ferrous-iron-oxidizers because of the ferric oxide-hydroxide and oxide encrustations around them, are metabolically involved in the electron transfer process, however, is still controversial.

The *Beggiatoa*-mats function as a biological diffusion barrier at the anoxic-oxic transition zone. Under conditions of balanced H_2S- and O_2-supply the bacteria oxidize all the H_2S; none will diffuse into the hypolimnion. They keep the water free of the toxic H_2S and prevent the development of an anoxic hypolimnion. It is probable that the benthic *Beggiatoa* also scavenge nutrients for growth thereby functioning as temporary nutrient traps and relieving the lake from cyclic self-eutrophication. At high rates of H_2S-produc-

tion in the hypolimnion *Beggiatoa* cannot establish themselves at the sediment-water interface. The hypolimnion becomes anoxic up to the zone where H_2S is oxidized abiotically or by planktonic sulfur bacteria.

Thus cycling of nutrients between sediment and water is governed to a large extent by the environmental conditions which allow bacterial populations to establish themselves at the interface as well as by their metabolic abilities and activities as a biological «diffusion filter».

10. Conclusions

Sediments offer a wealth of information for sciences as diverse as geology, chemistry, and biology.

- From stratigraphic studies one may derive the biological, chemical, and geological history of a lake and its surroundings.
- Studies of the sediment surface reveal an immediate picture of the processes going on momentarily in the water and in the underlying deposits.
- Analyses of processes in the sediment-water interface enable one to make predictions on the contribution of the sediments to future lake development.

Information from sediment studies will eventually lead to a more complete understanding of the lake ecosystem which may aid in deciding on appropriate lake management and restoration programs.

The microbes which inhabit sediments sometimes have to cope with conditions that are anything but encouraging for metabolism and growth. They have to exist under elevated pressures in the dark at temperatures that seldom exceed 4 °C; the richness in organic matter leads to rapid depletion of oxygen; the supply of alternative oxidants is regulated by the flux into the sediment and in eutrophic lakes is always insufficient to completely oxidize the organic matter. Biological and chemical stratifications within sediments are a consequence of these conditions. Bacteria belonging to many different genera and representatives from almost all metabolic types act synergistically in the breakdown of biomolecules. Acetate is a main intermediate; it plays a key role in the coupling between the carbon cycle and the oxidant cycles. Ecologically important acetate-producing and -utilizing reactions deserve to be studied further.

Because of the geochemical composition of our prealpine lakes, they offer model systems for studies on microbial interactions in oxidant limited ecosystems and on the behavior of microbes under transient conditions.

I have limited the number of topics discussed to only a few. Most of them reflect naturally our present interest in sediment

Table 7. Acid-base pairs important in aquatic microbial habitats.

