ISSN 0026-2617, Microbiology, 2012, Vol. 81, No. 1, pp. 90–97. © Pleiades Publishing, Ltd., 2012.
Original Russian Text © G.A. Dubinina, A.Yu. Sorokina, M.V. Gapeeva, A.V. Dolotov, 2012, published in Mikrobiologiya, 2012, Vol. 81, No. 1, pp. 96–104.

EXPERIMENTAL ARTICLES

Communities of Neutrophilic Iron-Oxidizing Microorganisms of Ferruginous Springs of Various Types and Their Involvement in Fractionation of Stable Iron Isotopes

G. A. Dubinina[a, 1], A. Yu. Sorokina[a], M. V. Gapeeva[b], and A. V. Dolotov[c]

[a] *Winogradsky Institute of Microbiology, Russian Academy of Sciences, pr. 60-letiya Oktyabrya 7, k. 2, Moscow, 117312 Russia*

[b] *Papanin Institute of Biology of Inland Waters, Borok, Yaroslavl region, 152742 Russia*

[c] *Borok Geophysical Observatory, Branch of the Schmidt Institute of Physics of the Earth, Russian Academy of Sciences, Borok, Yaroslavl region, 152742 Russia*

Received May 30, 2011

Abstract—Abundance and structure of the communities of neutrophilic lithotrophic iron-oxidizing bacteria (FeOB) inhabiting four low-mineralized ferruginous springs of the Marcial Waters Resort (South Karelia, Russia) and the brackish chalybeate spring of the Staraya Russa Resort (Novgorod region, Russia), were investigated, as well as the physicochemical conditions of these environments. In fresh iron-containing precipitates collected near the spring outlets and within the spring-discharge areas, as well as along the spring watercourses, the numbers of unicellular FeOB enumerated on nutrient media ranged from 10^5 to 10^7 cells per 1 mL of sediments irrespective of the initial Fe(II) concentration (11–126 mg L^{-1}). In the spring waters and along the spring watercourses inhabited by iron-oxidizing bacteria, the concentration of dissolved oxygen did not exceed 0.05–0.1 mg L^{-1} . Unicellular FeOB were predominant in three springs, while in the springs with relatively low Fe(II) concentrations (11–22 mg L^{-1}), various morphological forms of *Gallionella* and uncultured forms of the iron-oxidizing bacterium *Toxothrix trichogenes* prevailed. In the model experiments with the water samples collected in the ferruginous springs and bogs under controlled conditions, the fractionation of stable iron isotopes and the rate of iron oxidation were found to depend on the oxygen regime and, to a lesser extent, on the initial Fe(II) concentration. The maximum enrichment of the iron oxides formed during the simulation experiments with the light ^{54}Fe isotope was observed during bacterial oxidation under microaerobic conditions at O_2 concentrations of 0.1–0.3 mg L^{-1} and in the cultures of iron-oxidizing bacteria. During the abiogenic oxidation of Fe(II), the extent of stable isotope fractionation was 1.5–2 times lower. Enrichment of Fe(III) oxides with the light ^{54}Fe isotope (3- to 5-fold) was considerably lower at high rates of both the biogenic and abiogenic processes of iron oxidation under aerobic conditions; however, it was more intense during the bacterial processes. Comparison of the rates of enrichment of Fe(III) oxides with the light isotope during the model experiments with pure and enrichment cultures of iron-oxidizing bacteria from the sediments of ferruginous springs and bogs revealed that the biogenic factor plays a key role in the oxidation processes of the iron cycle, as well as in the differentiation of the composition of stable iron isotopes in the studied ecosystems.

Keywords: neutrophilic iron-oxidizing bacteria, stable iron isotopes, fractionation.

DOI: 10.1134/S0026261712010055

In nature, oxidative transformation of Fe(II) compounds at neutral pH may occur at high rates both via biogenic pathways (mediated by neutrophilic iron-oxidizing microorganisms) and abiogenic chemical reactions. Due to the high rates of oxidative chemical reactions in the presence of oxygen, as well as to the ambiguity of the results of investigations and the scarcity of relevant data, evaluation of the relative role of biogenic and abiogenic processes in the precipitation of oxidized Fe(III) in the sediments of modern water bodies is among the most complex problems [1, 2].

In the last decade, a great deal of attention has been devoted to the study of the involvement of stable iron isotopes in geochemical and biogeochemical processes. This interest is, first of all, due to the possibility of using iron isotopes as biosignatures for analyzing and understanding the origin of modern and ancient (Precambrian) sedimentary iron ores on Earth, as well as for studying Martian iron deposits [3–8]. However, the present knowledge of the fractionation of stable iron isotopes is insufficient for reliable interpretation of the obtained results. This is primarily due to the scarcity of data on the mechanisms of the abiogenic and biological processes of differentiation of the isoto-

[1] Corresponding author; e-mail: gdubinina@mail.ru

pic composition of compounds in the course of transformations of various forms of iron. Data on the factors controlling differentiation of the isotopic composition of iron compounds under natural conditions are scarce as well.

