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Some Mineralogical Approaches to Study the Biocarbonate and the Carbonate-Siliceous Nodules

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Abstract Nodules of different compositions from Paleozoic sedimentary rocks and those deposited by microbial communities in laboratory-scale experiments were studied by the use of electron paramagnetic resonance, X-ray diffraction, and scanning electron microscopy with energy-dispersive spectrometer. The study of the mineral composition of fossil nodules showed that they have monomineral composition of dolomite or chalcedony, mixed composition of dolomite-chalcedony or of opal-dolomite, and finally nodules can be composed of alternating opal and chalcedony layers cementing fine dispersed dolomite grains or clusters of irregular shape. Similarity in dolomite crystal lattice defects in both the nodules and the host rocks confirms their formation during synsedimentary early diagenesis. Bacterial activity during sedimentary nodules growth is evidenced by the presence of paramagnetic carbon-centered free radicals of fossilized protein substances and findings of fossil bacteria. Experimental laboratory-scale modeling of natural carbonate deposition by microbial communities confirms that bacteria can promote nodules formation.

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Keywords Nodules · Bacterial activity · Biogenic carbonates and flints · Methods of investigation · Experiments

Sedimentary and volcanic-sedimentary rocks are formed in the water-saturated upper part of the lithosphere. Water-saturated conditions can be considered to be the most favorable for biogenic–abiogenic interaction when the biota affects both upwelling gas–fluid flows from the Earth's interior and surface deposits resulting in the formation of biominerals. Current reassessment of the role of bacterial assemblages in sedimentogenesis and lithogenesis creates a new direction in the lithology called as "bacterial lithogenesis" (Antoshkina 2012). Since almost all sedimentary rocks formed in varying degrees with the participation of the microbiota, one of the tasks of lithogenesis is to develop models of sedimentation in ancient epicontinental basins taking into consideration the essential role of bacterial factor. The study of nodules can be useful for solving these tasks. The problem is that the participation of bacteria in sedimentogenesis and lithogenesis should be proved using a complex of physical research techniques.

Lithological syngenetic nodules are often found in sedimentary rocks of different geological age formed in various facies conditions. Chemical and mineral compositions of the nodules and the surrounding rock may be the same or different from one another: carbonates, phosphates, silica oxides (opal, chalcedony, quartz), oxide-hydroxides of iron and manganese, and mixed iron-manganese oxides. There are microscopic and giant, monomineralic or mixed mineral nodule, including clays and sandstones. Often, nodules are spheroid or disk-shaped but sometimes form a variety of more complex shapes. Nodules of similar shape but of different mineral composition are presented in our collection (Fig. 1a–f): a dolomite (gypsum-dolomite stratum P_2 kz_2, right bank of the Volga river, v. Klyuchischi); b vernadite-lithiophorite-quartz mineral association (jasper-silicite deposits with manganese ore interlayers D_2 ef, Southern Urals, Baimaksky district, v. Fayzulino); c sandstone with quartz cement (jasper-silicite deposits D_2 ef-zv, Southern Ural, Chelyabinsk region, v. Kizilskoe); d opal-dolomite association (gypsum-dolomite deposits P_2 kz_2, right bank of the Volga river, v. Krasnovidovo); e gray jasper (jasper-silicite deposits D_2 ef-zv, Southern Ural, Sibai); f quartz sandstone with vernadite-lithiophorite cement (jasper-silicite complex D_2 zv, Southern Ural, Sibai district, v. Khasanovo).

Outcrops of the nodules occur locally in the vertical section of a given sedimentary horizon, forming meadows in landscape views, and often traced from one layer to another. Their presence indicates local geological events in paleobasin development—localized gas/fluid flow associated with seafloor seeps. However, the question of the nodules genesis up to this time predominates the model of chemogenic consolidation in the late diagenesis and catagenesis, implying the presence of permeable media and migrating solutions with a high degree of mineralization

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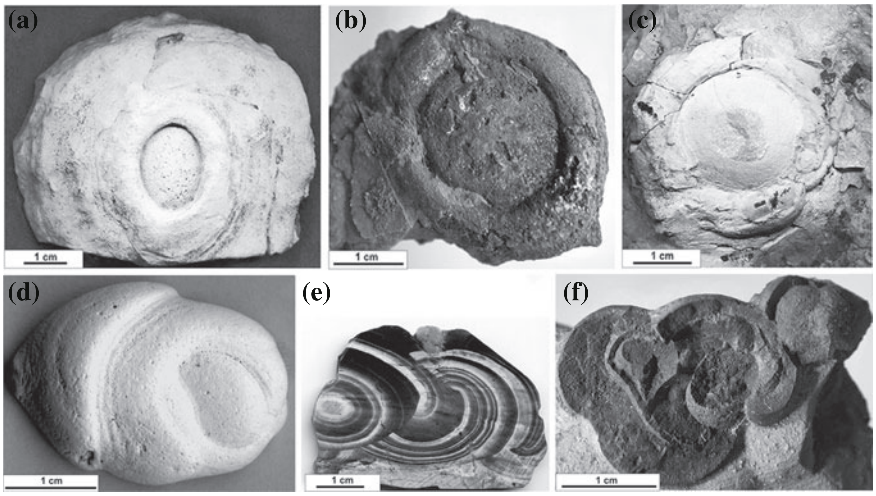

Fig. 1 **a–f** Nodules with similar morphology and differing in mineral composition: **a** dolomite (gypsum-dolomite stratum P_2 kz_2, *right* bank of the Volga river, v. Klyuchischi); **b** vernadite-lithiophorite-quartz mineral association (jasper-silicite deposits with manganese ore interlayers D_2 ef, Southern Urals, Baimaksky district, v. Fayzulino); **c** sandstone with quartz cement (jasper-silicite deposits D_2 ef-zv, Southern Ural, Chelyabinsk region, v. Kizilskoe); **d** opal-dolomite association (gypsum-dolomite deposits P_2 kz_2, *right* bank of the Volga river, v. Krasnovidovo); **e** *gray* jasper (jasper-silicite deposits D_2 ef-zv, Southern Ural, Sibai); **f** quartz sandstone with vernadite-lithiophorite cement (jasper-silicite complex D_2 zv, Southern Ural, Sibai district, v. Khasanovo)

(Strakhov 1962). But this model cannot explain the genesis of those nodules which are the same in the mineral composition with surrounding rocks, or whose specific shapes (Fig. 1a–f) do not follow the mechanism of chemogenic consolidation.

