

Ferritization of Industrial Waste Water and Microbial Synthesis of Iron-Based Magnetic Nanomaterials From Sediments

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The precipitation of iron and associated heavy metals in industrial waste water and in model solution using ferritization and aerobic bacterial culture was investigated. Magnetic sediments extractable by magnetic separation (specific saturation magnetization of 16–36.8 G cm³/g) were produced by precipitation of iron by ferritization method at pH (8–10) and 60–80°C for 15–30 min. Nanoparticles of ferrihydrite or ferric hydroxide doped with associated metals (Co, Ni) were produced under precipitation of ferric iron in model solution with bacteria at the temperature 26–34°C. The radii of synthesized particles are 1–5 nm and nanoparticles of ferrihydrite are superparamagnetic in both un-doped and doped (Co, Ni, Zn) sets. Dispersed structure of biogenic nanoparticle sols, their magnetic and other properties were studied by atomic force microscopy, X-ray small-angle scattering, X-ray diffraction, electron magnetic resonance, Mössbauer and X-ray photoelectron spectroscopy. © 2016 American Institute of Chemical Engineers Environ Prog, 35: 1407–1414, 2016

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INTRODUCTION

Nanostructured materials have attracted considerable attention in recent years because they exhibit useful and unusual properties compared to conventional polycrystalline materials [1]. The physical and chemical properties of metal nanoparticles are mainly determined by its size, shape, composition, crystallinity and structure [2]. Control over these parameters is crucial for a successful utilization of the size-dependent properties that are unique to nanoparticles like assembly of monolayer-protected nanoparticles into crystalline arrays of one-, two- or three-dimensions. These nanocrystalline particles have a high surface/volume ratio leading

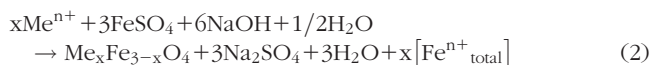
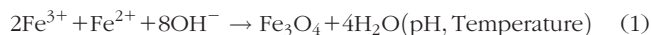
to magnetic properties different from those of bulk materials [1]. The controlled synthesis of magnetic nanoparticles is of high scientific and technological interest. Biomineralization, the processes by which organisms form minerals, is widespread, with more than 60 biologically formed minerals identified [3].

Synthesis of iron-based nanoparticles based on the physico-chemical methods by chemicals has now grasped the attention of biotechnology. The synthesis of minerals by bacteria has been classified according to the degree of control over the mineralization process, namely those that are formed passively or actively. Microbes are involved in the biomineralization of many minerals [4]. However, most of these minerals are produced by biologically induced mineralization (BIM) processes, and only a few good examples of minerals biomineralized by both BIM and BCM (biologically controlled mineralization) processes have been reported. Magnetite remains as the best characterized of these examples and it is interesting and important that magnetite produced by both processes can be formed almost side by side in certain environments [5,6]. Ferric oxides and hydroxides are found in natural materials—soil, sediments, often with the participation of microorganisms. They are found within cells and in the extracellular space. Microorganisms require specific conditions for their growth owing to which biogenic nanoparticles may have characteristics significantly distinguish them from synthetic physicochemical methods [5]. Taking into consideration the significance of microbially produced magnetite in natural environments [3], lack of information on the mechanisms on biologically controlled magnetite formed in magnetotactic bacteria, and unexplained aspects of biomineralization resulting in metal hydroxides (ferrihydrite and other) formation, it is likely that the biomineralization of magnetite by microbes is a new niche area [3,4]. Formation of magnetite and ferrite in an aqueous medium can be represented as follows [4]:

Table 1. Metal content in initial waste water and after ferritizing.

Element	Concentration of metals in initial waste water, mg/L						
	Fe	Cu	Zn	Pb	Mn	Cr(III)	Ni
Variation limits	3840–11665	00.5–3.6	37.2–227.2	20.4–777.7	17–36.9	0.9–6.3	21.9–253.2
	Residual concentrations of metals, mg/L						
Variation limits	n/d–1.1	n/d–0.19	0.03–3.1	n/d–0.3	0.03–1.57	n/d–1.1	0.05–3.4
Mean values with overshoot (N=10)	0.356	0.03	0.343	0.01	0.22	0.032	0.642

n/d – not detected (below sensitivity of the method).



The basic principle of the ferrite process consists in adding Fe(II) ions to a heavy metal containing solution. At high pH, mixed metal hydroxides are precipitated out of the solution, and under oxidizing conditions ferrite formation takes place [5].