In the present work, the results of comparative analysis of the rates of bacterial and abiogenic processes and their role in the transformation of the composition of stable iron isotopes ^{56}Fe and ^{54}Fe in low-temperature mineral ferruginous springs are presented. For this purpose, the effect of the oxygen content and the initial Fe(II) concentration on the rates of the oxidative processes, as well as on differentiation of the isotopic composition of the resultant Fe(III) oxides, was determined in situ during a series of model experiments with natural water samples. The isotopic composition of water and Fe(III) oxides in the samples of bottom sediments of mineral ferruginous springs and adjacent bogs fed by the springs with various types of water genesis and with different chemical compositions was determined. The composition of the microbial community involved in the reactions of the oxidative branch of the biogeochemical iron cycle was determined; the processes of differentiation of the stable iron isotopic composition in bacterial cultures were analyzed as well.

MATERIALS AND METHODS

Sources of isolation. The subjects of study were four low-temperature mineral ferruginous springs of the Marcial Waters Resort (South Karelia, Russia), as well as the brackish chalybeate spring of the Staraya Russa Resort (Novgorod region, Russia).

The Marcial Waters Resort is located in the southern part of the Baltic Sea basin, in the zone of the regional Archean–Proterozoic tectonic fault. Its mineral springs with pressure filtration are confined to the zone of intense fracturing. Two water-bearing systems were studied, one of Quaternary deposits and another of Proterozoic rocks (responsible for the formation of mineral waters with high iron content). Water discharges from the springs into the gutters and then to the surrounding bogs (springs nos. 2–4), or directly to the adjacent bog (spring no. 1). Ochreous sediments consisting of Fe(III) oxides are deposited near the capped spring outlets and along the spring watercourses. In the studied springs, the total mineralization of water varied from 0.3 to 0.9 g L^{-1} . The concentration of dissolved oxygen did not exceed 0.05–0.1 mg L^{-1}; the concentration of Fe(II) ranged from 16 to 126 mg L^{-1} . In summer, the water temperature near the spring outlets ranged from 6.1 to 9.1°C; the temperature of fresh Fe(III) oxide precipitates was 12–15°C. In addition to water samples and fresh iron-containing precipitates, samples were collected from the sediments of the gutters and the surrounding bogs. While located at a short distance (about 200 m) from each other, the springs are fed by deep waters from various horizons of different geologic ages and differ considerably in the chemical composition of their waters, primarily in the Fe(II) content. Due to this fact, comparative studies of these springs may serve as a convenient natural "laboratory" for model experiments aimed at elucidation of the effect of the chemical composition (especially of the concentrations of dissolved Fe(II) and O_2) on the rate of bacterial and abiogenic oxidative processes and on the differentiation of the isotopic composition of iron.

Spring no. 8 of the Staraya Russa Resort is allied with the water-bearing bed of the ancient Devon sea. The total mineralization of its water is 20 g L^{-1}; the concentration of dissolved oxygen is 0.1 mg L^{-1} . The Fe(II) content is 11 mg L^{-1} . The water temperature near the spring outlet was 11–14°C.

Nitrates were not detected in the ground waters of the studied springs.

Quantitative assessment of iron-oxidizing bacteria, their isolation and cultivation conditions. Quantitative assessment and cultivation of iron-oxidizing bacteria inhabiting low-mineralized springs were carried out using the medium containing the following (g L^{-1}): $(NH_4)_2SO_4$, 0.3; $CaCl_2 \cdot 6H_2O$, 0.05; $MgSO_4 \cdot 7H_2O$, 0.1; $NaHCO_3$, 0.3; 10% phosphate buffer (pH 7.0), 0.1; Hepes buffer (pH 7.0), 3.0; KNO_3, 0.3; CH_3COONa, 0.15; vitamins and trace elements [9]; Difco agar, 5.0; pH 6.8.

Isolation and enumeration of iron-oxidizing bacteria from the brackish spring were performed using the medium containing the following (g L^{-1}): NaCl, 20; NH_4Cl, 0.3; $CaCl_2 \cdot 6H_2O$, 0.3; $MgCl_2 \cdot 7H_2O$, 3; $NaHCO_3$, 0.5; 10% phosphate buffer (pH 7.0), 0.1; Hepes buffer (pH 7.2), 3.0; KNO_3, 0.3; CH_3COONa, 0.15; vitamins and trace elements [9]; Difco agar, 5.0; pH 7.0.