An alternative hypothesis of the dissolved substances concentration and of forming consolidated nodules is related to the leading role of microbial communities, the part of which can promote mineral precipitation (Berner 1971; Skinner and Fitzpatrick 1992; De Craen et al. 1999; Green and Madgwick 1991; Tazaki 2000; Raiswell 1976; Stocks et al. 1999). This is supported by the findings of mineralized remains of the fossil cyanobacteria and the bacteria buried in nodules substrate. Unfortunately, in carbonate formations such finds are rare. Therefore, this paper focuses on the study of carbonate nodules. Probably, the depositions of such mineral aggregates are biochemogenic in nature and occur as a result of catalytic influence of bacterial activity. In these cases it is necessary to adapt the analytical research techniques used in the physics and chemistry of minerals, which may reveal the implicit role of bacteria in concretion formation. And of course, the experimental evidences are the best way to prove the hypothesis.

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1 Materials

For this study, we collected 40 samples from gypsum-dolomite Upper Permian (P_2 kz_2) deposits on the right bank of the Volga river (Russia, the western part of the Republic of Tatarstan, 50–100 km downstream of Kazan: Pechischi, Krasnovidovo, Antonovka, Yashelcha, Tenishevo piers) (Fig. 2). The carbonate sediments, formed in the shallow marine-salinated basin, are dominating this area. The brachyanticlinal uplift and the small folds characterize the structural features of this area, but the rocks show no signs of regional metamorphism, inheriting the outlines of the relief on the roof of the Upper Devonian (Geology 2007). However, some local changes of the rocks due to the penetration of hydrocarbon fluids are observed in the vicinity of brachyanticlinal uplifts (Syukeevskoe, Kama-Ustyinsky, Krasnovidovskoe, Verkhneuslonsky, Sviazhsky at alias) (Geology 2007; Korolev et al. 2014).

Nodules in the outcrops occur at several stratigraphic levels of upper Kazanian horizon: Prikazanskaya, Pechishchinskaya, Verkhneuslonskaya, and Morkvashinskaya stratas (Geology 2007). They are composed of dolomite, silica, dolomite-silica (chalcedony) or dolomite-opal. Moreover, silica (chalcedony) appears in different forms: fine-dispersed, lumpy, or localized in the nucleation center of nodules (Fig. 3a). The color of the silica component of gray or black depends on ultrafine and nano-sized inclusions of pyrite (melnikovite), magnetite, and maghemite. The maximum size of up to 60 cm is typical to purely dolomite nodules, whereas the dolomite-opal nodules are of the minimum size of 1–3 cm. The dominating shapes are spheroids (Fig. 3b) or flattened spheroids (Fig. 3c), rarely—spheroids with rim (Fig. 1a).

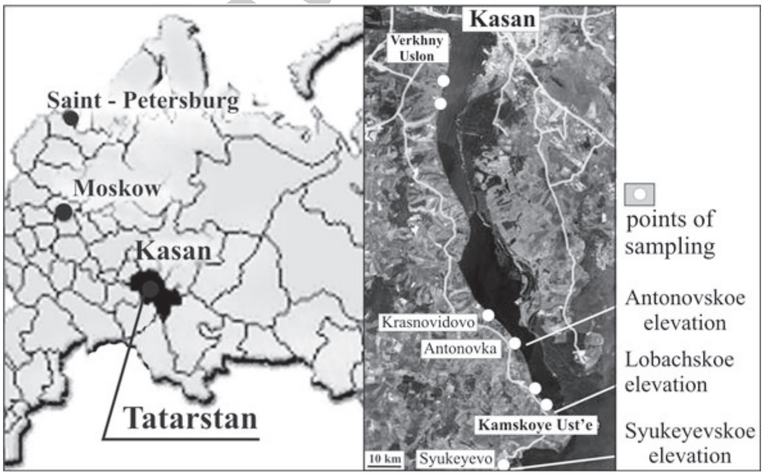


Fig. 2 Survey maps of studied area

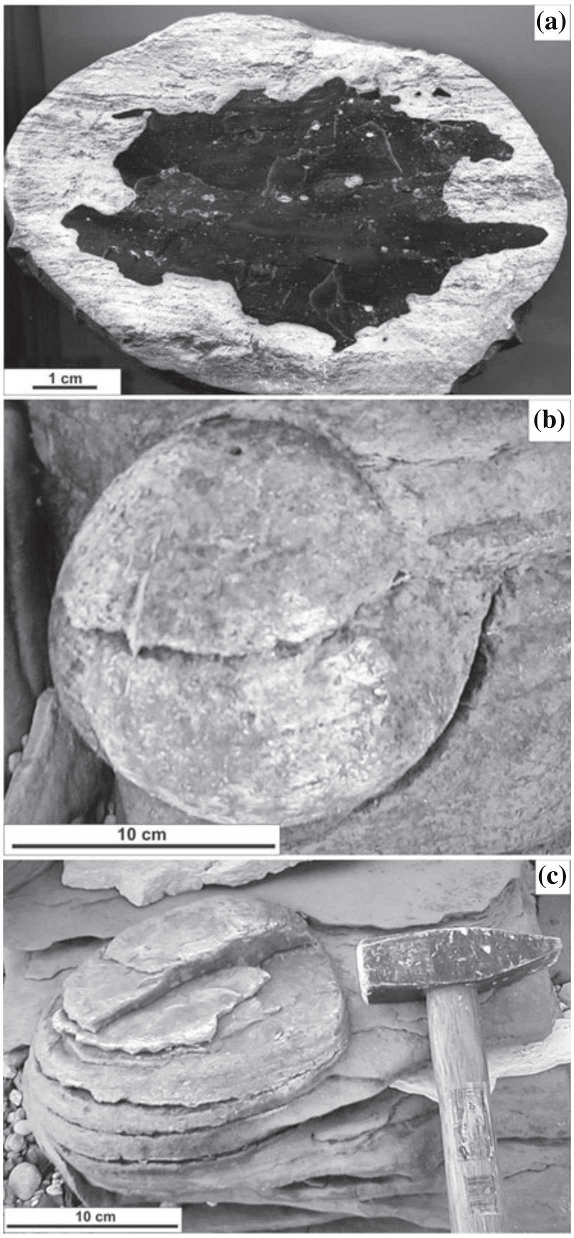


Fig. 3 **a–c** Nodules from *right* bank of the Volga river, v. Krasnovidovo: **a** chalcedony component is localized in the central part of dolomite concretion; **b** spheroidal dolomite concretion in dolostone; **c** flattened-spheroidal dolomite concretion in dolostone

