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REVIEWS

Neutrophilic Lithotrophic Iron-Oxidizing Prokaryotes and Their Role in the Biogeochemical Processes of the Iron Cycle

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Abstract—Biology of lithotrophic neutrophilic iron-oxidizing prokaryotes and their role in the processes of the biogeochemical cycle of iron are discussed. This group of microorganisms is phylogenetically, taxonomically, and physiologically heterogeneous, comprising three metabolically different groups: aerobes, nitrate-dependent anaerobes, and phototrophs; the latter two groups have been revealed relatively recently. Their taxonomy and metabolism are described. Materials on the structure and functioning of the electron transport chain in the course of Fe(II) oxidation by members of various physiological groups are discussed. Occurrence of iron oxidizers in freshwater and marine ecosystems, thermal springs, areas of hydrothermal activity, and underwater volcanic areas are considered. Molecular genetic techniques were used to determine the structure of iron-oxidizing microbial communities in various natural ecosystems. Analysis of stable isotope fractionation of $^{56/54}$Fe in pure cultures and model experiments revealed a predominance of biological oxidation over abiotic ones in shallow aquatic habitats and mineral springs, which was especially pronounced under microaerobic conditions at the redox zone boundary. Discovery of anaerobic bacterial Fe(II) oxidation resulted in development of new hypotheses concerning the possible role of microorganisms and the mechanisms of formation of the major iron ore deposits during Precambrian era until the early Proterozoic epoch. Paleobiological data are presented on the microfossils and specific biomarkers retrieved from ancient ore samples and confirming involvement of anaerobic biogenic processes in their formation.

Keywords: neutrophilic lithotrophic iron-oxidizing bacteria, Fe(II) oxidation coupled to anoxygenic photosynthesis and nitrate reduction, biogeochemical cycle of iron

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INTRODUCTION

Prokaryotes belonging to various taxonomic and physiological groups within the domains *Bacteria* and *Archaea* are able to oxidize reduced ferrous iron (Fe(II)) compounds. Fe(II) oxidation by microorganisms may carry out a number of physiological functions, either associated with energy metabolism, with Fe(II) acting as an electron donor, or those not related directly to any energy gain.

Several groups of iron-oxidizing microorganisms (Fe-OM) may be discerned based on their physiological properties and the role of Fe(II) oxidation. Acidophilic microorganisms able to use Fe(II) oxidation in an acidic environment, in which it is resistant to chemical oxidation by oxygen, in dissimilation processes, have been discovered in 1950 [1] and, due to their immense role and widespread application in the biotechnological processes of leaching heavy and noble metals from ores, are a subject of numerous studies. Neutrophilic Fe-OM are physiologically heterogeneous and may be subdivided into four groups according to the type of their metabolism (electron donors and acceptors utilized) [1]: (1) aerobic organoheterotrophs; (2) aerobic lithotrophs; (3) facultative anaerobic and anaerobic lithotrophs; and (4) anaerobic phototrophs.

Characterization of a numerous group of organoheterotrophic Fe-OM is beyond the scope of this review.

The first neutrophilic iron-oxidizing prokaryote, discovered in the 19th century, was described as *Gallionella ferruginea*, a bacterium catalyzing Fe(II) oxidation with abundant precipitation of Fe(III) hydroxides [2, 3]. During the subsequent period of almost two centuries, the information concerning the physiological and morphological diversity of iron-oxidizing microorganisms has been accumulated. Although a number of *Gallionella* pure cultures had been obtained in the 1960s–1970s, *Gallionella ferruginea* was isolated in pure culture only in 1991. This culture was used to confirm Winogradsky's concept of anorgoxidation and ability of this microorganism to use Fe(II) at near-neutral pH as an electron donor for chemolithoautotrophic growth [4, 5]. Intense research on neutrophilic lithotrophic Fe-OM commenced in mid-1990s. This group is of interest not only due to its role in the global processes of the iron cycle now and in the geological past, as well as for

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Table 1. Lithotrophic obligately aerobic iron-oxidizing bacteria

Name*	Morphology	Source of isolation	Type of metabolism	Year, reference
Gallionella ferruginea	Bean-shaped cells with iron-encrusted stalks	Freshwater chalybeate spring	Autotrophic, mixotrophic	1836 [2]; 1991 [4]; 1993 [5]
Gallionella capsiferriformans	Bean-shaped cells without stalks	"	Autotrophic	1997 [6]; 2013 [7]
Sideroxydans lithotrophicus	"	"	"	1997 [6]; 2013 [7]
Strain TW-2	Curved rods	Freshwater sediments	Autotrophic, mixotrophic	2004 [8]
Sideroxydans paludicola BrT	Bean-shaped cells	Plant rhizosphere	Autotrophic	2007 [9]
Ferritrophicum radicicola	Bean-shaped cells	"	"	2007 [9]
Mariprofundus ferrooxidans PV-1	Bean-shaped cells	Deep-water marine hydrotherms	"	2007 [10]
Mariprofundus sp. GSB2	"	Littoral marine sediments	ND	2011 [11]
Strain R1	"	Underground freshwater springs	Autotrophic	2012 [12]

Here and further on "*" indicates the phylogenetic position presented on Fig. 1; "ND" stands for no data.

astrobiological research, but also due to emergence of fundamentally new data on the group and to discovery of new types of metabolism.

The biology of three groups of neutrophilic lithotrophic Fe-OM, their occurrence in various aquatic ecosystems, and their involvement in the oxidative processes of the biogeochemical cycle of iron presently, as well as their hypothetical role in the past geological epochs, were studied.

