Production and magnetic properties of biogenic ferrihydrite nanoparticles

S. V. STOLYAR[a,b*], O. A. BAYUKOV[b], D.A. BALAEV[a,b], R. S. ISKHAKOV[b*], L. A. ISHCHENKO[b], V.P. LADYGINA[c], R.N. YAROSLAVTSEV[a]
[a]*Siberian Federal University, Svobodny 79, Krasnoyarsk, 660041, Russia*
[b]*Kirensky Institute of Physics, SB RAS, Akademgorodok, 50/38, Krasnoyarsk, 660036, Russia*
[c]*International scientific centre for organism extreme states research attached Presidium of KSC, SB RAS Akademgorodok 50, Krasnoyarsk, 660036, Russia*

In present work the magnetic properties of biomineral ferrihydrite nanoparticles (biomass), produced by *Klebsiella oxytoca* strain and the nanoparticles obtained by drying the sol (sol), made on the basis of biomineral ferrihydrite nanoparticles have been studied. Ferrihydrite nanoparticles are formed on the surface of the bacterial cell.

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1. Introduction

The search for new methods of nanoparticles synthesis at the present time is conditional on unique physical characteristics of these objects and therefore a broad spectrum of their feasible application [1]. There are a lot of microorganisms synthesizing nanopacticles. For example, bacteria which can orient to the Earth's magnetic field synthesize magnetite crystals, sulfate-reducing bacteria produce palladium nanopacticles in the presence of electron donors, the bacteria Pseudomonas stutzeri synthesizes silver nanoparticles [2], etc. Much attention is given to biological synthesis of magnetic iron based nanoparticles what is due to a biological compatibility of these objects and possibility of their control by the external magnetic field. These advantages make it possible to consider nanoparticles as suitable applicant for clinical usages for drug (pharmaceutical composition) delivery to appropriate targets. It is known that four iron based compounds are formed as result of bacterial vital functions. These are magnetite Fe_3O_4, maghemite γ-Fe_2O_3, pyrrotine $Fe_{1-x}S_x$ and ferrihydrite $5Fe_2O_3*nH_2O$ ($n\approx9$). Up to now greater attention was attracted by magnetite showing ferrimagnetic properties and formed for example in magnetotactic bacteria. Ferrihydrite is an antiferromagnetic hydroxide. But if sizes of antiferromagnetic (AF) particles decrease to the nanometer scale their magnetic properties change drastically [3]. Small AF particles obtain uncompensated magnetic moment and this result in superparamagnetic behaviour of the system. Therefore, on the nanosized scale the AF particles can compete with ferromagnetic and ferrimagnetic ones in various practical applications inclusive of purposeful transfer of pharmaceutical composition in organism. Among a sufficiently wide variety of magnetic materials including ones of biological origin the ferrihydrite particles are absolutely nonhazardous for using in biomedicine since this mineral is located exactly in a core of ferritine which is an iron hydroxide particle contained in the protein capsule with an external diameter equal to 12 nm and internal diameter equal to 5-8 nm [4]. Practically all living organisms have these compounds with the purpose of iron storage.

2. Technology of obtaining

The microorganisms used in this study were isolated from sapropel of the Lake Borovoe (Krasnoyarsk region, Russia). This lake was characterized by absence of sulphate-reducing processes and presence of denitrification and iron-reducing processes. The sapropel sampled from the lake was passed through a magnetic separator. The microorganisms isolated in that way were inoculated onto an agarized Lovley medium [5]. Bacteria were grown in a batch culture without aeration and agitation on the mineral medium. Glucose, benzoic acid, citrates of iron and potassium were investigated as sole of carbon and energy. Under cultivation the bacteria had the maximum specific growth rate equal to 0.144 h^{-1} on the medium with glucose, 0.08 h^{-1} on the medium with potassium citrate, 0.06 h^{-1} on benzoic acid under aerobic and 0.02 h^{-1} under microaerophilic conditions. It turned out that in the terms of energy from tested substrates the potassium citrate was the most acceptable to synthesize the biomass of *Klebsiella oxytoca* and the iron citrate was required to store magnetic nanoparticles. Synthesis of nanoparticles in an one-stage culture was during period from 7 to 30 days that was a period of active reproduction and steady state of bacterial culture. Synthesis of bacterial biomass and nanoparticles was also in a two-stage process. Bacteria were grown on mineral medium with potassium

