



Mössbauer study of iron minerals transformations by *Fuchsiella ferrireducens*

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Abstract Biogenic transformations of iron-containing minerals synthesized ferrihydrite, magnetite and hydrothermal siderite by anaerobic alkaliphilic bacterium *Fuchsiella ferrireducens* (strain Z-7101^T) were studied by ⁵⁷Fe Mössbauer spectroscopy. Mössbauer investigations of solid phase samples obtained after microbial transformation were carried out at room temperature and at 82 K. It was found that all tested minerals transformed during bacterial growth. In the presence of synthesized ferrihydrite, added as an electron acceptor, a mixture of large (more than 100 nm) and small (~5 nm) particles of magnetically ordered phase and siderite was formed. Synthesized magnetite that contains both Fe³⁺ and Fe²⁺ forms could serve as electron acceptor as well as an electron donor for *F. ferrireducens* growth. As a result of its biotransformation, no siderite formation was observed while small particles of magnetite were formed. In the case of the addition of siderite as an electron donor formation of a small amount of a new phase containing Fe²⁺ caused by recrystallization of siderite during bacterial growth was detected.

Keywords Mössbauer spectroscopy · Anaerobic alkaliphilic bacterium · *Fuchsiella* · Synthesized ferrihydrite · Siderite · Synthesized magnetite

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1 Introduction

Dissimilatory iron-reducing bacteria are able to conserve energy by reducing of different Fe(III)-containing compounds including insoluble minerals using ferric iron as an electron acceptor [1] and organic (alcohols, organic acids, peptides) or inorganic (molecular hydrogen) substrates as an electron donors. This process leads to the formation of new mineral phases containing ferrous iron. Investigations revealed that dissimilatory iron-reducers predominate in a wide diversity of soils, marine and continental sediments and subsurface biosphere [1, 2]. These microorganisms may have a significant influence on the geochemistry of modern sedimentary environments. Moreover, understanding the biogeochemical process active by iron-reducing microorganisms may provide insights into biogeochemical processes of iron cycle on early Earth [3]. Investigation of this physiological group is actual because of the possibility of their practical applications such as the harvesting energy in the form of electricity from marine sediments [4] or biochemical utilization of pollutant including the degradation of contaminants in groundwater [5] and bioremediation of uranium [6].

In spite of intensive investigation of these microorganisms since 1980th, the possibility of biological iron reduction process in alkaline environments has been subject to doubt due to the low mobility of Fe^{3+} under these conditions [7]. Nevertheless, several species of the alkaliphilic anaerobic bacteria capable to reduce insoluble Fe^{3+} in the presence of wide range of electron donors have been isolated and described during last decade [8–13]. Synthesized ferrihydrite (SF) was the model mineral using as an electron acceptor in all these works.

Fuchsiella ferrireducens is the acetogenic bacterium that is capable to reduce carbonate, producing acetate as the sole metabolic product. It is also first described alkaliphilic homoacetogen capable to reduce iron from SF [13] in the presence of some organic compounds or molecular hydrogen as an electron donor. In our previous study the ability of alkaliphilic dissimilatory iron reducing bacterium *Geoalkalibacter ferrihydriticus* for anaerobic oxidation of Fe^{2+} in biotite and glaukonite during carbonate reduction was shown [14]. Here, we present Mössbauer study of transformations of iron minerals by *F. ferrireducens* containing only Fe^{3+} – SF, both Fe^{3+} and Fe^{2+} —synthesized magnetite and only Fe^{2+} —natural hydrothermal siderite. These minerals could serve electron acceptor, electron acceptor or donor, electron donor correspondingly. During our investigation, we planned to check the ability of *F. ferrireducens* to oxidize Fe^{2+} during carbonate reduction.

2 Objects and methods

Fuchsiella ferrireducens (strain Z-7101^T) [13] was isolated from the bottom sediments of soda lake Tanatar III (Altay, Russia). It is anaerobic, mesophilic (the growth temperature range is from 25 to 45 °C) and alkaliphilic (the pH growth range is from 8.5 to 10.2) microorganism. It was cultivated under anaerobic conditions (100% N_2 in gas phase) in the liquid medium with an optimal composition containing the follow main salts (g/l): NH_4Cl - 0.5; KH_2PO_4 - 0.2; $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ - 0.1; Na_2CO_3 - 68; NaHCO_3 - 38; NaCl - 60; pH was 9.5 and temperature was 35 °C in the dark. Ethanol (50 mM) was used as an electron donor. All experiments were carried out in duplicate.