PHYSIOLOGICAL AND PHYLOGENETIC DIVERSITY OF LITHOTROPHIC NEUTROPHILIC IRON-OXIDIZING PROKARYOTES

Iron-oxidizing aerobes. Morphology and certain physiological properties of members of the small group of iron-oxidizing aerobic lithotrophs are presented in Table 1. All members of this group are obligate microaerophiles and are capable of lithoautotrophic growth. *Gallionella ferruginea* and strain TW-2 are capable of mixotrophic growth in the presence of acetate. All known freshwater lithotrophic iron-oxidizing bacteria belong to the class *Betaproteobacteria* (*Gallionella ferruginea, G. capsiferriformans, Sideroxydans lithotrophicus, S. paludicola, Ferritrophicum radicicola*, strain TW-2, strain R1). Members of the first two genera, *Gallionella* and *Sideroxydans*, have been recently included into the new order *Gallionellales* [7]. Marine bacteria *Mariprofundus ferrooxidans* and *Mariprofundus* sp. GSB2, which are morphologically similar to freshwater *Gallionella* species, belong to the class *Zetaproteobacteria* (Fig. 1).

The morphologically conspicuous freshwater and marine aerobic Fe-OM have bean-shaped cells with iron-encrusted stalks, where Fe(III) oxides are precipitated. For some species, formation of iron-encrusted stalks was not observed, and amorphous Fe(III) oxides precipitated in the medium.

Apart from the organisms listed in Table 1, some psychrophilic marine Fe-OM were described. The strains belonging to the classes *Alphaproteobacteria* and *Gammaproteobacteria* were, however, not identified taxonomically [13].

Anaerobic iron-oxidizing prokaryotes. The group of anaerobic microorganisms capable of Fe(II) oxidation coupled to nitrate reduction is more numerous and more diverse taxonomically and phylogenetically than the group of aerobic neutrophilic Fe-OM (Fig. 1). This group comprises members of the classes *Alpha-, Beta, Gamma-*, and *Deltaproteobacteria*, as well as one hyperthermophilic archaeon, *Ferroglobus placidus* (growth range of 65–95°C), belonging to the order *Archaeoglobales* within the phylum *Crenarchaeota* (Table 2). This is the only strict anaerobe within this group, while all other species are facultative anaerobes.

The terminal electron acceptors used for anaerobic Fe(II) oxidation are nitrate, nitrite, nitrous oxide, and for some species, also perchlorate or chlorate.

Dinitrogen or the intermediate products of nitrate reduction (nitrites and traces of nitrous oxide) are the terminal products of this process. One nitrate reducer, *Geobacter metallireducens*, reduces nitrate to ammonium. Apart from anaerobic Fe(II) oxidation, *Geobacter metallireducens* is capable of Fe(III) reduction with H_2 as an electron donor. In the course of bacterial Fe(II) oxidation, perchlorate and chlorate are reduced to chlorine [33].

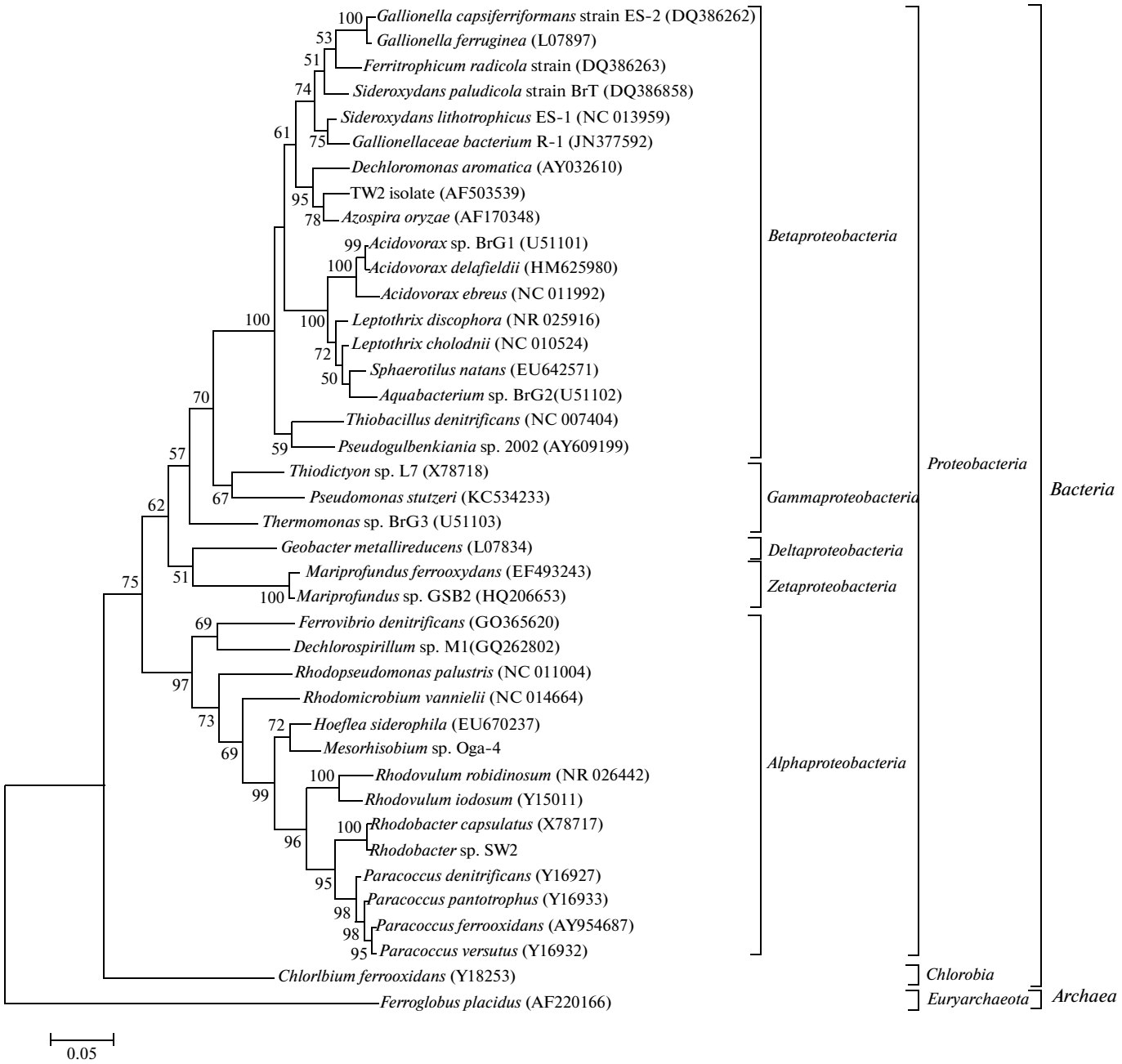

Fig. 1. Phylogenetic tree constructed using the 16S rRNA gene sequences. The scale is 5 nucleotide replacements per 100 nucleotides. The branching order determined by bootstrap analysis of 1000 alternative trees is indicated by the numerals.