In the early 80s, the removal of heavy metals from waste water was proposed by a method ferritization. The method consists in the formation of the magnetic iron-based particles in waste water under certain conditions where iron in the liquid medium can be in soluble and insoluble forms. Insoluble amorphous iron hydroxide and crystal formation gets associated with various heavy metals, non-metals and organic compounds contaminating the wastewater, thereby treating them. This method renders the advantages of transfer of water pollutants into a compact, insoluble form, which is easily removed by magnetic separation, and then the reuse of sludge produced as a sorbent. Keeping this in view, the contribution of aerobic bacteria in synthesis of magnetic nanoparticles by induced mineralization is studied in this work using sediment synthesized by ferritization. It particularly emphasized on the dispersed structure of biogenic nanoparticles and characteristics of these compounds by atomic force microscopy, X-ray small-angle scattering, X-ray diffraction, electron magnetic resonance, Mössbauer and X-ray photoelectron spectroscopy.

MATERIALS AND METHODS

Ferritization and Design of Experiments

Synthesis of disperse particles by ferritizing [7] was analyzed on model waste waters containing iron and ions of manganese, zinc and cobalt and in industrial waste water of non-ferrous metal processing plant. Industrial wastewater contained iron and, in the form of impurities—copper, nickel, chromium, lead, zinc and manganese. Environment conditions in precipitation of metal ions were evaluated in experiments carried out in compliance with design matrix of complete factorial experiment (CFE) 2³ and orthogonal central composition design (OCCD) [7]. Controlled variables were: temperature; concentration ratio of ferrous and ferric iron [Fe²⁺]/[Fe³⁺]. Total concentration of iron was 5 g/L in CFE and 1 g/L in OCCD experiment. Ferritizing was carried out at pH of the medium 8–10 and temperature 60–80°C for 15–30 min duration. Iron-attendant metals were set in the form of water-soluble salts. Ion concentrations in CFE were (mg/L): Zn—35, Mn—160, Ni—380. In OCCD experiments, impurity elements were set in lower concentration—18, 80 and 190 mg/L, respectively. All multifactorial experiments per-

formed in compliance with design matrices, were carried out in two versions: in the first, the source of trivalent iron was chemical reactant of ferric sulphate; in the second, ferric iron was produced by bacterial oxidation of the ferrous form. Residual concentration of metal in water was taken as measured response. Efficiency and reproducibility of extraction of iron and impurity metals in the form of magnetic precipitation by ferritizing were evaluated on industrial waste water while fluctuating composition of wastes [8]. Multifactorial experiments showed precipitation of iron and other metals to depend heavily on unidentified factor of bacterial origin.

Bacteria

Bacteria *Pseudomonas moorei* and *Delftia tsurutensis* were isolated from autochthonous microflora North Onega and the Middle Timan deposits (Russia), and halotolerant *Pseudomonas argentinensis* from salt lake Shira (Khakassia, Russia). Bacteria were cultured in a batch process with stirring and aeration with air at 26–34°C, initial pH 6.0–6.3 in 60 h in mineral medium with iron citrate as sole carbon and energy source and a precursor of iron precipitates (Eq. 3). The bacteria(s) used in this process were believed to help in biologically controlled mineralization.

Analysis and Characterization

The nanoparticles doped with metals (cobalt, nickel and zinc), were washed from bacteria and isolated as a colloidal solution with an iron concentration of about 0.5–1.5 g/L, then dried at 60°C and powders were prepared for characterization by XRD, XPS and Mössbauer spectroscopy. Residual concentrations of metals in water after precipitation and isolation of the desired solid phases were evaluated by atomic absorption spectrophotometer. Iron status was defined by the data of Mössbauer spectroscopy with source Co⁵⁷(Cr) (MC1104EM spectrometer). Morphological characteristics of nanoparticles were evaluated on small-angle X-ray diffractometer S3-MICRO (Hecus, Austria) and multi-mode scanning probe microscope Solver P47 (NT-MDT, Russia). Spectra of electronic magnetic resonance were studied with X-range spectrometer SE/X – 2544 with nitrogen attachment.