SF was prepared by titrating of a solution of $\text{FeCl}_3 \times 6\text{H}_2\text{O}$ with 10% NaOH to pH 9.0. Synthesized magnetite was prepared by titrating of equimolar mixture of $\text{Fe}_2(\text{SO}_4)_3 \times 9\text{H}_2\text{O}$ and $\text{FeSO}_4 \times 7\text{H}_2\text{O}$ salts with 10% NaOH to pH 9.0. Both minerals were added to the

medium before sterilization. Contents of Fe(III) was 45 mmol l^{-1} . Nature hydrothermal siderite (Bakalskoe deposit, Ural) was crushed into powder and 200 mg of the mineral was added into the flasks before sterilization. Abiological controls with each mineral adding to the sterile medium and incubated at the same conditions were done.

After the end of experiments samples of solid phase were at first dried in acetone and then completely dried under N_2 gas flow and then put into glass ampoules sealed under vacuum.

^{57}Fe Mössbauer investigations of solid phase samples obtained after bacterial growth were carried out at room temperature and $T = 82 \text{ K}$. The calibration was given relatively to $\alpha\text{-Fe}$. The program SPECTR from MSTools Complex [15] was used for spectra processing.

3 Results and discussion

3.1 Mössbauer study of synthesized ferrihydrite transformation

Mössbauer investigations of iron minerals formed during the process of SF reduction by *F. ferrireducens* in the presence of ethanol were carried out after lysing of the bacterium cells (10 days after inoculation). Reduction of SF was apparent from its color change from brown to black.

The influence of liquid medium on the SF was studied in abiological control to find out whether the environment conditions affect the initial structure of SF. The Mössbauer spectrum of SF after their interaction with the liquid medium consists of two quadrupole doublets (Fig. 1a). Their Mössbauer parameters are similar to the parameters of initial SF spectra [16].

Firstly solid phases formed during *F. ferrireducens* growth were measured at room temperature immediately after opening anaerobic glass ampoules. For a more detailed analysis Mössbauer investigations at $T = 82 \text{ K}$ were carried out. Since these measurements were made several months after the previous studies, the spectrum of the studied sample was repeatedly measured at room temperature to reveal the oxidation processes of the sample. The samples were stored in opened ampoules in the air.

Room temperature Mössbauer investigations of solid phases formed during *F. ferrireducens* growth in the presence of ethanol showed formation of the siderite. A relative intensity of quadrupole doublet ($\delta = 1.23 \pm 0.02 \text{ mm/s}$, $\varepsilon = 0.92 \pm 0.02 \text{ mm/s}$) corresponding to Fe^{2+} atoms in the siderite structure is $\sim 15\%$. Moreover, the presence of sextets in the fitting model leads to the conclusion that large particles (more than 100 nm) of magnetically ordered phase containing Fe^{3+} atoms were formed in the sample. Analysis of the spectrum of the oxidized sample revealed that in the sample the relative content of Fe^{3+} atoms also grew slightly (by $\sim 8\%$) due to the oxidation of the Fe^{2+} atoms in the siderite structure.

Mössbauer spectrum of solid phases measured at $T = 82 \text{ K}$ is typical for superparamagnetic behavior of small particles. Fitting model for processing 82 K spectrum consisted of five subspectra: two quadrupole doublets, two Zeeman sextets, and hyperfine field distribution in a many-state superparamagnetic relaxation model. Quadrupole doublets correspond to Fe^{2+} atoms in the siderite structure and Fe^{3+} atoms that could be associated with both the initial SF and superparamagnetic particles of magnetically ordered phase. Analysis of the parameters of sextets suggests that magnetically ordered phase contains a mixture of large particles of hematite and maghemite. It can be assumed that the Fe^{3+} atoms in the SF structure were biologically reduced, resulting in the formation of a mixture of siderite and