Most of the nitrate-reducing Fe-OM can grow only in the presence of an organic cosubstrate in the medium and possess mixotrophic or lithoheterotrophic metabolism. For some species, however, capacity for lithoautotrophy was shown. These are the obligate autotroph *Thiobacillus denitrificans*, a facultative anaerobic bacterium carrying out the oxidation of reduced sulfur compounds with nitrate or oxygen as reducers. *T. denitrificans* was among the first microorganisms for which autotrophic growth due to nitrate-dependent Fe(II) oxidation was shown [18]. *Geobacter metallireducens, Ferroglobus placidus, Paracoccus ferrooxidans*, and *Pseudogulbenkiania* sp. 2002 are also facultative autotrophs.

Molecular genetic confirmation of capacity for autotrophy via the reductive acetyl–CoA pathway have been obtained only for *Ferroglobus placidus* (Fig. 1) [34]. Specific RuBisCO genes associated with the reductive pentose phosphate cycle were identified in *Dechloromonas aromatica* cells growing by Fe(II) oxidation coupled to nitrate reduction, although

Table 2. Anaerobic iron-oxidizing bacteria

Name*	Morphology	Source of isolation	Electron acceptors		Type of metabolism	Year, reference
			for Fe(II) oxidation	alternative		
Geobacter metallireducens	Curved rods	Freshwater sediments	NO_3^-	ND	Autotrophic, mixotrophic	1993 [14]; 2001 [15]; 2002 [16]
Ferroglobus placidus	Cocci	Deep-water marine hydrotherms	NO_3^-	$S_2O_3^{2-}$	Autotrophic, mixotrophic	1996 [17]
Thiobacillus denitrificans	Curved rods	ND	NO_3^-	O_2	Autotrophic	1996 [18]; 2009 [19]
Strain HidR2	Rods	Marine sediments	NO_3^-, NO_2^-	O_2	Mixotrophic	1998 [20]
Azospira oryzae	″	Wastewater	NO_3^-, ClO_4^-	ND	″	2001 [21]
Dechloromonas aromatica RCB	″	ND	NO_3^-, ClO_4^-	O_2	″	2001 [22]
Acidovorax sp. BrG1	″	Freshwater sediments	NO_3^-, NO_2^-, N_2O	O_2	″	2004 [23]
Aquabacterium sp. BrG2	Curved rods	″	ND	O_2	″	″
Thermomonas sp. BrG3	Rods	″	NO_3^-, NO_2^-	ND	″	″
Acidovorax sp. BoFeN1	″	″	NO_3^-, NO_2^-, N_2O	ND	″	2005 [24]
Paracoccus ferrooxidans BDN-1	Curved rods	Bioreactor	NO_3^-, NO_2^-, N_2O, $[Fe(II)EDTA \cdot NO]_2^-$	O_2, $S_2O_3^{2-}$	Autotrophic, mixotrophic	2009 [26]
Paracoccus denitrificans	″	Freshwater	NO_3^-	O_2,	Mixotrophic	2006 [25]
Paracoccus pantotrophicus	″	ND	″	O_2,	″	″
Paracoccus versutus	″	ND	″	O_2,	″	″
Pseudomonas stutzeri	″	Human biological fluids	″	O_2,	″	″
Pseudogulbenkiania sp. 2002	Curved rods	Freshwater sediments	NO_3^-, NO_2^-, N_2O	O_2,	Autotrophic, mixotrophic	2006 [27]
Acidovorax ebreus TPSY	Rods	Freshwater underground spring	NO_3^-, NO_2^-, N_2O	O_2,	Mixotrophic	2010 [28]
Acidovorax sp. 2AN	″	Freshwater sediments	NO_3^-	O_2,	″	2011 [29]
Dechlorospirillum sp. M1	″	ND	″	O_2,	″	2011 [30]
Hoeflea siderophila	Curved rods	Brackish mineral spring	NO_3^-, N_2O	O_2,	Mixotrophic, organoheterotrophic	2012 [31]
Ferrovibrio denitrificans	Vibrio-shaped	Freshwater spring	″	O_2,	″	2012 [32]
Mesorhizobium sp. Oga-4	Rods	″	NO_3^-	O_2,	″	ND

Table 3. Neutrophilic phototrophic iron-oxidizing bacteria

Name*	Morphology	Source of isolation	Alternative electron donors	Type of metabolism	Year, reference
Thiodictyon sp. L7	Rounded-end rods	Bog	H_2, org. comp.	Photoautotrophic, photoheterotrophic	1994 [36]
Rhodobacter sp. SW2	ND	Freshwater spring	"	"	1994 [36]
Rhodomicrobium vanniellii BS-1	Ovoid cells with prosthecae	Freshwater spring	HS, H_2, org. comp.	"	1998 [37]
Rhodovulum robiginosum	Curved rods	Marine silts	$S_2O_3^{2-}$, HS, S^0, H_2	Photoautotrophic	1999 [38]
Rhodovulum iodosum	"	"	"	"	1999 [38]
Chlorobium ferrooxidans	"	Wastewater	ND	Photoheterotrophic	1999 [39]
Thiodictyon sp. F4	Rounded-end rods	Bog	ND	Photoautotrophic, photoheterotrophic	2004 [40]
Rhodopseudomonas palustris TIE-1	Curved rods	Freshwater spring	H_2, $S_2O_3^{2-}$	"	2005 [41]
Rhodobacter capsulatus	"	"	ND	Photoheterotrophic	2009 [42]

capacity for autotrophic growth was not confirmed for this organism.

Apart from its role as the terminal electron acceptor for growth, Fe(II) oxidation with nitrate, an intermediate of nitrate reduction, may have other functions which will be discussed further.