RESULTS AND DISCUSSION

Formation of hydroxides and iron oxides with magnetic properties in aqueous media is well known as per Eqs. 1,2 [4]. This phenomenon is used to purify industrial waste waters from heavy metal ions by ferritizing [7]. Ferritizing is carried out at pH of the medium 8–10 and temperature 60–80°C for 15–30min duration. It was observed that the process is highly sensitive to temperature, pH, Fe²⁺/Fe³⁺ ratio and total iron concentration. An example of real variability of ferritizing process in industrial waste water treatment is presented in Tables 1 and 2. Precipitation was most complete with lead and copper (in 7 and 4 out of 10 experiments

Table 2. Specific magnetization of sediment.

Sample No.	1	2	3	4	5	6	7	8	9
Specific magnetization [Gauss-cm ³ /g]	36.82	36.20	36.20	33.00	26.26	24.66	19.52	15.97	4.30

Table 3. CFE 2³ design matrix and experimental results.

Factor	T	pH	Fe ²⁺ /Fe ³⁺	Results of Experiments							
Denotation	X ₁	X ₂	X ₃	Residual concentration (mg/L)							
Units of measurement	⁰ C	pH	Relative units	Initial concentrations: Zn = 35, Mn = 160, Ni = 380, Fe _{total} = 5000							
Lower level (-)	60	8	0.50								
Medium level (0)	65	9	0.75								
Upper level (+)	70	10	1.00								
Factors	T	pH	Fe ²⁺ /Fe ³⁺	Zn ^{ch}	Zn ^b	Mn ^{ch}	Mn ^b	Co ^{ch}	Co ^b	Fe ^{ch}	Fe ^b
Experiments	Coded values			Zn ^{ch}	Zn ^b	Mn ^{ch}	Mn ^b	Co ^{ch}	Co ^b	Fe ^{ch}	Fe ^b
1	-	-	-	0.10	n/d	23.0	n/d	5.70	n/d	94.0	n/d
2	+	-	-	0.05	0.03	15.2	n/d	1.95	n/d	24.6	n/d
3	-	+	-	0.06	0.05	96.0	n/d	25.0	n/d	226	n/d
4	+	+	-	0.05	0.05	86.0	n/d	19.5	n/d	160	n/d
5	-	-	+	0.05	0.05	64.0	n/d	10.0	n/d	112	n/d
6	+	-	+	0.12	n/d	112	n/d	33.0	n/d	314	n/d
7	-	+	+	0.03	0.03	4.16	n/d	0.02	n/d	n/d	n/d
8	+	+	+	0.05	0.03	32.0	n/d	21.0	n/d	360	n/d
9	0	0	0	0.05	n/d	15.0	n/d	1.80	n/d	11.6	n/d

Note: indices “ch” and “b” denote experiment versions in which iron (III) was used in the form of chemical reactant of ferric sulfate and bacterially oxidized ferrous sulfate, respectively; n/d – not detected.

Table 4. Parameters of Mossbauer spectrum of iron hydroxide powder obtained in the culture of *P. argentinensis*.

IS	QS	W	A	Position
0.388	0.49	0.36	0.43	Fe1
0.385	0.81	0.31	0.32	Fe2
0.372	1.22	0.39	0.25	Fe3

Note: IS – isomer chemical shift relative α-Fe (± 0.01 mm/s), QS – quadrupole splitting (±0.02 mm/s); W – width of the absorption line (±0.02 mm/s), A – fractional site occupancy (± 0.03).

respectively; residual concentration was below evaluation threshold). The solid phase produced (crystalline sediment) is magnetically susceptible (Table 2) and can be extracted by magnetic separator. Measurements of specific magnetization of sediments produced in treatment of industrial waste water under above conditions are presented in Table 2.

Operational parameters of ferritizing were invariable in all experiments. However, the quality of water treatment and magnetic characteristics of the sediment were observed to vary considerably. The process seems to be very sensitive to the accuracy of maintaining operational parameters. Meanwhile, total content of iron and impurity metals in industrial waste water varies. In some situations organic compounds found in the water can have considerable impact [7]. To evaluate admissible variability of temperature, pH, ratio of reduced and oxidized forms of iron and its total content, factorial experiments were carried out. Factorial experiment design matrix and the results (residual concentration of zinc, manganese, cobalt and iron) are presented in Table 3.