2 Methods

In this study, we used the following physical techniques:

- electron paramagnetic resonance (EPR) (spectrometer EPR-PS100.X operating at 9.772 GHz), used to reveal the remains of fossil organic matter in mineral matrices of concretions, and to study point defects in crystal structure (radiation centers) of carbonate minerals of host rocks and nodules;
- experimental laboratory-scale modeling of natural biocarbonate deposition by microbial communities;
- scanning electron microscopy (SEM) (JSM-6390LV JEOL) with energy-dispersive spectrometer (EDS—Inca Energy 450), used to study mineral aggregates and microfossils micromorphology, and to determine the chemical composition of the minerals. The fractured and polished surfaces of samples were carbon coated for microscopic purposes.
- powder X-ray diffraction (XRD) (X-ray Diffractometer Shimadzu XRD-6000, CuKα, Ni filter, 30 kV, 20 mA, scanning in 2θ range from 02° to 35° and from 15° to 55°), used to characterize the crystal structure determination of mineral composition of nodules and microbial carbonates, deposited in laboratory.

3 Justification of Selected Methods

A prerequisite for the use of EPR technique in our work were the previously obtained spectral characteristics of carbon radicals in organic matter (OM) for the most common fossil residues of different age, genesis, and manifestation forms in the sedimentary rocks (Votyakov et al. 2005; Murav'yev 2007; Soroka et al. 2007; Conard 1984). Depolymerization and repolymerization products of proteins, lignin, and cellulose, such as fragments of aminoacids and polysaccharides, usually contain uncompensated bonds which can be locally stabilized in the form of carbon-centered free radicals (R_C-org) with paramagnetic properties. Being fossilized, these radicals can be preserved within the mineral matrix for a long period of geological time (Votyakov et al. 2005). The concentration of these paramagnetic centers in fossils can be changed during the specific heat treatment in laboratory conditions (Fig. 4a). The characteristic parameters of their EPR spectra are the g-value, the line width (ΔH, mT), and the line shape (mainly Lorentzian).

By measuring the paramagnetic properties and their temperature dependence, one can distinguish the following types of fossil OM:

- humus-sapropel matter metamorphized at early stages of diagenesis. EPR spectra with $g \sim 2.0031$ and $\Delta H \sim 0.5 \div 0.9$ mT are observed in the raw samples and gradually disappear when treated up to 350 °C (Fig. 4b).

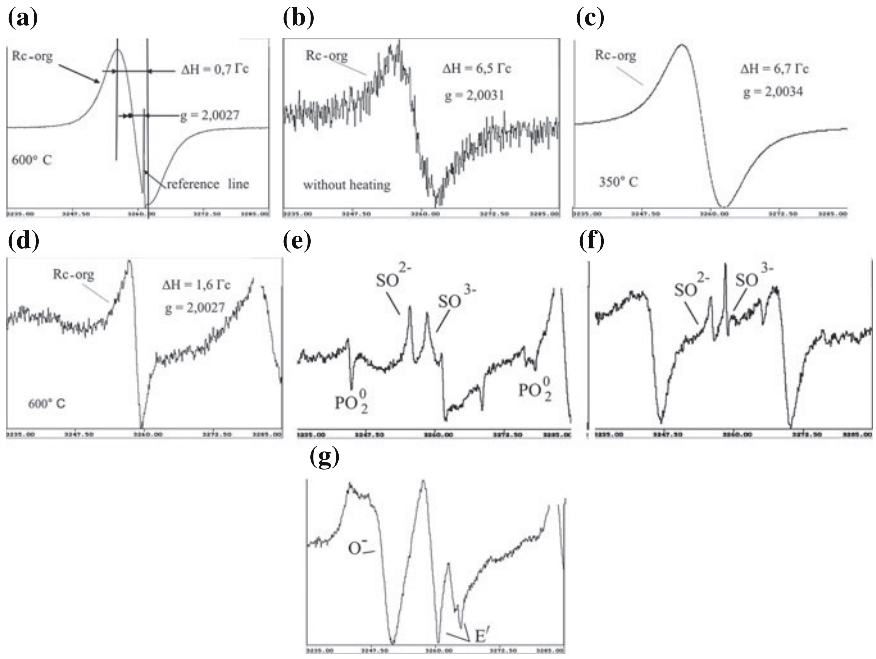


Fig. 4 **a**–**g** EPR spectra in the range of radical lines: **a** characteristics of the resonance lines in spectrum of carbon-centered organic free radicals R_C-org (g-factor is a dimensionless parameter, defining the Zeeman splitting of spin multiplets for a given atoms, ΔH—line width in mT ($\sim$10 Gs), and the upper temperature limit of stability range for paramagnetic properties of radicals); **b** typical R_C-org EPR spectrum ($g \sim 2.0031$, $\Delta H \sim 0.5 \div 0.9$ mT) of the humus-sapropel organic matter which is observed in the raw sample and disappears after heat treatment; **c** R_C-org spectrum of fossil OM of plant origin ($g \sim 2.0030 \div 2.0038$, $\Delta H \sim 0.4 \div 0.7$ mT, 350 °C); **d** typical R_C-org EPR spectrum of organic residues of animal, including bacterial, protein (collagen) ($g = 2.0027 - 2.0028$, $\Delta H \approx 0.5 \div 0.4$ mT, upper temperature limit of stability range is 600 °C); **e** radiation centers SO_2^-, SO_3^-, PO_2^0 in dolomite; **f** radiation centers SO_2^-, SO_3^- in calcite; **g** radiation centers O^- и E′ in silica components

- plant matter (lignin, cellulose). EPR spectrum with $g \sim 2.0030 \div 2.0038$ and $\Delta H \sim 0.4 \div 0.7$ mT can be observed in the raw samples and gradually increases when treated with maximum intensity at 350 °C and then disappear (Fig. 4c).
- OM of animal origin (collagen), including bacteria, is characterized by EPR spectra with—$g = 2.0027 - 2.0028$, $\Delta H \approx 0.05 \div 0.4$ mT, which disappears at treatment above 600 °C (Fig. 4d).

At high degree of natural coalification of OM, all the above-mentioned signals R_C-org can be observed in raw samples and disappear when treated to 350 °C or 600 °C depending on the original nature (Murav'yev 2007; Khasanov and Galeev 2008; Conard 1984).