Phototrophic iron-oxidizing prokaryotes. Light-dependent anaerobic Fe(II) oxidation was first observed in 1993 by German researchers studying the phototrophic purple bacterium *Rhodomicrobium vanniellii* [35, 36]. Unlike chemotrophic microorganisms, Fe(II) oxidation by phototrophic bacteria is carried out in the absence of alternative electron acceptors and uses the energy of light according to the equation

$$4Fe^{2+} + CO_2 + 11H_2O + h\nu$$

$$\rightarrow CH_2O + 4Fe(OH)_3 + 8H^+.$$

Phototrophic Fe-OM have been found within the phyla *Chlorobia* and *Proteobacteria* (Table 3). Most of them belong to the family *Rhodobacteriaceae* of the class *Alphaproteobacteria*. *Rhodovulum robiginosum, Rhodovulum iodosum, Rhodobacter* sp. SW2, *Rhodopseudomonas palustris, Rhodopseudomonas capsulatus*, and *Rhodomicrobium vannielli* belong to the family *Hyphomicrobiaceae* of the class *Alphaproteobacteria*. Two *Thiodiction* strains, L7 and F4 belong to the class *Gammaproteobacteria*. *Chlorobium ferrooxidans* (class *Chlorobia*) is the only known member of green bacteria capable of phototrophic Fe(II) oxidation.

Fe(II) oxidation in the light in the absence of alternative electron donors (H_2 or reduced sulfur compounds) supports growth of most phototrophic bacteria only in the presence of organic compounds (Table 3). Among phototrophic Fe-OM, only *Rhodobacter* sp. SW2 [43], *Rhodopseudomonas palustris* [44], and two *Rhodovulum* species, *Rh. robiginosum* and *Rh. iodosum* are capable of photoautotrophic growth with Fe(II) as the only electron donor (Table 3).

Apart from its role in energy metabolism, Fe(II) oxidation by phototrophs may have other functions. For instance, *Rhodopseudomonas capsulatus* probably gains advantages from Fe(II) oxidation not associated with energy metabolism [42]. The hypothetical detoxication mechanism is, however, not completely understood.

PHYSIOLOGICAL AND MOLECULAR BIOCHEMICAL ASPECTS OF Fe(II) OXIDATION

Compared to other inorganic energy sources, Fe(II) provides for the lowest yield of free energy (ΔG). In an acidic environment, it is 29 kJ/mol iron. Chemical oxidation of Fe(II) with oxygen at neutral pH results in twice higher values, while at low partial oxygen pressure ΔG increases to 90 kJ/mol Fe [45]. Thus, neutrophilic lithotrophic iron-oxidizing bacteria using Fe(II) as an energy substrate have an obvious advantage compared acidophilic iron oxidizers.

Kinetics of Fe(II) oxidation at neutral pH is another factor affecting the growth of neutrophilic

lithotrophs under aerobic conditions. While abiotic Fe(II) oxidation in oxygenated water does not exceed 1 min, it is more than two orders of magnitude (300 times) lower under microaerobic conditions. Chemical processes of Fe(II) oxidation dominate over the microbiological ones only at O_2 concentrations above 50 μM (≈1.5 mg/L) [43]. These factors are responsible for development of aerobic neutrophilic Fe-OM under microaerobic conditions when competition with the chemical process is suppressed. Comparative study of the rates of Fe(II) oxidation by *Sideroxydans paludicola, S. lithotrophicus*, strain TW-2, and the facultative aerobe *Hoeflea siderophila* revealed that at low O_2 concentrations not exceeding 3–10 μM (0.1–0.3 mg/L), bacterial activity was responsible for up to 90% of Fe(II) oxidation [46–48]. Investigation of comparative rates of abiotic and biological processes in model systems, ferriferous mats, and in laboratory cultures using specific inhibitors of cell respiration or nonspecific inhibitors of bacterial processes yielded similar results [46, 48, 49].

Formation of insoluble products of Fe(II) oxidation (amorphous or crystalline structures of protoferrihydrite and ferrihydrite) is one of the serious problems encountered by neutrophilic Fe-OM. Fe(III) hydroxides formed as a result of Fe(II) oxidation encrust the cells, impairing the contact between cell surface and the environment. Microorganisms use a number of strategies in order to prevent accumulation of Fe(III) hydroxides on the cells. Production of extracellular polysaccharides forming stalks or sheaths and binding the newly produced Fe(III) compounds is the most common way of hydroxide removal. Such polysaccharide encrustation is characteristic of freshwater and marine species of *Gallionella, Sideroxydans, Ferritrophicum, Mariprofundus*, strain R1 belonging to the family *Gallionellaceae* (Fig. 1), and many organohe-terotrophic organisms. Some aerobic and anaerobic species not excreting polysaccharide sheaths or stalks (e.g., strain TW-2) were reported to produce organic chelating agents, which bind Fe(III) transferring it into a soluble form [8, 43, 50, 51]. Excretion of low-molecular organic compounds into the microzone surrounding the cell in order to remove Fe(III) by binding with formation of soluble compounds was suggested for anaerobic nitrate reducers and phototrophs [51, 52]. In some environments, naturally occurring organic compounds, e.g., humic acids, may probably play a similar role. No accumulation of insoluble Fe(III) hydroxides was observed in laboratory cultures with organic complexes, such as [Fe(II)-EDTA], used as Fe(II) source, since iron oxidation was accompanied by formation of the soluble [Fe(III)-EDTA] complex. However, growth of many known anaerobic nitrate-reducing and phototrophic Fe-OM both in natural conditions and in laboratory media is accompanied by encrustation of the cells with the products of Fe(II) oxidation, often resulting in formation of extensive iron-encrusted mats or sediments consisting of iron-encrusted cells [6, 24, 52].

Interaction of the cells with insoluble Fe(II) compounds in the course of nitrate-dependent Fe(II) oxidation is another challenge encountered by Fe-OM. Washed cells of *Azospira oryzae* were able to oxidize insoluble compounds, including such silicate minerals as almandine $Fe_3Al_2(SiO_4)_3$ [21]. The mechanisms of interaction between the cells and insoluble electron donors in such biological reactions have not been elucidated.