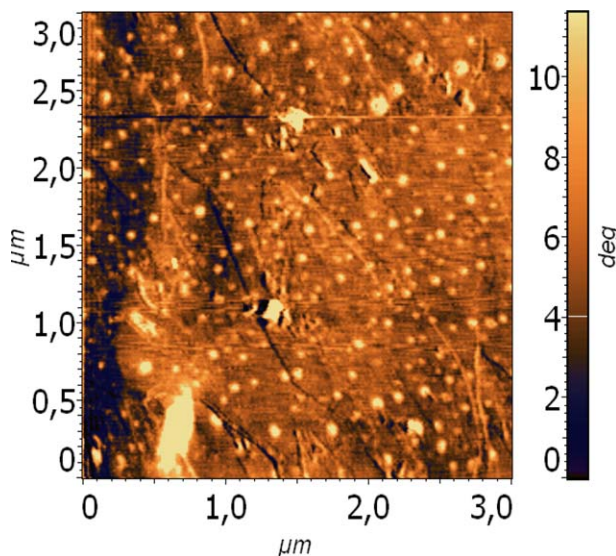


Figure 1. AFM image of microbial suspension with synthesized particles. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Quantitatively the effect of environment parameters [9] is described by regression equation (Eq. 4),

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 \quad (4)$$

where Y is the residual concentration of metal; b_0, b_1, b_2, b_3 are the regression coefficients.

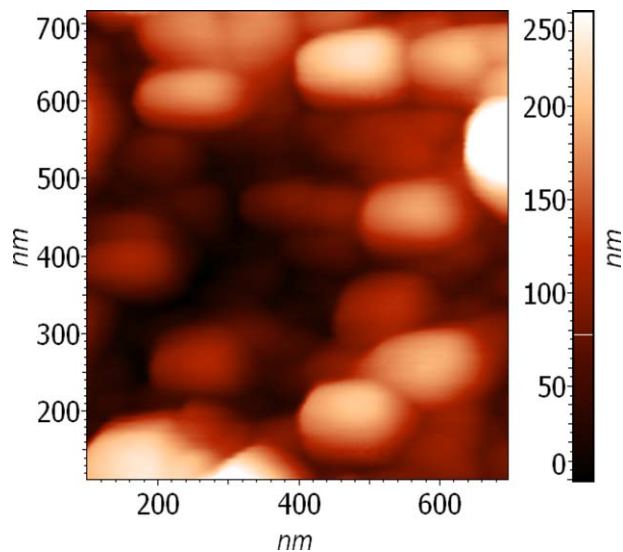


Figure 2. AFM image of biogenic nanoparticles deposited on pyrographite (tapping mode image) from colloidal solution. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

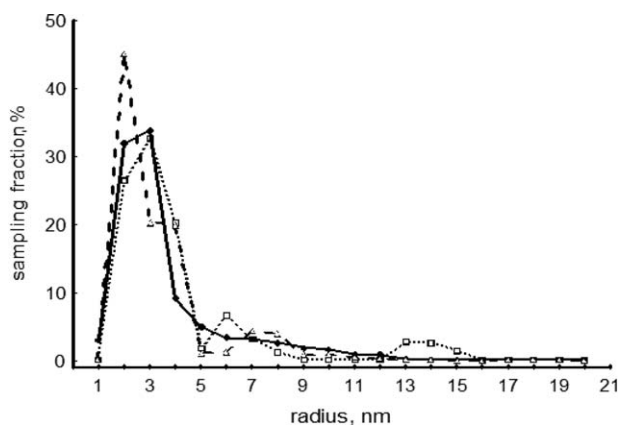


Figure 3. Size distribution of nanoparticles of doped iron hydroxide (solid line – Zn, dashed line – Co, dotted line—Ni).

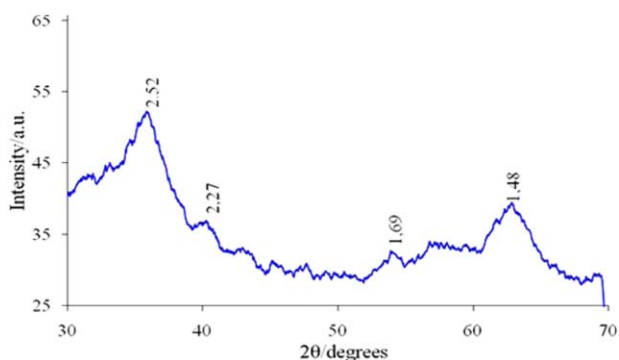


Figure 4. Typical X-ray powder diffraction pattern of un-doped biogenic ferrihydrite. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