acid	base	pK_a	$K_{eq.(298)}$	Ref.
$H_3O^{\oplus}$	H_2O	0	1.0	Def.
H_2O	$OH^{\ominus}$	14.00	10^{-14}	[a]
H_2CO_3	$HCO_3^{\ominus}$	6.36	$4.37 \cdot 10^{-7}$	[a]
$HCO_3^{\ominus}$	$CO_3^{2\ominus}$	10.33	$4.68 \cdot 10^{-11}$	[a]
HNO_3	$NO_3^{\ominus}$	≈ 0	≈ 1	[a]
HNO_2	$NO_2^{\ominus}$	3.23	$5.89 \cdot 10^{-4}$	[a]
$NH_4^{\oplus}$	NH_3	9.25	$5.62 \cdot 10^{-10}$	[a]
H_2SO_4	$HSO_4^{\ominus}$	− 1.99	> 1	[a]
$HSO_4^{\ominus}$	$SO_4^{2\ominus}$	1.99	$1.02 \cdot 10^{-2}$	[a]
H_2SO_3	$HSO_3^{\ominus}$	1.77	$1.70 \cdot 10^{-2}$	[a]
$HSO_3^{\ominus}$	$SO_3^{2\ominus}$	7.22	$6.03 \cdot 10^{-8}$	[a]
H_2S	$HS^{\ominus}$	7.00	$1 \cdot 10^{-7}$	[a]
$HS^{\ominus}$	$S^{2\ominus}$	12.92	$1.20 \cdot 10^{-13}$	[a]
$Fe^{2\oplus}aq$	$FeOH^{\oplus}$	6.77	$1.70 \cdot 10^{-7}$	[a]
$Fe^{3\oplus}aq$	$FeOH^{2\oplus}$	2.17	$6.76 \cdot 10^{-3}$	[a]
$FeOH^{2\oplus}$	$Fe(OH)_2^{\oplus}$	5.00	$1.00 \cdot 10^{-5}$	[a]
$Mn^{2\oplus}aq$	$MnOH^{\oplus}$	10.55	$2.82 \cdot 10^{-11}$	[a]
$Mn^{3\oplus}aq$	$MnOH^{2\oplus}$	?		[d]
$MnOH^{2\oplus}$	$Mn(OH)_2^{\oplus}$	?		[d]
H_3PO_4	$H_2PO_4^{\ominus}$	2.14	$7.24 \cdot 10^{-3}$	[a]
$H_2PO_4^{\ominus}$	$HPO_4^{2\ominus}$	7.20	$6.31 \cdot 10^{-8}$	[a]
$HPO_4^{2\ominus}$	$PO_4^{3\ominus}$	12.36	$4.37 \cdot 10^{-13}$	[a]
$Al^{3\oplus}aq$	$AlOH^{2\oplus}$	4.98	$1.05 \cdot 10^{-5}$	[a]
$AlOH^{2\oplus}$	$Al(OH)_2^{\oplus}$	3.56	$2.75 \cdot 10^{-4}$	[a]
$Si(OH)_4$	$SiO(OH)_3^{\ominus}$	9.5	$3.16 \cdot 10^{-10}$	[b]
$SiO(OH)_3^{\ominus}$	$SiO_2(OH)_2^{2\ominus}$	12.6	$2.51 \cdot 10^{-13}$	[b]
HCl	$Cl^{\ominus}$	0	≈ 1	[a]
HCOOH	$HCOO^{\ominus}$	3.75	$1.772 \cdot 10^{-4}$	[c]
CH_3COOH	$CH_3COO^{\ominus}$	4.75	$1.754 \cdot 10^{-5}$	[c]
CH_3CH_2COOH	$CH_3CH_2COO^{\ominus}$	4.87	$1.336 \cdot 10^{-5}$	[c]

[a] Calculated from the ΔG^0 of the dissociation reaction; [b] W. Stumm, J. J. Morgan[13]; [c] R. C. Weast et al.[18]; [d] G_f^0-values not available.

Table 8. Some microbially mediated dissolution and precipitation reactions occurring in aquatic environments.