Products of Fe(II) oxidation. The products of Fe(II) oxidation by aerobic and anaerobic microorganisms are amorphous Fe(III) hydroxides, weakly crystalline protoferrihydrite, or crystalline mineral ferrihydrite. These Fe(III) compounds are precursors of a number of minerals (goethite, hematite, and magnetite) [18, 21, 48]. Extracellular magnetite may be responsible for up to 25% of the oxidized Fe(II). In *Azospira oryzae*, the ratio of the terminal products (maghemite and magnetite) in the medium depended upon the kinetics of Fe(II) oxidation: accumulation of the latter prevailed when the rate of the oxidative processes was lower.

MOLECULAR BIOCHEMICAL MECHANISMS OF Fe(II) OXIDATION

Data on the electron transport chain (ETC) structure and functioning in the course of Fe(II) oxidation by some aerobic and anaerobic microorganisms became available recently. Although the structure and components of the systems of electron transfer from Fe(II) to the electron acceptor differ in different species, the overall organization of their electron transport systems shares some features not found in other groups of microorganisms. For example, instead of the ETC components located horizontally along the cytoplasmic membrane, in Fe-OM they are oriented vertically inside the cell. Due to such ETC orientation, electron transfer from Fe(II) via specific Fe oxidases results in retention of Fe(III), the oxidation product, at the outer cell surface. Thus, Fe(III) does not penetrate into the cytoplasm, making it possible to prevent both oxidative stress resulting from reactive oxygen species (ROS) formation and intracellular accumulation of insoluble Fe(III) mineral compounds.

Analysis of the genome sequences of investigated Fe-OM used in the study of the mechanisms of Fe(II) oxidation made it possible to identify the gene clusters involved in the process, as well as the genes encoding the electron-transporting proteins and specific cytochrome oxidases. The enzyme complexes isolated from *Sideroxydans lithotrophicus* were used for investigation of kinetics of oxidation of the soluble iron complexes and the effect of physicochemical parameters on the oxidative processes [44, 53]. While not all ETC components of Fe-OM have been studied in detail, some hypothetical pathways of Fe(II) oxidation have

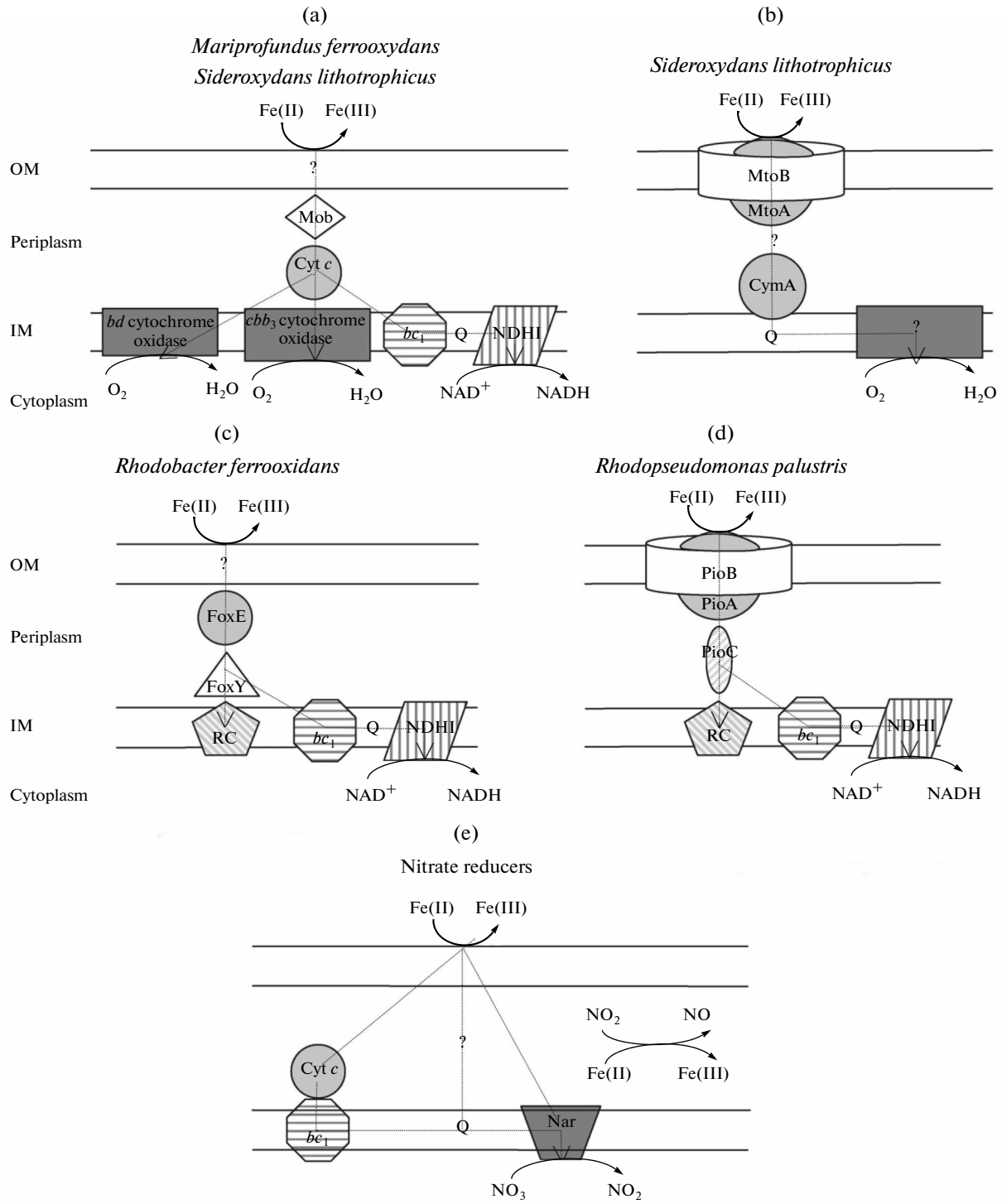

Fig. 2. Pathways of iron oxidation by lithotrophic aerobic (a, b), anaerobic phototrophic (c, d), and anaerobic nitrate-dependent (e) microorganisms: *Mariprofundus ferrooxidans* and *Sideroxydans lithotrophicus* (a); *Sideroxydans lithotrophicus* (b); *Rhodobacter ferrooxidans* (c); *Rhodopseudomonas palustris* (d); and nitrate reducers (e). Each family of the proteinaceous redox complexes is schematically represented by a geometric figure [54].