The presence of radiation paramagnetic centers in the EPR spectra of minerals indicates that these minerals were not subjected to recrystallization because defect structure of minerals is unique for a given sediment's formation conditions. Similarity in a set of radiation paramagnetic centers for nodules and surrounding rocks may indicate their syngenetic origin.

The sedimentary carbonate rocks contain relatively minor quantities of radioactive constituents in comparison with others. The detectable concentration of radiation-induced defects in crystal lattice of carbonate minerals may appear only in the early stages after deposition, before burial compaction, due to the influence of cosmogenic radioisotopes dissolved in shallow marine or pore waters. Primarily, the radiation-induced defects appear as metastable recharging of host lattice ions CO_3^-, CO_3^{3-}, and in part CO_2^-. Over geological time, these excess charges are redistributed in lattice and accumulated in the vicinity of impurities to form radicals SO_2^-, SO_3^-, PO_2^{2-} (or PO_2^0), and CO_2^- (Fig. 4e and f), which are commonly observed in Mesozoic, Paleozoic, and Proterozoic carbonates. For silica oxide minerals there are well-known radiation-induced centers related with oxygen ions O^- and E′ (Fig. 4g).

Experimental laboratory-scale modeling of natural biocarbonate deposition by microbial communities allows one to study not only the stability of community structure itself and pure cultures of bacteria, but also to estimate their role in carbonate deposition. As a primer, we used natural microbial community, combined with the habitat of various geo ecosystems from Shulgan-Tash cave (State Nature Reserve, Republic of Bashkortostan, Irgizly village). The aim of the experiment no. 1 was to identify the nutrient media, the most favorable for the development of bacterial communities and carbonate deposits. A Petri dish with bacterial colonies on an agar-based growth medium Variant 3 were inoculated by mineral pieces of needle-fiber (NFC), pool fingers (lake aggregates of calcite), and soil from the cave hall "Raduzhny" where gypsum crystals are growing. The most productive variants of culture media were used for the following experiments. Experiment no. 2 was conducted to study the newly formed biocarbonates and possible intermediate phases by the use of analytical research techniques: SEM, EDS, and XRD. Microbial isolates for this series of experiments were grown in medium variant 3 (Danielli and Edington 1983), g/l (modified): KNO_3(0.5); Na_2HPO_4*12H_2O (0.25); Ca succinate (5.0) (succinic acid (3.54); $CaCl_2$*2H_2O (3.3)); Oxoid agar (15.0); pH 7.1, and also in Grans medium (Mason-Williams 1969) g/l (modified): Ca malate (5.0) (malic acid (4.02); $CaCl_2$*2H_2O (3.3)); KNO_3 (0.5); Na_2HPO_4*12H_2O (0.25); Oxoid agar(15.0); pH 7.2. The isolates were grown in the growth medium peptone water g/l (modified) (Antipchuk 1979): Peptone (5.0); K_2HPO_4 (1.0); KH_2PO_4 (1.0); $MgSO_4$*7H_2O (0.5); $CaCl_{2-3}$; NaCl a very small content, were studied only by XRD.

The samples were prepared as follows: microbial community and isolates were plated on solid culture media (the crops for XRD were duplicated in liquid media) and cultured for 30 days. The specimens were prepared from these substrates including the spent culture medium with biofilm and the crystalline phase of newly formed carbonate minerals of different dimensions.

The substrate was dried at a temperature not higher than +25 °C and then the piece cut out film with crystals was mounted on carbon adhesive tape and placed in vacuum coating systems for SEM. To study the newly formed carbonates by XRD, the bacterial biomass were scraped from the culture media and frozen in eppendorf polypropylene tubes at −30 °C. Grown in liquid media carbonates and microbial biomass were centrifuged at 5000 rpm and then frozen for transport to XRD laboratory. It is assumed that in this manner the transformation of uncontrolled hydrocarbonate into carbonate will proceed slowly that allows to reveal all the phases of the newly formed carbonates. Unoriented samples for XRD analyses were prepared by drying the gel on rectangular glass plates. Directly before the analytical procedure ethyl alcohol was added to the glass slide with the sample for accelerated drying.

4 Results

The study of the mineral composition of fossil nodules showed that they can have monomineral composition of dolomite or chalcedony, mixed composition of dolomite-chalcedony or of opal-dolomite, and finally nodules can be composed of alternating opal and chalcedony layers cementing fine dispersed dolomite grains or clusters of irregular shape.

5 Electron Paramagnetic Resonance

The results of the EPR study are present in Table 1 for the samples of most representative mineral associations:

No. 1—dolomite, no. 2—black chalcedony concentration in middle of the dolomite concretions, no. 3—opal-dolomite mineral association, and no. 4—black pure chalcedony in dolomite host rock. EPR spectra of radiation centers SO_2^-, SO_3^- of dolomite are registered both in nodules and host rocks, the similar pattern observed for E′ centers of chalcedony. This indicates that the investigated objects were formed together with surrounding rocks, and never were subjected to recrystallization during diagenesis and regional metamorphism in subsequent periods. Furthermore, the signals R_C-org with $g = 2.0027$, $\Delta H \approx 0.14$–0.16 mT, typical for nonmetamorphized residual organic matter of animal protein (collagen), occur in EPR spectra only after treatment at 600 °C, and there were no any spectra registered which can be related with metamorphized OM, as well as with humus-sapropel or plants matters. It should be noted that measured concentration of paramagnetic centers R_C-org in concretion was sometimes considerably higher than in host rock. The well-preserved fossil OM within mineral matrices can be the evidence of high mineralization speeds and microbial activity during concretion growth because no faunal remains were found neither in concretions or in nearby host rocks.