citrate as source of carbon and energy in the first stage. Cultivation in second stage was on the medium with iron citrate. Bacteria titer in this case exceeded by a factor of ten – hundred than in one-stage culture. Sampling was carried out in 3-28 days after an introduction of microorganisms into the nutrient medium. Produced sediments were dried at the room temperature and as result powders were obtained. The identification of crystal structure of the synthesized nanoparticles was carried out in the works [6,7]. It was shown that obtained nanoparticles are ferrihydrite. There are some crystal structures with chemical formula of ferrihydrite $Fe_2O_3 \cdot nH_2O$ (the Fe^{3+} ion is in octahedral environment of ligands) what is determined by different arrangement of ligand layers (oxygen and OH groups) [6,7].

To isolate ferrihydrite from sediment and to obtain sol the bacterial biomass was separated from supernatant by centrifugation (10 min, 10000 rpm), then bacteria cells were destroyed by ultrasound (ultrasonic disintegrator USDN – 1 min, 44 kHz, 20 w) 3 times per 3 min in distilled water at 10 min interval. Then obtained sediment was incubated in acetone during 30 min to remove fatty acids and was washed thoroughly with distilled water. Then sediment was placed in 20% NaOH solution, incubation period was 1 hour. Obtained sediment was washed thoroughly with distilled water with NaCl addition till obtaining neutral pH. Sol obtained in this way was dried at the room temperature. Sizes of ferrihydrite nanoparticles were studied in the work [8].

3. Magnetic measurements

Mössbauer measurements were carried out with Co^{57}(Cr) gamma source with a full width at half maximum of 0.24 mm/s for a sodium nitroprusside powder absorber. The sample thickness was 5–10 mg/cm^2 in terms of iron content, which ensured a linear relation between absorption and the Fe content of the phase of interest. Values of chemical isomeric shifts were plotted against the metallic iron α-Fe. Mössbauer spectra were measured at 300 K and 4.2 K. Magnetic measurements were carried out with vibrating sample magnetometer [9]. Investigated powder was fixed in measuring capsule in paraffin. Data were corrected to the diamagnetic signal of capsule with paraffin. The temperature dependences of magnetic moment M(T) were measured under the zero field cooling (ZFC) and field cooling (FC) conditions. Hysteresis loops of magnetic moment at the temperature T =4.2 K were measured under conditions ZFC.

4. Results and discussion

To verify an assumption that biogenic ferrihydrite nanoparticles were located on the surface of bacterial cells when iron citrate was consumed some samples were prepared. Cultures were grown in the Lovley medium. Volumes of culture in every flasks were 0.25 l. Beginning from 7th day, when stable sediments were formed, 0.25 l of sterile citrate buffer solution pH 5 (40 g/l citric acid, pH 5.0 was established by using KOH) was added in every flasks, except control, during 25 hours. So, incubation periods were from 1 till 25 hours. The citrate buffer solution must provide a separation of nanoparticles from the cell surfaces. In control sample the 0.25 l of the sterile tap water was added. The incubation periods of bacterial culture samples with citrate buffer solution are shown in table 1. As follows from the submitted data the addition of citrate buffer solution into the cultivation media has insignificant effect on the growth of bacterial culture and therefore the citrate buffer solution has no destructive influence on cells.

Table 1. Numbers of growing bacterial colonies after the addition of citrate buffer pH5 to the basic nutrient medium

Sam-ples	The incubation period of bacterial culture with citrate buffer solution, hours	Volume of added citrate buffer pH5, l	Numbers, cfu/ml
A	0	0.25(H_2O)	$0.52 \cdot 10^8 \pm 0.17 \cdot 10^8$
B	25	0.25	$1.88 \cdot 10^8 \pm 0.12 \cdot 10^8$
C	21	0.25	$1.72 \cdot 10^8 \pm 0.07 \cdot 10^8$
D	6	0.25	$3.67 \cdot 10^8 \pm 0.23 \cdot 10^8$
E	4	0.25	$3.91 \cdot 10^8 \pm 1.9 \cdot 10^8$
F	3	0.25	$2.01 \cdot 10^8 \pm 0.09 \cdot 10^8$
G	2	0.25	$1.995 \cdot 10^8 \pm 0.21 \cdot 10^8$