The cultivation temperature was 20°C. Before inoculation, the media were supplemented with the freshly prepared sterile FeS suspension (according to Kucera and Wolfe, 1957, modified by Hallbeck and Pedersen, 2005) [10] (0.2 mL per 10 mL of the medium) as a Fe(II) source. Inoculated media were incubated for 2–3 weeks.

The cultures were grown in Hungate tubes filled to capacity with freshly boiled agarized or liquid medium (see below). Bacterial growth was assessed by the formation of ochreous colonies or zones of Fe(III) oxide formation, as well as by microscopic examination.

The numbers of iron-oxidizing bacteria were determined by tenfold dilutions on the above-described media. Pure cultures of iron-oxidizing bacteria were obtained by transfers of individual colonies to agarized or liquid media.

Microscopic analysis. Fe(III) oxides from bacterial cultures and fresh iron-containing precipitates from the studied springs, as well as Fe oxides precipitated on

the surfaces of submerged glass slides and in slit peloscopes incubated for 1–2 days in the surface horizons of the sediments of ferruginous springs, were studied under a CX31 phase contrast microscope (Japan) and under a JEM-100C transmission electron microscope (JEOL, Japan) at the accelerating voltage of 80 kV. In the latter case, the preparations were fixed with 2% glutaraldehyde, placed on formvar-coated grids covered, and examined without staining.

Modeling of the microbiological and chemical oxidation of Fe(II) in the waters of the studied springs. Glass vials (500 mL) were filled to capacity with water within the spring-discharge areas using a rubber tube. To avoid contact with oxygen, the flasks were refilled several times and sealed with rubber stoppers. In each series of experiments, the control vials were supplemented with freshly boiled NaCl solution to the final concentrations of 5 and 10% for the water samples collected in springs nos. 1, 3, and 4 (Marcial Waters Resort) and spring no. 8 (Staraya Russa Resort), respectively, in order to inhibit the biological processes [11]. To assess the effect of the concentration of dissolved oxygen on the rates of iron oxidation, as well as on fractionation of the isotopic composition of iron, in each series of experiments the vials filled halfway with argon were supplemented with a known amount of sterile air according to the scheme described in [12]. Argon and air were filtered through 0.22-μm sterile ultrafilters (Millipore, United States). During 45-day (springs nos. 1, 3, and 8) and 90-day (spring no. 4) experiments, the ratio between the liquid and gas phases in the vials was 1 : 1. After 2–3 days from the onset of the experiments, the oxygen content in the gas phase was determined on a gas chromatograph. The samples were taken with a microsyringe; if the oxygen concentration was lower than the specified one, air was added in the amount required to maintain the specified O_2 concentration. At the beginning and the end of the experiments, the content of dissolved Fe(II) was determined in the samples fixed with acetate buffer. These samples were also used to determine the isotopic composition of residual dissolved Fe(II) and the newly formed Fe(III) oxides. During the experiments, the decrease in the concentration of dissolved Fe(II) was determined at regular intervals.

Analysis of the isotopic $^{56}/^{54}$Fe composition. The iron isotope ratio with the average standard deviation of 0.48% was measured on a DRC-e ICP-MS mass spectrometer (Perkin-Elmer, United States) under the conditions optimal for analysis of the $^{56/54}$Fe ratios, as described in [13]. The standard $^{56/54}$Fe ratio of the Earth crust is 15.8138 (Perkin-Elmer, United States).

Other analytical methods. To determine the contents of Fe(II) and Fe(III), water samples (in situ and during the model experiments) were fixed with acetate buffer and analyzed on a KFK-3 spectrophotometer (Russia) using α,α'-dipyridyl [14].

The concentrations of dissolved oxygen were determined with a Hanna HI 9142 oximeter (Germany) at the sampling site, or in the gas phase using a Kristall 5000.1 gas chromatograph (Chromatec, Russia); the pH of the water was determined with a portable Hanna Checker 1 pH meter (Germany).

RESULTS AND DISCUSSION

Investigations into the species composition of the communities of iron-oxidizing microorganisms and enumeration of unicellular iron-oxidizing bacteria in the water and sediments of ferruginous springs. Microscopic analysis of fresh iron-containing precipitates collected near the spring outlets and along the spring watercourses in the gutters, as well as of the iron oxide particles suspended in bog water, revealed that they were represented by formations of biogenic origin, i.e. by iron-encrusted structures of the known morphotypes of iron-oxidizing bacteria and rod-shaped bacteria with iron-encrusted capsules. Amorphous iron oxides not associated with bacterial cells represented only a small part of the analyzed sediments (Fig. 1a). Microscopic examination of submerged glass slides and slit peloscopes exposed vertically in the sediments and water horizons of the studied springs, gutters, and bogs revealed a similar pattern. Comparative microscopic analysis revealed the predominant species and the differences in the qualitative compositions of the communities of iron-oxidizing bacteria isolated from freshly formed precipitates from different springs. For instance, various *Gallionella* species, with their unique structures of iron-encrusted stalks (Fig. 1b), and, to a lesser degree, unicellular iron-oxidizing bacteria, were involved in iron oxide precipitation in the fresh sediments of spring no. 1 and the adjacent bog. Cells arranged in chains and iron-encrusted sheaths of *Leptothrix* sp. were occasionally detected. A similar species composition of iron-oxidizing bacteria, with the exception of *Leptothrix* sp., was observed in spring no. 8 (Staraya Russa). Amorphous Fe(III) oxides were not detected at all. In springs nos. 2 and 3, unicellular iron-oxidizing bacteria with iron-encrusted capsules (Fig. 1c) were predominant; iron-encrusted structures of the uncultured iron-oxidizing bacterium *Toxothrix trichogenes* were a minor component. In spring no. 4, in addition to unicellular forms, *T. trichogenes* represented the predominant morphotype.