Table 1 EPR spectral characteristics of studied nodules

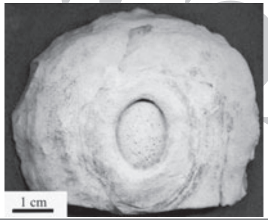
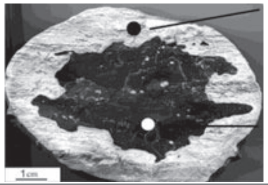
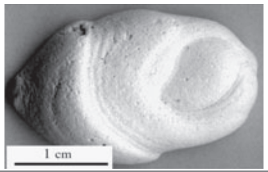
No.	Sample	Mineral composition	The presence of microfossils	The EPR characteristics of the signals in the field of radicals				
				Radiation centers		Radicals of organic carbon		
				E′	SO_2^- SO_3^-	Without heating	350 °C	600 °C
1		Dolomite	−	−	+	−	−	+
2		Dolomite	−	−	+	−	−	+
		Black flint	+	+	−	−	−	+
3		Dolomite-opal	+	+	−	−	−	+

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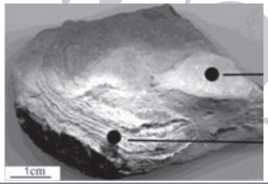
Table 1 (continued)

No.	Sample	Mineral composition	The presence of microfossils	The EPR characteristics of the signals in the field of radicals				
				Radiation centers		Radicals of organic carbon		
				E′	SO_2^- SO_3^-	Without heating	350 °C	600 °C
4		The host rock dolomite	–	–	+	–	–	+
		Black flint	–	+	–	–	–	+

6 Scanning Electron Microscopy

SEM and EDS analyses show that most nodules are composed by flocculant, drusy aggregations, and carbonate microcrystals (Fig. 5a) with a morphology different from rhombohedral grains of host rocks. Crystals and druses are characterized by similarity in size and by repeatability in shape. However, microbial fossils in the nodules are rare (Fig. 5b). Opal-dolomite nodules are always fine-grained mineral association, sometimes forming hollow spheroids (Fig. 5c), which possibly are mineralized remains of bacterial colonies.

Samples deposited in the laboratory experiment no. 1 contain carbonate crystals (Fig. 5d) similar in morphology to those in fossils or have a specific shape (Fig. 5e, f). There are crystals with biofilm on their surface (Fig. 5g). Microbial-induced precipitates form microscopic nodules (Fig. 5h and i). In experiment no. 2, we established that in pool fingers community, the abundance of microorganisms able to grow in media with organic acids (malic, succinic—Grans,

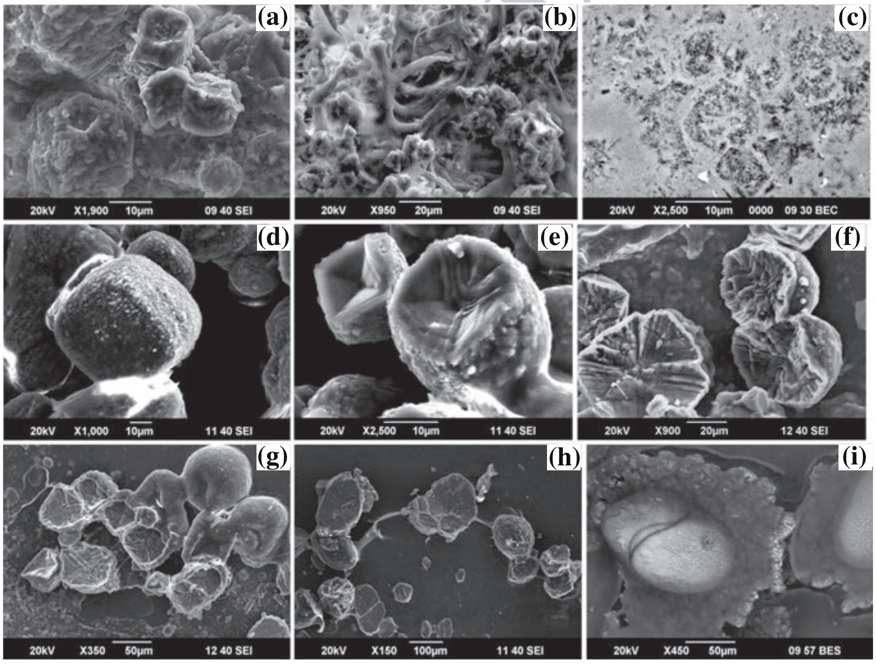


Fig. 5 **a**–**i** SEM images of carbonate nodules: **a** specific crystals of carbonates (dolomite and high-Mg-calcite) that constitute nodules in the Upper Permian deposits; **b** bacterial microfossils in the dolomite nodules; **c** microstructure in opal-dolomite nodules from the Upper Permian deposits; **d** bacterial controlled carbonate sediments in experiment No. 1; **e**, **f** bacterial controlled carbonate formation of a specific habitus, experiment No. 1; **g** subsequent stages of carbonate crystals encapsulation by biofilm, which deposits calcium carbonate; **h**, **i** carbonate microconcretions in experiment No. 1

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Table 2 Colony of microorganisms selected to study the mineral sedimentation in bacterial mats

Strains	Culture media	The shape of the cell	Description colony: color, colony size	Color gram*	The consumption of glucose	Aerobic growth	The habit of the crystals, Plate 1
1–2a	Variant 3	Sticks	Colorless with yellow paint small colony	+	+	±	a
1–2b	Variant 3	Sticks	Colorless small colony	−	+	±	b
1–3	Variant 3	Cocci	Yellow large colony	−		+	c
1–3a	Variant 3	Cocci	Yellow small colony	+	+	+	d
1–4	Variant 3	Sticks	Colorless large butyrous colony	−	+	+	e
1–5	Variant 3	Cocci	Orange small colony	−	−	+	f
1–6	Variant 3 + peptone	Sticks	Colorless large butyrous colony	−	+	±	g
2–2	Grans medium	Sticks	Colorless large butyrous colony	−	−	±	h
2–5	Grans medium	Cocci	Yellow large colony	−	+	±	i
2–6	Grans medium	Sticks	Colorless large colony	−	+	+	j
2–7	Grans medium	Sticks	Colorless small colony	−	+	+	k

* Test: + Positive, − Negative, ± Optional gone anaerobic

variant 3, and variant 3 + peptone) was about 1.8 ÷ 4.6 × 10^6 KFU/g. From this broad microbial diversity after several passages and exceptions (loss of ability to precipitate minerals, pollution by fungi at alias), we studied the physiological and biochemical properties of the remaining 11 isolates in order to identify them and to estimate their ability to precipitate minerals. Selected cultures were represented by aerobes and facultative anaerobes, mainly of gram-negative rod-shaped bacteria (6 isolates) and of cocci (3 isolates), and only by two gram-positive isolates (Table 2). The difference in morphology of biominerals precipitated by different isolates was established from SEM analyses. The microscopic images of biocarbonate dominating forms are shown in plate (Plate 1a–l). The influence of nutrient medium on nodules morphology was negligible. As it is seen in Fig. 3, the different isolates 1–5 (Plate 1f), 2–5 (Plate 1i), 2–7 (Plate 1k) precipitate aggregations of similar morphology, even if the isolate 1–5 was cultivated on the medium Variant 3, whereas the isolates 2–5 and 2–7 on Grans medium (Table 2). When observed at high magnification (more than ×4300), certain bacterial cells were found to be buried among crystal particles (Plate 1l).