Biomass was separated from supernatant by centrifugation (30 min, 5000 rpm). Dried biomass was investigated using the Mössbauer method to detect a presence of ferrihydrite. On the basis of the Mössbauer analysis data it was established that after treatment of bacterial cultures with iron citrate and washing sediments with solution of 0.9% NaCl there was no ferrihydrite in biomass, therefore all ferrihydrite nanoparticles were separated from the bacterial cells. The adduced arguments resulted in the conclusion that ferrihydrite nanoparticles were synthetized on the surfaces of the bacterial cells of *Klebsiella oxytoca*.

According to the classical thermodynamics a nanoparticle nucleation in the cultural medium must take place by heterophase way. Figs. 1and 2 show that the increase in the numbers of the bacterial cells grown in batch culture on the Lovley medium with addition of 0.57g/l iron citrate was accompanied by the decrease in quantity of iron ions in the nutrient medium.

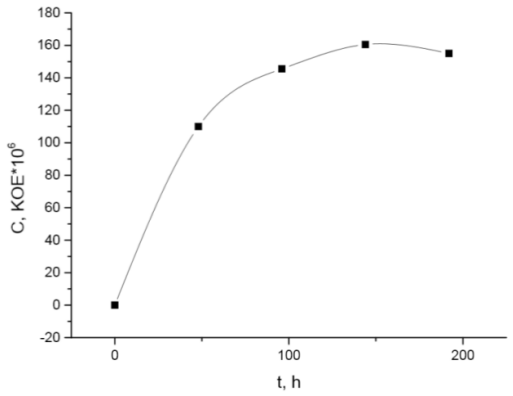

Fig. 1.The growth curve of the bacterial culture Klebsiella oxytoca. The bacteria number (10^6 cfu/ml) against time (hours)

The growth curve of microorganisms represented S-shaped curve characteristic of batch culture. The cultivation period was about 10 days. Fig. 1 showed distinctly that the growth curve had typical phases: the phase of an exponential growth, the phase of the growth retardation and in 7 days the stationary growth phase appeared. In this phase the number of living microorganisms remained constant, in this period the complete absorption of iron ions on the bacterial cell surfaces occurred, this is shown in fig.2. Dynamics of iron concentration changes in the nutrient medium was characterized by two features.

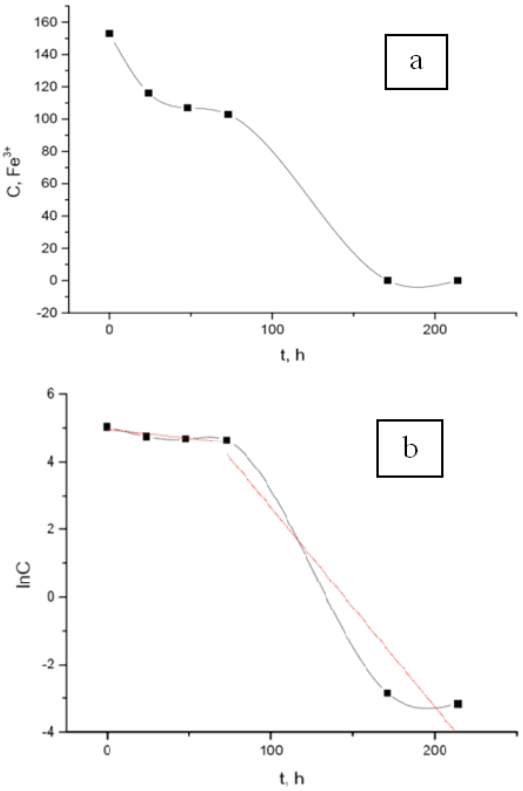

Fig. 2. Changes of Fe^{3+} ions in the culture medium: a) concentration of iron ions (g/l) against time of cultivation (h); b) logarithms of iron ion concentration against time (h).