The quantitative assessment of lithotrophic neutrophilic iron-oxidizing bacteria on nutrient media revealed 10^5–10^7 of viable cells per 1 mL of sediment samples from all the studied springs, including spring no. 8 (Staraya Russa Resort).

A pure culture of an iron-oxidizing bacterium (Fig. 1d) capable of oxidizing Fe(II) with oxygen under microaerobic conditions, as well as of nitrate-dependent growth under anaerobic conditions, was isolated from the sediments of this spring. Strain Hf1 was identified as a novel species, *Hoeflea siderophila*

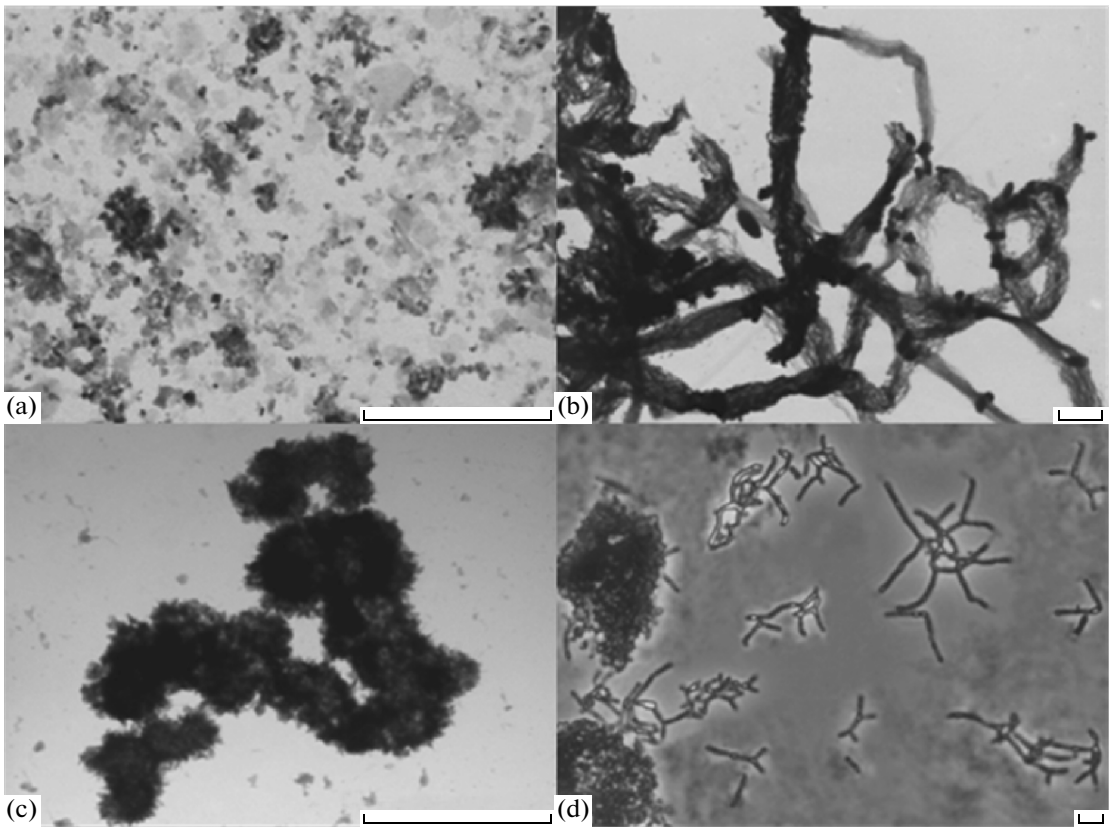

Fig. 1. Iron oxides of the biogenic and abiogenic origin in the fresh iron-containing precipitates from the mineral springs: amorphous Fe(III) oxides (a); iron-encrusted stalks of *Gallionella* spp. from the bog adjacent to spring no. 1 (b); iron-incrusted unicellular iron-oxidizing bacteria from spring no. 3 (a)–(c), electron microscopy; scale bar, 1 μm) (c); strain Hf1 isolated from spring no. 8 (phase contrast microscopy) (d).