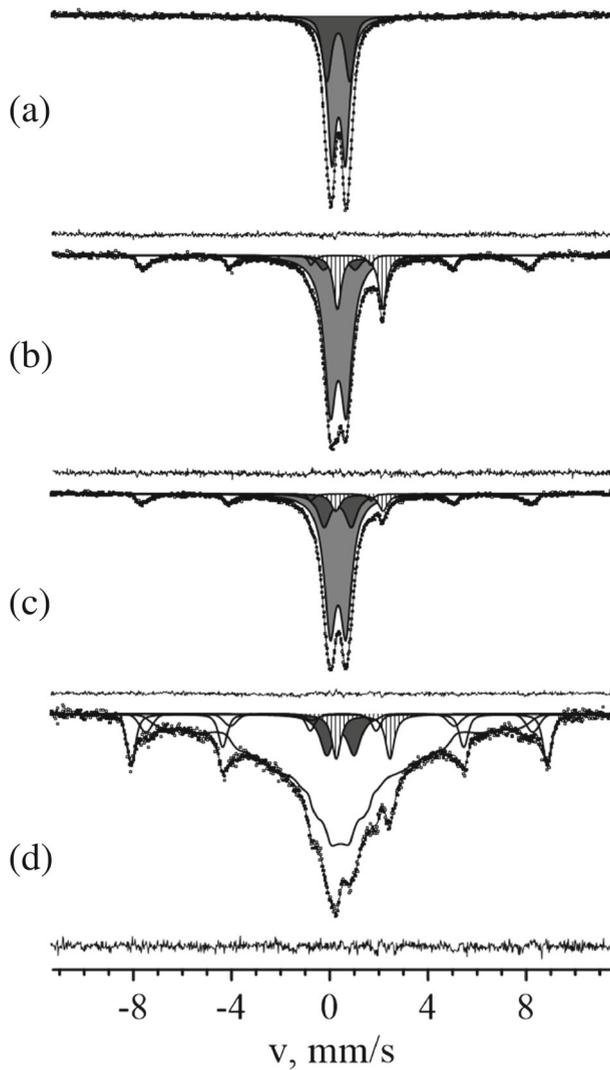


Fig. 1 Mössbauer spectra of **a** abiological control obtained after interaction of SF with the medium, **b** experimental sample formed during the bacterium growth, **c** oxidized experimental sample measured at 300 K and **d** oxidized experimental sample measured at 82 K

magnetite [17]. Then magnetite was oxidized, resulting in the formation of hematite and maghemite. Hyperfine field distribution with a relative intensity of $\sim 70\%$ corresponds to the Fe^{3+} atoms in the structure of nanoparticles of magnetically ordered phase.

Therefore, these studies showed that the Fe^{3+} atoms in the SF structure were reduced during the growth of *F. ferrireducens* in the presence of ethanol. Siderite and magnetically ordered phase were formed during this process. The resulting magnetically ordered phase containing Fe^{3+} is a mixture of large (more than 100 nm) and small (~ 5 nm) particles.

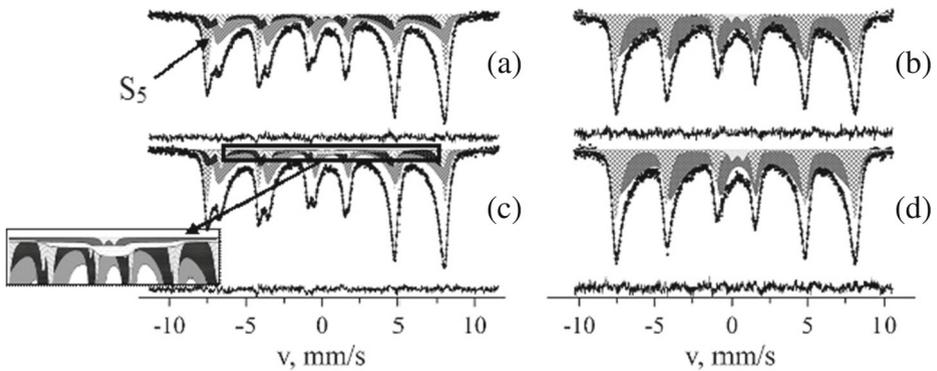


Fig. 2 Mössbauer spectra of **a** control sample, **b** oxidized control sample, **c** the experimental sample obtained after *F. ferrireducens* growth, **d** oxidized experimental sample measured at 300 K