7 X-ray Diffraction

XRD data indicate that calcite is the major crystalline component in sediments for all the analyzed samples. The amount of noncrystalline material (gel) evidenced by the XRD patterns of random mounts which exhibited high background levels and two humps with very broad maxima near 3.5 and 2.2 Å (Fig. 6). Nevertheless, distinct calcite peaks were detected on XRD patterns of such samples. As the gel in the samples dry, the calcite reflections on XRD patterns become more intensive and

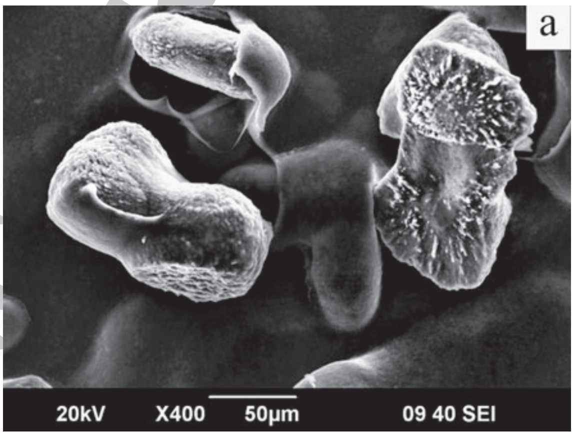

Plate 1 **a–l** SEM images of biocarbonate precipitates in experiment no. 2

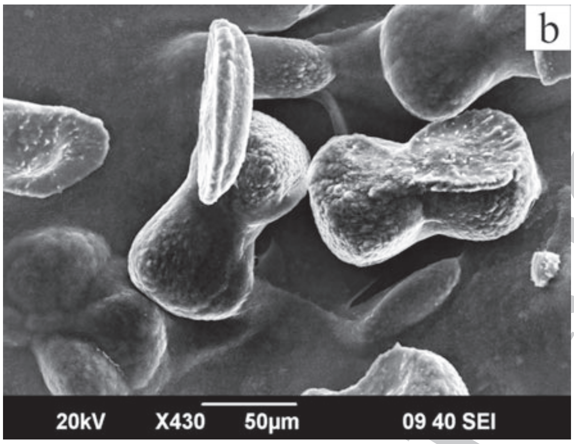

Plate 1 (continued)

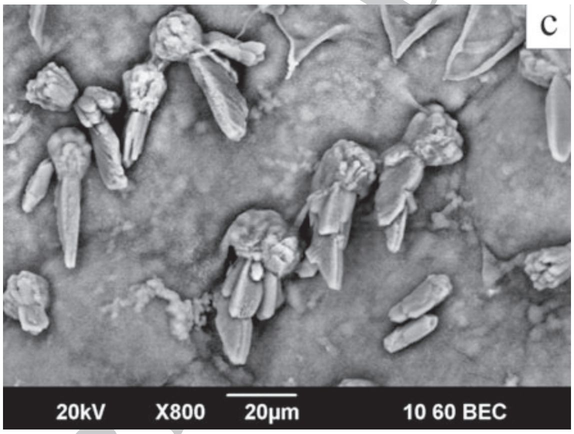

Plate 1 (continued)

narrow. Cell parameters have been determined for the biogenic calcites from the investigated samples (where possible, with Si as internal standard). Values of these parameters are very close to one another and have minimal deviations (Table 3).

It should be noted that in experimental precipitation induced by bacteria (Wei et al. 2011), the calcite was a dominant mineral phase. Some crystals after experimental interaction with bacteria had bacterial imprints on crystal surfaces. In spite of differences in the tasks of experiments, strains, and mediums chosen, calcite

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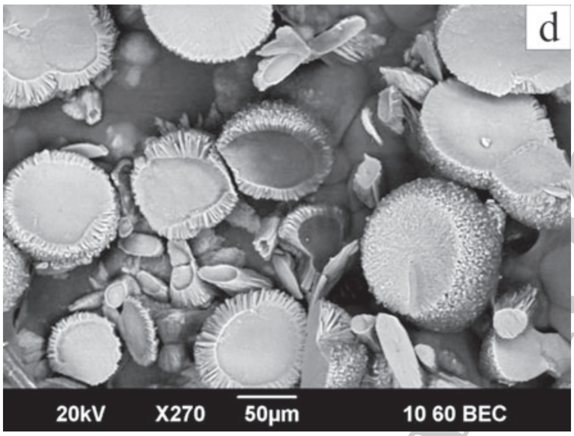

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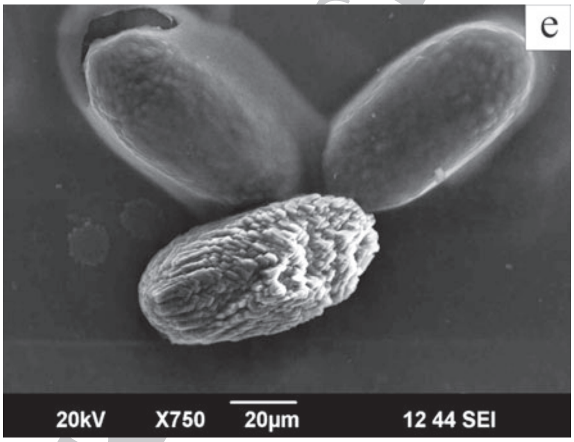

Plate 1 (continued)

precipitates regularly with increasing the pH of the medium. The presence of Mg salts in the nutrient solution leads to the precipitation of Mg-calcite.

Presence of Mg-calcite was determined in the sample A formed on Peptone nutrient solution ($MgSO_4$*$7H_2O$—0.5; $CaCl_2$—3 g/l). Cell parameters of this mineral are in good agreement with other data for biogenic Mg-calcite (sample A in Table 3) (Bischoff et al. 1983; Paquette and Reeder 1990).