Reaction: dissolution → / precipitation ←		$\Delta H^{\oplus}$ →	ΔC_T →	Δ ANC →	$\Delta G^0_{(298K)}$ [kJ/mol]	log K_{eq} (25 °C)	K_{eq}
$MnCO_{3(s)}$	$\rightleftharpoons Mn^{2\oplus} + CO_3^{2\ominus}$	0	+ 1	+ 2	+ 60.79	− 10.65	$2.24 \cdot 10^{-11}$
$MnS_{(s)}$	$\rightleftharpoons Mn^{2\oplus} + S^{2\ominus}$	0	0	+ 2	+ 76.17	− 13.35	$4.47 \cdot 10^{-14}$
$Mn(OH)_{2(s)}$	$\rightleftharpoons MnOH^{\oplus} + OH^{\ominus}$	(− 1)	0	+ 1	+ 52.75	− 9.24	$5.75 \cdot 10^{-10}$
$Al(OH)_{3(s)}$	$\rightleftharpoons Al(OH)_2^{\oplus} + OH^{\ominus}$	(− 1)	0	+ 1	+ 70.71	− 12.39	$4.07 \cdot 10^{-13}$
$FeCO_{3(s)}$	$\rightleftharpoons Fe^{2\oplus} + CO_3^{2\ominus}$	0	+ 1	+ 2	+ 59.95	− 10.51	$3.09 \cdot 10^{-11}$
$FeS_{(s)}$	$\rightleftharpoons Fe^{2\oplus} + S^{2\ominus}$	0	0	+ 2	+ 104.5	− 18.31	$4.90 \cdot 10^{-19}$
$Fe(OH)_{2(s)}$	$\rightleftharpoons FeOH^{\oplus} + OH^{\ominus}$	(− 1)	0	+ 1	+ 51.91	− 9.10	$7.94 \cdot 10^{-10}$
$Fe(OH)_{3(s)}$	$\rightleftharpoons Fe(OH)_2^{\oplus} + OH^{\ominus}$	(− 1)	0	+ 1	+ 101.29	− 17.75	$1.78 \cdot 10^{-18}$
$FePO_4 \cdot 2\,H_2O_{(s)}$	$\rightleftharpoons Fe^{3\oplus} + PO_4^{3\ominus} + 2\,H_2O$	0	0	+ 3	+ 159.94	− 28.03	$9.33 \cdot 10^{-29}$
$Ca_3(PO_4)_{2(s)}$	$\rightleftharpoons 3\,Ca^{2\oplus} + 2\,PO_4^{3\ominus}$	0	0	+ 6	+ 186.58	− 32.70	$2.00 \cdot 10^{-33}$
$CaHPO_{4(s)}$	$\rightleftharpoons Ca^{2\oplus} + HPO_4^{2\ominus}$	0	0	+ 2	+ 38.46	− 6.74	$1.82 \cdot 10^{-7}$
$CaCO_{3(s)}$ (Calcite)	$\rightleftharpoons Ca^{2\oplus} + CO_3^{2\ominus}$	0	+ 1	+ 2	+ 47.40	− 8.31	$4.90 \cdot 10^{-9}$
$CaCO_{3(s)}$ (Aragonite)	$\rightleftharpoons Ca^{2\oplus} + CO_3^{2\ominus}$	0	+ 1	+ 2	+ 46.36	− 8.12	$7.59 \cdot 10^{-9}$
$CaMg(CO_3)_{2(s)}$	$\rightleftharpoons Ca^{2\oplus} + Mg^{2\oplus} + 2\,CO_3^{2\ominus}$	0	+ 2	+ 4	+ 99.41	− 17.42	$3.80 \cdot 10^{-18}$
$CaSO_4 \cdot 2\,H_2O_{(s)}$	$\rightleftharpoons Ca^{2\oplus} + SO_4^{2\ominus} + 2\,H_2O$	0	0	+ 1	+ 24.92	− 4.37	$4.27 \cdot 10^{-5}$
$CaSO_{4(s)}$	$\rightleftharpoons Ca^{2\oplus} + SO_4^{2\ominus}$	0	0	+ 1	+ 23.86	− 4.15	$7.08 \cdot 10^{-5}$

$\Delta H^{\oplus}$, ΔC_T, Δ ANC, and ΔG^0 for the dissolution reaction. For thermodynamic values see ref.[18].

microbiology of lakes. Closely related subjects like microbial involvement in manganese and iron cycling, in erosion and corrosion processes, in the behavior towards recalcitrant molecules in connection with oil formation, and the possible role of bacteria in ancient sediment-ecosystems would also deserve to be discussed extensively. I also have chosen to link the subjects to geochemical and hydrochemical consequences rather than to physiological and ecological implications for the living bacterial cell. With this approach I intended to address myself primarily to geologists and chemists. I tried to fulfill their expectation that certain geochemical observations can be explained by microbially mediated processes and I hope to have aroused an appreciation of the microbe's contribution to biogeochemistry.

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