3.2 Mössbauer study of synthesized magnetite transformation

Firstly, Mössbauer spectrum of the control abiological sample of synthesized magnetite was measured at room temperature (Fig. 2a). A fitting model consisted of five sextets and a quadrupole doublet was used for its processing. Under the assumption that the spectrum of magnetite consists of two components corresponding to Fe^{3+} and $\text{Fe}^{2.5+}$ ions four sextets were added because of different particle size in control sample. Parameters ($\delta = 0.299 \pm 0.022$ mm/s, $\varepsilon = -0.01 \pm 0.01$ mm/s, $H = 486 \pm 1$ kOe) of the fifth sextet S_5 (Fig. 2a) might correspond to Fe^{3+} atoms in maghemite structure. The presence of the doublet ($\delta = 0.32 \pm 0.01$ mm/s, $\varepsilon = 0.35 \pm 0.01$ mm/s) in the spectrum suggests that the sample contains a small amount of ferrihydrite ($I \sim 1\%$). The particle size of magnetite in the control sample lies in the range $d = 13 \div 17$ nm. Mössbauer spectrum of the oxidized control (stored in opened ampoules in the air) sample measured at room temperature is shown in (Fig. 2b). One can see the line responsible for ferrous atoms had disappeared. This spectrum was fitted with the use of two sextets ($\delta = 0.42 \pm 0.01$ mm/s, $\varepsilon = 0.014 \pm 0.010$ mm/s, $H = 498 \pm 1$ kOe), ($\delta = 0.28 \pm 0.01$ mm/s, $\varepsilon = 0.001 \pm 0.010$ mm/s, $H = 493 \pm 1$ kOe). Subspectra with such parameters correspond to ferric atoms. When stored in opened ampoules the control sample is likely to oxidize in air.

Mössbauer investigations of solid phases formed during *F. ferrireducens* growth in the presence of ethanol were carried out at room temperature (Fig. 2c). To improve the quality of processing the spectra of prototypes, two sextets were added to the fitting model. These relaxation type sextets correspond to smaller, than in the control sample, particles of a mixture of magnetite and maghemite. The oxidized sample was also investigated. Mössbauer spectrum seems like the spectrum of the oxidized control sample (Fig. 2d).

As magnetite contains both Fe^{2+} and Fe^{3+} atoms it could serve as electron acceptor, as well as an electron donor. Mössbauer investigations of magnetite transformations showed only minimal changes ($\sim 7\%$) connected with the formation of small particles of magnetite due to reduction of ferric atoms in the maghemite and ferrihydrite structure that was present as the admixture in the initial magnetite. Magnetite is stable phase in the conditions of our experiments (low Eh and high pH) [18] and its transformation is thermodynamically unfavorable. That's why in this case *F. ferrireducens* practically did not transform mineral

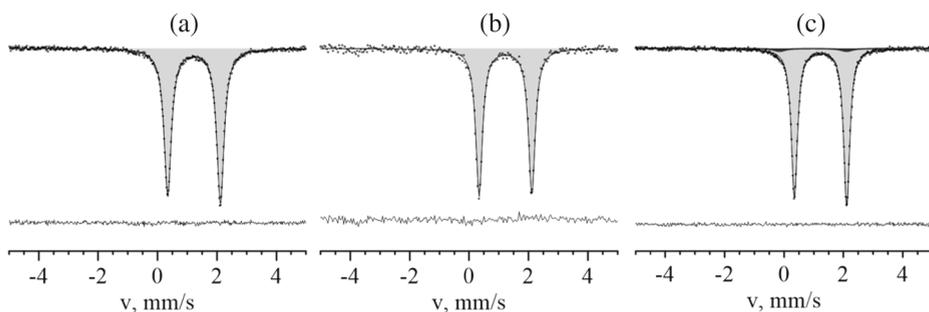


Fig. 3 Mössbauer spectra of **a** the abiological control obtained after interaction of siderite with the medium, **b** the abiological control obtained after interaction of siderite with the medium ($\theta = 54.74^\circ$), **c** the experimental sample obtained after *F. ferrireducens* growth in the presence of ethanol, measured at 300 K

and grew as true acetogen and iron-reducer: it reduced carbonate and ferric admixtures, oxidized ethanol and produced acetate.