(DSM 21587, VKM A7094) [15], and used in this work for comparative assessment of the rates of bacterial and abiogenic Fe(II) oxidation, as well as to assess its participation in fractionation of stable iron isotopes.

Fractionation of stable iron isotopes in the cultures of lithotrophic nitrate-dependent neutrophilic iron-oxidizing bacteria. To elucidate the role of bacterial oxidative reactions in the differentiation of the isotopic composition of iron, the isotopic composition of iron oxides produced by the pure and enrichment cultures of iron-oxidizing bacteria isolated from different ferruginous springs (Table 1) was analyzed under conditions of anaerobic nitrate-dependent growth. The experiments were carried out with strain Hf1 and the enrichment cultures of iron-oxidizing facultative anaerobic bacteria isolated from the low-temperature ferruginous spring of the Psekups mineral water deposit (Northern Caucasus, Russia).

In columns of agar medium, bacteria grew at the bottoms of the tubes above the FeS precipitate, forming ochreous layers containing Fe(III) oxides or separate ochreous colonies throughout the layer of the medium (Fig. 2).

The growth of bacteria oxidizing FeS in liquid medium was accompanied by the formation of loose precipitates of Fe(III) oxides at the bottom of the vial. In the control bacteria-free variants, FeS oxidation was not detected; no significant changes in the isotopic composition of the iron of the amorphous FeS suspension added to the medium were detected. At the same time, a shift in the isotopic composition of the newly formed iron oxides, resulting in a 3–5-fold increase in the concentration of the light ^{54}Fe isotope as compared to the control variants with chemical oxidation (Table 1), was detected in the pure and enrichment cultures of iron-oxidizing bacteria.

Isotopic composition of iron in the samples of spring water and sediments and in the iron-containing sediments of adjacent bogs. Table 2 shows the $^{56/54}Fe$ ratios in the waters and iron-containing precipitates of the studied springs, gutters, and adjacent bogs. Comparison of the isotopic compositions of dissolved Fe(II) and the newly formed ochreous Fe(III) precipitates undoubtedly indicates that the isotopic composition of the latter was significantly lighter. The newly precipitated iron oxides from the adjacent bogs and from the brackish spring no. 8 had the highest $\delta^{54}Fe$ values; these values were lower in the uppermost layers of bot-

Table 1. Fractionation of stable iron isotopes in the cultures of lithotrophic nitrate-dependent neutrophilic iron-oxidizing bacteria under anaerobic conditions

Isolation source	Cultivation conditions		Sample	δ, ‰
Spring no. 8 of the Staraya Russa Resort, strain Hf1	Agar medium + FeS	Test (stain Hf1)	Ochreous colonies	−35
		Control	Trace amounts of Fe(III) oxides in the upper layers of the medium	−13.5
Spring no. 4 of the Psekups mineral water deposit	Agar medium + FeS	Test (Enrichment culture of iron-oxidizing bacteria)	Fe(III) oxides in bacterial colonies above the FeS layer	−49
			Fe(III) oxides from the microzones of bacterial growth above the FeS layer	−71
		Control	Trace amounts of Fe(III) oxides on the surface of agar medium	−15
	Liquid medium + FeS	Test (Enrichment culture of iron-oxidizing bacteria)	Fe(III) oxides of bacterial origin precipitated to the bottoms of the flasks	−55
		Control	Trace amounts of Fe(III) at the bottoms of the flasks	−18

Note: (here and elsewhere in the tables): "Test" indicates biological and abiogenic oxidation; "Control" indicates abiogenic oxidation. The control variants were supplemented with NaCl to inhibit bacterial oxidation (see Materials and Methods).

tom sediments within the spring-discharge areas and along the spring watercourses.

Fractionation of stable Fe(II) isotopes during the model experiments. Table 3 shows the results of our model experiments carried out in order to elucidate the effect of oxygen concentrations in the water, as well as the effect of the initial Fe(II) concentration in the studied mineral springs, on the rates of oxidation processes and fractionation of stable iron isotopes. Comparison of these data indicates a direct relationship between the differentiation of the isotopic composition in the newly formed Fe(III) oxides and that of the dissolved Fe(II) of the initial water. The highest level of enrichment of the newly formed iron oxides with the light ^{54}Fe isotope was observed during the biological oxidation of iron under controlled microaerobic conditions at the lowest concentrations of dissolved oxygen (0.1–0.3 mg L^{-1}; springs nos. 1, 4, and 8); the level of enrichment was lower in the presence of nitrates. In the latter case, the rates of both chemical and biogenic oxidation were the lowest. Under free access of air (oxygen concentration at air saturation of 9.5 mg L^{-1} and temperature ~10–12°C), the rates of Fe(II) oxidation were high, while the values of isotope fractionation were lowest both during the bacterial and abiogenic processes. This may be because, under aerobic conditions at neutral and slightly acidic pH values of the spring water, the rates of chemical oxidation were quite high as compared with the test flasks with bacterial materials, leveling the effect of the biological differentiation of iron isotopes. At high rates of oxidation, the efficiency of differentiation of the isotopic composition and the relative lightening of iron oxides was much lower in all variants; however, it was 1.1–1.3 times lower in the case of chemical, rather than bacterial, oxidation. Unlike aerobic oxidation, when the effect of lightening of the isotopic composition of the final products, Fe(III) oxides represent the overall outcome of both biological and chemical reactions; the results of nitrate-dependent bacterial oxidation of Fe(II) represent the values of only biogenic isotopic differentiation. It is known that nitrite causes sponta-