To explain an origin of the curve features in figure 2 the thermodynamical approach indicated that an inhomogeneity of the curve of the iron ion concentration changes appeared due to a separation of the process into two stages with different rates. One of them was a nucleation. In this period the iron ions filled all free vacancies on the bacterial cells. The second period was a particle growth. The application of this approach became complicated due to increase in cell numbers simultaneously with particle growth and as result new centres of nucleation appeared. The correlation of dynamics of these processes demanded a further investigation.

The Mössbauer spectra of biomass and sol measured at the temperature 300K and 4.2K were showed on figure 3. The results of interpretation of spectra were tabulated in table 2 where positions with population more than 5% were given.

Table 2. Mössbauer parameters of nanoparticles

	IS	H	QS	W	A
biomass, 300 K	0.403	-	0.43	0.32	0.39
	0.402	-	0.70	0.28	0.32
	0.394	-	0.96	0.28	0.18
	0.394	-	1.23	0.30	0.11
biomass, 4.2 K	0.580	243	0	4.00	0.90
	0.537	-	1.24	1.02	0.10
Sol, 300 K	0.263	-	0.38	0.38	0.09
	0.361	-	0.46	0.29	0.28
	0.349	-	0.71	0.30	0.34
	0.341	-	0.98	0.29	0.19
	0.341	-	1.28	0.36	0.10
Sol, 4.2 K	0.489	519	0	0.43	0.17
	0.471	499	-0.04	0.48	0.30
	0.458	478	-0.06	0.46	0.20
	0.462	458	0.01	0.44	0.14
	0.463	437	0.08	0.43	0.12
	0.461	408	0	0.52	0.07

IS is chemical isomer shift relative to α-Fe, ±0.01 mm/s.
QS is quadrupole splitting, ± 0.02 mm/s.
W is the linewidth at half maximum, ± 0.02 mm/s.
S is the fractional occupancy of a position, ± 0.05.

Fig. 4 shows the temperature dependences of magnetic moment M(T) of investigated samples in the field H = 1 kOe measured under conditions ZFC and FC. There are no differences of M(T) data for biomass under these two conditions. The M(T) dependences of sol under conditions ZFC shows distinct maximum at the temperature T ≈ 23 K. In the vicinity of this temperature a bifurcation of M(T) dependences with different thermomagnetic prehistory is observed. This behaviour is characteristic of systems of superparamagnetic (SP)

particles with blocking temperature T_B, corresponding to the maximum of M(T) dependence under the ZFC conditions. The other distinguishing characteristic of SP system is the significant shift of the blocking temperature to the low temperature range under an increase of external field.

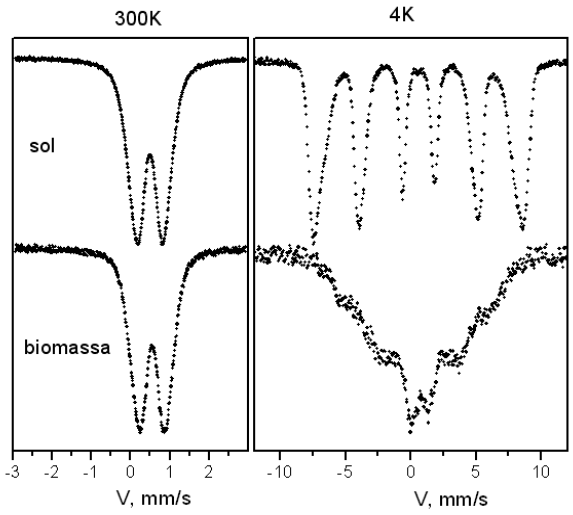

Fig. 3. Mössbauer spectra of investigated nanoparticles.

The inset of figure 4 illustrates this behavior; here the values of T_B (Y-axis) are plotted against different external fields (X-axis). At temperatures above 150 K the dependences 1/M are linear in T, their extrapolation to 1/M = 0 gives the value of the asymptotic paramagnetic Curie point equal to ~ –200 K. This indicates the presence of antiferromagnetic contribution, since ferrihydrite is an antiferromagnet [10].