3.3 Mössbauer study of siderite transformation

To study biogenic transformations of siderite it was firstly necessary to investigate charge states of iron atoms in its initial structure. Room temperature Mössbauer spectrum of abiological control obtained after the interaction of hydrothermal siderite with liquid medium is shown in Fig. 3a. It is clear that components of quadrupole doublet have unequal intensities ($I_2/I_1 = 1.06 \pm 0.01$). It was suggested that it was caused by the texture of the sample. To eliminate this effect Mössbauer spectrum was measured in such geometry that the angle between normal to the surface of the sample and the direction of gamma-rays is $\theta = 54.74^\circ$. That spectrum is shown in Fig. 3b. The fitting model consisted of one quadrupole doublet with components of equal intensity was used for its processing. Mössbauer parameters of the quadrupole doublet ($\delta = 1.23$ mm/s, $\varepsilon = 0.89$ mm/s) are typical for atoms Fe^{2+} in siderite structure which confirms the suggestion that the sample is texturing.

During bacterial growth in the presence of ethanol siderite sediment changed its color from light brown to the dark gray. However, Mössbauer investigations of solid phases showed that spectra of duplicate samples are nearly identical. That's why only one spectrum of the sample is presented in Fig. 3c. A fitting model consisted of two quadrupole doublets was used for processing all spectra. Parameters of the first doublet including intensity ratio are similar to the parameters of Fe^{2+} in initial siderite structure. The second quadrupole doublet corresponds to Fe^{2+} atoms in a structure of a new phase formed during bacterial growth. Its parameters are close to the parameters of iron atoms in siderite structure. Its intensity varies from 1.7% to 3.4% in duplicate samples. For a more detailed study Mössbauer investigations of one of the samples were carried out at $T = 82$ K. As expected, these measurements showed no significant changes in the spectrum. It is worth noting that no subspectra corresponding Fe^{3+} atoms were detected in control and experimental samples. So it can be argued that the possibility of the direct reduction is absolutely excluded.

In this case carbonate was the sole electron acceptor for *Fuchsiella ferrireducens*, while siderite as well as ethanol could be used as an electron donor. Visible color changes of siderite sediment and its Mössbauer clearly showed that some biogenic transformation took place. However the amount of new phase was small that allows us to state that in this case

ethanol was the main electron donor. From the other hand Mössbauer study revealed formation new phase containing only Fe^{2+} atoms. The only explanation is that during bacterial growth siderite was at first oxidized, but then this new phase was reduced to biologically formed siderite. The ability of acetogenic bacteria to oxidize Fe(II) as well as Fe^0 in anaerobic conditions was demonstrated recently in several study for alkaline [14] and neutral environments [19, 20].

4 Conclusions

^{57}Fe Mössbauer investigations of biogenic transformations of iron-containing minerals by *Fuchsiella ferrireducens* were carried out.

The Fe^{3+} atoms in the SF structure were reduced during the growth of *F. ferrireducens* into mixture of siderite and magnetically ordered phase. The resulting magnetically ordered phase containing Fe^{3+} is a mixture of large (more than 100 nm) and small (~ 5 nm) particles.

During *F. ferrireducens* growth in the presence of synthesized magnetite no changes of this mineral was observed due to its stability at the experimental conditions. However, by results of the study in this case, small particles of magnetite formed. Moreover, it was found that synthesized magnetite is likely to oxidize in the air.

During biogenic transformations of siderite some changes in the Mössbauer spectra were fixed caused by recrystallization of siderite during bacterial growth. This may be due to the fact that after the process of bacterial oxidation of iron bacterial reduction of new phase took place, which makes impossible to detect this process by Mössbauer methods. To prove this suggestion it is necessary to perform some additional experiments.

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