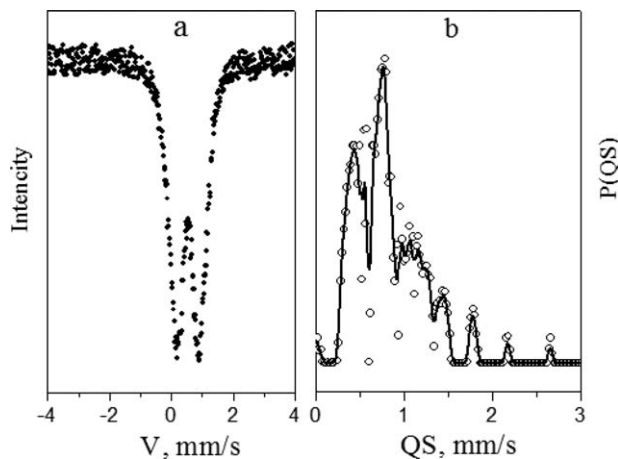


Figure 5. The room temperature Mossbauer spectrum (a) and QS probability (b) of un-doped iron hydroxide, resulting in the culture of bacteria *P. argentinensis*.

Table 5. Parameters of spectrum of electronic magnetic resonance of biogenic ferrihydrite doped with nickel and cobalt.

Sample	Hrez (mT)	g-factor	Width of resonance line (mT)
Fe + Ni	329.3	2.036	71
	323.0	2.070	191
Fe + Co	320.5	2.090	92
	270.0	2.480	369

For residual concentration of iron in the version of experiments Fe^{ch} , Eq. 4 is modified as Eq. 5 is generated (in coded variable values)

$$Y = 161.3 + 65X_1 + 36.9X_2 + 46.9X_3 \quad (5)$$

Iron precipitation in the center of design (11.6 – experiment 9, version Fe^{ch}) was considerably below the calculated values ($b_0 = 161.3$). This means that the degree of purification and residual concentrations of metal ions in water considerably and non-linearly depend on precipitation conditions (temperature, pH and Fe^{2+}/Fe^{3+} ratio). Another important conclusion is that minimum residual concentrations of metal ions (less than 0.1–0.2 mg/L) are attained in the case of ferrous sulfate oxidized by bacteria (variants with index “b” in Table 4). This indicates the higher efficiency of metal precipitation with ferric iron sulfate produced by oxidation of its divalent form by bacteria making the role of bacteria evident. From the data of Table 3, identical conclusions can be made for zinc, manganese and cobalt. To have a better idea about dependence of metal precipitation on operating parameters of the process, OCCD-design experiment was carried out which shows high and complex dependence of metal precipitation on environment parameters. In each specific case, the maximum effect can be achieved only under strictly defined values of the said operating parameters. Moreover, significant positive effect was obtained in variant with ferrous iron oxidized by bacteria. According to results of electron microscopy, the precipitates obtained with Fe^{bio} were aggregates of nanoparticles. The precipitates in this case are ferrites. Metal ions precipitated more efficiently in the presence of unidentified factor of bacterial origin. Keeping this in view,

Table 6. Comparative outlook on ferrihydrite biosynthesis from ferritized sediments by various researchers.

Source	Template used	Experimental Conditions	Results/Inference	Reference
0.2 M FeCl ₂	<i>Acidithiobacillus ferrooxidans</i> LX5 cells	pH <2.0, 28°C, in modified 9K medium	200nm spindle shaped akaganite [Fe ₈ O ₈ (OH) _{7.1} (Cl) _{0.9}]	20
0.1 M of FeCl ₂ /FeSO ₄ solution	<i>Acidithiobacillus ferrooxidans</i>	pH: 2.0-3.3, 28-35°C	<200nm Pure akaganite (Fe ₈ O ₈ (OH) _{7.1} (Cl) _{0.9}), Pure schwertmannite (Fe ₈ O ₈ (OH) _{4.42} (SO ₄) _{1.79} precipitates	21
Seafloor incubations of Juan de Fuca Ridge (Fe: 0.007-25 mmol/kg)	<i>Fe-encrusted biofilms of Fe oxidizing microbes</i>	pH: less than 3, by ageing	<10nm dia Ferrihydrite	22
Myron Lake, Rainbow Lake and Spring Lake, WA [Fe: 450.63 ± 17.73 mg/L at Myron Lake; 431.67 ± 109.58 mg/L at Spring Lake]	<i>Leptothrix ochracea</i>	pH: 7.1-7.8, 8-16°C, by ageing	<10nm dia δ ferrihydrite	23
Iron-alginate suspensions [Fe: 30-300 M]	<i>Gallionella ferrugina</i> from Pb-Zn mine (Tennyson, WI)	pH 5.8-6.4, 37°C, 20d	5 nm wide spherical clumps of ferrihydrite	24
Iron-sucrose and iron-gluconate suspension [Fe: 12-100mg]	Sucrose and Gluconate	pH 7.7-11.1, 20d	2nm and 7nm β FeOOH from Iron sucrose and iron gluconate respectively	25
Iron sulfate suspension	<i>Thermophillic Fe(III)-reducing bacteria</i>	45-70°C in 1-2d	30-100 nm (6.74 Gcm ³ /g)	26
Sediment of Deferritized waste water rich in iron	<i>Psychrotolerant strains P. moorei, P. argentinensis and D. isurubattensis</i>	0-37°C in 30d 26-34°C, pH 6, 2-3d	10-30 nm 1-5 nm (16-36.8 G cm ³ /g)	Present work