been proposed for the members of various physiological groups. These results are summarized on Fig. 2 [54]. In marine aerobic bacteria [55], the electrons obtained from Fe(II) oxidation by specific oxidases localized at the cell surface are transferred further by the periplasmic transport proteins and periplasmic

cytochromes *c*, and subsequently to the terminal cytochrome oxidases cbb_3 and/or *bd* at the inner membrane surface for O_2 reduction and to the bc_1 complex for NADH synthesis in *Mariprofundus ferrooxidans* or to the quinone pool in *Sideroxydans lithotrophicus* [53]. *Gallionella capsiferriformans* was shown to have a similar composition of the ETC components [53]. The electron transport pathways of the phototrophic species *Rhodobacter* sp. SW-2 [56] and *Rhodopseudomonas palustris* [44], which exhibit certain peculiarities, are shown on Figs. 2c and 2d.

The ETC components in Fe(II)-oxidizing nitrate reducers is insufficiently studied. Coupling of cytochrome *c* reduction to Fe(II) oxidation was shown for *Dechloromonas agitata* [33], *Azospira oryzae* [21], and *Pseudogulbenkiania* sp 2002 [58, 59]. Four mechanisms of Fe(II) oxidation were proposed based on the generalized results of physiological and biochemical investigations of nitrate reducers [60]. They include participation of (1) nonspecific Fe oxidoreductases, (2) nonspecific nitrate reductases, (3) the *bc* complex transferring electrons from Fe(II) to the quinone pool, and (4) abiotic reactions between Fe(II) and nitrite (Fig. 2e) [59–61]. The degree to which the latter mechanism (chemical reduction of ferrous iron with nitrite) is pronounced depends on a number of factors, including Fe(II) concentration, pH, and the presence of chelating compounds in the medium. The relative contribution of abiotic oxidation is rather variable, so that it may be responsible for up to half Fe(III) formed. Since no nitrite accumulation occurs in the cultures of nitrate reducers during organotrophic growth in the absence of reduced iron, two explanations were suggested for its accumulation associated with Fe(II) oxidation. The first one is competition for the electron donor in a branched ETC: electrons from Fe(II) are transported to nitrate, rather than to nitrite. The alternative mechanism suggests diffusion of Fe(II) into the periplasm and its sorption on the periplasmic nitrite reductase, resulting in its inhibition [61]. The mechanisms of biochemical reactions of Fe(II) oxidation involving nitrate and intermediate products of nitrate reduction are presently insufficiently understood and should probably attract more attention in the future.

ROLE OF NEUTROPHILIC IRON-OXIDIZING LITHOTROPHS IN THE OXIDATIVE PROCESSES OF THE BIOGEOCHEMICAL CYCLE OF IRON

Occurrence and abundance in various ecosystems. Aerobic Fe-OM are presently most common in aquatic ecosystems with elevated Fe(II) concentrations. In iron-enriched natural waters, they have been found at the redox zone of the hypolimnion, in the sediments of chalybeate springs, subterranean springs, in marine zones of hydrothermal and volcanic activity at the sites of Fe(II) influx, in oceanic deep-sea basalts, in industrial water treatment installations, and in plant rhizosphere [62]. At the hydrothermal sites in the oceans, mass development of aerobic Fe-OM may occur at temperatures of up to 50°C [63–67].

Development of microaerophilic lithotrophic Fe-OM results in the formation of iron-encrusted mats or bottom sediments, which sometimes become tens of centimeters thick. The biological origin of these sediments may be easily confirmed by microscopic analysis: they contain the morphologically conspicuous structures of the known members of this group (*Gallionella* and *Mariprofundus*). At the sites of oceanic hydrotherms, biogenic ochreous sediments may spread over hundreds square meters.

Due to the difficulty of laboratory cultivation of microaerophilic iron-oxidizing bacteria, with the cultivation techniques for many morphotypes observed in fresh samples still undeveloped, inoculation of selective media is practically unsuitable for quantitative assessment of these organisms. Laboratory cultivation in selective nutrient media may be, however, successfully used for enumeration of anaerobic iron-oxidizing bacteria.

Analysis of the 16S rRNA clone libraries is widely used to determine the species diversity, abundance, and composition of freshwater and marine communities of iron-oxidizing bacteria. In freshwater communities, the overwhelming majority was found to belong to the class *Betaproteobacteria*; 25–57% and 10–25% of the clones were closely related to *Gallionella* and *Sideroxydans* species, respectively [68–70]. According to the results of analysis of the clone libraries, up to 45% of the iron-oxidizing microorganisms from the iron-rich (chalybeate) sediments collected at various areas of the Pacific Ocean were identified as members of the class *Zetaproteobacteria* related to *Mariprofundus ferrooxidans*; they constituted up to 22% of the total cell number, while the *Betaproteobacteria* were not revealed [67].

The data on occurrence and abundance of anaerobic Fe-OM are scarce. These organisms are associated with the sediments of lakes, chalybeate springs, and seas, including the areas of high-temperature hydrotherms. In the populations from the bottom sediments of freshwater lakes, up to 10^5 cells/mL sediment was revealed by enumeration on nutrient media, while in situ hybridization with specific probes showed that their number did not exceed 0.2% of the total number of nitrate reducers. Analysis of the clone libraries obtained from bottom sediments revealed bacteria closely related to the genera *Geobacter* and *Dechloromonas* (>95% similarity) [71–73].