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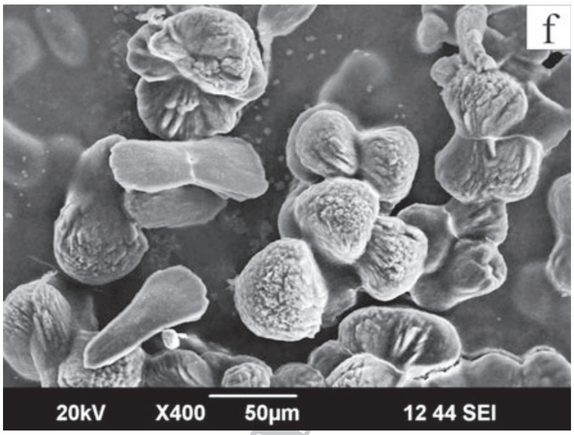
Plate 1 (continued)



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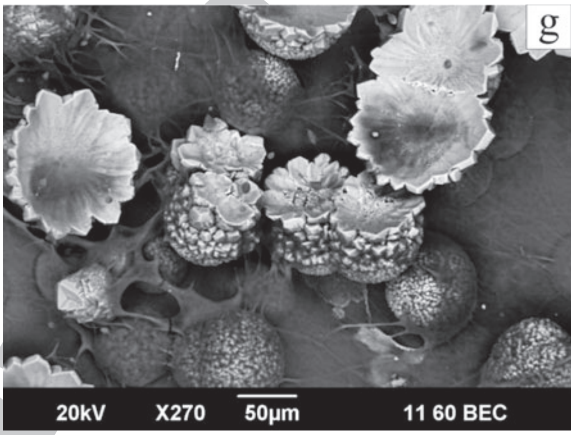

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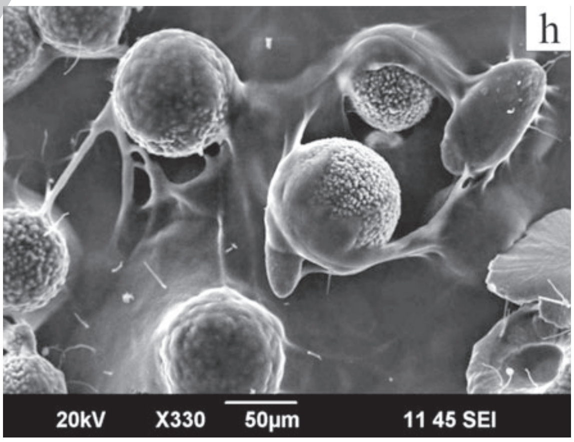

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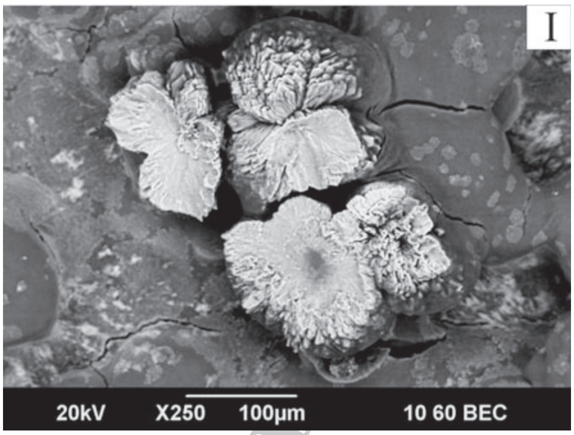
Plate 1 (continued)



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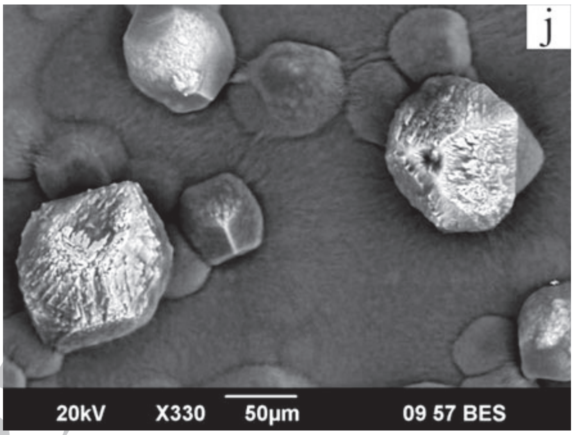

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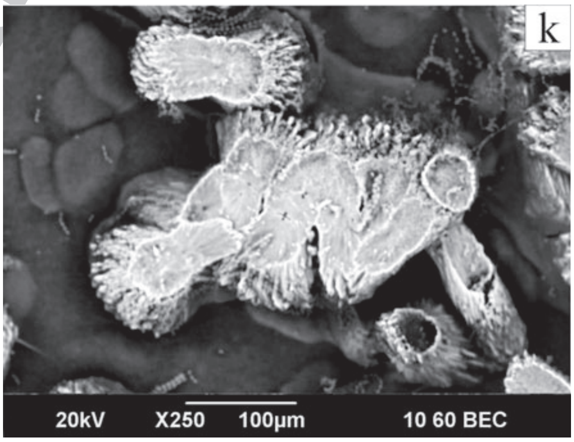

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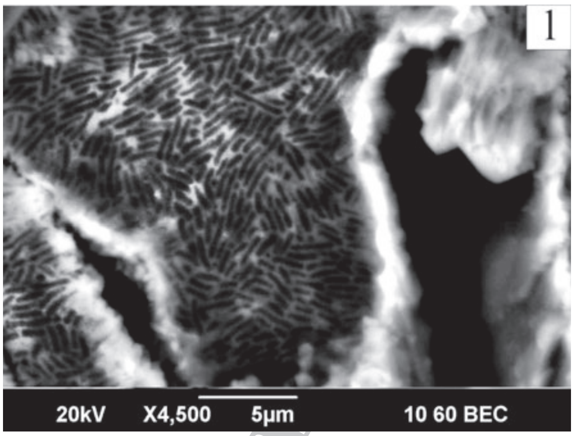

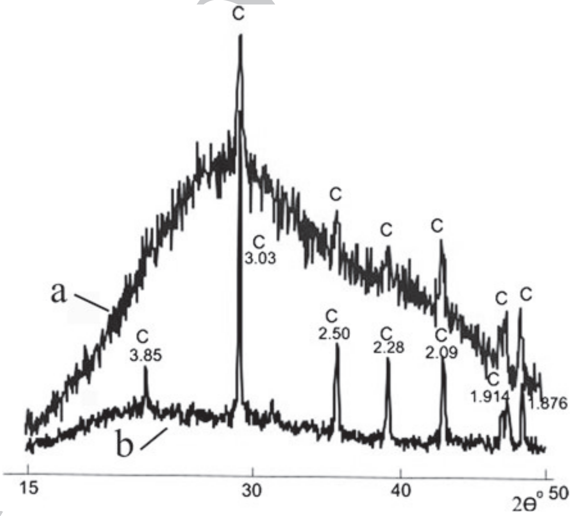

Fig. 6 X-ray diffraction patterns of sample 2–6: *a* gel state, *b* air-dried. *c* calcite. Spacing in Å

8 Conclusions

The complex study of structural and spectral properties of minerals in carbonate nodules evidences on microbial involvement in the genesis of studied natural and laboratory grown samples.