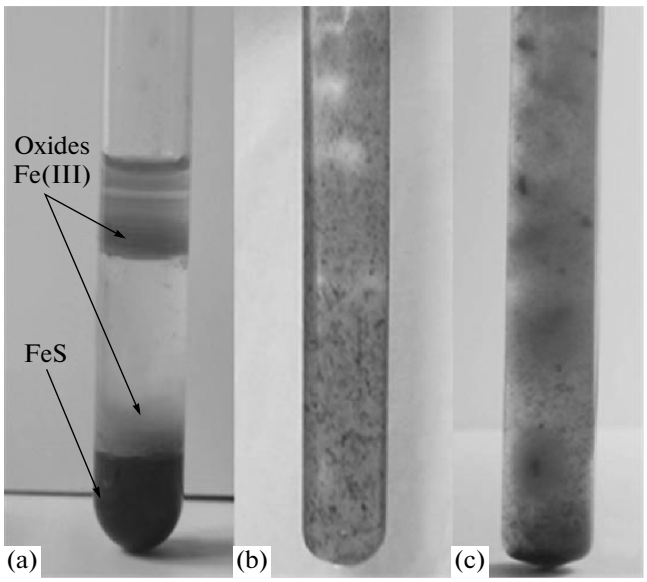

Fig. 2. Growth of iron-oxidizing bacteria in agarized media: ochreous layers of Fe(III) oxides in the medium with 0.2% agar under microaerobic conditions (arrows indicate the location of FeS suspension and Fe(III) oxides) (a); colonies of iron-oxidizing bacteria in the anaerobic agar medium (b), (c).

Table 2. Changes in the $^{56/54}$Fe ratio of the sediment samples collected in the investigated springs, as well as the adjacent bogs, of the Marcial Waters Resort and the Staraya Russa Resort, as compared to the dissolved Fe(II) of the initial water

Sampling site		Sample	δ, ‰
Spring no. 1	Spring	Upper layer of iron-containing sediments in the pipe near the spring outlet	–75
	Bog	Upper layer of the fresh iron-containing precipitate collected along the spring watercourses	–67
		Loose iron-containing precipitate	–98
Spring no. 2	Spring	Fresh iron-containing precipitate collected within the spring-discharge area	–81
Spring no. 3	Spring	Iron-containing precipitate collected near the spring outlet	–77
	Bog	Fresh iron-containing precipitate	–97
Spring no. 4	Spring	Newly formed precipitate collected within the spring-discharge area	–78
Spring no. 8	Spring	Fresh suspension of iron-containing precipitate	–96

neous Fe(II) oxidation only at high concentration above 0.5–1 mM [8].

It should be noted that, according to the results of the analysis of the energetic metabolism of strain Hf1, the enzymatic reaction of Fe(II) oxidation coupled to nitrate reduction does not result in accumulation of nitrites responsible for the chemical oxidation of Fe(II) [14].

The initial concentrations of dissolved Fe(II) ranging from 11 to 126 mg L^{-1} had no significant effect on the efficiency of isotope fractionation both during bacterial and chemical oxidation. At the final stages, Fe(II) oxidation in the experiments with water from springs nos. 3 and 4 was accompanied by acidification of the medium, with pH values decreasing to 2.7–2.9 during the biogenic oxidation of iron due to formation of Fe(III) hydroxides in the following reaction:

$$Fe^{3+} + 3H_2O \longrightarrow Fe(OH)_3 + 3H^+.$$

In the control variants, when the rates of iron oxidation were low, the pH of the water decreased insignificantly (Table 3).

The patterns of differentiation of stable iron isotopes revealed during the biogenic and abiogenic processes of iron oxidation in experiments with the samples collected at the Marcial Waters Resort were similar to those revealed in experiments with the samples from spring no. 8 of the Staraya Russa Resort (Table 3).