Numbers of anaerobic Fe-OM in the bottom sediments of freshwater and saline chalybeate springs was 10^5–10^7 cells/mL. The facultatively anaerobic nitrate-reducing Fe-OM isolated recently from low-temperature and moderately thermal chalybeate springs

belonged to both known and new taxa within the class *Alphaproteobacteria* [31, 32, 48].

Anaerobic phototrophic Fe-OM are presently restricted to the hypolimnion and a thin upper layer of the bottom sediments in stratified lakes. Their numbers are comparatively low, ~0.01% of the bacterial populations [71].

Role in Fe(II) oxidation in modern aquatic ecosystems. In natural environments, oxidative transformation of Fe(II) compounds at neutral pH may occur rapidly due to both biological processes carried out by neutrophilic Fe-OM and abiotic chemical reactions. Kinetics of these oxidative processes is determined by the concentrations of Fe(II) and oxygen. The few works on the rates of bacterial Fe(II) oxidation in slightly contaminated subterranean waters and underground springs revealed them to be, on average, 45% of the rate of chemical oxidation [74–76], varying from 28 to 75% depending on O_2 concentration in the water [47, 76]. In model systems imitating the Fe(II) and O_2 concentrations of natural waters, the highest rate of bacterial oxidation was observed under microaerobic conditions, while under aerobic conditions chemical oxidative processes prevailed due to dependence of the kinetics of abiotic oxidative reactions from O_2 concentration [48]. High rates of chemical Fe(II) oxidation under oxic conditions and insufficient data available make it difficult to assess the relative contribution of the biological and abiotic processes in formation of ferric iron deposits in present-day sediments.

No data are available concerning the rates of biological Fe(II) oxidation in anoxic ecosystems.

Fractionation of the $^{56/54}$Fe stable isotopes is one of the approaches to assessing the role of the biological factor in the oxidative reactions of the geochemical iron cycle. This technique makes it possible to determine the rates and scales of the microbial and abiotic processes. Measurement of the rates of oxidative reactions in combination with iron isotope fractionation in pure cultures and model aquatic systems depending on the oxygen regime and the presence of an alternative electron acceptor (nitrate) revealed that Fe(III) was most enriched with the light isotope ^{54}Fe under anoxic conditions (4.7–8.8 times compared to the isotopic composition of the original ferrous iron) and under microaerobic conditions at 0.1–0.3 mg/L O_2 (2–8.8 times). At high rates of oxidative processes occurring under free access of oxygen, iron isotope fractionation was less pronounced, although in the variants of biological Fe(II) oxidation it remained 1.3–2.9 times higher. The rates of the biological reactions in water samples were also 1.2–2.5 times higher than the rates of the chemical processes. Enrichment of the sediments in chalybeate springs and surrounding wetlands with light ^{54}Fe also indicates the major role of biological processes in their formation. These results suggest that under microaerobic conditions at the redox zone boundary and in the deeper anoxic horizons, the contribution of biotic Fe(II) oxidation should be much more pronounced than that of abiotic oxidation.

Role of neutrophilic FeOB in formation of ancient deposits of iron ores. The data presented above show that the ability to oxidize Fe(II) is widespread among lithotrophic prokaryotes of the domains *Bacteria* and *Archaea*. This process may be carried out within a broad range of physicochemical conditions, including extreme ones. It was suggested that utilization of Fe(II) in dissimilation processes as an energy source could be an ancient type of catabolism, which probably developed independently more than once in the course of evolution [54].

Discovery of new types of metabolism of Fe-OM—those which do not require oxygen—is important for the fields of Earth science investigating the origin of the most ancient deposits of iron-silicate ores, the so-called Banded Iron Formation (BIF). According to the results of paleoecological investigation, their formation in the ancient ocean occurred 2.5–2.7 Ga ago in the late Archaean and early Proterozoic eons (Fig. 3) [35]. These ancient iron ore formations are found on all continents; the Kryvbas deposit (Ukraine) is an example. Early models associated BIF formation with Fe(II) oxidation in abiotic processes of photochemical oxidation. Latter models implied significant role of oxygenic photosynthesis by cyanobacteria. Later, since free oxygen appeared in the atmosphere later (~2.5 Ga ago), and its production by aerobic phototrophs (cyanobacteria) was insufficient at this early stage, microbial processes of anaerobic oxidation of Fe(II) dissolved in ocean water were considered responsible for BIF formation. While aerobic lithotrophic microorganisms, as well as cyanobacteria, could participate in formation of the ancient iron ores, these processes were local, restricted to the zones of light penetration and oxygen production in the shallow areas of the ocean (Fig. 3). The model of BIF formation with iron precipitation due to the activity of anoxygenic phototrophs [24, 35] and anaerobic nitrate-dependent iron-oxidizing bacteria [17, 21] is presently considered more sound. Nitrate-dependent Fe(II) oxidation is accompanied by the formation of magnetite Fe_3O_4 and hematite Fe_2O_3, the major minerals forming the ancient ore deposits [17, 21, 24, 35, 48]. The electron acceptor (NO_3^-) probably arrived as a result of abiotic reactions of disproportionation of the atmospheric nitrogen compounds [77]. Although Fe(II) oxidation by phototrophs results in formation of other minerals (goethite and lepidocrocite, as well as amorphous Fe(III) hydroxides) [43], these minerals are known to undergo subsequent diagenetic transformations by iron reducers, another important metabolic group of microorganisms of the iron cycle. Microbial reduction of Fe(III) oxides and crystalline minerals was shown to result in magnetite formation [78].