From the results obtained, one can suggest the following conclusions. (1) Studied natural nodule, as well as surrounding rocks, was not subjected to significant regional metamorphization over time after sedimentation. This is evidenced by the presence of stable paramagnetic radiation centers in the crystal lattices of minerals forming nodules and host rocks, as well as by the presence of

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Table 3 The results of XRD analysis of crystalline material precipitated by the bacterial isolates

No.	No. of sample	Content	Cell parameters	
			a, Å	c, Å
1	1–2a	Calcite	4.9810 (0.0012)	17.0367 (0.0081)
2	1–2б	Calcite	4.9784 (0.0010)	17.0324 (0.0146)
3	1–3	Calcite	4.9805 (0.0005)	17.0158 (0.0040)
4	1–4	Calcite	4.9817 (0.0009)	16.9963 (0.0082)
5	1–5	Calcite		
6	1–6	Calcite	4.9816 (0.0007)	17.0237 (0.0103)
7	2–2	Calcite	4.9763 (0.0012)	17.0529 (0.0201)
8	2–5	Calcite		
9	2–6	Calcite	4.9800 (0.0007)	17.0086 (0.0052)
10	2–7	Calcite		
11	A	Calcite	4.9808	17.0295
11	1–6-liquid	Calcite, halite	4.9816 (0.0009)	16.9952 (0.0075)
12	Control	Halite		

animal protein (collagen) residues in fossil OM of low metamorphization stage. (2) Similarity in crystal lattice defects for both the dolomite in nodules and host rocks confirms their formation during synsedimentary early diagenesis; (3) Since the rocks nearby sampling areas are depleted in fossil macrofauna, one can assume that the fossilized remains of animal protein occured as a result of bacterial activity, while nodules themselves are the lithified microbial constructions. Findings of fossil bacteria additionally support this suggestion. (4) Experimental laboratory-scale modeling of natural carbonate deposition by microbial communities confirms that bacteria can promote nodules formation.

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Reference

Antipchuk AF (1979) Microbiological control in ponds.M. Food Industry
Antoshkina AI (2012) Bacterial lithogenesis. In: Obzor konceptual'nykh problem litologii. In: A Review of Conceptual Problems of Lithology, Moscow: GEOS, pp 89–105
Berner RA (1971) Bacterial processes effecting the precipitation of calcium carbonate in sediments. Johns Hopkins Univ Stud Geol 19:247–251
Bischoff WD, Bishop FC, Mackenzie FT (1983) Biogenically produced magnesian calcite: inhomogeneities in chemical and physical properties comparison with synthetic phases. Am Mineral 68:1183–1188
Conard J (1984) EPR in fossil carbonaceous materials. In: Petrakis L, Fraissard JP (eds) Magnetic resonance. Introduction. D. Reidel Publishing Company, Hingham, pp 441–459

Danielli HMC, Edington MA (1983) Bacterial calcification in limestone caves. Geomicrobiol J 3:1–15

De Craen M, Swennen R, Keppens EM, Macaulay CI, Kiriakoulakis K (1999) Bacterially mediated formation of carbonate concretions in the Oligocene Boom Clay of northern Belgium. J Sediment Res 69(5):1098–1106

Geology of Near-Kazan region (2007) Guidebook for field student geological practice. In: Shevelev AI (ed) “Novoie znanie”, Kazan

Green AC, Madgwick JC (1991) Microbial formation of manganese oxides. Appl Environ Microbiol 57:1114–1120

Khasanov RR, Galeev AA (2008) Evolution of sin-genetic organic material in Paleozoic deposits of central part of Volga-Ural anteclise. Science. Proceedings of Kazan University. Series of Natural Sciences, vol 150, 3:152–161

Korolev EA, Khuzin IA, Galeev AA, Leonova LV (2014) Epigenetic transformation of dolomite rocks under the influence of hydrocarbonaceous fluids (by the example of Syukeevskoe bituminous deposit). Geol Oil Gas 5:28–32

Mason-Williams MA (1969) Microorganisms in relation to food and energy sources in caves. Proc Br Speleol Assoc 4:69–74

Murav’yev FA (2007) Lithological-mineralogical characterization of Permian marking calcareous horizons in Tatarstan. PhD thesis. Kazan

Paquette J, Reeder RJ (1990) Single-crystal X-ray structure refinements of two biogenic magnesian calcite crystals. Am Mineral 75:1151–1158

Raiswell R (1976) The microbiological formation of carbonate concretions in the Upper Lias of NE England. Chem Geol 18:227–244

Skinner HCW, Fitzpatrick RW (eds) (1992) Biomineralization processes of iron and manganese-modern and ancient environments. CATENA, (Supplement 21)

Soroka EI, Leonova LV, Galeev AA, Gulyaeva TY (2007) EPR properties of organic component of some high-aluminous rocks of Urals. Lithosphere 4:125–128

Stocks-Fischer Sh, Galinat JK, Bang SS (1999) Microbiological precipitation of $CaCO_3$. Soil Biol Biochem 31:1563–1571

Strakhov NM (1962) Foundation of lithogenesis. vol 2

Tazaki K (2000) Formation of banded iron-manganese structures by natural microbial communities. Clays Clay Miner 48(5):511–520

Votyakov SL, Galeev AA, Leonova LV, Galakhova OL, Il’inykh AS (2005) EPR as method of investigation of organic component at biogenic calcareous rocks (Riphean stromatolite-containing rocks from South Urals as an example). Yearbook, Ekaterinburg, pp 39–47

Wei L, Li-Ping L, Peng-Peng Zh, Long C, Long-Jiang Y, Shi-Yun J (2011) Calcite precipitation induced by bacteria and bacterially produced carbonic anhydrase. Res Art Curr Sci 100 (4):502–508