Since the rates of fractionation of the isotopic composition of Fe(III) oxides in the abiogenic processes of iron oxidation were lower than those detected in the bacterial processes, it should be noted that the obtained values of the isotopic composition of the newly formed iron oxides in the variants of the modal experiments were lower that the true ones, and represented only the cumulative effect of fractionation in the course of biological and chemical processes.

The results obtained indicate that differentiation of the $^{56/54}$Fe composition in the studied water ecosystems—water from chalybeate springs and adjacent bogs where the iron-containing ground waters discharged—depends on at least two main factors: the mechanism of the oxidation reactions (biological or chemical), and ambient oxidative conditions. The highest rates of fractionation of the stable iron isotopes $^{56/54}$Fe, as well as of enrichment of the newly formed insoluble Fe(III) oxides with the light ^{54}Fe isotope, were detected during the biological processes of iron oxidation carried out under microaerobic conditions at low concentrations of dissolved oxygen not exceeding 0.1–0.3 mg L^{-1}, or under anaerobic conditions via nitrate respiration. Similar conditions of microaerobic and anaerobic growth are optimal for bacterial oxidation of Fe(II), as was shown during the studies of the metabolism of pure cultures of neutrophilic iron-oxidizing bacteria [15]. These findings were supported by the microscopic analyses of the composition of freshly

Table 3. Results of modelling experiments on the effect of the oxygen regimes and the initial Fe(II) concentration in the water on bacterial and abiogenic processes of Fe(II) oxidation in the waters of the ferruginous springs of the Marcial Waters Resort and the Staraya Russa Resort

Oxygen regime	Experimental variant	Fe(II) content, mg/L								O_2 content in the gas phase, %							pH		$^{56/54}$Fe ratio	δ, ‰
		Initial concentration, mg/L	Duration of the experiment, days						Total iron oxide produced, mg/L	Initial concentration, %	Duration of the experiment, days						Initial	End	Fe(III) in the sediments	
			15	30	37	44	50	90			15	30	38	45	54	90				
Spring no. 1																				
Microaerobic	Test	16	9.1	3	1.2	0.5	0		16	0.25	0.3	0.32	0.33	0.4	0		6.4	5.8	11.1064	–122
	Control		9.2	8	8	8	8		8		0.3	0.36	0.3	0.38	0			5.9	11.8153	–66
Spring no. 3																				
Aerobic	Test	63					6		57	21	–	15	16	–	5.8		6.3	2.97	11.7679	–78
	Control						17		46		–	16.5	17	–	7			5.5	11.9989	–61
Anaerobic	Test						60		2.5	0.03					0			5.9	11.4526	–103
	Control						62		1						0			6.05	11.8366	–73
Spring no. 4																				
Aerobic	Test	126						1	125	21	–	14	–	–	–	3.2	6.2	2.75	11.7905	–59
	Control							47	79		–	17	–	–	–	5.5		4.8	11.8618	–53
Microaerobic	Test		84	50	40	35	–	1	125	0.5	0.4	0.44	0.32	0.38	0.5	0.45		2.8	11.7029	–66
	Control		125	120	115	115	–	83	43		1	1.05	1	0.8	0.6	0.45		6.13	12.0279	–40
Aerobic	Test							116	10	0						0		5.8	11.7875	–59
	Control							122	4							0		5.9	12.0929	–35
Spring no. 8																				
Microaerobic	Test	11					0		11	0.1					0		7.0	6.4	11.7579	–75
	Control						7		4						0			6.7	12.0745	–50

Note: In all experiments, the O_2 concentrations in the gas phase under microaerobic incubation were maintained by periodical injections of oxygen with a sterile microsyringe, with ultrafilter Millipore 0.2 m.

formed Fe(III) oxides collected within the spring-discharge areas and bogs (where the oxygen concentration did not exceed 0.1 mg L^{-1}). They were represented only by iron-encrusted *Gallionella* cells or stalks and fine *Toxothrix trichogenes* structures covered with amorphous Fe(III) oxides. In natural ecosystems, these biogenic structures may eventually transform into dense goethite and hydrogoethite (to a lesser extent) deposits, which are accumulated in the deep layers of iron-containing deposits along the spring watercourses, or are subjected to diagenetic transformations (in which bacterial reduction processes are involved) in bottom sediments with formation of amorphous or crystalline precipitates consisting of FeS, magnetite, etc. Formation of magnetite crystals under the gradient conditions of the redox zone of the sediment samples collected in the bog adjacent to spring no. 1, as well as in anaerobically grown pure cultures of neutrophilic iron-oxidizing bacteria isolated from the ferruginous spring of the Psekups mineral water deposit, was detected by X-ray structure analysis and electron microscopy (data not presented).

