Mössbauer study of formation of iron oxides and carbonate by dissimilatory alkaliphilic bacterium

N I Chistyakova, V S Rusakov, K A Nazarova, A A Shapkin, T N Zhilina and D G Zavarzina

1 Faculty of Physics, M.V. Lomonosov Moscow State University, Leninskie Gory, Moscow 119992, Russia
2 Winogradsky Institute of Microbiology, Russian Academy of Sciences, Prospect 60-letiya Oktyabrya 7/2, 117312 Moscow, Russia

E-mail: nchistyakova@yandex.ru

Abstract. The process of amorphous Fe(III)-hydroxide (AFH) reduction by anaerobic alkaliphilic bacterium – Geoalkalibacter ferrihydriticus (strain Z-0531) was investigated by Mössbauer spectroscopy methods. Strain Z-0531 was isolated from the sediments of soda Lake Khadyn (Tuva, Russian Federation). The influence of AFH concentration and the concentration of anthraquinone-2,6-disulfonate added to the cultivation medium as well as the incubation time influence on the reducing process were studied. It was found that increase in the time of cultivation of bacteria led to an increase in the relative content of phases containing ferrous atoms that could be explained by the adaptation of bacterium to the cultivation medium.

1. Introduction
One of the possible ways of iron mineral formation is an extracellular reduction of amorphous Fe (III) oxides and hydroxides by the group of microorganisms that has been found in the 1980's and named dissimilatory iron-reducing bacteria [1]. A microbiological pathway of a mineral formation has been intensively studied during recent decades. Investigations of the kinetics of iron mineral formation by bacterium Thermicola ferriacetica (strain Z-0001) and a study of different physical and chemical conditions of the formation of biogenic minerals were carried out [2]. The possibility of the reduction of amorphous and weakly crystalline iron oxides in alkaline environments has been subject to doubt [3] due to the low mobility of Fe(III) under these conditions. However, the iron-reducing process with magnetite and siderite formation may occur in alkaline medium for anaerobic bacterium Geoalkalibacter ferrihydriticus (strain Z-0531) [4].

The goal of this work is to investigate the iron mineral formation process by alkaliphilic dissimilatory iron-reducing bacterium (strain Z-0531) by $^{57}$Fe Mössbauer spectroscopy.

2. Experimental
Anaerobic alkaliphilic bacterium Geoalkalibacter ferrihydriticus (strain Z-0531) was isolated from a bottom sediment sample from the weakly mineralized soda Lake Khadyn (Tuva, Russian Federation) [5]. It is a mesophilic representative of alkaliphilic microbial community. Optimal conditions for strain

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3 To whom any correspondence should be addressed
growth are pH 8.6, T = 35°C. The strain uses the amorphous Fe(III)-hydroxide as an electron acceptor
and acetate CH₃COO⁻ as an electron donor. The bacterium can also reduce sulfur S⁰ resulted in the
formation of H₂S.

Amorphous Fe(III) hydroxide (AFH) was prepared by titration of a FeCl₃ solution with 10% NaOH. AFH and acetate were added to a mineral medium prepared under anaerobic conditions. The medium was poured into flasks, and then inoculated flasks were incubated at 35°C. In order to reduce errors we prepared two series for each experiment, and the second series (b) was the repetition of the first one (a).

Mössbauer investigations of all samples were carried out at 300K on a spectrometer operated in constant acceleration mode and equipped with a ^⁵⁷Co-source into an Rh-matrix.

3. Results and discussion
Mössbauer investigations carried out at room temperature showed that the addition of anthraquinone-2,6-disulfonate (quinone) to the cultivation medium of the strain Z-0531 leads to a more intensive reduction of AFH and an increase in the relative content of magnetite and siderite unlike the strain growth with citrate addition and without any additional electron acceptors [4].

Therefore we studied the iron mineral formation by strain Z-0531 with quinone in the cultivation medium. The samples were obtained during the process of bacterium growth with different concentrations of AFH and quinine. The influence of incubation time and time of bacterium cultivation on biomineralization process was also studied.

3.1. AFH concentration influence
We investigated the influence of AFH concentration \( n_{Fe(III)} \) on iron reducing process. The content of initial AFH varied from 5 to 150 mM, quinone concentration was 0.1 g/l, and incubation time was 1 month. Typical Mössbauer spectra of obtained samples are shown in figure 1. It was found that the formation of siderite FeCO₃ was observed at a small concentration of AFH. Sufficiently large particles of magnetically ordered phases were formed under the content of AFH above 70 mM. Two hyperfine field distributions were extracted from these spectra. The analysis has shown that distribution with a lower isomer shift (\( \delta \sim 0.3 \) mm/s) could be attributed to cations Fe³⁺ located in A-positions of magnetite Fe₃O₄ and in both positions of maghemite \( \gamma \)-Fe₂O₃. Distributions with a large isomer shift value (\( \delta \sim 0.6 \) mm/s) could be attributed to cations Fe².⁵⁺, located in the B-positions of magnetite. The relative intensity of the subspectrum corresponding to B-position of magnetite was noticeably larger for b-series than for a-series. Thus, the more intensive reduction of AFH was observed in the case of b-series.