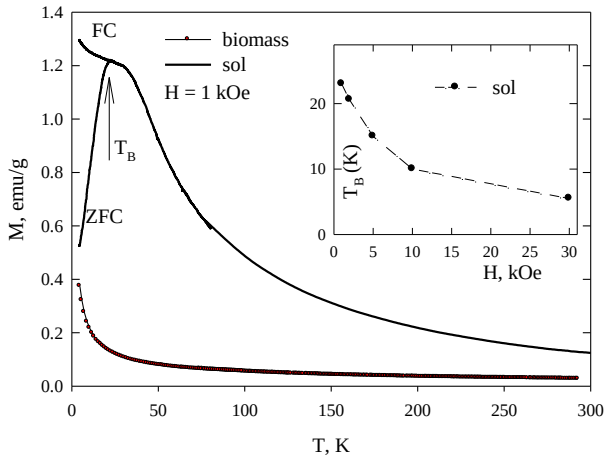

Fig. 4. The M(T) dependences in the field of H = 1 kOe under the ZFC and FC conditions (for biomass there are no differences between ZFC and FC conditions). The blocking temperatureT_B of the SP sol particles is shown. The inset shows the relationship between T_B and external field H for sol.

Fig. 5 shows the magnetization curves of investigated samples in low temperature range. For sol sample the M(H) dependences at $T < T_B$ are hysteretic, the value of coercive force at T = 4.2 K is equal to ≈ 1.9 kOe. For the investigated biomass sample the magnetization curves at the temperature above 4.2 K are completely reversible. These dependences like M(H) ones obtained for the sol sample at $T > T_B$ are superposition of SP contribution described in the simplest case by the Langevin function and linear function $M(H) = \chi_{AF} \times H$ (where χ_{AF} is AF susceptibility). Analysis of the magnetization curves permits to estimate the sizes of the ferrihydrite particles ~ 2÷3 nm [11,12,13]. The presence of magnetic moment in AF nanoparticles is caused by defects in the ferrihydrite structure [14].

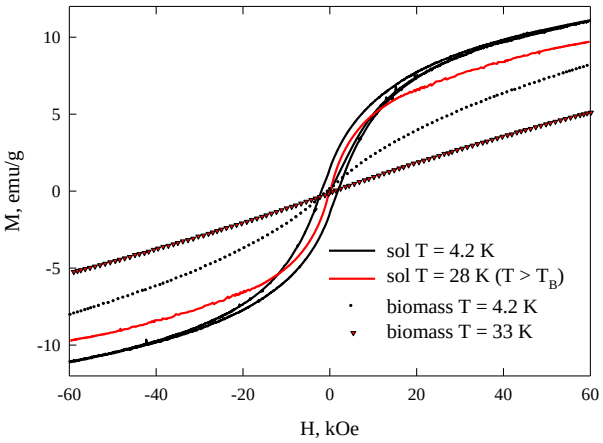

Fig. 5. The magnetization curves of investigated samples in the low temperature range.

5. Conclusion

The laboratory experimental technology to obtain biomass of bacteria producing the ferrihydrite nanopacticles was developed. The technological conditions of separation of nanoparticles from bacterial culture and obtaining the water sol were worked out. It was established that the ferrihydrite nanoparticles produced by bacteria *Klebsiella oxytoca* were located on the surface of bacterial cells. Magnetic features of the ferrihydrite nanoparticles in obtained sol changed considerably in comparison to initial nanoparticles. The blocking temperature $T_B \approx 23$ K and magnetic hysteresis at $T < T_B$ appeared. The magnetization curves of initial biomass nanoparticles are completely reversible. The Mössbauer spectra of investigated nanopacticles also considerably modified, in particular, the chemical isomeric shift in obtained sol is lower than in the initial biomass nanoparticles synthesized by microorganisms. This indicates changes of ligands surrounding the Fe^{3+} ions. The amount of oxygen in comparison with OH-group is increased or we can say that the amount of water in the ferrihydrite nanoparticles $5Fe_2O_3*nH_2O$ decreased.

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*Corresponding authors: rauf@iph.krasn.ru, stol@iph.krasn.ru