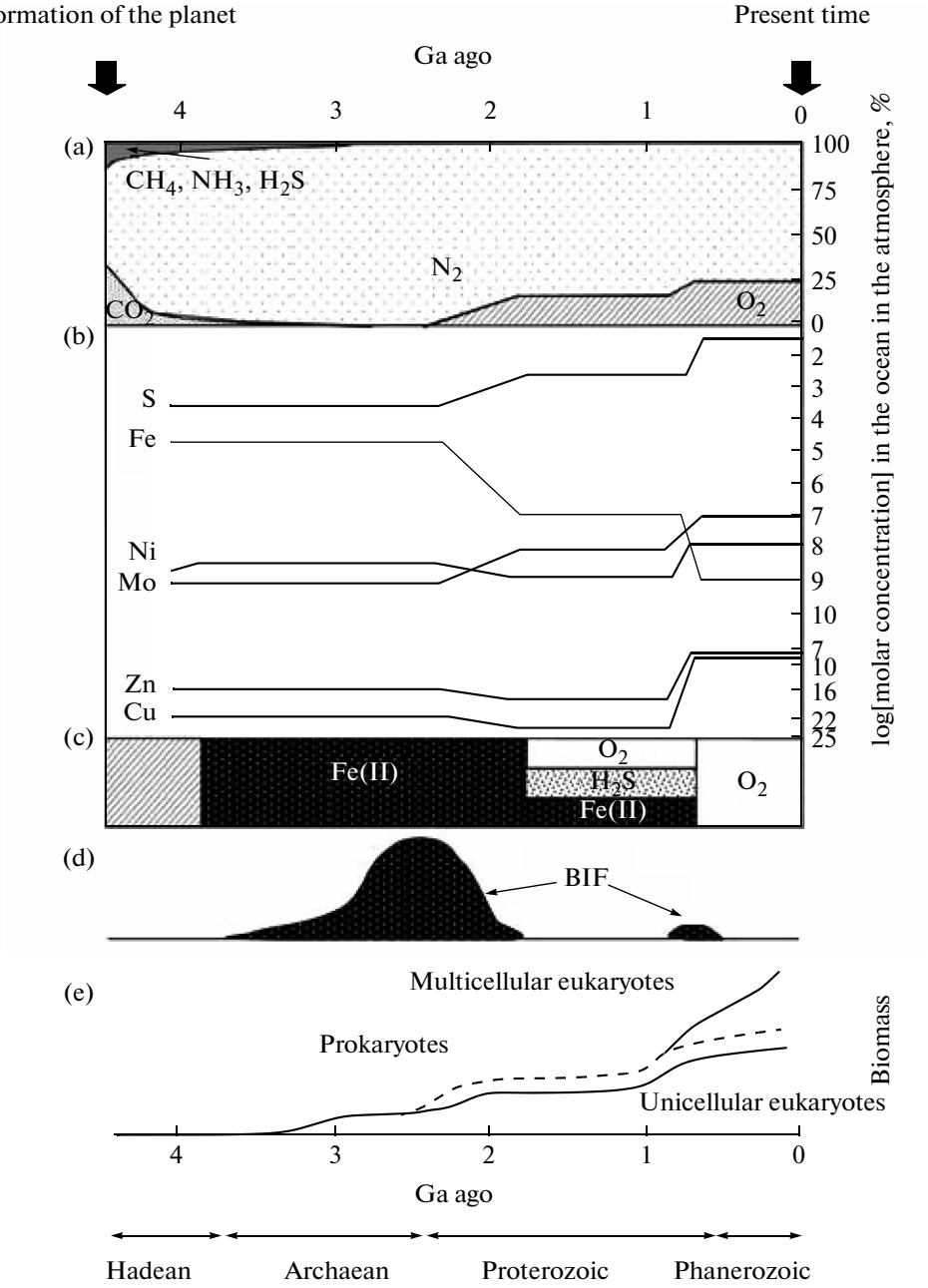

Fig. 3. Biogeochemical evolution of Earth. Occurrence of some elements in the atmosphere (a) and in the ocean (b); chemical composition of oceanic water (c); formation of sedimentary iron ores, BIF (d); evolution of life from prokaryotes to multicellular eukaryotes (e) [54, 81, 82].

It is reasonable to suggest that formation of ancient iron deposits of the oxygen-free time was a biogenic process involving the communities of phototrophs, nitrate-dependent anaerobic Fe-OM, and iron reducers, each of which contributed to BIF formation.

Detection of microfossils may be a confirmation of the role of microorganisms in formation of the ancient sedimentary iron ores. Thus, well-preserved microscopic structures typical of the morphologically conspicuous aerobic marine iron-oxidizing bacteria of the genus *Mariprofundus*, twisted iron-encrusted stalks, were found in the samples of iron ores of various ages: in ancient (0.5 Ga) deposits of hydrothermal areas [79], in Phanerozoic rocks associated with marine hydrotherms in Ireland (Fig. 3) [80], and in the Gunflint Range Proterozoic deposits [81, 82]. Apart from microfossils, paleobiological data, such as detection of the biomarkers, organic compounds with structural

similarity to the molecules found in modern organisms—which may be used for identification of specific groups—also confirm the biogenic origin of iron deposits. For example, lipid derivatives 2-α-methylhopanoids have been initially considered an evidence of aerobic photosynthesis and were used as indicators of cyanobacteria. These organic compounds were recently found in anoxygenic phototrophs [83] and in strictly anaerobic bacteria *Geobacter surfurreducens* [84].

Anaerobes were also found to produce steranes via the anaerobic pathway of H_2O_2 utilization [54, 85, 86]. These compounds have been previously considered specific mediators of aerobic organisms. Molecular fossils of steranes were found in Archaean iron ores.

Okenane, a biomarker for precursors of the carotenoid pigment okenone, has been found only in purple sulfur bacteria, including the phototrophs capable of anaerobic Fe(II) oxidation [24]. It was identified in the ancient iron ores of Australian BIF [87].

Paleontological investigation of a broader range of subjects may be required for better understanding of the evolution and biogeochemistry of the iron cycle.

In conclusion, it should be noted that low energy efficiency of the reactions of biogenic iron oxidation prevents neutrophilic lithotrophic Fe-OM from contributing considerably to the primary production in aquatic ecosystems. Their role in large-scale geochemical processes of the iron cycle both presently and in the ancient (Proterozoic and late Archaean) oceans is beyond doubt. Although the mechanisms and relative importance of the biological processes contributing to formation of Precambrian iron ores are as yet poorly understood, the paleobiological data presented above (microfossils and specific biomarkers) indicate the mostly biogenic origin of these ores.

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