experiments were carried out to synthesize ultrafine iron particles in bacterial culture as template [10–13].

Initial conditions of processes of precipitation of metal ions (ferritization method) [7] or chemical synthesis of nanoparticles by precipitation from solution precursors [11,12] differ on principle from the nanoparticle synthesis in bacterial culture. In first variant, the initial concentration of metal ions is maximal and in second variant one is minimal.

Different bacterial species were assumed to create essentially different conditions for precipitation of iron and accompanying metals. Originally, we synthesized biogenic nanoparticles of iron hydroxide in microaerophilic culture of capsulated bacteria *Klebsiella oxytoca*. In the present study, we used aerobic bacteria cultures *P. moorei*, *D. tsurubatusensis*, and halotolerant bacteria *P. argentinensis* without capsules. In the culture of *P.*

argentinensis, precipitation was carried out in the medium with high ionic strength of the solution (80 g/L NaCl) where iron nanoparticles are formed in the extracellular space as seen in Figure 1. These particles were precipitated from the colloidal solution on a pyrographite and visualised to have a spherical shape (up to 100 nm and about 300 nm diameter), and an elongated shape (about 40x80 and 60x160 nm). It should be noted that in case of extracellular production of nanoparticle, centrifuge could be used for extraction and purification of nanoparticles, but aggregation might happen as observed here too. To avoid aggregation and improve dispersion, the nanoparticles were doped with cobalt and visualised by scanning force microscopy in tapping mode as shown in Figure 2. The sols

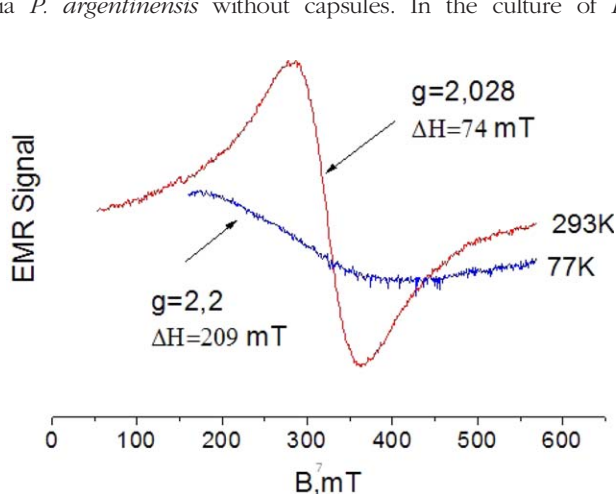


Figure 6. Electron magnetic resonance spectra of un-doped iron hydroxide. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

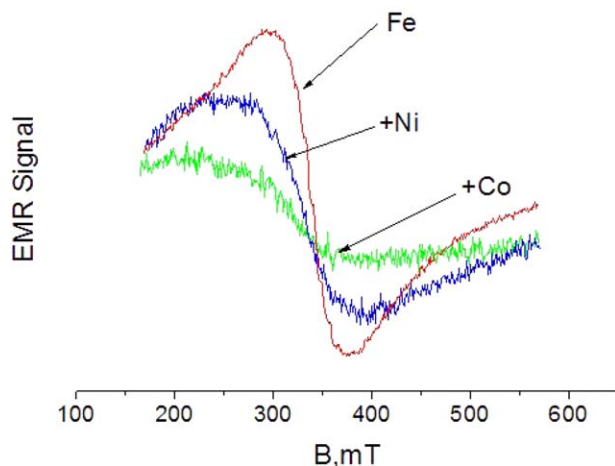


Figure 7. Electron magnetic resonance spectra of doped and un-doped iron hydroxides. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