The monotonic change of relative subspectrum intensities corresponding to the initial AFH, siderite and magnetically ordered phases was not observed within the range of 30-70 mM of AFH concentration for both series (figure 1). This behavior can be explained by the following considerations. In applying the method of series, when one flask corresponds to one experimental point, the fluctuations in bacterium cell number are inevitable. We suspect that biomineralization process becomes sensitive to the ratio between AFH concentration and the bacterium cell numbers.

3.2. Quinone concentration influence
We are interested in the influence of the quinone concentration on iron reducing process. The content of initial AFH was 100 Mm and quinine concentration was changed from 0 to 1 g/l. The typical Mössbauer spectra of obtained samples of both series for different quinine concentration are shown in figure 2. In general Mössbauer spectra are a superposition of three subspectra. Two quadrupole doublets correspond to AFH and siderite. The sextet with broad asymmetrical lines corresponds to magnetically ordered phases.

The increase in the sextet intensity is observed with an increase in \( n_q \) in the range of quinone concentration \( n_q = 0 \) g/l ÷ 0.05 g/l; further this intensity remains virtually unchanged up to \( n_q = 0.3 \) g/l. The relaxation character of the Mössbauer spectra of the samples for both series (a and b) becomes
more pronounced for \( n_q \geq 0.4 \text{ g/l} \), reflecting an increase in the relaxation rate of magnetic moments of iron atoms due to reducing of superparamagnetic particle size.

Thus, the quinone concentration affects the size of particles of magnetically ordered phases formed in the process of the AFH reduction by strain Z-0531. The sharp changes in the relative intensities of subspectra corresponding to obtained mineral phases were observed for b-series in the concentration range \( n_q = 0.7 \text{ g/l} \div 1.0 \text{ g/l} \). Apparently, the biomineralization process in this concentration range is sensitive to fluctuations in bacterium cell number as it was observed in the previous series.

### 3.3. Incubation time influence

We have studied how the incubation time would affect the process of mineral phase formation. Samples were synthesized by the strain Z-0531 in a mineral medium with the addition of AFH (100 mM) and quinone (0.05 g/l). Incubation time was varied from 1 month to 30 months. This experiment is still in the progress.

Analysis of the Mössbauer spectra showed that the relative intensity of the quadrupole doublet corresponding to siderite virtually had not been changed with the increase in incubation time (figure 3). Herewith the increase in the relative intensity of the subspectrum corresponding to cations Fe\(^{3+}\) located in the B-positions of magnetite and the decrease – to cations Fe\(^{3+}\) located in A-positions of magnetite and in both positions of maghemite were observed. Thus, an increase in the degree of initial AFH reduction occurs with the increase in the incubation time.
3.4. The influence of bacterium cultivation time

We found that the different times of bacterium cultivation connected with the number of bacterium inoculations and number of consecutive transfers of the culture affected the ratio of the mineral phases formed in the process of iron reduction under the same conditions. Thus, the relative amount of magnetically ordered phase was 75% for our first experiment with the addition of AFH (90 mM) and quinone (0.1 g/l) and 100% for the last one. Similar results were also obtained for another strain Z-0001. An increase in cultivation time led to a more intensive reduction of AFH which could be explained by the adaptation of bacteria to the culture medium.

4. Conclusion

The Mössbauer investigations of the process of amorphous Fe(III)-hydroxide reduction by the strain Z-0531 have shown that the increase in AFH concentration leads to a decrease in siderite content and to an increase in the content of the magnetically ordered phase. The phase is not a well-crystallized magnetite, or a mixture of magnetite and maghemite. The concentration range of AFH $n_{\text{Fe(III)}} = 30 \text{ mM} \div 70 \text{ mM}$ and quinone $n_q = 0.7 \text{ g/l} \div 1.0 \text{ g/l}$ is the region of unstable phase formations by the strain Z-0531. For this strain the process of biomineralization is sensitive to fluctuations in bacterium cell number. Quinone concentration has a significant influence on the size of the particles of magnetically ordered phases. The increase in the degree of initial AFH reduction is observed with the increase in the incubation time and the time of cultivation in iron-containing mineral medium.

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