Comparison of the effects of isotopic fractionation of iron and of enrichment of iron oxide with the light isotope ^{54}Fe in the natural sediments of the studied springs of the Marcial Waters Resort and the Staraya Russa Resort, as well as in the samples collected from the adjacent bogs, with those observed in the model experiments and cultures of iron-oxidizing bacteria, demonstrates that the biogenic factor plays the key role in the differentiation of the isotopic composition of iron oxide precipitates, as well as in the processes of the oxidative branch of the geochemical iron cycle. Our data on the dependence of the rates of oxidative processes and of the transformation of the iron isotopic composition on the oxygen regime demonstrate that, under microaerobic conditions, at the boundary of the redox zone and in the deep horizons, the effect of the biogenic factor was several times more pronounced than that of the abiotic factors.

ACKNOWLEDGMENTS

This work was supported by the Russian Foundation for Basic Research, project nos. 07-05-00336 and 10-04-01500.

REFERENCES

1. Balci, N., Bullen, T.D., Witte-Lien, K., Shanks, W., Motelica, M., and Mandernack, K.W., Iron Isotope Fractionation during Microbially Stimulated Fe(II) Oxidation and Fe(III) Precipitation, *Geochem. Cosmochem. Acta*, 2006, vol. 70, pp. 622–639.

2. Anbar, A.D., Iron Stable Isotopes: Beyond Biosignatures, *Earth Planet. Sci.*, 2004, vol. 217, pp. 223–236.

3. Anbar, A.D., Roe, J.E., Barling, J., and Nealson, K.H., Nonbiological Fractionation of Iron Isotopes, *Science*, vol. 288, pp. 126–128.

4. Beard, B.L., Johnson, C.M., Cox, L., Sun, H., Nealson, K.H., and Aguilar, C., Iron Isotope Biosignatures, *Science*, 1999, vol. 285, pp. 1889–1892.

5. Beard, B.L., Johnson, C.M., Skulan, J.L., Nealson, K.H., Cox, L., and Sun, H., Application of Fe Isotopes to Tracing the Geochemical and Biological Cycling of Fe, *Chem. Geol.*, 2003, vol. 195, pp. 87–117.

6. Bullen, T.D., White, A.F., Childs, C.W., Vivit, D.V., and Shulz, M.S., Demonstration of Significant Abiotic Iron Isotope Fractionation in Nature, *Geology,* 2001, vol. 29, pp. 699–702.

7. Croal, L.R., Johnson, C.M., Beard, B.L., and Newman, D.K., Iron Isotope Fractionation by Fe(II)-Oxidizing Photoautotrophic Bacteria, *Geochem. Cosmochem. Acta*, 2004, vol. 68, pp. 1227–1242.

8. Tangalos, G.E., Beard, B.L., Johnson, C.M., Alpers, C.N., Shelobolina, E.S., Xu, H., Konishi, H., and Roden, E.E., Microbial Production of Isotopically Light Iron(II) in Modern Chemically Precipitated Sediment and Implications for Isotopic Variation in Ancient Rocks*, Geology*, 2010, vol. 8, pp. 197–208.

9. Pfennig, N. and Lippert, K.D., Über das Vitamin B_{12}-Bedürfnis phototropher Schwefelbakterien, *Arch. Mikrobiol.*, 1966, vol. 55, pp. 245–256.

10. Hallbeck, E.L. and Pedersen, K., The Genus *Gallionella*, in *Bergey's Manual of Systematic Bacteriology*, Brener, D.J., Krieg, N.R., and Staley, J.T., Eds., Berlin: Springer, 2005, vol. 2, pp. 880–886.

11. Kuznetsov, S.I. and Dubinina, G.A., *Metody izucheniya vodnykh mikroorganizmov* (Methods of Investigation of Aquatic Microorganisms), Moscow: Nauka, 1989.

12. Podkopaeva, D.A., Grabovich, M.Yu., and Dubinina, G.A., Oxidative Stress and Antioxidant Cell Protection Systems in the Microaerophilic Bacterium *Spirillum winogradskii, Microbiology*, 2003, no. 5, vol. 72, pp. 534–541.

13. Dolotov, A.V., Gapeeva, M.V., Dubinina, G.A., and Sorokina, A.Yu., Possible Application of an ELAN DRC-e ICP-MS Quadrupole Mass Spectrometer for Determination of Iron Isotopic Composition in Model Experiments, *Issledovano v Rossii*, 2008. http://zhurnal.gpi.ru/articles/2008/095.pdf

14. Reznikov, A.A., Mulikovskaya, E.P., and Sokolov, I.Yu., *Metody analiza prirodnykh vod* (Methods of Analysis of natural Waters), Moscow: Nedra, 1970.

15. Sorokina, A.Yu., Chernousova, E.Yu., and Dubinina, G.A., *Hoeflea siderophila* sp. nov., a New Neutrophilic Iron-oxidizing Bacterium, *Microbiology*, 2012, no. 1, pp. 64–71.