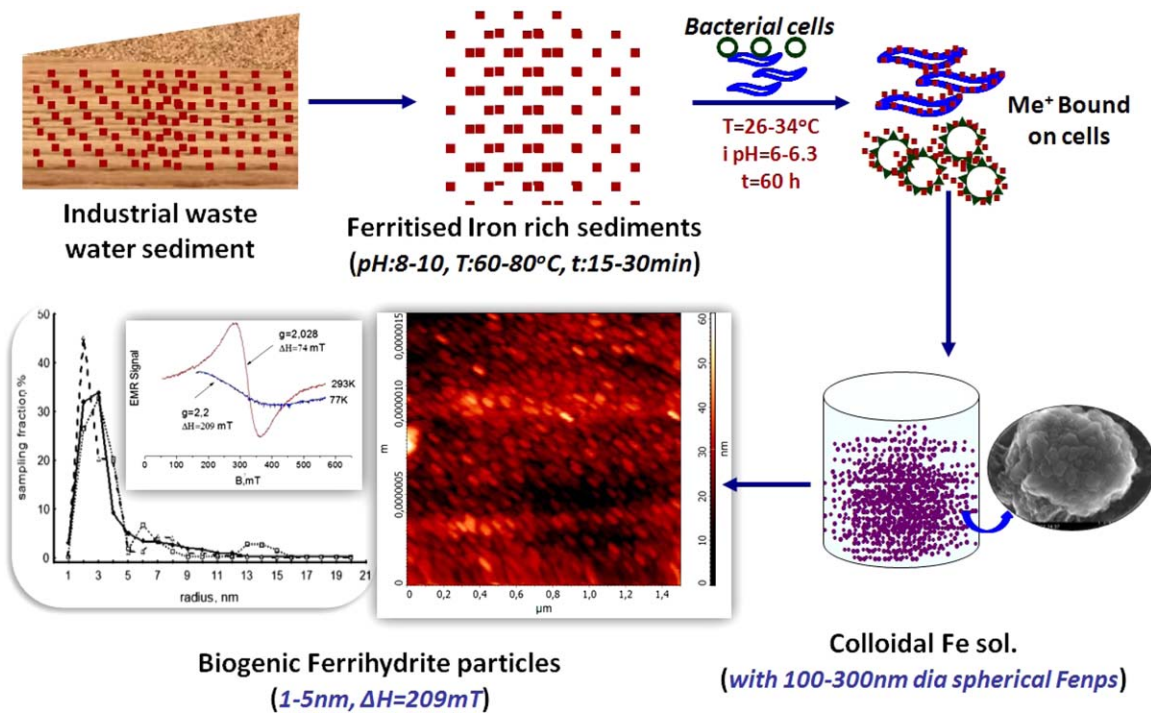


Figure 8. Mechanistic representation of microbial synthesis of ferrihydrite magnetic nanoparticles from ferritized sediment. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

dispersed structure was analyzed also by small-angle X-ray scattering. About 90% of the nanoparticles have a radius in the range 1–5 nm. Figure 3 shows size distribution of iron nanoparticles doped with zinc (solid line, *P. moorei*), cobalt (dashed line, *D. tsurubatusensis*) and nickel (dotted line, *P. moorei*). Distribution of nickel-doped nanoparticles somewhat shifted towards smaller size relative to two other samples which practically coincide. Comparing data in Figures 1–3, it can be said that observed AFM particles are aggregates of nanoparticles which have a much smaller size.

X-ray diffraction pattern obtained for samples without introducing cobalt and nickel, are shown on Figure 4. As evidenced by two broad diffraction peaks in Figure 4 in the aerobic bacteria culture, 2-line ferrihydrite was formed. These data complement the results of Mössbauer spectroscopy. The Mössbauer spectrum of the sample of nanoparticles synthesized in the culture of halophilic bacteria *P. tsurubatusensis* is shown in Figure 5a. Analysis of the probability distribution of the quadrupole splitting (QS) (Figure 5b) indicates that the iron in the structure of nanocrystals is in three nonequivalent positions (Fe1, Fe2, Fe3), which are characterized by the values of the QS 0.50, 0.82 and 1.13 mm/s respectively (Table 4). The same type of iron states was observed in the structure of the nanoparticles from cultures *P. moorei* and *D. tsurubatusensis*. The values of isomeric chemical shifts are typical of the ferric hydroxides in the range 0.322–0.388 mm/s [14]. Four nonequivalent positions of iron were previously found in nanocrystals from *Klebsiella oxytoca* culture.

To interpret forms of iron, it is necessary to carry out additional investigations. Content and ratios of Fe, O and C, and content of minor elements depend on purification efficiency of nanoparticles under isolation them from culture liquid. Analysis by electron magnetic resonance spectra (EMR) in Figure 6 showed the material produced in cultures of aerobic bacteria exhibited superparamagnetic properties. As the temperature drops, the resonance field (Hrez) decreases and the line broadens which corresponds to magnetic moment. The value of g-factor (2.028) corresponds to nanoparticles of iron hydroxide.

Analysis of EMR spectrum of the doped ferrihydrite nanoparticles shows the amount of nickel and cobalt in the latter is insignificant resulting in a shift of the resonance field and in considerable broadening of the line (Figure 7). Doping results in particles with pronounced magnetocrystalline anisotropy in line with EMR parameters is presented in Table 5. The modeling of experimental spectra was carried out with help of two lines of Lorentz form that indicates definitely a presence of magnetic anisotropy which is more significant in the case of cobalt. One can assert that Ni²⁺ and Co²⁺ ions are included in structure of biogenic ferrihydrite.

XPS was used to determine the content of elements, their ratio and to specify the chemical state in the layer which had thickness about one nanometer [15,16]. According to the data of XPS, the ground state of iron biogenic nanoparticles was ferrihydrite. Dominant elements on the surface of sample were iron (12.5% at), oxygen (45.7% at) and carbon (33.8% at) with traces of nitrogen, sodium and impurities like calcium and chlorine. The O/Fe ratios in examined samples of iron hydroxide were 2.38 and 1.89. For ferrihydrite with its varying content, there were suggested a number of chemical formulae—Fe₁₀O₁₄(OH)₂ and Fe_{8.2}O_{8.5}(OH)_{7.4} [17,18], 5Fe₂O₃·9H₂O [19] with the O/Fe ratios equal to 1.6, 1.94 and 2.4, accordingly. Carbon and oxygen in spectra can be in hydrocarbon compounds (C—H group), hydroxyl (C—OH) and carboxyl or carbonyl groups (COOH or C=O). A comparative representation of various works published elsewhere is presented in Table 6, where in the major crux emphasized is the application of microbes from mine sediments as the sole template for synthesis of ferrihydrite. The mechanistic approach followed in this paper for producing

the ferrihydrite super-paramagnetic nanoparticles from sediments is represented in Figure 8.

CONCLUSIONS

- Ferritization at pH 8–10 and 60–80°C for 15–30 min followed by chemical precipitation of metal ions (Fe, Co, Mn, Ni, Pb) at pH 7–10 and temperature 70–90°C resulted in the formation of ultrafine particles of magnetite and ferrite in 15–30 min.
- The temperature, pH, Fe²⁺/Fe³⁺ ratio and oxidation of iron by bacteria exert an essential influence on the efficiency of metal precipitation.
- It was shown that extracellular superparamagnetic particles of ferrihydrite and ferric hydroxide doped with Co, Ni and Zn ions are generated in cultures of aerobic bacteria (*P. moorei*, *P. argentinisensis* and *D. tsurubatusensis*) at 26–34°C, pH 6 in 60 h.
- The values of radii of biogenic ferrihydrite (about 90%) are within the range 1–5 nm. The aggregates of spherical and elongated forms in liquid culture and colloid solutions are about 40–60 nm, 90–120 nm and more large ones about 300 nm in size.
- Analyses performed by Mössbauer spectroscopy, small-angle X-ray scattering, X-ray diffraction and electron paramagnetic resonance, showed that in all test cultures of bacteria, relatively uniform superparamagnetic nanoparticles of iron hydroxides are formed, and the production process of nanoparticles is reproducible. In all samples, the iron is in the ferric state. Particle size can vary depending on process parameters culturing bacteria and isolation of the nanoparticles.
- The biogenic ferrihydrite can serve as a potential adsorbent for multi-metal effluents, which shall be depicted in the later part of this study.

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ABBREVIATIONS

BIM	Biologically induced mineralization;
CFE	Complete factorial experiment;
OCCD	Orthogonal central composition design;
QS	Quadrupole splitting

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